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Ecology and Management of Invasive Plants

2nd Edition

Edited by
Congyan Wang and Hongli Li

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Guest Editors

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Review

Understanding the Influence of Secondary Metabolites in Plant Invasion Strategies: A Comprehensive Review

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Abstract: The invasion of non-native plant species presents a significant ecological challenge worldwide, impacting native ecosystems and biodiversity. These invasive plant species significantly affect the native ecosystem. The threat of invasive plant species having harmful effects on the natural ecosystem is a serious concern. Invasive plant species produce secondary metabolites, which not only help in growth and development but are also essential for the spread of these plant species. This review highlights the important functions of secondary metabolites in plant invasion, particularly their effect on allelopathy, defense system, interaction with micro soil biota, and competitive advantages. Secondary metabolites produced by invasive plant species play an important role by affecting allelopathic interactions and herbivory. They sometimes change the soil chemistry to make a viable condition for their proliferation. The secondary metabolites of invasive plant species inhibit the growth of native plant species by changing the resources available to them. Therefore, it is necessary to understand this complicated interaction between secondary metabolites and plant invasion. This review mainly summarizes all the known secondary metabolites of non-native plant species, emphasizing their significance for integrated weed management and research.

Keywords: allelopathy; invasive species; herbivory; integrated weed management; phytochemicals

1. Introduction

In recent years, along with the development of traffic networks, the increase of human activity and the strengthening of international trade, the interaction among biological species from diverse habitats has significantly increased. Some of these species have shown a high ability to adapt to their new environments, allowing them to flourish and spread quickly. This trend has adversely affected the local economy, ecology, and society [1]. Plant species have been unintentionally or purposefully brought to other continents for ornamental purposes. Due to climate change, species distributions have shifted over the last few decades [2]. Biological invasions are caused by species reorganization and climate change, which allow exotic species to grow progressively in native ecosystems [3]. Plant invasion is a primary threat to natural ecosystems and causes global issues [4]. As plants invade new areas, the introduction of invasive species may have a significant negative impact

on local plant species. Decreasing the richness or diversity of native plants potentially leads to limited diversity in numerous portions of the invaded range [5]. In fact, similar ecological effects have been seen in invaded communities by invasive species, including *Centaurea maculosa*, *Solidago canadensis*, *Mikania micrantha*, *Alternanthera philoxeroides*, *Eupatorium odoratum*, and *Fallopia japonica* [6]. However, the specific mechanisms that lead to this dominance remain elusive and require further investigation for clarification [7].

1.1. Invasion Mechanism

Several theories exist on the potential for exotic plants to become invasive [8]. Plant invasions are major mechanisms described in the literature, such as:

1.1.1. The Enemy Release Hypothesis

The enemy release hypothesis is known as the predator escape or ecological release. The herbivore escape hypothesis refers to the absence of competitors, predators and pathogens in the introduced range as the main advantage for an introduced species [9]. The absence of predators allows the plant to allocate energy and resources towards competitive traits [10]. The enemy escape hypothesis suggests that: (a) natural enemies play a crucial role in shaping and controlling plant populations; (b) native plant species are generally more susceptible to biocontrol agents than non-native species; and (c) the reduced presence or absence of natural enemies contributes to the accelerated growth and proliferation of exotic species [11]. Based on these assumptions, the main method for controlling exotics was the introduction of biocontrol agents, which has not always been successful [12].

1.1.2. The Novel Weapons Hypothesis

The novel weapons hypothesis suggests that plants use biochemical mechanisms to conquer new environments [13]. Invasive species not only escaped from their natural enemies but also brought novel mechanisms of interaction to invade plant communities [14]. These exotics possessed phytochemicals, unique metabolites, and biochemical mechanisms that recipient communities have not previously encountered. Invasive plant secondary metabolites (chemical weapons) have interfered with native plants and were successfully established in the native range [15]. Nowadays, recent research has shown a diversity of phytochemical differences between native and invasive plant species [14], and these differences can be distinguished by metabolomics and metabolic profiling. Allelopathy is also an important phenomenon in which the invasive plant releases some phytochemicals and retard the growth and development of neighboring plants [16]. Most invasive plant species release allelochemicals (phenolic compounds, alkaloids, and terpheniods) into the nearby environment by litter or root exudation [15]. These phytochemicals influence the growth attributes of nearby plants, such as root growth, germination, and nutrient intake. It is necessary to figure out why invasive plant species spread quickly in an area and how to manage them. We need to know how allelopathy works.

1.1.3. Resistance Against Herbivores

The main factor in the success of invasive plant species is the resistance against herbivores. Invasive plant species use different strategies to compete with native plants and protect themselves from herbivores [17]. These defense mechanisms against herbivores are the production of secondary metabolites (phenolic compounds, terpheniods, and alkaloids) that might be poisonous or repel herbivores [18]. In a new environment, the invasive plant species can decrease the herbivory attack and focus on more resources to grow and reproduce by becoming dominant over the native plant species.

1.1.4. Secondary Metabolites

Invasive plant species utilize various secondary metabolites, which enhance their competitive ability over native plants and help in nutrient uptake. Malic acid and citric acid are the organic acids produced in invasive plant species' rhizosphere. The function of

these compounds is to solubilize nutrients, such as phosphorous, so that it is easy for the plant roots to absorb [19].

1.1.5. Antimicrobial Abilities

Antimicrobial abilities are another mechanism of invasive plant species that enables them to invade successfully [20]. Invasive plant species produce secondary metabolites such as phytoalexins, alkaloids, and essential oils, which are rich in compounds like limonene, pinene, terpinene, and caryophyllene [21]. These metabolites possess antimicrobial properties that alter the microbial ecosystem, creating favorable environmental conditions that support the growth and establishment of invasive plants while inhibiting the growth of native plant species [22].

1.1.6. Mutualistic Interactions

During the invasion process of invasive plants, secondary metabolites played important mutualistic interactions and served different functions. According to [23], flavonoids, such as kaempferol and quercetin, are important signaling molecules that promote mutualistic relationships with mycorrhizal fungi. These flavonoids act as chemo attractants, facilitating the growth and development of mycorrhizal hyphae and enhancing the invasive plant's nutrition intake. Isoflavonoids, like genistein and daidzein, are produced by leguminous invasive species and play a key signaling role in attracting and forming symbiotic relationships with nitrogen-fixing bacteria in root nodules [24]. The precise role of these secondary metabolites facilitates the beneficial interactions that sustenance the growth of invasive plant species.

In invasive plant species, the production of secondary metabolites is important and gives advantages over native flora, aiding in establishment within new habitats. These phytochemical compounds are used by the invasive plant as a defense against the herbivory. Also, they inhibit the germination, growth, root development, and intake of nutrients of native plant species. Alkaloids and phenolic compounds are examples of these compounds [25]. This enables invasive plant species to invade and grow quickly, making the environment unsuitable for the native plant species. Secondary metabolites from the invasive plant species affect the soil microbial population by preventing beneficial microorganisms and enhancing pathogenic growth [26]. These interruptions in the microbial balance in the soil further strengthen the ability of invasive plant species to control the resources, and the competition they face from native plant species decreases. Invasive plant species produce secondary metabolites that play an important role in determining the ecological interactions within invaded ecosystems, supporting the plant establishment. The generation and discharge of secondary metabolites by invasive plants are crucial in determining the nature of ecological interactions in invaded ecosystems, which, in turn, help these plants establish and proliferate successfully.

Investigating the mechanisms and functions of phytochemical compounds in the invasion of invasive plant species is an important and significant field, highlighting a notable research gap. Much research has provided valuable insights into specific functions, like defense mechanisms, stress tolerance, and allelopathy [27]. It has also been reported that secondary metabolites in the invasive plant species strengthen the plant against herbivory and infection [28]. There is a clear gap in the literature where a comprehensive review combines and explores all these results across various invasive plant species. A comprehensive review is necessary to scientifically evaluate the diversity of secondary metabolites and their complex roles in assisting invasion success. A comprehensive review designed within an integrated framework would reveal both shared and different elements among invasive species, contributing to more integrated interpretations of secondary metabolites' roles in plant invasion dynamics. The current literature commonly focuses on the biochemical roles of secondary metabolites, but there is a scientific gap about the physiological and essential molecular mechanisms regulating these roles. A comprehensive review should not only collect the existing research studies but also focus on the functions

of these aspects. This will guide future research and provide a better understanding of the role of secondary metabolites in plant invasions. Filling this gap will greatly improve our knowledge and help construct effective management strategies.

2. Secondary Metabolites in Invasive Plants

Plants released secondary metabolites, which may have affected the growth and development of nearby plant species. When organic matter breaks down in plants, secondary metabolites are produced and released, which may be reached by precipitation and influence interactions between soil microbes and plants [29]. Phytochemical compounds may be divided into defense compounds and signal substances [30]. Signal substances facilitate allelobiosis, while defense compounds, i.e., allelochemicals, facilitate allelopathy. A maximum of the secondary metabolites is made up of various organic compounds, which may be volatile or non-volatile. The phytochemicals include alkaloids, glycosides, organic acids, phenols, and terpeniods [30]; these compounds are important for regulating the rhizosphere microbiota, proper plant growth, and defense systems [31]. It is essential to understand what makes a plant invasion successful [8,32]. Research in the past showed that to improve their effectiveness, invasive plants produce secondary metabolites [33]. These secondary metabolites have influenced the cycling of nutrients, native plants, and soil microbe [34]. To mediate these effects, both direct and indirect routes can be used. Observing how chemicals from invasive plants affect plant competition—by limiting the growth of nearby plants—provides a straightforward way to understand their effects [35]. According to [36], the growth of the native plants is inhibited immediately by the chemical phytotoxin (2)-catechin emitted from the roots of the invading *Centaurea maculosa*. Also, the seeds of native plants are prevented from germination due to the secondary metabolites produced by invaded invasive plant species [37]. It is clear that the increase in competitiveness of the invasive plant may be due to the inhibition of these direct impacts [34]. Invasive plant species release some phytochemicals, such as alkaloids, phenols, terpeniods, and volatile compounds, which indirectly affect the competition. By changing the cycling nutrients—specifically, soil inorganic and organic nutrients flows and pools—these metabolites might unintentionally promote the growth of invaders [38]. Additionally, invasive plants may compete indirectly by using natural soil microbe. Plant pathogens and symbionts, along with secondary metabolites, have the ability to change the soil's microbial community [39]. These microbes ensure the survival of plants in challenging environments [40]. The reason behind the success of invasive plant invasion over the native plant is the presence of secondary metabolites. Secondary compounds include many substances like growth inhibitors, poisons, and allelopathic chemicals [41]. The following are several secondary metabolites that are mostly present in invasive plants and can harm native plant species.

2.1. Phenolic Compounds

Among the phenolic compounds are tannins, phenolic acids, flavonoids, and coumarins (Figure 1). When present in high concentrations, they can prevent seeds' α-amylase from working, which reduces germination by 18% [14]. Phenolics, which are oxygen radical producers and feeding deterrents, can have adverse effects. It has been discovered that the creation of hydrogen or covalent connections between phenolic chemicals in herbivores' intestinal tracts and food proteins or digestive enzymes inhibits digestion [42]. Fall webworm (*Hyphantria cunea* Drury) digestion and food consumption can be greatly impacted by tannic acid [43]. According to [44], flavonoids not only prevent weed growth and fungal pathogen spore germination but also serve as chemical cues for legumes to modulate, and the root exudates of barley (*Hordeum vulgare* L. cv. 'Barke') contain phenylpropanoids that have antifungal properties. When discharged in high quantities, phenolic compounds can have negative impacts on animals, soil, and groundwater, even if low concentrations of the same chemicals may discourage pests, prevent infections, or promote beneficial species [45].

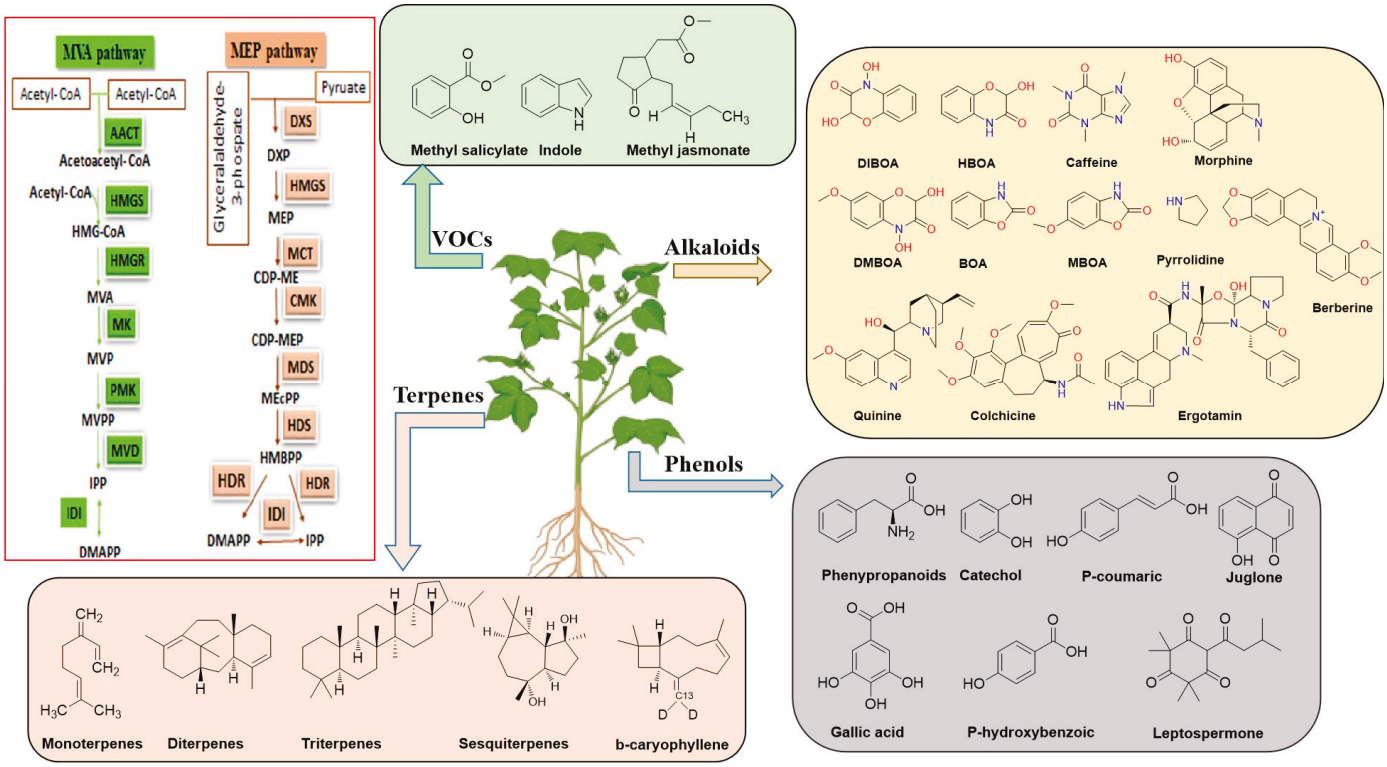

Figure 1. Secondary metabolites in invasive weeds, for the synthesis of isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP), methylerythritol phosphate (MEP), and mevalonate (MVA) pathways responsible. Acetoacetyl-CoA, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), acetyl-CoA, acetoacetyl-CoA, acetyl-CoA, MVA, 5-phosphomevalonate (MVP), and 5-diphosphomevalonate (MVPP) are the intermediaries of the MVA pathway. Acetyl-CoA acetyltransferase (AACT), 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS), mevalonate kinase (MK), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), phosphomevalonate kinase (PMK), diphosphomevalonate decarboxylase (MVD), and isopentenyl diphosphate isomerase (IDI) are the enzymes involved in the MVA pathway. Relatively to the MEP pathway, its intermediaries are D-glyceraldehyde 3-phosphate (G3P), pyruvate, 1-deoxy-d-xylulose 5-phosphate (DXP), MEP, 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol (CDP-ME), 2-phospho-4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol (CDP-MEP), 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (MEcPP), and 1-hydroxy-2-methyl-2-butenyl 4-diphosphate (HMBPP). The enzymes involved in the MEP pathway are 1-deoxy-d-xylulose-5-phosphate synthase (DXS), 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR), 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (MCT), 4-diphosphocyt-idyl-2-C-methyl-D-erythritol kinase (CMK), 2-C-methyl-D-erythritol 2,4-cyclodi-phosphate synthase (MDS), 4-hydroxy-3-methylbut-2-enyl-diphosphate synthase (HDS), and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR).

Eucalyptus species, such as *E. microtheca*, *E. polycarpa*, *E. tereticornis*, and *E. camaldulensis*, have been found to contain some of the possible phenolic allelochemicals in new leaves, bark, and leaf litter leachates. Studies revealed the existence of catechol, p-coumaric, gallic, and p-hydroxybenzoic, which have detrimental effects on crops in the ecosystem, such as black gram (*Phaseolus mungo* L.), by reducing and delaying germination, seedling mortality, and growth and yield reduction [46]. Black walnuts (*Juglans nigra* L.) produce a phenolic chemical called juglone (5-hydroxy-1,4-naphthalenedione) (Figure 1). It is widely recognized to have a detrimental effect on the growth of other plants. The primary enzyme in the manufacture of plastoquinone, hydroxyphenylpyruvate dioxygenase (HPPD), is strongly inhibited by juglone. It also affects the respiratory and photosynthetic electron transport systems [47]. The natural triketone leptospermone (1-hydroxy-2-isovaloryl-4,4,6,6-

tetramethyl cyclohexen-3,5-dione) (Figure 1), produced by the roots of the bottlebrush (*Callistemon citrinus* Curtis), inhibits p-hydroxyphenylpyruvate dioxygenase, contributing to its herbicidal properties. This inhibition causes chlorophyll loss and disrupts carotenoid production. Because of its strong herbicidal effects, commercial development of leptospermone is not possible. Nonetheless, the structure of leptospermone served as a foundation for the creation of synthetic analogs that were utilized to suppress broadleaved weeds in maize. For example, [46] highlight manuka oil, which contains leptospermone as its main active ingredient, can enhance the herbicidal effects of other essential oils. Redroot pigweed, barnyard grass, velvet leaf, and hairy crabgrass showed markedly reduced growth and dry weight when manuka oil (1%) was sprayed on them after they emerged. This type of application offers an additional avenue for using this allelopathic molecule without modifying its chemical structure [48].

2.2. Alkaloids

Alkaloids are vital secondary chemicals in plants with significant physiological and biological effects. Citronella (*Cymbopogon nardus* (L.) Rendle) root extract includes N-octanoyl tyramine, which can prevent Italian ryegrass (*Lolium multiflorum* Lam.), lettuce (*Lactuca sativa* L.), barnyard grass (*Echinochloa crus-galli* L.), and cress (*Lepidium sativum* L.) from maturing [49]. Caffeine (1,3,7-trimethixanthine) is a purine alkaloid found in about 100 plant species. It can be utilized directly as an allelopathic toxin or indirectly as an activator of plant defense systems. It can also strengthen a plant's capacity for defense. Additionally, caffeine can be excreted actively through primary roots, influencing nearby microbial populations [50]. *Echium plantagineum* L. produces harmful pyrrolidine alkaloids to defend against herbivores; naphthoquinone has an effect on insects and livestock and can reduce competition among weeds, insects, and pathogens [51]. Several gramineous species release benzoxazinoids into the rhizosphere to change the bacterial and fungal populations linked with roots and inhibit nearby plants' growth [52]. Moreover, gramineous species' roots may release benzoxazinoids to prevent nearby plants from growing [53].

Still, it has been established that certain alkaloids, such as quinine, colchicine, morphine, berberine, ergotamine, and allyl isothiocyanate, demonstrate phytotoxicity and prevent neighboring plants' seeds from germinating or growing into seedlings. An investigation was carried out in which the alkaloid fraction of *Crotalaria retusa* was gathered and examined for *Phaseolus vulgaris* allelopathic potential at different concentrations. Allelochemicals caused oxidative stress and prevented bean seed germination as concentrations increased [54]. Their phytotoxicity and allelopathic efficacy on weeds have been extensively investigated. Cereals create a variety of benzoxazinoids and hydroxamic acids, which are then exuded into the surrounding soil solution from plant tissues and residues during decomposition and root exudation (from root hairs or secondary roots). These compounds include benzoxazolin-2(3H)-one (BOA), benzoxazinones 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA), 2-hydroxy7-methoxy-1,4-benzoxazin-3-one (HMBOA), 2-hydroxy-1,4-benzoxazin-3-one (HBOA), 6-methoxy-benzoxazolin-2-one (MBOA), and 2,4-dihydroxy-(2H)-1,4-benzoxazin-3(4H)-one (DIBOA (Figure 1) [52]. It has been demonstrated that they exhibit physicochemical and microbiological changes after release, which results in modifications to phytotoxicity mediated by microbes [55]. When external stimuli are released into the cytoplasm, benzoxazinones are retained in vacuoles in the glucosidic form, where they are digested by β-glucosidases to increase their reactivity and biological activity [56]. While the benzoxazolinone breakdown products, MBOA and BOA, are thought to be less bioactive than the initial molecules, the unstable benzoxazinone aglucones, DIBOA and DIMBOA, are poisonous. Nevertheless, research has demonstrated that the glucosides of DIBOA and DIMBOA, along with their corresponding aglycones and degradation products, control weeds such as redroot pigweed, barnyard grass, and crabgrass [57]. Alkaloids, such as pyrrolizidine alkaloids in *Chromolaena odorata*, act as deterrents to herbivores and other natural enemies, reducing the impact of predation and facilitating the plant's establishment in new environments [58]. Also, these compounds

exhibit allelopathic properties, inhibiting the germination and growth of surrounding plant species, which helps invasive plants like *Chromolaena* and *E. adenophorum* dominate native flora [59]. In essence, alkaloids and other allelopathic substances give invasive species a competitive edge by suppressing native plants and enhancing their resilience against herbivory and other environmental stresses, contributing to their successful spread and establishment [60]. Understanding the specific mechanisms and concentrations of these compounds in the environment is crucial for developing strategies to manage invasive plant species effectively

2.3. Terpenes

Terpenoids are vital substances found in nature that are divided into four groups according to the number of isoprene units in their carbon structure: monoterpenes, sesquiterpenes, diterpenes, and triterpenes [61]. These two distinct mechanisms produce isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP), essential for the synthesis of terpenoid compounds. Figure 1 shows the pathways for mevalonic acid in the cytosol, endoplasmic reticulum, peroxisomes, and methylerythritol phosphate (MEP) in plastids. Learning these complex systems and how they function in biological processes helps us better understand how these compounds are made [62]. Terpeniods function not only as allelochemicals and reproductive hormones but also provide photoprotection, which is vital to plants because they mediate polysaccharide assembly. The literature has highlighted the inhibitory effect of seedling germination and growth, exhibiting an autotoxic and allelopathic nature. These changes are the results of complex interactions relating ATP (Adenosine triphosphate) production alteration, endocrine activity, protein complexation, and respiratory blockage. According to [46], terpenoids are important not only for plants' environment but also for defense and communication. Terpeniods have tremendous characteristics that help invasive plants attract pollinators and protect the plant from herbivores and microbes. A lot of research work was conducted on how certain chemicals from invasive plants can affect other plants, revealing compelling results. There is one chemical called β-caryophyllene, which is mainly found in plant aromas. It can hinder the seeds from growing into plants in *Brassica napus* L. and *Raphanus sativus* L. [63]. Researchers have thoroughly examined how certain plant compounds, i.e., monoterpenes and sesquiterpenes, have phytotoxic effects against other plant species. In many cases, invasive species produce higher concentrations of terpenes, which can deter generalist herbivores commonly found in new environments, making the invaders less vulnerable to predation compared to native plants [64]. Terpenes can have allelopathic effects, releasing chemicals into the soil to inhibit the growth of neighboring plants. This can significantly alter the composition of native plant communities, providing invasive species with more access to critical resources like nutrients, light, and space [65]. Beyond their defensive role, terpenes also enable invasive species to adapt to a range of abiotic stresses, such as temperature extremes, drought, and salinity. These compounds help maintain the plant physiological functions under challenging conditions, allowing them to thrive in diverse environments where native species may struggle [25]. Many invasive plants adopt a strategy of producing low-cost chemical defenses, like terpenes, which allows them to allocate more energy toward growth and reproduction. This balance between rapid growth and effective chemical defense often results in higher reproductive success and faster spread of invasive species in new habitats [66]. The ecological impact of terpenes extends beyond direct competition, as their presence can modify interactions within the ecosystem, influencing pollinators, herbivores, and soil microbial communities. These alterations can lead to shifts in biodiversity and ecosystem function, often disadvantaging native species. Understanding the role of terpenes in plant invasions is thus essential for developing strategies to manage invasive populations and protect native ecosystems [67]. By shedding light on these biochemical mechanisms, research can inform more effective management practices aimed at mitigating the ecological impacts of invasive species.

2.4. Volatile Organic Compounds

The compounds in the air are volatile organic compounds (VOCs), which may be ethylene, methyl jasmonate, methyl salicylate, and indole (Figure 1). These VOCs help the plant species interact with the environment comprising other plant species, herbivores, natural enemies, pollinators, and microbes [68]. Ethylene activates genes through air diffusion, which helps plants defend themselves. In tobacco (*Nicotiana tabacum* L.), small quantities of ethylene can cause characteristics associated with shade avoidance [69]. According to [70], methyl jasmonate in *Artemisia tridentate* activates the defense genes, as shown in Table 1. Moreover, defense genes can be activated by methyl jasmonate via spreading through the environment [71]. An excellent way to attract the green lacewing (*Chrysopa nigricornis* Burmeister) is with methyl salicylate [72]. Indole acetic acid functions as a quick and efficient aerial priming agent to prime neighboring plants' tissues for defense [53].

Table 1. Secondary metabolites in invasive plant species and their mechanism.

Plant Species	Category	Compounds	Mechanism	References
Artemisia tridentata Nutt.	Volatile organic compounds	Methyl jasmonate	Activates expression of defense genes	[70]
Alliaria petiolate (M.Bieb.) Cavara & Grande	Phenolic compounds	Glucosinolates (sinigrin)	Mycorrhiza are suppressed by sinigrin, which breaks their mutualistic relationships with native plants.	[73]
Ageratum conyzoides L.		P-coumaric acid, gallic acid, ferulic acid, p-hydroxybenzoic acid, and anisic aci	Rice growth was adversely influenced by phytotoxins released into the soil rhizosphere by *A. conyzoides* residues and root exudates.	[74]
Cymbopogon nardus (L.) Rendle		N-Octanoyl tyramine	Inhibits ripening of *Lepidium sativum*, *L. sativa, Echinochloa crusgalli, Lolium multiflorum*	[49]
Juglans nigra L.		Juglone	Inhibitor of the essential enzyme for the formation of plastoquinone, hydroxyphenylpyruvate dioxygenase (HPPD), as well as other plants' photosynthetic and respiratory electron transport systems	[47]
Secale cereale L.	Alkaloid compounds	Benzoxazinoid	Boosts benzoxazinoids' synthesis and exudation from roots in reaction to nearby plants	[75]
Echium plantagineum L.		Pyrrolidine and Naphthoquinones	Provide a competitive edge over weeds and protect against livestock and insect herbivory.	[75]
Senecio jacobaea L.		Pyrrolizidine	Increased alkaloids produced in non-native range compared to native range; protection against generalists	[75]
Imperata cylindrica (L.) P. Beauv.	Tarpenes	Tabanone, 4-(2-butenylidene)-3,5,5-trimethyl-2-cyclohexen-1-one; cogongrass,	Impeded the growth of the garden onion's roots, the lesser duckweed's frond area, and the garden lettuce's fresh weight gain.	[76]

2.5. Phytochemicals Reported in Invasion Mechanisms

One of the most economically damaging alien invaders in North America is the *Centaurea* species, which is suspected of displacing native species Catechins quickly through allelopathic mechanisms [77]. (−)-catechin (Figure 2) from *C. maculosa* (Asteraceae), often known as spotted knapweed, was found to be a phytotoxic root exudate, while (+)-catechin (Figure 2) exhibited antibacterial qualities. The idea that *C. maculosa* invasiveness is enabled by (−)-catechin release is supported by the abundance of racemic catechin found in soil extracts from fields where the plant has invaded. In soils sustaining invasive *C. maculosa* in North America, the natural content of (−)-catechin was more than double that in Europe. The findings offer compelling evidence that *C. maculosa* root exudation of (−)-catechin is responsible for the displacement of native plant communities, at least partially. Additionally, they used an integration of ecological, physiological, biochemical, cellular, and genetic techniques to show the allelopathic effects of *C. maculosa*. The findings demonstrated that natural field soil conditions inhibited the growth and germination of native species [78]. The natural content of 8-hydroxyquinoline (8HQ, Figure 2), an allelochemical that has never before been described as a natural product, varies biogeographically from *C. diffusa* (Asteraceae) root exudates [79]. It has larger phytotoxic effects on North American grass species than on Eurasian grass species, and it is at least three times more concentrated in soils invaded by *C. diffusa* in North America than in the native Eurasian soils of this plant. Moreover, regardless of the biogeographical origin of the soil biota, experimental communities constructed from North American plant species are considerably more vulnerable to invasion by *C. diffusa* than communities constructed from Eurasian species. More so than Eurasian soils, North American soil biota sterilization inhibited *C. diffusa*, suggesting that the latter may be encouraged to invade North American soils. Since North American plants have not developed a natural resistance to 8-HQ, it is possible that Eurasian plants and soil microbes have. This suggests a remarkable potential for evolutionary compatibility and homeostasis among plants within natural communities, as well as a mechanism by which exotic weeds ruin these communities. *C. maculosa* (Asteraceae) was shown to have a phytotoxic chemical called cnicin (Figure 2), a sesquiterpene lactone (spotted knapweed) [80]. It can hinder larval growth and development [81]. *M. micrantha* (Asteraceae), commonly known as Mile-a-Minute, is one of the top 100 worst invasive alien species in the world. Deoxymikanolide (Figure 2) and other sesquiterpene lactones were identified from this plant [82], revealing a high level of phytotoxicity to the family Brassica (Brassicaceae). These compounds might significantly impact how well the weed invades [83]. Throughout the plant's life cycle in a natural population, ocimenones (Figure 2), the predominant terpenes in the essential oils of the leaves and reproductive structures of *Tagetes minuta* L. (Asteraceae) were investigated. Ocimenones' phytotoxic impact on germination was assessed. According to bioassays, *T. minuta* fruit material and pure ocimenones slowed and prevented coexisting species from germinating. Regarding *T. minuta's* chemical ecology, a connection between allelopathy, biosynthesis, catabolism, and terpene release is suggested [84]. In field conditions, it was discovered that methyl jasmonate (Figure 2), a trace amount exuded by sagebrush (*Artemisia tridentate* ssp. tridentate, Asteraceae), inhibited *Nicotiana attenuata* seed germination [85,86]. Anthraquinones: in a recirculating system, the root exudates of *Polygonum sachalinense* F. Schmidt ex Maxim. (Polygonaceae) considerably slowed down the growth of lettuce seedlings. Emodin (Figure 2) and physcion's (Figure 2) inhibitory effects on the growth of seedlings of various tested plant species were demonstrated using TLC agar plates [87]. Emodin and physcion were present in the rhizome, roots, and fallen leaves in comparatively high proportions. This plant community's soil also contains emodin, with autumnal effective concentrations being the highest. These powerful allelopathic compounds, known as anthraquinones, are therefore likely responsible for the interference seen [16]. The plant parthenium yields a wide range of allelochemicals, which can be classified into many chemical classes. A thorough description of these kinds of allelochemicals emitted by parthenium weed and its residues was provided by [88]. Perthenin (Figure 2) is a sesquiterpene lactone secondary metabolite released from parthenium plants,

and this compound affects other plant species [89]. Besides perthenin, parthenium plants also produced a variety of hydrophilic phenols comprising ferulic, anicic, fumaric, vanillic, and caffeic acids. These phenolic compounds exhibit phytotoxicity in water extracts from parthenium species [90]. According to [91], several additional sesquiterpene lactones, flavonoids, and tannins are possible allelochemicals produced by parthenium plant species.

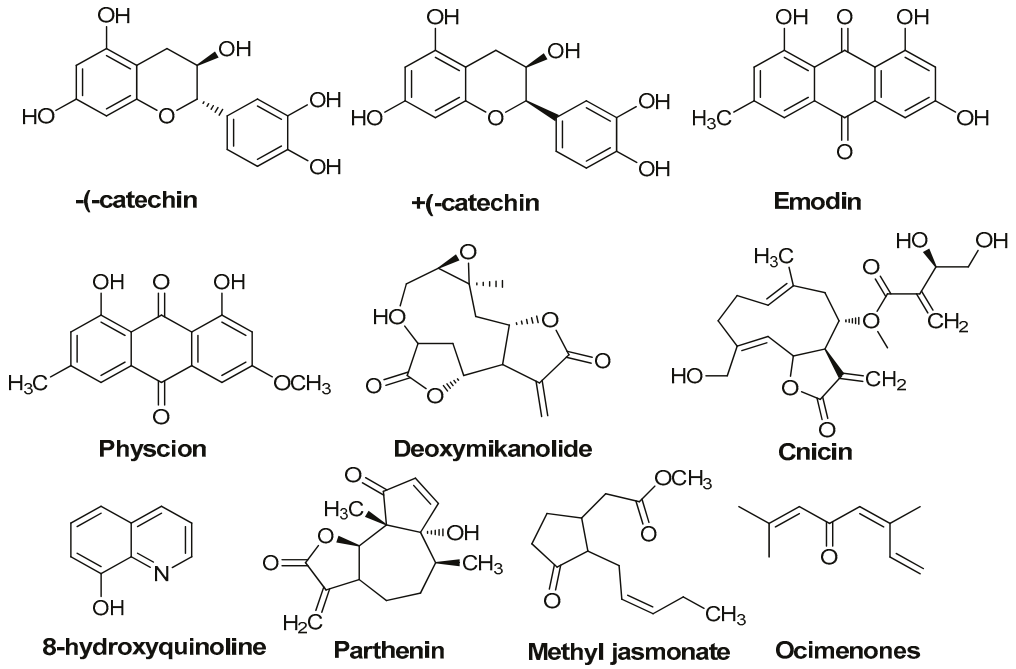

Figure 2. The figure shows a range of secondary metabolites like (−)-catechin, (+)-catechin, cnicin, ocimenones, 8-hydroxyquinoline, deoxymikanolide, emodin, methyl jasmonate, physcion, and parthenin. These compounds are known to play significant roles in plant invasion strategies through allelopathic interactions, where they inhibit native plant growth, disrupt beneficial mycorrhizal fungi associations, and alter the microbial dynamics in the soil. For example, catechins released by invasive species can suppress native vegetation, while methyl jasmonate and emodin may influence plant defense mechanisms and stress responses, enhancing the competitive ability of invasive plants. These biochemical strategies give invasive species a significant ecological advantage.

3. Functions of Secondary Metabolites

3.1. Allelopathy of Invasive Plants

Allelopathy is the chemical exchange of allelochemicals between recipient and donor plants. Certain plant components produce allelochemicals, which are then released into the soil around donor plants, including their rhizosphere, through a variety of mechanisms such as root exudation, rainfall leachates, volatilization of plant parts, or plant residue decomposition [92]. Plant allelopathy has significantly impacted how scarce resources are used and how competition for them exists. The chemical makeup, mechanism of action, and effects of plant root exudates differ, and plants appear to be largely resistant to the allelochemicals they generate. Aqueous extracts from the above-ground portions of *S. canadensis* inhibited the development and germination of *Digitaria sanguinalis* (L.), *Amaranthus retroflexus* L., and *Lactuca sativa* L. [93]. The germination and growth of *Zoysia japonica* (Steud) were suppressed by water-based extracts of *S. canadensis* roots and above-ground parts, while the above-ground parts' extracts markedly increased malondialdehyde and peroxidase activity [94]. *Raphanus sativus* L. germination was also postponed, and growth was inhibited by preparations of Japanese and Bohemian knotweed using aqueous rhizome. *R. sativus* roots exhibited signs of oxidative stress, including aberrant nuclear, plasma membrane, mitochondrial, and endoplasmic reticulum shapes [95]. The finding suggests that some allelochemicals may infiltrate the seeds and prevent the germination and growth of the

seeds. Aqueous extracts of *C. odorata* inhibit the germination of *Ageratum conyzoides* L., *Crassocephalum crepidioides* (Benth.) *S. Moore*, and *Cynodon dactylon* L. [96], and the growth of *Eleusine indica* (L.) Gaertn., *Cyperus iria* L., and *Ageratum conyzoides* L. [97]. According to [98], the growth and germinations of *Amaranthus viridis* L. and *Echinochloa crus-galli* (L.) P. Beauv was inhibited when exposed to aqueous extracts of *C. odorata*. Also, the plant height, leaf area, root length, and plant masses of *Amaranthus spinosus* L. and *Amaranthus spinosus* were decreased when methanol extract of *C. odorata* was sprayed on them [99].

Methanolic extracts of *Mimosa pigra* used against *Ruellia tuberosa*, *Echinochloa crus-galli* (L.) P. Beauv., and *Lactuca sativa* L. affects their growth and development. *M. pigra* extracts are concentration dependent; they interrupt the root mitosis and decrease their cell viability. The allelochemicals from *M. pigra* prevented the native plants from regenerating in the area where these invasive plant species spread [100]. *P. hysterophorus* extract inhibited the germination and growth of *Cyperus iria* L. and showed the same toxic potency as the glyphosate and glufosinate-ammonium synthetic insecticides [101]. Parthenium leaf extracts and residues inhibit the early seedling development and germination of *Phalaris minor* Retz and wild oats *(Avena fatua* L.) in the Petri dish and soil bioassays [102]. The seedling growth and germination of seeds in *Oryza sativa*, *Raphanus sativus*, and *Triticum aestivum* were also delayed by aqueous leaf extract of *M. micrantha* [103]. Aqueous leachates of *M. micrantha* exhibited allelophatic effect against *Aphanus sativus*, *Lactuca sativa*, *Trifolium repens*, and *Lolium multiforum* [104]. The allelochemicals found in *M. micrantha* leaf extracts include vanillic acid, resorcinol, caffeic acid, and p-hydroxybenzaldehyde [105]. *Biden pilosa* L. and *L. perenne* were likewise inhibited from germinating when applying aqueous extracts of *Pueraria montana's* litter. *B. pilosa* and *L. perenne's* root and shoot growth was suppressed by the mixture of pure soil and *P. montana* extracts. When compared to the non-infested soil, in the soil infested with *P. montana*, the total phenolic concentration was 30- to 50-fold greater [106]. The results of the experiments point to the possibility that these phenolics are responsible for the inhibition brought on by *P. montana* soils and litter is a new agrochemical tool that has gained recognition for its ability to manage weeds. Growth of *Amaranthus caudatus*, *Amaranthus spinosus*, *Digitaria sanguinalis*, *Lactuca sativa*, *Echinochloa crus-galli*, and *Monochoria vaginalis* was inhibited by plant extracts [107]. When intercropped in citrus orchards, *A. conyzoides* greatly suppressed weeds, including *Cyperus difformis*, *B. pilosa*, and *Digitaria sanguinalis*, [108]. Table 2 summarizes the biological properties of invasive plant species and their allelopathic effect.

Table 2. Biological properties of invasive plant species and their allelopathic effect.

Invasive Plant Species	Allelochemicals	Mode of Action	Effected Plants	References
Solidago canadensis L.	Kaempferol-3-O-d-glucoside	Growth	*Arabidopsis thaliana* (L.) Heynh., *Echinochloa colona* L.	[93]
Ageratina adenophora (Spreng).	Propan-2-ylidene (4,7-dimethyl-1-) tetrahydronaphthalene-1,4,4a, 8a, 2(1H, 7H) DTD and 6-hydroxy-5-isopropyl-3	Growth and development	*Osbeckia stellate buch.* HAM. EX D. DON and *Elsholtzia blanda* Benth.) Benth.	[109]
Polygonum cuspidatum Sieb. et Zucc	(−)-catechin, (−)-epicatechin, resveratroloside, and piceatannol	Growth	*Lepidium sativum* L.	[110]
Chromolaena odoratum L.	Globulol, α-cadinal, 1-hexadecanol, caryophyllene, (−)-spathulenol, and caryphyllene oxide hexadecane	Growth	*Eleusine indica* (L.) Gaertn, *Cyperus iria* L., and *Ageratum conyzoides* L.	[111]

Table 2. *Cont.*

Invasive Plant Species	Allelochemicals	Mode of Action	Effected Plants	References
Ambrosia artemisiifolia L.	α-pinent, β-pinene, cineole, camphene, spanthueol	Germinations and root growth	*Zea mays* L. (Corn), *Triticum aestivum* L. and *Oryza sativa* L.	[112]
Ageratum conyzoides L.	Precocenes, sesquiterpenes, Gallic acid, proteocatechins acid and coumaric acid,	Germination up to 89%	*Parthenium hysterophorus* L.	[113]
Conyza bonariensis (L.) Cronquist	(4Z)-lachnophyllum lactone	Suppression of growth	*Cuscuta campestris* L.	[114]
Eucalyptus camaldulensis Dehnh.	Syringic acid, vanillic acid, gentisic, gallic, p-coumaric, p-hydroxybenzoic, and catechol	Suppression of germination and growth	*Portulaca oleracea* L.	[46]
Eichhornia colona L.	Tricin	Inhibit germination and seedling growth	*Glycine max* L. and *Oryzae sativa* L.	[16]
Eucalyptus globulus Labill.	Kaempferol 3-O-glucoside, hyperoside, and shikimic-succinic acids	Inhibit germination, growth and physiological parameters	*Agrostis stolonifera* L.	[115]
Mikania micrantha Kunth.	Dihydromikanolide, deoxymikanolide, 2,3-epoxy-1-hydroxy4,9-germacradiene12, 8:15,6-diolide.	Limit the length of the radicle and shoot.	*Trifolium repens* L., *Raphanus sativus* L., and *Lolium perenne* L.	[104]
Parthenium hysterophorus L.	Caffeic acid, parthenin	Suppress the growth of seedlings and germination	*Digitaria sanguinalis* (L.) Scop. and *Eleusine indica* (L.) Gaertn	[116]
Asystasia gangetica L.	(6R,9S)-3-oxo-α-ionol and indole-3-carboxaldehyde	Cause 10% yield reduction	*Cucumis sativus* L.	[117]
Artemisia annuas L.	Artemisinin	Prevent development and expansion of the roots	*Ipomoea lacunose* L., *Lactuca sativa* L., *Portulaca oleracea* L.	[118]
Bidens pilosa L.	Terpenes, phenolic acids, polyacetylenes, flavonoids, and fatty acids	Inhibit the growth	*Zea mays* L., *Sorghum bicolor* (L.) Moench., *Lactuca sativa* L, and *Vigna radiate* (L.) R. Wilczek	[119]
Brachiaria mutica (Forssk.) Stapf	Tannin, saponin	Germination and growth suppression	*Mimosa pudica* L.	[120]
Cyperus rotundus L.	Quercetin, luteolin, chrysin, rutin, myricitrin, catechin, apigenin, and chlorogenic acid	Lowers yield by 93% and 86%	*Oryza sativa* L.	[121]
Pueraria montana (Lour.) Merr.	12(13)-dien-bisabolene, 7-carboxy-8-hydroxy-1(2), and (-)-hamanasic acid A	Germination and Growth	*Lactuca sativa* L. and *Raphanus sativa* L., *Bidens pilosa* L. and *Lolium perenne* L.	[122]
Datura stramonium L.	Tropane alkaloids, Scopolamine, Hyoscyamine	Germination and growth	*Tagetes minuta* L. and *Amaranthus hybridus* L.	[123]
Juglans nigra L.	Juglone	Herbicidal activities	*Sonchus arvensis* L., *Cirsium arvense* L, *Papaver rhoeas* L., *Lamium amplexicaule* L.	[124]

3.1.1. Plants Interspecific Allelochemicals

Utilization and competition for scarce resources have been significantly impacted by plant allelopathy and allelobiosis. The chemical makeup, mechanism of action, and effects of plant root exudates differ, and plants appear to be comparatively resistant to the allelochemicals they generate. Furthermore, certain non-allelopathic plants are resistant to the allelochemicals that allelopathic plants produce. The allelochemicals of spotted knapweed (*Centaurea maculosa* Lam.) do not harm eight of the twenty-three grassland species as much as the plant itself does [53]. Strong allelochemicals secreted by certain Asteraceae species can be utilized as "novel weapons" to encroach on new environments, such as *Parthenium hysterophorus* L. [125], *Ambrosia trifida* L. [126], and *C. diffusa* Lam. [127]. However, plants can also use their root exudates to decrease the allelopathy of other plants. *Cunninghamia lanceolata* (Lamb.) Hook., for example, inhibits its growth by releasing cyclic dipeptides (6-hydroxy-1,3-dimethyl-8-nonadecyl-[1,4]-diazocane2,5-diketone) into the soil [128]. By reducing these cyclic dipeptides and lowering their autotoxicity, *M. macclurei* provides chemical signals in a mixed system of *C. lanceolata* and *Michelia macclurei* [129]. The parasite *Striga asiatica* (L.) O. Kuntze's seeds can undergo allelobiosis for years without finding a host plant. *S. asiatica* parasitizes a host when it recognizes strigolactone released by the host's roots [130].

The development of two Asian original plant species, namely *Gnaphalium affine* D. Don and *Xanthium sibiricum* Patrin ex Widder, as well as two tropical species, *Aster subulatus* Michx. and *Sesbania cannabina* (Renz.) Poir., and a cosmopolitan species, *Eclipta prostrata* (L.), was significantly suppressed by root exudates of *S. canadensis* gathered from its aeroponic culture. Moreover, *Arabidopsis thaliana* (L.) Heynh's growth was inhibited by *S. canadensis* root exudates [131]. These data indicated that some allelochemicals that may restrict growth would be released into the rhizosphere soil as *S. canadensis* root exudates and that the amount of these released allelochemicals may be higher in invading ranges than in native ranges. It is well-recognized that *Ageratina adenophora* negatively affects natural vegetation [132]. It influences species diversity, abundance, and the composition of plant communities. This plant is responsible for the decline in the diversity of native species in Nepal's overrun areas [133]. Allelopathy is one of the ways that *A. adenophora* affects other plants. *A. adenophora's* allelopathy occurred by leachates; three compounds were isolated and determined to be the primary allelochemicals: 6-hydroxy-5-isopropyl-3, 8-dimethyl-4a, 5, 6, 7, 8, and 8a-hexahydronaphthalen2(1H)-one (HHO), 4,7-dimethyl-1-(propan-2-ylidene)-1, 4,4a, and 8a tetrahydronaphthalene-2, 6(1H, 7H)-dione (DTD) [134,135].

Under field conditions, bohemian knotweed decreased the growth and survival rate of native plants, such as *Acer saccharinum* L. and *Eupatorium perfoliatum* L. Although the knotweed's inhibitory effects were partially reversed, only a small amount of the native plants' development conditions were improved with additional nutrients and light. Thus, bohemian knotweed's allelopathy may partially account for the decline in the growth and survival rate of native plant species [136]. *Centaurea stoebe* L., an invasive plant species, was thought to use (−)-catechin as an allelochemical to continue its invasion of North America because its inhibitory activity was greater than that of (+)-catechin. According to their theory [137], this substance may be released into the soil from the roots of *C. stoebe*, inhibiting the germination and growth of native plant species and disrupting their regeneration. Nonetheless, considerably less catechin was discovered in the field soil to prevent the establishment of local plant species [138]. *Cuscuta chinensis* Lam. seedlings have the ability to discriminate between volatile compounds emitted by the host tomato and non-host wheat. They also grow more selectively toward the tomato plant and successfully parasitize it [53].

3.1.2. Plants Intraspecific Allelochemicals

Plants of the same species have the ability to poison surrounding individuals by producing autotoxic allelochemicals [53]. This phenomenon inside the natural ecosystem is self-thinning. Furthermore, plants have the ability to suppress their own seed germination

and seedling growth in order to control the population in both space and time. This allows them to avoid internal competition and increase their geographic range [139]. Autotoxicity is also found in agroecosystems [140] and medicinal plants [141], resulting in a drop in output, inadequate seedling growth, and lower-quality leaves. Chinese fir roots release autotoxic compounds that inhibit the plant's ability to regenerate [43]. Kin recognition is the ability of plants to recognize and react to their neighbors thanks to intraspecific allelobiosis [142], as shown in Figure 3. Plants identify their relations by subterranean chemical signals, which helps them control community competitiveness and growth [143]. According to evolutionary theory, kin selection will favor individuals with the same genes, providing a higher chance of survival in a changing environment when relatives are recognized [144]. Furthermore, to ensure that outcrossing plants can effectively complete pollination, *Brassica para* L. var *nipposinica* (L. H. Bailey) Hanelt's root exudates can control both aboveground flowering time and flowering duration [53]. Chemical signals from plants that evaporate into the atmosphere also trigger chemical defense responses in nearby plants or the evaporating plants themselves, regulating population density [145]. Higher levels of tetradecane are released by *Holotrichia parallela*-infested maize roots. In order to create protective jasmonic acid and BX in the roots of maize plants, Motschulsky sends a chemical signal to nearby uninfected plants [146].

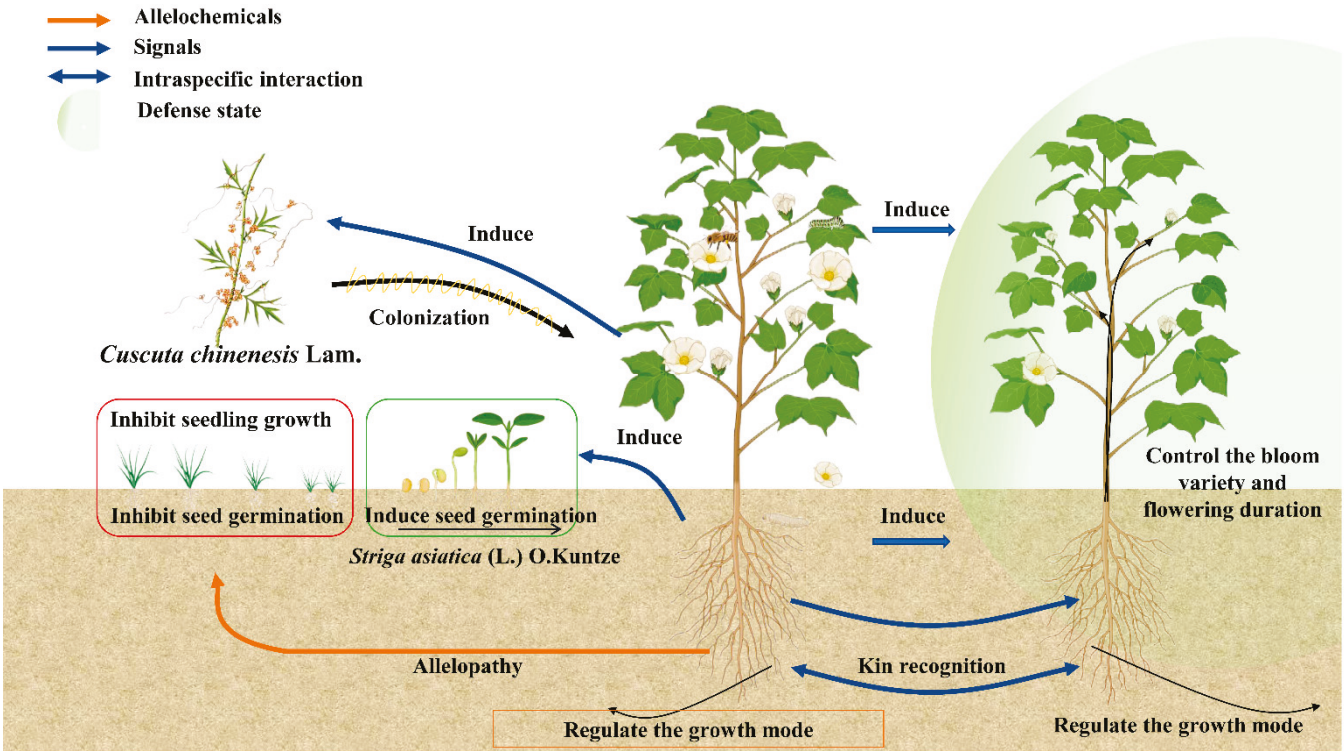

Figure 3. Shows the roles of allelopathy and allelobiosis in plant interactions, focusing on interspecific and intraspecific relationships. Allelopathy, represented by chemical signals, affects the growth of nearby plants, either inhibiting or promoting seed germination. This interaction influences interspecific dynamics, as seen in the impact of *Cuscuta chinensis* on different host species. Allelobiosis involves signaling between plants, including kin recognition, which allows intraspecific regulation of growth and adaptation. Together, these processes shape how plants, both within the same species and among different species, adapt to parasitic pressures and competition.

Certain plant parts manufacture allelochemicals, which are then released into the environment around the plants through leachates from rainfall, volatilization from the plants, exudation of roots, or the breakdown of plant debris and litter [100]. Allelochemicals, the byproducts of secondary metabolism, are found in every part of the plant, including the leaves, stems, flowers, seeds, fruits, and/or roots. There are various ways in which

the producing plant can release these products: plant waste volatilization, foliar leaching, root exudations, and decomposition (Figure 4). VOCs are widely distributed plant allelochemicals and secondary metabolites that plants volatilize [147]. Mevalonic acid (MVA), methylerythritol phosphate (MEP), lipoxygenase (LOX), and shikimate/phenylalanine are the four basic mechanisms for the production of volatile organic compounds (VOCs). Terpenoids, phenylpropanoids/benzenoids, and fatty acid derivatives are among the VOCs that plants can create and release [148]. According to [149], these plants release volatile organic compounds (VOCs) that serve a variety of ecological purposes, including chemical communication, kin recognition, insect attraction or repulsion, and many more. While most studies on volatile organic compounds (VOCs) in plants focus on aboveground chemical signals, an increasing body of research indicates that VOCs are also crucial for belowground plant-plant interactions [150]. Volatile oil of *C. odoratum* at 800 mg/L inhibits the growth of *Pyricularia grisea*, *Phytophthora nicotianae*, and *Fusarium axysporum*.

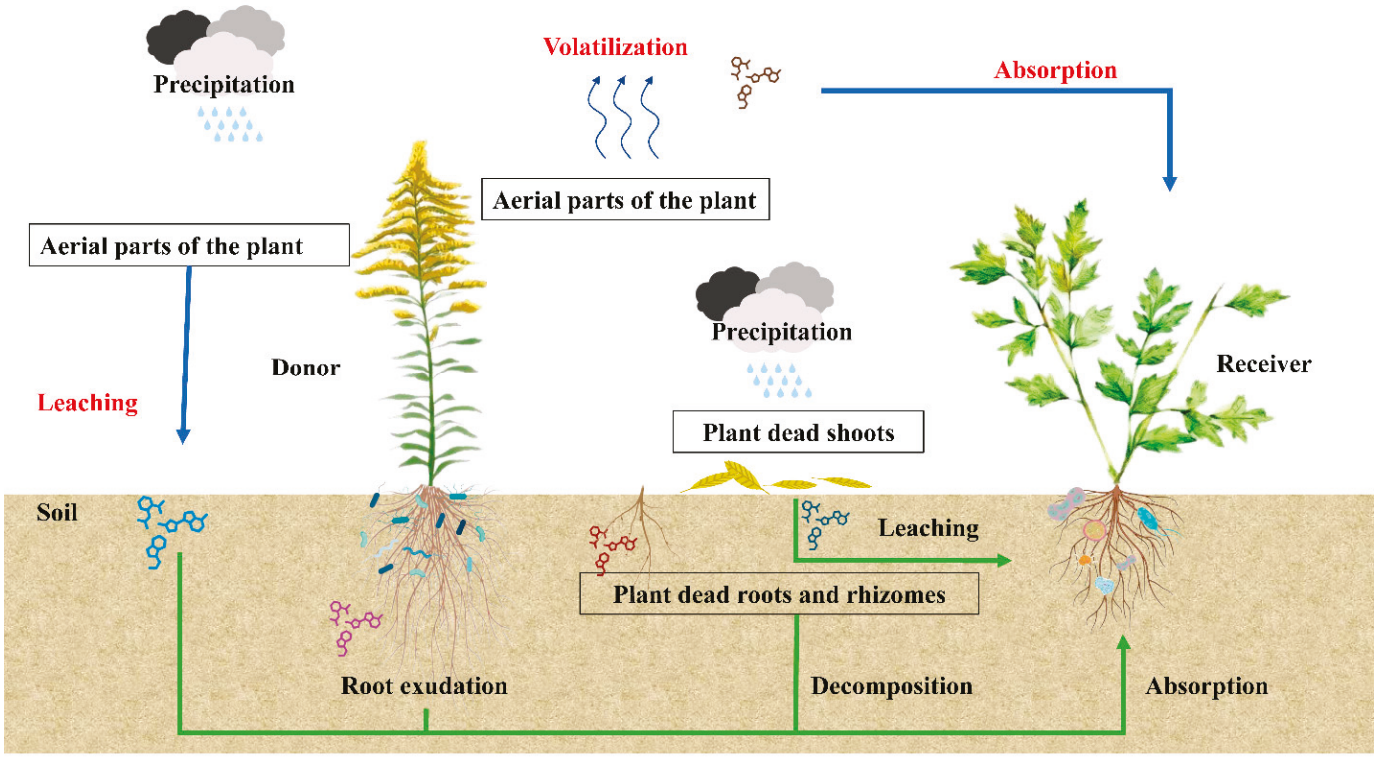

Figure 4. Illustrates the transfer of allelopathic compounds from a donor plant to a receiver plant through various pathways, including leaching, volatilization, root exudation, and decomposition. Compounds are released into the soil via precipitation or root exudation, volatilized into the atmosphere, or deposited through decomposing plant material, where they can be absorbed by neighboring plants. This chemical exchange plays an important role in shaping plant interactions, influencing competitive dynamics and ecosystem structure.

Table 3 summarizes studies during the last decade reporting VOC-mediated allelopathic effects of invasive plants. According to these findings, invading species may have a significant negative impact on native plants' chemical habitats by releasing volatile allelochemicals into the environment that prevent or lessen native species' ability to germinate and flourish. Allelopathic substances can accomplish this by lowering the photosynthetic efficiency of recipient plants or interfering with the mechanisms involved in cell division (mitosis) [53]. The release of allelochemicals appears to be the fundamental mechanism, causing the receiver to emit reactive oxygen species, which set off a chain of signals and ultimately alter gene expression across the entire genome [151]. To clarify the mechanism(s) of action of volatile allelochemicals, more research is required. Litter is another

way that volatile allelochemicals from invasive species might enter the rhizosphere. It is well known that litter volatile chemicals are tenacious and can be found years after litter deposition [152]. A pioneering study outside the scope of this review [27] indicates that an invasive species' ability to spread is greatly influenced by volatile chemicals found in its litter. We therefore recommend further research to ascertain the allelopathic potential of the volatile organic compounds (VOCs) that invasive plants leave behind on native species and the length of time that residues remain bioactive after invasive plants have been removed.

Table 3. Research has been performed in the last few decades on the adverse effects of VOCs from invasive plant species on recipient plants.

Invasive Plants Species	Negative Effect on Receiver Plant Species	Receiver Plants Species	References
Phytolacca americana	Adverse effects on reproductive and morphological features	*Phytolacca acinosa*	[153]
Prunus serotina	Prevented the elongation of the roots, shoots, and germination	*Pinus sylvestris*	[154]
Mikania micrantha	Decreased rate of germination reduced levels of chlorophyll and reduced levels of malondialdehyde and reduced activity of superoxide dismutase	*Abutilon theophrasti, Bidens pilosa, Chrysanthemum coronarium* and *Lactuca sativa*	[155]
Ageratina adenophora	Reduced germination rate and limited height of seedlings reduced biomass of the shoots and roots	*Schima wallichii*	[132]
Acacia longifolia	Reduced biomass, shoot length, and root length	*Lolium multiflorum, Plantago lanceolata* and *Trifolium subterraneum*	[156]

3.2. Herbivory and Invasive Plant Species Interactions

3.2.1. Insects

Invasive plant species have a major impact on the insect ecosystem due to the actions of secondary metabolites. These compounds perform different roles in influencing insect ecology, behavior, and community dynamics. Secondary metabolites have defensive functions. For example, invasive plant species produce phytochemical compounds that work as feeding deterrents and stimulants for herbivores, influencing their choice of host and foraging [157]. Invasive plant compounds change the nutritional quality, affecting herbivore performance and fitness [158]. These secondary metabolites also affect the interactions between invasive plants and their mutualistic or antagonistic insect companions, including pollinators or natural enemies [159,160]. Actually, invasive plants use these secondary metabolites as defense strategies against herbivory. This may include direct avoidance of feeding or toxicity to herbivores [158], as shown in Figure 5. These might change in the abundance and distribution of insects and modification in the tropic relationships that exist within the ecosystem. The chemical signals facilitated by secondary metabolites affect the mutualistic relationship between the herbivores, pollinators, or predators and invasive plant species, which may affect the reproductive rate and spread the invasive plant population [146]. In order to improve management and conservation strategies for invasive species, researchers can gain a better understanding of the mechanisms underlying the success of invasive plants and their ecological impacts on insect communities by clarifying the roles that secondary metabolites play in mediating plant-insect interactions in natural settings. Table 4 lists the invasive plant species utilized to control insect infestations. Cotton aphids are poisoned by *E. adenophorum* chloroform extracts. The primary allelochemicals found to be harmful were *Aphis gossypii* and *eupatorin A*. Within 48 h, *eupatorin A* at 2 mg/mL can eliminate 81% of cotton aphids [161,162]. This compound also inhibits the enzymatic activity of AChE and NaK-ATPase of the cotton aphids in vitro and in vivo. A-1, P-1, Zi-2, and the leachates of *E. adenophorum* had anti-feeding activity to the fourth instars

of *Pieris rapae*, as reported by [163]. The weed extracts have strong insecticidal activity against four stored grain insects: rice weevil, maize weevil, Chinese bean weevil, and European bean weevil [164]. Epifriedelinol, stigmasterol, octacosanoic acid, 8-daucos tero1, 2-isopropeny1-5-acetyl-6-hydrxybenzofuran aceate, and o-hydroxy einnamic acid were isolated from the *E. adenophorum* [165]. The ethanolic extract from *Alternanthera brasiliana* (L.) Kuntze's leaves was evaluated for its insecticidal activity against the Hamburg strain of *Drosophila melanogaster* [166]. After exposed for 24 to 48 h, researchers discovered that the ethanolic extract at the studied quantities had a slight insecticidal impact. Phytomolecules like kaempferol and kaempferol analogs [167], quercetin and quercetin analogs [168], stigmasterol [169], β-sitosterol [170], spinasterol [171], and ferulic acid [172], which were isolated earlier from *Alternanthera brasiliana* (L.) Kuntze, might be in charge of this insecticidal characteristic. When *A. adenophora* was extracted in methanol, it showed a significant toxic effect against mites, *Sarcoptes scabiei* and *Psoroptes cuniculi* [173].

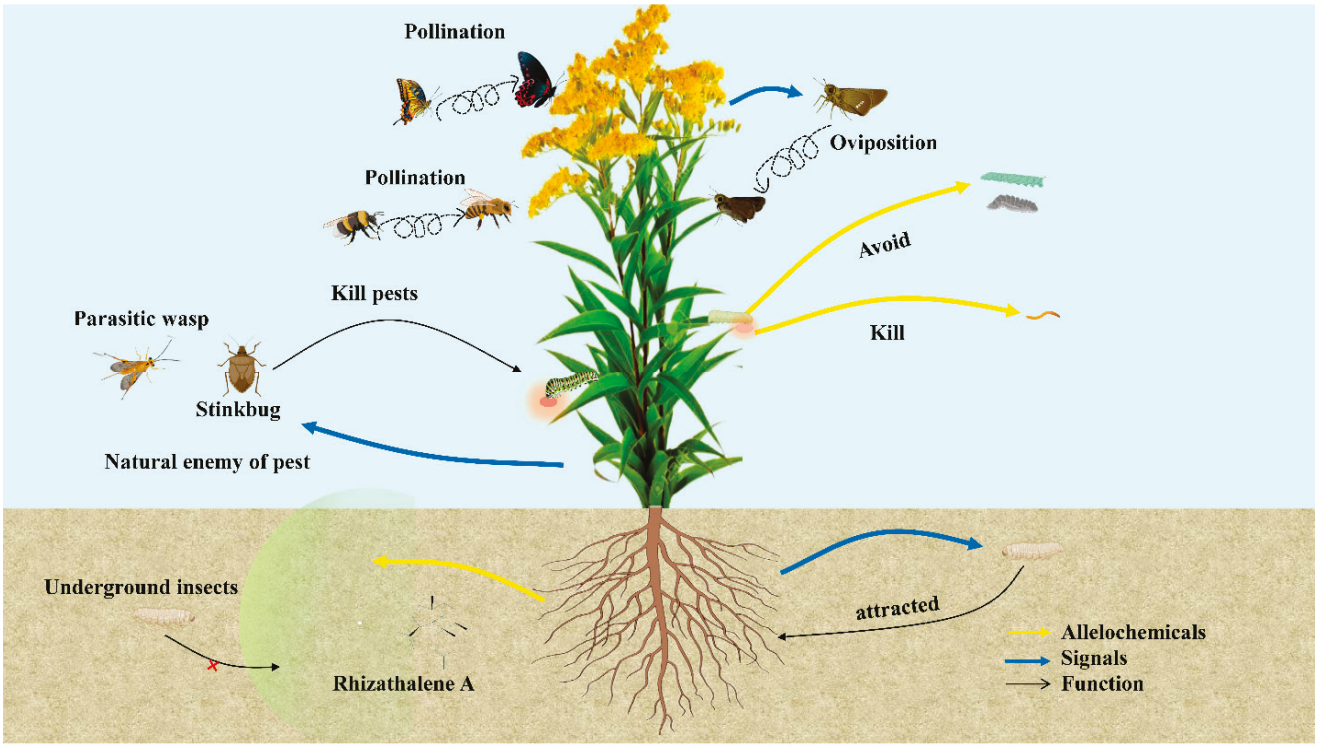

Figure 5. Role of secondary metabolites in plant–insect interactions.

When the leaves of *A. adenophora* were extracted in ethyl acetate, the compounds were identified using gas chromatography-mass spectrometry. 5,6-dihydroxycadinan-3-ene-2,7-dione was found to be most effective against *Meloidogyne incognita* [174]. The essential oils called precocenes from A. conyzoides affect the digestive system, and the anti-juvenile hormones of the oil caused abnormalities in metamorphosis [175]. According to [176], the oil extracts also exhibited genotypic or phenotypic abnormalities in the immature *Aedes*, *Anopheles*, and *Culex* species. Also, the secondary metabolites in this invasive plant showed promising results against many insect pests, such as *Helicoverpa armigera*, *Phytophthora megakarya*, *Rhipicephalus microplus*, *Tribolium castaneum*, *Diaphania hyalinata*, and *Plutella xylostella* [177]. The methanolic extracts of *M. micrantha* significantly repelled Oriental fruit flies in the field [135,178]. According to [179], *Plutella xylostella*, *Phyllotretast riolata*, and *Phaedon brassicae* showed oviposition deterrence when volatile oils of *M. micrantha* were used at a dose of 5–10 µL/plant. The antecedent effects of crude extract of *M. micrantha* on l–2 instars of *Pieris rapae* and 2–3 instars of *Plutella xylostella* were 80% and 70%, respectively [180]. Allelochemicals such as mikanin, eupalitin, eupafolin, (3,4′,5,7-tetra-hydroxy 6- methoxyflavone 3-O-β-D-glucopyranoside, luteolin, 3,5-

di-O-caffeoylquinic acid n-butyl ester, and 3,4-di-O-caffeoylquinic acid n-butyl ester were identified from *M. micrantha* [105]. β-cubebene, terpinolene, β-caryophyllene, 1imonene, β-farnesene, ocimene, δcadino1, γ-terpinene, ethylnaphthalene, a-caryophy11ene, β-cadinene + isocaryophyllene, δ-bisabolene, and β-bisabolene+cubebo1 were determined as the main compounds in essential oil of *M. micrantha* [180]. The volatile oil of *Chromolaena odoratum* is a strong oviposition deterrent of striped flea beetle (*Phyllotreta striolata*) and diamondback moth (DBM) (*Plutella xylostella*) at dose of 10–20 µL/plant [181]. The alcohol extract and its chloroform fraction exhibited strong repellent effects (80%) against DBM [182]. The alcohol extracts of *C. odoratum* effectively deterred the oviposition of DBM, and the active compounds were identified as chalcones and flavonols [183].

Table 4. List of invasive plants used against Insects.

Invasive Plant	Extract	Phytochemical	Target Insect	Mode of Action	References
Ageratina adenophora (Spreng).	Aqueous	Epifriedelinol, stigmasterol, octacosanoic acid, 8-daucos tero1, 2- isopropeny1-5-acetyl-6-hydrxybenzofuran aceate and o-hydroxy einnamic acid	Rice weevil, maize weevil, Chinese bean weevil and European bean weevil	Toxicity	[161]
Alternanthera brasiliana (L.) Kuntze	Ethanolic extract	Kaempferol and kaempferol analogs, quercetin and quercetin analogs, stigmasterol, β-sitosterol, spinasterol and ferulic acid	*Drosophila melanogaster*	Toxicity	[184]
Ageratina adenophora (Spreng).	Ethyl acetate	Cadinene sesquiterpenes, 5,6-dihydroxycadinan-3-ene-2,7-dione	*Meloidogyne incognita*	Antinemic activity	[174]
Ageratum conyzoides L. Lemmon grass	Crude extracts	PONNEEM	*Aedes*, *Anopheles*, *Culex* spp.	Affects the oviposition rate and increases the deterrence percentage	[176,185]
	Methanol extracts	6-demethyoxyageratochromene (precocene I) and ageratochromene (precocene II)	*Preris rapae* and *Plutella xyloaella*	Antifeeding effects	[125]
Mikania micrantha Kunth.	Methonal extract	Mikanin, eupalitin, eupafolin, (3,4′,5,7-tetra-hydroxy 6- methoxyflavone 3-O-β-D-glucopyranoside, luteolin, 3,5-di-O-caffeoylquinic acid n-butyl ester and 3,4-di-O-caffeoylquinic acid n-butyl ester were identified from M. micrantha	Oriental fruit fly	Repellent effects	[105]
	EOs	β-cubebene, terpinolene, β-caryophyllene, 1imonene, β-farnesene, ocimene, δcadino1, γ-terpinene, ethylnaphthalene, a-caryophy11ene,	*Plutella xylostella*, *Phyllotretast riolata* and *Phaedon brassicae*	Oviposition deterrent	[179,180]
Chromolaena odoratum L.	Alcohol extracts	Chalcones and flavonols	*Plutella xylostella*	Repellent	[183]
	Crude extracts		*Helicoverpa armigera*	Antifeeding effects	[181]
	EOs	Trans-caryophyllene, β-cadinene, a-copaene, caryophyllene oxide, germacrene-D and n-humuhne	*Phyllotreta striolata*	Oviposition deterrent	[186]
Parthenium hysterophorus L.	Flower, leaf stem powders	Parthenin ageratochromene, precocene I, and precocene II have strong insecticidal effects, endo-borneol, farnesol, quercetin, kaempferol, and its glucosides	*Callosobruchus chinensis*	Repellency, inhibit cholinesterase	[187]
	Aqueous leaf and stem		*Aedes aegypti*, *Sitophious oryzae*	Toxic and oviposition deterrent	[188,189]
Melia azedarach L.	Aqueous extract Fruits	Azadirachtin	*Callosobruchus maculatus*	Toxicity and repellency	[190]

3.2.2. Soil Microorganism

Invasive plant species often produce secondary metabolites—organic compounds not directly involved in the primary metabolic processes of growth, development, and reproduction but play important roles in interactions with other organisms. These secondary metabolites not only modulate soil microbial populations but also defend against herbivores, pathogens, and allelopathy. Invasive plant species, plant-soil microbes, and secondary metabolites, there is a complex interaction among them, which impacts ecosystem dynamics. When invasive plant species invade an area, the interaction between the native below-ground and above-ground plant species changes. These interactions affect not only the structure of soil inputs derived from plants but also the quality, quantity, and timing [191]. In this situation, invasive plant species invasion may change the timing of litter formation, types, quantities, and the nutrients that are absorbed by the soil. Sometimes, the frequency and intensity of fire increase due to the increased litter production from some invasive plant species [192]. When invasive plants excrete unknown exudates (roots of plant species exude secondary metabolites), they may change the composition and role of the soil community [193]. Invasive plant species produce allelochemicals from the roots, primarily affecting plant-to-plant interactions, which describes the success of invasive plants [194]. However, recently studies suggest that allelochemicals change the interaction between native plant species and soil ecosystems. For example, in Western North America, *Centaurea diffusa* spreads extensively in its environment, with the roots of *C. diffusa* releasing the allelochemicals 8-hydroxyquinoline, which functions as an antibacterial agent [195]. According to [196], *C. diffusa* alters the soil microbial community due to these allelochemicals. Invasive plant species also release some novel chemicals which can change the soil microbial community. There is a diverse research gap in this case. For example, in Hawaii, *Myrica faya* invaded nitrogen-limited areas along with its nitrogen-fixing root symbionts (*Frankia* spp.), affecting nitrogen cycling and changing the composition of the plant community [197]. Invasive plant species directly change the physical properties of the soil environment, initiating ecosystem modifications that lead to control of soil functions and composition. For example, in the western United States, *Halogeton glomeratus,* a plant species that invades rangeland, accumulates sodium from the below soil to its biomass. In the invaded soils, this invasive plant species increases the sodium concentrations, which creates problems and modifications in microbial communities [197]. Invasive plant species produce some chemicals in the soil that prevent the growth of nearby plants and microbes. The compositions and activity of soil microbial communities changed due to the ability of allelochemicals, which selectively promote the growth of some microbial taxa while inhibiting others [28]. Nitrogen-fixing bacteria or mycorrhizal fungi might be prevented from growing by the phenolic compounds from invasive plant species, which alter the plant and microbial interactions and the mechanisms involved in the cycling of nutrients [36]. Also, Ref. [198] studied that some invasive plant species produced allelochemicals that affected the rhizosphere microbiome by serving as nutrient sources or signaling molecules for specific microbial populations. Some invasive plant species produced flavonoids, which change the functions and structure of the soil microbial community. The growth of rhizobacteria is influenced by these flavonoids, which promote the growth of plants or suppress diseases [199]. Some invasive plant species directly inhibit the growth of soil-borne pathogens or competing microorganisms through secondary metabolites with antimicrobial properties [73]. The presence or activity of harmful microbes is decreased by the invasive plant species, allowing them to outcompete the native plants, further exacerbating their impact on ecosystem structure and function. Table 5 summarizes the impacts of invasive plant species on native soil microbe communities.

Table 5. Summary of the impacts of invasive plant species on native soil microbe communities.

Invasive Plant Species	Novel Compounds	Impact on Soil Microbe	References
Solidago gigantean Aiton.	Sesquiterpene lactones	Affect soil microbial communities and inhibit microbial activity.	[151]
Lantana camara L.	Lantadene A	Disrupt microbial symbioses and alter soil microbial communities.	[200]
Rubus armeniacus Focke.	Ellagic acid	Allelopathic and antimicrobial effects on soil microbial populations.	[201]
Centaurea maculosa L.	Cnicin	Antifungal and antibacterial properties, affecting soil microbial composition.	[202]
Alliaria petiolate (M.Bieb.) Cavara & Grande	Glucosinolates (sinigrin)	Sinigrin suppresses mycorrhiza, therefore disrupting their mutualistic associations with native plants	[73]
Phragmites australis (Cav.) Trin. ex Steud.	Catechins	Influence microbial decomposition processes and soil nutrient cycling.	[203]
Chromolaena odorata L.	Acutellerin-40, 6,7-trimethy ether, 40, 5,6,7- tetramethoxyflavone, isosakuranetin	Greater amounts of flavonoids in the non-native range provide competitive advantages and better defense against soil borne pathogens	[204]

3.3. Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with a diverse range of plant species and are important and prevalent soil microorganisms in terrestrial environments [205]. The competition of invasive plant species is influenced by AMF [206]. The invasive plant species *Centaurea maculosa* in North America utilize the mycorrhizal network that connects the roots of native plants [137]. In a new environment, an invasive plant, *S. canadensis,* in China has the ability to change AMF composition, making it more successful in invaded areas than native plant species [207]. Invasive plant species disrupt the symbiotic relationship between native plant species and AMF through secondary metabolites. For example, the invasive plant *Alliaria petiolate* cannot form symbiosis with AMF due to the secondary metabolites, which cannot develop symbiosis with AMF [208]. The specific flavonoids from *A. petiolate* cause invaded soil to have a significantly larger inhibitory action on AMF compared to its native soil [209]. Furthermore, secondary compounds derived from *A. petiolata* change the AMF community linked to native sugar maple seedlings and inhibit AMF hyphal and spore germination [210], despite abundance research showing invasive plants can disrupt local hosts' AMF symbioses through secondary metabolites [207].

3.3.1. Symbiotic Relationship Between Invasive/Native Plants and AMF Communities

Plant and fungal metabolites mediate the plant–AMF symbiosis. Primary, specific, and phytohormone metabolites facilitate partner recognition, colonization, and the development of a symbiotic relationship in the plant–AMF symbiosis. Root-released quercetin and 2-hydroxy fatty acids trigger compound-specific morphological AM fungal responses during pre-symbiotic communication. Next, hyphopodium formation on the root surface is triggered by strigolactone and cutin monomers (1,16-hexadecanediol and 16-hydroxyhexadecanoic acid). It is known that the pre-symbiotic phase of the association between AMF colonies and plants determines the specificity of such a relationship [211,212]. Phytohormones play an important role between AMF and plant species as a signaling molecule. According to [212,213], strigolactones, auxins, abscisic acid, brassinosteroids, and gibberellic acid are involved in their function from the first detection of AMF in the soil to the ultimate development of mycorrhiza. Auxin is necessary for both the early stage of fungal development and the differentiation of arbuscules, while the synthesis of arbuscules is controlled by gibberellic acids [213]. During the early colonization stage, plant–AMF interactions

are facilitated by carotenoid pathways and control of signaling. Phytohormones boost gibberellic acid production by varying the ratio of the salicylic acid and jasmonate signaling pathways and affect plant immunity. Through the symbiotic relationship, plants produce maximum sugar and metabolites for the tricarboxylic acid cycle, boosting photosynthetic sharing. Changes in the primary metabolites also influence arbuscular mycorrhiza growth, affecting the synthesis of specialized metabolites [214]. AM has a beneficial impact on the synthesis of specialized metabolites by boosting metabolite biosynthesis pathways or increasing plant biomass. According to [215], changing plant immunity through plant hormones enables mycorrhizal fungi to influence key plant chemicals that deter pests. For example, Senecio genus plants produce pyrrolizidine alkaloids, their primary defense compounds, in response to AMF invasion.

3.3.2. Mechanisms by Which Invasive Plants Affect Native Plant Mycorrhizal Fungi

Ecological Mechanisms

Invasive plants can affect native plants through a variety of ecological mechanisms. At present, relevant research mainly focuses on species competition, soil nutrient changes, and allelopathy. As shown in Table 6, invasive plants tend to compete for resources (light and nutrients, etc.). For example, *Solanum carolinense* has a strong ability to reproduce asexually and can spread rapidly [216]. Invasive plants can also inhibit photosynthesis in native plants; reduced photosynthates may inhibit native plant mycorrhizal fungal infection [217] (Figure 6). Invasive plants tend to have strong nutrient competitiveness and a large amount of root exudates [218]. Invasive plants can also improve and enhance soil nutrients through litter and root exudates, forming a positive feedback loop between plant and soil [219]. This process reduces the infection rate of mycorrhizal fungi in native plants (such as *Solidago decurrens* and *Andropogon gerardii*) and alters the community composition of mycorrhizal fungi [220] (Figure 6). Invasive plants can also direct affect native plant mycorrhizal fungi through allelopathy [221] (Figure 6). Allelopathic substances produced by invasive plants (such as flavonoids and glucosides, etc.) inhibit the germination, growth, and infection of mycorrhizal fungi spores, thereby inhibiting the growth of native plants dependent on these fungi [222]. These allelopathic substances may also inhibit mycorrhizal fungal infection by inhibiting the growth of native plants, thereby reducing the carbon supply of plants to mycorrhizal fungi [223]. In addition, allelopathic substances secreted by foreign plants may also affect the non-mycorrhizal fungi of native plants, thereby indirectly affecting the mycorrhizal fungi of native plants (Figure 6). In fact, changes in native plant mycorrhizal fungi may result from a combination of mechanisms [224]. The influence of different invasive plants on native plant mycorrhizal fungi may be different, and the response of native plant mycorrhizal fungi to each mechanism may also be different, resulting in varied trends in the influence of foreign plant invasion on different native mycorrhizal fungi.

Table 6. Ecological mechanisms of invasive alien plant species.

Sr. No	Examples	Mechanism	References
1	*Parthenium hysterophorus* L., an invasive plant, may develop far more quickly than crops like *Sorghum bicolor* L. Moench) and *Zea mays* L.	Species competition	[20]
2	When 19 paired invasive and native plants in Hawaii were compared for resource usage efficiency, it was found that invasive plants had better rates of carbon absorption, light use, immediate nitrogen, and energy use.		[225]
3	Invasive plants have larger leaf nitrogen contents are less damaged by herbivores, according to comparisons between 47 paired invasive and non-invasive species' leaf herbivore resistance and nutrient content.		[226]

Table 6. *Cont.*

Sr. No	Examples	Mechanism	References
4	When 125 invasive plants and 196 non-invasive plants are compared physiologically, that invasive plants are more advantageous in terms of growth rate, resource allocation, and stress resistance.	Species competition	[227]
5	*Plantanum carolinense* L., *Solanum carolinense* L. is an exotic plant with great cold resistance and asexual reproduction.		[228]
6	*Solidago canadensis* L. is an invasive plant that can benefit from increasing nitrogen deposition and climate warming by acquiring more leaf resources.		[229]
7	Leachate of the invasive plant *Bothriochloa ischaemum* L. Keng prevents native species *Schizachyrium scoparium* (Michx.) Nash and *Andropogon gerardii* L. from germinating and growing	Allelochemicals	[230]
8	*Lactuca sativa* L., a native plant, seed germination and seedling growth inhibited by allelochemicals released by *S. canadensis* L. invasion		[155]
9	*Crystals of solanine* and oxalate are found in the exotic plant *Solanum carolinense* L.		[104]
10	To aid in its invasion, *P. hysterophorus* L. can release parthenin, vanillic acid, caffeic acid, and other allelochemicals		[88]

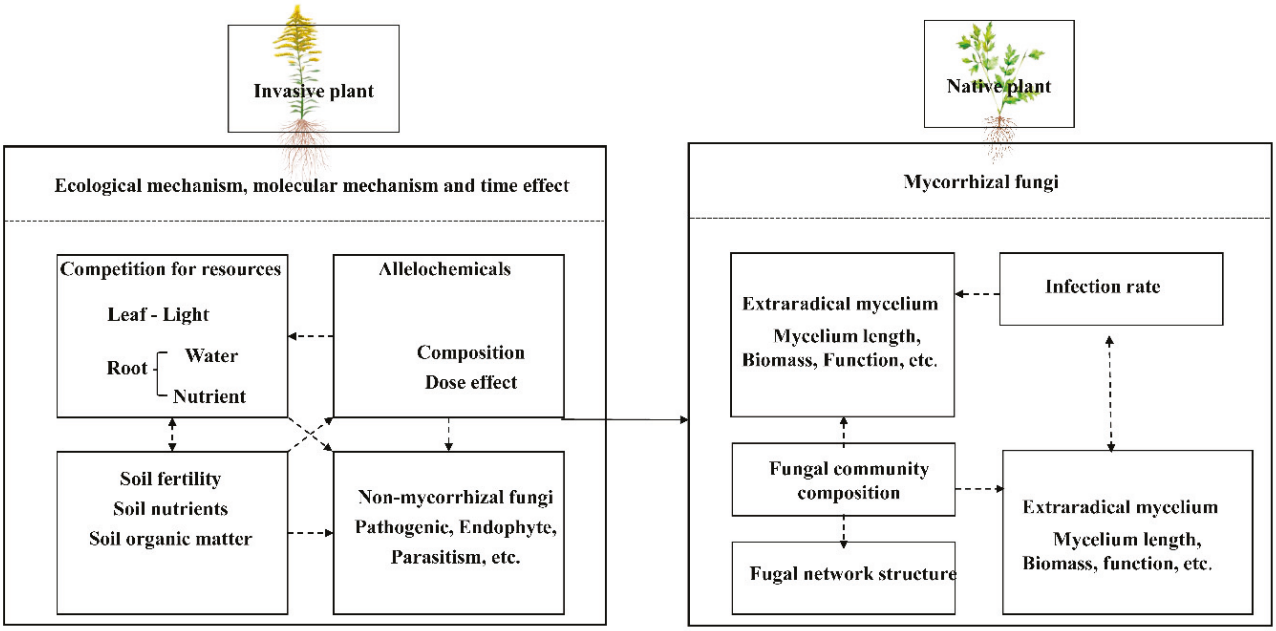

Figure 6. Conceptual illustration showing how invasive plants affect the symbiotic mycorrhizal fungi in native plant roots Solid arrows indicate change; dotted arrows indicate possible relationships.

Molecular Mechanism

The symbiotic relationship between plants and mycorrhizal fungi is a complex signal transduction process. In the creation of arbuscular mycorrhizal fungi, signal exchange between the root system and AMF is the first step [231]. According to [232], strigolactones are signaling molecules secreted by the root system (e.g., *Lotus japonicus*) that not only encourage the AMF spore germination but also enhance the chance of hyphae contact with the root system. Mycorrhizal fungi secreted an array of signaling molecules (i.e., mycorrhizal factors, like LCOs, CO4/CO5, and PsMiSSP10b). As shown in Figure 7, corresponding receptors in the root system recognize these molecules and activate the calcium ion signaling pathway, which in turn produces an infection line and initiates the mycorrhizal fungi's infection process [233]. For example, short chitosan oligosaccharide (CO4/CO5) and lipochitosaccharide

(LCOs) secreted by rice AMF are recognized by heteromers of LysM receptor-like kinases in rice, including OsMYR1/OsLYK2 and OsCERK1 [234]. LCOs and CO4/CO5 factors released by AMF are recognized by NFR1/LYK3H in leguminous plants [235]. Mycorrhizal fungi also produce plant cell wall decomposition enzymes (PCWDEs) by producing small secreted proteins (MiSSPs) to recognize hosts [127], disrupting the cell wall of the host plant and removing the "barrier" to the establishment of symbiotic relationships. The important relationship between the mycorrhizal fungi and roots lies in the exchange of nutrients and carbon (lipids and sugars) [234], which comprises a sequence of lipid synthases and nutrient transporters. Mitogen-activated protein kinases (MAPKs) are key pillars in AMF and plant signaling. According to [236], it is the STR transporters that carry fatty acids through alfalfa *Medicago truncatula*. Invasive plant species inhibit native plant mycorrhizal fungal infections by changing these transport enzymes, ultimately impacting native plant growth. For example, lipid synthase (FatM and RAM2) mutations in alfalfa roots, which control the supply of lipids needed by mycorrhizal fungi in plants, were found to reduce the infection rate of AMF [236].

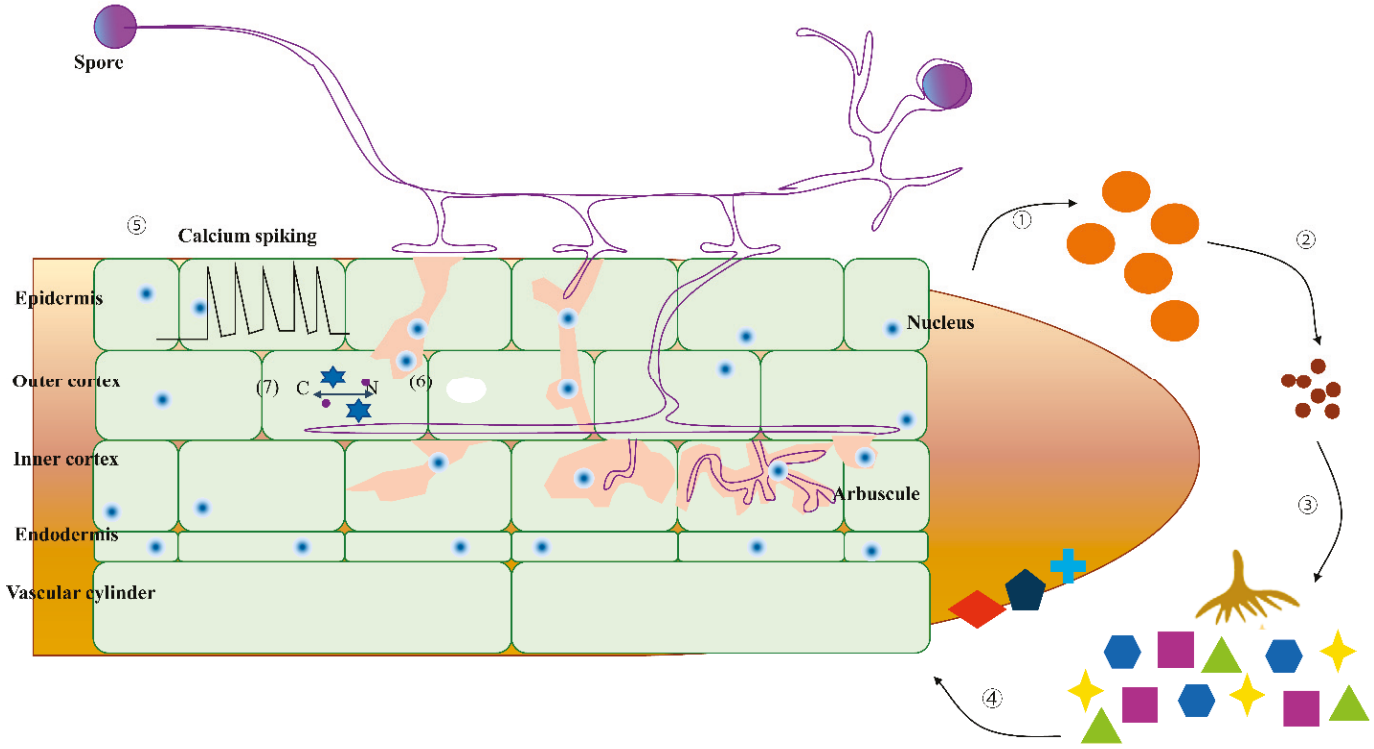

Figure 7. Conceptual framework illustrating the symbiotic relationship between plants and mycorrhizal fungi (adapted from Martin et al [236] Roots secrete signaling molecules (1) that enhance AMF spore germinations (2) and mycelium branching in the soil. AMF secretes mycorrhizal factors (3), which are recognized by receptor proteins in root cells (4) and then stimulate the calcium signaling pathway (5) to pledgee creation of invasion lines of the mycorrhizal fungi (6). After that, nutrient and carbon exchange between mycorrhizae also requires a series of enzymes and transport proteins at the root–mycorrhiza interface (7).

4. Management of Invasive Plant Species

In order to manage the invasive plant species, we should focus on the secondary metabolites responsible for their invasiveness. Here are a few methods to elaborate specific secondary metabolites that significantly enhance the plant species' invasiveness. According to [237], there should be a focus on plant species which exhibit allelopathic effects or competitive advantages. Biological control refers to managing invasive plant species by introducing another living organism, such as insects, diseases, or herbivores, to prevent invasive plant species from spreading and growing. Introducing natural enemies

from the exotic plant species' habitat can reduce the growth and reproduction of specific plant species. Weed scientists and entomologists carefully select those natural enemies from exotic habitats to ensure they target only the invasive plant species without causing harm to other living organisms. According to [238], biological control of invasive weeds offers environmentally friendly and sustainable strategies through careful observation and adaptive management. To reduce the impact of invasive plant species, biological control is often combined with other control techniques. To stop the production or release of harmful secondary metabolites from invasive plant species, targeted chemical management strategies are needed [239]. To reduce the effectiveness of secondary metabolites of invasive plant species, land management strategies can be used to change the soil properties and microbial population [237]. Reducing soil-borne allelopathic chemicals' effects use cover crops or targeted plant techniques [240]. Developing native cultivar plant species through genetic modification or selective breeding can enhance tolerance or resistance to secondary metabolites of invasive plant species. Additionally, exploring options to modify invasive plants to produce fewer secondary metabolites that confer invasiveness may be beneficial [241].

To reduce the impact of invasive plant species' secondary metabolites in invaded ecosystems, integrated pest management (IPM) strategies should be used to decrease dependence on a single control method and enhance the diversity of native plant species. While taking the ecological environment into consideration, IPM approaches combine different control methods [242]. According to [243], IPM concentrates on identifying invasive plant species and their secondary metabolites in newly invaded area. Before invasive plant species establish dominance, IPM strategy can manage them effectively. Educating stakeholders, land managers, and the general public regarding stopping the introduction and spread of invasive plant species, as well as selecting appropriate plant species for gardening and landscaping, is essential [242]. Efficient and quick management strategies for invasive plant species should incorporate multiple tactics to minimize their negative effects on ecosystems. A good management strategy integrates methods for early detection and quick response. Strict biosecurity protocols and public awareness campaigns can prevent the introduction and spread of invasive plant species. Early detection and timely identification enable immediate actions to prevent the invasive plant species from growing and spreading. Management strategies may include biological control through the introduction of natural enemies, mechanical control (e.g., manual removal or mowing), chemical control with weedicides, culture control through vegetation with native plant species, and microbial control. Implementing a combination of these available techniques can open new avenues for managing invasive plant species, preserving biodiversity, and promoting ecosystem health. Regular monitoring to evaluate the efficacy of these measures is also crucial.

5. Conclusions and Future Perspectives

Plant invasions highlight the significant impact of non-native species on ecosystems, biodiversity, and human activities. Understanding the mechanisms of plant invasions is essential for effective management and conservation efforts. Secondary metabolites in invasive plant species play a pivotal role in facilitating plant invasions by influencing various stages of the invasion process. Phenolic compounds, alkaloids, terpeniods, and other phytochemicals are important volatile organic compounds that serve as major contributors to the success of invasive plants. Invasive plants outcompete native ones due to their diverse chemical properties, alter soil composition, and modify ecological interactions. Due to the presence of secondary metabolites, invasive plants possess chemical defense mechanisms against herbivores, pathogens, and competing vegetation. The complex functions of these compounds not only support the establishment and spread of invasive species but also impact ecosystem dynamics and functions. In the future, biological control methods for invasive plant species will involve harnessing living organisms such as insects, pathogens, or herbivores to effectively manage the proliferation and spread of invasive plant species.

In the context of secondary metabolites and plant invasion, future research will likely focus on clarifying the molecular process controlling the production, release, and ecological functions of these compounds. Understanding secondary metabolites in plant invasion is pivotal for shaping the dynamics of invasive species and their interactions with native flora and fauna. By elucidating the mechanisms underlying the production and function of these metabolites, we can develop targeted management strategies to mitigate the impact of invasive plants on ecosystems. Future developments in omics technology will offer deeper insights into the intricate interactions between secondary metabolites and plant invasion, opening the door for more successful conservation and management initiatives. These insights will be paired with ecological modeling and field research.

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Growth Allocation Shifts in the Invasive *Hydrilla verticillata* Under Interspecific Competition with Native Submerged Macrophytes

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Abstract: Communities with high native species diversity tend to be less susceptible to the establishment of invasive species, especially in studies that test their local impact. This study investigated the impact of competition between native submerged aquatic macrophytes (SAMs) (*Egeria najas* and *Egeria densa*) and the exotic *Hydrilla verticillata*, recognized for its invasive potential in aquatic ecosystems, through a mesocosm experiment conducted over six months. Two treatments were evaluated: the intraspecific competition of *H. verticillata* and an interspecific competition involving all three species. The plants were cultivated under controlled conditions, with the foliar and subterranean biomass being monitored to analyze resource allocation patterns. The results showed that, under interspecific competition, the mean foliar biomass of *H. verticillata* was significantly higher compared to the intraspecific treatment, while the subterranean biomass was reduced in the presence of native species. We conclude that native species alter the biomass allocation pattern of the invader, favoring foliar structures over subterranean ones. Among the mechanisms of invasiveness, differential resource allocation represents an important strategy for the adaptation and competitiveness of invasive species influenced by environmental factors and resource competition. These findings contribute to the understanding of competitive interactions in aquatic ecosystems and have implications for the management and control of invasive species, highlighting the importance of promoting native diversity to mitigate invasibility. Future studies should investigate the impacts of reduced subterranean structures on the persistence and spread of submerged invasive species.

Keywords: submerged macrophytes; invasive exotics; natives as barriers; competition

1. Introduction

Aquatic macrophytes play a structural role in lotic and lentic environments, promoting habitat heterogeneity and diversity in natural and artificial ecosystems and contributing to ecosystem services [1–4]. However, their overabundance can cause environmental impacts such as biodiversity loss, eutrophication, and economic and recreational losses, in addition to compromising the very ecosystem services they support [5–7].

The dominance of macrophytes is often associated with damming using hydroelectric reservoirs, which alters aquatic ecosystems and facilitates biological invasions [8–10]. In lentic environments, favorable conditions can accelerate the growth and spread of exotic species, driving physicochemical and biological changes in the habitat, as well as species composition shifts [11–13].

While communities with greater native diversity tend to resist invasive species establishment, the effectiveness of this barrier remains uncertain, varying with environmental changes and species interactions [14–16]. Experiments suggest that low native species diversity facilitates the occurrence of exotic species and that exotic species closely related to natives are potentially more invasive [17,18]. When the invader is functionally similar, it may become a naturalized member of the community [19]. For a successful plant invasion, the exotic species must be functionally different from the natives, but not too much [20]. On the other hand, other studies emphasize that both similarities and differences can be determinants of invasive plant success [21,22].

Moreover, there is still uncertainty regarding whether abundance and species richness act as a barrier [14,15], the extent to which the barrier effect is due to abiotic factors [16], or the development patterns of the involved species [23]. For an invasive plant species to establish and spread, it is necessary for the community to allow the exotic species to thrive initially [24]. Over time, coexistence between the exotic species and the native community would increase spatially, causing the biotic resistance of even functionally diverse communities to weaken. This absence of a biodiversity barrier to plant invasion suggests that coexistence between the invader and the native community is more important than exclusion through competition [25].

The coexistence between exotic and native macrophytes, in addition to being influenced by phylogenetic proximity, can also be influenced by phylogenetic and morphological differences. An example is the invasive macrophyte *Hydrilla verticillata* (L.f.) Royle, which, in a specific community in the Rosana Reservoir in southern Brazil, did not face biotic resistance and established alongside species that were less phylogenetically and morphologically similar [26]. However, it is not excluded that the invader could facilitate the occurrence of some dissimilar native species [26].

Various competitive interactions can affect species biomass and contribute to invasion processes [27]. In stressful situations, such as competition, plants may exhibit differential biomass allocation, prioritizing either aerial (or aquatic, in the case of aquatic organisms) or underground parts [28]. For macrophytes, differential resource allocation is related to the pursuit of greater plant development, such as nutrient assimilation and leaf growth, in response to stressful environmental variations [29–31].

H. verticillata is an invasive submerged aquatic macrophyte [32] that grows in a variety of environments, from lentic to lotic, with great adaptability [3,33]. It has higher energy density compared to native species occupying the same niche, becoming a resource for herbivores [34]. Native to Asia and Australia, it is found on every continent, causing problems for aquatic ecosystems worldwide [35]. In Brazil, it exhibits significant dominance in several river basins [36]. It is often confused with the native species *Egeria najas* Planch. and *Egeria densa* Planch. due to phylogenetic and functional similarities, as all three belong to the Hydrocharitaceae family [3]. *E. najas* and *E. densa* are submerged macrophyte species native to Brazil. Frequently confused with the invasive *H. verticillata*, these natives also cause significant impacts due to excessive growth in reservoirs [37]. They have simple adventitious roots [38] and respond negatively to reservoir disturbances during dry periods [39]. *E. densa* has a higher light requirement and is more affected by water turbidity, while *E. najas* shows greater tolerance and thrives better in reservoirs [40,41]. *H. verticillata* possesses great phenotypic plasticity and disperses efficiently via propagules [42–44]. The large biomass exhibited by this species in dominant situations can displace native species and alter their occurrence in invaded areas [26,36].

Understanding the interactions between native and invasive exotic species is imperative for controlling their spread and developing more effective mitigation strategies [45]. Exploring functional traits related to biomass and differential resource allocation capabilities can reveal important competitive abilities for plant dominance and are crucial for the persistence of exotic species after invasion [46]. Studies on submerged macrophytes conducted in tropical mesocosms, which allow for the control and isolation of desirable or undesirable variables in near-natural conditions, are essential to advancing knowl-

edge about the biology and ecology of these plants [47,48]. This approach facilitates the study of complex species interactions in a controlled environment that simulates natural conditions [49,50].

In this study, we aim to evaluate the effects of native submerged aquatic macrophyte (SAM) species on the invasibility of *H. verticillata*. In a mesocosm experiment (i.e., exploring local climatic conditions), we subjected the invader to growth under (A) intraspecific competition (Control) and (B) interspecific interaction with two native SAM species. Our hypothesis is that competition will negatively influence the development of invasive exotic species. Thus, *H. verticillata* would exhibit changes in leaf and subterranean biomass patterns when subjected to competition with different native SAM species, compared to its development under intraspecific competition.

2. Results

Throughout the experiment period, the daily average temperature was 17.71 °C, with a minimum average of 13.45 °C and a maximum average of 20.42 °C, average humidity of 79.40%, and average precipitation of 5.05 mm, according to data from the meteorological station of Simepar [51], located near the Piracema Park Station, Itaipu Hydroelectric (25°25′46″ S, 54°34′50″ W). The initial biomass of 10 *H. verticillata* ramets was 0.58 g (SD = 0.09), and therefore, the initial biomass of each ramet was 0.06 g.

2.1. Total Biomass of Submerged Aquatic Macrophytes (SAMs)

In terms of total biomass (sum of aquatic and subterranean biomass) (Figure 1), the intraspecific treatment had an average biomass of 122.93 g (SD = 30.65), and the interspecific treatment had an average biomass of 113.40 g (SD = 13.36). These results suggest that, although interspecific competition with native species slightly reduces the total biomass of *H. verticillata*, the effect is not strong enough to result in significant differences. This indicates that the invasive species maintains its competitive potential even in the presence of native species. Further analysis of foliar and subterranean biomass components provides additional insights into resource allocation patterns (Table 1).

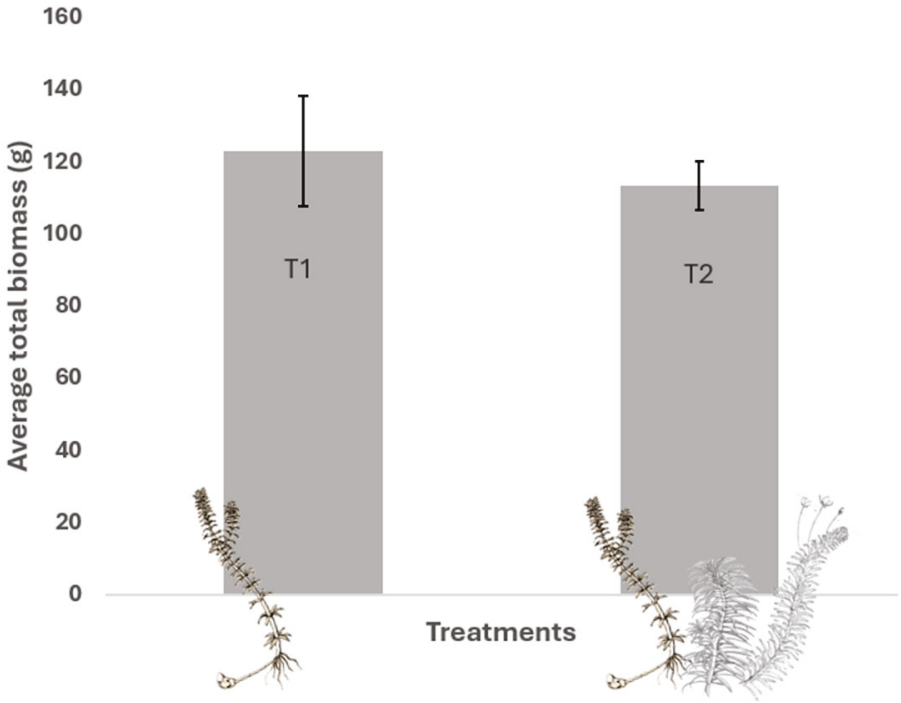

Figure 1. Mean total biomass (foliar + subterranean) of SAMs at the end of the experiment under different competitive treatments. T1 = control with intraspecific competition of *H. verticillata* only; T2 = with interspecific competition of *H. verticillata*, *E. najas*, and *E. densa*.

Table 1. Post hoc Tukey pairwise test results for the generalized linear model (GLM) comparing the biomass growth of SAMs (total, subterranean, aquatic, and biomass increment) in different competitive treatments, focusing on the invasive species *H. verticillata*. T1 = control with intraspecific competition of *H. verticillata*; T2 = with interspecific competition of *H. verticillata*, *E. najas*, and *E. densa*.

Treatment	Total Biomass (Tukey Model)		Subterranean Biomass (Tukey Model)		Foliar Biomass (Tukey Model)		*H. verticillata* Biomass Increment (Tukey Model)	
	Estimate	z-Value	Estimate	z-Value	Estimate	z-Value	Estimate	z-Value
T1 × T2	−0.08	−0.63	−0.63	−3.07 **	0.37	3.29 **	2100.2	9.782 ***

** < 0.01; *** < 0.001.

2.2. Subterranean Biomass of Submerged Aquatic Macrophytes

The subterranean biomass of SAMs (Figure 2) showed that the intraspecific treatment had an average biomass of 70.58 g (SD = 19.22), compared to the interspecific treatment, which had an average biomass of 37.35 g (SD = 11.39; GLM; z = −3.073; $p = 0.006$) (Figure 2, Table 1). These results indicate that interspecific competition with native species reduced the subterranean biomass of *H. verticillata* compared to intraspecific competition, highlighting the impact of the presence of native species on the allocation of subterranean biomass. The significant difference between the treatments suggests that competition with native species has a greater effect on subterranean biomass than on the total or foliar biomass of *H. verticillata*.

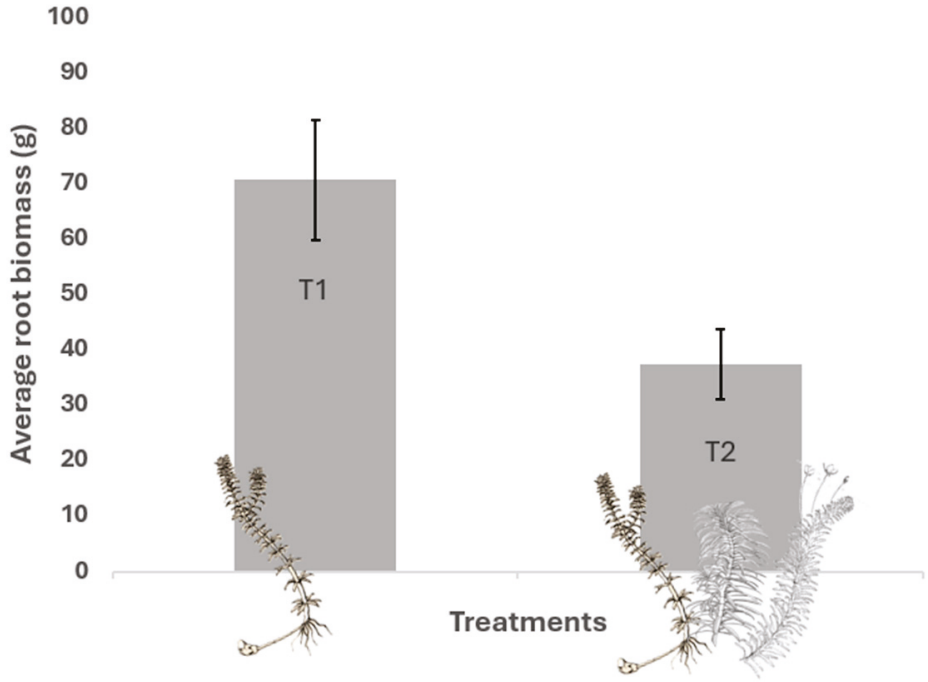

Figure 2. Mean subterranean biomass of SAMs at the end of the experiment under different competitive treatments. T1 = control with intraspecific competition of *H. verticillata* only; T2 = with interspecific competition of *H. verticillata*, *E. najas*, and *E. densa*.

2.3. Total Foliar Biomass of Macrophytes

As for the total foliar biomass of SAMs (Figure 3), the interspecific treatment had an average biomass of 76.05 g (SD = 13.75), presenting a higher mean value than the intraspecific treatment, which had an average biomass of 52.35 g (SD = 10.45; GLM; Z = 3.293; $p = 0.002$). These results suggest that interspecific competition with native species promoted the allocation of more biomass to foliar structures in *H. verticillata* compared to intraspecific competition. The statistical analysis confirmed a significant difference between

the two treatments, highlighting the role of interspecific competition in shaping the biomass distribution towards foliar structures.

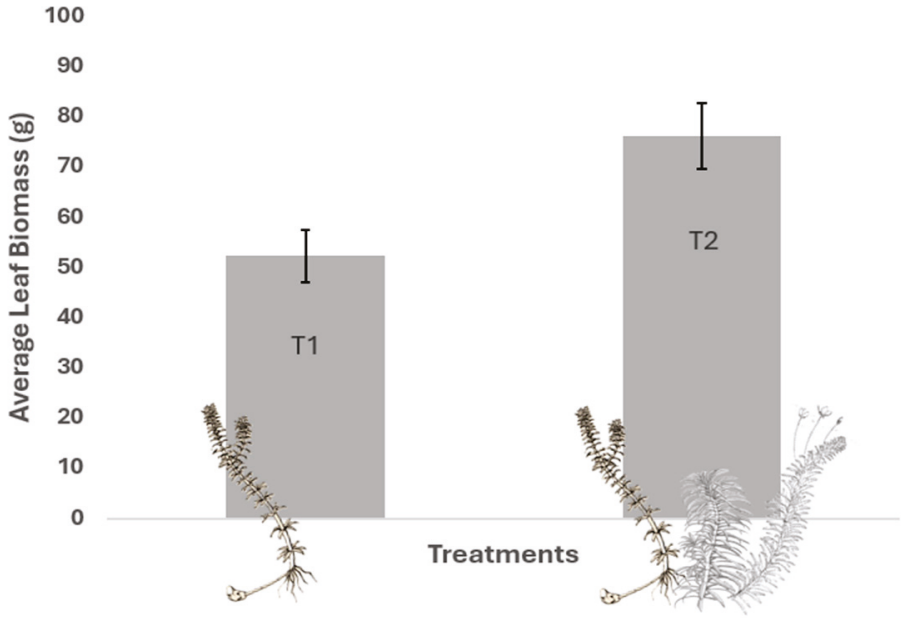

Figure 3. Mean foliar biomass of SAMs at the end of the experiment under different competitive treatments. T1 = control with intraspecific competition of *H. verticillata* only; T2 = with interspecific competition of *H. verticillata*, *E. najas*, and *E. densa*.

2.4. Foliar Biomass Increment of H. verticillata

In terms of *H. verticillata* biomass increment (Figure 4), the interspecific treatment (3103%; SD = 519) had a higher mean value than the intraspecific treatment (1003%; SD = 200; GLM; Z = 9.782; $p = 0.000$). These data indicate that, under interspecific competition, *H. verticillata* had a substantial increase in its foliar biomass, suggesting a competitive adaptation response to maximize leaf growth at the expense of root growth.

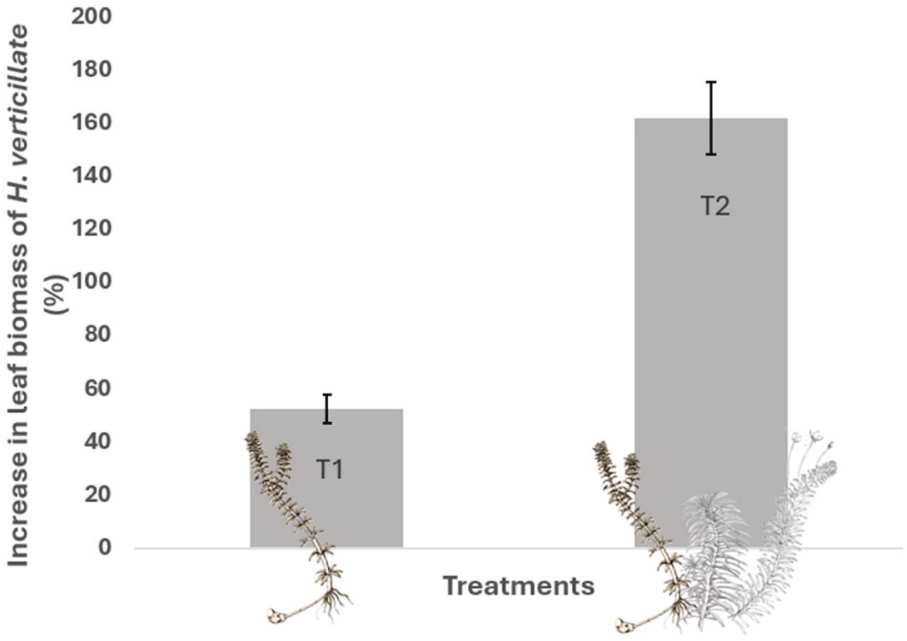

Figure 4. Foliar biomass increment (in percentage) of *H. verticillata* at the end of the experiment under different competitive treatments. T1 = control with intraspecific competition of *H. verticillata* only; T2 = with interspecific competition of *H. verticillata*, *E. najas*, and *E. densa*.

3. Discussion

The study tested the effects of native submerged macrophytes' competitiveness on the growth of the invasive exotic species *H. verticillata*. Our results demonstrate that *H. verticillata* altered its development by promoting differential biomass allocation in response to changes in intraspecific and interspecific competition in the mesocosm treatments. When subjected to interspecific competition, this invasive species allocated biomass to foliar structures at the expense of root structures. It was observed that the native submerged species did not act as a barrier but influenced a reduction in root biomass and an increase in foliar biomass. This effect may be attributed to interspecific competitive interactions, which influence the performance of invasive plants [52–56].

Communities with higher diversity tend to be more competitive and resistant to invasion, which results in greater success in suppressing invasive species [14,54,55]. Furthermore, it is highlighted that communities with high diversity may contain a significant proportion of exotic species, with this diversity being maintained by environmental heterogeneity and interactions between native and exotic species [56]. In other cases, even with diversity, these areas may suffer from invasive species suppression, which can affect small spaces without exerting pressure on large-scale diversity [57], affecting species richness and functional diversity, though with a certain resilience of seeds when confronted by invasive species [58].

Spatial scales must be considered in invasion models, as abiotic factors influence the success of the invasive macrophyte *H. verticillata* at different spatial scales. At a smaller spatial scale, organic matter becomes important, and competitive interactions may inhibit invasion [11]. Therefore, biodiversity loss can increase the vulnerability of native communities to invasions [14], affecting the structure and functioning of ecosystems.

Resource allocation is the foundation for adopting different behavioral strategies in response to environmental pressures [59]. Morphological variations and decisions related to biomass allocation are crucial for plants' developmental success, allowing them to efficiently obtain resources during competition [60,61] and extend their residence time [62]. In this context, we believe that the invasive species prioritized vegetative growth in photosynthetic and dispersal structures upon detecting competition with native plants. Limiting factors, such as light and space, may intensify competition between species, leading to the adoption of strategies for resource acquisition, thereby promoting a positive differential allocation towards the growth of photosynthetic structures [63].

However, it is important to emphasize that this differential resource allocation can favor certain structures at the expense of others [64]. It is not yet certain whether this strategy will have positive or negative effects on the invasive species in the long term, as available results come from six-month experiments conducted with perennial plants [32]. This highlights the need for longer-duration experiments that consider environmental variations with different complexities. It is essential to understand that ecosystem changes may be the main factor influencing interactions related to differential allocations, impacting communities by modifying the availability of limited resources and the allocation priorities of each species [65].

Differential allocation has been evidenced in invasive exotic species, which demonstrate biomass allocation towards foliar areas to enhance light acquisition and photosynthetic capacity [66]. During the invasion process, this differential allocation primarily occurs when species are in interspecific competition, increasing their growth regardless of correlated environmental issues [67]. In some cases, the invasive species adopts this strategy in response to the synergistic effect of interspecific competition with native species and environmental gradients [68]. In other cases, interspecific competition may result in reproductive allocations due to changes in abiotic conditions caused by interactions with native species [69]. This functional characteristic is one of the determinants of the potential for macrophyte species to become invasive [70,71].

Our results confirm the differential allocation of biomass by the invasive species under interspecific competition. With increased competitive potential in seeking vegetative growth to surpass native species, the invasive species compromised its root structures, resulting in reduced resource allocation for anchoring and storage. The increase in foliar biomass of *H. verticillata* at the expense of its subterranean structures indicates that the invasive species exhibited a differentiated growth strategy in the presence of natives. The presence of natives differentially influenced the development of the invasive species, which indeed recognized a competitor. Despite the limitation of its root structure, the greater foliar biomass under interspecific competition may confer competitive advantages to the invasive species in terms of light acquisition and vegetative propagation. During the experiment period, under ideal light and substrate nutrient conditions, the exotic species maintained significant biomass growth above the sediment despite the reduction in root growth, suggesting a trade-off. Continuing this growth pattern could eventually negatively impact the available aquatic space for native species. Thus, the invasive species could gain advantages in vegetative dissemination. However, this potential advantage is inconclusive. The reduction in *H. verticillata* roots may have consequences for its ability to anchor to the substrate and obtain nutrients, especially in more challenging environments [72–74]. As such, the balance of advantages and disadvantages promoted by differential allocation should be explored in future studies.

The 50% shading used in the mesocosm experiments to avoid algae proliferation and water overheating also deserves attention. This experimental choice, based on the literature, may have influenced the development of *H. verticillata* and native species, suggesting the need to consider its possible effects on the competitive dynamics between species. Thus, we suggest that future studies consider different shading levels in small-scale experiments in tropical regions and their potential interactions with root growth dynamics, as well as evaluate the impact of other abiotic factors (i.e., nutrient availability and temperature variations).

Despite these considerations, our contributions are relevant for understanding the competitive interactions between *H. verticillata* and native species, both above and below the substrate. By identifying that *H. verticillata* increases foliar biomass but reduces root growth, management practices focusing on root structures are suggested. The removal of ramets could offer promising results, as the potential for regeneration through root structures may be compromised due to the significantly reduced biomass. The study reaffirms the importance of environmental management strategies that consider these multiple factors, as well as the need for further research exploring the complex interactions between invasive and native species under different ecological conditions, the effects of root structure reduction on invasiveness and competition in submerged aquatic plants, considering temporal variations, and incorporating greater complexity into experimental communities and other native community parameters, such as species composition, abundance, and functional traits, which should be explored as potential barriers to invasion under different environmental factor variations.

4. Materials and Methods

4.1. Collection Area of SAMs

The Itaipu Reservoir, located on the Paraná River between Brazil and Paraguay, has relatively stable hydrometric levels on its eastern side (Brazilian side), with an average depth of 22 m and water quality ranging from oligotrophic to eutrophic [75]. Its large expanse of shallow areas (between 0.5 and 4.0 m deep) favors the development of submerged macrophytes [11]. The macrophytes were collected from two of the eight tributary rivers formed by the Itaipu reservoir that had already been monitored [76], the Passo Cuê River (25°20′19″ S, 54°26′41″ W) and the São João River. (25°04′21″ S, 54°22′27″ W) (Figure 5). Using a rake, we collected samples of the native species *E. najas*, *E. densa*, and the exotic invasive *H. verticillata*, Gathering the collection from both arms for the mesocosm experiment. These species are among the main submerged macrophytes found in the reservoir,

where four species of Hydrocharitaceae can be found, contributing to a total diversity of 87 macrophyte species, five of which are non-native [37].

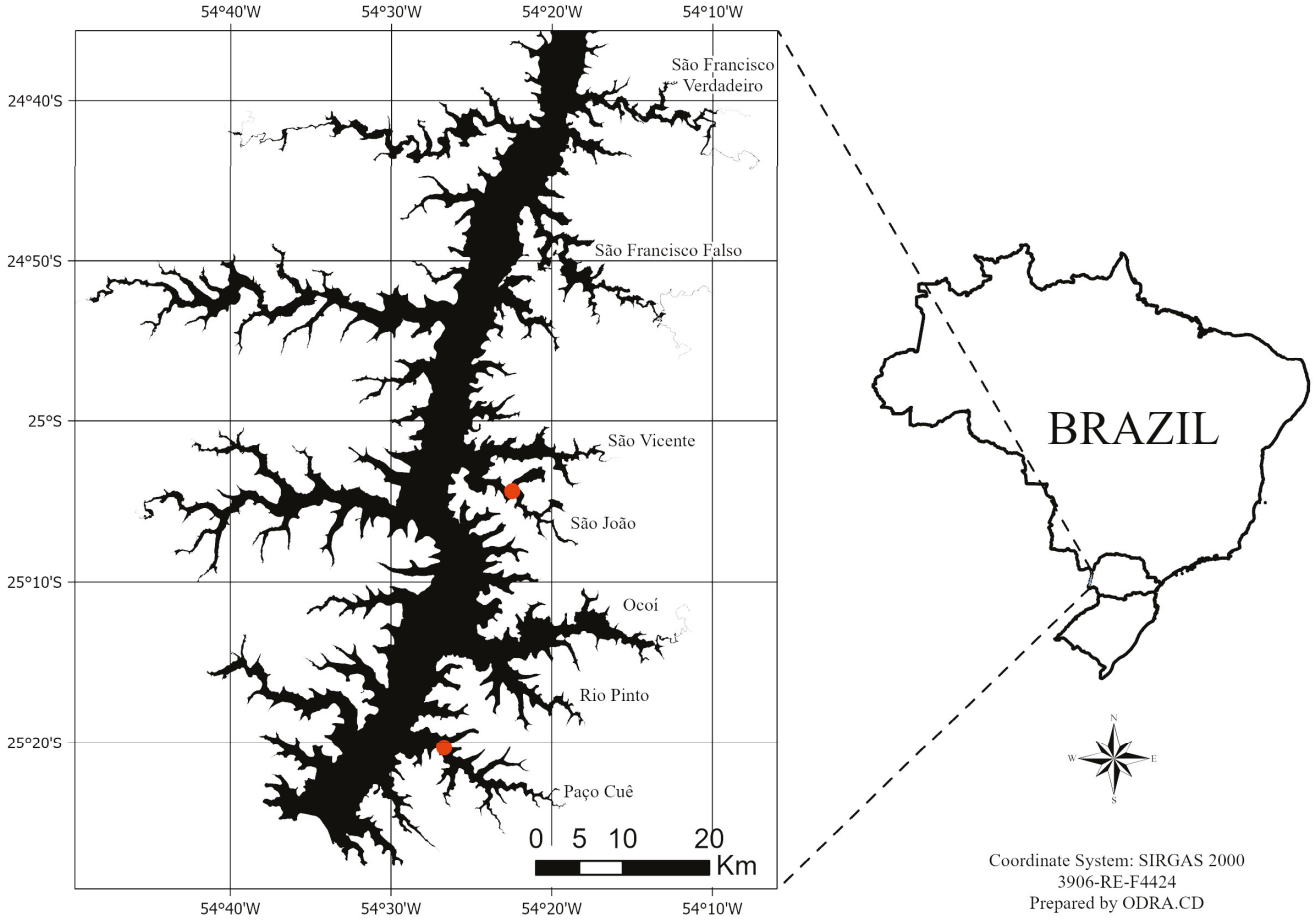

Figure 5. Collection area in the Itaipu Reservoir, located in the Paraná River basin. The marked points are the Passo Cuê and São João arms, where the invasive exotic *H. verticillata* and the native species *E. najas* and *E. densa* were collected.

4.2. Plant Processing and Mesocosm Setup

The collected macrophytes were stored in plastic bags with reservoir water. In the laboratory, the macrophytes were carefully washed to remove macroinvertebrates, algae, and debris. The experiment was conducted over six months at the Parque da Piracema station, located at the Itaipu Hydroelectric (25°25′46″ S, 54°34′50″ W), in 10 cylindrical tanks of 500 L capacity, 60 cm in height, and 1 m in diameter, installed outdoors with 50% shading, ideal for cultivating submerged macrophytes [69]. Each tank contained a 25 kg layer of industrialized organic substrate and a 2 cm layer of coarse sand and was filled with well water (without the addition of artificial chemical components). The macrophyte ramets were standardized into 15 cm fragments for planting, all on the same day. During the experiment, partial water exchanges (50%) were performed weekly to prevent algae growth (Figure 6). To ensure that the observed effects were solely due to plant competition, the experiment was conducted using the same substrate, space, and number of initial ramets across the different treatments, controlling the main environmental variables responsible for plant development and providing standardized conditions across treatments. However, the initial number of *H. verticillata* ramets varied for each treatment. To address this analytically, we worked with the biomass acquired throughout the experiment, allowing us to make balanced comparisons of invasive development, regardless of the treatment and initial number of ramets, while maintaining standardized treatments.



Figure 6. Mesocosms in 500 L tanks; newly planted macrophytes; macrophytes after five months; 50% shading setup.

To assess the influence of different species quantities on the invader's development, the experiment was conducted with treatments involving different interactions, randomly distributed across the 10 tanks (5 replicates per treatment) as follows: (1) Control treatment of intraspecific interaction, planting 90 ramets of *H. verticillata*, to evaluate the invader's development without the influence of interspecific competition; and (2) Interspecific interaction treatment, planting 90 ramets, with 30 ramets of *H. verticillata*, 30 ramets of *E. najas*, and 30 ramets of *E. densa*.

To estimate the initial biomass of *H. verticillata* ramets, 50 standardized ramets (15 cm) were separated and dehydrated in an oven at 45 °C until constant weight was achieved (about 2 days). The dry mass (g) was obtained gravimetrically by separating the ramets into five sets of 10. Thus, the estimated initial biomass of each *H. verticillata* ramet is the average dry mass obtained (average of five sets of 10 dried ramets) divided by 10. The initial biomass value of *H. verticillata* in each treatment is the estimated initial weight of each ramet multiplied by the number of ramets of the invader at the start of the experiment.

4.3. Data Collection

The experiments were dismantled after six months of growth of the macrophyte plants. All plant material was removed from the mesocosms, and the ramets (aquatic biomass) were separated from the roots (subterranean biomass) in each replicate for each treatment. The ramets were also sorted by species. It was not possible to separate the roots by species, as by the end of the experiment, all root structures were entangled. In the laboratory, the biomass (ramets and roots) was thoroughly washed to remove algae and invertebrates and then dried in an oven at 45 °C until a constant weight was achieved (about 4 days). The dry mass (g) of each set (total roots, ramets by species in each replicate of each treatment) was obtained gravimetrically.

4.4. Data Analysis

To assess the biomass increase in *H. verticillata* ramets in each treatment over the course of the experiment, we divided the dry biomass of the invader obtained gravimetrically at the end of the experiment by the initial biomass and calculated the percentage increase. To assess the effects of competitive relationships of *H. verticillata* on preferential biomass allocation (above the sediment or subterranean biomass) in the competitive treatments, we structured the data analysis by comparing (1) differences in total biomass per treatment (biomass of ramets and root structures combined for all species per treatment); (2) differences in subterranean biomass per treatment (biomass of all root structures combined); (3)

differences in foliar biomass per treatment (biomass above the sediment, combined for all species); and (4) differences in the increase in foliar biomass of the invasive species per treatment (increase in the biomass of *H. verticillata* ramets above the sediment).

For this, a generalized linear model was used with the glm function from the R software 2024.04.1+748 [77]. To compare each treatment pairwise, we used a multiple comparisons test for general linear hypotheses by Tukey with the glht function from the multcomp package [78]. The graphs were generated using the Excel software [79].

5. Conclusions

The results indicated that the presence of native species influenced the biomass allocation pattern of *Hydrilla verticillata*, prioritizing the growth of foliar structures at the expense of root structures, particularly under interspecific competition. Although the native species did not function as a complete barrier to the dominance of the invasive species, they induced a reduction in the root biomass of *H. verticillata*, which may compromise its anchorage and ability to exploit nutrients in the substrate. These findings suggest that, while the hypothesis was partially supported, further studies are needed to fully understand the long-term implications of reduced root structures and their impact on aquatic plant invasion. Therefore, the effect of native species diversity on invasion dynamics remains complex and dependent on factors such as competition intensity and environmental conditions.

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Article

Adaptive Benefits of Antioxidant and Hormone Fluctuations in *Wedelia trilobata* Under Simulated Salt Stress with Nutrient Conditions

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Abstract: Salinity is one of the most significant environmental factors limiting plant development and productivity. Invasive plants could quickly respond to environmental changes, thus successfully achieving invasion. However, there is limited research on the mechanism of salt responses in invasive plants under different nutritional conditions. This study evaluated and compared the impact of salinity stress and nutrient application on physiological responses in the invasive plant *Wedelia trilobata* and native plant *Wedelia chinensis*. Mild salinity stress disrupted the growth of these two plants, significantly reducing their leaf and stem node number under a low nutrient condition. *W. trilobata* showed notable decreases in height and leaf number with high salinity stress regardless of nutrient levels, whereas it was observed only in the low nutrient state in *W. chinensis*. The negative effects of high salinity on both species were most evident in nutrient-poor environments. Under low salinity and nutrient stress, *W. trilobata*'s leaves exhibited increased levels of proline, MDA, CAT, and ABA, with decreased GA and IAA content. A low-salt environment favored *W. trilobata*'s competitive advantage, and nutrient enrichment appeared to enhance its invasive potential, in which process the plant antioxidant system and endogenous hormones contribute greatly. This study provides a theoretical foundation for predicting suitable growth areas for *W. trilobata* referring to the salt condition, guiding future strategies for preventing and controlling its invasive spread.

Keywords: invasive plant; simulated salt stress; nutrient conditions; plant antioxidant system; hormone contents

1. Introduction

Salinity has increasingly come under scientific scrutiny as a critical factor impeding the normal growth of plants, with approximately 8.7% of the world's land being endangered by salinization, and China, in particular, experiencing a disproportionately severe soil salinization issue, constituting one-eighth of the global total [1,2]. Elevated salt concentrations under stressful conditions often lead to physiological disruptions in most plants, including impediments in different physiological activities [3]. These adverse effects may culminate in outcomes such as leaf abscission, reduced biomass, and, in extreme cases, plant mortality [4]. Salt stress raises intracellular osmotic pressure and leads to sodium ion toxicity, necessitating plant adaptations including ion balance regulation, phytohormone

signaling, and antioxidant enzyme control [5]. The global proliferation of invasive plant species into new habitats underscores a pressing environmental challenge, as these invaders swiftly adapt to unfamiliar conditions, thereby enhancing their invasive potential through intricate interactions with a range of biotic and abiotic factors [6,7]. Many invasive plants show rapid adaptability to environmental stress [8,9]. Understanding the mechanisms behind invasive plant success is a primary focus in invasion ecology [10]. Invasive weeds consequently find themselves vulnerable to physiological disorders, a marked reduction in photosynthetic rates, and compromised growth [11]. Conversely, in nutrient-rich environments, invasive weeds gain a competitive edge over their native counterparts, flourishing amidst the challenges of saline ecosystems [12].

In the realm of invasive weeds, the paramount influence of salinity and nutrient application takes center stage, orchestrating a sophisticated interplay of enzymatic activities and endogenous hormones within plants [13]. Particularly in the challenging terrain of saline environments, salinity emerges as a formidable adversary, disrupting the operation of vital enzymes engaged in metabolic processes crucial for the very foundation of plant growth and development [14]. In this hostile setting, the disruption of enzymatic activities by salinity poses a substantial hurdle, affecting essential pathways such as photosynthesis and respiration, and thereby challenging the overall health and productivity of the plant [15,16]. Moreover, nutrient availability takes on the role of a linchpin, driving the efficiency of enzymatic reactions and wielding influence over endogenous hormones [17,18]. Specific nutrients serve as indispensable cofactors and substrates, forming the fundamental basis for achieving optimal enzymatic performance and regulating the delicate balance of hormones [19]. This nuanced interplay underlines the significance of precise nutrient provision and management, pivotal to ensuring robust enzymatic activities and hormone equilibrium, ultimately enhancing the resilience and performance of plants in various environmental contexts [20]. Endogenous hormones, the masterminds behind plant growth and development, are finely attuned to the persuasive influences of salinity and nutrient availability [21,22]. They govern the intricate orchestration of hormones, including auxins, gibberellins, and abscisic acid, through their synthesis and distribution [23]. In saline conditions, the closure of stomata is prompted to conserve water, while nutrient-rich environments encourage the activation of hormone pathways that expedite growth and mold the plant's form and function [24]. This intricate interplay, entailing salinity, nutrient application, enzymatic activities, and endogenous hormones, carries profound significance within the realm of agriculture [25].

The strategies developed to oversee and alleviate the effects of salinity on enzymatic processes and hormone regulation are undeniably crucial, particularly in regions plagued by the enduring challenge of soil salinity [26]. The precise delivery of nutrients and skilled management of salinity levels emerge as pivotal tools to enhance plant resilience. *W. trilobata*, native to tropical America, is a highly invasive species found in southern China, notably in the Guangxi and Hainan provinces [27,28]. It is listed among the "World's 100 most malignant invasive species" by the IUCN [29]. Many studies have explored diverse facets of *W. trilobata* in the context of abiotic stresses, yet its adaptation to salt stress under varying nutrient conditions remains notably unexamined and unreported. In this study, we used *W. chinensis*, a closely related Asteraceae plant in China, as the native control plant [28], and compared how simulated salt stress affects the growth, antioxidant enzyme activities, and endogenous hormone ratios of *W. trilobata* and *W. chinensis* under varying nutrient conditions. Our specific objectives were to answer the following questions: (1) Might *W. trilobata* outcompete native species under simulated salt stress with various nutrient conditions? (2) In this process, what are the differences in antioxidant systems and plant hormone responses between these two species? The findings will offer insights

into predicting suitable habitats for *W. trilobata* and inform strategies for future invasion prevention and control.

2. Materials and Methods

2.1. Experimental Materials

The invasive *W. trilobata* was collected from the urban precincts of Haikou city, Hainan province (19°31′~20°04′ N, 110°07′~110°42′ E). Concurrently, *W. chinensis* was gathered from the greenhouse of School of Environment and Safety Engineering at Jiangsu University. The stem segments of *W. trilobata* and *W. chinensis*, exuding remarkable vigor and steadfast uniformity in both length and girth, were handpicked to ensure the retention of two nodes for each stem segment. These selected cuttings were cultured in round plastic pots, measuring 90 millimeters (mm) in diameter, 60 mm in width, and 80 mm in height as botanical specimens. As the experiment's foundation, the river sand was measured with exactitude, allotting 360 g for each individual pot. The chosen stem segments were arranged in a vertical posture within their respective pots, watered with deionized water, and housed within the greenhouse. After a span of 4 to 5 days, the nascent buds were ushered into the realm of emergence, marking the moment for subsequent treatments.

2.2. Experimental Method and Design

The bifactorial design in this experiment was as follows: factor 1—three different salt stress levels: no salt (0 mM), low salt (100 mM, equivalent to a salt content of 0.117%), and high salt (200 mM, corresponding to a salt content of 0.234%); and factor 2—three divergent nutrient levels: low nutrient level (0.1 × Hogland standard nutrient solution to replicate circumstances of nutrient deprivation), normal nutrient level (0.5 × Hogland standard nutrient solution to mimic the requisites for typical plant growth), and high nutrient level (1.0 × Hogland standard nutrient solution to imitate a nutrient-rich milieu). For each experimental treatment, there were 5 replicates and 2 plant species, resulting in a total of 90 pots. Three plants of each experimental treatment replicates were randomly selected for data analysis. Simultaneously, in order to mitigate the salinity-induced repercussions stemming from the 200 mM salt treatment, incremental escalations from 100 mM NaCl were implemented at intervals of 24 h, with a concomitant addition of 100 mL on each occasion to reach the desired concentration. Following the salt stress intervention, the aforementioned triad of nutrient levels was applied every alternate day, with each plant receiving a 100 mL aliquot of water.

3. Data Collection

3.1. Phenotypic Growth Indicators

After a growth period of 30 days post-cultivation, the plants grown in each receptacle were carefully harvested, and relevant parameters underwent assessment. The evaluation encompassed a range of growth indices, including plant height, leaf number, stem node number, and root length. In the interest of precision, each index underwent triplicate measurements, and the resultant data were subsequently averaged for statistical rigor. The enumeration of leaf and stem nodes was carried out manually, while plant height and root length were quantified using Image J 1.38e (https://imagej.nih.gov/ij/) (accessed on 15 October 2021), which was employed after capturing digital images with a specialized camera apparatus. The freshly harvested leaves, stems, and roots were thoroughly cleansed with pristine water, briefly exposed to a temperature of 105 °C for a duration of 10 min, and thereafter transferred to a drying oven, where they underwent desiccation at 65 °C for a span of 72 h. The determination of leaf dry weight, stem dry weight, and root dry weight was carried out with utmost precision, employing an analytical balance of exactitude. The

aggregate of these individual measurements constituted the overall plant biomass, whereas the ratio of root dry weight to aerial dry weight delineated the root–shoot ratio.

3.2. Biochemical Indicators

Measurements of the antioxidant system include the determination of proline, malondialdehyde (MDA), peroxidase (POD), and catalase (CAT) content. Where proline was measured by the acid ninhydrin method [30], MDA was determined using the thiobarbituric acid method [31], POD activity was determined by the guaiacol chromogenic method and CAT activity was measured by the UV absorption method [32].

3.3. Endogenous Hormones

The content of gibberellin (GA), auxin (IAA), and abscisic acid (ABA) in *W. trilobata* and *W. chinensis* was determined using an enzyme-linked immunosorbent assay (ELISA) kit [33]. The endogenous hormone kit was purchased from the Crop Chemical Control Laboratory, China Agricultural University (Beijing, China).

4. Data Analysis

The analytical software SPSS (version 22.0) was duly employed to carry out both univariate and multivariate analyses of variance (ANOVA) as well as correlation analyses for each individual factor under consideration. The two-way analysis of variance of the two factors was shown in Tables 1 and 2, and the Pearson correlation analysis was shown in Tables 3 and 4. Concurrently, the software Origin (version 8.1) was harnessed to graphically represent the outcomes derived from the rigorous statistical examination of the dataset. In the pursuit of comprehensive insights, multiple comparisons were meticulously executed through the application of the Student–Newman–Keuls test, facilitating a detailed exploration of the observed disparities. It is noteworthy that, throughout this intricate investigative process, a threshold of statistical significance was meticulously set at a value of $p \leq 0.05$ for all the biomarkers, thereby ensuring that only the most robust and substantiated findings were considered noteworthy and, by extension, actionable.

Table 1. Analysis of variance results of the effects of salt and nutrition on *Wedelia trilobata*.

***Wt* Parameters**	**Salt**		**Nutrition**		**Salt × Nutrition**	
	F	***p***	**F**	***p***	**F**	***p***
PH	9.653	0.001	42.472	<0.001	3.427	0.03
LN	0.419	0.664	32.012	<0.001	1.882	0.157
NN	2.188	0.141	10.256	0.001	0.251	0.905
RL	15.22	<0.001	2.436	0.116	1.209	0.341
TB	76.457	<0.001	49.404	<0.001	2.871	0.053
RSR	3.289	0.061	21.522	<0.001	1.173	0.356
Proline	29.772	<0.001	37.071	<0.001	10.639	<0.001
MDA	5.597	0.013	45.933	<0.001	0.851	0.511
POD	41.974	<0.001	17.317	<0.001	0.821	0.529
CAT	20.634	<0.001	18.081	<0.001	3.917	0.019
GA/ABA	46.861	<0.001	36.61	<0.001	12.993	<0.001
GA/IAA	7.473	0.004	43.59	<0.001	0.219	0.924
IAA/ABA	48.753	<0.001	25.182	<0.001	12.206	<0.001

Notes: $p < 0.05$ indicates a significant difference, $p > 0.05$ means no significant difference.

Table 2. Analysis of variance results of the effects of salt and nutrition on Wedelia chinensis.

Wc Parameters	Salt		Nutrition		Salt × Nutrition	
	F	*p*	F	*p*	F	*p*
PH	7.339	0.005	33.814	<0.001	2.838	0.055
LN	4.41	0.028	6.304	0.008	1.675	0.199
NN	17.397	<0.001	10.691	0.001	2.132	0.119
RL	22.25	<0.001	7.416	0.004	1.879	0.158
TB	84.638	<0.001	17.916	<0.001	0.966	0.45
RSR	0.135	0.875	8.182	0.003	1.656	0.204
Proline	0.895	0.426	9.356	0.002	0.555	0.698
MDA	21.139	<0.001	1.558	0.238	1.731	0.187
POD	114.331	<0.001	20.27	<0.001	7.878	0.001
CAT	24.65	<0.001	3.56	0.05	13.604	<0.001
GA/ABA	9.234	0.002	3.158	0.067	1.23	0.333
GA/IAA	32.582	<0.001	9.064	0.002	14.803	<0.001
IAA/ABA	17.049	<0.001	0.451	0.644	10.295	<0.001

Notes: $p < 0.05$ indicates a significant difference, $p > 0.05$ means no significant difference.

Table 3. Pearson correlation analysis of simulated salt stress on growth indicators, antioxidant system, and endogenous hormone ratio of *Wedelia trilobata* under different nutrient conditions.

	PH	LN	NN	RL	TB	RSR	Pro	MDA	POD	CAT	G/A	G/I	I/A
PH	1	0.737 **	0.755 **	0.368	0.767 **	−0.728 **	−0.419 *	−0.584 **	−0.288	−0.686 **	0.680 **	0.555 **	0.700 **
LN		1	0.658 **	0.007	0.517 **	−0.651 **	−0.288	−0.590 **	−0.150	−0.490 **	0.291	0.233	0.341
NN			1	0.316	0.694 **	−0.557 **	−0.414 *	−0.594 **	−0.144	−0.553 **	0.504 **	0.419 *	0.563 **
RL				1	0.610 **	−0.296	−0.355	−0.265	−0.460 *	−0.577 **	0.474 *	0.306	0.536 **
TB					1	−0.552 **	−0.494 **	−0.520 **	−0.653 **	−0.792 **	0.778 **	0.513 **	0.809 **
RSR						1	0.601 **	0.779 **	0.327	0.626 **	−0.501 **	−0.504 **	−0.532 **
Pro							1	0.684 **	0.275	0.374	−0.273	−0.480 *	−0.323
MDA								1	0.075	0.496 **	−0.387 *	−0.422 *	−0.467 *
POD									1	0.547 **	−0.450 *	−0.083	−0.489 **
CAT										1	−0.762 **	−0.374	−0.801 **
G/A											1	0.588 **	0.949 **
G/I												1	0.390 *
I/A													1

The PH: plant height; LN: leaf number; NN: number of nodes, RL: root length, TB: total biomass, RSR: root–shoot ratio, Pro: proline, MDA: malonaldehyde, POD: peroxidase; CAT: catalase; G/A: GA/ABA value; G/I: GA/IAA value; I/A: IAA/ABA value. The symbol of "*" indicates significant correlations ($p < 0.05$) and "**" signifies exceedingly momentous correlation ($p < 0.01$).

Table 4. Pearson correlation analysis of simulated salt stress on growth indicators, antioxidant system, and endogenous hormone ratio of *W. chinensis* under different nutrient conditions.

	PH	LN	NN	RL	TB	RSR	Pro	MDA	POD	CAT	G/A	G/I	I/A
PH	1	0.639 **	0.465 *	0.400 *	0.701 **	−0.417 *	−0.415 *	0.175	−0.507 **	−0.380	0.509 **	0.366	0.608 **
LN		1	0.694 **	0.362	0.674 **	−0.389 *	−0.232	0.183	−0.632 **	−0.310	0.501 **	0.123	0.652 **
NN			1	0.379	0.742 **	−0.354	−0.225	0.485 *	−0.800 **	−0.440 *	0.595 **	0.355	0.692 **
RL				1	0.672 **	−0.29	0.127	0.321	−0.684 **	−0.482 *	0.255	−0.134	0.476 *
TB					1	−0.293	−0.050	0.649 **	−0.831 **	−0.587 **	0.589 **	0.306	0.711 **
RSR						1	0.437 *	0.130	0.180	0.007	−0.258	−0.322	−0.293
Pro							1	0.141	0.097	−0.099	−0.384 *	−0.516 **	−0.259
MDA								1	−0.487 **	−0.268	0.301	0.192	0.281
POD									1	0.674 **	−0.702 **	−0.253	−0.829 **
CAT										1	−0.496 **	−0.187	−0.707 **
G/A											1	0.753 **	0.901 **
G/I												1	0.484 *
I/A													1

The PH: plant height; LN: leaf number; NN: number of nodes, RL: root length, TB: total biomass, RSR: root–shoot ratio, Pro: proline, MDA: malonaldehyde, POD: peroxidase; CAT: catalase; G/A: GA/ABA value; G/I: GA/IAA value; I/A: IAA/ABA value. The symbol of "*" indicates significant correlations ($p < 0.05$) and "**" signifies exceedingly momentous correlation ($p < 0.01$).

5. Results and Analysis

5.1. Effects of Different Salinity Level on the Growth of Wedelia trilobata and W. chinensis Under Different Nutrient Conditions

In the absence of salinity stress, incremental or decremental alterations in nutritional levels exhibited no discernible influence on the growth parameters of *W. trilobata* in comparison to standard nutrient conditions (Figure 1a–d). However, for *W. chinensis*, the decrease in leaf number and stem nodes was notably significant under low nutrient conditions (Figure 1b,c). Under conditions of low salinity stress, the plant height and leaf number of both *W. trilobata* and *W. chinensis* exhibited substantial reductions to varying degrees in response to low nutrient levels (Figure 1a,b). When subjected to high salinity stress, low nutrient levels manifested distinct impacts on plant height, as contrasted with standard nutrient conditions (Figure 1a). Furthermore, low nutrient levels had a noticeable effect on the leaf number of *W. trilobata* (as exemplified in Figure 1b). However, for *W. chinensis*, the pronounced influence of high salinity stress on plant height, leaf number, and root length was only observed under conditions of low nutrient availability (Figure 1a,b,d). In the absence of salinity stress, low nutrient levels demonstrated a significant effect on the total biomass and root–shoot ratio of *W. trilobata* in comparison to standard nutrient conditions (Figure 2a,b; $p < 0.05$), resulting in a 21.6% decrease in the total biomass. Conversely, *W. chinensis* appeared unaffected (Figure 2a). Under both high and low salinity stress, low nutrient levels led to a substantial reduction in the total biomass of *W. trilobata* compared to standard nutrient conditions (Figure 2a; $p < 0.05$). Conversely, the total biomass of *W. chinensis* only declined under low nutrient conditions in the presence of high salinity stress (Figure 2a; $p < 0.05$). Irrespective of high salinity stress conditions, Low nutrition reduced the root–shoot ratio of both plant species when contrasted with standard nutrient conditions (Figure 2b). Correlation analysis results indicated a positive association between the total biomass of *W. trilobata* and all corresponding growth indicators. The root–shoot ratio exhibited a notably negative correlation with plant height, leaf number, and stem node number (as presented in Table 4; $p < 0.01$). In the case of *W. chinensis*, the total biomass was significantly positively correlated with all the pertinent growth indicators, while the root–shoot ratio displayed a significant negative association with plant height and leaf number (Table 4; $p < 0.05$).

5.2. Effects of Different Salinity Levels on the Antioxidant Enzyme Activities of Wedelia trilobata and W. chinensis Under Different Nutrient Conditions

As depicted in Figure 3, in the absence of salt-induced stress, the concentrations of proline, MDA, and CAT in *W. trilobata* exhibited a notable increase under conditions of diminished nutrient availability, in stark contrast to standard nutrient levels, while the levels of POD did not change significantly and remained unaltered (Figure 3a–d). Within the same treatment framework, solely the POD content displayed a substantial increment (Figure 3c), while the remaining antioxidant indices exhibited no significant deviations for *W. chinensis*. Under low salt stress, heightened nutrient levels resulted in a significant reduction in MDA content in *W. trilobata* compared to the standard nutrient levels, while *W. chinensis* remained relatively unchanged. Notably, both elevated and reduced nutrient levels elicited an augmentation in the POD content for *W. trilobata*, with this effect being solely observable at diminished nutrient levels for *W. chinensis* (Figure 3c). In the context of low salt stress, the nutritional status impacted the CAT content of the two species disparately, giving rise to a substantial decline in CAT content for *W. trilobata* under heightened nutrient levels, while *W. chinensis* experienced a marked increase in CAT content, irrespective of whether nutrient levels were elevated or reduced (Figure 3d, $p < 0.05$). Under high salt stress conditions, heightened nutrient levels engen-

dered a pronounced reduce in proline and MDA concentrations for *W. trilobata*, whereas these fluctuations did not significantly influence *W. chinensis*. For both *W. trilobata* and *W. chinensis*, the levels of POD and CAT underwent substantial modifications in response to diminished nutrient availability, with POD content experiencing a notable increment under low nutrient levels for *W. trilobata*, while for the same species, CAT content exhibited a significant reduction under reduced nutrient conditions (Figure 3). The outcomes of the correlation analysis unveiled that proline content in *W. trilobata* exhibited a notable and adverse relationship with plant height, node count, total biomass, and the root–shoot ratio, while the MDA content revealed a remarkably strong inverse correlation with plant height, leaf number, stem count, total biomass, and the root–shoot ratio. Furthermore, the POD content demonstrated a significant and negative association with root length and total biomass, whereas the CAT content exhibited a substantial and negative relationship with all the growth indicators (Table 3, $p < 0.05$). Conversely, the proline content in *W. chinensis* exhibited a significant association with plant height and the root–shoot ratio. The MDA content displayed a noteworthy positive correlation with the stem node count and total biomass. Intriguingly, the POD content exhibited an extremely strong and inverse relationship with all the growth indicators, while the CAT content showcased a significant and negative association with the stem node count, root length, and total biomass (Table 4, $p < 0.05$).

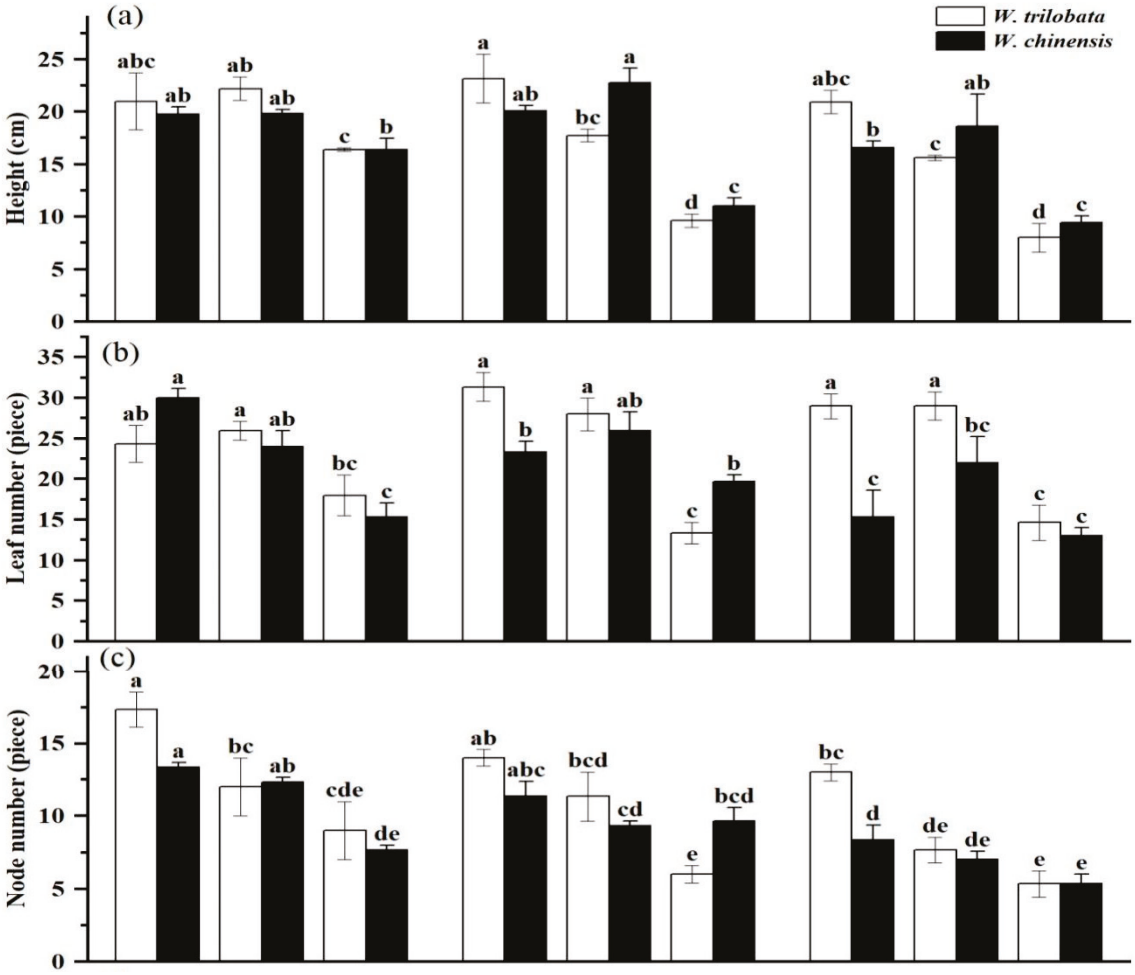

Figure 1. *Cont.*

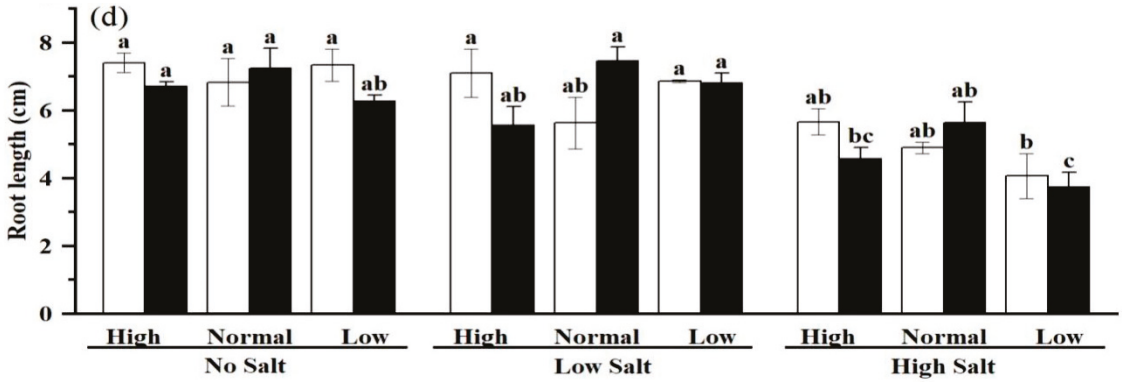

Figure 1. Effects of simulated salt stress on plant height (**a**), leaf number (**b**), nude number (**c**), and root length (**d**) of two plants under different nutrient conditions. The data are presented as the mean ± SE (n = 3), and sharing the different letters indicates significant differences between treatments ($p < 0.05$).

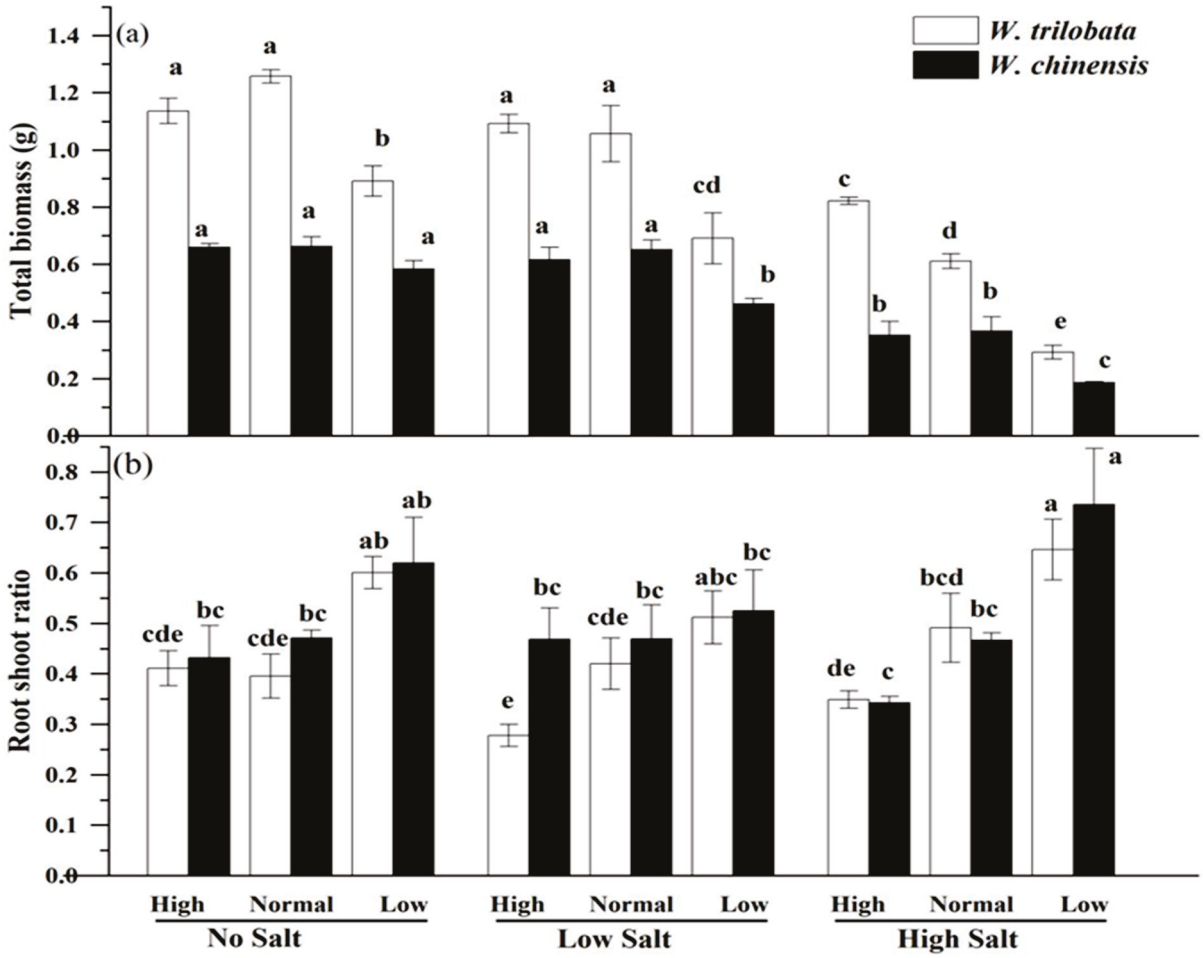

Figure 2. The impact of simulated salt stress on the total biomass (**a**) and root–shoot ratio (**b**) of two plants under different nutrient conditions. The data are presented as the mean ± SE (n = 3), and sharing the different letters indicates significant differences between treatments ($p < 0.05$).

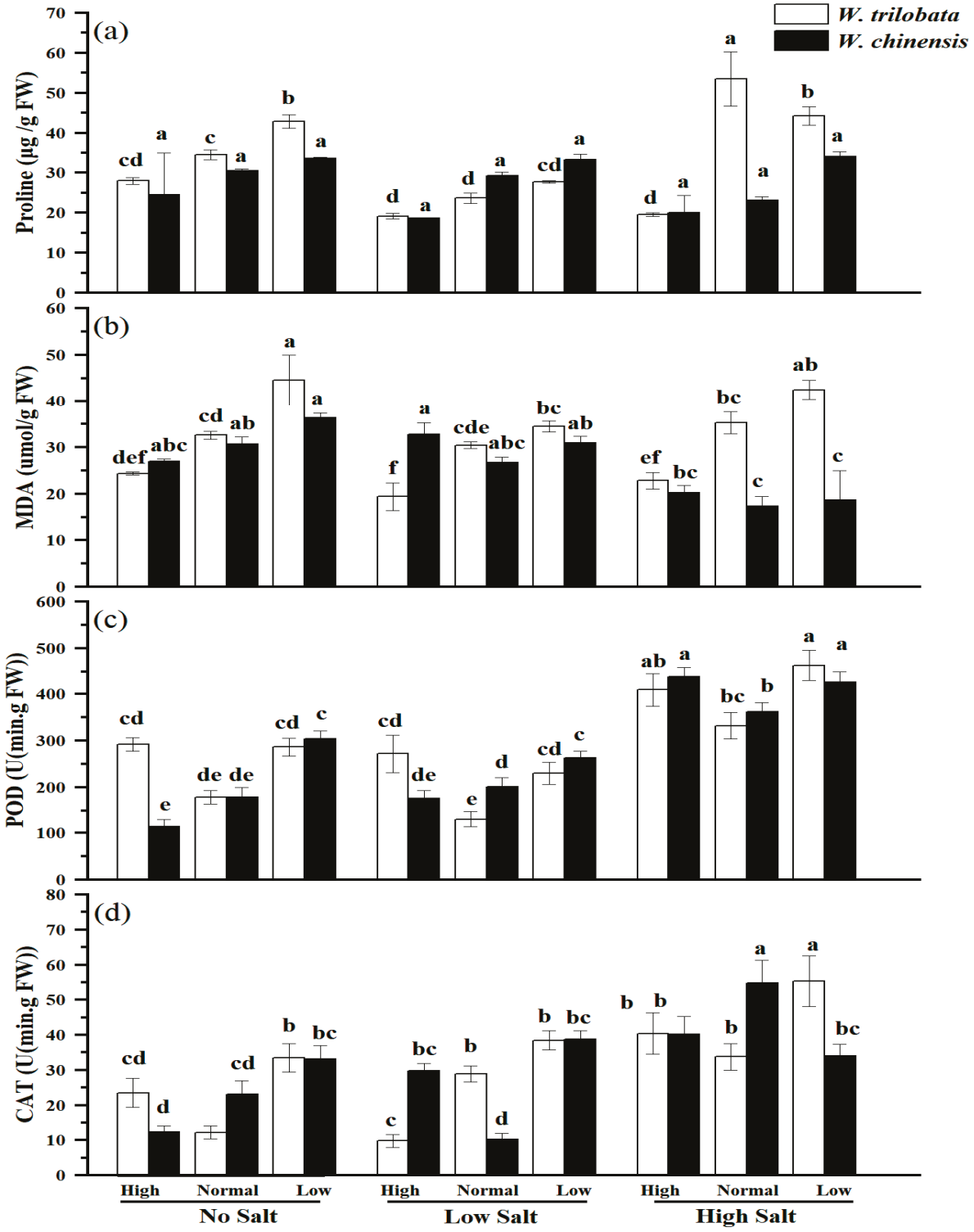

Figure 3. The impacts of simulated salt stress on proline (**a**), malondialdehyde (**b**), peroxidase (**c**), and catalase (**d**) of two plants under different nutrient conditions. The data are presented as the mean ± SE (n = 3), and sharing the different letters indicates significant differences between treatments ($p < 0.05$).

5.3. Effects of Simulated Salinity Stress on Endogenous Hormone Ratio of Wedelia trilobata Under Different Nutrient Conditions

As shown in Figure 4, in the absence of salt stress, high or low nutrient levels significantly reduced the GA/ABA and GA/IAA values of *W. trilobata* compared to normal nutrient levels (Figure 4a,b, $p < 0.05$), while the IAA/ABA values increased at high nu-

trient levels and were significantly reduced at low nutrient levels (Figure 4c, $p < 0.05$). In the absence of salt stress, low nutrient levels significantly reduced the GA/ABA and IAA/ABA values for *W. trilobata* (Figure 4a,c, $p < 0.05$). At low salt stress, high nutrient levels significantly increased the GA/ABA and GA/IAA values, while low nutrient levels significantly reduced the GA/ABA and IAA/ABA values of the *W. trilobata* (Figure 4a,c, $p < 0.05$). Low nutrient levels significantly reduced the ratio of three endogenous hormones for *W. chinensis* (Figure 4a–c, $p < 0.05$). Under high salt stress, the GA/IAA values in the leaves of both plants increased significantly with high and low nutrient levels (Figure 4b, $p < 0.05$). The results of the correlation analysis showed that the GA/ABA values were significantly positively correlated with plant height, node number, root length, and total biomass for *W. trilobata* and negatively correlated with the root–shoot ratio, MDA, POD, and CAT content. The GA/IAA values showed a significant positive correlation with plant height, stem node number, and total biomass, and a significant positive correlation with the root–shoot ratio, proline, and MDA content. The IAA/ABA values showed significant positive correlations with plant height, stem node number, root length, and total biomass, and a significant negative correlation with MDA, POD, and CAT (Table 3, $p < 0.05$). For *W. chinensis*, the GA/ABA values were positively associated with plant height, stem node number, leaf number, and total biomass, while there was a significant negative correlation with proline, POD, and CAT content. The IAA/ABA values showed a significant positive correlation with all growth indicators and showed a significant negative correlation with POD and CAT content (Table 4, $p < 0.05$).

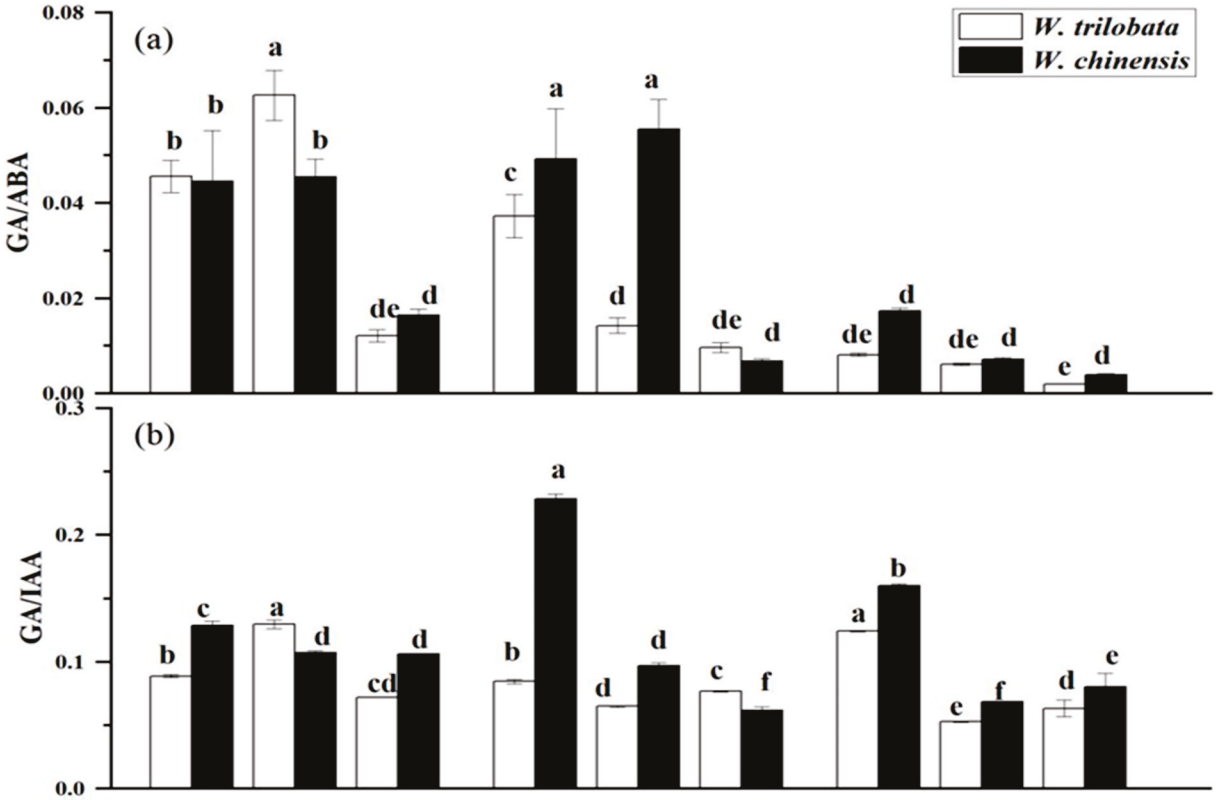

Figure 4. *Cont.*

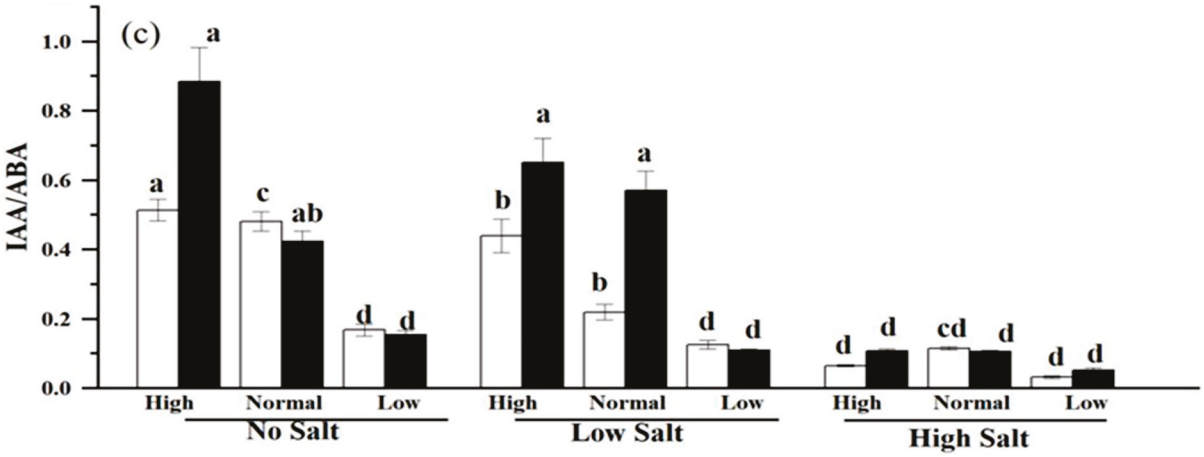

Figure 4. Influence of simulated salt stress on the ratios of GA to ABA (**a**), GA to IAA (**b**), and IAA to ABA (**c**) of two plants under different nutrient conditions. The data are presented as the mean ± SE (n = 3), and sharing the different letters indicates significant differences between treatments ($p < 0.05$).

6. Discussion

Elevated salinity, as a potent environmental determinant, exerts a profound impact on the intricate orchestration of plant growth and developmental processes, precipitating adverse consequences and unsettling the established norms of vegetative expansion [34,35]. Concurrently, it is imperative to acknowledge that the nutritional context in which plants reside can wield a significant influence over the colonization and proliferation of invasive plant species [36]. Within the scope of this scientific inquiry, we have meticulously scrutinized the implications of simulated salt stress on the growth dynamics of two distinct plant species across a spectrum of nutritional scenarios. Our all-encompassing analysis, encompassing both morphological metrics and biomass evaluations, has unveiled compelling insights. Under salt stress, seed priming with SWE increased root length, shoot length, root dry weight, chlorophyll content, and antioxidant enzymes activity compared with non-treated plants [37]. Under no salt stress, nutritional variations primarily manifested as an increase in leaf number and stem nodes for *W. chinensis*, whereas for *W. trilobata*, these variances were chiefly evident in the aspects of total biomass and the root–shoot ratio [38]. In the presence of high salt stress, changes in nutrient levels had a significant impact on both plants; high and low nutrient levels inhibited plant height and total biomass in *W. trilobata*, while in *W. chinensis*, only plant height, leaf number, and root length significantly decreased under low nutrient conditions. This suggests that higher nutrient levels can partially mitigate the negative effects of salt stress on plants under low salt stress conditions [39]. Enhancing nutrient utilization across varying salinity levels, as demonstrated in our study, ameliorates the effects of abiotic stress in both plants, as evidenced in the case of the invasive plant *Alternanthera philoxeroides*, underscoring the potential of nutritional conditions to alleviate stress-induced inhibition [40]. In resource-rich habitats, lower root–shoot ratios signify heightened competitiveness, aligning with the expectation of optimized biomass allocation for efficient resource capture [41,42]. In a high nutrient environment, salt reduced the root–shoot ratio of *W. trilobata*, indicating a shift in plant priorities toward optimizing aboveground growth for enhanced photosynthesis and improved survival [43]. The augmentation of nitrogen and phosphorus, coupled with the utilization of various Hogland nutrient solutions, fosters the growth and competitive advantage of invasive weeds [44,45]. Our study highlights the impracticality of this premise under high-salinity conditions, potentially arising from the adverse impact of high salt stress on plant cells, leading to metabolic disruptions and the impairment of the plant's antioxidant system [46]. In the absence of salt stress, *W. trilobata* showed increased proline, MDA, and CAT levels in

low nutrient conditions, with no significant change in POD. Under low salt stress, both *W. trilobata* and *W. chinensis* had lower proline, MDA, POD, and CAT levels in high nutrient environments, while proline and MDA increased in low nutrient conditions [47]. In high salt stress, elevated nutrition levels significantly raised proline and MDA in *W. trilobata* but had no significant impact on *W. chinensis*. The changes in the antioxidant system indexes were not obvious, as only the content of POD increased significantly [48]. Overall, plants can efficiently mitigate the detrimental effects of excessive Reactive Oxygen Species (ROS) by harnessing the synergistic capabilities of POD and CAT [49]. Under low salt stress, both *W. trilobata* and *W. chinensis* had lower proline, MDA, POD, and CAT levels in high nutrient conditions, but higher proline and MDA in low nutrient conditions. However, under high salt stress, high nutrition significantly increased proline and MDA in *W. trilobata*, with no notable effect on *W. chinensis*. These results highlight the intricate interactions between salt stress, nutrient availability, and plant antioxidant system responses [50]. In response to high salt stress, *W. trilobata* elevates proline content to enhance cellular osmotic adjustment, thereby stabilizing plant proteins and mitigating the damage inflicted by salt ions on plant cells. The alteration in MDA content in plants further lends support to this hypothesis [51]. Under the influence of drought stress, the ABA levels in the leaves and roots of *W.trilobata* were significantly increased compared with the control group [52]. When we measured the endogenous plant hormone levels under different conditions, we noticed a marked reduction in the GA/ABA ratio in the leaves of both plants when the nutrient levels were altered, particularly in comparison to the normal nutrient level [53]. Low salt stress had the greatest impact on hormone ratios in *W. chinensis* with low nutrient levels, causing a decrease in all three hormone ratios in this specific condition [54]. High salt stress increased the GA/IAA ratio in both plant species under both high and low nutrient conditions. The GA/ABA and IAA/ABA ratios under salt stress were positively correlated with plant height and total biomass [55]. The hormone ratios in *W. trilobata* strongly correlate with growth parameters, highlighting the roles of GA, IAA, and ABA in salt adaptation. In changing environments, increasing ABA helps regulate stomatal closure during drought, reducing water loss and enhancing drought tolerance [56].

7. Conclusions

This study demonstrates that the impact of simulated salt stress on *Wedelia trilobata* and *W. chinensis* is significantly influenced by nutrient levels. Low nutrient levels exert a more pronounced effect on both plants compared to high nutrient levels, regardless of salt conditions. Higher salt concentrations exacerbate the negative effects on plant growth and development. Under low salt stress, *W. trilobata* adapts by increasing proline, MDA, and CAT content in leaves while reducing GA/IAA content, indicating that a low-salt environment is more favorable for its competitiveness. Additionally, nutrient enrichment appears to enhance *W. trilobata*'s invasive potential. In this process, plant antioxidant system and endogenous hormones contribute greatly. To control and prevent the spread of *W. trilobata*, the application of saline water solutions and artificial soil salt enrichment are recommended as effective measures. These strategies can inhibit the plant's propagation and mitigate its invasive impact. Further study on the competition with multi-species, which are coexisting and competing with *Wedelia trilobata* in practice, will provide more support for our research findings. Overall, this study provides a theoretical foundation for predicting suitable growth areas for *W. trilobata* and offers valuable insights for guiding future efforts in invasion prevention and control.

Author Contributions: P.H. and D.D. conceived and designed the experiments; H.Y. (Hong Yang), B.L. and B.Z. conducted the experiments; H.Y. (Hong Yang), B.L., B.Z., Z.X. and H.Y. (Huilei Yin) analyzed the data; P.H., H.Y. (Hong Yang), B.Z. and A.A. wrote the manuscript; P.H., A.A. and D.D. were involved in the related discussion; A.A. helped to improve the quality of the manuscript; P.H. provided supervision and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Review

A Synthetic Review of Feedbacks and Drivers of Shrub–Grass Interaction in the Process of Grassland Shrub Encroachment

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Abstract: Many grasslands around the world are affected by shrub encroachment. The essence of shrub encroachment into a grassland habitat is a change in the direction and intensity of shrub–grass interactions, which leads to an alteration in the grassland community structure. Recent research progress can be summarized as encompassing the primary factors influencing shrub encroachment and the physical, biological, and chemical ways through which they affect grassland community succession and shrub–grass interactions. The purpose of this study was to explore how shrub–grass interactions and relationships change under the influence of various environmental factors and their impact on grassland communities to provide a theoretical basis for grassland restoration and the management of shrubs within grassland from the perspective of shrub–grass interaction.

Keywords: shrub–grass interaction; stress gradient hypothesis; shrub encroachment; community succession

1. Introduction

Plant–plant interactions are generally considered to be one of the most important parts of the mechanism by which environmental change drivers affect species composition and the community structure [1]. Due to climate change and anthropogenic disturbances, the abundance of shrub species in grassland ecosystems is increasing and the phenomenon of grassland shrub encroachment is exacerbated [2]. The essence of grassland shrub encroachment can be understood as a change in the interaction between shrub and grass plant species, which alters the structure and appearance of the community. Shrub–grass interactions include positive (facilitation), negative (competition), and neutral interactions [3]. The balance between positive and negative interactions hinges on the intensity of abiotic stress [4]. This conceptual model of the "stress gradient hypothesis" (SGH) effectively predicts and reveals the patterns of plant–plant interactions in various ecosystems in response to abiotic stress gradients [4].

In recent years, there has been an increase in studies of interactions between shrubs and other plants [5,6]. Shrub–grass interactions are considered an important mechanism for maintaining ecosystem functions (such as biodiversity and productivity) in grasslands undergoing shrub encroachment [7]. The effects of shrub–grass interaction on communities

can be modified by external drivers such as climatic conditions or biological disturbances. At present, although scholars have conducted comprehensive studies on the drivers of shrub encroachment [8,9], there are few studies examining shrub encroachment from the perspective of the environmental impact of shrub–grass interactions. The influence of the indirect shrub–grass interaction mechanism on shrub encroachment also needs to be further explored. There is no consensus regarding the mechanism of shrub–grass interactions and their impacts on community succession in the process of grassland shrub encroachment. Considering the important position of shrub–grass interaction and the current background of global climate change, it is of great significance to explore the mechanism of shrub–grass interaction and how the environmental change process influences it.

This review focuses on three aspects of recent advancements: the primary drivers; the occurrence and mechanisms of physical, chemical, and biological factors; and their influence on community succession and shrub–grass interaction. Based on recent advancements related to shrub–grass interaction, the problems and research directions that need to be further explored in future research are outlined and recommended. The purpose of this review is to explore the changing trends of shrub–grass interactions in the presence of various environmental factors and their influence on grassland communities to provide a reference for dynamic changes in shrub–grass presence and interaction.

2. Major Drivers Affecting Shrub–Grass Interactions

Shrub–grass interactions may be disturbed by biological and abiotic stresses such as grazing, drought, temperature, salinity, fire, and the atmospheric CO_2 concentration in the survival environment (Figures 1 and 2). According to the stress gradient hypothesis (SGH), the intensity of plant–plant interactions is closely related to the degree of stress and disturbance of the external environment in which the plant is located. The original hypothesis indicated that facilitation increases monotonically with the degree of environmental stress [10]. However, a multitude of empirical studies have shown that facilitation results in a unimodal pattern as environmental stress increases. When the degree of environmental stress is low, plants mostly experience competition. Under moderate stress, facilitation dominates, but under higher levels of stress, facilitation decreases or even changes to competition. The changing pattern of shrub–grass interactions under environmental stress provides an important theoretical basis for understanding the construction of shrub encroachment in grassland communities [11].

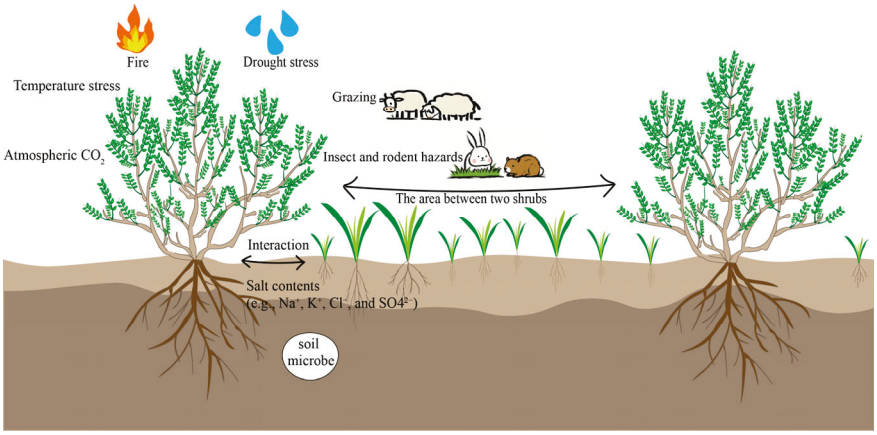

Figure 1. Factors affecting shrub–grass interaction.

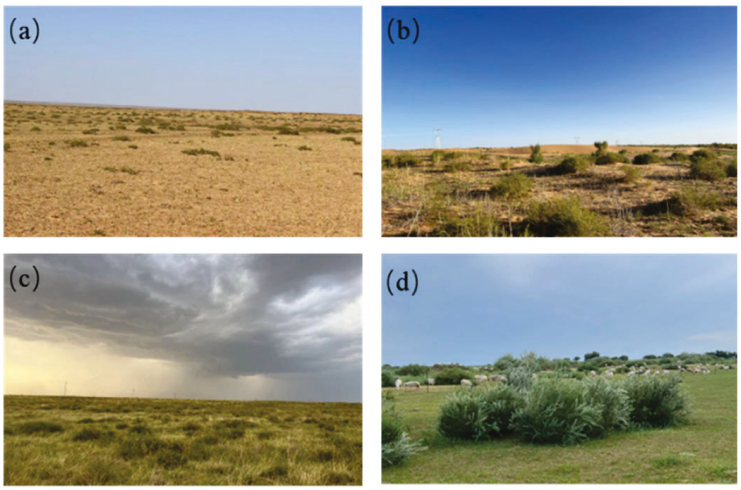

Figure 2. Examples of shrub-encroached grassland landscapes: (**a**) shrub-encroached grassland under drought stress; (**b**) shrub-encroached grassland after desertification; (**c**) shrub grassland under abundant precipitation; and (**d**) shrub grassland under grazing disturbance.

2.1. Biological Stress

2.1.1. Grazing Disturbance

Grazing causes changes in the species compositions and structures of original communities primarily by altering the strengths and directions of species interactions driven by environmental change. Shrub–grass interactions may be influenced by grazing intensity, livestock species, and plant palatability. The facilitation effect of shrubs on grass may show a monotonic increase or a unimodal increase following grazing, consistent with the prediction of the SGH. For example, the survival of a species that is characteristically a facilitation plant, *Caragana stenophylla* (Leguminosae), increased with grazing intensity in the Inner Mongolia Plateau region [12]. As grazing pressure increases, the role of shrubs in protecting seedlings from grass becomes more important. A study conducted in the Xilin River region of Inner Mongolia found that the facilitation effect of *Caragana stenophylla* on soil nutrients showed a unimodal trend along the grazing intensity gradient [13]. This ameliorative effect on nutrients may imply that the facilitation effect of shrubs on grasses also shows a unimodal trend with the grazing gradient. It is generally believed that overgrazing or selective foraging of grass species by livestock leads to a massive reduction in grass individuals and taxa. This elucidates their disadvantage in competing with shrubs, which gradually become the dominant grassland vegetation [14]. Shrubs with better palatability are also susceptible to livestock foraging and share part of the foraging pressure of grass species, and their competition with grass taxa is weakened, which means that appropriate grazing may slow down the process of shrub encroachment. Long-term livestock exclusion experiments in alpine grasslands on the Qinghai–Tibet Plateau showed that shrub encroachment induced by grazing exclusion was not only related to the elimination of direct interference from livestock but also to the competitive advantage of shrubs due to plant–plant interactions and the improvement of the deep soil resources of shrubs [12,15].

2.1.2. Insects and Social, Burrowing, and Herbivorous Mammals

Social, burrowing, and herbivorous mammals are the key functional groups that shape grassland ecosystems, which helps maintain grasslands and open habitats [16]. For example, vizcachas, burrowing bettongs, and prairie dogs prevent invasion and establishment of shrubs through foraging and clipping [16,17]. Shrubland expansion in semi-arid regions of North America and Australia has been attributed, in part, to reductions in the populations of prairie dogs and bettongs, respectively [17,18]. Small burrowing mammals and

megaherbivores can have mutualistic relationships. Grazing by megaherbivores facilitates increases in the population densities of burrowing mammal species that prefer more open grassland, thereby increasing their overall impact on a grassland ecosystem [16]. Many burrowing mammals preferentially forage on grasses, thereby facilitating the establishment of forbs [16,17]. However, some research has shown that rodent feeding, mowing, grass storage, and digging holes to create mounds reduce the aboveground and belowground biomass of herbaceous plants, thus weakening their competitiveness. Soil is pushed to the surface by wind and water erosion, which contributes to the degradation of grassland environments and is more conducive to the expansion of shrubs [19]. Some of the grassland rodents that feed on the fruits of leguminous shrubs can be used as vectors to assist in the dispersal of shrub seeds. Studies conducted in South Africa have found that rodents contribute to the spread of shrub seeds in grasslands, which plays an important role in grassland shrub encroachment [20].

Arthropods are important factors that alter the structures of plant communities. Invasions by exotic shrubs into habitats are associated with changes to insect communities [21]. Feeding by insect herbivores may have some effects, such as changing plant metabolism, hindering plant growth and reproduction, reducing plant community biomass, and changing plant community species diversity. In grassland ecosystems around the world, orthopterans including grasshoppers (Acrididae) and katydids/bush crickets (Tettigoniidae) are common and important herbivores [22,23]. Recent studies conducted in Europe and the continental United States found that plant biomass increases with grasshopper functional diversity [24,25]. This indicates that the effect of herbivore functional composition on grassland plant communities cannot be ignored. Insects may slow down grassland shrub encroachment to a certain extent by feeding on woody shrubs. Studies conducted in the Horqin Sandy Land found that seed pests reduced the competitiveness of *Caragana microphylla*, which affected the natural regeneration of its populations [26]. Similarly, it is speculated that some pests that cause shrub death or slow growth may also cause shrubs to occupy a weaker position in the competition with herbaceous species, which in turn slows shrub encroachment or promotes the succession of shrub-encroached grassland to grassland.

2.2. Abiotic Stress

2.2.1. Shrub–Grass Interactions Under Drought Stress

The pattern of plant–plant interactions along stress gradients in semi-arid environments elucidated by Maestre et al. has been widely recognized [27]. As drought increases, the facilitation effect of shrubs on grasses may diminish or change to being competitive, thus contributing to the succession of grassland to shrub-encroached grassland. In southeastern Spain, the effects of *Retama sphaerocarpa* (a shrub) on grass biomass and species richness shifted from facilitation to competition as drought intensified [28]. A study conducted in an Oregon grassland found that the promoting effect of the shrub *Artemisia tridentata* on the reproductive potential of grasses increased then decreased along the drought gradient [29]. Weakening drought stress may help slow the process of grassland shrub encroachment. The research in the Inner Mongolian shrub-encroached grassland found that increased precipitation significantly increased the mean height, cover, and aboveground biomass of grasses, and as the degree of drought decreased, the relative cover of shrubs decreased while that of grasses increased, indicating that increased precipitation was beneficial to the growth of grass species but had no significant effect on shrub growth [30]. The reasons for this phenomenon may be that the maximum photosynthetic rate of the locally dominant herb *Leymus chinensis* is more sensitive to changes in precipitation and that its photosynthetic nitrogen use efficiency and water use efficiency are significantly higher than those of

the invasive shrub *Caragana microphylla* under high soil moisture conditions. Most previous studies have centered on changes in indicator traits such as shrub-to-grass multiplicity, richness, density, biomass, survival, and reproductive rates along the drought gradient. From the perspective of leaf nutrients, the cumulative effects of *Caragana microphylla* on Ca, Cu, Fe, and Zn in leaves of plants from the non-grass functional group and P in leaves of plants from the grass functional group shifted from negative or neutral to positive with drought intensification in the Inner Mongolian grassland region [31]. This revealed drought effects on shrub–grass interactions in terms of deeper element utilization.

2.2.2. Shrub–Grass Interactions Under Temperature Stress

The argument that climate warming affects shrub–grass interactions arises from the observation that some encroaching shrub species are sensitive to low temperatures. For example, *Larrea tridentata* is prone to death from freezing-induced xylem cavitation [32]. Studies conducted in Spain have shown that due to rising temperatures, *Juniperus communis* has gradually replaced *Festuca aragonensis* and has become the dominant species. Shrubs have begun to expand to high altitudes and high latitudes [33]. There may be two explanations for the change in shrub–grass interaction caused by the temperature increase: (1) The degree of stress in suitable-temperature areas is elevated, and the competitive capacity of shrubs is increased. (2) Stresses decrease in low-temperature areas, as they are cooler. Thus, the effect of shrubs on grasses weakens. Liancourt et al., in the Ladakh region in India, found that the facilitation effect of the shrub *Caragana versicolor* on grasses increased then decreased with decreasing altitude (increasing temperature and drought) at the community level. There was an enhancing effect on the dominant companion grasses, *Elymus jacquemontii* and *Kraschenninnikovia pungens*, whose presence and prevalence increased with decreasing elevation [34]. This research only focused on one shrub species, which ensured that the results were not affected by the facilitation of other shrub plants along the entire study gradient. It also suggested that the trend of plant interactions along the temperature gradient varied across study levels. Shrubs can alleviate the growth inhibition and distribution limitations of grass species at low temperatures. Amy et al. found that the promotion of the grass *Chamerion angustifolium* by the shrubs *Salix glauca* and *Salix brachycarpa* increased the grass's winter survival and favored the expansion of its distribution range into high mountain areas where it otherwise would not survive [35].

2.2.3. Shrub–Grass Interactions Under Salt Stress

Higher salt contents (e.g., Na^+, K^+, Cl^-, and $SO_4{}^{2-}$) on vegetation patches beneath shrubs may interfere with the presence and conditions of grasses, thus modifying their spatial pattern and changing shrub–grass interactions [36]. Some salt-tolerant shrubs can alleviate a saline environment by reducing soil salinity and improving soil fertility, thus facilitating the survival and growth of grasses in the canopy. Rumbough found that the shrub *Atriplex canescens* ameliorated the physicochemical properties of saline soils, reduced their salinity, and increased the soil nutrient content, thus facilitating the growth of *Agropyron cristatum* and significantly increasing the grass production in the canopy [37]. Qi et al. studied the interactions between the shrub *Tamarix chinensis* and the grass species *Phragmites australis* and *Suaeda salsa* and found that, within the physiological limits of salt-tolerant plants, with the aggravation of salt stress, the plant interactions changed from competition to facilitation. When the salt stress exceeded the physiological tolerance of the salt-tolerant plants, the facilitation collapsed [38]. These results suggest that the changes in shrub–grass interactions along the salt stress gradient are in general agreement with the predictions of the SGH.

2.2.4. Effects of Fire on Shrub–Grass Interactions

The degree to which fire affects shrub–grass interactions is related to the intensity and frequency of fires. Lower-frequency and -intensity fires are less damaging to shrubs than to grasses, making grasses less competitive. Studies conducted in the grassland of South Africa have found that small grassland fires are more conducive to shrub expansion. On the contrary, large fires are more conducive to the recovery of grass species [39]. The reasons for the suppression of shrub encroachment after a fire may include the following: (1) Compared with shrubs with deep roots and a long growth cycle, shallow-rooted grasses are more likely to obtain water and nutrients in the surface soil and have a stronger recovery ability in the short term. (2) Shrub seedlings are sensitive to fire, so severe fires have greater impacts on shrub plants. In the long-term process of community succession, other factors may lead to reductions in grasses and combustibles, thus reducing the intensity of fire disturbances, which is conducive to the expansion of shrubs and makes shrubs further replace the original grass plant community. In North America, as the degree of shrub encroachment is reduced, grass plants decrease, fire frequency decreases, and shrubs obtain a more stable living environment, further promoting shrub expansion, while at the same time the dominance of grasses and their biomass and cover decrease again, providing more favorable habitat conditions for shrubs and forming a positive feedback loop of shrub expansion [40]. Thus, fire management, controlling the frequency and sizes of fires, may affect the process of shrub encroachment. A fire plan can be formulated according to the degree of grassland shrub encroachment and the plant species, and periodic fire management can be carried out to control the number of shrubs and inhibit shrubs.

2.2.5. Effects of Atmospheric CO_2 Concentration on Shrub–Grass Interactions

An elevated atmospheric CO_2 concentration may be a driver of grassland shrub encroachment. A 50-year experimental study conducted in the semi-arid grassland of Kruger National Park in South Africa found that with the passage of time, the increase in the CO_2 concentration was the driving factor for the increase in shrub density in the region when other environmental driving factors such as rainfall remained unchanged [41]. On the one hand, studies have shown that an elevated CO_2 concentration can enhance deep soil water storage. Compared with shallow-rooted grasses, shrubs with deep roots can obtain deep soil water [42]. On the other hand, shrubs and grasses have different physiological characteristics, and the differences in the respiratory responses between the two may lead to the expansion of shrubs. Most of the shrubs with increased abundance in shrub-encroached grasslands in tropical, subtropical, and temperate regions of North America are C_3 species, while most of the replaced species are C_4 species and grasses. Under low-CO_2 conditions, the photosynthetic efficiency of C_4 grasses is higher, while a high CO_2 concentration can increase the net photosynthetic rate of C_3 shrubs by inhibiting photorespiration. Therefore, the increase in the atmospheric CO_2 concentration makes C_3 shrubs more advantageous with respect to physiological activity, growth, and competitiveness [43,44]. In some dry cold desert steppes, even C_3 grasses are replaced by C_3 shrubs [45]. Therefore, the increase in the atmospheric CO_2 concentration may not be the main factor driving changes in local vegetation.

2.2.6. Species Specificity and Life-History Stages

The results of changes in plant–plant interactions with the degree of environmental stress are also related to the species specificity and life-history stages of the plants themselves. For example, shrubs provide some shade to grasses in the understory canopy, but the effect depends on the morphological characteristics of the shrub and the shade tolerance of the grass taxa. In the southwestern Iberian Peninsula, it was found that shrubs

with scattered structures facilitated plant seedling survival and growth more than shrubs with clumped structures [46]. In the northern part of India, the shrub *Caragana versicolor* exerted a positive effect on erect-growing graminoid species while showing a negative effect on prostrate-growing grass species [47]. From the life-history point of view, during the seed germination stage, the facilitation of shrubs is mainly characterized by the provision of a microenvironment suitable for seed germination. After plant germination, shrubs can provide effective protection for seedlings, reducing their probability of being trampled and nibbled by phytophagous animals and reducing the negative effects of high temperatures and intense light on seedlings. As grasses grow, their competitive advantage related to water and other environmental resources increases and shrub–grass interactions are transformed from facilitation to competition. For example, in southeastern Spain, the facilitation effect of the shrub *Cistus clusii* on the grass *Stipa tenacissima* diminished as the grass matured, with *S. tenacissima* showing a neutral effect on the shrub during the seedling stage, then competition with the shrub after maturity, which was caused by the mature grass's increased ability to access water [48].

3. Mechanisms of Shrub–Grass Interactions

3.1. Main Competition Mechanisms

The grass components of a plant community are influenced by a variety of mechanisms, such as allelopathy and competition for resources. Shrubs produce and release allelochemicals, which may hinder the growth and reproduction of grass species [49]. Shrubs and grasses compete for living space, light, water, nutrients, and other resources. This competition can lead to decreases in the species diversity of grass communities, expediting the expansion of shrubs and significantly altering the compositions and structures of the original grassland ecosystems.

Allelopathy and Competition

Allelopathy and competition possess different mechanisms. Chemically mediated plant–plant interactions are represented by allelopathy and allelobiosis [50]. By releasing toxic compounds (allelochemicals or signaling chemicals), a shrub may have a negative impact on the germination, growth, and survival of grasses. Competition is imposed by the environment, where plants draw from the same resource pools (i.e., shrubs consume water and nutrients and provide shade). Furthermore, allelopathy is a species-specific behavior, but competition occurs invariably during plant coexistence irrespective of the plant species. Importantly, competition occurs solely during the coexistence of living plants, but allelopathic potential can affect subsequent plant growth by the release of allelochemicals from deceased plants and their decomposing remnants. Plants biosynthesize a wide variety of allelochemicals, including terpenoids (monoterpenes, sesquiterpenes, diterpenes, and steroids), phenolics (simple phenolic acids, flavonoids, coumarins, and quinones), nitrogen-containing chemicals (alkaloids, benzoxazinoids, and cyanogenic glycosides), and other chemical families [51,52]. These allelochemicals are controlled by a range of genes, including those responsible for encoding transcription factors, which ultimately shape their allelopathic potentials [53]. In the Neotropical Savanna, leaf extracts from the shrub *Lepidaploa aurea* reduced the growth of other grasses [54]. Wagner et al., in an arid grassland dominated by perennial grasses, found that shrubs consistently competed with grasses for growth, and light-sensitive tufted grasses were affected by shrub shading, resulting in declining populations [55]. An overview of the types of shrub–grass interactions is shown in Table 1.

3.2. Main Facilitation Mechanisms

3.2.1. Habitat Amelioration

The nurse effects provided by shrubs can help with the regeneration of native grasses by alleviating the detrimental effect that limits their growth and reproduction in degraded habitats. Shrubs can reduce the solar radiation reaching the understory or nearby grasses through their shading effect, can prevent or slow down the occurrence of plant photoinhibition, and can reduce the probability of high-temperature burns on leaves [56]. At the same time, the existence of shrubs is conducive to local maintenance and aggregation of water. On the one hand, shading can reduce temperatures, maintain higher soil humidity, and lower the transpiration of grasses. On the other hand, shading reduces the intensity of solar radiation and soil evaporation and maintains a high soil moisture content near the shrub. In desert environments, shrubs can protect and facilitate understory grasses by reducing the wind speed and flow abrasion. Studies have shown that *Nitraria tangutorum* shrubs play important roles in breaking the wind and sand fixation by reducing the air velocity to form shrub sand piles [57]. All the above processes increase the survival rates of grass species.

3.2.2. Resource Enrichment

The precipitation interception capacity of shrub branches can increase the soil water content in shrubs, thus alleviating drought [58]. A study conducted in the Horqin Sandy Land of Inner Mongolia showed that the soil water content inside a *Caragana microphylla* shrub was higher than that outside the shrub [9]. In addition to live shrubs, after death the residual parts (branches, leaves, etc.) of shrubs can still play a role in increasing the soil water content and reducing the negative effects of high temperatures and water shortages on grass seedlings [59]. The hydraulic redistribution of shrub roots may also facilitate the growth of surrounding grass plants. Grass facilitation by woody plants through the process of hydraulic lift allows savannas to persist stably in mesic regions [60]. However, the process of hydraulic redistribution can result in hydraulic descent and hydraulic lift, and depending on inter-annual variations in precipitation. The redistribution of woody species can deplete or enhance soil moisture at graminoid rooting depths [61]. Hydraulic lift within overstory neighbors might be a more important driver of grass growth in drier regions where water is more limited or regions with pronounced dry seasons [62,63]. Shrub branches and leaves block and intercept large amounts of dust and litterfall so that more organic matter is deposited beneath the shrub canopy. To a certain extent, this increases the soil nutrient content in the shrub area. This phenomenon of significant accumulation of soil nutrients in local spaces caused by the formation of shrubs is called the fertile island effect (fertile islands). In a study conducted in northwestern Spain, it was found that the soluble nitrogen content, nitrification rate, NO concentration, and nitrogen absorption efficiency of plants beneath the shrub *Erica umbellata* were significantly higher than those outside the shrub [64].

3.2.3. Protection Against Herbivory

Plant resistance to any biotic or abiotic stressor is generally assigned to two major categories: tolerance and avoidance traits [65–67]. Tolerance mechanisms increase the rate of leaf replacement through meristematic activity and several physiological processes, such as compensatory photosynthesis and increased carbon allocation to leaves [65–67]. Avoidance mechanisms minimize the frequency and/or intensity of herbivory by reducing plant palatability and accessibility [65–67]. Species that are grazed less severely (i.e., avoidance mechanisms), are capable of growing more rapidly following defoliation (i.e., tolerance mechanisms), or possess a combination of these two mechanisms realize a com-

petitive advantage within a community [65–67]. Shrub avoidance mechanisms involve structural elements such as thorns, pubescence, raphides, and sclerophylly, all of which play important roles in protecting herbs from herbivores [68]. Chemical defenses such as the production of phenolics can deter herbivores from continued feeding following an initial bite [69]. Poor palatability or thorns on shrubs can protect herbaceous plants from herbivore predation. Studies conducted under grazing and fencing conditions have found that *Caragana intermedia* indirectly facilitates the growth of *L. chinensis* beneath shrubs as a 'herbivore shelter' [70]. Palatability is multi-faceted. Reductions in palatability can be facultative and induced both in terms of tissue quality and structural defences or obligate and always expressed. These responses may reflect co-adaptive responses to the selective pressures of drought and grazing. Although both mechanisms are known to occur, how variations in climate drivers, as well as the intensity and duration of herbivory, affect the expression of these traits and subsequently modulate plant–plant interactions is not well understood.

3.2.4. Introduction of Beneficial Organisms

Shrubs can indirectly facilitate grass species by introducing seed communicators [71], pollinators, mycorrhizal fungi [72], parasitic plants, and other beneficial soil microorganisms [73–75]. Shrub encroachment can lead to soil bacteria and fungal biomass increasing in shrub canopies compared to interspace grasses, and the composition of fungal communities has been observed to respond to shrub encroachment. For instance, arbuscular mycorrhizal fungi (AMF) can establish a symbiotic relationship with *Amorpha fruticosa*, a perennial leguminous shrub, forming arbuscular mycorrhiza (AM) [76]. Advances in molecular biology techniques now allow for quantitative analysis of protein expression differences during the colonization process of these symbiotic microorganisms, aiding in the understanding of plant interaction mechanisms. In one study, iTRAQ (Isobaric Tags for Relative and Absolute Quantification) combined with 2-D LC-MS/MS (two-dimensional liquid chromatography–tandem mass spectrometry) (GE Healthcare, Chicago, USA) was used to examine the expression of *A. fruticosa* mycorrhizal proteins at maturity [73]. The findings revealed that genes involved in plant metabolism and stress defense are crucial for the symbiotic relationship between AMF and *A. fruticose* [73]. Ericaceous shrubs can establish root–fungus associations with phosphate-solubilizing soil microorganisms (PSMs) involved in P-solubilization processes [77]. Fungal and actinomycete isolates obtained from a habitat of the shrub *Calluna vulgaris* can release soluble phosphate and exhibit diverse plant growth-promoting (PGP) activities [78]. The facilitation effect of soil available nitrogen fixed by legume shrubs on grasses is closely related to the symbiotic nitrogen fixation of rhizobia [74].

3.2.5. Allelopathic Shrubs

It is commonly thought that allelopathic plants adversely affect the growth of nearby species. They can play a crucial role in determining the species diversity within a community by creating microenvironments where species interactions vary. For example, research conducted in the St. Martin de Londres valley in southern France revealed that the aromatic shrub *Thymus vulgaris* enhances species diversity both locally and at the community level [79]. This is achieved through the production of the monoterpene carvacrol, which creates a mosaic of thyme-modified and unmodified microhabitats differing in richness and species composition. Similar effects may be observed in other aromatic plants prevalent in Mediterranean ecosystems.

Table 1. Mechanisms of shrub–grass interaction.

Potential Mechanisms That May Occur Within Shrub–Grass Interactions		References
Habitat amelioration	Relieving solar radiation.	[56]
	Reducing soil salinity.	[36,37]
	Reducing wind erosion and water erosion.	[57]
	Enhancing the soil infiltration capacity.	[80]
	Providing soil humus.	[57]
Resource enrichment	Increasing the water content and nutrients in the soil in the microenvironment.	[9,58]
	Changing the microbial processes that affect soil carbon storage and nutrient cycling.	[81]
Association defense	Reducing eating and trampling by herbivores.	[70]
Introduction of beneficial organisms	Attracting pollinating animals and improving the pollination rate.	[82]
	Increasing soil microorganisms, such as mycorrhizal fungi, etc.	[8,55,72,73,83]
	Attracting seed-spreading animals (birds, etc.).	[71]
Competition for resources	Reducing soil moisture, reducing compaction, and enhancing aggregate stability.	[80]
	Different plant traits.	[84]
Allelopathy	Inhibitory effects of plants producing and releasing allelochemicals.	[49,53,54]

4. Effects of Shrub–Grass Interactions on Community Succession

Plant community succession is the result of synergistic interactions between a community's internal relationships (species interactions and others) and external environmental factors. Shrub–grass interactions are one of the endogenous factors that drive grassland community succession and influence the direction of community succession. There are two possible outcomes due to changes in shrub–grass plant interactions after shrub encroachment. One is that shrubs can increase grass plant abundance, productivity, and ecosystem stability after shrub encroachment. The second is that the productivity and species diversity of grassland communities can be reduced, as an increase in shrub fertilization may cause the degradation of grassland ecosystems. The reasons for these results may be related to the types of plant species, the degree of shrub encroachment, climatic conditions, and other factors, so the impact of shrub fertilization on species composition has not yet been unanimously determined.

4.1. Effects of Shrub–Grass Interaction on Community Restoration Succession

The positive interaction between shrubs and grasses is one of the important mechanisms leading to community succession and driving community dynamics. This positive interaction may accelerate the restoration and succession of vegetation communities in degraded ecosystems by changing the spatial patterns of communities, which is more conducive to species coexistence, increasing community diversity and productivity. As nurse plants that can facilitate the survival and growth of other plant species under their canopies, shrubs are often used in the community restoration of degraded grasslands. At present,

the facilitation of shrubs is often used to carry out ecological restoration research in deserts, alpine ecosystems, grasslands, and other harsh ecosystems. *Haloxylon ammodendron* is an effective tool for the restoration of arid desert vegetation, as it can reduce the wind erosion on the surfaces of dunes, provides protection for grasses, and effectively controls desertification [85]. Soil resources are enriched around the shrub *Salix cupularis* [86]. *Salix cupularis*, as a suitable pioneer shrub for vegetation restoration, contributes to the emergence of associated grasses in the desert alpine meadow of the Qinghai–Tibet Plateau [86]. In the typical steppe of Xilingol, Inner Mongolia, a study found that grassland with moderate shrub encroachment has higher species and functional diversity than grassland with mild or severe shrub encroachment, which means that a certain degree of shrub encroachment is beneficial for the maintenance of diversity and the restoration of degraded grassland [87]. The facilitation effect of grasses on shrubs can also accelerate the restoration of degraded grasslands. In the open grasslands of southeastern Spain, the survival rate of shrubs near *Stipa tenacissima* (grass) was significantly higher than that outside the grass, and *S. tenacissima* had a direct facilitation effect on the introduced shrubs [88]. This facilitation is of great significance for the effective restoration of degraded semi-arid ecosystems.

4.2. Effects of Shrub–Grass Interaction on Community Retrograde Succession

Shrub encroachment may be one of the causes of grassland ecosystem degradation. After grassland shrub encroachment, colonizing shrubs compete with the grass species that are the primary component of grassland habitats. The original uniformity of the grassland surface is destroyed, and wind and water erosion cause the deterioration of environmental conditions. Most shrub species have deep roots and strong drought resistance. Therefore, shrubs have higher viability and more competitive advantages than grasses in harsh environments. The survival of shrubs gradually changes the species compositions of plant communities, from grasses to shrub dominance. Knapp et al. found that the net primary productivity and species richness of grasses were significantly reduced by the competitive effect of shrubs compared with native communities at the scale of the North American steppe transect, thereby accelerating grassland degradation [89]. A study conducted in the New Mexico grasslands of the United States showed that the invasion of the shrub *Larrea tridentata* reduced the species diversity of grassland plant communities, and the stability of communities in shrub invasion areas decreased with an increase in shrub coverage [90]. Different functional groups of grass plants have different responses to the presence of shrubs. Zhang et al. found that shrub encroachment has a negative impact on the structure and function of grass plant communities [91]. Shrub invasion drives community succession by causing the extinction and colonization of non-random grass plant species. Low-lying grasses are more likely to become eradicated, while tall and shade-tolerant grasses benefit from shrubs. In the later stage of shrub invasion, there may be a high level of spatial heterogeneity of grassland soil nutrients and community composition. Xiong Xiaogang et al. divided the shrub invasion process into three relatively stable ecological states, namely native grassland, shrub grassland, and dune shrub encroachment [92]. After the emergence of shrub dunes, surface degradation is more serious. There are no shrubs in the surrounding area to block the strong wind and sand. The grasses are greatly reduced, and the function of the ecosystem tends to be lost. The dominance of shrubs in shrub–grass competition further aggravates grassland shrub encroachment, which provides environmental conditions for the invasion of shrubs. In Inner Mongolia, a study of different degrees of shrub grassland found that the soil nutrient content in the shrub *Caragana stenophylla* was significantly higher than that outside the shrub and gradually decreased when moving outward. This trend became more and more obvious with increases in the shrub degree [13]. The nutrient status of the soil around a shrub is more unfavorable to the survival of grasses outside the

shrub. The soil outside the shrub is more likely to be eroded with the appearance of more bare land, eventually leading to the degradation of the grassland ecosystem. Considering the relationship between shrub–grass interactions, the degree of shrub encroachment, and the degradation of grassland ecosystems, controlling the number of shrubs and the degree of shrub encroachment may be an important measure to inhibit the degradation of shrub grassland.

5. Perspectives and Outlook

Shrub encroachment has become a widespread and increasing ecological phenomenon in grassland ecosystems around the world. Clarifying the factors that drive shrub encroachment and finding ways that the conversion of grassland to shrubland can be managed is currently an important research area. The role of shrub–grass interactions is clearly variable within the context of numerous environmental change drivers. At present, most of the research on shrub encroachment and shrub–grass interactions have been conducted through the observation and analysis of natural phenomena and processes, and there is a lack of research on their mechanisms. A material challenge for plant community ecology is determining how mathematical modeling could and should be applied to aid our understanding of the shrub–grass interaction mechanisms. This study summarizes and presents new information regarding the aboveground and underground parts of shrub and grass plants and the factors influencing their interactions. The other findings are as follows:

(1) The causes of shrub encroachment are complex, as there has been insufficient research on shrub encroachment caused by multi-factor coupling. It is necessary to study these factors and their combined effects on grassland shrub encroachment and shrub–grass interaction.

(2) From the perspective of the mechanism of the shrub–grass interaction, it is necessary to carry out more accurate simulation studies and establish corresponding models to clarify the phenomenon of grassland shrub encroachment caused by multiple mechanisms under different backgrounds. The influence of the indirect shrub–grass interaction mechanism on shrub encroachment needs to be further explored. Further assessments of allelopathy and allelobiosis in the context of environmental fluctuations and global shifts are needed. Future studies of the molecular pathways involved in allelopathy and allelobiosis in shrub–plant interactions, focusing on gene expression patterns, protein receptors, and their transport mechanisms are important research needs.

(3) Long-term, multi-dimensional monitoring and meta-analysis were conducted at a large scale to study the effects of shrub–grass interactions on community succession. The practical application of positive shrub–grass effects in a wide area needs to be further explored. It will be necessary to give full play to the positive effects of shrub–grass interactions on community succession to restore degraded grassland ecosystems.

(4) Although the SGH is indeed broadly tested in studies of plant community ecology, there is a lack of research on how the duration of an interaction affects the detected patterns, so we suggest this as a future research direction.

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The Role of Mycorrhizal Fungi in the Inter and Intraspecific Competition of *Nicotiana glauca* and *Vachellia gerrardii*

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Abstract: A competition experiment between *Vachellia gerrardii* and invasive *Nicotiana glauca* Graham was conducted to assess the impact of Arbuscular Mycorrhizal Fungi (AMF) symbiosis on the inter and intraspecific competition between the two species. Seedlings were established under mono and mixed plantations with different species proportions (3:1, 2:2, 1:3) and plant densities (1, 2, 3, and 4 plants/pot) for mixed and mono planting respectively, with and without AMF. The vegetative growth parameters (height, leaf area and number, total dry weight/plant, relative yield, relative yield total), roots characteristics (length, surface area, volume, tips number), competitive interaction (aggressivity), and physiological traits (chlorophyll *a*, chlorophyll *b*, photosynthesis, stomatal conductance) were measured to evaluate plant responses to AMF symbiosis and competition. The results revealed that AMF symbiosis significantly enhanced the vegetative parameters (leaf area, height, and total dry weight) in both species under mono and mixed plantations compared to plants without AMF. Under AMF treatment, in the interspecific competition, most vegetative and root parameters of *N. glauca* were higher than *V. gerrardii*. At inoculant and species proportions, the relative yield of *N. glauca* exceeded that for *V. gerrardii*; however, *N. glauca* was more aggressive towards *V. gerrardii*. *N. glauca* root indices were higher than *V. gerrardii* under inter and intraspecific competition. Simultaneously, for both species, in monoculture plantations, most parameters decreased as plant density increased, wherein the decrease was higher for plants grown without AMF. Photosynthesis increased in AMF treatment, particularly for *N. glauca*. In conclusion, AMF promoted the growth of invasive *N. glauca* more than native *V. gerrardii*, particularly in terms of the root system. Our results provide a critical perspective that the AMF has the potential to contribute and facilitate the invasion of *N. glauca*, as well as support it with a competitive advantage over *V. gerrardii*, thus highlighting its potential role in shaping plant–plant interaction in invaded habitats.

Keywords: root parameters; photosynthesis; competition; relative yield; aggressivity

1. Introduction

Plant invasion usually results in a clear reduction in plant biodiversity and community stability, leading to an impact on indigenous plant species [1,2]. In addition, invasive plants can change soil physiochemical properties through competition and interactions with soil microbial communities [3]. Therefore, competition is considered one of the key processes determining plant community structure and dynamics [4,5], which is often arbitrated by nutrients and water availability [6]. Competition is caused due to the limited

availability of resources such as water or nutraingredients to any individual plant during its growth. Intraspecific competition occurs between individuals of the same species, and the intensity of competition depends on the level of the limiting resource. However, interspecific competition depends on the species density [7]. In general, many studies indicate that intraspecific competition in plants is stronger than interspecific competition due to similar needs for resources [8]. But, in some cases (competition between native and invasive plants), interspecific competition becomes more intense than intraspecific competition [9]. In general, studies indicate that competition reduces the ability of plants to acquire their essential elements for growth, and thus the degree of intra and interspecific competition can change the competitive balance between invasive and native plants [10].

Vachellia gerrardii and invasive *Nicotiana glauca* Graham plants are found in several parts of the southwestern region of Saudi Arabia, such as Khamees Mushait, Al-Baha, Al-Mandag and Al-Karrar [11,12]. *N. glauca*, as an invasive species, is widely considered one of the most destructive plants, resulting in ecological problems worldwide. This plant has been increasing in terms of both population and area, dominating and competing with the natural habitats of native wild plants [13,14]. *N. glauca* has invaded large areas of the Southwestern region of Saudi Arabia [15] and became a dangerous plant in that region over the last few years [12]. *N. glauca* is among the numerous species that have the potential to damage biodiversity or alter ecosystems [13,16]. The presence of toxic substances in the different parts of this plant is believed to contribute to its allelopathic impacts, allowing it to dominate over native plant species [17].

Plant canopies and root areas are overlapped by other plants and living organisms, which creates competitive interaction and environmental stress that challenges their survival [6]. Interactions between plants, soil, and microbes play an important role in determining the structure of plant communities [18]. Moreover, in some cases, complex feedback loops may help plants become more invasive and competitive [19]. The success of invasive plant species is usually related to their competitiveness [20]. Different competitive interactions can impact plant biomass production and facilitate the invasion process [21]. Scientists have proposed that the competitive advantages of native and invasive plants mainly depend on their relation with associated microorganisms [22]. Soil microorganisms act as one of the key drivers in different plant interactions and their community structures. Such interactions not only affect the local species but also endanger the whole ecosystem as long as invasive plants have a mutualistic relationship with native soil microorganisms, e.g., Arbuscular Mycorrhizal Fungi (AMF), where invasive plants receive benefits from AMF symbiosis rather than native plants [23]. AMF can provide several benefits to the host plant such as increased access to water and important nutrients like nitrogen, phosphorus, potassium, carbon, and other micronutrients [24]. AMF interaction might play an important and direct role in shifting the balance of competition between invasive and native plant communities [25]. The competitive dynamics among plants can be altered by the presence of AMF, which plays a crucial role in improving plant nutrition and enhancing their ability to tolerate stress. Different types of plants can either enhance or suppress the AMF association of neighbouring plants, thereby impacting the results of plant interactions [26]. In addition, invasive plants greatly affect the richness and diversity of AMF [27], whereas invasive plants that are not dependent on AMF or have less dependence inhibit mycorrhiza, thereby reducing the competitive advantage of native mycorrhizal plants [28]. Invasive plants that depend on AMF have a selective effect on the formation of the fungal community by promoting their preferred symbiotic species and inhibiting other, less preferred fungi [29]. The preference of the invasive plant for more efficient AMF may alter the richness and abundance of other symbiotic mycorrhizal fungi, and their composition thus facilitate the growth of the invasive plant and reduce the performance

of the native plant species [28]. Although not all invasive species cause environmental changes, some can selectively disrupt microbial communities to gain an advantage over native competitors [30]. Furthermore, invasive plants may accumulate pathogens that are more harmful to competitors' plants [31].

Accordingly, this study attempts to assess the role of AMF in the inter and intra-specific competition of *V. gerrardii* and invasive *N. glauca* and their interaction in various planting densities in greenhouse conditions. Furthermore, the study aims to offer a critical perspective on the potential role of AMF in facilitating the invasion of *N. glauca* by providing it with a competitive edge over *V. gerrardii*.

2. Results

2.1. Effects of Plant Species Proportion on the AMF Colonization Rate (In the Root) and Spore Number (In the Rhizosphere) of V. gerrardii and Invasive N. glauca Seedlings Grown in Mixed Plantations

Table 1 shows the infection rates of AMF in the roots of *V. gerrardii* and *N. glauca* seedlings grown in mixed planting. The results revealed that species proportion did not show a significant effect on the AMF (mycelium; $p = 0.5$, arbuscular; $p = 0.11$, vesicular; $p = 0.15$) infection rates of *V. gerrardii* and *N. glauca* seedlings. Also, no significant difference was found in the AMF infection rate between the two plant species grown in mixed planting, where p values were 0.18, 0.15, and 0.65 for the mycelium, arbuscular, and vesicular, respectively.

Table 1. AMF % in the roots of *V. gerrardii* and *N. glauca* grown in mixed culture with different species proportion.

Species	Plant Species Proportion (*V. gerrardii*:*N. glauca*)	AMF Structural Colonization%		
		Mycelium%	Arbuscular%	Vesicular%
V. gerrardii	3:1 (0.75:0.25)	40.0 ± 5.7 a	50.3 ± 5.7 a	53.3 ± 3.3 a
	2:2 (0.50:0.50)	56.6 ± 6.0 a	43.0 ± 3.3 a	45.0 ± 2.8 a
	1:3 (0.25:0.75)	53.3 ± 3.3 a	40.0 ± 5.7 a	71.6 ± 4.4 a
N. glauca	3:1 (0.75:0.25)	60.0 ± 10 a	35.0 ± 2.8 a	53.0 ± 3.3 a
	2:2 (0.50:0.50)	50.0 ± 5.7 a	36.6 ± 3.3 a	56.6 ± 3.3 a
	1:3 (0.25:0.75)	61.6 ± 4.4 a	45 ± 5.0 a	54.0 ± 3.4 a
LSD		29.6	21.4	27.8

Note: Same letters within each column represent no significant differences at $p < 0.05$ (LSD test).

In the soils of the mixed plantations of *V. gerrardii* and *N. glauca*, no significant differences were observed in the abundance of AMF spores between treatments of plant species proportion ($p = 0.34$). The average spore number was 80, 70, and 78 spores/100 g of soil in the species proportions 1 *V. gerrardii* (V):3 *N. glauca* (N), 2 *V. gerrardii*:2 *N. glauca*, and 3 *V. gerrardii*:1 *N. glauca*, respectively (Figure 1).

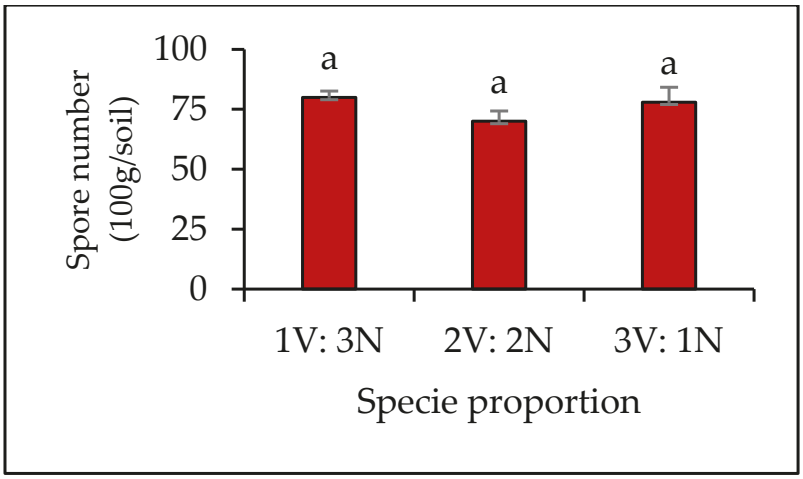

Figure 1. AMF spore number for soils of *V. gerrardii* and *N. glauca* grown in mixed plantations with different species proportion. Note: V = *V. gerrardii*; N = *N. glauca.* Similar letters above bars indicate no significant difference at $p < 0.05$ (LSD test).

2.2. Effect of AMF and Species Proportion/Plant Density on Vegetative Part of V. gerrardii and N. glauca Grown in Mixed Plantations

The height of *V. gerrardii* increased in AMF inoculum treatment and increased with a decrease in the proportion of *N. glauca* seedlings (Table 2) (Figure 2). On the other hand, *N. glauca* showed a slight reduction in height with increasing species proportion of *V. gerrardii* (3 *V. gerrardii*:1 *N. glauca*). Under the non-AMF-treated seedlings, species proportion did not significantly affect the heights of *V. gerrardii* and *N. glauca* seedlings (Table 2).

Table 2. Effect of AMF and species proportion on height, leaf number, and leaf area of *V. gerrardii* and *N. glauca* grown in mixed plantations.

Species	Plant Species Proportion (*V. gerrardii*:*N. glauca*)	AMF Inoculant	Height (cm)	Leaf Number	Leaf Area (cm^3)
V. gerrardii	3:1 (0.75:0.25)	+AMF	37.2 ± 3.86 a	42.0 ± 5.19 a	128 ± 11.47 c
		−AMF	19.8 ± 1.28 bc	21.4 ± 1.60 bc	63 ± 5.49 cd
	2:2 (0.50:0.50)	+AMF	24.6 ± 0.74 b	30.2 ± 0.73 b	88 ± 2.17 cd
		−AMF	18.8 ± 3.52 bc	16.0 ± 2.16 cde	41 ± 5.42 cd
	1:3 (0.25:0.75)	+AMF	17.0 ± 1.44 bc	20.2 ± 1.98 bcd	46 ± 4.34 cd
		−AMF	18.0 ± 2.70 bc	15.8 ± 2.8 cde	33 ± 4.98 d
N. glauca	3:1 (0.75:0.25)	+AMF	12.20 ± 1.24 c	10.8 ± 0.73 de	478 ± 34.7 a
		−AMF	12.4 ± 1.50 bc	9.8 ± 0.58 e	215 ± 12.9 b
	2:2 (0.50:0.50)	+AMF	18.4 ± 2.87 bc	11.40 ± 1.02 cde	289 ± 41.3 b
		−AMF	10.8 ± 2.28 c	8.6 ± 0.92 e	124 ± 15.5 c
	1:3 (0.25:0.75)	+AMF	16.4 ± 3.82 bc	10.6 ± 1.36 de	104 ± 14.6 cd
		−AMF	15.2 ± 2.35 bc	10.8 ± 0.80 de	82 ± 5.7 cd
LSD			12.25	10.14	86.51

Note: Different letters within each column represent significant differences at $p < 0.05$ (LSD test).



Figure 2. Effect of AMF and species proportion on vegetative growth of *V. gerrardii* and *N. glauca* seedlings established in mixed plantations. Species proportion of *N. glauca* to *V. gerrardii*.

The leaf number of both species was significantly affected by AMF inoculant ($p = 0.0001$), species ($p = 0.0001$), and species proportion ($p = 0.0002$) (Table 2). The number of leaves of the *V. gerrardii* seedlings decreased as the density of *N. glauca* increased in the presence of AMF. However, the number of leaves on *N. glauca* remained constant across all mixed plantations in the presence of AMF (Table 2). The number of leaves of *V. gerrardii* decreased with increasing levels of *N. glauca* in the absence of AMF treatment. Regarding *N. glauca* in the non-AMF treatment, seedlings showed an increase in leaf number as its proportion increased (Table 2).

V. gerrardii seedlings treated with AMF had a greater leaf area at all plant densities compared to non-treated seedlings. Seedlings with a higher proportion of species had a greater leaf area compared to seedlings with a lower proportion, irrespective of the inoculant treatment. For *N. glauca*, seedlings treated with AMF exhibited a noticeable increase in leaf area compared to seedlings without AMF at all proportions. Seedlings with lower species proportion had a higher leaf area compared to seedlings with a higher proportion at both inoculant levels. This means that the increase in the number of *N. glauca* had led to intraspecific competition among the seedlings; nevertheless, the intraspecific competition in the AMF treatment was lower than in seedlings without AMF (Table 2) (Figure 2).

2.3. Effect of AMF and Species Proportion/Plant Density on Total Dry Weight of V. gerrardii and Invasive N. glauca Grown in Mixed Plantations

In mixed plantations, total dry weight per seedling was significantly affected by AMF inoculant ($p < 0.0001$), species ($p = 0.002$), plant proportion ($p < 0.0001$), and the interaction between AMF inoculant and species ($p = 0.0007$) (Figure 3). The average total dry weight per plant for *V. gerrardii* in the AMF treatment decreased with an increasing proportion of *N. glauca* (Figure 3A). *V. gerrardii* and *N. glauca* seedlings had dry weights of 4.08 g and 3.1 g, respectively, at a ratio of 2:2 plants (0.50:0.50). Under the non-AMF treatment conditions, *N. glauca* seedlings exhibited a slight decrease in dry weights as the species proportions increased in the pot. In contrast, *V. gerrardii* seedlings maintained consistent dry biomass levels regardless of species proportions (Figure 3B). However, in the species proportion (0.75 A:0.25 N), the average total dry weights were 4.94 g for *N. glauca* and 2.6 g for *V. gerrardii*, indicating that *N. glauca* was affected more with intraspecific competition.

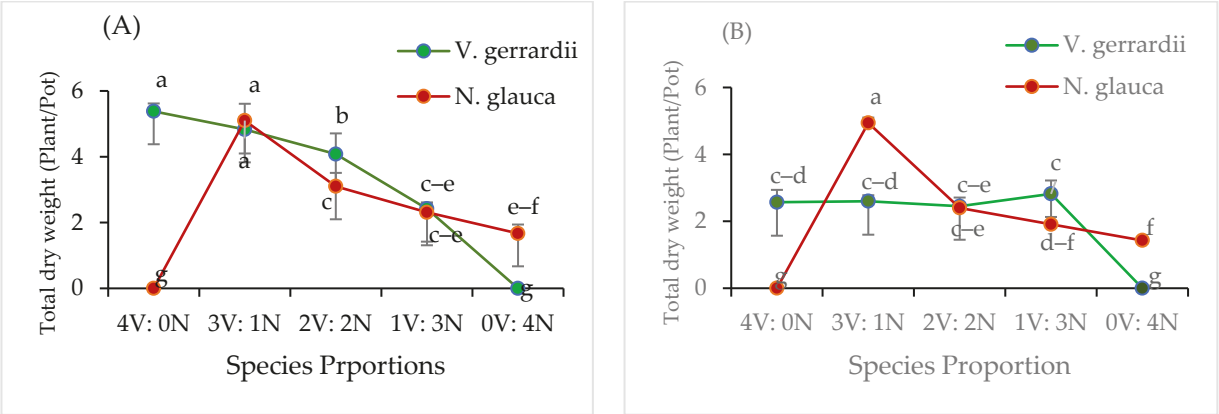

Figure 3. Replacement diagram displaying dry weight for *V. gerrardii* and *N. glauca* in a replacement series with AMF inoculant (**A**) and without AMF inoculant (**B**). Note: V = *V. gerrardii*; N = *N. glauca*. Different letters above graphs indicate a significant difference at $p < 0.05$ (LSD test).

2.4. Effect of AMF on Relative Yield and Relative Yield Total of V. gerrardii and N. glauca Seedlings

Relative yield was significantly affected by plant species ($p = 0.0002$) and the species proportion treatment ($p < 0.0001$) (Table 3). The increase in species proportion of *N. glauca* resulted in a decrease in the relative yield of *V. gerrardii* in the AMF treatment. In *N. glauca*, the relative yield also decreased as its proportion increased. The relative yield of *N. glauca* was consistently higher than that of *V. gerrardii* across all species proportions. Relative yield totals in AMF treatment were higher than in the non-AMF treatment. The relative yield of *V. gerrardii* showed a decrease in the non-AMF treatment as the proportion of *N. glauca* increased, particularly in the 1 *V. gerrardii*:3 *N. glauca*. However, the decrease is lower in comparison to that in AMF-treated seedlings of the same species ratio. (Table 3).

Table 3. Relative yield and relative yield total of *V. gerrardii* and *N. glauca* grown with and without AMF inoculant.

Species	Plant Species Proportion (*V. gerrardii*:*N. glauca*)	AMF Inoculant	Relative Yield (RY)	Relative Yield Total (RYT)
V. gerrardii *N. glauca*	3:1 (0.75:0.25)	AMF+ AMF+	3.81 ± 0.29 b 5.79 ± 0.7 a	5.29
V. gerrardii *N. glauca*	2:2 (0.50:0.50)	AMF+ AMF+	3.21 ± 0.50 bcd 3.26 ± 0.43 bcd	3.235
V. gerrardii *N. glauca*	1:3 (0.25:0.75)	AMF+ AMF+	1.90 ± 0.14 e 2.44 ± 0.32 cde	2.035
V. gerrardii *N. glauca*	3:1 (0.75:0.25)	AMF− AMF−	3.25 ± 0.23 bcd 5.46 ± 0.22 a	4.095
V. gerrardii *N. glauca*	2:2 (0.50:0.50)	AMF− AMF−	3.06 ± 0.33 bcd 3.31 ± 0.14 bc	3.185
V. gerrardii *N. glauca*	1:3 (0.25:0.75)	AMF− AMF−	2.15 ± 0.60 de 2.64 ± 0.31 cde	2.27

Note: Different letters within each column represent significant differences at $p < 0.05$ (LSD test).

2.5. Aggressivity Index of V. gerrardii and N. glauca Grown with and Without AMF Inoculant

The effect of the AMF inoculant on the aggressiveness of the two species was not significant ($p = 0.78$), implying a similar response from both species. However, species proportion had a significant effect on species aggressivity ($p = 0.004$) (Table 4). The aggressivity index of the AMF treatment reveals that *N. glauca* displays a higher level of

aggression towards *V. gerrardii*. Nevertheless, as the proportion of *N. glauca* in the mixture increases to 2:2 plants, its aggressivity decreases. *N. glauca* displayed increased aggressiveness towards *V. gerrardii* in the absence of AMF treatment, particularly in the ratio of 3 *V. gerrardii*:1 *N. glauca*. *N. glauca* demonstrated a high level of aggression towards *V. gerrardii*, regardless of the presence or absence of AMF. However, the presence of AMF resulted in a reduction in aggression, except for the proportion 1 *V. gerrardii*:3 *N. glauca* (Table 4).

Table 4. Aggressivity index for *V. gerrardii* and *N. glauca* grown in mixed plantations with and without AMF inoculant.

Plant Species Proportion (*V. gerrardii*:*N. glauca*)	**Aggressivety Index**	
	+AMF	**−AMF**
3:1 (0.75:0.25)	−1.98 ± 0.87 bc	−2.21 ± 0.39 c
2:2 (0.50:0.50)	−0.05 ± 0.64 a	−0.24 ± 0.32 a
1:3 (0.25:0.75)	−0.53 ± 0.38 ab	−0.48 ± 0.50 ab

Aggressivity index was identified as (RY of *V. gerrardii*- RY of *N. glauca*). The negative values indicate that the RY of *V. gerrardii* are less than the RY of *N. glauca*; thus, negative values indicate that *N. glauca* is more aggressive than *V. grrardii.* Different letters within each column represent significant differences at $p < 0.05$ (LSD test).

2.6. Effect of AMF and Plant Proportion/Density on the Root Parameters of V. gerrardii and N. glauca Seedlings Grown in Mixed Plantations

The total root length in the diverse plantation was significantly influenced by plant species ($p = 0.0001$), AMF inoculant ($p < 0.0001$), species proportion ($p = 0.005$), the interaction between AMF inoculant and species ($p < 0.0001$), and the interaction between species and proportion ($p = 0.001$). The AMF-treated seedlings of *V. gerrardii* in the mixed plantations displayed a slight augmentation in root length at the species proportions of 3 *V. gerrardii*:1 *N. glauca* and 1 *V. gerrardii*:3 *N. glauca*, in comparison to their non-AMF-treated counterparts. However, in the 2 *V. gerrardii*:2 *N. glauca* ratio, seedlings that were not inoculated with AMF exhibited a longer root length compared to the seedlings that received AMF treatment. The total root length of *N. glauca* seedlings showed a marked increase when treated with AMF as opposed to those that were not treated, across all proportions (Figure 4A).

The total root surface area was significantly impacted by plant species ($p = 0.004$), AMF inoculant ($p = 0.0016$), and the interaction between plant species and AMF ($p = 0.0098$). In *V. gerrardii*, the application of AMF resulted in an increase in the root surface area in the treated seedlings. However, this increase was not significantly different from the non-AMF-treated seedlings across all species proportions. On the other hand, in *N. glauca*, the seedlings experienced significant advantages from AMF compared to the non-AMF-treated seedlings, especially in the proportion of 3 *V. gerrardii*:1 *N. glauca* (Figure 4B).

Root volume was notably influenced by species ($p = 0.03$), whereas the presence of AMF inoculant ($p = 0.05$) and species proportion did not have a significant effect ($p = 0.95$). The root volume of *V. gerrardii* seedlings showed an increase after being inoculated with AMF, regardless of the species proportion, although this difference was not statistically significant when compared to the non-AMF-treated seedlings (Figure 4C). In the case of *N. glauca* seedlings, the application of AMF led to a noticeable increase in root volume when compared to its counterpart and *V. gerrardii* seedlings grown with and without AMF inoculant. This suggests that *N. glauca* derives greater advantages from AMF association than *V. gerrardii*, as shown in Figure 4C.

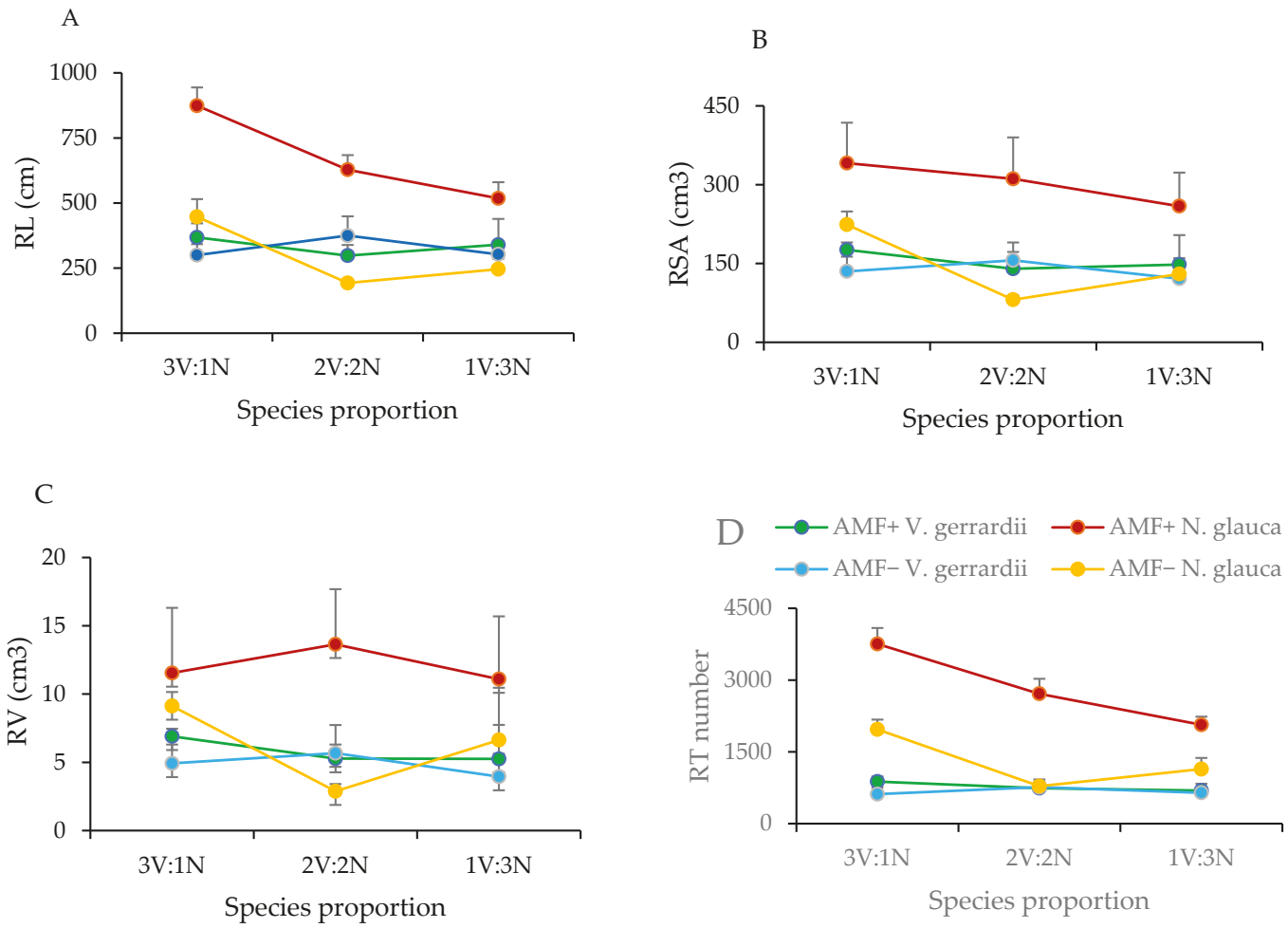

Figure 4. Total RL (**A**), RSA (**B**), RV (**C**), and RT number (**D**) for *V. gerrardii* and *N. glauca* grown in mix-culture in different species proportions with and without AMF. V = *V. gerrardii;* N = *N. glauca*.

Root tip numbers were significantly affected by AMF ($p < 0.0001$), plant species ($p < 0.0001$), species proportion ($p = 0.0003$), and the interaction between species and AMF inoculant ($p < 0.0001$). Despite being inoculated with AMF, the root tips in *V. gerrardii* seedlings did not show a considerable increase along the entire species proportion. However, *N. glauca* showed the highest root tip numbers in the AMF-treated seedlings compared to the non-AMF-treated plants (Figure 4D).

2.7. Effect of AMF and Plant Proportion on the Chlorophyll a and b Levels of V. gerrardii and N. glauca Seedlings Grown in Mixed Plantations

Plant species in the mixed plantation had a significant impact on chlorophyll *a* content ($p = 0.0001$). Furthermore, chlorophyll *a* content was influenced by AMF ($p = 0.01$) and the proportion of species ($p = 0.008$) (Figure 5A). The chlorophyll *a* content of *V. gerrardii* seedlings treated with AMF displayed a slight increase throughout proportions compared to the non-AMF-treated seedlings, although the difference was not statistically significant. In contrast, the application of AMF to *N. glauca* seedlings resulted in a significant elevation in chlorophyll *a* content. This effect was observed across all species proportions, indicating the positive impact of AMF treatment. Notably, *V. gerrardii* displayed higher levels of chlorophyll *a* content in both AMF inoculant levels compared to *N. glauca* plants (Figure 5A).

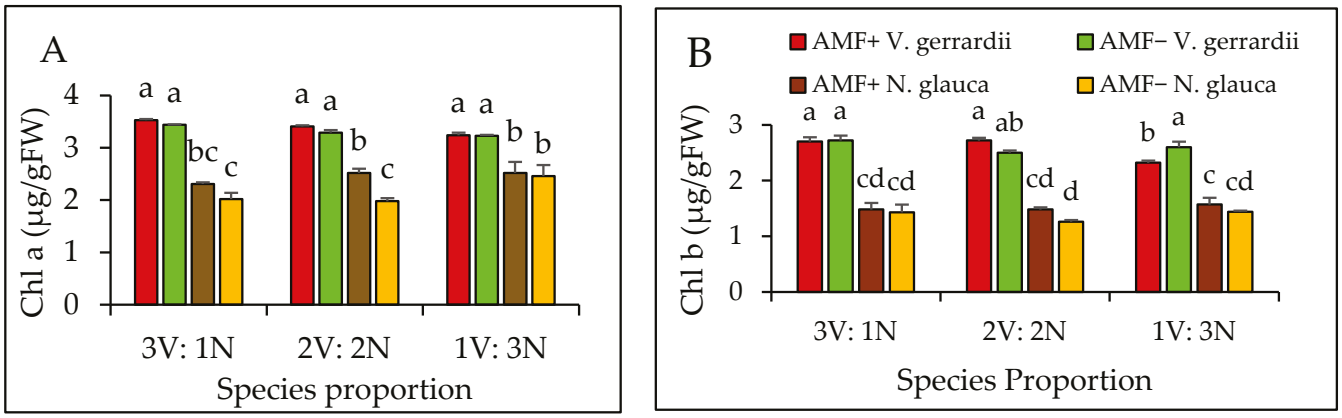

Figure 5. Chlorophyll *a* (**A**) and chlorophyll *b* (**B**) for *V. gerrardii* and *N. glauca* grown in mixed plantations. Note: V = *V. gerrardii;* N = *N. glauca.* Different letters above bars indicate a significant difference at $p < 0.05$ (LSD test).

Plant species had a significant impact on the content of chlorophyll *b* ($p = 0.0001$), while no significant effects were observed for AMF inoculant ($p = 0.33$) and species proportion ($p = 0.07$); however, their interactions ($p = 0.056$) were marginally affected chlorophyll *b*. *V. gerrardii* seedlings treated with AMF in all proportions showed a slight increase in chlorophyll *b* content compared to those without AMF, but the difference was not statistically significant (Figure 5B). In contrast, *N. glauca* seedlings had higher levels of chlorophyll *b* content in the AMF-treated group across all species proportions, yet the difference was not significantly different from the seedlings without AMF in the same proportions (Figure 5B).

2.8. Effect of AMF and Plant Proportions on the Photosynthesis and Stomatal Conductance of V. gerrardii and N. glauca Seedlings Grown in Mixed Plantations

The photosynthesis rate in the mixed plantations was found to be significantly influenced by various treatments. These include species ($p = 0.004$), AMF inoculant ($p = 0.00001$), and plant proportion ($p < 0.0001$) (Table 5). In the case of *V. gerrardii* seedlings, AMF treatment resulted in significantly higher photosynthesis rates compared to the seedlings that were not inoculated with AMF, even when considering the same species proportion. Furthermore, as the proportion of *V. gerrardii* decreased in the pot, the photosynthesis rate also decreased, primarily due to interspecific competition. However, the seedlings treated with AMF maintained the highest photosynthesis values in comparison to their non-treated counterparts. The highest photosynthesis rate was observed in *N. glauca* seedlings that were treated with AMF at a species ratio of 3 *V. gerrardii*:1 *N. glauca*. A decrease in the photosynthesis rate was noted with an increase in the proportion of *N. glauca* (Table 5).

Stomatal conductance in the mixed plantations was significantly influenced by plant species ($p = 0.0004$), species proportion ($p < 0.0001$), and their interaction ($p = 0.02$). In the case of *V. gerrardii* seedlings, the highest stomatal conductance value was observed in the presence of AMF seedlings. As the proportion of *V. gerrardii* per pot decreased, the stomatal conductance decreased in both AMF-treated and non-AMF-treated seedlings. Nevertheless, the seedlings treated with AMF exhibited a higher stomatal conductance compared to the non-AMF-treated seedlings (Table 5). Stomatal conductance in *N. glauca* was significantly higher in the species proportion 3 *V. gerrardii*:1 *N. glauca* when treated with AMF, as indicated by the recorded values. Additionally, an increase in the proportion of *N. glauca* per pot led to a noticeable decrease in stomatal conductance, especially in seedlings without AMF (Table 5).

Table 5. Effect of species proportion and AMF inoculant on photosynthesis and stomatal conductance for *V. gerrardii* and *N. glauca* in mixed plantation.

Species	Plant Species Proportion (*V. gerrardii*:*N. glauca*)	AMF Inoculant	Photosynthesis (μmol $CO_2/m^2/s$)	Stomatal Conductance (mol $H_2O/m^2/s$)
V. gerrardii	3:1 (0.75:0.25)	AMF+	16.1 ± 0.8 ab	207 ± 25 ab
		AMF−	8.1 ± 1.1 cde	213 ± 19 ab
	2:2 (0.50:0.50)	AMF+	13.5 ± 1.3 abc	198 ± 11 b
		AMF−	6.4 ± 0.4 e	178 ± 9 b
	1:3 (0.25:0.75)	AMF+	9.8 ± 0.8 cde	144 ± 11 b
		AMF−	7.7 ± 1.2 de	151 ± 12 b
N. glauca	3:1 (0.75:0.25)	AMF+	18.5 ± 0.9 a	289 ± 22 a
		AMF−	12.7 ± 1.6 bcd	288 ± 25 a
	2:2 (0.50:0.50)	AMF+	13.5 ± 1.3 abc	214 ± 20 ab
		AMF−	10.4 ± 1.3 cde	197 ± 11 b
	1:3 (0.25:0.75)	AMF+	10.6 ± 0.9 cde	167 ± 16 b
		AMF−	7.5 ± 0.6 de	167 ± 12 b
LSD			5.4489	85.3

Different letters within each column represent significant differences at $p < 0.05$ (LSD test).

Figure 6 illustrates that the first two principal components (PCs) on the X and Y axes accounted for a total of 76.7% variance, with PC1 and PC2 showing a variance of 53.8% and 22.9%, respectively. Our results in principle component analysis (PCA) indicated that the root length (RL), relative yield (RY), and stomatal conductance (C) of *N. glauca* are negatively correlated to aggressivity at a proportion of 1:3 with and without AMF treatment. In the lower left quadrant, root volume (RV) and root tip number (RT) are highly correlated under the AMF treatment at a 1:3 plant/pot proportion. Additionally, RL and leaf area were positively correlated under both AMF and non-AMF treatments at a species proportion of 1:3 plant/pot in the same quadrant. Similarly, chlorophyll *a* and chlorophyll *b* showed a positive correlation under AMF at species 2 *V. gerrardii*:2 *N. glauca* in the upper right quadrant, while height and leaf number are closely related in AMF treatment at 1 *V. gerrardii*:3 *N. glauca* plant/pot.

2.9. Effects of Plant Densities on the AMF Colonization Rate and Spore Number of V. gerrardii and Invasive N. glauca Seedlings Grown in Mono Plantations

In the monoculture plantations of *V. gerrardii*, plant density has a marginal effect on the infection rate of arbuscular ($p = 0.05$) and did not affect mycelium ($p = 0.11$) and vesicular mycorrhiza ($p = 0.24$). Arbuscular mycorrhiza had a higher value, being recorded in the density 1 plant/pot. However, the lowest infection rates were recorded by mycelium (at 1, and 2 plant densities) and arbuscular (at 3 and 4 plant densities) (Table 6).

For *N. glauca*, plant densities did not impact the infection rates of mycelium ($p = 0.86$), arbuscular ($p = 0.84$), and vesicular ($p = 0.67$) mycorrhiza. Nevertheless, arbuscular mycorrhiza showed the highest infection rate along all plant densities. However, the lowest infection rates were recorded for vesical along all plant densities (Table 6).

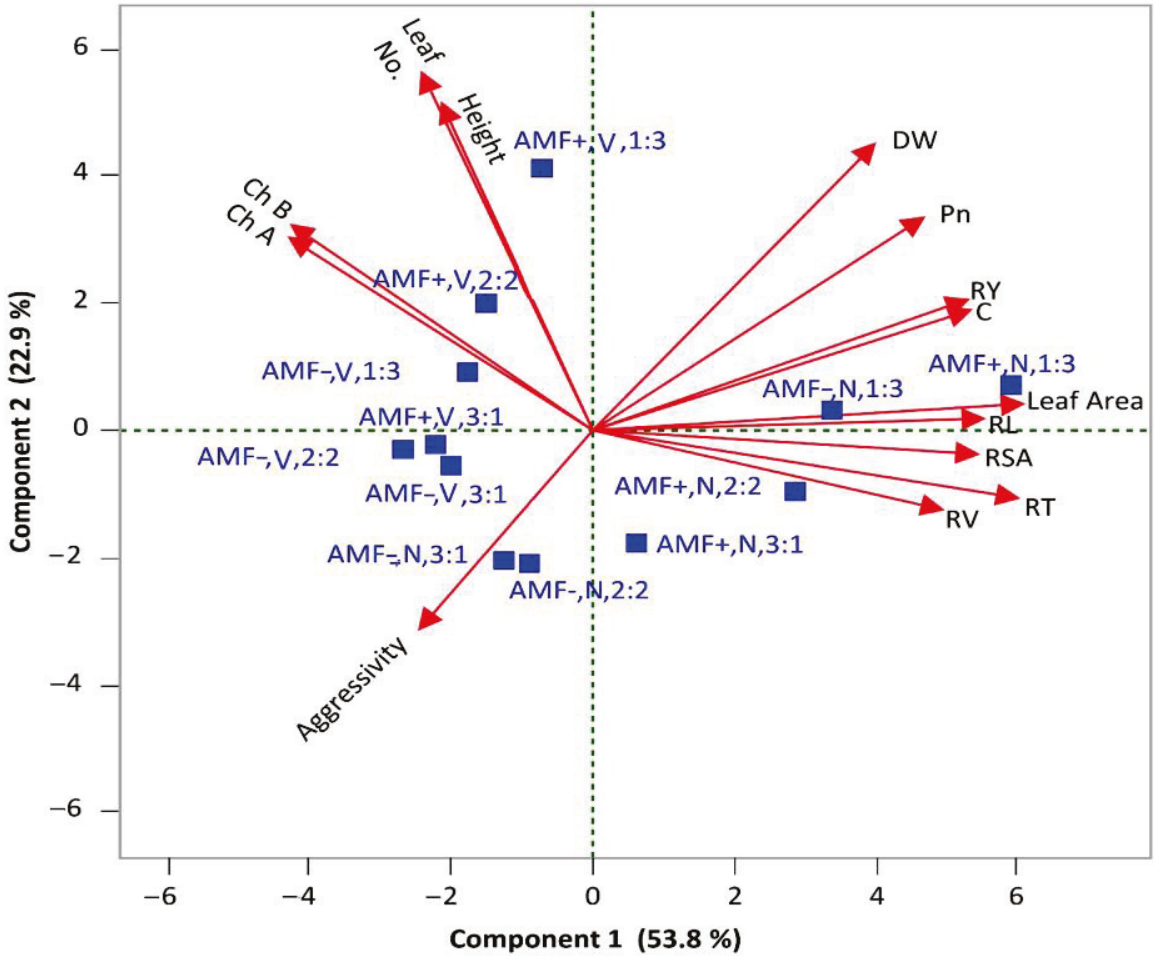

Figure 6. PCA for parameters of *V. gerraradii* and *N. glauca* seedlings established at mixed plantations with and without AMF. AMF+, V, 1:3 = *V. gerrardii* seedlings treated with AMF at species proportions of 1:3, 2:2, and 3:1 *V. gerrardii*: *N. glauca*; AMF−, V, 1:3 = *V. gerrardii* seedlings without AMF at species proportions of 1:3, 2:2, and 3:1 *V. gerrardii*:*N. glauca*; AMF+, N, 1:3= *N. glauca* seedlings treated with AMF at species proportions of 1:3, 2:2, and 3:1 *V. gerrardii*: *N. glauca*; AMF−, N, 1:3 = *N. glauca* seedlings without AMF at species proportions of 1:3, 2:2, and 3:1 *V. gerrardii*: *N. glauca*.

Table 6. AMF infection rates for *V. gerrardii* and *N. glauca* seedlings grown in monoculture plantations with different plant densities.

Plant Density	*V. gerrardii*			*N. glauca*		
	AMF Structural Colonization%			AMF Structural Colonization%		
	Mycelium%	Arbuscular%	Vesicular %	Mycelium%	Arbuscular%	Vesicular%
1	50.0 ± 0.0 a	62.6 ± 9.3 a	63.3 ± 6.6 a	53.3 ± 6.6 a	86.6 ± 3.3 a	48.3 ± 4.4 a
2	41.6 ± 6.0 a	43.3 ± 3.3 ab	45.0 ± 5.0 a	56.6 ± 8.8 a	70.0 ± 11 a	43.3 ± 8.8 a
3	45.0 ± 5.0 a	41.6 ± 7.2 ab	48.3 ± 1.6 a	60.0 ± 10 a	66.6 ± 12 a	46.0 ± 3.3 a
4	33.3 ± 3.3 a	30.0 ± 5.7 b	53.4 ± 8.8 a	46.6 ± 6.6 a	80.0 ± 5.7 a	45.0 ± 2.8 a
LSD	19.2	30.73	27.7	26.6	29.2	17.6

Different letters within each column represent significant differences at $p < 0.05$ (LSD test).

Also, no significant differences were found in the spore numbers between plant densities for both plant species established in mono plantings ($p = 0.58$ and 0.31 for *V. gerrardii* and *N. glauca* respectively). AMF spores in *V. gerrardii* soils were 152, 140, 144, and 123 for plant densities 1, 2, 3, and 4 respectively. However, AMF spores in *N. glauca* soils were 98, 89, 103, and 85 at densities 1, 2, 3, and 4, respectively (Figure 7A, B).

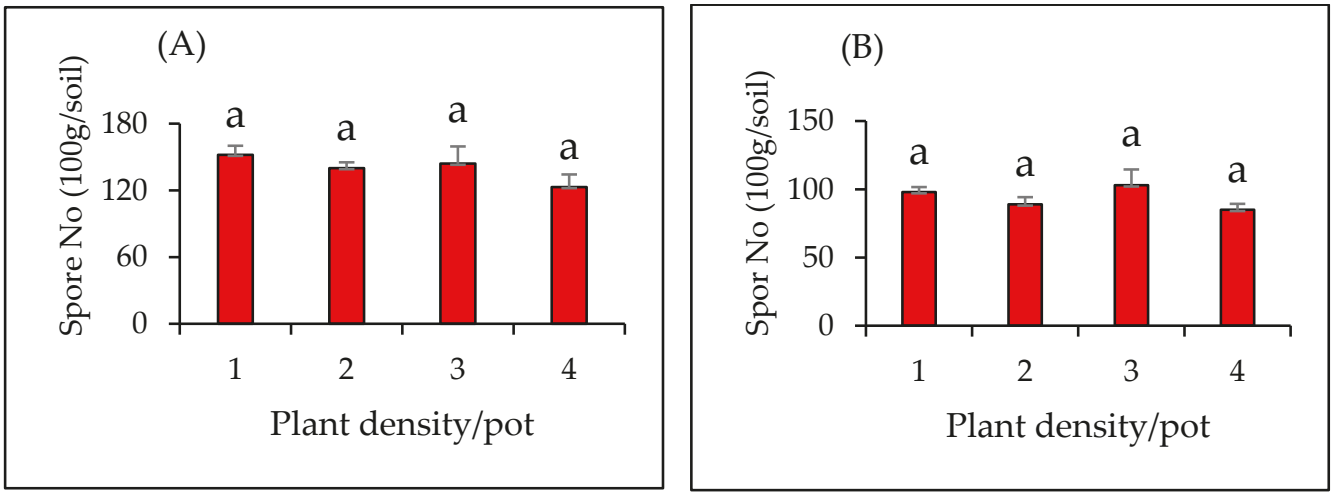

Figure 7. AMF spore in soils of (**A**) *V. gerrardii* and (**B**) *N. glauca* seedlings grown in mono plantings with different plant densities. Similar letters above bars indicate no significant difference at $p < 0.05$ (LSD test).

2.10. Effect of AMF and Plant Density on Height, Leaf Number, and Leaf Area of V. gerrardii and N. glauca Grown in Mono Plantations

The height of *V. gerrardii* in monoculture plantations was significantly influenced by both AMF inoculant ($p = 0.0001$) and plant density ($p = 0.03$). In the seedlings treated with AMF inoculant, height exhibited an increase as the density decreased. The height of plants grown without AMF experienced a significant decrease as plant density increased (Table 7; Figure S1).

Table 7. Effect of AMF and density on height, leaf number, and leaf area of *V. gerrardii* grown in mono plantations.

Plant Density	AMF Inoculant	*V. gerrardii*			*N. glauca*		
		Height (cm)	Leaf Number	Leaf Area (cm^3)	Height (cm)	Leaf Number	Leaf Area (cm^3)
1	AMF+	50.5 ± 6.5 a	72.2 ± 14.3 a	239 ± 50 a	54.5 ± 12.3 a	23.7 ± 2.9 a	577 ± 59 a
	AMF−	36.2 ± 2.7 bc	43.7 ± 2.9 bcd	134 ± 12 b	27.0 ± 1.4 bcd	16.7 ± 1.2 bc	321 ± 34 bc
2	AMF+	49.0 ± 2.6 a	53.7 ± 3.9 b	164 ± 15 ab	35.7 ± 5.0 ab	18.2 ± 2.5 ab	410 ± 33 ab
	AMF−	34.0 ± 1.5 bcd	36.2 ± 2.0 bcd	111 ± 10 b	23.0 ± 1.7 bcd	14.2 ± 1.1 bc	189 ± 15 cd
3	AMF+	42.2 ± 2.9 ab	45.7 ± 2.2 bc	136 ± 13 b	36.7 ± 9.6 ab	18.7 ± 2.0 b	322 ± 46 bc
	AMF−	27.7 ± 2.2 cd	29.7 ± 1.6 cd	84 ± 4 b	13.7 ± 1.6 cd	12.5 ± 1.0 bc	136 ± 8 d
4	AMF+	42.0 ± 5.4 ab	45.0 ± 6.0 bcd	129 ± 15 b	36.7 ± 7.1 abc	17.2 ± 1.3 bc	231 ± 19 cd
	AMF−	24.7 ± 2.4 d	28.0 ± 2.9 d	72 ± 9 b	17.5 ± 1.5 d	14.5 ± 1.0 c	124 ± 12 d
LSD		10.79	17.56	98	18.82	5.31	156

Different letters within each column represent significant differences at $p < 0.05$ (LSD test).

The height of *N. glauca* at monoculture plantations was significantly affected by AMF inoculant ($p = 0.0001$). Increasing plant density led to a reduction in the height of the seedlings, irrespective of whether they were AMF or non-AMF seedlings. Seedlings grown

without AMF experienced a 35.18% decrease in height at a density of four plants per pot, while those with AMF showed a slightly lower reduction of 32.66% (Table 7; Figure S2).

The leaf number of *V. gerrardii* was significantly affected by AMF inoculant ($p = 0.005$) and plant density ($p = 0.0001$). Seedlings with AMF showed the highest values of leaf number in all plant densities compared to the non-AMF-treated seedlings (Table 7). A reduction in leaf number was observed with an increase in plant density for both treated and non-treated seedlings. However, the presence of AMF resulted in a higher leaf number compared to the untreated seedlings (Table 7).

The number of leaves on *N. glauca* seedlings in monoculture was significantly influenced by the AMF inoculant ($p = 0.0007$), while density ($p = 0.057$) and the interaction between AMF and density did not have a significant effect on leaf numbers ($p = 0.63$). Seedlings treated with AMF exhibited a remarkable increase in leaf number when contrasted with untreated seedlings (Table 7).

The monoculture plantations displayed significant variations in leaf area per plant for *V. gerrardii* seedlings under AMF treatment and the chosen plant density ($p = 0.0002$, $p = 0.001$), respectively (Table 7). The increase in plant densities resulted in a decline in leaf area, regardless of whether the plants were associated with AMF or not. The presence of AMF alleviated the influence of plant density on leaf area, contrasting with the non-AMF-treated seedlings (Table 7).

Leaf area in *N. glauca* was significantly impacted by plant density and the presence of AMF ($p < 0.0001$) (Table 7). Seedlings with AMF treatment exhibited higher leaf area compared to the seedlings without AMF in the same density. The increase in plant density resulted in a decrease in leaf area for both AMF and non-AMF-treated seedlings; however, seedlings inoculated with AMF exhibited increased leaf area when compared to those without AMF (Table 7).

2.11. Effect of AMF and Plant Density on Total Dry Weight of V. gerrardii and Invasive N. glauca Grown in Mono Plantations

In the context of monoculture planting, findings demonstrated significant impacts for AMF ($p = 0.003$) and plant density (0.00001) on the total plant dry weight of *V. gerrradii* seedlings. In both AMF and non-AMF-treated seedlings, dry weight decreased progressively as plant density increased. Nevertheless, the decline in dry weight was more moderate in AMF-treated seedlings than in non-inoculated seedlings at the corresponding density (Figure 8A).

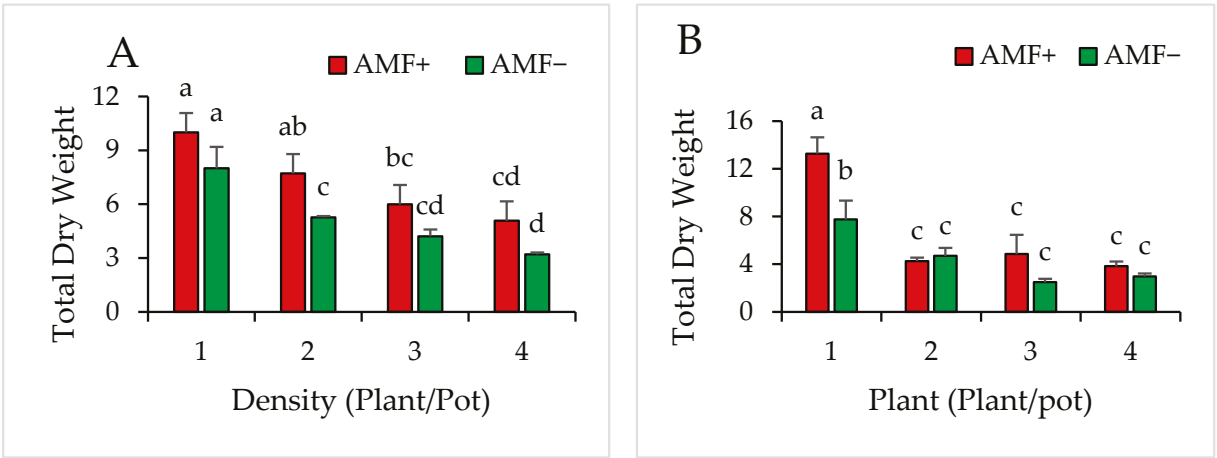

Figure 8. Total dry weight for *V. gerrardii* (**A**) and *N. glauca* (**B**) grown in monoculture in different densities with and without AMF. Different letters above bars indicate a significant difference at $p < 0.05$ (LSD test).

The total dry weight of *N. glauca* cultivated in monoculture was found to be significantly influenced by the AMF inoculant ($p = 0.006$), density ($p < 0.0001$), and the interaction between the two factors ($p = 0.026$). The presence of AMF effectively reduced the intraspecific competition among *N. glauca* seedlings caused by the higher plant densities. In the case of non-AMF (four plants per pot) seedlings, the average dry weight was measured at 2.98 g. However, the AMF-treated seedlings at the same density displayed a significantly higher average dry weight of 3.83 g. This indicates that *N. glauca* seedlings derived benefits from the AMF inoculant (Figure 8B).

2.12. Effect of AMF and Plant Densities on the Chlorophyll a and b of V. gerrardii Seedling Grown in Monoculture Plantations

In monoculture plantations, the content of chlorophyll *a* in *V. gerrardii* seedlings was not significantly influenced by the AMF inoculant ($p = 0.12$), plant densities ($p = 0.75$), or their interaction ($p = 0.67$) (Figure 9A).

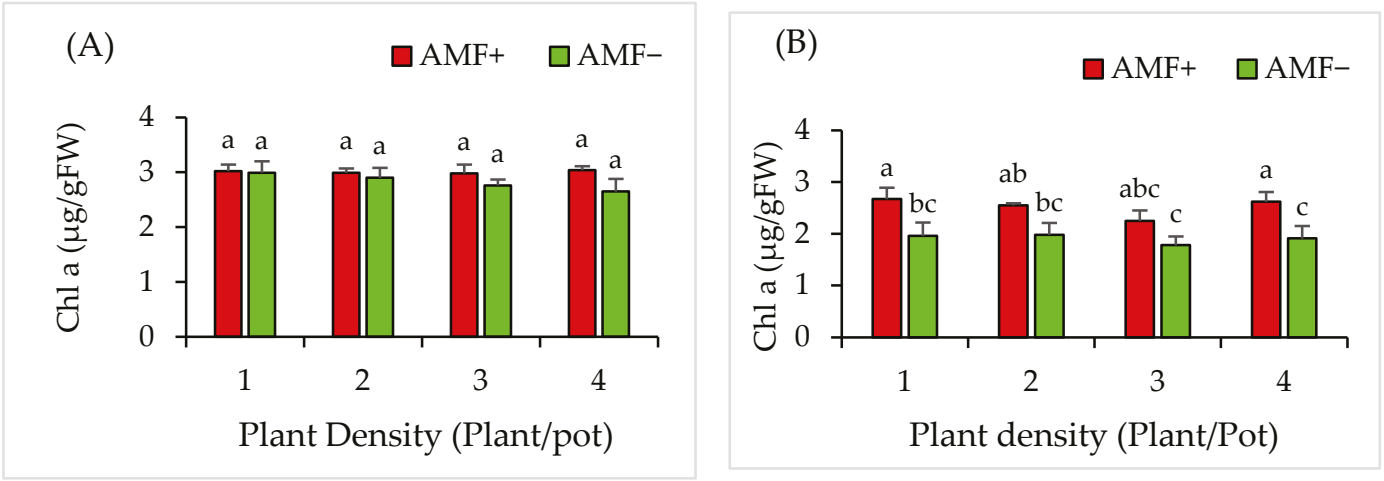

Figure 9. Chlorophyll *a* (**A**) and *b* (**B**) for *V. gerrardii* grown in monoculture plantations. Note: Different letters above bars indicate a significant difference at $p < 0.05$ (LSD test).

For chlorophyll *b*, AMF had a significant impact on the content of chlorophyll *b* ($p = 0.0004$) in *V. gerrardii* seedlings, whereas plant density did not show a significant effect on chlorophyll *b* ($p = 0.49$). Furthermore, the interaction between the AMF inoculant and plant density did not show a significant effect on chlorophyll *b* content ($p = 0.92$ (Figure 9B)).

2.13. Effect of AMF and Plant Densities on the Chlorophyll a and b of N. glauca Seedling Grown in Monoculture Plantations

The chlorophyll *a* content in *N. glauca* monoculture plantations was significantly influenced by the AMF ($p = 0.01$) and plant densities ($p = 0.0006$), while their interaction did not yield a significant effect ($p = 0.601$). The AMF treatment had a profound impact on the levels of chlorophyll *a*. Notably, the seedlings treated with AMF exhibited significantly higher values of chlorophyll *a* when compared to their counterparts at the same density level (Figure 10A).

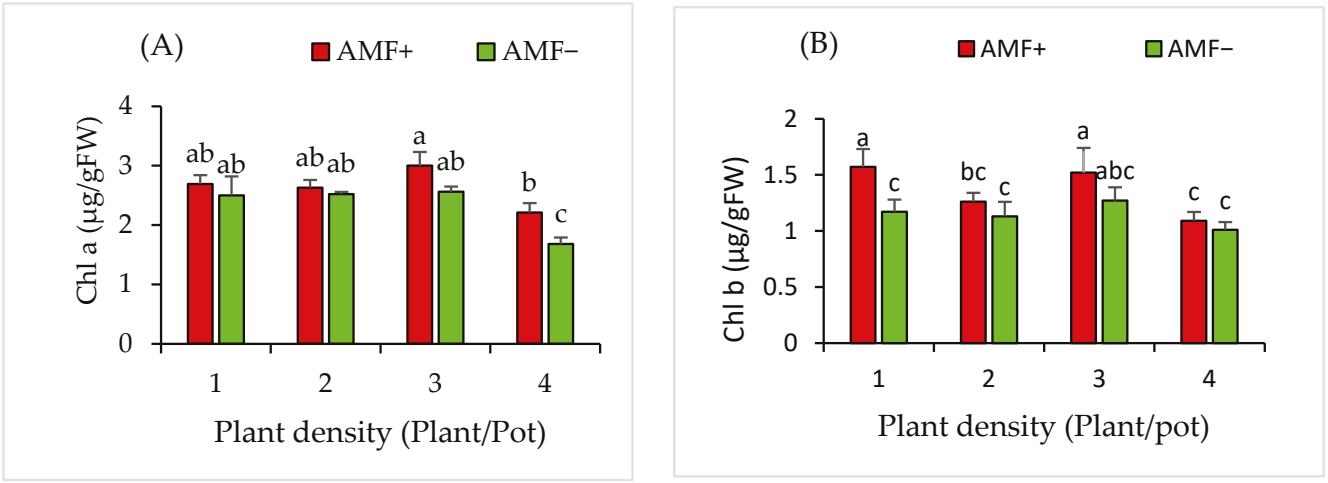

Figure 10. Chlorophyll *a* (**A**) and *b* (**B**) for *N. glauca* seedlings grown in monoculture plantation. Note: Different letters above bars indicate a significant difference at $p < 0.05$ (LSD test).

The chlorophyll *b* of *N. glauca* seedlings grown in monoculture plantation were significantly impacted by the AMF treatment, as demonstrated ($p = 0.0006$). The density of plants did not show a statistically significant effect on chlorophyll *b* levels in *N. glauca* seedlings ($p = 0.49$). AMF-treated seedlings exhibited the highest chlorophyll *b* content across all plant densities, in contrast to the untreated seedlings. Specifically, the density of one plant per pot resulted in the highest chlorophyll *b* content for AMF-treated seedlings, while the lowest value was observed in non-AMF-treated seedlings at a plant density of four plants per pot (Figure 10B).

2.14. Effect of AMF and Plant Densities on the Photosynthesis and Stomatal Conductance of V. gerrardii and N. glauca Seedling Grown in Monoculture Plantations

The photosynthesis rate of *V. gerrardii* seedlings grown in monoculture plantations was significantly influenced by AMF inoculant ($p = 0.04$). Conversely, no significant impact was detected for the plant density ($p = 0.11$) or its interaction with AMF ($p = 0.49$). The photosynthesis rate of *V. gerrardii* seedlings was found to be higher with AMF treated seedlings, as opposed to the seedlings without AMF, despite having a similar plant density (Table 8).

Table 8. Effect of AMF on photosynthesis and stomatal conductance in *V. gerrardii* and *N. glauca* growing in monoculture plantations.

Plant Density (Plant/Pot)	AMF Inoculant	*V. gerrardii*		*N. gerrardii*	
		Photosynthesis (µmol $CO_2/m^2/s$)	**Stomatal Conductance (mol $H_2O/m^2/s$)**	**Photosynthesis (µmol $CO_2/m^2/s$)**	**Stomatal Conductance (mol $H_2O/m^2/s$)**
1	AMF+	13.8 ± 2.05 a	320.0 ± 67.6 a	18.7 ± 0.9 a	230.0 ± 24.6 a
	AMF−	10.5 ± 0.82 bc	238.0 ± 39.4 abc	14.3 ± 3.1 abc	210.5 ± 28.2 ab
2	AMF+	13.2 ± 0.47 ab	302.7 ± 9.07 ab	16.3 ± 2.4 ab	169.5 ± 25.4 abc
	AMF−	10.9 ± 1.2 abc	248.0 ± 26.9 abc	8.2 ± 1.1 bc	181.7 ± 48.87 abc
3	AMF+	9.9 ± 1.07 c	264.2 ± 19.1 abc	16.9 ± 1.9 ab	168.5 ± 15.4 abc
	AMF−	9.8 ± 1.08 c	213.5 ± 4.83 bc	7.5 ± 1.4 c	168.0 ± 18.7 abc
4	AMF+	10.9 ± 0.35 abc	228.0 ± 18.5 bc	11.1 ± 1.7 abc	146.5 ± 19.5 bc
	AMF−	9.97 ± 0.83 c	188.2 ± 6.04 c	5.9 ± 0.7 c	115.0 ± 7.93 c
LSD		5.2057	145.44	8.70	122.48

Different letters within each column represent significant differences at $p < 0.05$ (LSD test).

The stomatal conductance of *V. gerrardii* seedlings was significantly influenced by AMF treatment ($p = 0.01$). In contrast, the plant density ($p = 0.10$) did not show any significant effects ($p = 0.9$). Stomatal conductance was observed to be higher in the AMF treatment as compared to the non-AMF treatment within each plant density. It was observed that as the plant density increased, there was a decrease in stomatal conductance. However, this decrease was not significantly different and was more pronounced in the non-AMF-treated seedlings compared to the seedlings with AMF (Table 8).

N. glauca plants exhibited significant differences in photosynthesis rate as a result of the AMF inoculant ($p = 0.0001$) and plant density ($p = 0.002$), with no significant impact observed for the interaction between plant density and AMF inoculant ($p = 0.49$). AMF significantly improved the rate of photosynthesis in all seedling densities when compared to seedlings that did not receive AMF treatment (Table 8). As the density of plants increased, there was a significant reduction in the photosynthesis rate, particularly observed in seedlings lacking AMF (Table 8).

Stomatal conductance in monoculture plantations of *N. glauca* seedlings was not significantly affected by AMF inoculant ($p = 0.60$). However, plant density had a significant effect ($p = 0.02$) on stomatal conductance. Despite the lack of a significant impact on stomatal conductance in *N. glauca*, the presence of AMF inoculant resulted in higher stomatal conductance in treated seedlings compared to those without AMF. Notably, an increase in plant density per pot was observed to decrease stomatal conductance in the seedlings (Table 8).

For the mono plantings of *V. gerrardii*, heatmap analysis revealed three distinct clusters, which included seedlings without AMF for the plants with densities of 3 and 4 (blue color), seedlings with AMF at densities of 1 and 2 plants (red color), and three seedlings with AMF at plant densities of 3 and 4 and seedlings without AMF at plant densities of 1 and 2. Most of the measured traits showed a positive correlation with AMF treatments at all plant densities, where correlation increases as plant density decreases. However, in untreated plants, the studied parameters showed a negative correlation, particularly at plant densities of 3 and 4 (Figure 11A).

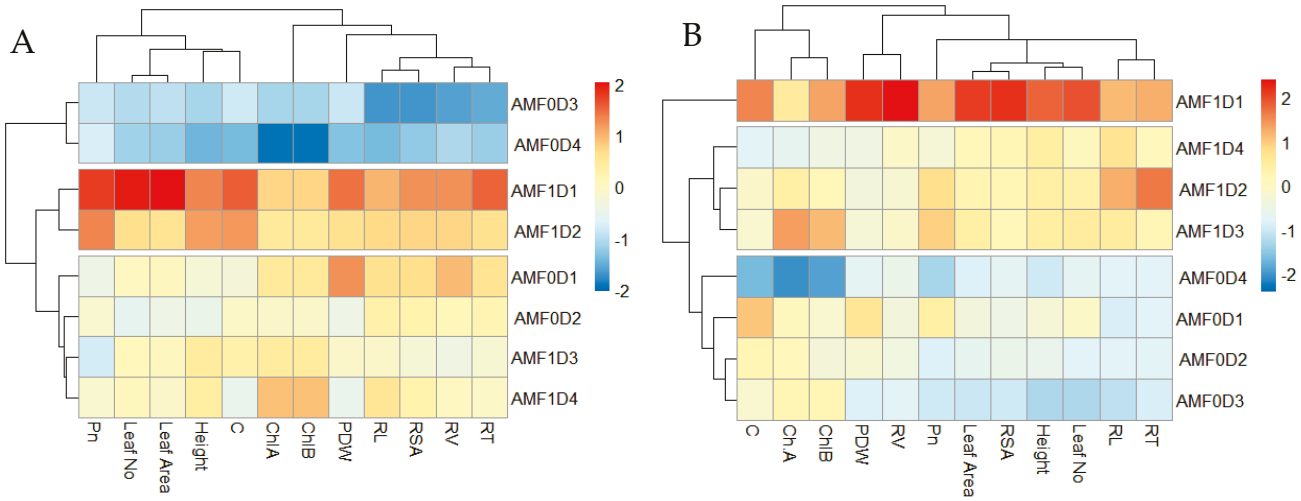

Figure 11. Heatmap for the parameters (root tips number RT; root volume RV; root surface area RSA; plant dry weight PDW; chlorophyll *a* ChA; chlorophyll *b* ChB; stomatal conductance C; photosynthesis rate P) of *V. gerrardii* (**A**) and *N. glauca* (**B**) grown in mono plantings. AMF1D1, AMF1D2, AMF1D3, AMF1D4 = seedlings treated with AMF at plant densities 1−4; AMF0D1, AMF0D2, AMF0D3, AMF0D4 = seedlings without AMF at plant densities 1−4.

For *N. glauca* plantings, most parameters correlated positively (red color) with AMF treatment at the different plant densities, particularly at 1 plant/pot. The positive correlation decreases with the increase in plant density. On the other hand, seedlings without AMF showed lower values in the most measured traits with no or negative (blue color) correlation, particularly in the higher plant densities. In general, seedlings with AMF treatments showed a positive correlation in studied parameters, while seedlings without AMF correlated negatively with the most studied traits (Figure 11B).

3. Discussions

3.1. AMF Spores and Colonization Rate in the Roots of V. gerrardii and N. glauca Grown in the Mono and Mixed Plantations

In this study, both plant species (*V. gerrardii* and invasive *N. glauca*) attracted AMF and made a symbiosis relationship with it, with no statistical variation in AMF infection% and spore numbers. The consistency of the AMF colonization rate between *V. gerrardii* and *N. glauca* could be attributed to the ability of both species to form a symbiotic relationship with AMF, and both species were cultivated in a uniform environment. In this regard, the authors of [32] have indicated that *N. glauca* plants have the ability to form associations with AMF in various environments. Also, *V. gerrardii* has been reported for its ability to establish symbiotic relationships with AMF. According to [33], AMF colonization was observed in the roots of *V. gerrardii* across different environments in Saudi Arabia. Furthermore, a competition study conducted by the authors of [34] revealed that the rate of AMF colonization was similar between invasive and native plants in numerous cases. Also, several studies have indicated that the abundance of AMF spores is influenced by the host plant species as well as other factors such as soil pH and moisture content [35]. Conversely, several studies have reported either a decrease [28] or an increase [36] in AMF spores between co-concurrent invasive and native plants growing in invaded soils, as reported in [37].

3.2. Responses of Vegetative Parameters of V. gerrardii and N. glauca Grown in Mixed and Monoculture Plantations to AMF and Competition

The performance of *V. gerrardii* and *N. glauca* in the greenhouse condition showed different responses to the AMF inoculant levels, which indicates that AMF inoculant plays an important role in the interference and interactions between the two species. Our results demonstrated that *N. glauca* has great capability of competition (due to high total yield) and maintains a dominant growth pattern in both conditions (with and without AMF). These findings follow the results of the authors of [38], who stated that the total yield of invasive and native plants can increase with AMF colonization. This could be attributed to the stronger mutualistic relationship between invasive species and AMF compared to native species, resulting in a high competitive advantage for invasive plants [39]. Interestingly, the highest level of aggression was observed when the plant proportion was 3 *V. gerrardii*:1 *N. glauca*, highlighting the ability of *N. glauca* to dominate in various environmental settings. In mixed plantations, it was observed that *V. gerrardii* experienced a higher impact from the interspecific competition, while *N. glauca* was more influenced by intraspecific competition, as evidenced by the dry weight production and vegetative parameters per plant. In *N. glauca*, it is also observed that the population density is effectively controlled by the intense intraspecific competition that occurs within the species. In this regard, studies explained that non-native plants produce extensively (double to four times) higher biomass and showed more aggressive growth than that of indigenous plants, suggesting their better ability to exploit accessible resources [40]. The ability of plant competition for the available resources in the soil is an important issue because competition

can determine their growth, reproduction, and biomass. According to the findings reported by [41], competition between *Proteus vulgaris* and invasive *Ipomoea purpurea* was greatly impacted by AMF.

In our study, the interspecific competition between seedlings was extensive in reducing relative yield (RY) for *V. gerrardii* when the two species are grown together, suggesting that *N. glauca* gains more RY and advantages from the AMF inoculant. These findings are in line with the results of a competition study between invasive and native plants, which stated that AMF can change the competitive relationship between native and invasive plants [42]. Moreover, the AMF role is linked with the inter and intraspecific competition between plant species [43]. In a study conducted by the authors of [41], *Iopmoea purpurea* weed showed positive AMF growth responses in mono and mixed plantation, and its growth biomass in the mixed planting was significantly superior to those in monocultures, demonstrating that the growth of *I. purpurea* under the mixed situations was better improved by AMF compared to the growth of the *Proteus vulgaris* plain crop. Regardless of the supportive role of AMF in increasing the competitiveness of invasive *N. glauca* over *V. gerrardii*, this superiority can also be linked and attributed to the allelopathic substances that may be exuded by invasive *N. glauca* roots. Scientists have reported that the invasive plant may compete with native plant species by releasing allelochemical compounds [44] and growing rapidly, thereby suppressing neighbouring native plants [45]. This can be a crucial mechanism, allowing them to invade and colonise new environments [46]. Therefore, the allelochemical compounds released from invasive plants may provide the species with a competitive advantage against native plant species [47].

The outcomes of the effect of AMF and species proportion on vegetative parameters in mixed and mono plantations resulted in different patterns of interspecific and intraspecific competition between the two plants. For example, in the mixed plantations, *N. glauca* had a higher leaf area at all proportions regardless of AMF compared to *V. gerrardii*, thereby exerting stronger interspecific competition against *V. gerrardii*. This could be attributed to the morphological variations between the two species. In this context, the authors of [48] stated that differences in morphological structure are linked to biomass allocation, which is considered important for plant development, helping them to acquire resources during competition. Additionally, our findings align with the research conducted by the authors of [39], who reported a reduction in the shoot biomass of indigenous plants due to competition, while simultaneously enhancing the shoot biomass of invasive plant species. The competitive character of invasive plants that benefit from their symbiotic relation with AMF may also be ascribed to improved N and P uptake, which has already been facilitated by AMF hyphae [49]. Many studies have indicated that invasive plants have greater advantages in many different trait groups such as fast growth rate, vigour, size, leaf area, and shoot allocation than native plants [50]. It is well known that AMF increases the ability of plants to absorb water and nutrients. Therefore, the suppression of AMF inoculants reduces the competitive ability and vigour status of native plant species [51].

3.3. Responses of Root Parameters of V. gerrardii and N. glauca Growing in Mixed and Monoculture Plantation to AMF and Competition

The presence of AMF in mixed plantations had a favourable effect on the growth and development of *N. glauca* in interspecific competition, particularly in terms of root traits. Our study confirmed that *N. glauca* exhibited increased root parameters compared to *V. gerrardii* under the influence of AMF throughout the entire species proportion. Conversely, the root parameters of native *V. gerrardii* did not demonstrate the same degree of benefits as *N. glauca*. This result is consistent with the findings documented in [52], which demonstrated that, in the mixed plantations of the invasive *Rhus typhina* and the native *Acer truncatum*, root competition from the invasive species significantly reduced

the space available for the root system of *A. truncatum*. As a result, this led to more pronounced detrimental effects on the growth of root parameters in the native *A. truncatum*. Furthermore, [42] revealed that, in the presence of the interspecific competition between invasive and native plants, AMF influenced root parameters such as length, surface area, volume, and tips in both plant species, and, in most cases, invasive plants derived greater advantages compared to native plants.

Scientists reported that the low growth of root systems in plants directly limits the plant's nutrient and resource assimilation [53]. Therefore, the native *V. gerrardii*, probably absorbed little amounts of nutrients when competing with the invasive *N. glauca*. As a result, the exploitation of soil resources by *V. gerrardii* roots decreased more prominently, which may indirectly have resulted in a competitive advantage for *N. glauca* in resource utilisation. This is in line with the fact that non-native invaders are known to influence nutrient fluxes, availability, and their uptake [54], which can change the physiology and growth rate of native species, as well as alter the abundance of species within plant communities [55].

In this study, the invasive species *N. glauca* shows a greater ability to outcompete the native species *V. gerrardii* in utilising soil resources through its increased root and symbiotic relationships with AMF. This symbiosis further enhances the invasive potential of *N. glauca*. Our findings are in agreement with results reported by the authors of [52], who stated that invasive *R. typhina* attracted more AMF when competing with the native *A. truncatum*. Also, AMF decreased the root network of the invasive *R. typhina* compared to the native *A. truncatum*. Moreover, invasive plants can produce allelochemical compounds that inhibit neighbouring native plants via the disruption of beneficial belowground microbial mutualisms, or altered soil resources [56]. Allelopathy could have a large impact on native species across the globe [57]. Allelopathy and resource competition are considered to be the second primary mechanisms responsible for the loss of biodiversity in forest ecosystems [58]. Allelopathy was reported to be more important than resource competition in mediating the reduction in plant biodiversity [59]. A study conducted by the authors of [60] stated that the root traits of the introduced invasive species *Chromolaena odorata* populations were significantly different from those of the native populations. Many results have confirmed the role of AMF in increasing the roots of invasive plants when grown singly or mixed with native plant species [38]. The differences in the roots and morphological traits between invaders and native plants can impose interspecific competition advantages for invaders rather than the native neighbours [61]. The fine root demonstrates that the plant root system has a better ability to uptake soil resources (nutrients, water) [62]. This may suggest the competitive advantages of invasive *N. glauca* over the native *V. gerrardii*.

In this study, the presence of AMF in monoculture plantations significantly increased root system traits, such as root length, root surface area, root volume, and root tip, for both plant species across varied densities when compared with their counterparts. This suggests that the invasive *N. glauca* exhibits a higher capacity to attract AMF through its fine root structure compared to the native *V. gerrardii*. According to the authors of [63], AMF inoculant increases the length of root hyphae, which offers help in soil nutrient absorption and, therefore, encourages plant growth [64]. The growth of root parameters in invasive species, such as *Baptisia alba*, and the native species *Petrophile biternate*, has been significantly enhanced by the application of AMF inoculant in monoculture plantation, as reported by several researchers. This improvement can be attributed to the facilitation provided by AMF, as highlighted in the study conducted by the authors of [43].

3.4. Responses of Physiological Parameters of V. gerrardii and N. glauca Grown in Mixed and Monoculture to AMF and Competition

The differences in chlorophyll *a* and chlorophyll *b* content in plants under AMF inoculant have been described in different plant species [65]. Studies have proposed that the use

of AMF may lead to better plant biomass, plant water content, as well as other vegetative parameters, in addition to allowing the enhanced quality and quantity of chlorophyll contents [66]. In our study, the chlorophyll content among seedlings in mixed plantations was found to be consistent regardless of the presence of AMF or species proportion. However, variations in chlorophyll content were observed between the two plant species. Despite the lack of a significant effect on chlorophyll content from the presence of AMF in *N. glauca* within mixed plantations, seedlings treated with AMF demonstrated increased levels of chlorophyll *a* and chlorophyll *b* compared to their untreated counterparts. On the other hand, *V. gerrardii* had superior chlorophyll *a* and chlorophyll *b* compared to *N. glauca*. The results from a study on *Z. mays* showed that the plant showed increased chlorophyll content when inoculated with AMF [65]. On the other hand, a reduction in the total chlorophyll content was reported in *Thymus daenensis* and *T. vulgaris* plants when inoculated with two different types of AMF (*Rhizophagus intraradices* and *Funneliformis mosseaea*) [67]. In general, the differences in the content of plant chlorophyll are considered an important aspect that indirectly identifies the plant's photosynthesis capacity [68].

AMF might enhance the photosynthetic assimilation in plants by enhancing their various vegetative parameters, finally resulting in the improvement of photosynthesis efficiency [69]. This finding is in accordance with our results, which revealed that the AMF inoculant showed a slight increase in the photosynthesis rate, particularly for invasive *N. glauca* compared to *V. gerrardii* in mixed and mono plantations. AMF plants may display enhanced stomatal conductance. Therefore, the plants may have the ability to keep their stomata open for a longer time than non-AMF-treated plants [70].

4. Materials and Methods

4.1. Plant Materials and Growth Conditions

Newly produced seeds of *V. gerrardii* and *N. glauca* were collected from the AL-Baha region (19°53′56″ N, 41°34′17″ E), located in the southwestern part of Saudi Arabia. Additionally, soil samples under both species were collected from the same site for the purpose of preparing the mycorrhizal inoculum. All the collected materials were taken to the lab of Forestry and Range Science, Faculty of Food and Agriculture Sciences, King Saud University (KSU).

4.2. Interspecific Competition Between V. gerrardii and N. glauca

To measure the intensity of competition between *V. gerrardii* and invasive *N. glauca* plants, the seeds of both species were germinated in a mixture, and the total density was 4 plants per tube (16 cm diameter and 50 cm height). The density levels were 3:1, 2:2, and 1:3 (*V. gerrardii*:*N. glauca*). Seedlings were cultivated with (+) and without (–) AMF. Twenty-five grams of inoculum soil containing AMF spores (100 spore/10 g) were added to each AMF treatment. Young seedlings were established for a specific density in experimental tubes (16 cm diameter and 50 cm height), filled with a mixture of soil consisting of sand and silt (3:1) with five replicates per treatment. For the tubes that contain AMF, the soil was inoculated before seedlings were established. The total number of tubes in this experiment was 30, and the experimental design was factorial (3 levels of density* $\pm$ AMF$\times$5 replicates) in a Complete Randomised Design (CRD). Each tube received a weekly intake of 250 mL of water, and 250 mL of Hogland's solution was added every 2 months. The experiment was conducted in a greenhouse (College of Food and Agriculture Sciences, King Saud University (KSU)) and lasted for 7 months.

4.3. Intraspecific Competition for V. gerrardii and N. glauca

To measure the intensity of competition, the seedlings of each species (*V. gerrardii* and *N. glauca*) were established in monoculture with (+) and without (–) AMF in the densities of 4, 3, 2, and 1 plant per tube. The seeds of each species were sown separately in the experimental tubes (16 cm diameter and 50 cm height) and filled with a mixture of soil consisting of silt and sand (3:1) with four replicates per treatment. After germinating, seedlings were thinned for a specific density. For the tubes that contain AMF, soils were inoculated before sowing the seeds. Next, 25 g of inoculum soil containing AMF spores (100 spore/10 g) was added in each AMF treatment. Hoagland solution was added to the experiment during the growth period (7 months). This experiment was set as a randomised complete block design with four replicates for every density/species, and tubes were set randomly within blocks in the greenhouse. The experiment consisted of 4 levels of density (for each species) * $\pm$ AMF $\times$ 4 replicates. In both experiments, all tubes were placed in a greenhouse at 30 °C under natural daylight.

4.4. Data Collection

4.4.1. AMF Colonization in Seedlings Root and Spore Extraction

AMF was extracted from the root sample of each treatment for both species using the method described in [71] and modified by that in [72]. The roots of seedlings were cleaned with tap water (4 °C) to remove the attached soil and then cleaned with potassium hydroxide (KOH, 10%) solution and, consequently, staining with trypan blue in lactophenol as stated in [73]. According to this method, roots were cut for segments and checked for AMF occurrence using a light microscope at 400× magnification. The infection rate of AMF (mycelium, vesicles, and arbuscules) in the roots of both species was calculated as stated in the formula below:

$$\% \text{ Colonization } = \frac{\text{Total number of AM positive segments}}{\text{Total number of segment studied!}} \times 100 \quad (1)$$

The spores of AMF were extracted from the soil of mono and mixed plantations using the wet sieving and decanting protocol [74]. According to this method, 100 g of soil from each sample was kept in a bucket (1500 mL capacity) containing 1 litre of water. The soil sample was shaken and mixed with water to create soil suspension. The resulting soil suspension was left for 6 min and then sieved using gradual sieves (ASTM-60, ASTM-100, and ASTM-240) for spore extraction. Soil suspension was filtered via Whatman filter paper No. 1. The filter papers that contained AMF spores were examined under an electronic microscope (with 2.5 $\times$ 10 magnification) and then spores were counted. Moreover, AMF spores and their species were identified according to their morphological features under the microscope used [74].

4.4.2. Relative Yield (RY)

The relative yields of *V. gerrardii* and *N. glauca* were measured using the formula below, according to the protocol used in [75].

$$\text{Relative Yield (RY) of } \textit{V. gerrardii} = Y_{AN}/(pY_{AA}) \quad (2)$$

$$\text{Relative Yield (RY) of } \textit{N. glauca} = Y_{NA}/(qY_{NN}) \quad (3)$$

where Y_{AN} indicates the biomass of the *V. gerrardii* seedlings that were established in the mixed planting and p is the primary proportions (75%, 50%, and 25%) of *V. gerrardii* seedlings, duplicated by the Y_{AA}, which is the average production of *V. gerrardii* seedlings

that was established in monoculture plants (4 plant per tube). Likewise, Y_{NA} indicates the production of *N. glauca* seedlings that was established in mixture with *V. gerrardii* seedlings, and "q" is the amounts of the species (75%, 50%, and 25%), multiplied by the average production of *N. glauca* that was established in monoculture planting (4 plant per pot). The relative yield of the *V. gerrardii* and *N. glauca* seedlings (2 plant species) was used to measure the relative yield total per tube.

4.4.3. Relative Total Yield (RTY)

Measuring RTY is an indicator that shows whether the two plant species participate in resources or interfere with each other by comparing their biomass (per tube) in monoculture plantations to their biomass (per tube) in mixed plantations [76]. The formula below is applied to identify RTY:

$$RTY = pRY_A + qRY_P \quad (4)$$

where p = initial proportion of *V. gerrardii* seedlings in a mixture plant and q is the initial amount of *N. glauca* seedlings in a mixture such that

$$p + q = 1. \quad (5)$$

The data of this experiment (replacement series experiments) were analysed following a replacement series diagram. In the case of the competition between the two plant species, each species participates in the total production in the direct ratio according to its proportion in the mixture culture. The yield for each species increases linearly with its proportion in the mixture plants. In the unequal competition, the aggressive plant species will receive more biomass than expected. The aggressivity index for *V. gerrardii* and *N. glauca* seedlings was identified using the formulas below:

$$\text{Aggressivity of } \textit{V. gerrardii} = RY_A - RY_N \quad (6)$$

$$\text{Aggressivity of } \textit{N. glauca} = RY_N - RY_A \quad (7)$$

4.4.4. Measurement of Chlorophyll (a, b) and Photosynthesis Parameters

To determine chlorophyll content, fresh leaves from *V. gerrardii* and *N. glauca* under each treatment were collected separately. Samples weighing 0.5 g from each treatment were collected in the early morning, brought to the lab directly and weighed by digital balance. The samples were then crushed by mortar and pestle and transferred to test tubes. Each tube was filled with dimethyl formamide (5 mL) and left at room temperature for 24 h. The resulted leaf extracts were filtered via Whatman filter paper No. 1. and placed in a cuvette to measure chlorophyll content using a spectrophotometer (SHIMADZU, Kyoto, Japan, UV1800). Absorbance was read at different wavelengths (664 nm for chlorophyll *a*, and 620 nm for chlorophyll *b*) [77].

The net photosynthesis rate and stomatal conductance were measured using a portable system device (CI-340 handheld photosynthesis system, CID Bio-Science, Camas, WA, USA). The measurements were conducted between 9.00 and 11.00 am in the greenhouse on the healthy leaves of the plant.

4.4.5. Measurements of Above Ground Parameters

The height of both seedling species (*V. gerrardii* and *N. glauca*) was measured separately from soil surface to seedling apex using a 1-m ruler. Also, the leaf area (cm^2) was measured using a portable leaf area meter (CI-202). Leaf number per seedling was counted and the plant dry biomass (g) was determined after drying the whole plant at 70 °C for 48 h.

4.4.6. Measurements of Bellow Ground System (Root Parameters)

After harvesting, the roots of both plant seedlings were separated from the vegetative part and gently washed with tap water to remove soil particles. The roots were then stained using a red-coloured dye and spread gently over a computer scanner for imaging. The roots were scanned at 600 dpi, and the root images were saved in Tiff format. The root characteristics such as total root length, total root surface area (cm^2), root volume, and root tip number were analysed using a PC equipped with WinRHIZO version 2004a (Regent Instruments Inc., Québec, QC, Canada) software.

4.5. Data Arrangement and Statistical Analysis

All data were subjected to analysis of variance (ANOVA); moreover, upon finding significance, means were separated using a Least Significant Difference (LSD) test at $p < 0.05$. Principal component analysis (PCA) was applied for the data of mixed plantation to capture the maximum variation along X and Y axes, thereby facilitating the interpretation of experimental results. The results were projected in the factor space, and their relationships were interpreted based on the angle and distance from the origin. Additionally, heatmap analysis was performed based on the correlation matrix for mono plantations. Statistical analysis was conducted using Statistix 10.0 software program and JMP Pro.16. Also, the replacement diagram was generated graphically to show species replacement dynamics in mixed plantations.

5. Conclusions

Biological invasion is a critical problem facing the world today, leading to damage to ecosystems and biodiversity. The relationship between AMF and plants can affect the competition dynamics between invasive and native plants. In our study, AMF was associated with the changes and growth of *V. gerrardii* and invasive *N. glauca* and enhanced vegetative and root parameters for the two plant species in mono and mixed plantations via the connected AMF hyphae. Meanwhile, the two plant species responded differently to AMF and to the interspecific competition, where the performance of *V. gerrardii* in interspecific competition was lower than *N. glauca*. AMF induced an improvement in several growth parameters including vegetative (leaf area, leaf number, height, plant dry weight, relative yield, relative yield total), physiological (photosynthesis, stomatal conductance), and root traits for *N. glauca* and *V. gerrardii*. The increase in these studied parameters was generally more pronounced and statistically more significant for *N. glauca* than for *V. gerrardii*. Furthermore, *N. glauca* presented with a greater relative yield than *V. gerrardii* under interspecific competition, making the *N. glauca* more aggressive and a stronger competitor than *V. gerrardii*. In conclusion, our results suggest that the invasive *N. glauca* gained higher assistance scores from AMF and more competitive advantage over the native *V. gerrardii*, particularly in the development of the root system. These findings may provide evidence that AMF increases the likelihood of *N. glauca* invasion, giving a perspective with which to recognise the mechanisms of plant biological invasion.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants14060858/s1, Figure S1: Growth of *V. gerrardii* with and without AMF in monoculture plantations in 1 (A), 2 (B), 3 (C), and 4 (D) plants densities.; Figure S2: Growth of *N. glauca* with and without AMF in monoculture plantations in 1 (A), 2 (B), 3 (C), and 4 (D) plants densities.

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Review

Smart Chip Technology for the Control and Management of Invasive Plant Species: A Review

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Abstract: Invasive plant species threaten biodiversity, disrupt ecosystems, and are costly to manage. Standard control methods, such as mechanical and chemical (herbicides), are usually ineffective and time-consuming and negatively affect the environment, especially in the latter case. This review explores the potential of smart chip technology (SCT) as a sustainable, precision approach tool for invasive species management. Integrating microchip sensors with artificial intelligence (AI) into the Internet of Things (IoT) and remote sensing technology allows for real-time monitoring, predictive modelling, and focused action, significantly improving management effectiveness. As one of many examples discussed herein, AI-driven decision-making systems can process real-time data from IoT-enabled environmental sensors to optimize invasive species detection. Smart chip technology also offers real-time monitoring of invasive species' life processes, spread, and environmental effects, enabling artificial intelligence-powered eco-friendly control strategies that minimize herbicide usage and lessen collateral ecosystem damage. Despite the potential of SCT, challenges remain, including cost, biodegradability, and regulatory constraints. However, recent advances in biodegradable electronics and AI-driven automation offer promising solutions to many identified obstacles. Future research should focus on scalable deployment, improved predictive analytics, and interdisciplinary collaboration to drive innovation. Using SCT can help make invasive species control more sustainable while supporting biodiversity and strengthening agricultural systems.

Keywords: artificial intelligence; biosensors; internet of things; invasive plant control; precision agriculture; remote sensing; sustainable ecosystem management

1. Introduction

The rapid spread of invasive plant species is a major ecological and economic challenge worldwide, as they alter soil chemistry and disrupt native biodiversity by outcompeting indigenous plants [1,2]. Invasive species are defined by the FAO (2015) as being "Species that are non-native to a particular ecosystem and whose introduction and spread cause, or are likely to cause, socio-cultural, economic, or environmental harm, or harm to human health" [3], while the Convention on Biological Diversity (CBD) describe invasive alien species as "species whose introduction and/or spread outside their natural past or present distribution threatens biological diversity" [4] and the International Union for Conservation of Nature (IUCN) as "Animals, plants or other organisms introduced by man into places

out of their natural range of distribution, where they become established and disperse, generating a negative impact on the local ecosystem and species" [5]. What is known is that invasive species exploit environmental changes, climate variability, and human-mediated disturbances to establish ecosystem dominance [6–8]. Conventional management strategies, including mechanical removal, chemical control (herbicides), and biological control, often fall short due to the cost of implementation, labour-intensive processes, and unintended environmental consequences such as soil degradation, water contamination, and harm to non-target species [9].

Heringer et al. [10] estimated that the cumulative economic cost of invasive species in urban areas reached approximately US$326.7 billion between 1965 and 2021, while other studies estimate that global losses due to invasive species across all sectors likely exceed US$2.2 trillion [11–14]. Two good examples are Japanese knotweed (*Fallopia japonica*, syn. *Reynoutria japonica*), which has caused significant infrastructure damage in the UK, leading to estimated losses of £166 million per year [15], and common ragweed (*Ambrosia artemisiifolia* L.), which damages agriculture and human health. Ambrosia-related agricultural losses are estimated at €13.5 million per year, while Ambrosia-induced allergies cost €7.4 billion annually in Europe [16].

Addressing the problem of invasive species and their impact on agriculture, forestry, and nature reserves will require innovative, technology-driven solutions for better detection, monitoring, and targeted eradication efforts [17]. Integrating advanced digital technologies such as embedded chip systems, biosensors, and AI offers a ground-breaking approach to invasive species management to achieve sustainable control.

Smart chip technology (SCT) has already demonstrated its potential in precision agriculture, forestry, and conservation [18]. For example, SCT can be used to monitor, visualize and generate digital data to control the application of resources and improve productivity. They are also used in precision farming, such as helping to determine precisely when and how much water a given crop needs or in devices that monitor nutrient levels and disease outbreaks [19,20]. In Europe, the Tyndall National Institute is conducting a collaborative research project called 'HALY.ID' with Teagasc. This project aims to monitor the highly invasive insect species *Halyomorpha halys* (Stål) (brown marmorated stink bug). Here, AI and drones automate the monitoring process, replacing traditional manual monitoring methods with AI technology [21]. Importantly, when combined with AI-driven predictive modeling, these chips support targeted interventions, helping land managers optimize decision-making [22]. A similar strategy could be adapted for managing invasive plant species, where bioengineered microchips embedded in targeted plants would track their physiological traits and spread dynamics, offering valuable insights for controlling their environmental impact.

Integrating SCT into ecological conservation, sustainable agriculture, and biodiversity restoration offers transformative potential. This review focuses on the role of SCT in invasive species management, highlighting its advantages, challenges, and future directions. Smart sensors installed in invasive species such as *Eichhornia crassipes* (Mart.) Solms., (water hyacinth) have been used to gather real-time information on growth rate and nutrient uptake [23]. This information can help land managers implement timely eradication strategies before real environmental harm occurs. In another study, sensors connected to an invasive plant were used to detect its tolerance to biotic and abiotic stresses, helping predict its survival under climate change scenarios [24]. Drones equipped with remote sensors could target affected regions with eco-friendly herbicide sprays or biological control agents, reducing the reliance on large-scale chemical treatments [25]. Invasive species eradication programs in Australia and the United States, for instance, are increasingly exploring automated drone-based monitoring

systems, which could be further enhanced by integrating smart chips for data collection, allowing real-time management decisions [26,27].

Despite SCT's potential, many obstacles remain, such as the cost of deployment, especially on a large scale, issues relating to the environmental risks of electronic waste if disposal is not correctly managed, and regulatory challenges regarding introducing such devices in the natural environment. However, ongoing research in nanotechnology and biodegradable electronic materials could result in eco-friendly smart chips. Researchers are developing bioresorbable electronics based on micro-chip technology capable of transmitting plant health data before naturally decomposing into the environment [28–30].

2. Smart Chip Technology: An Innovative Approach

A smart chip is an integrated circuit (IC) that can process data, interact with its environment, and communicate with other devices. It is designed with embedded sensors, processors, and communication technology, enabling it to collect, process and transmit data for various applications and make decisions or take actions based on their input, often in real-time [31]. Smart chip technology is a broader term for using these chips in various applications, including environmental monitoring, agriculture, healthcare, and more (Figure 1). In invasive species management, smart chip technology can include sensors to detect invasive species or environmental conditions and communicate data to a central system for analysis and decision-making. Integrating SCT into invasive plant management represents a novel approach to crop management. Farmers can use embedded SCT to monitor their real-time physiological responses, growth dynamics, and environmental impact (Figure 1). These chips can collect data on plant health indicators such as moisture levels, nutrient uptake, and stress responses, allowing for early detection and targeted intervention [32,33].

Chip-based systems have already demonstrated their effectiveness in monitoring crop conditions by providing farmers with real-time data on soil moisture and nutrient availability, enabling optimized irrigation and fertilization strategies [34]. A comparable approach can be applied to invasive species, where chips embedded in invasive plants can track their spread patterns, aiding in developing precision control measures.

Bioengineered smart chips, in conjunction with biosensors, can also detect biochemical changes and trigger targeted responses, such as the release of allelopathic compounds, through embedded reservoirs or actuator technologies. Bioengineered smart chips typically operate as part of an integrated biosensor system. These chips embedded with biosensors, often based on enzymes, antibodies, or nucleic acid probes, are specifically designed to detect biochemical signals such as the release of allelopathic compounds, stress-induced metabolites, or hormonal changes in invasive plant species [35]. After detecting the targeted biochemical markers, the chip transmits signals to a processing unit via wireless or microcontroller-based systems. This ability allows for real-time monitoring and potentially triggers pre-programmed responses, such as activating countermeasures or alerts for manual intervention [35,36]. This information can be used to prioritize removing high-impact invasive species before they cause significant damage and for large-scale tracking and predictive modeling to identify high-risk areas for early interventions [37].

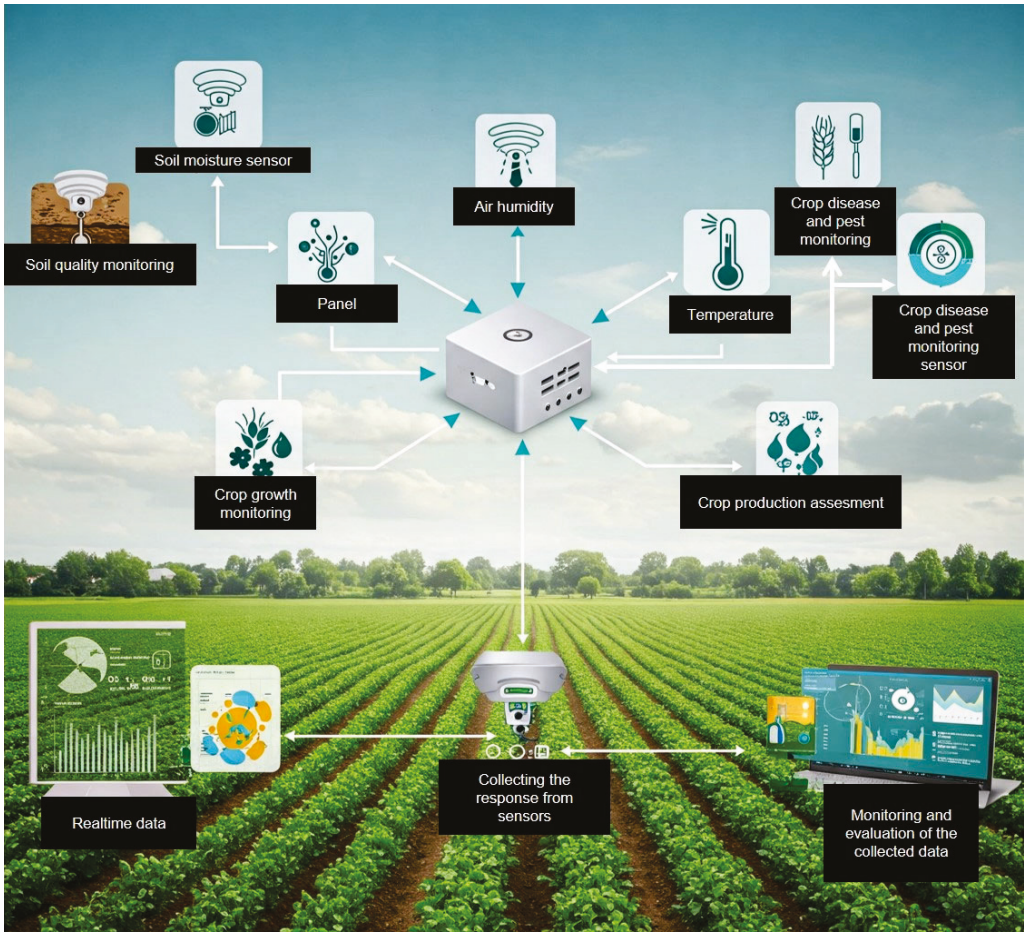

Figure 1. Conceptual representation of a smart farming system integrating IoT devices and various sensor technologies to monitor key environmental and crop health factors for improved agricultural outcomes. The system includes soil moisture and quality sensors, air humidity and temperature sensors, and crop disease and pest monitoring sensors. These sensors help monitor crop growth, assess production, and detect early signs of stress or infestation. Data collected from the field is transmitted in real-time to central processing units and digital analytics platforms, enabling informed decision-making and sustainable precision agriculture practices.

In smart agriculture, IoT-based sensors are widely used to detect crop diseases and environmental stressors [38,39]. For example, in vineyards, AI-integrated biosensors are being used to monitor the presence of fungal infections such as powdery mildew, alerting farmers before significant damage occurs [40]. Similarly, chip-based technology is used in citrus orchards to detect Huanglongbing (citrus greening disease), allowing for removing infected trees before the disease spreads [41]. Biosensors also have the potential to identify genetic markers unique to invasive species, enabling their precise detection even at early growth stages. Moreover, drone-assisted monitoring systems combined with smart chip-based data collection and AI autonomously target and treat affected areas using eco-friendly herbicide sprays or biological control agents. Detailed information about different types of sensors embedded in smart chips and their application is given below (Table 1), while specific commercial and research-based sensor models and chips commonly used in weed control and precision agriculture are provided in Table S1.

Table 1. Types of sensors, applications, and data collection methods in smart chip systems for precision agriculture and environmental monitoring.

Sensor Type	Application in Field	Data Collected	Data Collection Method	Studied Crop/Plant/Species	References
Biosensors	Detect plant diseases, stress responses, and allelopathic interactions.	Enzyme activity, secondary metabolites, stress markers	Embedded microchips with biochemical detection, wireless transmission	*Zea mays* L. (Maize), *Oryza sativa* L. (Rice)	[42]
GPS Sensors	Track the movement and spread of invasive plant species.	Geolocation, plant movement patterns	GPS-embedded chips, satellite-based tracking.	*Parthenium hysterophorus* L., *Lantana camara* L	[43,44]
IoT-Based Smart Sensors	Monitor soil parameters for precision agriculture.	Soil moisture, temperature, pH, salinity, humidity	Wireless IoT network, real-time monitoring.	*Triticum aestivum* L. (Wheat), *Gossypium hirsutum* L. (Cotton)	[45,46]
Electrochemical Sensors	Measure soil nutrient levels and heavy metal contamination.	pH, nitrogen, phosphorus, potassium levels, contaminants.	Integrated electrochemical probes, automated soil analysis	*Brassica napus* L. (Canola), *Glycine max* (L.) Merr. (Soybean)	[47,48]
AI-Integrated Image Sensors	Identify plant diseases and classify species using machine learning.	Leaf shape, disease symptoms, chlorophyll content	AI-driven image analysis, UAV and drone-based monitoring	*Solanum lycopersicum* L. (Tomato), *Vitis vinifera* L. (Grapevine)	[49,50]
Dendrometers	Monitor tree and shrub growth rates.	Stem diameter, biomass accumulation	Sensor-equipped microchips attached to plant stems.	*Quercus robur* L. (Oak), *Pinus sylvestris* L. (Scots Pine)	[51]
Thermal Infrared Sensors	Detect plant stress and drought conditions.	Leaf temperature, transpiration rates, canopy stress	UAV-based infrared thermography, real-time heat mapping	*Citrus* × *sinensis* (L.) Osbeck. (Orange), *Capsicum annuum* L. (Pepper)	[52,53]
Hyperspectral Sensors	Differentiate between native and invasive species based on spectral signatures.	Reflectance indices, vegetation health data	UAV and satellite-based spectral imaging.	*Alternanthera philoxeroides* (Mart.) Griseb. (Alligator weed)	[54,55]
Microfluidic Sensors	Analyze plant sap flow, water use efficiency	Xylem conductivity, nutrient transport rates	Embedded chip with microfluidic flow analysis	Woody plants	[56]
Chlorophyll Fluorescence Sensors	Detect photosynthetic efficiency and plant health	Fv/Fm ratio, electron transport rate	Portable fluorescence imaging, automated leaf-level analysis	*Phaseolus vulgaris* L. (Common bean), *Coffea arabica* L. (Coffee)	[57]
Drones with Multispectral Cameras	Conduct large-scale monitoring of crop health and invasive weeds	NDVI, LAI, canopy cover, disease identification	UAV-based remote sensing, automated GIS mapping	*Cicer arietinum* L. (Chickpea)	[58]
LIDAR Sensors	Assess plant canopy structure, biomass estimation	Canopy height, vegetation density	Airborne LIDAR scanning, 3D mapping	*Populus tremuloides* Michx., *Eucalyptus globulus* Labill	[59]
Nano-Sensors	Detect chemical stressors, pollutant levels in soil and plants	Heavy metals, pesticide residues, soil contamination	Nanoscale biosensor technology, colorimetric detection	*Zea mays* L. (Maize), *Nicotiana tabacum* L. (Tobacco)	[60]
Radio Frequency Identification (RFID) Sensors	Track movements of fishes	Identification tags, plant movement patterns	RFID-embedded plant tracking, real-time monitoring	Fishes	[61]

3. Smart Chip-Enabled Invasive Plant Management

Real-time data collection is crucial for the early detection and ongoing monitoring of invasive plants (Figure 2). Technologies such as satellite imagery, drones, and ground-based sensors equipped with chips allow continuous tracking of invasive species across large areas, providing up-to-date insights into their spread and environmental impact [61,62].

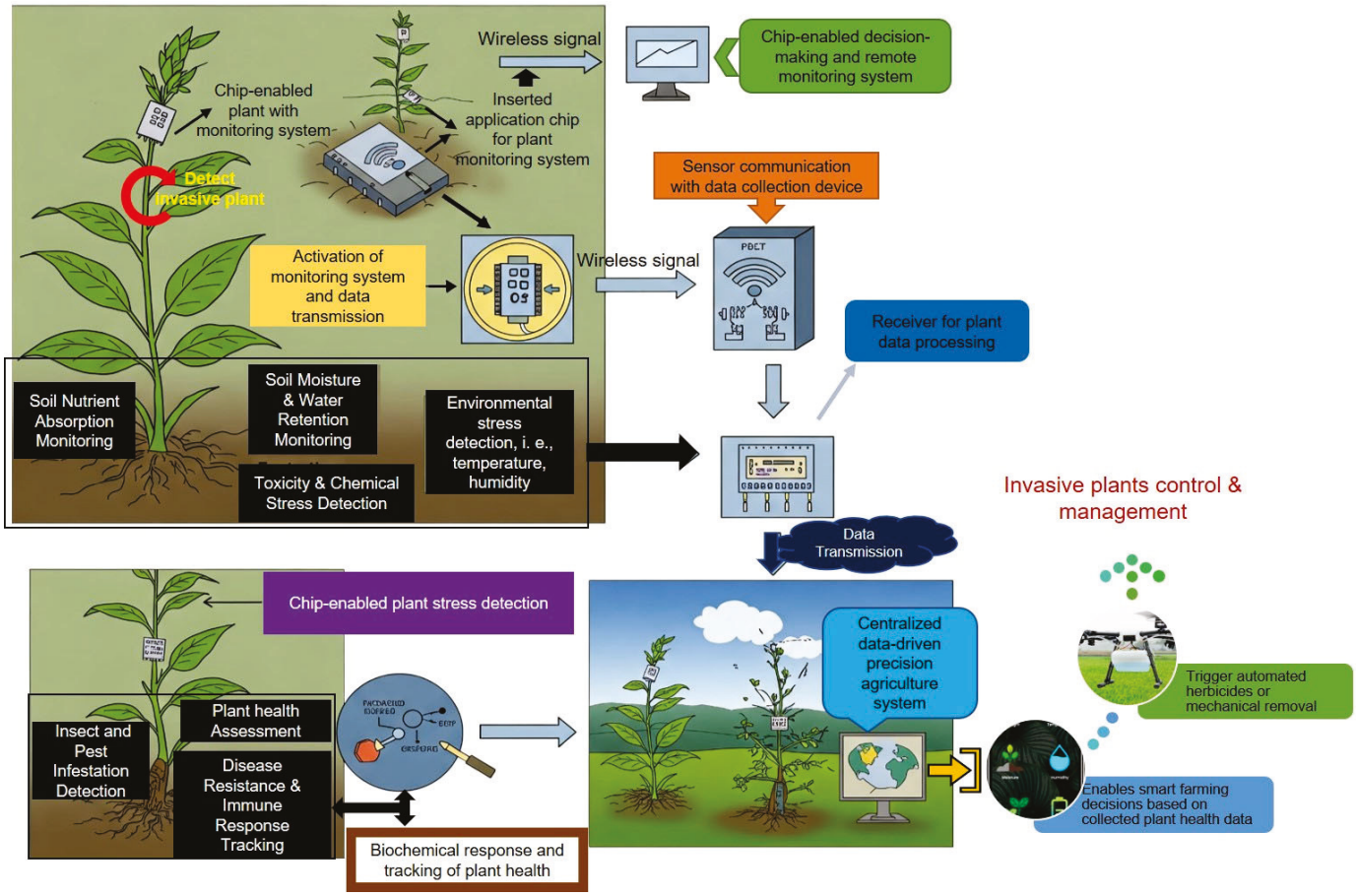

Figure 2. Smart plant monitoring system using chip-enabled technology for invasive species management. The diagram illustrates how embedded sensors monitor plant health, detect invasive species, and assess environmental stressors. The system enables real-time data collection and remote decision-making by tracking soil conditions, pest infestations, and chemical responses. This technology supports early detection and precise management of invasive species.

For instance, agricultural landscapes, where crops like corn and soybean are frequently threatened by invasive weeds such as *Amaranthus tuberculatus* (Moq.) (waterhemp) and *Amaranthus palmeri* S. Watson [63], benefit from satellite-based monitoring systems. These systems enable farmers to identify affected areas quickly and prevent further spread in riparian zones where Japanese knotweed is a persistent invasive species. Drones equipped with GPS and sensors could monitor its spread along riverbanks, allowing land managers to develop targeted control strategies [64] similar to how Australia uses drones equipped with infrared sensors to locate and target the invasive Cane toads (*Rhinella marina Linnaeus*, 1758), delivering traps or biological agents to control the population with minimal human intervention [65].

Real-time data collection helps optimize resource allocation and response times. Recent research in Slovenia developed an automatic detection system for Japanese knotweed using a One-Class Support Vector Machine (SVM) connected to multiple Sentinel-2 satellites. The input data for this system included red-green-blue band composites, infrared bands (IR), and additional satellite images using the Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI). The K-means algorithm was used on the IR band to group the samples. As a result, 83% accuracy was recorded on the aerial images, and 90% was recorded on satellite data for detecting Japanese knotweed. Such information made it possible to detect Japanese knotweed at the municipality level and can help understand the distribution of this species [66]. Similarly, Catala-Roman et al. [67]

used an unmanned aerial system (UASYS) equipped with neural networks (CNNs) based on YOLOv10 for detecting and geo-locating two invasive species, *Araujia sericifera Brot.* and *Cortaderia selloana* in the citrus orchard. The system significantly enhanced productivity, reduced the costs associated with manual weeding in organic farming and promoted environmental sustainability, offering a scalable method for organic and conventional agriculture.

In addition to the widespread use of satellites, drones, and AI-based detection methods, several innovative smart systems have emerged, each offering unique solutions for monitoring invasive species. For example, Nature Metrics utilizes environmental DNA (eDNA) analysis to detect invasive species in soil, water, or air samples, providing a non-invasive, highly accurate monitoring method [68]. Similarly, Trellis Data has developed AI-powered cameras designed for port biosecurity inspections, capable of identifying small invasive pests on cargo containers, thus reducing the need for time-consuming manual checks [69]. Meanwhile, Wildlife Drones offers a radio-telemetry system integrated with drones, allowing real-time tracking of up to 40 radio-tagged invasive animals across difficult terrain, which enhances targeted control efforts [70]. Fulcrum's customizable mobile app for field data collection simplifies invasive plant species monitoring by enabling geo-tagged photos, offline data entry, and cloud-based synchronization for easier reporting. SGS has developed global eDNA-based services, including sample collection, DNA sequencing, and species identification, facilitating early detection of invasive species from a single environmental sample [71]. All these technologies show the growing trend toward precision, automation, and scalability in invasive species management. They also offer a range of options that vary in spatial scale, deployment costs, and application.

AI-driven analysis and predictive modelling further enhance invasive species management by enabling the processing of large datasets. Analyzing real-time and historical data, such as temperature, soil moisture, and land cover, where and when an invasive species might spread could be predicted, and areas for treatment could be prioritized. This information will allow land managers to take proactive measures before any invasion becomes uncontrollable. For example, the spread of *Pueraria montana* var. lobata (Willd.) (kudzu), an invasive plant in the south-eastern U.S., has been studied using AI models incorporating weather data and land-use patterns [72]. Similarly, AI models are being used to predict the spread of *Ailanthus altissima* (Mill.) (tree-of-heaven) in forest ecosystems based on satellite imagery, ground surveys, and climate data [73].

The application of automated removal strategies, including precision herbicide application and drones, has already dramatically improved the efficiency of invasive plant control [74,75]. For example, the application of precision herbicides using AI-enabled drones allows farmers to target invasive weeds in vegetable crops like potatoes [76,77], threatening crop health while sparing the surrounding plants. Reducing chemical usage minimizes runoff into nearby water systems [78]. The system also ensures that only affected zones are treated, significantly improving management and minimizing waste. In another study, the results showed that accurate and timely maps of *Sorghum halepense* (L.) Pers. (Johnsongrass) patches in maize can be key to achieving site-specific and sustainable herbicide applications for reducing spraying herbicides and costs [79].

Drones could play a key role in automated removal of invasive plant species, with high-resolution cameras and AI algorithms enabling rapid identification and mapping of invasive species [25]. In fields, invasive species like *Chromolaena odorata* (L.) R.M.King & H.Rob. (Siam weed) can be identified early by drones equipped with high resolution cameras [25]. Precision herbicide application can also minimize the impact on surrounding vegetation [80,81]. These systems can distinguish between target and non-target species, typically using Red, Green, and Blue (RGB) cameras, hyperspectral imaging, multispectral

sensors, and machine learning models [82]. RGB cameras are considered a good method for weed identification, which uses physical characteristics such as shape, colour, and size [83]. Despite its wide application, RGB cameras are currently limited in identification, especially when the alien and indigenous species have similar leaf shapes and sizes during the early growth stage [84].

Ferreira et al. [85] grouped image pixels rendering similar colour and spatial closeness using simple-linear-iterative-clustering, with the images being divided into different sections containing multiple soy and weed leaves using this technique. Then, CNNs were used for classification, giving the results with 99.5% higher classification accuracy than conventional machine learning (ML) classifiers, i.e., support vector machines.

Hyperspectral imaging provides comprehensive details about crop vegetation by capturing a wide range of spatial and spectral information. Hyperspectral imaging uses images in narrow bands with a visible light range of 400–1000 nm and 900−1700 nm near-infrared range of the electromagnetic spectrum [86]. This information helps to identify and differentiate between weed and crop species based on the chemical information, i.e., chlorophyll content and water absorption present within the cellular structures of plants because the chemical information is inherently unique among plant species [82]. At the same time, hyperspectral imaging generates large amounts of data, which also contains redundant data that can impact the spectral data. Machine learning and deep learning (DL) techniques offer promising solutions to these challenges.

In ML and DL techniques, discrete responses are used, and every pixel in the image is classified into a distinct class for weed identification. Algorithms then identify patterns, relationships, and features within the input data by iteratively adjusting predetermined parameters [86]. Mensah et al. [82] achieved accuracy between 75% and 95% by using ML and DL over traditional classifiers such as Partial Least Square Discriminant Analysis, Random Forest, Spectral Angle Mapping, Maximum Likelihood Classifier, Support Vector Machine, and Bayesian classifier have been utilized for weed identification. However, the same approach can be employed with the latest developments in hyperspectral remote sensing to differentiate between crops and invasive plant species in farmlands.

Robotic systems, in combination with SCT, are also being investigated for invasive species management [87]. These robots can, through the use of machine learning, autonomously identify and remove invasive plants with a strimmer of a robotic hand. This ability is especially beneficial in labour-intensive or hazardous environments [88]. Bakker et al. [89] used autonomous robots with real-time kinematics global positioning systems to complete the inter-row hoeing in corn crops without damage to the surrounding plants. Nørremark et al. [90] recorded 91% success rates by removing numerous inter-row weeds using a cycloid robotic, while Kunz et al. [91] used Robocrop, a camera-guided inter-row hoeing device and noted an 89% reduction in weed density in soybean crops and 87% in sugar beet crops. Other devices, such as AgBotII, are capable of accurately identifying up to 90% of selected weeds, including wild oats (*Avena fatua* L.) and common sowthistle (*Sonchus oleraceus* L.) [92]. BoniRob, produced by Bosch Deepfield Robotics, showed 94% success in monitoring various weeds [93]. These robots help reduce the need for costly and potentially harmful chemical herbicides (e.g., Glyphosate) while improving efficiency.

4. Challenges and Prospects of Smart Chip-Enabled Invasive Plant Management

Chapman and Brooke (2021) investigated various methods, including hand pulling, herbicide application, mowing, cutting, and daubing (Figure 3), to eradicate invasive plant species at the Santa Lucia Preserve [94]. They successfully removed invasive plants without

impacting native species and disrupting the habitat. While their techniques succeeded in controlling the invasive species, the more advanced technologies, drones, and AI-driven systems show great promise.



Figure 3. The ways to treat invasive species in natural areas at Santa Lucia Preserve, the Conservancy practices minimally intrusive methods for maximum effect. They use four tried and true methods to target invasive plants: hand pulling, spot-spraying herbicide, spot-mowing, and cut and daub [94].

Despite the numerous benefits, chip-enabled invasive plant management faces challenges, including cost, environmental impact, and ethical considerations. Among these, the main barrier remains the high cost of its implementation and the initial investment required for drones, sensors, AI-driven systems, and robotics, particularly for small-scale farmers, especially those without access to sufficient capital or subsidies. Also, they acquire technical knowledge in fly drones and robotics, software and data processing, maintenance and robustness of the equipment. In numerous studies, UASYS has been used to acquire image data for successfully sensing and monitoring invasive plants. However, these studies used high-end UASYS (e.g., Michez et al. [95]) and commercial image processing software, which cost 3000 US dollars or more for a single license. Fixed-wing UASYS, as an alternative, is being used widely with multirotor drones. SCompaniessuch as senseFly, QuestUAV, Gatewing, and Trimble offer ready-to-fly fixed-wing UASYS with full autopilot control and camera sensors. The system costs less than 2000 US dollars. Although this approach does not charge extra labour costs, it can be operated easily with only basic knowledge of image processing and GIS [96].

In another study, Takekawa et al. [97] used UASYS to monitor pepper weed more effectively (*Lepidium latifolium* L.) in Suisun Marsh in northern California, USA. In Suisun Marsh, a team of hand backpack sprayers commercially costs USD 456.95/ha to spray the herbicides, whereas tractor and spraying helicopter-spraying costs 97.50/ha USD and 140.45/ha USD. However, Takekawa et al. [97] reported operational costs of USD 36.79 /ha for the UASYS system, mainly attributed to technician labor, compared to the higher costs associated with drone spraying, which includes chemical mixing, drone operation, and

logistical management. The company "Leading Edge Aerial Technologies, Inc." commercially charged USD 125–150/ha (estimated) for spraying with drone application, including equipment and pilot, but it is still cheaper than contracting with backpack sprayers.

The ethical considerations surrounding autonomous technologies in invasive species management involve issues such as access to public and private lands and the potential impacts on agricultural land abandonment. In many regions, land abandonment occurs due to unfavourable cultivation conditions, low farm profitability, and broader socio-economic challenges [98]. These abandoned areas often become a good hotspot for invasive plant species, aggravating biodiversity loss, increasing fire risk, and accelerating soil erosion [99]. Although using AI-driven drones combined with remote sensing and GIS offers a promising solution for spotting, identifying, and managing invasive plants in such areas, implementing such SCT raises concerns about land rights, privacy, and regulatory oversight [99]. In community farms or gardens, questions may arise about the ethical implications of automation in decision-making, such as which species should be removed and the surety not to remove beneficial plants. For this reason, the ethical and practical deployment of these technologies should align with broader conservation goals while respecting landownership rights and local ecological dynamics.

Expanding these technologies to invasive species management presents another exciting opportunity. Many of the tools for controlling weeds in natural environments can be adapted to manage invasive plant species. For instance, AI and sensor networks in wheat fields can work with robotic systems to detect and uproot invasive species like wild oats competing with wheat for nutrients [100]. In rice paddies, the precision herbicide application systems developed for controlling waterhemp [75] could be applied to manage *Lemna* L. (duckweed), which can overtake rice fields if left unchecked [101]. In addition, existing models powered by AI used for invasion prediction for crops infested by Cheatgrass (*Bromus tectorum* L.) [102] may be modified for other invasive plants, assisting farmers in knowing when action is needed.

AI-based technologies could also greatly benefit ecological restoration [103]. Automating invasive species removal makes large-scale restoration projects more feasible [104]. Robotics and drones aiding in the restoration of native flora, such as in the U.S., the efforts to restore native grasslands, automated systems could target and remove invasive *Lespedeza cuneata* (Dum.Cours.) G.Don, allowing native grasses to regrow [105]. In wetland (invaded by *Lythrum salicaria* L. (purple loosestrife, [106]) restoration, these systems could be used to remove invasive purple loosestrife, facilitating the restoration of critical wetland habitats for species like waterfowl and native fish. The same technology could tackle bindweed in grapevine fields, preventing it from spreading, smothering the vines and reducing competition for nutrients and water [107]. Integrating SCT with remote sensing, robotics, and AI-driven predictive models holds significant potential for the future.

5. Conclusions

5.1. Summary of Key Findings

This paper looked at the ability of SCT to serve as a new methodology for invasive plant species management. Embedded microchip sensors with AI, IoT, and remote sensing technologies can enhance real-time monitoring and precision control measures. For example, they will allow land managers to monitor the invasive species' physiological traits, spread dynamics, and environmental interactions, making remedial measures more targeted and effective. Such proactive measures will allow early detection combined with rapid response, thereby reducing the negative impacts of broad-spectrum herbicides. The benefits of smart chip-enabled monitoring systems are already being used in precision agriculture, forest management, and ecological monitoring. Case studies show that using

GPS sensors integrated with bioengineered microchips and drones if applied to invasive species management, could significantly improve the efficiency and sustainability of invasive species management. However, challenges include unfavourable implementation costs, microchip biodegradability, environmental issues (e.g., chemical leaching, E-waste accumulation and wildlife interference) and controlling policies.

5.2. Call for Further Research and Technology Development

While some technologies have tried to incorporate smart chips in managing invasive species with little success, optimizing the technology's integration, cost, and environmental impact requires further investigation. This task should include looking into more affordable production methods, as well as other strategies that can be broadly implemented so that small farmers and conservation groups can benefit. As a starting point, future research should focus on developing more sophisticated AI models capable of predictive mapping so that invasions can be anticipated and addressed before they have started. Automating the drone-assisted precision treatment processes and data gathering will further improve the control of invasive species by efficient detection and mapping, targeted treatment application and high-resolution data collection and analysis. Achieving these goals will require a multidisciplinary approach by ecologists, engineers, and policymakers to design regulatory options and best practices to mitigate the risks associated with these new technologies.

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Abbreviations

The following abbreviations are used in this manuscript:

SCT	Smart Chip Technology
AI	Artificial Intelligence
IoT	Internet of Things
IR	Infrared Bands
RGB	Red, Green, and Blue cameras
CNNs	Convolutional Neural Networks
ML	Machine Learning
DL	Deep Learning
UASYS	Unmanned Aerial Systems

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Differences in the Response of Invasive *Solidago canadensis* and Native *Imperata cylindrica* to Glyphosate

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Abstract: Exotic invasive plant species can cause biodiversity loss by outcompeting and replacing native species. Herbicides are commonly used to control invasive plants owing to their low cost and high efficiency. However, herbicide use can have unintended effects on co-occurring native plant species by altering the competitive balance. We studied how herbicide application modifies the competition between an invasive and a native species. We examined the effects of applying glyphosate on the mortality, photosynthetic capacity, and growth of *Solidago canadensis*, an aggressive invasive species, and *Imperata cylindrica*, a native species that commonly co-occurs with *S. canadensis*. We also studied how applying glyphosate affected the competition between these species. Various glyphosate concentrations were applied to the two species grown either together or separately. The mortality rate increased while the photosynthetic capacity and growth decreased with increasing glyphosate concentration. Increasing the glyphosate concentration more negatively affected the parameters of *I. cylindrica* than those of *S. canadensis*. Plant growth, especially that of *I. cylindrica*, was more restricted by intraspecific competition than by interspecific competition as the glyphosate concentration increased. Furthermore, the relative competitive potential of the native species decreased with increasing glyphosate concentration. *S. canadensis* is more tolerant of glyphosate, which enhances its competitive advantage and hinders the proliferation, reintroduction, and success of native plant species. Future studies should focus on developing techniques to mitigate the negative impacts of invasive plant species, for example, via optimizing methods of spraying herbicides.

Keywords: exotic invasive species; herbicide control; competition; *Solidago canadensis*; native species restoration

1. Introduction

Global climate change has increased the invasiveness of exotic species in new areas and consequently leads to damages to local ecosystems [1–5]. In particular, many invasive plant species encroach on agricultural fields and natural reserves, where they directly compete with crops and native plants for space, nutrients, and pollinators during growth and reproduction, resulting in substantial yield and biodiversity losses [6,7]. Therefore, managing invasive species has become a major issue in agriculture and natural protection [8,9].

Various methods are used to control invasive species, with herbicides commonly used because of their efficiency and cost-effectiveness [10,11]. However, concerns have arisen

about the secondary effects of the methods used to manage invasive species, particularly those related to herbicide use [12,13]. Herbicides can negatively affect the biodiversity of plants, microorganisms, fauna, and overall ecosystem functioning [14–16]. Herbicides reach and affect nontarget plants by drifting through the air [17], diffusing into the water of wetland ecosystems [18], or being released from the roots of target plants and transferring to nontarget plants [19,20]. The residues of some herbicides in the soil can have long-lasting effects depending on the timing of application [21]. Therefore, herbicides can have serious negative nontarget effects on native plant species.

The herbicides used to control invasive species can have negative, neutral, or positive effects on native species [22,23], depending on the type and dosage of the herbicide, as well as the context of the ecosystem being managed. A single herbicide application can cause the populations of native forbs to decline, thereby allowing for the establishment and spread of invasive species [14]. The abundance of native plants is negatively affected by 2,4-D ester or glyphosate application, whereas invasive monocots may thrive with their application [18,23]. In contrast, the native plant community and representative native species were not affected and only modestly affected by herbicides applied to control invasive species in other studies [22,24]. The responses of native plant communities and species to herbicides vary widely. Therefore, the mechanisms underlying the different responses must be understood. The short-term effects of herbicide application are mainly determined by the sensitivity and tolerance of native species to the herbicide [18,25,26]. Some invasive species are highly tolerant to herbicides [27,28]. For example, their photosynthetic capacity and growth may be less affected by herbicide application than those of native species [18,25]. This stronger herbicide tolerance may increase their competitiveness and hinder efforts to restore native plants to these habitats [14,29]. Therefore, the differences in the impacts of herbicides on the growth and competition of invasive and native species must be better understood.

Determining the mechanisms underlying the tolerance of individual species to herbicides provides a foundation for understanding the responses of entire plant communities to herbicides applied to eradicate invasive species. However, previous studies have not compared the herbicide tolerance between invasive and co-occurring native species or linked the differences in their interactions during regrowth periods. Therefore, this study focused on the simultaneous effects of herbicide application on invasive and native plant species to understand how attempts to restore native plant species are affected by herbicide application. Specifically, we aimed to determine the differences in the effects of herbicide application on mortality, photosynthetic capacity, growth, and competition. In this study, we aimed to clarify how herbicide application alters the competitive balance between invasive and native plants. We hypothesized that invasive species might be more tolerant to herbicides, thereby enabling a competitive advantage over native species.

Solidago canadensis (Canada goldenrod) is native to North America and has become invasive in Asia, Europe, and Australia [30–32]. *S. canadensis* rapidly expands through prominent clonal growth and the dispersal of large numbers of seeds. As an invasive species, *S. canadensis* frequently replaces native plant species through competition [33,34]. Herbicide application is the most common and cost-effective method of controlling *S. canadensis* [31,35]. However, the effects of the herbicides used to control *S. canadensis* in ecosystems remain unclear. Field tests have shown that glyphosate, a widely used herbicide [36,37], effectively controls this species but also harms various native species. Glyphosate application may alter the interactions between *S. canadensis* and native plant species during growth recovery periods, involving the reestablishment of native species. However, the effects of glyphosate have not been examined.

In this study, *Imperata cylindrica* (Poaceae) was selected as a representative native grass species because *I. cylindrica* is the dominant native grass species that coexists with *S. canadensis* in eastern China [38]. *I. cylindrica* is a fast-growing pioneer species that rapidly occupies available habitats and exhibits early resistance to invasion by *S. canadensis*. *I. cylindrica* is often replaced by *S. canadensis* in the field, and controlling *S. canadensis* with herbicides does not enable the successful re-establishment of *I. cylindrica*, suggesting the off-target effects of glyphosate. This study highlights the importance of considering the nontarget effects of herbicide application and provides a theoretical basis for determining whether herbicides can be applied to control exotic invasive species when reestablishing native species.

2. Results

2.1. Plant Mortality Rate in Glyphosate and Competition Treatments

The plant mortality rate significantly differed between species, at different glyphosate concentrations, and under competition (Table 1, $p < 0.05$). The mortality rate increased with increasing glyphosate concentration in both species (Table 1 and Supplementary Table S1) and was higher for *I. cylindrica* than for *S. canadensis*. *I. cylindrica* mortality was 66.0% higher at 1.8 mL·L^{-1} glyphosate when averaged for all the competition treatments. The mortality rate between species significantly differed in the 1.2–1.8 mL·L^{-1} glyphosate treatments ($p < 0.05$). *S. canadensis* showed a higher mortality rate in the mixture treatment than in the monoculture treatment (e.g., 38.6% higher at 1.8 mL·L^{-1} glyphosate), whereas *I. cylindrica* mortality showed a higher mortality rate in the monoculture treatment (e.g., 8.4% higher at 1.8 mL·L^{-1}).

Table 1. Plant mortality rate (%) of *Solidago canadensis* or *Imperata cylindrica* treated with different concentrations of glyphosate.

Glyphosate Treatments (ml·L^{-1})	*S. canadensis* Monoculture	*S. canadensis* Mixture	*I. cylindrica* Monoculture	*I. cylindrica* Mixture
0	0.0 ± 0.0 bA	0.0 ± 0.0 bA	0.0 ± 0.0 cA	0.0 ± 0.0 cA
0.3	0.0 ± 0.0 bA	0.0 ± 0.0 bA	0.0 ± 0.0 cA	0.0 ± 0.0 cA
0.6	14.3 ± 10.7 bA	0.0 ± 0.0 bB	14.3 ± 9.2 bA	14.3 ± 9.2 cA
0.9	7.1 ± 4.6 bB	7.1 ± 2.1 bB	39.3 ± 12.0 bA	7.1 ± 7.1 cB
1.2	25.0 ± 14.4 abB	0.0 ± 0.0 bC	71.4 ± 12.7 aA	50.0 ± 15.4 bA
1.5	21.4 ± 8.5 abC	50.0 ± 10.9 aB	89.3 ± 7.4 aA	57.1 ± 14.9 abB
1.8	46.4 ± 14.9 aB	64.3 ± 14.3 aB	92.9 ± 7.1 aA	85.7 ± 9.2 aA

Different lowercase letters (a, b, and c) indicate significant differences ($p < 0.05$) among glyphosate concentration treatments within each species × competition combination, whereas different uppercase letters (A, B, and C) indicate significant differences among different species × competition combinations within each glyphosate concentration treatment.

2.2. Response of Photosynthetic Capacity to Glyphosate Application and Competition

The glyphosate concentration and species competition affected the photosynthetic capacity of both species (Table 2) and more strongly affected the maximum net photosynthetic rate (P_{nmax}) and light saturation point (LSP) of *I. cylindrica* (Supplementary Table S2). The stomatal conductance (G_s) of *S. canadensis* was more affected by glyphosate and competition than that of *I. cylindrica*. The effects of the interaction between glyphosate and competition on P_{nmax} and G_s were significant for *S. canadensis* ($p < 0.05$).

For both species, the P_{nmax}, LSP, G_s, and apparent quantum efficiency (AQY) generally decreased, the light compensation point (LCP) increased, and the R_d remained relatively stable with increasing glyphosate concentrations (Figure 1). The P_{nmax} of *I. cylindrica* was higher than that of *S. canadensis* in the absence of glyphosate in the monoculture treatment. However, the P_{nmax} of *S. canadensis* was higher when glyphosate was applied and was consistently higher when the species were grown together (Figure 1A). The LSP of *I. cylindrica* was higher than that of *S. canadensis* in the monoculture treatment, except at a glyphosate concentration of 1.8 $mL \cdot L^{-1}$. However, the LSP of *S. canadensis* was higher when the species were grown together (Figure 1B). The G_s and AQY were generally higher and the LCP was lower for *S. canadensis* than for *I. cylindrica*, regardless of the competition treatment (Figure 1C,D). Changes in the R_d of the species were inconsistent among the glyphosate concentrations (Figure 1E).

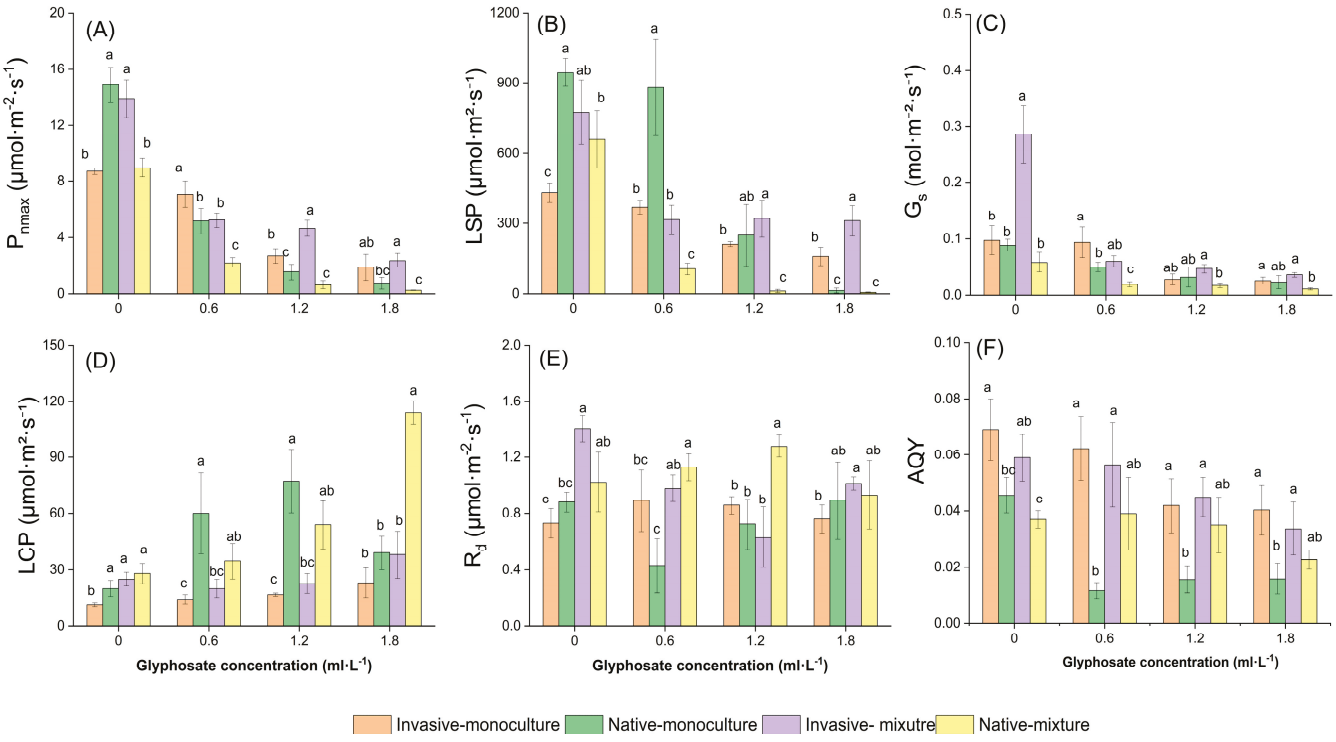

Figure 1. Effects of glyphosate application and competition on the photosynthetic parameters of the invasive *Solidago canadensis* and native *Imperata cylindrica*. Plants were grown either in monoculture (four *S. canadensis* plants or four *I. cylindrica* plants) or mixed (two *S. canadensis* plants + two *I. cylindrica* plants). (**A**) P_{nmax}, maximum net photosynthetic rate; (**B**) LSP, light saturation point; (**C**) G_s, stomatal conductance; (**D**) LCP, light compensation rate; (**E**) R_d, dark respiration rate; (**F**) AQY, apparent quantum yield. Only significant differences between different combinations of species × competition for each parameter are shown for clarity. Different lowercase letters (a, b, and c) indicate significant differences ($p < 0.05$).

Competition between species altered their photosynthetic capacities. The P_{nmax}, LSP, and G_s of *S. canadensis* were higher under interspecific competition (mixed treatment) than under intraspecific competition (monoculture treatment), except for the 0.6 $mL \cdot L^{-1}$ glyphosate treatment. In contrast, the P_{nmax}, LSP, and G_s of *I. cylindrica* were higher in the monoculture treatment than in the mixed treatment (Figure 1A–C). The LCP and R_d were generally higher under interspecific competition than under intraspecific competition for both species (Figure 1D,E). The AQY was lower for *S. canadensis* and higher for *I. cylindrica* under interspecific competition than under intraspecific competition (Figure 1F).

Table 2. Effects of competition and glyphosate treatment on photosynthetic parameters of *Solidago canadensis* and *Imperata cylindrica*.

Treatments	Species		Photosynthetic Parameters					
			P_{nmax}	LSP	G_S	LCP	R_d	AQY
Glyphosate (G)	*S. canadensis*	*F*	31.1	11.4	20.985	2.1	2.1	1.0
		P	0.000 **	0.000 **	0.000 **	0.047 *	0.146	0.423
	I. cylindrica	*F*	44.1	18.9	10.399	2.2	1.5	3.3
		P	0.000 **	0.000 **	0.000 **	0.026 *	0.25	0.046 *
Competition (C)	*S. canadensis*	*F*	7.9	8.8	8.05	3.5	4.5	1.7
		P	0.013 *	0.009 *	0.012 *	0.081	0.05	0.213
	I. cylindrica	*F*	10.9	17.8	7.8	0.03	19.8	11.5
		P	0.005 **	0.001 **	0.013 *	0.874	0.009 **	0.004 **
G × C	*S. canadensis*	*F*	6.1	2.5	8.986	0.64	4.1	0.8
		P	0.006 **	0.094	0.001 **	0.602	0.065	0.513
	I. cylindrica	*F*	2.3	2.9	0.417	2.6	0.5	1.7
		P	0.112	0.066	0.743	0.086	0.656	0.204

Asterisks indicate significant differences in the treatments (G, glyphosate; C, competition; and G × C, interaction between glyphosate and competition) for each photosynthetic parameter. *, $p < 0.05$, **, $p < 0.01$. P_{nmax}, maximum net photosynthetic rate; LSP, light saturation point; G_s, stomatal conductance; LCP, light compensation rate; R_d, dark respiration rate; AQY, apparent quantum yield.

2.3. Plant Growth Response to Glyphosate and Competition

Both glyphosate and competition affected the growth and biomass accumulation of *S. canadensis* and *I. cylindrica* (Table 3). The total, aboveground, and belowground biomass of both species was significantly affected by glyphosate, competition type, and their interaction ($p < 0.05$). However, the number of green leaves of *S. canadensis* and the total green leaf length of *I. cylindrica* were significantly affected only by glyphosate treatment. Glyphosate and the interaction between glyphosate and interspecific competition generally more strongly affected *I. cylindrica* than *S. canadensis*, whereas the effects of interspecific competition were generally stronger in *S. canadensis*.

Table 3. Effects of glyphosate treatment and competition on the growth and biomass accumulation of *Solidago canadensis* and *Imperata cylindrica*.

S. canadensis						
Treatments	Significance	Total biomass	Aboveground biomass	Belowground biomass	Shoot height	Number of green leaves
Glyphosate (G)	*F*	20.4	20.2	11.8	18.0	11.2
	P	<0.001	<0.001	<0.001	<0.001	<0.001
Competition (C)	*F*	69.7	82.6	22.4	18.5	0.6
	P	<0.001	<0.001	<0.001	<0.001	0.439
G × C	*F*	2.9	0.5	2.9	3.0	1.6
	P	0.011	0.03	0.013	0.01	0.151
I. cylindrica						
Treatments	Significance	Total biomass	Aboveground biomass	Belowground biomass	Number of ramets	Total length of green leaves
Glyphosate (G)	*F*	37.7	29.1	36.9	50.8	52.523
	P	<0.001	<0.001	<0.001	<0.001	<0.001
Competition (C)	*F*	37.8	32.2	36.9	13.2	1.379
	P	<0.001	<0.001	<0.001	<0.001	0.244
G × C	*F*	7.8	5.6	8.7	5.8	1.254
	P	<0.001	<0.001	<0.001	<0.001	0.288

The growth of both species generally decreased with increasing glyphosate concentration (Figures 2 and 3, Supplementary Table S1). This reduction was more pronounced in the monoculture treatment than in the mixed treatment and in *I. cylindrica* than in *S. canadensis* (Supplementary Table S2). The total plant biomass was lower by 33.3–81.8% and 18.5–54.9% in the monoculture treatment and by 14.1–65.6% and 3.4–55.7% than in the mixed treatment for *I. cylindrica* and *S. canadensis*, respectively. The reductions in the other growth parameters (e.g., aboveground biomass) with increasing glyphosate concentration were similar.

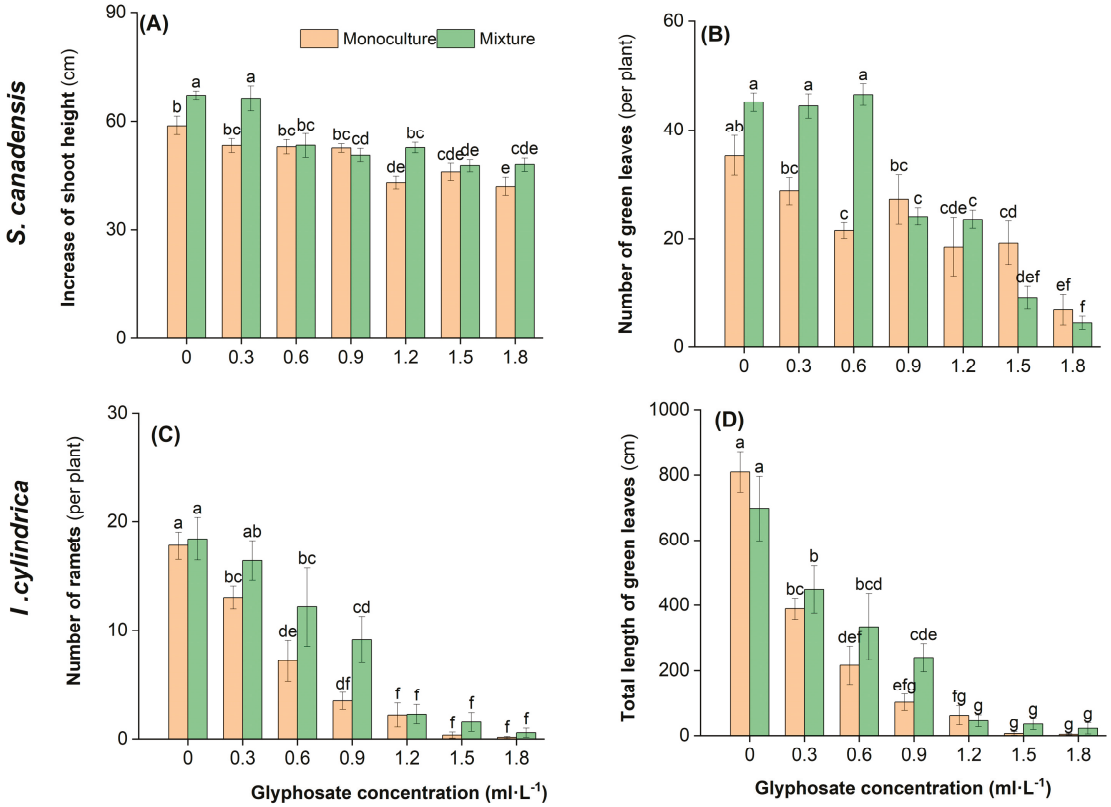

Figure 2. Effect of glyphosate application and competition on the growth of *Solidago canadensis* and *Imperata cylindrica*. Plants were grown either in monoculture (four *S. canadensis* plants or four *I. cylindrica* plants) or mixed (two *S. canadensis* plants + two *I. cylindrica* plants). (**A**) Increase in shoot height of *S. canadensis*; (**B**) number of green leaves of *S. canadensis*; (**C**) number of ramets of *I. cylindrica*; (**D**) total length of green leaves of *I. cylindrica*. Data are presented as the mean ± standard error ($n = 7$). Different letters indicate significant differences ($p < 0.05$) between treatments with different combinations of glyphosate concentration and competition.

Interspecific competition generally led to increased growth in both species compared with that observed with intraspecific competition. The relative yield (RY) differed significantly between the species and glyphosate concentrations ($F = 2.85$, $p < 0.05$, Figure 4A). In both species, RY was higher at lower glyphosate concentrations and decreased as the glyphosate concentration increased. The RY of *I. cylindrica* was lower at 0–0.3 mL·L^{-1} and higher at 0.6–1.8 mL·L^{-1} than that of *S. canadensis*. The ratio of *I. cylindrica* to *S. canadensis* biomass decreased with increasing glyphosate concentrations under both intra- and interspecific competition ($F = 11.828$, $p < 0.05$; Figure 4B).

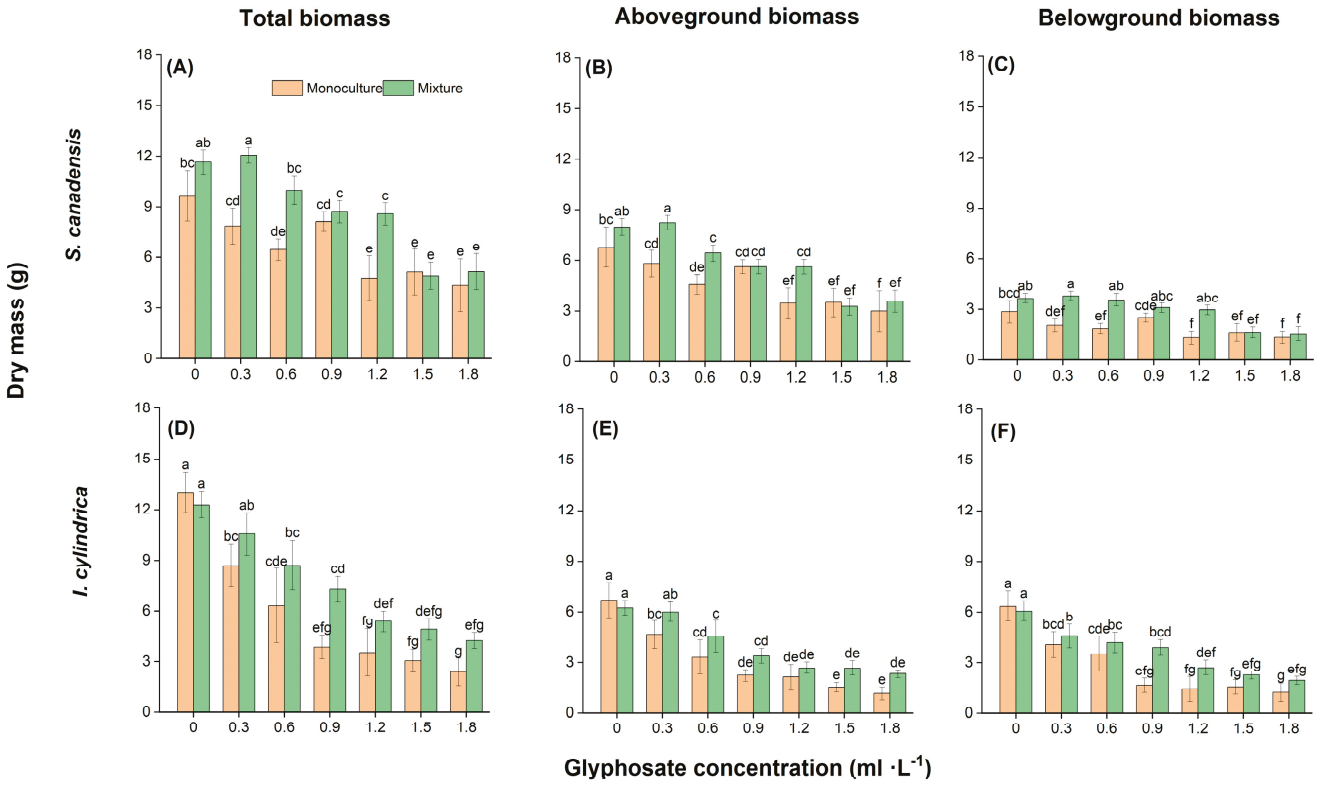

Figure 3. Effect of glyphosate application and competition on the biomass of *Solidago canadensis* and *Imperata cylindrica*. Plants were grown either in monoculture (four *S. canadensis* plants or four *I. cylindrica* plants) or mixed (two *S. canadensis* plants + two *I. cylindrica* plants). (**A**,**D**), (**B**,**E**), and (**C**,**F**) Total, aboveground, and belowground biomass of *S. canadensis and I. cylindrica*, respectively. Data are presented as mean ± standard error (n = 7). Different letters indicate significant differences ($p < 0.05$) between treatments with different combinations of glyphosate concentration and competition.

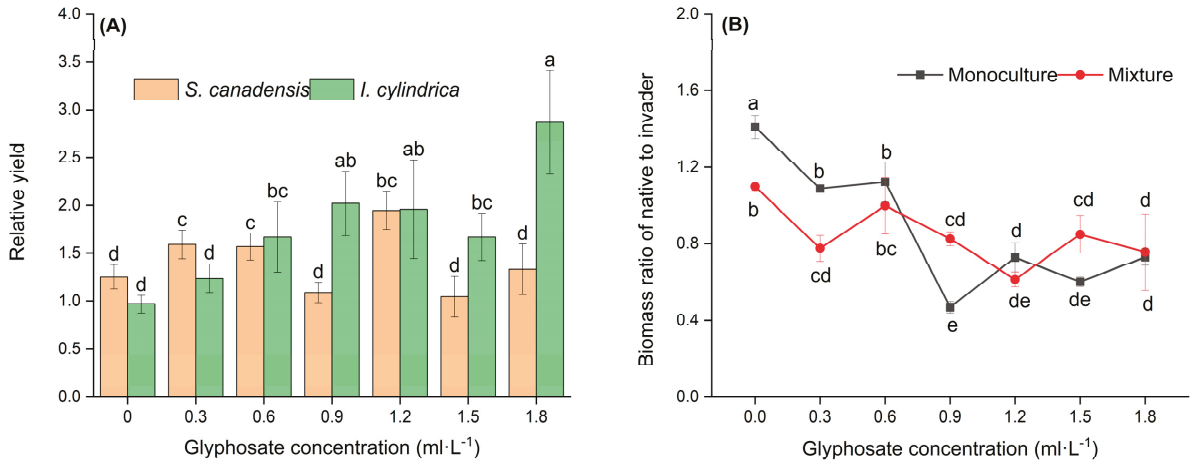

Figure 4. Effect of glyphosate application on competition between *Solidago canadensis* and *Imperata cylindrica*. The plants were grown in monoculture (four *S. canadensis* plants or four *I. cylindrica* plants) or mixed (two *S. canadensis* plants + two *I. cylindrica* plants). (**A**) Relative yield of *S. canadensis* and *I. cylindrica*; (**B**) Biomass ratio of *I. cylindrica* relative to that of *S. canadensis*, either in monoculture or mixed plots. Data are the mean ± standard error (n = 7). Different letters indicate significant differences ($p < 0.05$) between treatments with different combination of glyphosate concentration.

3. Discussion

The exotic invasive species *S. canadensis* was more tolerant to glyphosate than the native *I. cylindrica*. Applying glyphosate reduced the ability of *I. cylindrica* to compete with *S. canadensis*. Consequently, using glyphosate to control *S. canadensis* may suppress the populations of native *I. cylindrica* in the long term, particularly at sublethal doses. Therefore, strategies must be developed to mitigate these nontarget effects.

3.1. High Tolerance of S. canadensis to Glyphosate

The resistance of *S. canadensis* to glyphosate was much higher than that of *I. cylindrica*, as indicated by their photosynthetic and regrowth capacities. *S. canadensis* was not affected by moderate glyphosate application (below 0.6 $mL \cdot L^{-1}$) and was able to survive to a certain degree and regrow at a glyphosate concentration of 1.8 $mL \cdot L^{-1}$, indicating a high tolerance to glyphosate. Yanniccari et al. [39] reported that G_s is a sensitive indicator of glyphosate susceptibility in plants. However, our study showed that the P_{nmax} and LSP of *S. canadensis* and *I. cylindrica* were more sensitive than G_s to glyphosate application. The P_{nmax} and LSP of the invasive and native species showed different responses to glyphosate, and they were consistent with the trends in total biomass. The differences in the findings between studies could be due to differences in the duration between glyphosate treatment and photosynthesis measurement (three weeks in this study vs. 1–7 days in Yanniccari et al. [39]). Short-term photosynthesis changes may be more closely related to G_s, whereas long-term plant growth may be limited by the number of photosynthetic apparatuses, as indicated by P_{nmax} and LSP [40]. Glyphosate inhibits the biosynthesis of tyrosine, phenylalanine, and tryptophan, which are three essential amino acids found in all proteins [36]. The combined influence of decreases in P_{nmax}, LSP, G_S, and AQY and increases in the LCP may reduce the carbon assimilation capacity and growth of plants. In this study, the growth of the above- and belowground parts of the two species was similarly affected by glyphosate, which is transported from the leaves to the roots and rhizomes [36]. However, the systemic responses of plants to herbicides may not be caused by direct injury to each part but rather by resource allocation to the aboveground and belowground parts.

This study only evaluated the tolerance of one species; however, herbicide resistance varies widely among and within species [21,25,41]. *S. canadensis* might be more tolerant to herbicides than most co-occurring native species because many invasive species are more stress-tolerant and more quickly regrow after disturbances [42–44]. High herbicide tolerance is frequently observed in weeds and exotic plant species [27,28], which is likely due to the selection of resistant genotypes through herbicide application in these species [45]. Invasive plant species rapidly expand from their native range to an invasive range [46,47]. The high herbicide tolerance in some invasive species is due to mutations [45], as recorded in many weeds [41]. Polyploidization may also enhance tolerance to herbicides [30,48]. The mechanisms of herbicide tolerance differ within invasive species and may not be mutually exclusive. First, exotic invasive species may have a stronger capacity to biotransform, degrade, or detoxify herbicides, thereby reducing the associated damage [49,50]. Second, exotic invasive plant species tend to be larger than the native species they replace [51,52] and thus may require higher amounts of herbicides to cause equivalent levels of damage. Third, if both species have similar clonal growth forms, then the invasive plant species may benefit more from clonal integration than native plant species under various stress conditions [53,54]. In this study, the higher clonal integration of *S. canadensis* compared with that of *I. cylindrica* provided more protection against herbicide damage; however, in a previous study on the invasive plant *Carpobrotus eduli*, the level of clonal integration did not enhance the plant's performance after glyphosate treatment [55]. Finally, invasive

plant species that show stronger associations with soil microorganisms (such as arbuscular mycorrhizal fungi) than native species may be more tolerant to herbicides [56].

3.2. Glyphosate Treatment Altered Competition Between S. canadensis and I. cylindrica

Although native vegetation attempts to resist invasions by exotic plant species [57,58], invasive species outcompete native species for resources and have stronger reproductive capacities [51,59], resulting in the replacement of native species with invasive species [60,61]. We found that the application of herbicides to control invasive species may alter the interactions between invasive and native species, influencing attempts to re-establish native species. The higher RY of *S. canadensis* indicated that this species benefited more from reductions in intraspecific competition than the native species when herbicide was not applied. This partially explains the higher invasive capacity of native grasses [38,62]. Conversely, the higher RY of *S. canadensis* after glyphosate application indicates that the native *I. cylindrica* is less negatively affected when competing with *S. canadensis* due to reduced intraspecific competition intensity. A model study also indicated that native plant species experience a relief period after herbicide degradation and before invasive species re-establishment [29]. However, the competitive ability of *I. cylindrica* decreased with increasing glyphosate concentrations during the regrowth period, as indicated by the decrease in the ratio of *I. cylindrica* to *S. canadensis* biomass. This reduction in the competitive ability of *I. cylindrica* was attributed to its lower tolerance to glyphosate. These findings suggest that the application of herbicides to invasive species during the growing season further decreases the population of *I. cylindrica* at sites with *S. canadensis*. In addition to the differences in tolerance between these nontarget native and target invasive species, herbicide application may encourage secondary invasion. The failure of native vegetation to re-establish following herbicide application may occur because of re-invasion by the target invasive species or the establishment of other non-native plants [23,63]. Many invasive species can colonize disturbed sites more rapidly than native species because they have a stronger ability to regrow after disturbances [64] and greater number of propagules available for colonization [65,66].

This study was limited to two months, and only one dominant species was considered. Whether the differences in the effects of herbicide application persist for longer durations under field conditions remains unclear. Differences in herbicide tolerance and competitive capacity between invasive species can alter the response of the entire plant community. Long-term evaluations are important for quantifying the outcomes of attempts to restore native species [11,29]. Glyphosate application may temporarily alleviate competition intensity by suppressing the growth of both invasive and native species [67]; however, competition intensity may strengthen as invasive species recover from herbicide stresses, as predicted by the stress gradient hypothesis [68,69]. This may explain why some native plant species show neutral or positive dominance to herbicide application in the short term [22–24] but then exhibit decreased dominance in the long term [14,16]. Further long-term field studies of the community-level interactions between native and invasive species following herbicide application are essential for guiding the successful restoration of native vegetation.

3.3. Potential Approaches for Alleviating Nontarget Effects of Herbicides

Several approaches can be used for reducing the nontarget effects of herbicides on native plant species: (1) Spray methods can be optimized. Applying herbicides using spot or boom sprays based on the density of the target invasive species may reduce injury to native species [70]. (2) The application of control methods can be more precisely timed. Native and invasive plant species show distinct phenological niche separation in some cases,

which allows for the control of invasive species without harming native species through the precise timing of herbicide application. For example, the winter application of herbicides effectively suppressed the regrowth of winter-active *S. canadensis* but did not impact the growth of winter-dormant *I. cylindrica* in a subtropical climate [71]. (3) The propagules of native plant species can be protected. Providing sufficient propagules [35] and protecting seeds via pelleting with local soil or activated carbon after herbicide application can enhance the restoration ability of native species [72,73]. However, the efficiencies of these approaches are highly dependent on the context; therefore, further study is required.

4. Materials and Methods

4.1. Seedling Culture

The seedlings were collected from a nature conservation area in the Hangzhou Bay National Wetland Park (121°08′43″ E, 30°18′40″ N) in Ningbo, Zhejiang Province, China. Individual ramets with five mature leaves and a shoot height of 13–15 cm were chosen for *S. canadensis* and *I. cylindrica* on 20 March 2016. The rhizomes were uniformly trimmed to 2–3 cm in length. The plants were temporarily grown in river sand and watered with tap water for three weeks. Plants of similar sizes and growth vigor were transplanted into pots (23 × 17 cm). The pots were filled with soil collected from the same site where the seedlings were collected. The soil was silty, characterized by mild salinity (2.5‰), slightly alkaline conditions (pH 8.2), low fertility (0.45 $g \cdot kg^{-1}$ total N, 0.60 $g \cdot kg^{-1}$ total P, and 7.23 $g \cdot kg^{-1}$ total soil organic matter content), and a field water holding capacity of 28.9%. We added 2 g of urea and 2 g of potassium dihydrogen phosphate (KH_2PO_4) to each pot to mitigate nitrogen and phosphorus limitations from affecting plant growth. The plants were acclimatized to greenhouse conditions for three weeks prior to the start of the treatments.

4.2. Competition and Glyphosate Treatments

A two-factor experiment involving competition and glyphosate application was conducted in a greenhouse at the Institute of Subtropical Forestry of the Chinese Academy of Forestry. A replacement design [74] was used for the competition treatments: four *S. canadensis* plants (*S. canadensis* monoculture) or four *I. cylindrica* plants (*I. cylindrica* monoculture) were grown without interspecific competition within a plastic pot or two *S. canadensis* plants and two *I. cylindrica* plants were grown together (mixed treatment, with interspecific competition). The effects of applying different glyphosate concentrations on *S. canadensis* leaf chlorosis and withering were investigated. Applying 1.8 $mL \cdot L^{-1}$ glyphosate caused serious leaf injury and plant death. Each competition treatment included one of seven glyphosate concentration levels: 0, 0.3, 0.6, 0.9, 1.2, 1.5, or 1.8 $mL \cdot L^{-1}$ (41% Roundup [Monsanto Company, Saint Louis, MO, USA] in distilled water), with 5 mL of glyphosate solution applied per pot to examine the drift effects of sublethal glyphosate dosages on the two species. The glyphosate dosages were much lower than those applied to achieve effective control in the field [75] owing to the large differences in plant size between the species investigated in this study and those growing in field. Glyphosate solutions were evenly sprayed on the leaves of each plant using a 20 mL plastic sprayer on 12 June 2016. Seven replicates were conducted for each combination of competition and glyphosate treatment. A total of 147 pots were used (seven glyphosate concentration treatments × three competition treatments × seven replicates). The pots were rearranged once per week to minimize the effects of environmental heterogeneity within the greenhouse. The photosynthetically active radiation (PAR) intensity in the greenhouse was approximately 50–70% of the ambient PAR depending on the external PAR. The air temperature and relative humidity in the greenhouse followed external conditions. The temperature fluctuated between 20 and 38 °C during the day and between 20 and 26 °C at

night, and the relative humidity was 40–95%. The plants were watered regularly to avoid drought stress.

4.3. Photosynthesis Measurements

The growth of the treated plants was monitored starting on the first day of glyphosate treatment. Symptoms of injury and regrowth were observed 10 and 20 days after glyphosate spraying, respectively. Photosynthetic capacity was a major determinant of regrowth, and it was measured using a 6400 XT Portable Photosynthesis Measurement System (LiCOR, Lincoln, NE, USA) after three weeks of treatment. Before this, the first three fully expanded *S. canadensis* and *I. cylindrica* leaves of the same position were selected from each plant. Three individual plants of *S. canadensis* and *I. cylindrica* from either the monoculture or mixed treatment at each glyphosate concentration were selected for testing on sunny days between 7:30 and 11:30. The photosynthetic parameters were measured along a PAR gradient of 1500, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 20, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The PAR–$P_n$ curves were fitted using a nonrectangular hyperbolic equation to determine the P_{nmax}, LSP, LCP, AQY, and dark respiration rate (R_d) [76]. The Gs was recorded using the PAR at the LSP. The values of the photosynthetic parameters obtained from three leaves of the same plant were averaged before statistical analysis.

4.4. Measurement of Mortality, Growth, and Competition Effects

The growth and mortality rates were evaluated after two months of growth. Shoot height and number of green leaves were recorded for each *S. canadensis* plant, whereas the number of ramets and total length of green leaves were recorded for *I. cylindrica*. The mortality rate was calculated in each pot by assessing the presence of green leaves, and it was defined as the ratio of dead plants to the total number of plants (four in the monoculture treatment and two in the mixed treatment). Biomass of surviving live plants in at least three pots was analyzed. Mortality rates were averaged for each replicate of each combination treatment. Plants were harvested to determine the biomass on August 18–20, 2016. The belowground parts of each plant were washed and separated from the aboveground parts. All the harvested plants were dried at 80 °C to a constant weight. The RY was used to assess the relative intensity of interspecific competition with intraspecific competition based on total individual biomass [74,77]. The RYs of *S. canadensis* and *I. cylindrica* were calculated using the following formula:

$$RY = Y_{mix}/Y_{mono}$$

where Y_{mix} indicates the total *S. canadensis* or *I. cylindrica* biomass in each pot in the mixed treatment and Y_{mono} indicates the total biomass of the corresponding species in each pot in the monoculture treatment.

The ratio of *I. cylindrica* to *S. canadensis* biomass was calculated to evaluate the relative competitive capacities of the species. The *I. cylindrica* and *S. canadensis* plants grown in the same pot were paired in the mixed treatment. The *I. cylindrica* and *S. canadensis* plants from the same glyphosate treatment group were randomly paired in the monoculture treatment.

4.5. Data Analysis and Statistics

The normality of all data was tested using the Shapiro–Wilk test. Two-way analysis of variance (ANOVA) was conducted to analyze the general effects of glyphosate concentration, competition, and their interactions on photosynthetic parameters (P_{nmax}, LSP, Gs at LSP, LCP AQY, and R_d), growth (increase in shoot height and number of green leaves of *S. canadensis*; number of ramets and total length of green leaves of *I. cylindrica*), and biomass (aboveground, belowground, and total biomass of *S. canadensis* and *I. cylindrica*). One-way ANOVA was performed for the above parameters to determine the differences

between the combined glyphosate × competition treatments. The data were transformed using the square root, natural log, or arcsine when the assumption of homogeneity of variance was not met. One-way ANOVA was also used to test the effects of glyphosate concentration on the mortality rate and the ratio of *I. cylindrica* to *S. canadensis* biomass for each glyphosate concentration treatment in the mixed or monoculture treatment. Multiple comparisons were performed using the Duncan method to determine the significance of the differences between the different treatment combinations. The size of these effects was estimated using the partial eta squared (Supplementary Table S2). A regression analysis between the glyphosate concentration and mortality rate as well as the growth parameters (growth and biomass) was also performed to examine the trends in these parameters with increasing glyphosate concentration (Supplementary Table S1). Statistical analyses were conducted using IBM SPSS Statistics 27 (Armonk, NY, USA).

5. Conclusions

Glyphosate applied for the control of the invasive species *S. canadensis* has nontarget effects on native plant species. The lower tolerance of *I. cylindrica* to glyphosate was evidenced by its higher mortality rates, decreased photosynthetic capacity, and suppressed regrowth capacity compared with those of *S. canadensis*. Moreover, the lower tolerance decreased the relative competitive ability of *I. cylindrica* with increasing glyphosate dosage. This could ultimately result in a decline in the population of this native species, thereby contradicting the intended goal of restoring native species by controlling invasive species. These nontarget effects may become more pronounced over time. Therefore, long-term studies on the responses of entire plant communities to herbicide application are essential for ensuring the effective management of exotic invasive species. Strategies must be developed and implemented to mitigate these adverse effects and support efforts to re-establish native plant species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants14172640/s1, Table S1: Linear regression of the growth parameters with the glyphosate concentration for *S. canadensis* and *Imperata cylindrica*, Table S2: Effect size of the effects of the competition treatments and the glyphosate treatments on photosynthetic and growth parameters of *Solidago canadensis* and *Imperata cylindrica*.

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Data Availability Statement: The data are available upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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Does the Species Number of Invasive Plants Regulate the Intensity of Interspecific Interactions Among Multiple Plants Under Different Invasion Scenarios?

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Abstract: Multiple invasive plants (IPS) can coexist in the same community. The intensity of interspecific interactions among multiple plants may progressively alter with the differences in the species number of IPS (S_i) under different invasion scenarios. However, the correlation between plant taxonomic diversity, S_i, the invasion intensity of IPS, the community invasibility and the intensity of interspecific interactions among multiple plants under different invasion scenarios remains unclear. This study aims to estimate the differences in the intensity of interspecific interactions among multiple plants, the taxonomic diversity of plants, the invasion intensity of IPS and the invasibility of the plant community under different invasion scenarios along a gradient of S_i. This study used a comparative field survey method in four cities in Jiangsu (including Lianyungang, Yancheng, Nantong and Zhenjiang), China. The species number of plants and plant richness decreased under the mono-invasion achieved by one IP compared to the uninvaded communities. Plant taxonomic diversity was negatively associated with the invasion intensity of IPS and the community invasibility. Plant taxonomic diversity was positively associated with S_i. The intensity of interspecific interactions among multiple plants decreased across all invasion scenarios. The intensity of interspecific interactions among multiple plants showed a significant positive association with the ratio of the max and min relative coverage of all plants, but a significant negative association with plant evenness. Therefore, the ratio of the max and min relative coverage of all plants and plant evenness may be the main factor regulating the intensity of interspecific interactions among multiple plants under different invasion scenarios, rather than S_i.

Keywords: community invasibility; co-invasion; invasion intensity; plant taxonomic diversity; relative coverage

1. Introduction

Invasive plants (IPS) can significantly alter the structure and function of native habitats. Specifically, the invasion of these IPS can significantly reduce the diversity of native plants but significantly increase the community invasibility in the invaded habitats [1–3]. Therefore, explaining the mechanisms behind the successful invasion

of these IPS has become one of the major key scientific issues in the field of invasion ecology in recent decades [1,4,5].

It is worth noting that multiple IPS can coexist in a given plant community [1,6,7]. More importantly, one IP can increase the chances of another IP successfully invading via the facilitative interactions by changing relevant environmental conditions, such as altering the competitive relationship and relative advantage comparison between IPS and native plants, leading to the co-invasion of two or even more IPS in the same habitat [8–10]. Nevertheless, under different invasion scenarios, the differences in the structure and function of native habitats may gradually fluctuate with the differences in the species number of IPS (S_i). In particular, plant taxonomic diversity, the invasion intensity of IPS and the community invasibility may progressively alter with the differences in S_i under different invasion scenarios, mainly due to the difference in the intensity of interspecific interactions among multiple plants, especially those between IPS and native plants. Nevertheless, it is presently uncertain whether the correlation between plant taxonomic diversity, S_i, the invasion intensity of IPS, the community invasibility and the intensity of interspecific interactions among multiple plants under different invasion scenarios along a gradient of S_i. Therefore, it is crucial to determine the influences of plant taxonomic diversity, S_i, the invasion intensity of IPS and the community invasibility on the intensity of interspecific interactions among multiple plants under different invasion scenarios along a gradient of S_i. However, the research progress in this field is limited.

This study aims to estimate the differences in the intensity of interspecific interactions among multiple plants, plant taxonomic diversity, the invasion intensity of IPS and the community invasibility under different invasion scenarios along a gradient of S_i. Furthermore, the correlation between plant taxonomic diversity, S_i, the invasion intensity of IPS, the community invasibility and the intensity of interspecific interactions among multiple plants under different invasion scenarios were assessed. In addition, the influences of plant taxonomic diversity, S_i, the invasion intensity of IPS and the community invasibility on the intensity of interspecific interactions among multiple plants under different invasion scenarios were also evaluated. This study used a comparative field survey method in four cities in Jiangsu (including Lianyungang, Yancheng, Nantong and Zhenjiang), China. The measured plant communities, including the mono-invasion achieved by different IPS and the co-invasion achieved by two and three IPS, were compared to the uninvaded communities.

The current study examines the following hypotheses: (1) the invasion intensity of IPS, the community invasibility and the intensity of interspecific interactions among multiple plants may increase progressively as S_i increases, but plant taxonomic diversity may decrease gradually as S_i increases; (2) the intensity of interspecific interactions among multiple plants may be mainly under the influence of S_i.

2. Materials and Methods

2.1. Study Design

The plant communities in four cities in Jiangsu (including Lianyungang, Yancheng, Nantong and Zhenjiang), China, were examined from May 2024 to July 2025. The name and the corresponding information about the survey points are shown in Table S1. The principal reason for taking multiple cities as the samples in this study is to minimize the influence of geographical location on the results. All studied plant communities are herbaceous without trees or shrubs. The species composition, source area and life form of IPS in the surveyed plant plots are defined in Table S2. The foremost reason for taking multiple co-invasive IPS of the samples in this study is to minimize the influence of the species of IPS on the results.

In this study, four types of plant plots (size: 1 m × 1 m) were surveyed:

(1) The uninvaded plots (control; no invasion by any IPS) (n = 92);
(2) The mono-invasion achieved by one IP (n = 827);
(3) The co-invasion achieved by two IPS (n = 280);
(4) The co-invasion achieved by three IPS (n = 71).

Plant plots with the same invasion scenario were separated by more than 20 m.

The species number of plants (including IPS and native plants) and the relative coverage per plant species were verified for all examined plant plots.

2.2. Determination of the Intensity of Interspecific Interactions Among Multiple Plants

To measure the intensity of interspecific interactions among multiple plants in the plant community, the intensity index of interspecific interactions (*IIII*) among multiple plants was estimated as follows:

$$IIII = \sum_{i=1}^{S} \frac{RC_i}{RC_j} \times \frac{1}{S^2}$$

where S is the species number of plants in a given plot. RC_i and RC_j are the relative coverages of plant i and j in a given plot, respectively. The basic principle of designing this formula is as follows: since the interspecific interactions among two plants are mutual, *IIII* in a given plot is calculated as the ratio of the sum of the relative coverages of any two plant species to the square of the number of plant species in a given plot. The relative coverage of one plant species was calculated as the ratio of its coverage to the total coverage of all plant species in a given plot. The coverage was determined by the vertically projected area of the plant canopy to a large extent [11–13]. In particular, the plots with a higher value of *IIII* have a stronger intensity of interspecific interaction among multiple plants in comparison with those with a lower value of *IIII*.

The method for determining *IIII* described in this study was defined by the authors of the present study.

2.3. Determination of Plant Taxonomic Diversity, IPS' Invasion Intensity and the Community Invasibility

Plant taxonomic diversity for all evaluated plant plots was examined using the following: Shannon's diversity [14], Simpson's dominance [15], Pielou's evenness [16] and Margalef's richness [17] because these indices are often used to measure plant taxonomic diversity.

The invasion intensity index was appraised to assess IPS' invasion intensity of all invaded plant plots [18].

The community invasibility index was analyzed to quantify the community invasibility of all invaded plant plots [18].

The determination methods for the appraised indices of plant taxonomic diversity, IPS' invasion intensity and the community invasibility are labeled in Table S3.

2.4. Statistical Analysis

Deviations from normality and homogeneity of the raw data of the assessed variances were assessed via the Shapiro–Wilk's test and Bartlett's test, respectively, and the assumptions of the anova model were met.

Differences in the values of the appraised variances under different invasion scenarios were evaluated by using the one-way analysis of variance (ANOVA) with Tukey's test.

Correlation between the measured variances under different invasion scenarios was estimated by using the correlation analysis according to the values of the Pearson product-

moment correlation coefficient. Bonferroni corrections were also applied to correct the p values to decrease the occurrence probability of type I error.

Influences of S_i on the measured variances and influences of the evaluated variances on *IIII* under different invasion scenarios were quantified by using the path analysis according to the values of the path coefficient (i.e., the standardized regression coefficient).

Statistical analyses were performed using SPSS Statistics 26.0 (IBM, Inc., Armonk, NY, USA).

3. Results

3.1. Differences in the Measured Indices Under Different Invasion Scenarios

Compared to the uninvaded communities, the mono-invasion achieved by one IP decreased the species number of plants, the ratio of the max and min relative coverage of all plants, *IIII* and the Margalef's richness index ($p < 0.05$; Figure 1a,e,f,j), whereas it increased the min relative coverage of all plants ($p < 0.05$; Figure 1c).

Compared to the uninvaded communities, the co-invasion achieved by two IPS decreased the difference between the max and min relative coverage of all plants, the ratio of the max and min relative coverage of all plants and *IIII* ($p < 0.05$; Figure 1d–f), whereas it increased the Shannon's diversity index, the Simpson's dominance index and the Pielou's evenness index ($p < 0.05$; Figure 1g–i).

Compared to the uninvaded communities, the co-invasion achieved by three IPS increased the species number of plants and all estimated plant taxonomic diversity indices ($p < 0.05$; Figure 1a,g–j), whereas it decreased the max relative coverage of all plants, the difference between the max and min relative coverage of all plants, and the ratio of the max and min relative coverage of all plants and *IIII* ($p < 0.05$; Figure 1b,d–f).

In invaded communities, the species number of plants, the Shannon's diversity index, the Simpson's dominance index and the Margalef's richness index increased with S_i ($p < 0.05$; Figure 1a,g,h,j); the max relative coverage of all plants and the difference between the max and min relative coverage of all plants decreased with S_i ($p < 0.05$; Figure 1b,d).

Compared to the mono-invasion achieved by one IP, the co-invasion achieved by two and three IPS increased Pielou's evenness index ($p < 0.05$; Figure 1i); the co-invasion achieved by three IPS decreased the min relative coverage of all plants ($p < 0.05$; Figure 1c).

In invaded communities, the values of the invasion intensity index of IPS and the community invasibility index under the co-invasion achieved by three IPS were higher than those under the mono-invasion achieved by one IP and the co-invasion achieved by two IPS ($p < 0.05$; Figure 2a,b).

3.2. Correlation Between the Measured Indices Under Different Invasion Scenarios

The species number of plants was positively correlated with the ratio of the max and min relative coverage of all plants, all estimated plant taxonomic diversity indices and S_i ($p < 0.001$; Table 1). The species number of plants was negatively associated with the max and min relative coverage of all plants, the difference between the max and min relative coverage of all plants, the invasion intensity index of IPS and the community invasibility index ($p < 0.001$; Table 1).

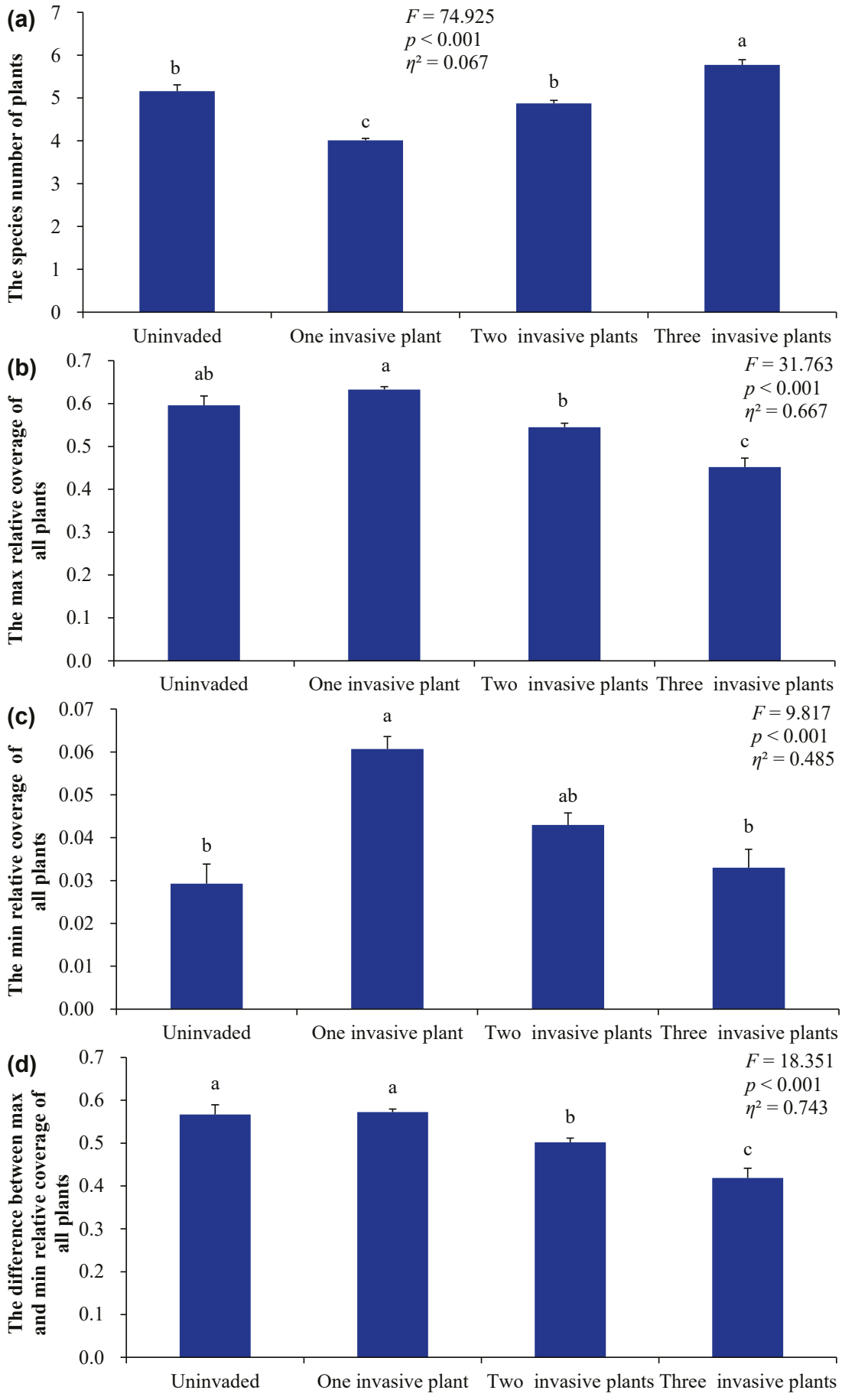
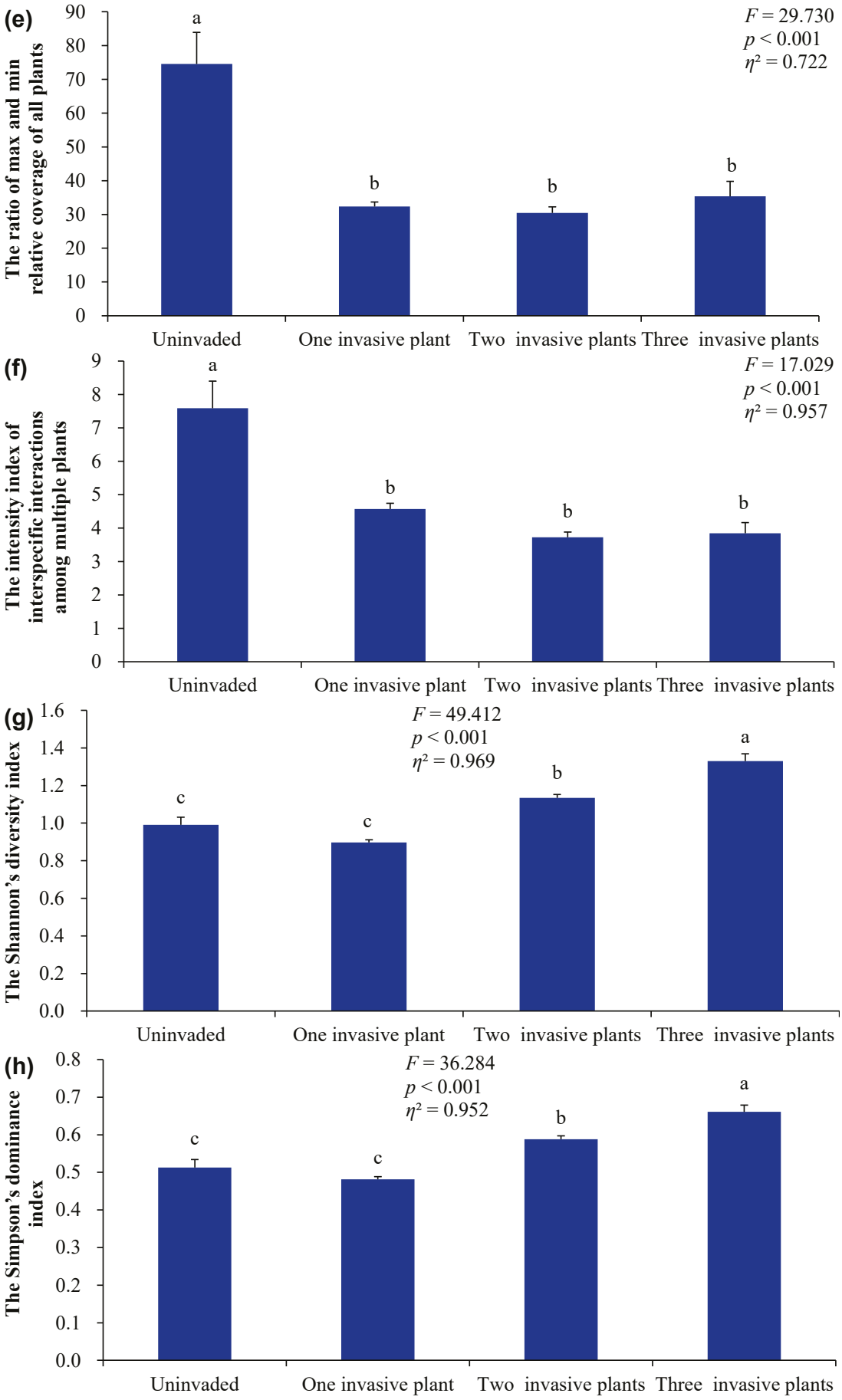


Figure 1. *Cont.*



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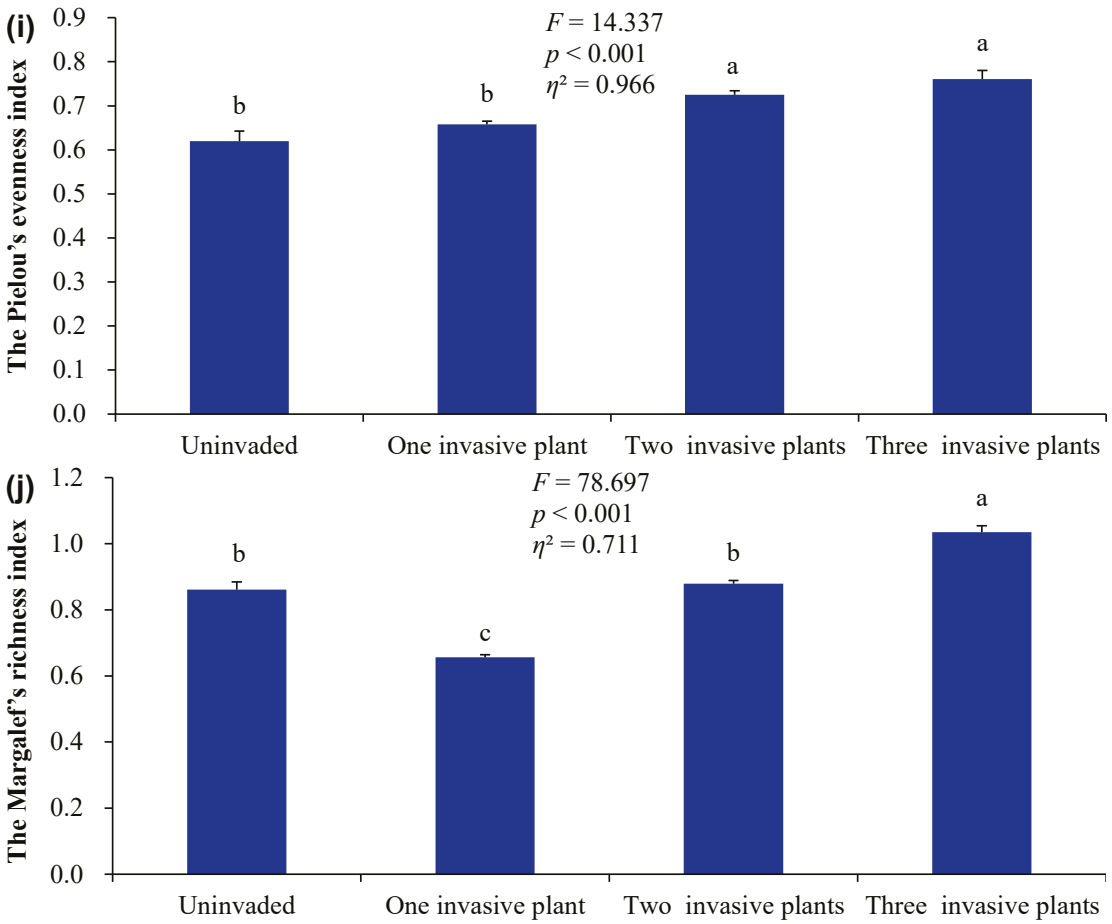

Figure 1. Differences in the measured indices under different invasion scenarios ((**a**), the species number of plants; (**b**), the max relative coverage of all plants; (**c**), the min relative coverage of all plants; (**d**), the difference between max and min relative coverage of all plants; (**e**), the ratio of max and min relative coverage of all plants; (**f**), the intensity index of interspecific interactions among multiple plants; (**g**), the Shannon's diversity index; (**h**), the Simpson's dominance index; (**i**), the Pielou's evenness index; (**j**), the Margalef's richness index). Data (mean and standard error) with different lowercase letters indicate statistically significant differences at 0.05 probability.

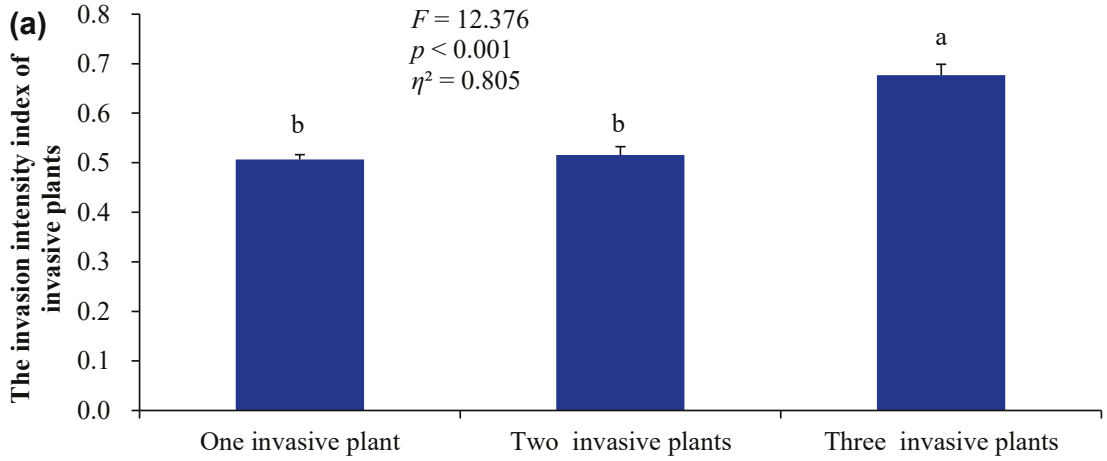

Figure 2. *Cont.*

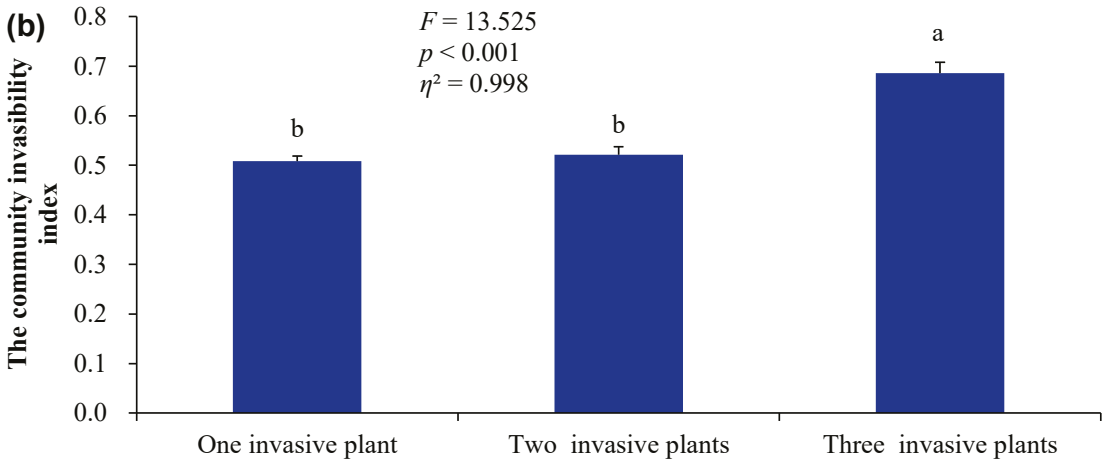

Figure 2. Differences in the invasion intensity index of invasive plants (**a**) and the community invasibility index (**b**) under different invasion scenarios. Data (mean and standard error) with different lowercase letters indicate statistically significant differences at 0.05 probability.

S_i was positively correlated with the Shannon's diversity index, the Simpson's dominance index, the Margalef's richness index, the invasion intensity index of IPS and the community invasibility index ($p < 0.001$; Table 1). S_i was negatively associated with the max and min relative coverage of all plants as well as the difference between the max and min relative coverage of all plants ($p < 0.001$; Table 1).

The invasion intensity index of IPS was positively correlated with the community invasibility index ($p < 0.001$; Table 1). The invasion intensity index of IPS and the community invasibility index were positively linked to the max relative coverage of all plants, the difference between the max and min relative coverage of all plants and the ratio of the max and min relative coverage of all plants ($p < 0.001$; Table 1). The invasion intensity index of IPS and the community invasibility index were negatively associated with the Shannon's diversity index, the Simpson's dominance index and the Margalef's richness index ($p < 0.001$; Table 1).

IIII was positively correlated with the max relative coverage of all plants, the difference between the max and min relative coverage of all plants, the ratio of the max and min relative coverage of all plants, the invasion intensity index of IPS and the community invasibility index ($p < 0.001$; Table 1). *IIII* was negatively associated with the min relative coverage of all plants and all estimated plant taxonomic diversity indices ($p < 0.001$; Table 1).

3.3. Influences of S_i on the Measured Variances as Well as Influences of the Measured Variances on IIII Under Different Invasion Scenarios

The ratio of the max and min relative coverage of all plants activated the max positive influence on *IIII* among the measured variances ($p < 0.05$; Figure 3).

Pielou's evenness index generated max negative influences on *IIII* among the measured variances ($p < 0.05$; Figure 3).

Table 1. Correlation (*r*, the Pearson's coefficient) between the measured indices under different invasion scenarios. *p* values equal to or lower than the corrected *p* value (corrected *p* value = 0.05/(13 × 12/2) = 0.000641) by using Bonferroni correction are shown in bold. Abbreviation: *S*, the species number of plants; $MaxRC_i$, the max relative coverage of all plants; $MinRC_i$, the min relative coverage of all plants; *IIII*, the intensity index of interspecific interactions among multiple plants; *H′*, the Shannon's diversity index; *D*, the Simpson's dominance index; E_H, the Pielou's evenness index; *F*, the Margalef's richness index; *III*, the invasion intensity index of invasive plants; *CII*, the community invasibility index; S_i, the species number of invasive plants.

r	*S*	$MaxRC_i$	$MinRC_i$	Difference Between $MaxRC_i$ and $MinRC_i$	Ratio of $MaxRC_i$ and $MinRC_i$	*IIII*	*H′*	*D*	E_H	*F*	*III*	*CII*	S_i
S	1	**−0.599**	**−0.474**	**−0.381**	**0.149**	−0.064	**0.787**	**0.661**	**0.303**	**0.936**	**−0.380**	**−0.378**	**0.374**
$MaxRC_i$		1	−0.022	**0.932**	**0.367**	**0.409**	**−0.928**	**−0.967**	**−0.864**	**−0.617**	**0.481**	**0.478**	**−0.276**
$MinRC_i$			1	**−0.383**	**−0.436**	**−0.365**	**−0.134**	**−00.015**	**0.390**	**−0.434**	0.033	0.033	**−0.122**
Difference between $MaxRC_i$ and $MinRC_i$				1	**0.497**	**0.510**	**−0.808**	**−0.888**	**−0.940**	**−0.412**	**0.432**	**0.430**	**−0.211**
Ratio of $MaxRC_i$ and $MinRC_i$					1	**0.845**	**−0.302**	**−0.356**	**−0.569**	00.041	**0.189**	**0.189**	<0.001
IIII						**1**	**−0.419**	**−0.449**	**−0.642**	**−0.146**	**0.235**	**0.234**	−0.080
H′							1	**0.955**	**0.794**	**0.789**	**−0.493**	**−0.490**	**0.335**
D								1	**0.865**	**0.677**	**−0.505**	**−0.502**	**0.292**
E_H									1	0.345	−0.424	−0.422	0.170
F										1	**−0.387**	**−0.384**	**0.395**
III											1	**1.000**	**0.110**
CII												1	**0.118**
S_i													1

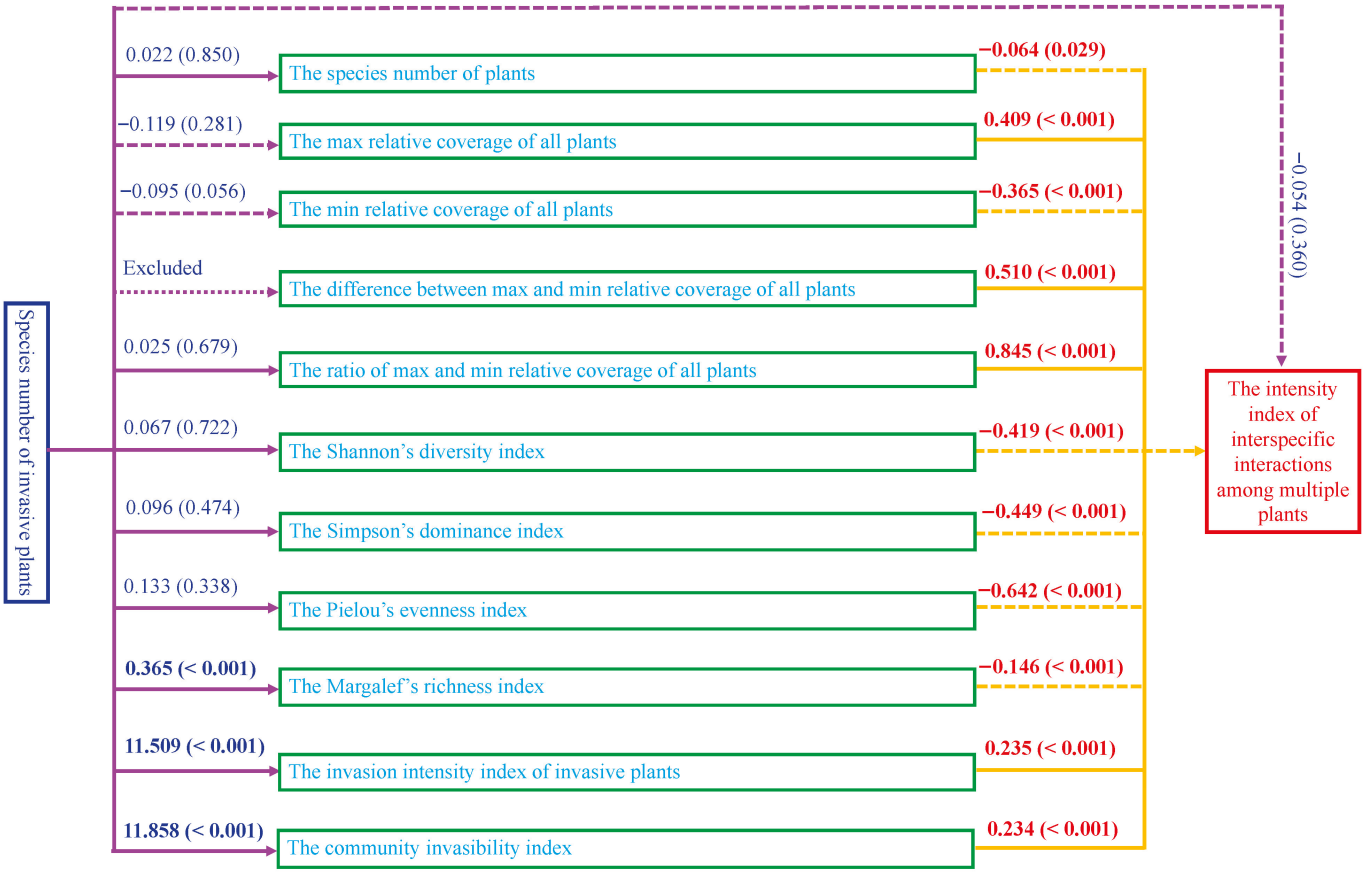

Figure 3. Schematic diagram of influences of the species number of invasive plants (dark blue numbers) on the measured variances and influences of the measured variances (fuchsia numbers) on the intensity index of interspecific interactions among multiple plants under different invasion scenarios. Positive values (solid lines) mean positive influences, while negative values (dashed lines) mean negative influences. The stronger the influence, the greater the deviation from 0, and vice versa. The p value is presented in parentheses. $p \leq 0.05$ are shown in bold.

4. Discussion

The mono-invasion achieved by one IP significantly decreased the species number of plants and plant richness in the invaded habitats compared to the uninvaded communities (Figure 1a,j). The finding may be attributed to the fact that IPS has stronger growth competitiveness compared to native plants [2,3,5]. More importantly, the results of the correlation analysis indicated that the invasion intensity of IPS was negatively correlated with plant taxonomic diversity (Table 1). This may mean that as the invasion intensity of IPS progressively upsurges, the growth competitiveness of native plants gradually declines, leading to the extinction of some species of native plants. This may further lead to a decrease in the resistance of native plant communities to IPS' invasion, while increasing their invasibility. In addition, the results of the correlation analysis showed that the invasion intensity of IPS was positively correlated with the community invasibility, but the community invasibility was negatively correlated with plant taxonomic diversity (Table 1).

In other words, high plant taxonomic diversity generates more stable plant communities that are more resistant to IPS' invasion but with lower community invasibility [19–21]. The main reason for the negative correlation between plant taxonomic diversity and community invasibility may be attributed to the fact that a higher level of plant diversity can increase the efficiency of acquisition of ecological resources, recognized as the complementary resource use, and then decrease the community invasibility, but increase the invasion

resistance [19,22–24]. The negative correlation between plant taxonomic diversity and the community invasibility may also be attributed to the asynchronism in the relative coverage of different plants, i.e., the reduction in the relative coverage of some plants may be counteracted by the amplification in the relative coverage of other functionally comparable plants [25–27]. Consequently, the mono-invasion achieved by one IP can cause a decrease in plant taxonomic diversity in the invaded habitats compared to the uninvaded communities, and plant taxonomic diversity may be negatively associated with the invasion intensity of IPS and the community invasibility.

However, the co-invasion achieved by two and three IPS recruited an increase in plant taxonomic diversity compared to the mono-invasion achieved by one IP (Figure 1a,g–j). In particular, plant taxonomic diversity increases with S_i under different invasion scenarios (Figure 1a,g–j). Furthermore, S_i was positively correlated with plant taxonomic diversity under different invasion scenarios (Table 1). Therefore, plant taxonomic diversity was linked to S_i under different invasion scenarios. This finding is inconsistent with the first hypothesis. The positive effects of S_i on plant taxonomic diversity under different invasion scenarios may be ascribed to the fact that the co-invasion of multiple IPS permits these IPS to act as a positive driver rather than a passenger [19,28,29]. In addition, this phenomenon may be attributable to the antagonistic effects rather than synergistic effects of multiple IPS on the native communities under the co-invasion scenarios compared to their mono-invasion; that is to say, multiple IPS may interfere with each other potentially when they invade together. This phenomenon is named invasion interference. Precisely, the invasion interference between multiple IPS when they invade together can lead to a reduction in their combined effects on the native plant community structure. Additionally, the invasion interference between multiple IPS under co-invasion scenarios has been identified in numerous earlier studies [18,30,31], and has been supposed to be attributed to the robust competitiveness to obtain limited ecological resources, which upsurges from the interspecific and intraspecific competition increasing gradually under different co-invasion scenarios. More importantly, based on the habitat filtering hypothesis [32–34], due to natural selection achieved through the environmental factors screening for stress, the ecological niches of plants in the same habitat may be close to each other. Nevertheless, similar ecological niches between multiple plants can cause a strong intensity in the intraspecific and interspecific competition for the acquisition of ecological resources according to the niche differentiation hypothesis and Gause's hypothesis [35–37]. Furthermore, the positive effects of the co-invasion achieved by two and three IPS may also be attributed to the fact that the co-invasion achieved by two and three IPS may trigger a certain degree of disturbance on the native plant community structure. More importantly, according to the intermediate disturbance hypothesis, the appropriate disturbance may maintain a high level of plant diversity [38–40].

The intensity of interspecific interactions among multiple plants decreased under IPS' invasion (Figure 1f). However, there was no significant difference in the intensity of interspecific interactions among multiple plants under different invasion scenarios (Figure 1f). Accordingly, the intensity of interspecific interactions among multiple plants may be decreased under IPS' invasion, regardless of the invasion scenario. This finding is inconsistent with the first hypothesis. This phenomenon may be attributed to the decreased ratio of the max and min relative coverage of all plants and the increased plant evenness under IPS' invasion (Figure 1e,i). The results of both the correlation analysis and the path analysis presented that the intensity of interspecific interactions among multiple plants was positively correlated with the ratio of the max and min relative coverage of all plants but was negatively associated with plant evenness (Table 1 and Figure 3). Hence, the result is inconsistent with the second hypothesis. Therefore, compared to the habitats

with lower ratios of the max and min relative coverage of all plants (but with higher plant evenness), habitats with higher ratios of the max and min relative coverage of all plants (but with lower plant evenness) have a stronger intensity of interspecific interactions among multiple plants. In other words, once the distribution of different plants becomes more non-uniform, gradually shifting from uniformity in a given habitat, the ratio of the max and min relative coverage of all plants will progressively increase, and the same changes will also occur in the intensity of interspecific interactions among multiple plants. In particular, the intensified interspecific interactions among multiple plants can lead to an increase in the relative coverage of plants with stronger competitive ability for ecological resources, but result in a decrease in the relative coverage or even loss of plants with weaker competitive ability for ecological resources, with gradually increase in the ratio of the max and min relative coverage of all plants but with progressively decrease in plant evenness. This will create a possible situation where the strong become stronger and the weak become weaker.

5. Conclusions

This study aimed to determine how the plant community structure affects the intensity of interspecific interactions among multiple plants under different invasion scenarios. The mono-invasion achieved by one IP decreased the species number of plants and plant richness in the invaded habitats compared to the uninvaded communities. Plant taxonomic diversity increased with S_i under different invasion scenarios. The invasion intensity of IPS and the community invasibility were negatively correlated with plant taxonomic diversity. IPS' invasion, regardless of the invasion scenario, decreased the intensity of interspecific interactions among multiple plants compared to the uninvaded communities. The intensity of interspecific interactions among multiple plants was positively correlated with the ratio of the max and min relative coverage of all plants but was negatively correlated with plant evenness under different invasion scenarios. Thus, the ratio of the max and min relative coverage of all plants and plant evenness may be the main factor regulating the intensity of interspecific interactions among multiple plants under different invasion scenarios, rather than the species number of invasive plants.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/plants14172767/s1, Table S1: The name and the corresponding information about the survey sites in this study; Table S2: Information about the species composition, source area and life form of invasive plants; Table S3: The determination methods and the corresponding references for the analyzed variables in this study.

Author Contributions: Y.D.: data curation, investigation, methodology and writing—review and editing; Y.L.: data curation, investigation, methodology and writing—review and editing; X.G.: data curation, investigation, methodology and writing—review and editing; Y.Z.: data curation, investigation, methodology and writing—review and editing; C.W.: conceptualization, formal analysis, funding acquisition, project administration, supervision and writing—original draft preparation; D.D.: funding acquisition, project administration writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Risk Assessment of Alien Woody Plants in China's National Nature Reserves Under Climate Change

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Abstract: Alien woody plants (AWPs) increasingly threaten biodiversity in China's national nature reserves, with climate change expected to intensify these risks. We used species distribution modeling (MaxEnt) and spatial prioritization (Zonation) to assess invasion risk for 251 AWP species across 479 national nature reserves under current and future climate scenarios (SSP245 and SSP585). Spatial prioritization revealed current hotspots in southern tropical–subtropical national nature reserves (e.g., Hainan, Fujian, Yunnan provinces), with significant northward and westward expansion projected under warming. A total of 71 species—such as *Quercus robur*, *Salix alba*, and *Robinia pseudoacacia*—pose consistently high risks, while some others (e.g., *Ficus benghalensis*) may become emerging threats under future conditions. These range shifts are driven by thermal constraint relaxation and longer growing seasons. To mitigate future impacts, we recommend region-specific strategies: containment and seed-source control in southern national nature reserves, and early detection and monitoring in northern and western regions. Our findings provide a spatially explicit framework for climate-informed invasive species management in protected areas.

Keywords: alien woody plants; climate change; national nature reserves; MaxEnt modeling; species distribution modeling

1. Introduction

Plant invasion is the process by which alien plant species spread rapidly and establish stable populations in their non-native habitats, negatively affecting local biodiversity and ecosystem function [1]. Alien woody plants (AWP) pose a profound and persistent threat to terrestrial ecosystems, and their longevity, structural dominance and ability to alter ecosystem functioning make them distinct from other invasive species forms [2]. Unlike herbaceous invaders, invasive trees often trigger cascading effects from canopy dominance to soil legacy effects [3] and its deep root system may disrupt the hydrologic cycle [4]. These invasions often lead to biodiversity loss in protected areas, as AWPs outcompete native species for resources such as light, water, and nutrients [5]. Additionally, they degrade ecosystem services critical for human well-being, including water regulation, carbon sequestration, and soil stabilization [6].

Climate change is reshaping global biodiversity patterns and invasive species are both drivers and beneficiaries of ecosystem change. Warmer temperatures and changes in precipitation are expanding the potential ecological niche for non-native plants, especially woody

invasive species, which threaten native forest ecosystems through competitive exclusion, disruption of the carbon cycle, and degradation of ecosystem services (e.g., water regulation and soil conservation) [7]. Climate change can intensify woody plant invasions worldwide by reshaping ecosystem conditions, including temperature, precipitation regimes, and seasonality [8,9]. Such shifts create more favorable environments for AWPs, influencing their growth and distribution [10]. For instance, warmer climates enhance survival and reproduction, enabling species previously constrained by low temperatures to expand into higher latitudes or colder regions [11]. Warming will result in a longer growing season, allowing AWP to grow and reproduce further [12]. These changes have not only altered the distribution of species, but also exacerbated pressures on protected areas, traditionally seen as strongholds of biodiversity, which now face the dual challenge of mitigating climate impacts while resisting homogenization [13]. However, the synergistic effects of climate change and biological invasions on these critical habitats remain under-quantified, especially in biodiversity-rich regions such as China, where complex topography and diverse climates create unique opportunities for invasive species to take advantage of changing environmental conditions.

China is one of the richest countries in the world in terms of biodiversity and also a hotspot for biological invasions [14]. China's unique geographic and climatic features—such as its broad latitudinal span ranging from tropical to cold-temperate zones—contribute to its exceptionally high biodiversity [15]. However, this biogeographic advantage also makes China highly vulnerable to the twin threats of climate change and biological invasions [16]. For example, the rate of invasion of AWPs in China has accelerated significantly in recent decades with the intensification of international trade and climate warming. Some of these species pose serious threats to the stability of native ecosystems [16]. As a cornerstone of biodiversity conservation, a nature reserve is legally defined as an area designated for the special protection and management of representative natural ecosystems; natural concentrated distribution areas of rare and endangered wildlife and plant species; and land, water, or sea areas housing natural relics of special significance [17]. These natural reserves provide essential habitat for endangered species, such as giant pandas and northeastern tigers [18,19]. Additionally, they play a crucial role in sustaining ecosystem services [17,20].

While earlier research has identified region-specific challenges of AWP invasions—such as elevated invasion risks in northern China and stronger competition in the south—systematic and comprehensive evaluations of these risks across national nature reserves under current and future climate scenarios remain scarce [21,22]. This study addresses this critical knowledge gap by providing the first large-scale, spatially explicit evaluation of invasion dynamics for 251 alien woody species across all national nature reserves in China under multiple climate change scenarios. By integrating species distribution models with conservation prioritization tools, our research offers novel insights into how climate change differentially affects woody plant invasions across biogeographical regions. These findings are essential for informing adaptive management strategies and serve as a scientific reference for biodiversity conservation and invasive species control in global biodiversity hotspots.

This study aims to answer the two questions:(1) Which regions in China's national nature reserves are at the highest risk of AWP invasion under current and future climates? and (2) Which AWP species pose the greatest threats to these protected areas? This study can reveal the combined effects of climate change and invasion of alien woody plants, fill the gap of multi-species and multi-scenario invasion risk assessment in China, and provide quantitative basis for the development of climate.

2. Results

2.1. Species Distribution Patterns and Trends in the Context of Climate Change

For the 251 Chinese alien woody plants, the average AUC was 0.909 (±0.071 standard deviation [SD]) for training data and 0.907 (±0.070 SD) for test data (Table S1, Figure S1). The results showed that the maxent model performed well, and all the results are applicable to further experiments involving Chinese alien woody plants. Under current and future climatic conditions, the high distribution areas of the 251 Chinese alien woody plants are concentrated in Hainan, Fujian, Zhejiang and Yunnan provinces (Figure 1). However, with increasing climate change, the alien woody plants will expand to the north (Figure 2).

Under the current climatic conditions, the average distribution probability alien woody plants of in national nature reserves shows obvious regional differences. The average distribution probability of alien woody plants in nature reserves in southern provinces such as Hainan is generally high (Figure 1), especially in nature reserves such as Diaoluoshan National Nature Reserve (Average distribution probability = 0.227), Zhangjiangkou Mangrove Forest National Nature Reserve (average distribution probability = 0.214), Wuzhishan National Nature Reserve (average distribution probability = 0.213), Dongzhai Harbor National Nature Reserve (average distribution probability = 0.201) and Cangshan Erhai National Nature Reserve (average distribution probability = 0.201). Some nature reserves in the central and eastern regions have intermediate distribution probabilities. The distribution probability of nature reserves in the northern and western regions is low.

However, under the future climate scenarios (SSP245 and SSP585), the distribution probability of alien woody plants increases significantly in national nature reserves in the northern and western regions (Figure 2). For example, the distribution probability of invasive woody plant species in national nature reserves in the northern region, such as Heilongjiang Langxiang National Nature Reserve and Heilongjiang Qixing Lazi Northeast Tiger National Nature Reserve, showed an increasing trend. Meanwhile, the distribution probability of invasive woody plants in nature reserves in western regions, such as Gansu Annanba Wild Camel National Nature Reserve and Ningxia Habahu National Nature Reserve, has also increased (Figure 2). In contrast, the distribution probability of invasive woody plants in national nature reserves in the southern and central regions generally decreased.

To address potential concerns regarding the interpretation of relatively low mean probability values (e.g., ~0.2), we conducted a correlation analysis between our continuous mean probability metric and an alternative risk indicator: the number of species predicted to occur using a binary threshold of 0.5. A strong and statistically significant positive correlation ($r = 0.90$, $p < 0.001$) was found between these two metrics. This high correlation demonstrates that areas identified as high-risk by our continuous probability approach are the same areas identified as high-risk by the binary threshold method. Therefore, our use of mean probability as a risk indicator is statistically robust and effectively captures the spatial pattern of multi-species invasion pressure, validating its application in our study.

2.2. Highest Risk Areas and Protected Areas

Zonation analysis showed that the invasion risk of alien woody plants in China's national nature reserves exhibited significant spatial differentiation (Figure 3). Under current climate conditions, southern reserves showed the highest invasion risk, concentrated in tropical–subtropical areas such as Hainan and Fujian. In contrast, northern reserves (e.g., Huzhong National Nature Reserve (Heilongjiang)) and western arid zones (e.g., Altun Shan National Nature Reserve (Xinjiang)) exhibited lower risks.

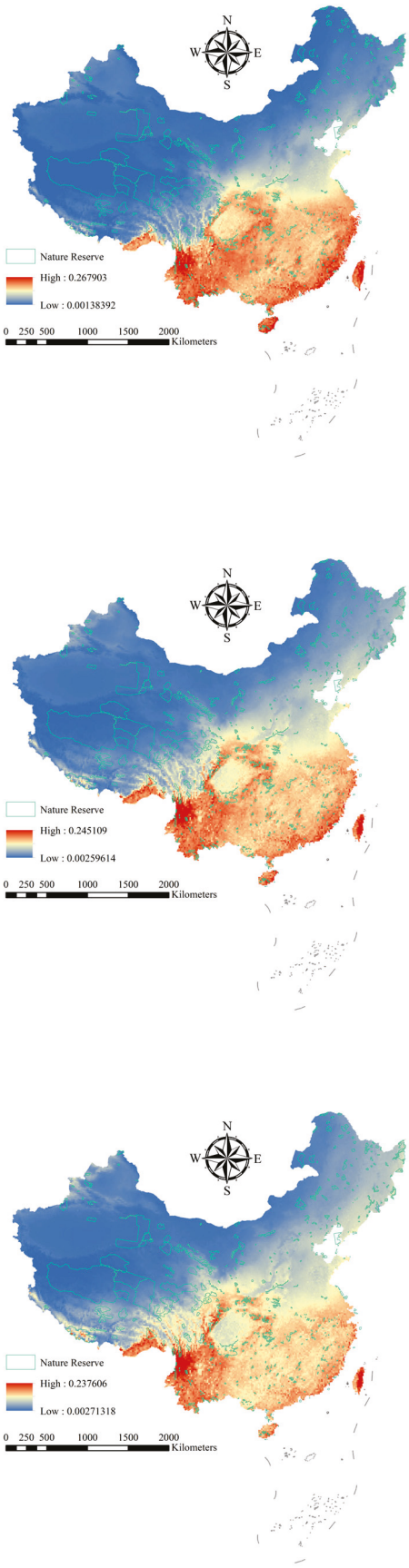

Figure 1. Average distribution probability of alien woody plants under different climate scenarios (current, SSP245, and SSP585). Red, yellow, and blue represent high, medium, and low probabilities of plant species distributions, respectively.

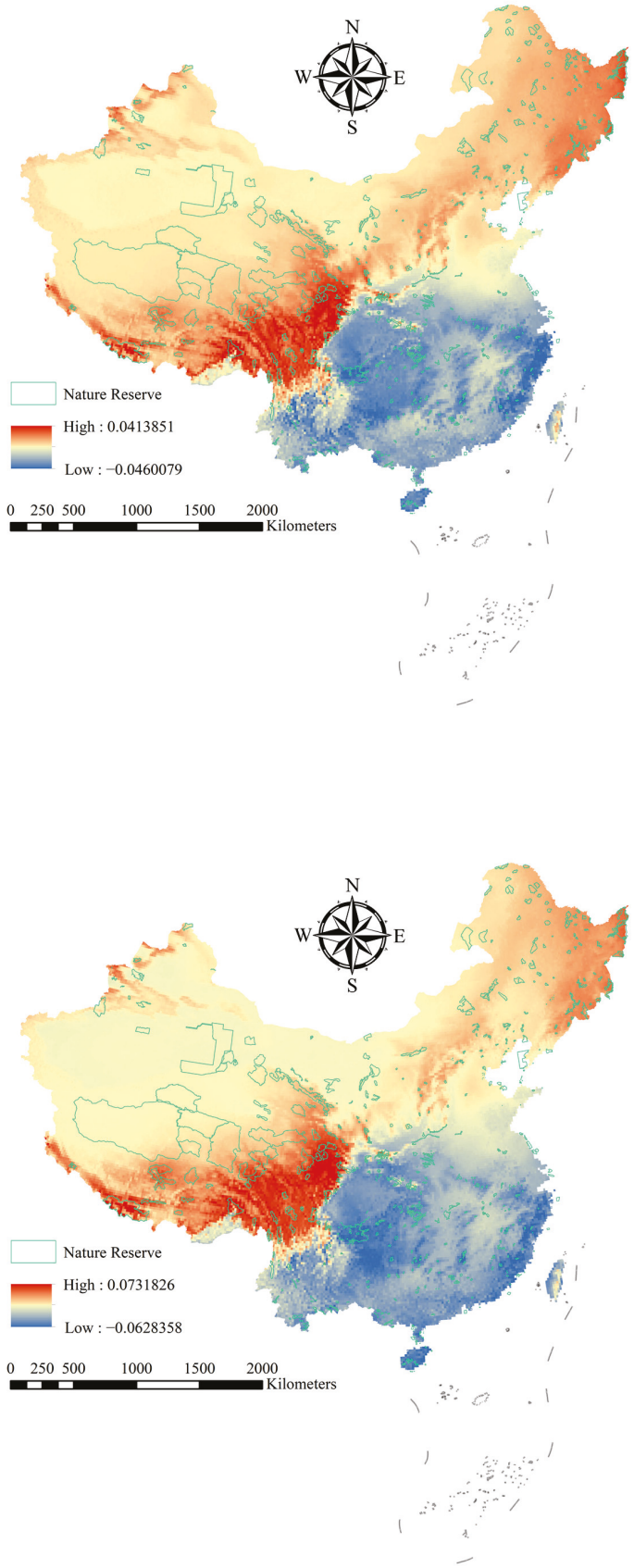

Figure 2. Change in the potential distribution of alien woody plants between current and future distributions. Red, yellow, and blue represent high, medium, and low probabilities of plant species distributions, respectively.

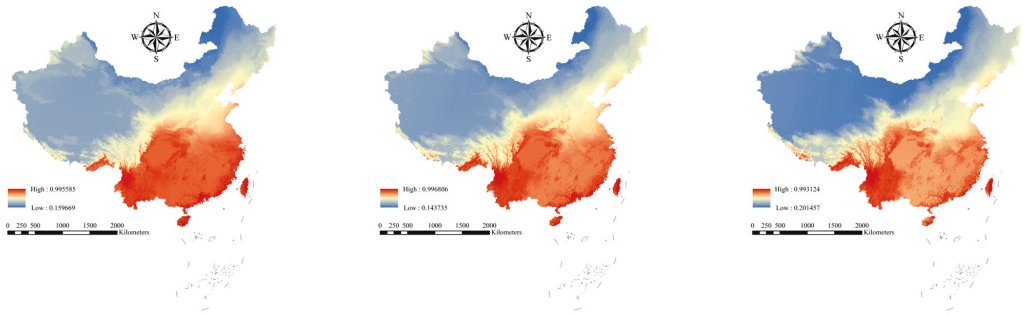

Figure 3. Spatial prioritization of AWP invasion risk in China under current and future climate scenarios (SSP245 and SSP585). Red, yellow, and blue represent high, medium, and low probabilities of plant species distributions, respectively.

Under the SSP245 scenario, the invasion risk increases in northern and western national nature reserves, mainly concentrated in Heilongjiang, Inner Mongolia, and other provinces (Table S2). Examples include Chonar River National Nature Reserve (Heilongjiang;
RR245 = 0.315) and Bila River National Nature Reserve (Inner Mongolia; RR245 = 0.294). The SSP585 scenario exacerbates the expansion trend, with heightened risks primarily in Heilongjiang, Inner Mongolia, Qinghai, and Tibet (Figure 4). For instance, the invasion risk in the Chonar River (Heilongjiang; RR585 = 0.436) is further increasing., Selin Co National Nature Reserve (Tibet; RR585 = 0.377) emerges as a new hotspot. While the southern coastal regions maintain high threats, some inland areas exhibit slight risk reduction, such as Honghu National Nature Reserve (Hubei; RR585 = −0.037).

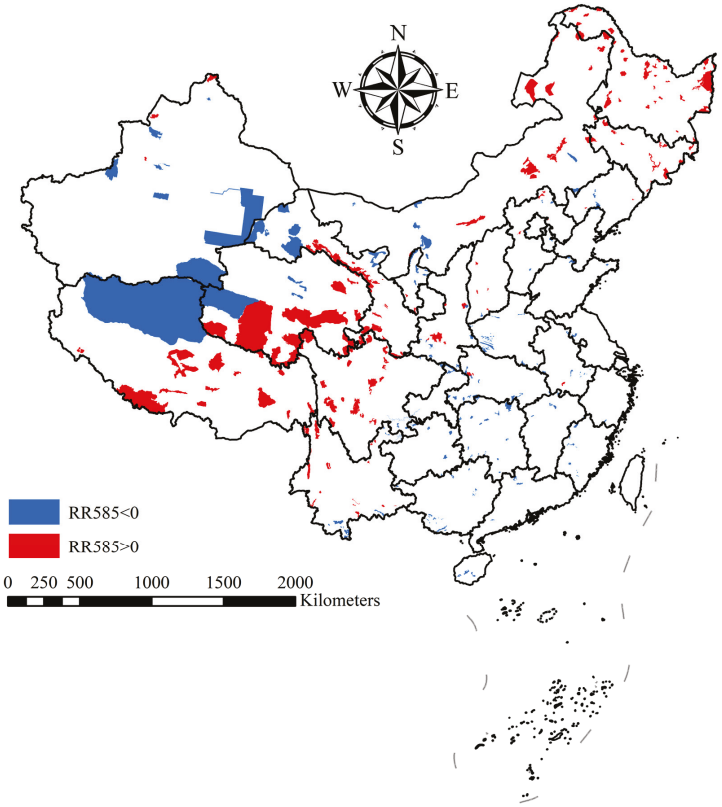

Figure 4. Logarithmic response ratios of alien woody plants in China's national nature reserves under the SSP585 climate scenario (RR > 0 represents an elevated risk of future intrusion, RR < 0 represents a reduced risk of future intrusion).

Despite the numerically low average distribution probabilities (e.g., <0.25), these values should not be interpreted as an indicator of low overall risk. The MaxEnt model outputs represent a relative index of environmental suitability rather than an absolute probability of occurrence. In large-scale ecological niche models encompassing vast and environmentally heterogeneous regions like China, absolute suitability values are naturally attenuated. The critical insight lies not in the absolute value, but in the relative spatial patterns and the temporal shifts they reveal. Our identification of high-risk areas and species is based on this relative framework. The designated hotspots (e.g., Diaoluoshan) exhibit suitability scores that are markedly higher than the vast majority of the landscape. Furthermore, the significant northward and westward expansion of suitability, quantified by the positive logarithmic response ratios (RR) in many nature reserves (e.g., $RR_{585} > 0.4$ for Chonar River), indicates a substantial increase in invasion potential due to climate change. For invasive species, even a modest shift from negligible suitability (e.g., ~0) to low suitability (e.g., 0.1–0.2) can be ecologically transformative, as it opens previously inaccessible regions to potential colonization.

2.3. List of Alien Woody Species with High Invasion Risk in National Nature Reserves

Species in the top 10% with a distribution probability higher than the threshold for alien woody species in national nature reserves are defined as high-risk species. Under both current and future climate conditions, the potentially high-risk species include *Quercus robur* L., *Salix alba* L., *Populus tremuloides* Michx., *Acer negundo* L., *Sorbaria tomentosa* (Lindl.) Rehder, *Ribes nigrum* L., *Pseudotsuga menziesii* (Mirb.) Franco, *Betula pubescens* Ehrh., *Robinia pseudoacacia* L., *Populus balsamifera* L., among a total of 71 species (Table S3).

Species that pose a low risk under the current climate but are predicted to become high-risk in the future include *Ficus benghalensis* L., *Genista tinctoria* L., *Tephrosia candida* DC., *Quercus petraea* (Matt.) LieBlume, *Abies alba* Mill., *Tilia platyphyllos* Scop., *Casuarina equisetifolia* L., *Acaciella glauca* (L.) L. Rico, *Cassia fistula* L., *Schinus molle* L., *Artocarpus heterophyllus* Lam., and *Azadirachta indica* A. Juss. (Figure 5). These species are projected to emerge as high-risk invaders within nature reserves in the future.

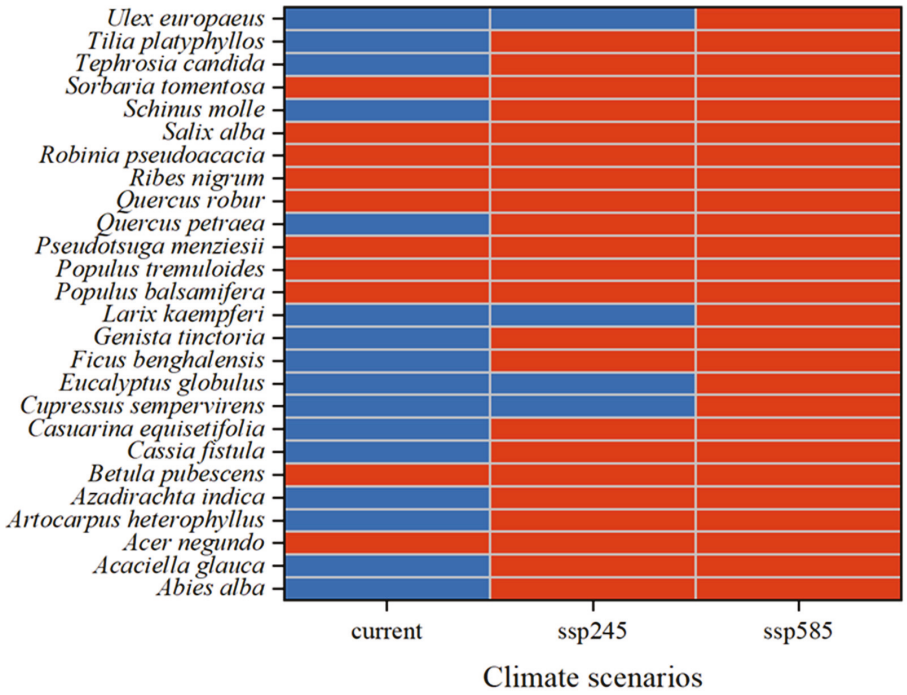

Figure 5. Alien woody plant species identified as high-risk in China's National Nature Reserves under current and future climate scenarios (current, SSP245, SSP585). Red indicates species whose

invasion probability in national nature reserves is at least 10% higher than the overall average across reserves. Blue indicates species with invasion probability in potential national parks $\leq$10% higher than in national nature reserves.

3. Discussion

3.1. High-Risk Regions for AWP Invasion Under Climate Change Scenarios

Our study demonstrates that under current climate conditions, the highest risk of AWP invasion is concentrated in tropical and subtropical national nature reserves in southern China, such as Dongzhai Port (Hainan), Zhangjiangkou Mangrove (Guangdong), and Wuyishan (Fujian). These areas exhibit high mean invasion probabilities, primarily due to favorable temperature and precipitation conditions. However, under future climate scenarios—especially the high-emission SSP585 scenario—a marked shift occurs: invasion risk expands significantly toward northern and western reserves (Figure 2).

This shift in high-risk zones may largely driven by climate-mediated release from abiotic constraints. Historically, low temperatures have limited the northward and altitudinal expansion of most AWPs, many of which are native to tropical or subtropical climates [23]. With warming, however, the mean annual temperature (Bio1) is projected to increase in northern and western China [24,25], effectively raising the cold tolerance threshold for many invasive species [26]. In addition, changes in precipitation seasonality (Bio15) may create temporal resource pulses [27]—especially in arid western regions. These climatic shifts extend the growing season and improve seedling survival rates, enhancing the likelihood of long-term establishment.

Our findings align with previous studies, suggesting an increased risk of biological invasions in high-latitude regions in the context of global warming [28,29]. Similar patterns have been observed in the Eastern United States [11] and Mediterranean Europe [10]. However, China's unique topography introduces regional complexity [30]. The rapid warming of the Tibetan Plateau creates novel ecological niches at high elevations [29,31]. Additionally, our multi-species approach—modeling 251 AWPs simultaneously—extends beyond prior work that focused on single species (e.g., *Robinia pseudoacacia*, *Quercus robur*), providing a broader landscape of emerging threats.

Given the climate-driven expansion of AWP distribution toward northern and western China, invasion prevention strategies must be regionally tailored and forward-looking. In southern reserves—such as Dongzhai Port (Hainan) and Wuyishan (Fujian)—which currently face high invasion pressure, management should emphasize the suppression of established seed sources and the prevention of secondary spread. This includes mechanical removal, habitat restoration, and the establishment of early detection and rapid response mechanisms to mitigate further ecological degradation [32]. In northern and western reserves—where risk is currently low but rapidly increasing—monitoring networks must be expanded to detect early signs of colonization, particularly during vulnerable phenological windows like the spring thaw [33]. In coastal reserves such as the Sanya Coral Reefs and Chongming Dongtan, the low probability of AWP invasion may be largely due to harsh abiotic conditions—such as high salinity, periodic inundation, and poor soil aeration—which are inherently unsuitable for the establishment and growth of most woody species [34,35]. These natural barriers, combined with effective habitat protection and quarantine regulations, help suppress potential invasions. While these coastal mechanisms are not directly transferable to inland ecosystems, their effectiveness underscores the value of targeted, context-specific management strategies for mitigating invasion risks under diverse ecological conditions [36]. Overall, adopting a spatially differentiated, climate-informed management framework is essential to safeguard biodiversity in China's national nature reserves under future global change scenarios [13,20,37].

3.2. High-Risk AWP Species in China's National Nature Reserves

Our multi-scenario analysis identified 71 AWP species as high-risk invaders in China's national nature reserves under current and future climate conditions. Among these, several species consistently exhibited high distribution probabilities across all scenarios, including *Q. robur*, *S. tomentosa*, *S. alba*, *P. tremuloides*, *A. negundo*, and *R. pseudoacacia.* These species currently have high establishment potential in southern and eastern reserves and are projected to expand into northern and western regions with increasing climatic suitability. Additionally, 12 species previously considered low risk under the current climate—such as *F. benghalensis*, *S. molle*, *T. platyphyllos*, and *A. indica*—emerge as high-risk invaders under future climate scenarios (SSP245 and SSP585). Their distribution probabilities exceeded the average risk threshold of all reserves by more than 10%, indicating a significant invasion potential under warming conditions.

The success of these invasive species is closely related to their functional traits. Many high-risk alien woody plants (AWP) exhibit strong phenotypic plasticity, rapid growth rates, prolific reproductive capacity, and deep root systems. A representative example is *R. pseudoacacia* (black locust), native to temperate and subtropical North America. Introduced to Europe in the 17th century and to China in the 19th century, it has since been widely cultivated across Eurasia due to its fast growth, strong adaptability, and high economic value [38,39]. *R. pseudoacacia* can improve soil nutrient conditions through nitrogen fixation, creating a positive feedback mechanism that favors its own growth [40–42]. In the past 30–40 years, owing to the transformation of land use from arable land to artificial forests in the Tisza floodplain as well as poor forest management, the spread of *R. pseudoacacia* has been exceptionally rapid [43]. *P. tremuloides* and *S. alba* demonstrate strong competitive advantages in riparian zones and disturbed habitats through clonal reproduction and rapid canopy establishment [44]. Our study found that *Q. robur* has a high distribution probability under current and future climate scenarios. *Q. robur* is native to France and Italy in Europe. It has been introduced and cultivated in Xinjiang, Beijing and Shandong, China [45]. *Q. robur* can grow on many types of soil, including sandy, clay and limestone soils, giving it a wide distribution. Its resistance to wind, soot, short-term water and humidity, and the presence of dormant buds in its branches and strong tillering ability are also important reasons for its wide distribution.

Notably, several of the 12 species identified as emerging high-risk invaders—such as *A. indica*, *A. heterophyllus*, and *F. benghalensis*—are native to tropical or subtropical regions. These species are currently constrained by low-temperature thresholds, but under future climate scenarios, their suitable habitats are projected to shift toward higher latitudes and elevations. Climate warming reduces cold-related mortality, enhances seedling establishment, and allows thermophilic species to invade areas previously climatically inaccessible [46]. For instance, Osland and Feher (2020) demonstrated that warming winters reduce freeze events, enabling tropical species like *Schinus terebinthifolius* Raddi to expand into temperate zones [47]. Similarly, Keller (2021) found that elevated temperatures accelerate the life cycle of invasive plants, increasing survival and growth rates, which can lead to higher reproductive output [48]. These ecological and physiological responses to warming climates suggest that tropical invasive woody plants may increasingly establish and spread in temperate ecosystems, posing heightened invasion risks under future climate scenarios.

Effective management of high-risk AWP species necessitates differentiated strategies tailored to their ecological characteristics and invasion dynamics. For established invaders like *R. pseudoacacia* (black locust), which has been extensively planted for purposes such as ecological restoration and erosion control, management practices should focus on mitigating its invasive potential. Recommended measures include site-specific planting, avoiding

monocultures, and integrating native species to promote biodiversity. In areas where *R. pseudoacacia* dominates, converting pure stands into mixed forests and implementing mechanical or biological control methods can help suppress its spread [49].

Beyond *R. pseudoacacia*, other high-risk AWP species identified in this study, such as *Q. robur*, *S. alba*, *P. tremuloides*, and *A. negundo*, require species-specific approaches based on their reproductive modes, dispersal vectors, and ecological niches [50]. For instance, clonal species like *S. alba* and *P. tremuloides* can rapidly colonize riparian zones and disturbed areas, necessitating the use of hydrological restoration or riparian buffer planting to limit establishment [51]. Wind-dispersed species such as *A. negundo* may require coordinated management at landscape scales, including seed source removal and the regulation of human-mediated dispersal pathways (e.g., roadside planting, horticulture) [52]. For species that establish dense canopies or alter soil properties (e.g., *Q. robur*), periodic understory monitoring and adaptive thinning practices may be employed to restore light availability and soil biota integrity [53]. Overall, integrated management frameworks that combine early detection, population containment, habitat restoration, and long-term monitoring are essential for curbing the expansion of high-risk AWP species, especially in climate-sensitive protected areas [7,54].

For emerging invasive species—many of which are currently undergoing climate-driven range expansions from tropical or subtropical regions—prevention and early detection are key [7,55,56]. A forward-looking strategy should involve the development of trait-based risk screening tools that evaluate attributes such as reproductive capacity, dispersal ability, and stress tolerance, which have been identified as reliable predictors of invasion success [57]. Moreover, strict regulation of horticultural introductions and commercial plant trade near the buffer zones of protected areas is needed to minimize propagule pressure [58]. Ecological restoration following invasive species removal should prioritize the reintroduction of native species with similar functional roles to prevent the emergence of vacant ecological niches and subsequent secondary invasions [59].

Our results also align with emerging international evidence that climate change is reshaping invasion risks in protected areas worldwide. In the eastern United States, climate warming of +2 °C is projected to drive invasive plant "hotspots" northeastward by ~200–250 km, exposing new reserves and natural areas to non-native species pressure [60]. In Europe, projections indicate that many alien deciduous tree species will expand their climatic niches northward or to higher elevations under 2061–2080 scenarios, while coniferous aliens may contract depending on local conditions [61]. In South America, case studies in Argentina show that woody invaders such as *Ligustrum lucidum* and *Acacia melanoxylon* are already threatening mountain forests, subtropical forests, and temperate grasslands, with risks amplified by climate change and land-use pressures [62]. These findings resonate with our China-wide analysis and reinforce that the methodological framework and adaptive management strategies we propose are transferable to other regions confronting climate-driven biological invasions.

This study is subject to several limitations. First, projections of invasion risk are inherently constrained by uncertainties in climate forecasts. While we utilized established GHG emission scenarios and GCM ensembles, unforeseen climatic shifts or deviations from these pathways could alter the projected distribution of suitable habitats for alien woody plants. Second, although the AUC statistic is widely used for model evaluation, its application over large geographic extents has recognized drawbacks. AUC values can be influenced by species prevalence and background sampling methods, potentially overestimating performance when applied across broad and heterogeneous landscapes. Third, the translation of continuous habitat suitability into binary presence-absence maps requires threshold selection, which introduces subjectivity. Different thresholding methods can

produce substantially different range estimates, adding uncertainty to spatial projections of invasion risk. Future work would benefit from employing multi-model ensembles to capture climate uncertainty, using complementary evaluation metrics beyond AUC, and conducting sensitivity analyses on threshold selection.

4. Material and Methods

4.1. Species Data

All 251 AWPs were selected strictly from China's National Invasive Species List, ensuring their documented impacts within Chinese ecosystems [63]. These AWP exhibit three defining attributes: rapid growth rates coupled with prolific reproductive potential, detrimental effects on native biodiversity and ecosystem functioning, and extensive geographical ranges associated with substantial invasive impacts [2,3,64]. The occurrence records of AWP were downloaded from the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/ (accessed on 24 September 2025)). To capture the full global climatic niche of each species, we used all available global occurrence records for each species, without geographic filtering. Data for the 251 species were retrieved through multiple download tasks (see Data Availability Statement for a representative DOI). Taxonomic standardization was performed using the 'Species Matching' tool via the GBIF API, which aligns all names with the Catalog of Life backbone taxonomy. We accepted only records resolved to 'ACCEPTED' species names, automatically merging synonyms under their accepted names. We removed records of occurrences with the same latitude and longitude before inclusion in the modeling, and we corrected coordinates that did not match in latitude and longitude to minimize errors. We removed duplicate records within a given spatial resolution region to minimize the effect of sampling bias on the results.

4.2. Climatic and Topographic Data

We have selected climate change models from the global general circulation model, which are primarily used to project and assess future climate change trends and impacts [65]. We downloaded 19 bioclimatic variables (bio1–bio19) at 10 arc-minutes spatial resolution from the WorldClim database (https://www.worldclim.org/) to represent both current and future climate conditions [66]. The current climate dataset represents the baseline period of 1970–2000. For future climate projections (2081–2100), we selected three general circulation models (GCMs) under the Coupled Model Intercomparison Project Phase 6 (CMIP6): MIROC6, MPI-ESM1-2-HR, and MRI-ESM2-0. These models were chosen for their wide use and complementary simulation performances in East Asia. Two shared socioeconomic pathway scenarios (SSPs) were applied: SSP245, representing a moderate emission trajectory with partial mitigation efforts, and SSP585, representing a high-emission pathway with continued increases in greenhouse gas emissions. Under these scenarios, global average temperature increases are projected to remain within 2 °C for SSP245 and potentially exceed 4 °C for SSP585 by the end of the century. For each of the three GCMs and two future scenarios, we extracted four key variables (bio1, bio4, bio12, and bio15) and calculated their weighted average to generate ensemble climate layers.

The selection of these four variables—mean annual temperature (bio1), temperature seasonality (bio4), annual precipitation (bio12), and precipitation seasonality (bio15)—was based on two criteria: (1) ecological relevance for plant growth and invasion dynamics, and (2) low multicollinearity ($|r| < 0.8$) among variables, assessed via Pearson correlation analysis using IBM SPSS Statistics (version 26). This ensured that the final model inputs captured climatic gradients critical to the potential distribution of AWPs, while minimizing redundancy. All environmental layers were spatially standardized and resampled to 10 km resolution using ArcGIS 10.8 to ensure consistency in modeling.

We used five terrain data factors from https://www.earthenv.org, which include elevation, roughness, terrain roughness index (TRI), vector roughness measure (VRM), and terrain position index (TPI), all at 10 km resolution [67].

4.3. National Nature Reserve Data

The spatial boundaries and administrative attributes of China's national nature reserves were obtained from the National Ecological Protection Database, managed by the Ministry of Ecology and Environment. The dataset includes 479 reserves designated before 2020, covering all major ecosystem types (e.g., forests, wetlands, grasslands) and endangered species habitats (e.g., giant pandas, Siberian tigers). The map of China's nature reserves was created using the Resource and Environmental Science and Data Center (https://www.resdc.cn/Default.aspx (accessed on 24 September 2025)) and World Database on Protected Areas (https://www.protectedplanet.net/).

4.4. Species Distribution Modeling

Species distribution modeling is a tool used to predict the potential distribution of species under different environmental conditions [68]. These models are often based on correlations between species and environmental factors, such as climate, topography, and soils. Using species occurrence data and environmental data, species distribution models can be constructed to simulate the distribution of species under current and future climatic conditions. MaxEnt models sometimes outperform other techniques in predicting the potential area of a species, requiring only a small sample size of presence-only data [69,70].

The distribution of 251 AWP was predicted using MaxEnt, which predicts the probability and suitability of the distribution of AWP using only presence-only species distribution data and environmental variables [71]. The results of the MaxEnt model yielded species distribution probabilities ranging from 0 to 1. We selected a fixed regularization multiplier (RM) value of 2.0 across all species based on preliminary sensitivity analyses. For a representative subset of species, we tested RM values ranging from 0.5 to 5.0 and evaluated model performance. We found that RM = 2.0 consistently yielded optimal or near-optimal performance for the majority of species, while effectively reducing overfitting—particularly for species with sparse or spatially clustered occurrence records. To ensure methodological consistency and facilitate cross-species comparisons, we applied this value uniformly across all models. Both the randomized training test data and test data for each dataset were repeated for four runs, with the regularization multiplier (beta) was 2.0 to produce a smooth and general response curve that represents the actual biological behavior and the other parameters at default values [72]. We used the logistic output format transformation to estimate the plant species distribution probability, which ranges from 0 (lowest) to 1 (highest) [73].

The area under the ROC Curve (AUC) value of the MaxEnt model can be used to evaluate the model's performance and prediction accuracy [70]. The AUC value ranges from 0 to 1, where 0.5 indicates that the model's prediction is equivalent to a random guess and 1 indicates that the model's prediction is completely accurate [74]. In general, the closer the AUC value is to 1, the better the performance of the model. In this study, we considered AUC values greater than 0.7 as good model performance and discarded those with AUC values less than 0.7 [75].

4.5. Zonation Spatial Prioritization Analysis

To identify priority areas for managing AWP under climate change, we employed Zonation GUI 4.0.0 for spatial conservation planning [76]. The framework integrates species distribution models (MaxEnt outputs) to generate hierarchical priority layers through iterative removal of grids with the lowest conservation value, emphasizing connectivity

and multi-species risk aggregation [69,77]. Current and future climate scenario (SSP245 and SSP585) distribution probability layers (.asc format) for 251 AWP were imported at 10 km resolution, with the "core-area" algorithm applied to prioritize high-risk invasion hotspots [78]. Zonation generated spatial priority layers for both climate scenarios, with values approaching 1 indicating regions requiring urgent management due to overlapping multi-species invasion risks [76].

To evaluate climate-driven shifts in risk, provincial-level monitoring priorities were derived by overlaying Zonation outputs with national nature reserve boundaries in ArcGIS 10.8. Mean priority scores were extracted for each reserve using Zonal Statistics, and provincial averages were calculated via Summary Statistics based on administrative attributes.

In order to assess the impacts of climate change on the risk of invasion, the logarithmic response ratio (RR) was calculated as RR = ln (Xf/Xc), where Xc and Xf represent the mean invasion risk scores for grid cells within each nature reserve under current and future climate conditions, respectively [79]. This metric is employed to quantify the proportional change in invasion risk, with positive RR values denoting elevated future risk and negative values indicating reduced vulnerability. The RR transformation provides a standardized measure of climate-mediated risk shifts across reserves while maintaining the ratio's original proportionality [80].

4.6. Statistical Analysis

To investigate the influence of climate change on woody plant invasions in protected areas, we conducted a regional statistical analysis using the information on protected areas and the potential distribution areas of AWP under different climate scenarios [28]. The probability of distribution of AWP in protected areas under the current climate scenario was compared with that under the future climate scenario [11]. If the distribution of AWP under the future climate scenario is higher than the current one, then climate change can exacerbate woody plant invasion in protected areas. If the distribution of AWP under the future climate scenario is lower than the current one, then climate change cannot exacerbate woody plant invasion in protected areas.

Finally, we used the average distribution of alien woody species in national nature reserves to calculate the distribution probability of each of the 251 invasive woody species in the nature reserves under different climate scenarios. Species were considered high-risk if their mean probability of occurrence in a potential protected area exceeded the average across national nature reserves by more than 10% [22]. Similar threshold-based classification approaches have been widely applied in invasion risk studies [81–83].

5. Conclusions

Understanding spatial–temporal invasion risks of alien woody plants (AWPs) under climate change is vital for China's nature reserves. Modeling 251 AWPs across 479 reserves revealed current invasion hotspots in the south, with future risks shifting north and west. Seventy-one high-risk species were identified, including *Q. robur*, *S. alba*, and *R. pseudoacacia*, while others like *F. benghalensis* are expected to spread further amid global change. Regional, climate-adaptive management is essential: southern reserves should prioritize containment, northern and western areas focus on early detection and monitoring. This study provides a practical framework for biodiversity conservation amid global change.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants14193006/s1, Table S1: Training AUC and test AUC for 251 Alien Woody Plants; Table S2: Logarithmic response ratios (RR) for national nature reserves under the ssp245 and ssp585 climate scenarios; Table S3: Alien woody plant species identified as high-risk

(≥10% above the mean invasion probability) in China's national nature reserves under current and future climate scenarios (Current, SSP245, SSP585); Supplementary Material S1: GBIF Data Citations.

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Data Availability Statement: Occurrence data for the 251 alien woody plants were obtained from the Global Biodiversity Information Facility (GBIF) through multiple download tasks conducted between 30 June and 2 July 2023. The data are publicly available through the following DOIs: https://doi.org/10.15468/dl.fyy9xv, https://doi.org/10.15468/dl.sayetr, . . . (and so on for all DOIs). The complete list of GBIF dataset citations is provided as Supplementary Material S1. Climatic data were sourced from WorldClim (https://www.worldclim.org), and topographic data from the EarthEnv project (https://www.earthenv.org).

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Seed Germination Ecology and Longevity of the Invasive Aquatic Plant *Sagittaria platyphylla*

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Abstract: *Sagittaria platyphylla* (Engelm.) J.G.Sm. is an invasive aquatic plant of concern in Australian freshwater systems. Understanding its seed germination ecology and seedbank longevity is critical for effective management. This study examined environmental influences on germination and longevity through three controlled experiments. Seeds germinated between 17 and 29 °C, with optimal germination (96 ± 2%) at 21 °C under a 12/12 h light/dark photoperiod. High germination (93–99%) also occurred under light in diurnal regimes of 15/5 °C, 25/15 °C, and 30/20 °C. In a burial experiment, seedlings emerged only from surface-sown seeds (76 ± 4%); no emergence occurred from buried seeds, though viability remained high, peaking at 98 ± 2% at 2.5 cm depth. A controlled aging test indicated a 50% viability loss (P_{50}) in 36 days under warm, moist laboratory conditions. Based on established criteria, *S. platyphylla* produces short-lived seeds, which are likely to persist in the substrate seedbank for <1 to 3 years. The strong light dependence of germination suggests that sediment disturbance, which exposes buried seeds to light, could significantly enhance recruitment, highlighting the importance of minimizing disturbance for effective long-term management.

Keywords: seed longevity; delta arrowhead; water weed; seedling emergence; seedbank dynamics; sagittaria

1. Introduction

Sagittaria or arrowhead [*Sagittaria platyphylla* (Engelm.) J.G.Sm.] is a highly invasive aquatic plant (IAAP) native to North America [1]. It has become naturalized and invasive in multiple regions globally, including in Australia, where it has been listed as a Weed of National Significance due to its severe ecological and economic impacts [2,3]. The species aggressively invades natural wetlands, irrigation channels, and drainage systems, forming dense monocultures that restrict water flow, degrade aquatic habitat quality, and outcompete native flora and fauna [4]. These dense stands pose a significant threat to biodiversity and waterway functionality; hence, *S. platyphylla* management is a priority in infested regions [5]. The success of *S. platyphylla* as an invader is largely attributed to its versatile reproductive strategies. It reproduces both asexually and sexually, allowing for rapid spread and population recovery after disturbance [1]. Asexual propagation occurs through fragmentation of shoots and the production of vegetative propagules such as

rhizomes, tubers, and stolons [3]. In addition to asexual propagation, the species produces large numbers of viable achenes (hereafter referred to as seeds), with estimates of up to 20,000 seeds produced per plant during a single season [6,7]. Despite the emphasis often placed on vegetative reproduction in IAAPs, seed-based recruitment from the substrate seedbank (SSB) is also playing a major role in population persistence and re-establishment following control interventions [8,9].

Seed-based recruitment from the SSB can serve as a persistent source of reinfestation [10,11]. Germination is a key stage in the plant life cycle, directly influencing population establishment, spread, and persistence [12,13]. For invasive species like *S. platyphylla*, understanding the environmental triggers that promote or inhibit germination is crucial for developing targeted control strategies. For example, synchronized germination events may allow for the application of accurately timed application of control measures (e.g., herbicides) that target all seedlings simultaneously, potentially depleting the seedbank over time [14,15]. Such synchronous germination offers an opportunity for uniform and potentially permanent control of IAAPs [15]. Conversely, understanding dormancy mechanisms and inhibitory environmental conditions may help suppress seed recruitment and prevent spreading. However, germination of aquatic plants from the SSB is influenced by numerous environmental factors such as light intensity, water temperature, seed burial depth, and seed dormancy [16].

Sagittaria platyphylla seeds have been shown to germinate year-round under favorable conditions, with a preference for silty substrates over clay [1,17]. It is believed that *S. platyphylla* germination is accelerated under anaerobic conditions, a response that is common in other Alismataceae species [1]. Insights from related species support this view: for example, *S. latifolia* Willd. requires light for germination and performs best when seeds are located near the substrate surface [18]. Similarly, *S. trifolia* L. exhibits light and temperature-dependent germination, particularly under warm (30/20 °C) and hypoxic conditions, with delayed but increasing germination after extended periods on the substrate surface [19]. In addition, *S. lancifolia* L. has been observed to reach 100% germination under light at 25 °C [20]. High seedling emergence in *S. montevidensis* Cham. & Schltdl. has been reported from seeds buried at depths of 0.5 to 1.0 cm, indicating a tolerance for shallow burial depth [21]. These observations suggest that *S. platyphylla* germination may be affected by burial depth, light, and temperature, but these factors remain poorly studied.

In addition to seed germination ecology, seed longevity in the SSB is a fundamental aspect of population dynamics and management planning [22,23]. Seed longevity or lifespan refers to the duration of seeds remain viable [24]. It is an important aspect of IAAP management as eradication cannot be deemed successful if viable seeds remain in the SSB [25]. Seed longevity is a complex characteristic that varies widely across species and even among different seed lots within the same species [24]. Moisture and temperature are key environmental factors that determine seed survival or mortality, influencing the timescale over which lifespan is measured [26]. Data on seed longevity of aquatic plants are limited, but some studies on germination and seed persistence suggested that the seeds need a special mechanism to maintain their seed viability in the natural habitat through many seasons or for ex situ storage [27]. Some IAAP seeds can remain viable for many years if buried, such as *Pontederia crassipes* Mart., which has been shown to survive up to 28 years [28], and *Gymnocoronis spilanthoides* (D.Don) DC., which has a seed longevity up to 16 years [29]. In contrast, other aquatic plant species such as *Myriophyllum petraeum* Orchard, *Myriophyllum balladoniense* Orchard, *Glossostigma drummondii* Benth., and *Rithuria submersa* Hook.f. produce seeds that are all short-lived (1 to 3 years) in the seedbank [30].

Currently, no empirical data/studies exist on the longevity of *S. platyphylla* seeds in the SSB, representing a critical knowledge gap [1]. It is difficult to estimate how long sites remain at risk of reinfestation following treatment without the understanding of seed longevity. Traditional seed longevity studies rely on long-term field burial experiments [31], which are labor-intensive and time-consuming. A more rapid and widely adopted alternative is the controlled aging test (CAT) [25]. In this method, seeds are exposed to elevated temperature (45 °C) and 60% relative humidity (RH) in a sealed chamber to accelerate the deterioration process [25]. At regular intervals, samples are removed and tested for germination to assess viability decline over time [25]. Although this method does not replicate natural conditions, it provides a practical and comparative approach for estimating relative longevity, particularly for invasive or problematic weed species where rapid data are needed [32]. Thus, this study aims to address critical gaps in our understanding of *S. platyphylla* seed ecology, focusing on factors that trigger germination and determine seed persistence in the SSB. This knowledge is essential for informing integrated control strategies that target both above-ground biomass and SSBs, thus reducing long-term reinvasion risks. Specifically, this study aims to (1) assess the influence of environmental variables (light, temperature, and burial depth) on germination, and (2) estimate seed longevity using a CAT.

2. Results

2.1. Seed Fill Analysis

The seed fill percentage was determined by analysis of the X-ray images, which revealed that the average seed fill percentage of the *S. platyphylla* seed lot was 95 ± 2%. Figure 1 presents a representative X-ray image from one replicate of the seed lot used to assess seed fill.

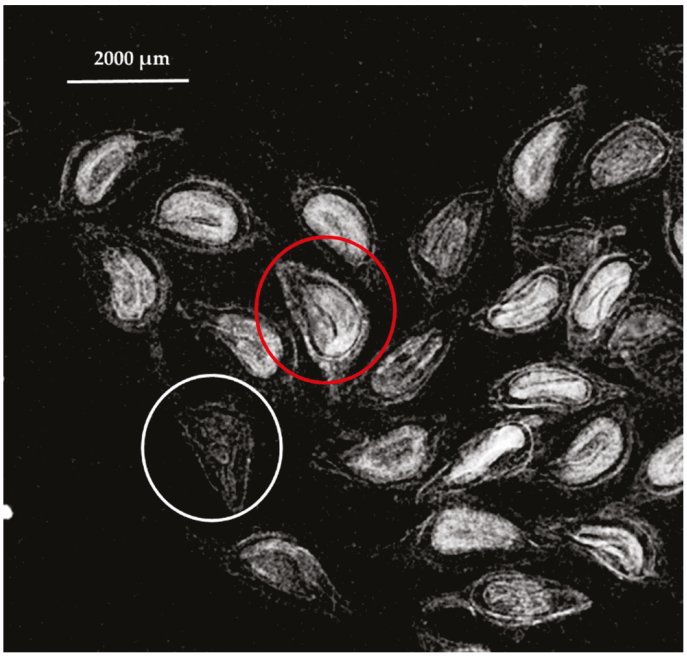

Figure 1. Seeds of *S. platyphylla* under X-ray examination (Faxitron MX-20), with the seed circled in red identified as filled and the seed circled in white considered to be unfilled.

2.2. Experiment 1—Effects of Light and Temperature on Seed Germination

A significant interaction was observed between incubation temperature and light conditions (Generalized Linear Model, GLM binomial, $p < 0.0001$, Figure 2). Germination predominantly occurred at warmer temperatures (17 to 29 °C) under both light and dark conditions, but with significantly higher germination rates under light (GLM binomial, $p < 0.0001$) (Figure 2). The average percentage germination achieved under both illumination conditions ranged from ca. 37 (dark) to 96% (light). Under light conditions, germination remained low at temperatures below 17 °C and then increased sharply, peaking at 21 °C with ca. 96 ± 2% (GLM binomial, $p < 0.0001$). Beyond 21 °C, germination declined. Under constant dark conditions, germination was low, below 17 °C, increased steadily between 17 °C and 21 °C (reaching 37 ± 8%), dropped to near zero at 23 °C, and then slightly increased again toward 29 °C (20 ± 11%). The results of a Type II analysis of deviance (ANOVA) are summarized in Table 1.

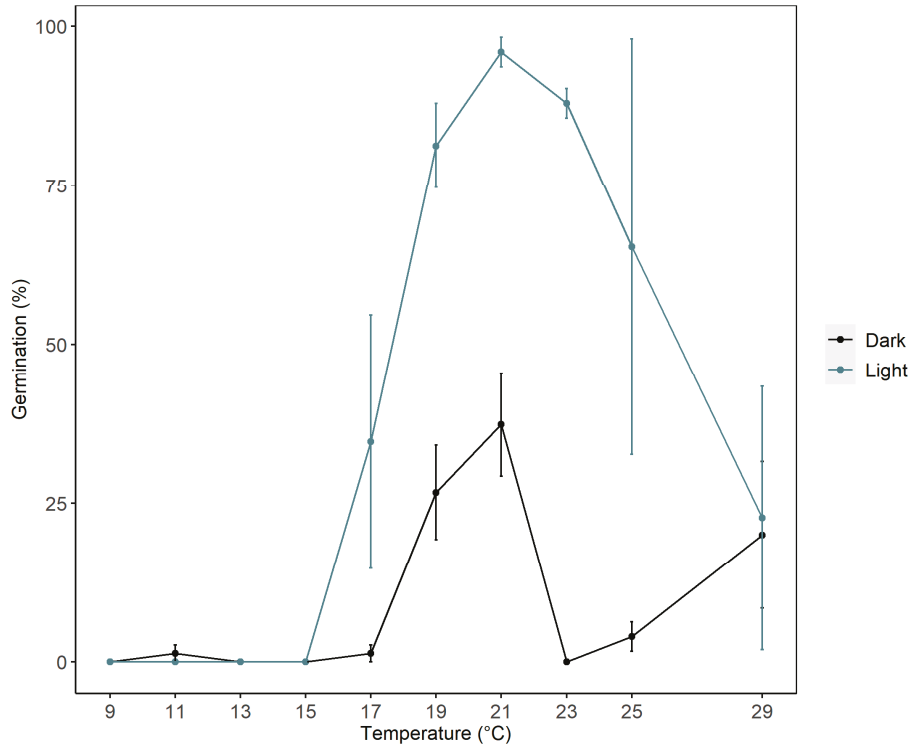

Figure 2. The 28-day germination percentage of *S. platyphylla* seeds under a range of constant incubation temperatures and using either a 12/12 h (light/dark) photoperiod or under constant darkness. The data are for the mean germination of three replicates of 25 seeds, and bars represent ± standard error.

Table 1. Type II ANOVA table (analysis of deviance) from a Generalized Linear Model of seed germination under different light conditions and temperatures.

Term	Likelihood Ratio Chi-Square	Df	*p*-Value
Light conditions (light vs. dark)	289.95	1	$<2.2 \times 10^{-16}$
Temperature (10 levels)	641.58	9	$<2.2 \times 10^{-16}$
Light condition x temperature	82.39	9	5.40×10^{-14}
Residuals	-	40	-
AIC (Akaike Information Criterion)	-	-	311.86

Under three contrasting alternating temperature regimes, the light/dark condition had a significant effect on seed germination (GLM binomial, $p < 0.0001$), while the effect was not significant for diurnally alternating temperature (GLM binomial, $p = 0.14$) and for the interaction between the two factors (GLM binomial, $p = 0.06$) (Figure 3). These results confirm that 24 h darkness significantly inhibits the germination of *S. platyphylla* seeds and does so across all three temperature regimes when compared to seeds exposed to light. Although temperature had no significant effect on germination, light had a substantial impact. Under the 12/12 h (light/dark) photoperiod, the mean germination rate across all three temperatures was ca. 96%, while in constant darkness, it dropped dramatically to just ca. 2% (Figure 3).

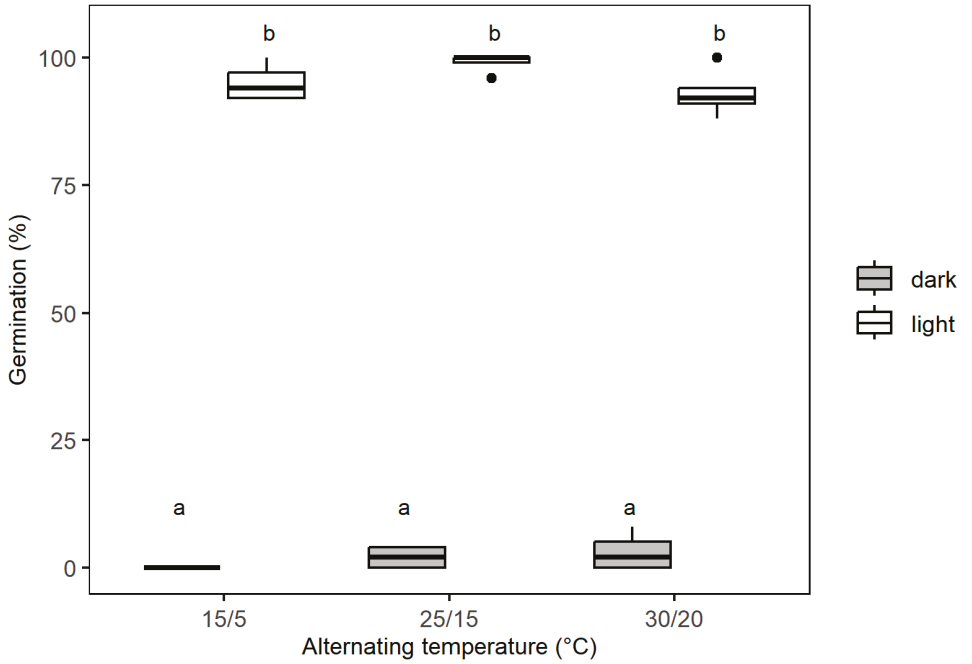

Figure 3. Effect of three different diurnal temperature regimes on germination of *S. platyphylla* seed under a 12/12 h (light/dark) photoperiod of white light and darkness after 28 days. The box plots span the 25th and 75th quartiles, the band in the box is the median, and the whiskers indicate the data range. Outliers are denoted as dots. Letters indicate the significant difference between the treatment means at $p < 0.05$ (TukeyHSD).

2.3. Experiment 2—Effects of Burial Depth on Seedling Emergence and Viability of Retrieved Seeds

Burial depth significantly inhibited seedling emergence as only non-buried seeds germinated (GLM binomial, $p < 0.0001$). Seedling emergence at the surface level reached 76 ± 4%, while there was no emergence recorded for the other four burial depths (Figure 4a). After 3 months, all remaining non-germinated seeds on the surface (non-buried) had decayed and could not be retrieved. Therefore, only the buried seeds were retrieved for viability testing. There was a significant difference in the germination of the retrieved seeds from the different burial depths (GLM binomial, $p = 0.0007$). The highest germination (98 ± 2%) was recorded for the seeds initially buried at 2.5 cm. However, this was not statistically different when compared to seeds buried at depths of 1.0 or 2.0 cm (83 ± 6% and 92 ± 3%, respectively; Figure 4b). At the 0.5 cm burial depth, the retrieved seeds showed the lowest germination percentage (71 ± 5%) which was significantly lower when compared to all other burial depths (TukeyHSD, $p < 0.05$).

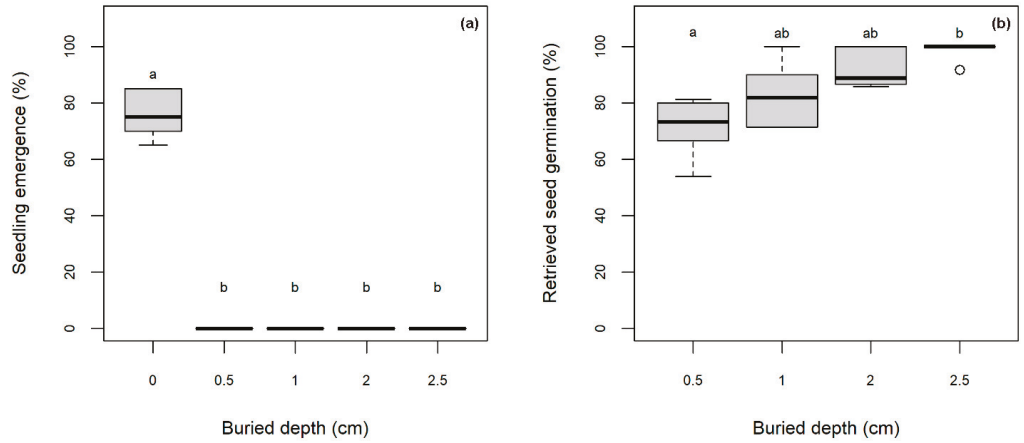

Figure 4. Effects of five different burial depths (0.0 to 2.5 cm) on *S. platyphylla* seedling emergence (**a**) and germination of retrieved seeds (**b**). In panel (**b**), all seeds from the substrate surface (non-buried treatment, 0 cm) had fully decomposed and could not be recovered. The box plots span the 25th and 75th quartiles, the band in the box is the median, and the whiskers indicate the data range. Outliers are denoted as dots. Letters indicate the significant difference between the treatment means at $p < 0.05$ (TukeyHSD).

2.4. Experiment 3—Prediction of Seed Longevity Using a CAT

The initial viability of the seed lot used was 98 ± 2%. As the aging test progressed, the germination percentage of the seed lot slowly reduced, before there was a rapid decline at around the 25th day (Figure 5). The fitted GLM model showed that the *S. platyphylla* seed lot lost about 50% of its viability (P50) within 36 days (Figure 5).

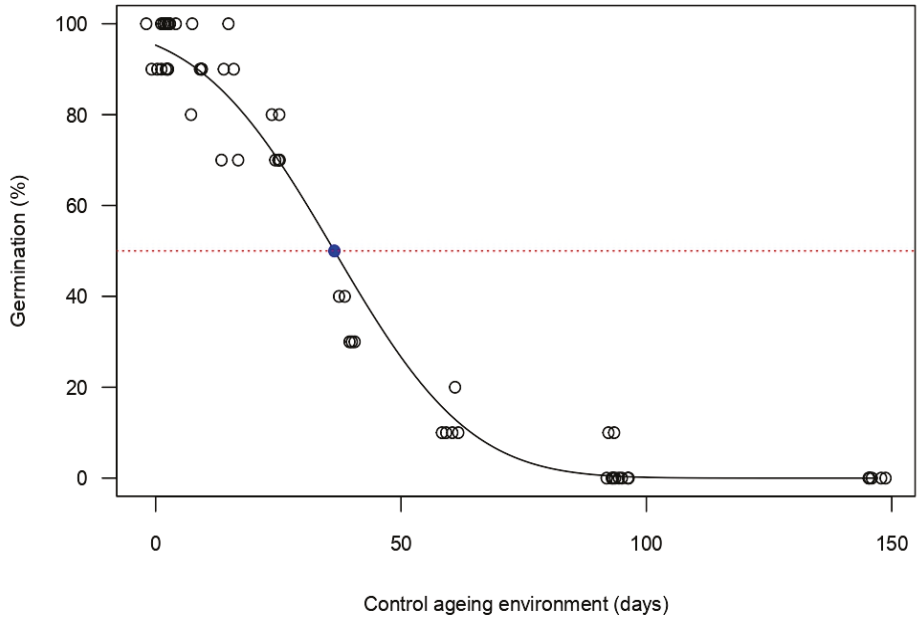

Figure 5. The response of a *S. platyphylla* seed lot under a controlled aging test run at 45 °C and 60% relative humidity. Upon sample retrieval from the test, the germinability was determined by incubation at 25/15 °C with a matching 12/12 h (light/dark) photoperiod. The blue dot indicates the predicted time when germination had fallen to 50% (P50). The red horizontal dashed line marks the 50% germination threshold. The open circles are the mean germination percentages of five replicate lots of 10 seeds, and overlapping germination values were slightly shifted to ensure all data points are visible.

3. Discussion

3.1. Seed Germination Affected by Light and Temperature

Seed germination in *S. platyphylla* was strongly influenced by both temperature and light. The highest germination percentage (up to 99%) was recorded at a constant 21 °C under a 12/12 h light/dark photoperiod, while germination remained low in continuous darkness. This confirmed earlier findings by Flower [33], who also identified 21 °C as the optimal germination temperature for this species. Light has been widely recognized as a critical trigger for germination in aquatic plants [16]. For example, several *Carex* wetland species required up to eight hours of light exposure to achieve maximum germination [34], and light similarly enhanced germination in *Zostera marina* L. and various *Nymphaea* species [35–37]. The consistently high germination observed under both constant (21 °C) and all fluctuating temperature regimes tested (Figures 3 and 4) suggested that *S. platyphylla* had a relatively broad thermal range for germination. However, germination appeared suppressed under colder (<15 °C) or hotter (>30 °C) conditions, implying that extreme seasonal temperatures may inhibit recruitment (Figure 2). Notably, alternating temperature regimes strongly promoted germination under light conditions even at relatively low mean temperatures (15/5 °C), whereas constant temperatures near these extremes inhibited germination. This pattern aligns with findings for *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha latifolia* L., where final germination was sensitive to the amplitude of daily temperature fluctuations, and higher amplitudes significantly enhanced germination under light conditions [38]. Similarly, studies on *Carex* species from freshwater wetlands and mesic habitats showed that seeds incubated under alternating temperature regimes germinated more effectively than under constant temperatures [39], indicating that diurnal temperature fluctuations act as ecological cues for breaking dormancy and signaling favorable conditions for seedling establishment [40].

Comparable responses have been reported in other species within the Alismataceae family. For instance, *S. montevidensis* germinated optimally at 26 °C but failed to germinate at 10 °C [21], while *S. lancifolia* only reached full germination at 25 °C when exposed to light [20]. Under a 33/20 °C thermoperiod with a matching 14/10 h (light/dark) photoperiod, *S. platyphylla* achieved a reduced 62% germination [41]. This suggested that higher thermoperiods may exceed the optimal range for *S. platyphylla* germination. Given these temperature–light requirements, there are clear implications for management strategies. In cooler regions of Australia, such as Victoria, the southern and tableland regions of New South Wales (NSW), and parts of South Australia [42], natural winter conditions may delay germination. This offers an opportunity to schedule control actions (e.g., herbicide application or mechanical removal) shortly after germination but before seed set, ideally one to two months after the onset of warmer temperatures. In contrast, in warmer climates such as Queensland, northern NSW, and parts of Western Australia [42], germination may begin earlier and persist over a longer seasonal window. These regions may require more frequent monitoring and earlier control efforts, possibly starting in late winter or early spring.

Notably, some seeds demonstrated the ability to germinate in darkness, achieving ca. 37% germination at 21 °C (Figure 2). This indicated that while light typically enhances germination, it is not always essential, and temperature may serve as a more dominant cue in some cases. These differences might be attributed to physiological or genetic variation among seed lots, influenced by factors such as maternal environment, seed maturity, or seed age [43,44]. Similar environmentally responsive germination behaviors have been observed in other species. For instance, *Arabidopsis thaliana* (L.) Heynh. seeds produced under warmer conditions exhibit reduced dormancy [45], while seeds of *Thlaspi arvense* L. formed in shaded environments show reduced dependence on light for germination [46]. These

patterns suggest a bet-hedging strategy, where variable germination responses increase the likelihood of successful establishment across fluctuating environmental conditions [47]. This highlighted the need for sustained, long-term control, as not all *S. platyphylla* seeds will respond uniformly to environmental triggers or treatment interventions. Very short light exposure during seed imbibition may have contributed to germination in the dark treatment; although overall germination was strongly light-dependent, stricter light exclusion could further refine assessments of true dark germination responses.

3.2. Seedling Emergence Affected by Burial Depth, and Seed Viability of Retrieved Seeds

Seedling emergence of *S. platyphylla* occurred only when seeds were placed on the substrate surface (Figure 4a), indicating that burial effectively inhibited germination. This is likely due to the absence of light reaching seeds beneath the surface, which aligns with findings from Experiment 1 where germination was significantly reduced in darkness. In aquatic habitats, light intensity is naturally reduced as it passes through the water column [48,49], further limiting the light available to buried seeds. Several aquatic and wetland species exhibit similar responses, showing reduced or no germination when seeds are buried. This has been observed in *Carex* spp. [34], *Myriophyllum spicatum* L., *Hydrocharias dubia* (Blume) Backer., *Ottelia alismoides* (L.) Pers., *Epilobium prostratum* Dougl. ex Hook. [50], *Vallisneria natans* (L.) H.B.K. [51], and *Zannichellia palustris* L. [52]. These findings suggest a widespread light requirement among aquatic macrophytes for successful germination.

The seeds used in this study were freshly harvested and likely exhibited strong dark-induced dormancy. It is well established that freshly shed seeds often have high levels of dormancy, which can be gradually alleviated through a process known as after-ripening [53]. This process leads to a progressive widening of the environmental conditions under which seeds can germinate, including the ability to germinate in darkness. For example, a small proportion of *Carex ferruginea* Scop. seeds buried for 3 to 15 months were able to germinate in darkness, although this species typically requires light [54]. Seeds buried under field conditions are known to continuously perceive environmental cues that regulate their dormancy status [55] and this dormancy cycling enables them to time their germination to coincide with favorable conditions [56,57]. Consequently, it is possible that *S. platyphylla* seeds, when buried over time, may gradually lose their dormancy and become capable of germinating even in the absence of light.

The high viability of seeds recovered after three months of burial (Figure 4b) suggests that burial may support seed persistence by protecting seeds until suitable germination conditions return. Seeds buried at 2.5 cm retained significantly higher viability than those at 0.5 cm, consistent with previous findings that deeper burial prolongs seed longevity. For example, a long-term study on arable weed seed survival revealed that seeds of *Avena fatua* L., *Polygonum aviculare* L., and *Orippa palustris* (L.) Besser buried at 15.0 cm maintained viability longer than those at shallower depths [58]. Although the present experiment only spanned three months, it demonstrated the potential for *S. platyphylla* to form a persistent soil seed bank. This highlights the importance of minimizing substrate disturbance, which could unearth viable seeds and expose them to light, potentially triggering germination. For example, in Victoria, spring and summer irrigation often involves refilling channels, which can disturb sediment and bring seeds back to the surface [59]. Likewise, mechanical or physical control efforts such as vegetation removal may also unintentionally expose buried seeds [60].

3.3. CAT to Estimate Seed Longevity of Sagittaria platyphylla

The results of the CAT indicated that *S. platyphylla* produces short-lived seeds (Figure 5), with an estimated field lifespan of one to less than three years (Table 1). Due

to their short longevity, *S. platyphylla* seeds must germinate within a relatively narrow window of opportunity. To improve the likelihood of successful establishment, the species compensates by producing large quantities of seeds, increasing the chances that some will encounter favorable conditions for germination. A similar approach is seen in members of the Amaranthaceae family, which also produce numerous seeds with rapid germination responses to suit dynamic or unpredictable environments [61]. This life-history strategy is characteristic of ruderal (r-selected) species such as *S. platyphylla*, which tend to produce many small seeds, exhibit rapid growth, and are well-adapted for colonizing frequently disturbed habitats [62].

Seed persistence in the field is known to vary depending on a range of factors, including population origin, soil type [63], seed size [64,65], and disturbance in environments, and even between seed lots of the same species [24,66]. Additionally, seed aging is shaped by environmental conditions such as temperature, relative humidity, and oxygen availability, in combination with intrinsic seed traits like structural composition, chemical makeup, and desiccation tolerance [22,24]. Although no direct studies have quantified the seed longevity of *S. platyphylla* under natural conditions, some insight can be drawn from its close relative, *S. montevidensis*, which has been recorded to maintain seed viability for up to 3 years [33]. Among IAAPs, seed longevity spans a wide spectrum. For instance, seeds of *P. crassipes* can persist in the soil for up to 28 years [28], while *Glossostigma drummondii* Benth. and *Myriophyllum petreaum* Orchard produce short-lived seeds [30]. However, as discussed in Experiment 2, *S. platyphylla* seeds that remain buried for extended periods may gradually lose dormancy and eventually gain the ability to germinate in darkness. This reinforces the importance of managing the seedbank within a defined timeframe. From a practical standpoint, sustained management over a period of 3 to 4 years is likely to significantly reduce the viable SSB, thereby curbing population recovery. This is consistent with broader research indicating that preventing seed input over multiple consecutive seasons can reduce IAAP seedbanks to less than 5% of their original size [67]. Moreover, by reducing the SSB, this will create ecological niches for native species to re-establish, thereby restoring biodiversity and ecosystem function. Integrated approaches such as using herbicides on mature plants followed by treatments targeting seedlings can enhance effectiveness and speed up population decline.

4. Materials and Methods

4.1. Seed Collection

Seeds of *S. platyphylla* were randomly collected from an infestation in an urban creek in Sinnamon Park (27°32′42.6″ S; 152°56′40.9″ E), Southeast Queensland, Australia, during the peak flowering season (summer; October to February). The first seed lot, collected in late November, was used in Experiments 1 and 2, while the second lot, collected in early February, was used in Experiment 3.

Mature seeds, characterized by a subtle brown coloration, were easily released from the inflorescence by gentle touch. Seeds were collected in paper bags and immediately transferred to the seed laboratory at the University of Queensland (UQ) for cleaning of floral debris and removal of non-filled and immature seeds using a series of three stainless steel sieves (2.00, 1.00, and 0.5 mm; Endecotts Ltd., London, UK) applied sequentially. Non-filled and immature seeds were smaller and passed through the sieves, whereas most of the filled seeds were retained on the sieves. The cleaned seeds were kept in a dedicated seed storage room at 15 ± 1 °C and 15 ± 3% RH until being used for experimentation ca. 4 days later. Seeds not immediately needed were air-dried and then placed into a laboratory fridge at 4 ± 1 °C in a sealed container [68].

4.2. Seed Fill Determination

To determine the seed fill percentage of the seed lot prior germination test, seeds were examined using X-ray imaging (Faxitron MX-20, Lincolnshire, IL, USA). Four replicates of 25 seeds were randomly selected and exposed to 18 kV for 20 s, with images captured using Bioptics software. Based on the X-ray images, seeds were classified as filled and viable if they displayed a healthy embryo or as non-viable if they lacked an embryo or had an abnormally developed embryo. The percentage of filled seeds was calculated as the number of filled seeds relative to the total number of seeds in the sample.

4.3. Experiment 1—Effects of Light and Temperature on Seed Germination

Seeds were placed into 20 mL glass vials (Wheaton® liquid scintillation vial, Millville, NJ, USA) filled with 15 mL distilled water and closed with its lid to minimize water evaporation and incubated at one of 10 constant temperatures (9, 11, 13, 15, 17, 19, 21, 23, 25, or 29 $\pm$ 1 °C) and under one of two illumination conditions (constant darkness or a 12/12 h light/dark photoperiod) on a thermogradient bar (Thermoline Ltd., Wetherill Park, Australia) to determine the optimal temperature for *S. platyphylla* germination. For the dark treatment, seeds were carefully handled to minimize or prevent light exposure. To maintain continuous darkness, half of the glass vials were quickly wrapped in two layers of aluminum foil after the seeds were imbibed. The remaining vials were exposed to cool white fluorescent light (with a light intensity of *ca*. 100 μmol m^{-2} s^{-1}) to simulate a 12 h day period. A series of data loggers (Tinytags, TGP 4017, Hastings Data Loggers, Port Macquarie, NSW, Australia) was used to monitor temperature and RH in each of the 10 chambers on an hourly basis.

The experiment was conducted in a completely randomized design (CRD) with three replications of 25 seeds each. Prior to placing seeds into the glass vials, the seeds were shaken for 10 min in a 2% (*v*/*v*) sodium hypochlorite (NaOCl) solution (White King Bleach, Melbourne, Victoria, Australia) containing two drops of 1% Tween 20 (Labchem, Zelienople, PA 16063, USA), followed by a wash under running distilled water for 1 min to surface sterilize the seeds and prevent contamination during germination [69]. The effect of alternating temperature on germination was determined by undertaking a further germination study in three incubators (TRIL-750 Illuminated Refrigerator Incubator, Thermoline Ltd., Wetherill Park, Australia) set at either 15/5, 25/15, or 30/20 $\pm$ 2 °C with a 12/12 h (light/dark) photoperiod or under constant darkness. The experiment followed a randomized complete block design with four replications of 25 seeds. Seeds were surface-disinfected and placed in glass vials, as described in the previous thermogradient bar experiment.

Germination was assessed every second day and defined as the protrusion of the radicle by $\geq$2 mm through both the testa and fruiting layers. Once germinated, seedlings were removed from the glass vials and discarded. Seeds in the dark treatment were observed for germination inside a darkened tent and under a green safety light. The experiment was run for 28 days [16]. At the conclusion of both experiments, the ungerminated and healthy-looking seeds, as well as those free from contamination, were subjected to a second X-ray analysis to indirectly assess their viability. Filled but ungerminated seeds were defined as viable but dormant, whereas unfilled seeds were deemed to be non-viable.

4.4. Experiment 2—Effects of Burial Depth on Seedling Emergence and Viability of Retrieved Seeds

This experiment was undertaken in a CRD with four replications, each replication consisting of 10 seeds. Six-substrate burial depths were used (*viz.* 0—sown on the surface, 0.5, 1.0, 1.5, 2.0, and 2.5 cm deep). To do this, black plastic pots (300 mL) were filled with a substrate mixture (three parts of quartz sand and one part of peat) containing an Osmocote slow-release fertilizer (Osmocote® Plus Trace Elements: Total All Purpose, Scots

Co, Marysville, OH, USA). The fertilizer was added to the bottom of the pot at a rate of 3.75 g N kg^{-1} of the substrate mixture to supply macronutrients. The outside of the pot was marked to show the position to which the seeds would be buried. Ten seeds were placed inside a bag (16 cm^2) made from 1.0 mm insect screen fiberglass mesh (Permastik™, Brisbane, QLD, Australia). The mesh bags helped in keeping the surface seeds in place and aided the retrieval of buried, ungerminated seeds at the termination of the experiment, while still permitting germinating seedlings to grow through the mesh. The mesh bags were buried at the designated depths, and the experiment ran for 90 days. To submerge the pots, a glass aquarium (216 L) was filled with a suitable culture solution following Smart and Barko [70] and kept constant at 25 ± 1 °C. The pH was adjusted to 6.5 ± 0.1 by using a carbon dioxide (CO_2) injection system to create ideal growing conditions for any emerging seedlings. The incubation temperature of 25 °C had been determined to be optimal in Experiment 1. White light-emitting diode (LED) aquarium lights (Fluval, freshwater plant light, Denver, CO, USA), set at ca. 120 mmol m^{-2} s^{-1}, were used to supply a 12/12 h (light/dark) photoperiod. The culture solution level was maintained at 40 cm above the level of the substrate surface in the pots. The seedling emergence was recorded weekly. After 90 days, all non-germinated seeds were retrieved and assessed for viability using a germination test in an incubator set at the optimum alternating temperature and lighting conditions, as determined in Experiment 1.

4.5. Experiment 3—Prediction of Seed Longevity Using a CAT

Prior to the CAT experiment, the second seed lot was tested for germination under optimal conditions (25/15 °C, 12/12 h light/dark photoperiod) as identified in Experiment 1 with four replicates of 25 seeds per replicate. The CAT was conducted in the seed laboratory at UQ, Gatton Campus. Prior to transferring the seeds to the CAT-controlled environment, a rehydration step was applied to minimize damage caused by sudden rehydration [25]. To pre-equilibrate the seeds, 12 replicates of 50 freshly harvested seeds (ca. 10 days-old) were placed in open-top glass vials (25 mL) and exposed to a lithium chloride (LiCl) solution (370 g L^{-1} H_2O), maintaining a RH of 47 ± 1% inside a sealable chamber, incubated at 20 ± 1 °C. The sealable chamber had a plastic grid stand inside to keep the glass vials with seeds above the LiCl solution (Figure 6). After 14 days of seed rehydration, all 12 glass vials containing seeds were transferred into a second identical sealable chamber containing a LiCl solution (300 g L^{-1} H_2O), producing a 60 ± 2% RH. The box was then placed into a laboratory oven set at 45.0 ± 0.5 °C to undergo the CAT under darkness. As the RH produced by the LiCl solution inside the box is known to decrease with time, distilled water was added weekly to readjust the RH back to 60% [25,71]. Glass vials were removed periodically (at 0, 1, 3, 8, 15, 24, 39, 60, 92, 95, 120, and 147 days after treatment), and their seeds were assessed for viability using a germination test. The germination test was conducted under optimal temperature and light conditions, as identified in Experiment 1, with five replications of 10 seeds per replicate, taken at random from each glass vial.

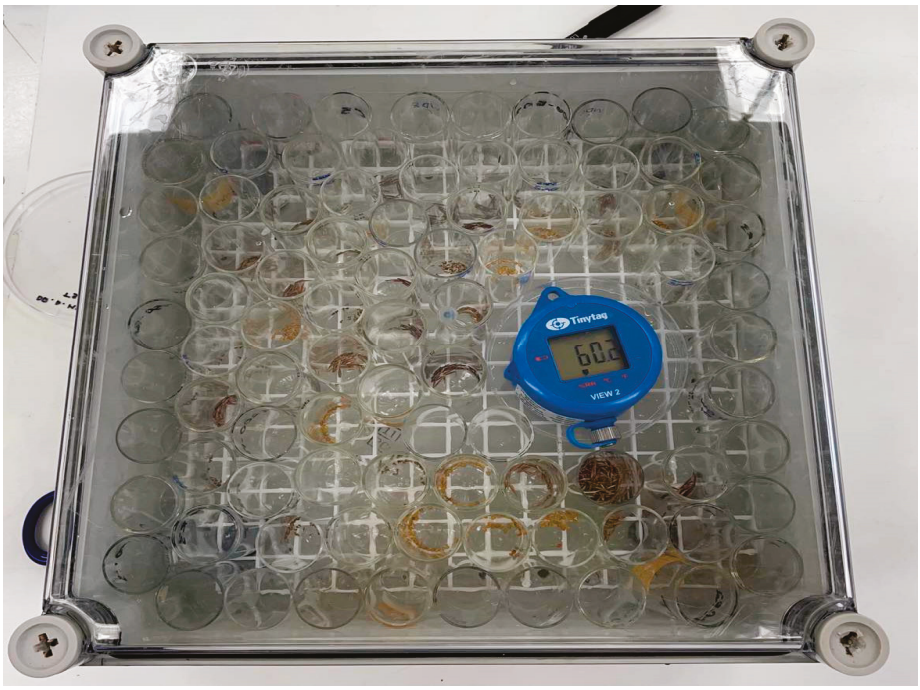

Figure 6. The airtight sealable chamber, containing lithium chloride to generate a 60.0% relative humidity environment, used to conduct the control aging test. Seeds in lots of 50 were placed into glass vials, then placed inside the box, and incubated in a laboratory oven set at 45 ± 1.0 °C, under constant dark conditions. Each glass vial was randomly removed at 0, 1, 3, 8, 15, 24, 39, 60, 92, 95, 120, and 147 days after treatment for the germination test of five replications with 10 seeds per replication.

4.6. Data Collection and Analysis

All data analyses were carried out in R version 4.2.2 using R-studio version 2023.09.1 + 494 [72,73]. Germination percentage was calculated using the method of Davies, Di Sacco, and Newton [69] and following adjustment for seed fill using a modification of the method of Merritt and Rokich [74].

Equation (1): Germination percentage

$$\text{Germination}(\%) = \frac{\text{Total seeds germinated}}{\text{Total seeds per replicate}} \times 100 \quad (1)$$

Equation (2): Final germination percentage adjusted by seed fill

$$\text{Final germination}(\%) = \frac{\text{Germination}(\%)}{\text{Seed fill}(\%)} \times 100 \quad (2)$$

To all germination data in Experiment 1, a GLM was fitted with a logistic link function and a binominal error to analyze the germination count data [75,76]. For the burial depth in Experiment 2, a GLM with the same binominal approach was also used to analyze seedling emergence to test the effect of different burial depths. The significance of model terms was assessed using Type II ANOVA with the "car" package [77]. Then a Tukey post hoc test using the "multcomp" package [78] was applied to compare the differences between the means of treatment in both experiments.

To estimate seed longevity in Experiment 3, a GLM model with a probit link function and binomial error was fitted to the seed longevity data. The response variable was final germination, with days in the CAT as explanatory variable [79]. The time taken for germination to decline by 50% in the CAT environment (P_{50}) was estimated by the fitted

GLM model. Therefore, viability was calculated using Equation 3, developed by Ellis and Roberts [80].

Equation (3): Seed longevity

$$v = Ki - (p/\sigma) \quad (3)$$

where v is the viability of seeds after p days in the aging environment, Ki represents the initial probit viability of the seed lot, and σ is the time taken for viability to decrease by one normal equivalent deviate, which is equivalent to the standard deviation of the frequency distribution of seed deaths over time (days). Seed classification based on the predicted P_{50} value from the model was determined as shown below (Table 2).

Table 2. The time for germination declining to 50% (P_{50}) in the controlled aging test (CAT) was used to classified the seeds into longevity categories and given a predicted lifespan under field conditions [25].

P50 Value (Days)	P50 Category	Lifespan Predicted In Situ (Years)
<20	Transient	<1
20 < P50 < 50	Short-lived	1 to 3
>50	Long-lived	>3

5. Conclusions

This study demonstrated that *S. platyphylla* seeds germinate optimally at 21 °C, with successful emergence across a wide temperature range (17 to 29 °C) under a 12/12 h (light/dark) photoperiod. High germination also occurred under fluctuating temperature regimes (15/5 °C, 25/15 °C, and 30/20 °C), indicating adaptability to variable natural conditions. A strong light requirement means that seeds buried even at shallow depths of 0.5 cm failed to germinate, although they remained viable for at least 1 to 3 years, highlighting short-term persistence in the seedbank. Practically, these findings suggest that minimizing sediment disturbance to keep seeds buried, combined with timing control interventions around seasonal temperature windows, can effectively reduce *S. platyphylla* recruitment and contribute to more strategic weed management.

Given these findings, future research should focus on how local habitat characteristics, such as sediment type, hydrology, and canopy cover, influence seed persistence and germination potential. Studies examining the interactive effects of sediment disturbance, mechanical control, and herbicide application on seedbank dynamics are urgently needed. Such work will be essential for developing more effective and integrated management strategies that not only target above-ground plant biomass but also address the regenerative potential held within the seedbank.

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Abbreviations

The following abbreviations are used in this manuscript:

IAAP	Invasive alien aquatic plant
SSB	Substrate seedbank
CAT	Controlled aging test
RH	Relative humidity
GLM	Generalized linear model
CRD	Completely randomized design
AIC	Akaike information criterion
P50	The time taken for germination to decline to 50%
NSW	New South Wales
UQ	University of Queensland
NaOCl	Sodium hypochlorite
CO2	Carbon dioxide
LED	Light-emitting diode
LiCl	Lithium chloride

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Distribution Characteristics and Adaptation Mechanisms of Exotic Spontaneous Plant Diversity in Urban Road Green Spaces of Changchun, China

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Abstract

Spontaneous plants are plants that occur in urban environments such as pavement gaps or cracks in walls without cultivation and are not remnants of historic native habitats. They are critical components of urban road green space vegetation, and their distribution is affected by multiple factors. Heavy traffic and frequent human disturbances on urban roads exacerbate exotic spontaneous plant invasions. Exploring the diversity of their distributions, adaptation mechanisms of these exotic plants and their relationship with native ones is vital for focused control of harmful invasives. Based on field surveys, this study analyzed the distribution of exotic spontaneous plants across habitat types, urbanization gradients and disturbance intensities in road green spaces, and their interactions with native counterparts. Our results indicated: (1) 425 spontaneous species were recorded (234 exotic, 191 native), with 71.8% cosmopolitan and 74.7% monotypic genera. (2) The spontaneous exotic plant community achieves extensive resource preemption by forming a structure dominated by a single super-dominant species (Setaria viridis) and characterized by a broader overall niche breadth. (3) Different habitats sustain a similar number of exotic spontaneous plant species (i.e., α-diversity), but their species compositions are highly differentiated, with such differences driven almost entirely by species turnover. At the urban scale, spontaneous exotic plants adapt to regional environments with varying urbanization intensities by maintaining extensive similarity in community composition and making only extremely weak adjustments to the pattern of individual distribution among species. (4) The spontaneous plant community exhibits a pattern dominated by weak interspecific associations and random assemblages, where ecological interactions among species are weak, and the community structure is more consistent with the stochastic processes described by the Neutral Theory. At the regional environmental gradient scale, the diversity of spontaneous native and exotic plants exhibited coordinated variation. The study provides a scientific basis for urban biological invasion control and biodiversity management.

Keywords: urban road green spaces; spontaneous plant diversity; exotic plants; distribution patterns; adaptation mechanisms

1. Introduction

Biological invasion can be summarized as a multi-stage dynamic process in which a species crosses geographical barriers through human activities to enter a new area and then sequentially overcomes multiple obstacles such as environmental constraints, reproductive challenges, and dispersal limitations, establishes populations, and ultimately exerts negative impacts on the local ecology, economy, and other aspects [1]. According to their spread status, they can be classified into naturalized plants, invasive plants, and escaped cultivated plants. They represent different states in the process of "introduction → escape (escaped species) → population establishment (naturalized species) → widespread spread (invasive species)" [2,3]. Driven by globalization, specific pathways such as international trade, horticultural introduction, and transportation networks have significantly increased the risk of exotic species spread. Meanwhile, climate change may further exacerbate this threat—for instance, rendering certain drought-adapted species that have reached their physiological tolerance limits more vulnerable. In recent years, scientific research has been improving the precision of risk management and control through data integration and mechanistic modeling. For example, distinguishing between the "invasion" and "expansion" processes of pine wood nematode disease and quantifying the contributions of anthropogenic and natural dispersal can provide direct evidence for regionalized prevention and control strategies; systematically integrating citizen science and traditional survey data can more comprehensively reveal species niches, thereby enhancing the reliability of large-scale risk prediction [4,5].

China attaches great importance to the issue of exotic species invasion. The National Strategy and Action Plan for Biodiversity Conservation (2011–2030), issued in 2010, incorporated the prevention and control of invasive alien species into the core goals of national biodiversity conservation. However, restricted by multiple objective factors, China remains one of the countries most threatened by biological invasions and suffering the most severe losses globally [6,7]. According to the Report on the State of the Ecology and Environment in China 2020, more than 660 invasive alien species have been recorded in China, ranking among the highest in the world; among the 100 most threatening invasive alien species globally listed by the International Union for Conservation of Nature (IUCN), 50 are present in China. These species cause direct economic losses exceeding 200 billion yuan annually, placing China in a grim situation regarding the prevention and control of invasive exotic species [8,9].

A large number of studies on urban exotic spontaneous plants have been conducted domestically and internationally, mostly focusing on aspects such as their diversity distribution patterns [10,11], driving factors (e.g., land use, transportation networks, socioeconomic factors) [12–23], and impacts on the diversity of native spontaneous plants. Foreign research on exotic spontaneous plants started earlier, and many hypotheses on biological invasions have been proposed, such as the "Darwin's Naturalization Hypothesis" and the "Adaptation Hypothesis" [24]. Compared with domestic research, foreign studies focus more on invasion causes [25,26] and invasion mechanisms [27]. In contrast, domestic research on interspecific relationships is mostly concentrated in forests, riparian zones, and other areas [28–30], with fewer studies on highly urbanized habitats. Existing studies have shown that, in addition to environmental influences, the interactions between exotic spontaneous plants and native spontaneous plants are also one of the factors affecting the naturalization or invasion of exotic plants [13,31].

However, most domestic studies have been carried out in southern provinces/cities or megacities (such as Guangdong Province and Beijing), with relatively insufficient attention paid to cities in northern temperate or frigid-temperate zones, especially those in the old industrial base of Northeast China [32]. Furthermore, there is a paucity of in-depth research

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on urban spatial heterogeneity, spontaneous plants, ecological processes, and the mechanisms of human disturbance in these regions. Existing research on exotic spontaneous plants in cold regions also mostly focuses on farmlands and forests, neglecting road green spaces as a "key corridor," resulting in a lack of scientific basis for the prediction and prevention of invasion risks in cold-region cities [33]. Combined with the habitat fragmentation effect of high-density road networks [34], and the superimposed impacts of human activities [35], road green spaces have the potential to become suitable habitats for exotic spontaneous plants. Therefore, studying the distribution patterns of exotic spontaneous plants in road green spaces is of great significance.

Spontaneous plants refer to plants that are not intentionally introduced or cultivated, and they establish and regenerate naturally through their own propagules. They have high sensitivity and wide adaptability, can quickly respond to urban environments, and are excellent research objects. Changchun City, a regional central city in Northeast Asia, has a well-developed transportation network. As a northern cold-region city, the spontaneous plants in its road green spaces are affected by the superposition of urbanization and the special habitats of cold regions, which may form unique distribution patterns and adaptation strategies [36,37]. However, existing studies have not specifically covered this area.

In summary, studying the distribution of exotic spontaneous plants and their interaction mechanisms with native spontaneous plants in the road green spaces of Changchun City is of great significance for the management of exotic plants and is also an important part of biodiversity conservation. Therefore, based on a series of scientific questions, such as the diversity distribution patterns of exotic spontaneous plants, how they adapt to the highly heterogeneous urban habitats, and their diversity interactions and interspecific relationships with native spontaneous plants, this study takes the spontaneous plants in the road green spaces of Changchun City as the research object and aims, through quadrat surveys, to: (1) reveal the distribution patterns of exotic spontaneous plants; (2) clarify their strategies for adapting to heterogeneous urban habitats; (3) explore their interspecific relationships with native spontaneous plants. The research results will provide a scientific basis for the precise management and diversity conservation of plants in cold-region cities.

To this end, we propose the following hypotheses: (1) The richness of exotic plants exhibits a regular variation along the environmental gradient from urban centers to rural fringes—characterized by a gradual decrease in the intensity of human disturbance—and is expected to show significant dominance in habitats subjected to high-intensity human disturbance; (2) Successfully established exotic plants adapt to urban habitats by exhibiting a suite of functional traits conducive to dispersal, rapid growth, and stress tolerance, and their ecological niche breadth is wider than that of native plants, enabling them to exploit a broad range of urban disturbed resources; (3) Based on the Biotic Resistance Hypothesis, the abundance of exotic plants is predicted to be suppressed in communities with high native plant diversity, and the interspecific association network between exotic and native plants is hypothesized to be dominated by competitive interactions.

2. Results

2.1. Characteristics of Species Diversity Composition of Exotic Spontaneous Plants

2.1.1. Analysis of Family-Genus Composition and Geographical Elements

A total of 54 families, 169 genera, and 234 species of exotic spontaneous plants were recorded in this floristic survey (Figure 1). The number of families is abundant (54 families), among which Asteraceae is the absolute dominant family, accounting for 18% of the total number of exotic spontaneous plant species. Poaceae, Rosaceae, Fabaceae, and other families are the main component families of exotic plants; oligotypic families and monotypic

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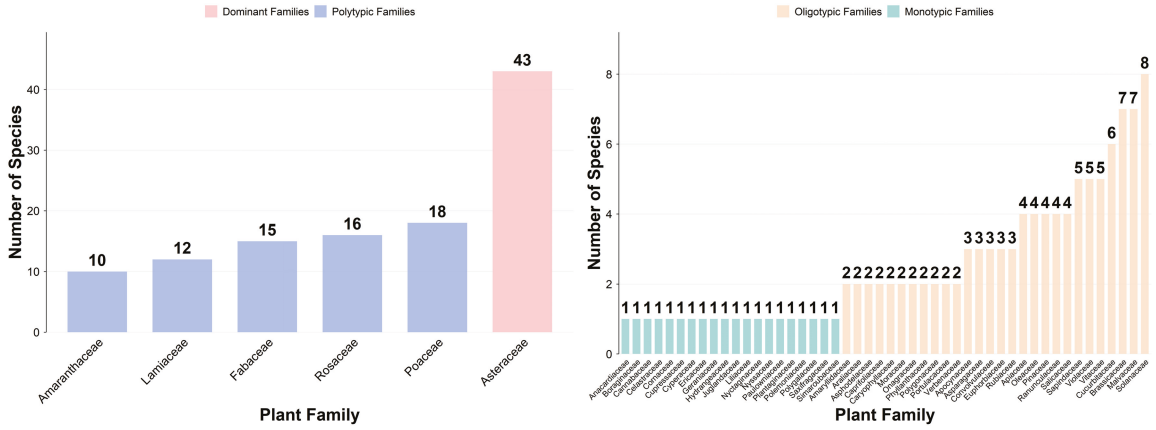
families together consist of 48 families, contributing 50% of the total number of species. This indicates that exotic spontaneous plants are scattered in distribution at the family level, and have strong diffusion and adaptability, with diverse family origins and multiple invasion pathways.



(**a**) (**b**)

Figure 1. Family Composition of Exotic Spontaneous Plants. (**a**) Number of species in dominant families and diverse families. (**b**) Number of species in oligotypic families and monotypic families.

Monotypic genera account for 74.7% of the total genera and contribute 54% of the total species, whereas polytypic genera (e.g., tetratypic and pentatypic genera) only account for 2.4% and 1.2% of the total genera, respectively (Table 1). The predominance of monotypic genera coupled with a severe lack of dominant genera results in an extremely scattered distribution of species at the generic level in the study area. This pattern may be jointly driven by continuous horticultural introduction, strong anthropogenic selection preferences, and the climatic filtering effect of the urban environment. These factors induce sustained and diversified propagule pressure, which enables a large number of genera to be successfully established and maintain populations with only a few individual species, thereby inhibiting the formation of a small number of dominant genera. This result is consistent with the global general pattern that urban floras tend to be internationalized and homogenized [38]: urban environments filter and assemble plant species from all over the world, yet typically only a small number of widely adaptable species can become dominant, while most species exist in a low-frequency and isolated manner. In addition, the evolutionary history of the flora in the study area (e.g., multiple independent immigration events) and local random colonization processes may also play a certain role in shaping this pattern.

Table 1. Genus Composition of Exotic Spontaneous Plants.

	Genus Type				
	Monotypic Genus	**Ditypic Genus**	**Trittypic Genus**	**Tetratypic Genus**	**Pentatypic Genus**
Genus/Species	127/127	29/58	8/24	4/16	2/10
Percentage (%)					
Genus proportion	74.7	17.1	4.7	2.4	1.2
Species proportion	54.0	24.7	10.2	6.8	4.3

Monotypic genus: genera with one species; Ditypic genus: genera with two species; Trittypic genus: genera with three species; Tetratypic genus: genera with four species; Pentatypic genus: genera with five species.

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In terms of geographical elements (Figure 2), cosmopolitan species account for 71.8% of the total number of species, tropical elements account for 17.1%, and temperate elements (mainly North Temperate Zone distribution, etc.) account for 6.4%. Elements such as the Mediterranean-Central Asia distribution type, pantropical distribution type, and East Asia-North America disjunct distribution type account for 2.2%. Exotic spontaneous plants are dominated by highly adaptable cosmopolitan species, which reflects that, under the influence of intense human disturbance and globalization, cosmopolitan species have strong environmental tolerance and are easy to establish communities in new habitats. The relatively large number of tropical elements indicates that road greening in Changchun tends to plant tropical plants, such as *Phyla nodiflora* (L.) Greene and *Mirabilis jalapa* L.

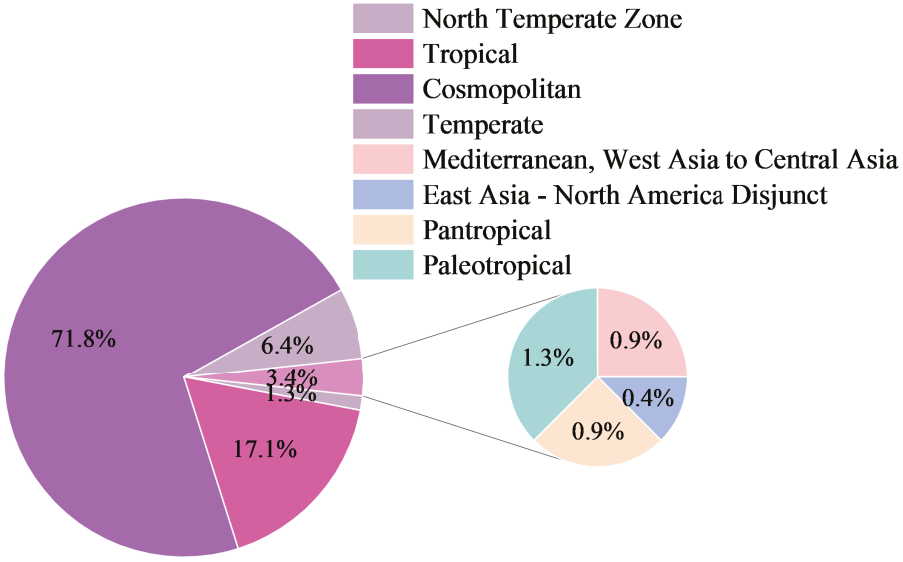

Figure 2. Floristic Composition of Exotic Spontaneous Plants.

2.1.2. Composition Characteristics of Dominant Species and High-Frequency Species

The results of this study indicate that there are fundamental differences in niche structure between spontaneous native plant communities and exotic plant communities. The spontaneous exotic plant community is dominated by a single super-dominant species—*Setaria viridis* (abbreviated as SetVir)—with a comprehensive importance value (IV) as high as 57.05, which is 2.5 times that of *Taraxacum mongolicum* (IV = 22.43), the dominant species of spontaneous native plants (Figure 3c). At the community level, the niche breadth of spontaneous exotic plants is significantly wider than that of spontaneous native plants (Wilcoxon test, $p < 0.05$; Figure 3a). The null model test confirmed that a higher proportion of spontaneous exotic species (17.2%) exhibit niche breadth significantly higher than the random expectation, compared with spontaneous native species (14.3%).

In addition, although the overall proportion of species pairs with significant niche overlap is similar between the two types of plant communities, the niche overlap intensity among dominant spontaneous exotic species is extremely high. The observed maximum niche overlap occurs between the dominant species *Setaria viridis* and *Galinsoga parviflora*, with an overlap index reaching 0.330 (Figure 3b). In contrast, the niche overlap network of dominant spontaneous native species exhibits a more moderate and dispersed pattern.

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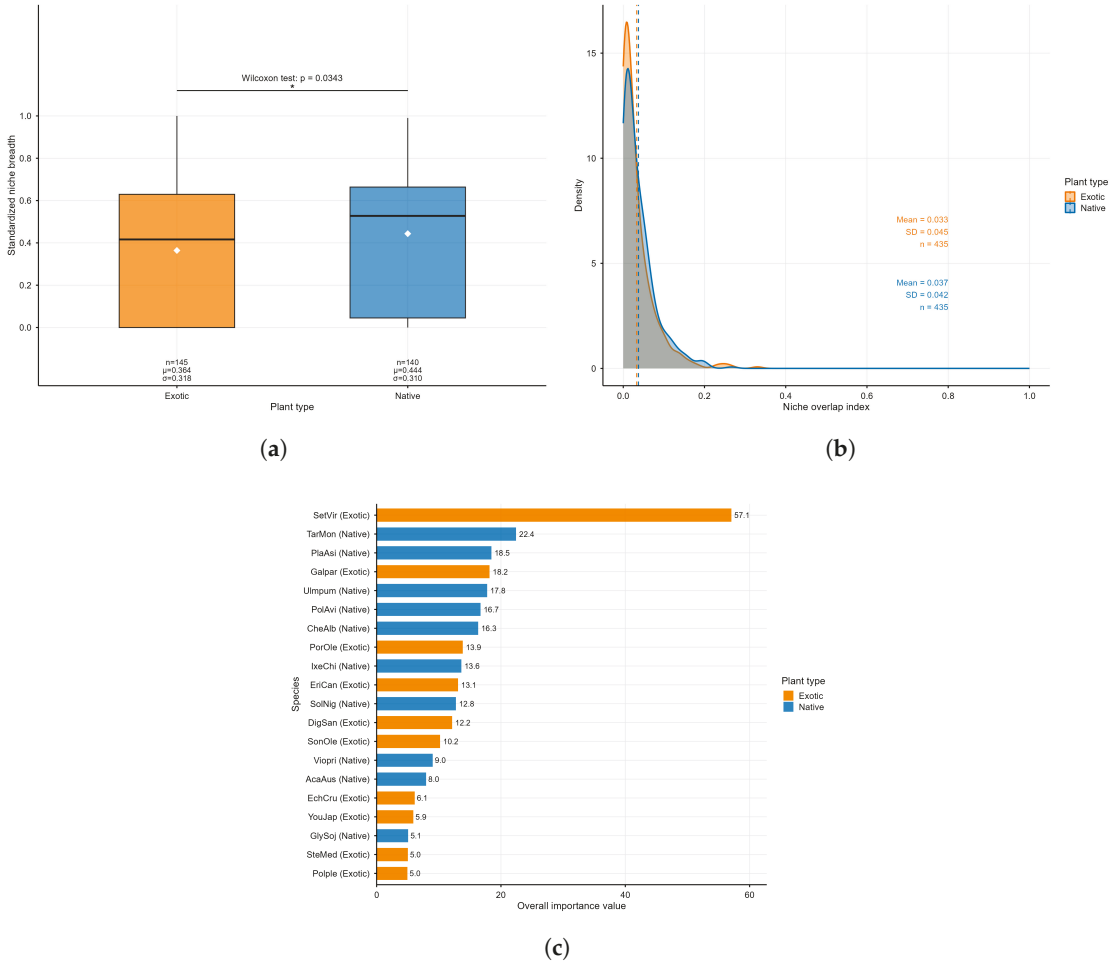

Figure 3. A Comparative Analysis of Ecological Niches and Importance between Spontaneous Native and Exotic Plants (**a**) Boxplot of Niche Breadth for Spontaneous Native and Exotic Plant Species. (**b**) Distribution of Niche Overlap Between Spontaneous Native and Exotic Plant Species. (**c**) Comparative Bar Chart of the Top 10 Species Ranked by Importance Value Among Spontaneous Native and Exotic Plants.

2.2. Distribution Pattern of Exotic Spontaneous Plant Diversity

2.2.1. Diversity Distribution Characteristics of Exotic Spontaneous Plants in Different Habitats

Based on the Kruskal–Wallis nonparametric test for the diversity of exotic spontaneous plants across different habitats (Figure 4), the α-diversity of exotic spontaneous plants showed no significant differences among different habitats (all $p > 0.05$), with an extremely small effect size ($\varepsilon^2 < 0.01$). This result was consistent between the observed data and the rarefaction-corrected data.

This indicates that each habitat supports a similar level of species diversity. This does not, however, indicate identical species composition; instead, there may be a scenario of "equal diversity but distinct species composition" across different habitats.

Non-metric Principal Coordinates Analysis (PCoA) analysis based on Bray–Curtis distances was conducted, and the results of PERMANOVA analysis revealed that $R^2 = 0.014$, $F = 2.931$, and $p = 0.001$ (Figure 5). To explore the components underlying such differences, we performed a β-diversity partitioning analysis. The Sørensen index indicated that the overall β-diversity reached 0.997, among which species turnover contributed 99.9%, while species nestedness accounted for only 0.1%. In habitats with high overall similarity, any detectable limited differences are driven almost entirely by species turnover.

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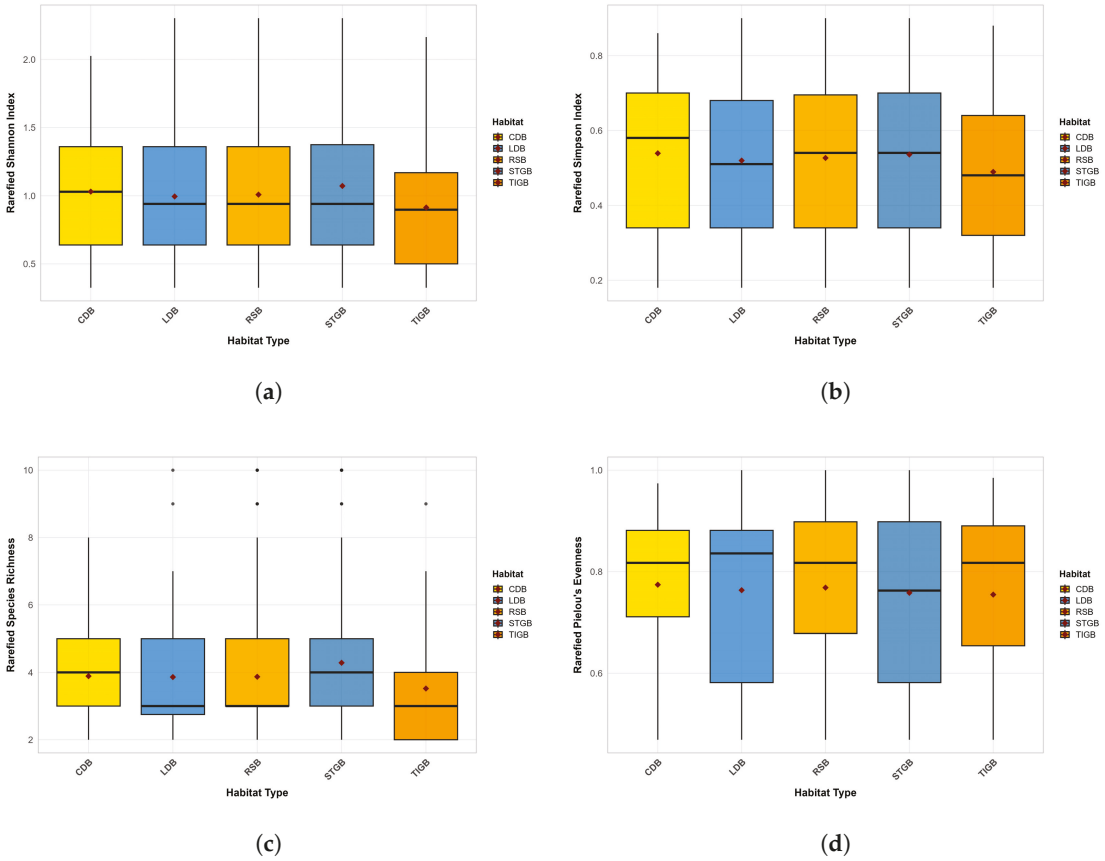

Figure 4. Correlation Analysis between Different Habitat Types and Exotic Spontaneous Plant Diversity. (**a**) Shannon–Wiener Index. (**b**) Simpson's Diversity Index. (**c**) Species Richness. (**d**) Pielou's Evenness Index.

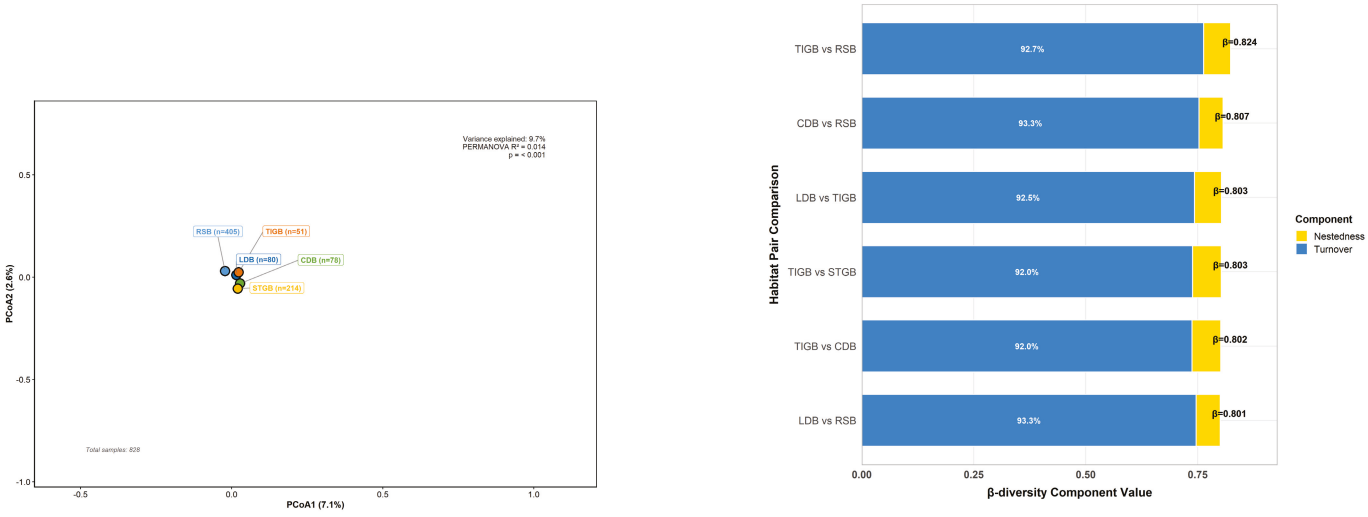

Figure 5. β-Diversity and Partitioning of Exotic Spontaneous Plants Across Habitat Types. (**a**) PCoA Ordination Plot Without Ellipses. (**b**) Stacked Bar Chart of β-Diversity Partitioning.

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2.2.2. Diversity Distribution Characteristics of Exotic Spontaneous Plants Across Different Urbanization Levels

For plant occurrence frequency, a binomial generalized linear model (GLM) was used to test the differences in α-diversity between exotic spontaneous and native plants across different habitat types (Figure 6).

Despite similarly weak effect sizes (ε^2 = 0.002–0.013), alpha diversity analysis revealed the following statistically identifiable spatial structures that may have limited practical implications: For spontaneous exotic plants, their Pielou's evenness exhibited a significant statistical gradient from the urban center (a) to the inner suburbs (ab) and further to the outer suburbs (b). This suggests that the evenness of individual distribution among exotic species may undergo a systematic yet extremely weak change along the urbanization pressure gradient. But for spontaneous native plants, their species richness, Shannon index, and Simpson index, the diversity levels in the urban center and inner suburbs were statistically similar (Group a), and both were significantly higher than those in the outer suburbs (Group b). This implies that the urban core and peripheral areas may represent two statistically distinguishable yet practically undifferentiated states in terms of maintaining native plant diversity.

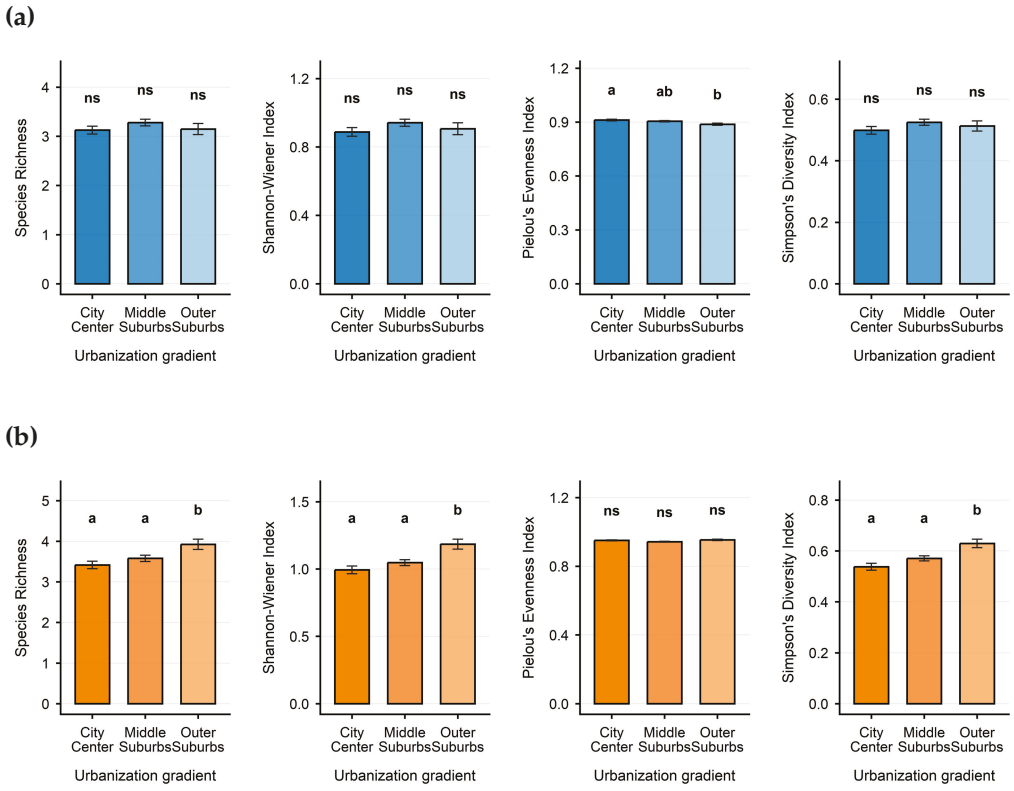

Figure 6. Effects of Disturbance Intensities on the Diversity Distribution Patterns of Native Spontaneous and Exotic Spontaneous Plants. (**a**) α diversity of spontaneous exotic plants along different urbanization gradients. (**b**) α-diversity of spontaneous native plants along different urbanization gradients. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$). "ns" = not significant. Error bars = SEM.

As can be seen from (Figure 7), Principal Coordinates Analysis (PCoA) showed that there were no extremely significant statistical differences in plant community composition among regions ($p > 0.05$). Meanwhile, the extremely low effect size ($\eta^2 = 0.002$) indicated that "region type" had a limited contribution to explaining the variation in overall community composition and failed to form a clear, stable spatial gradient or grouping pattern.

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Despite the a priori assumption of environmental filtering effects potentially induced by the urban gradient, the data analysis did not support the occurrence of systematic changes in plant community composition along the urban center-to-outer suburb gradient. The extremely low explanatory variance suggests that, compared with macro-scale urban gradient zoning, other local environmental factors or stochastic processes may play a more dominant role in the assembly of plant communities within the study area.

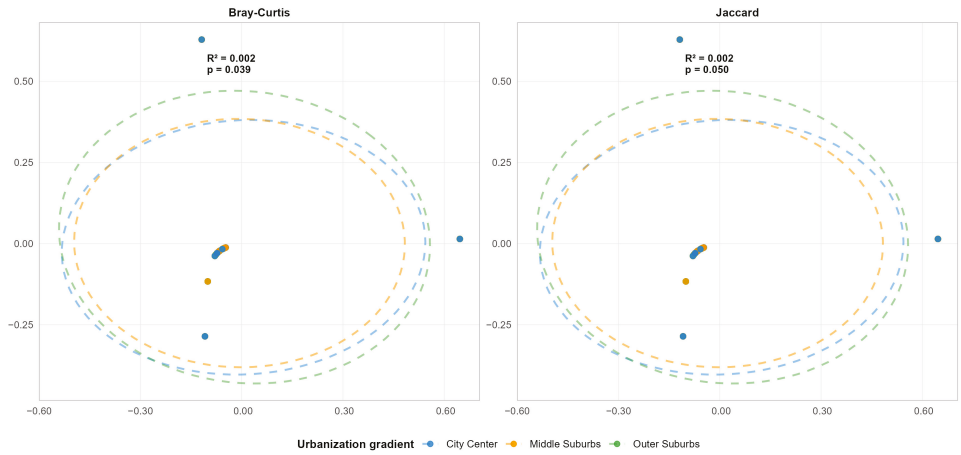

Figure 7. PCoA Confidence Ellipses of β-Diversity for Spontaneous Plants Along Urbanization Gradients.

2.3. Interspecific Relationships Between Exotic Spontaneous Plants and Native Spontaneous Plants

Analysis of the overall interspecific association of species spontaneous herbaceous plants showed that the variance ratio (VR) was 1.4557, which is greater than 1, indicating a positive association among dominant species. The test statistic W was 1844.363. At the 0.05 significance level, the upper critical value of the chi-square distribution was $\chi^2_{0.95} = 1350.921$, and the lower critical value was $\chi^2_{0.05} = 1185.352$. Since the W statistic fell within the right-tailed rejection region, there was an extremely significant overall positive association among the dominant spontaneous species in the road green spaces of Changchun City.

As shown in (Figure 8), we analyzed the 351 species pairs among the 27 dominant species using Fisher's exact test combined with FDR multiple comparison correction. The results showed that, after FDR correction, a total of 67 species pairs (19.09%) exhibited significant associations (corrected $p < 0.05$). Further analysis incorporating effect size criteria ($|V| > 0.3$, $|AC| > 0.3$) revealed only 1 species pair (0.28%), sp27–sp28, with a statistically significant positive association that also had ecological relevance; no ecologically meaningful negative associations were detected. These findings indicate that, although certain statistical associations exist among the dominant spontaneous species in the road green spaces of Changchun City, there are relatively few interspecific associations with strong ecological significance.

In Figure 9, there were **0 pairs** with strong positive association ($AC > 0.5$), **196 pairs (55.84%)** with positive association ($0 < AC \leq 0.5$), **155 pairs (44.16%)** with negative association ($-0.5 \leq AC < 0$), and **0 pairs** with strong negative association ($AC < -0.5$).

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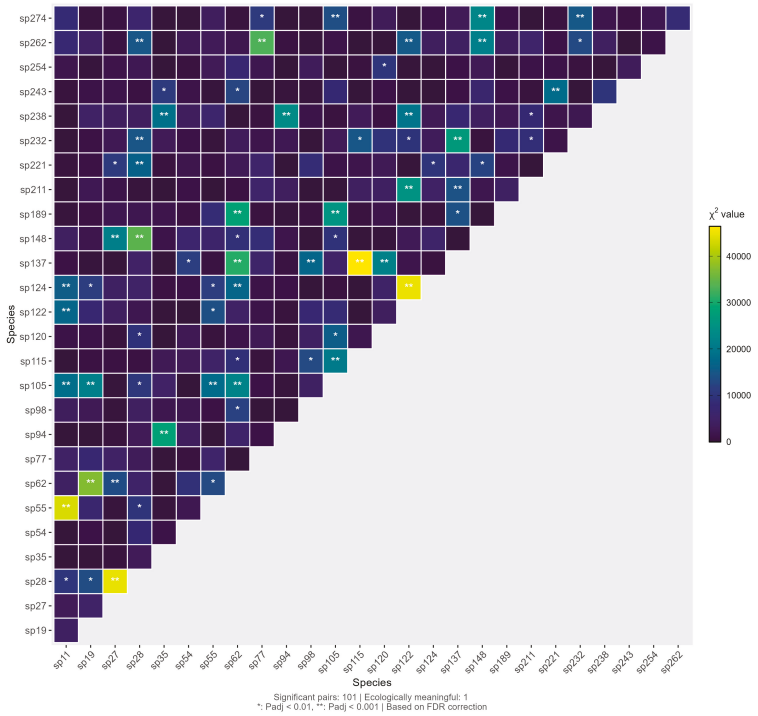

Figure 8. Semi-matrix Plot of χ^2 Test for Interspecific Association of Dominant Spontaneous Herbaceous Plant Species. For details on species, see the Appendix A Table A1.

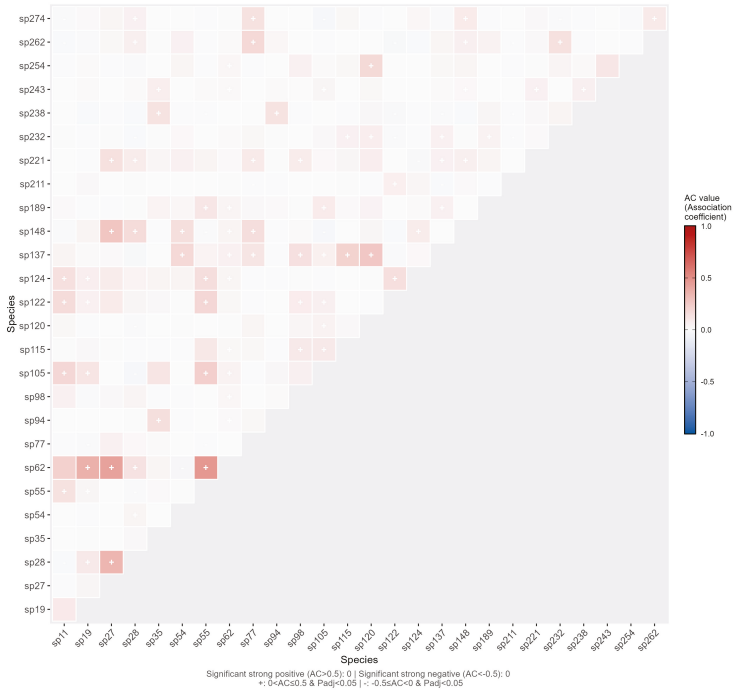

Figure 9. Semi-matrix Plot of AC Values for Interspecific Association of Dominant Spontaneous Plant Species.

As shown in (Figure 10), the median of the Pearson correlation coefficients was $-$**0.0232** and the mean was $-$**0.0173**, indicating a weak negative correlation overall. The absolute values of the correlation coefficients for all 351 species pairs were less than 0.3, meaning **100% of the pairs exhibited weak correlations** ($|r| \leq 0.3$). Among these pairs, 100 (28.49%) showed positive correlations, while 251 (71.51%) showed negative correlations, with negative correlations being dominant.

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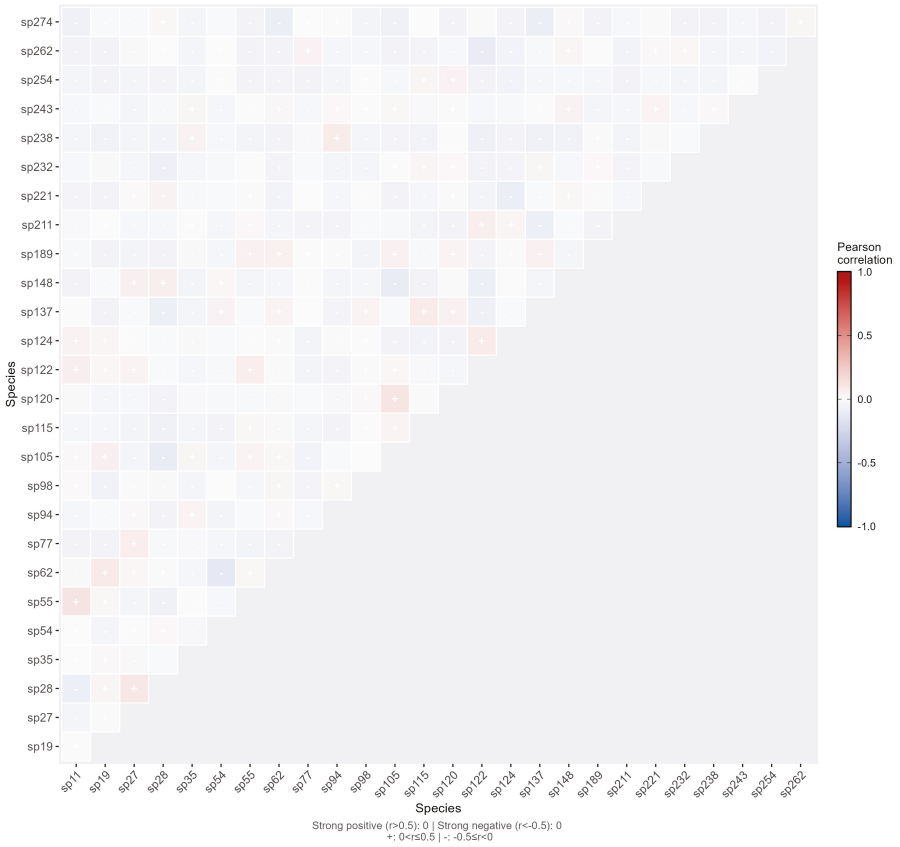

Figure 10. Semi-matrix Plot of Pearson's Rank Correlation Test for Interspecific Association of Dominant Spontaneous Plant Species.

The median of the Spearman correlation coefficients was −0.0017, and the mean was 0.0036, indicating that the overall correlation was close to 0 but slightly positive. Similarly, the absolute values of the Spearman correlation coefficients for all species pairs were less than 0.3, meaning 100% of the pairs showed weak correlations. Among these pairs, 169 (48.15%) exhibited positive correlations, and 182 (51.85%) showed negative correlations, with positive and negative correlations being nearly balanced (Figure 11). The correlation coefficient analysis revealed that the interactions among dominant spontaneous plants in the road green spaces of Changchun City were dominated by weak correlations ($|r| < 0.3$ for 78.35% of species pairs). This proportion was significantly higher than that in natural forest ecosystems (typically 30–50%), reflecting the uniqueness of species interactions in urban artificial ecosystems. Strong correlations were extremely rare ($|r| > 0.5$ for only 5.98% of species pairs) and were predominantly positive (with a positive-to-negative ratio of 20:1), indicating that coordinated changes existed but were limited in intensity. The Spearman correlation coefficients were highly consistent with the Pearson results (r = 0.97), which verified the robustness of the analysis outcomes. This pattern of weak interspecific associations may be attributed to the high heterogeneity of urban environments, frequent anthropogenic disturbances, and the fact that most spontaneous plants are r-strategist species.

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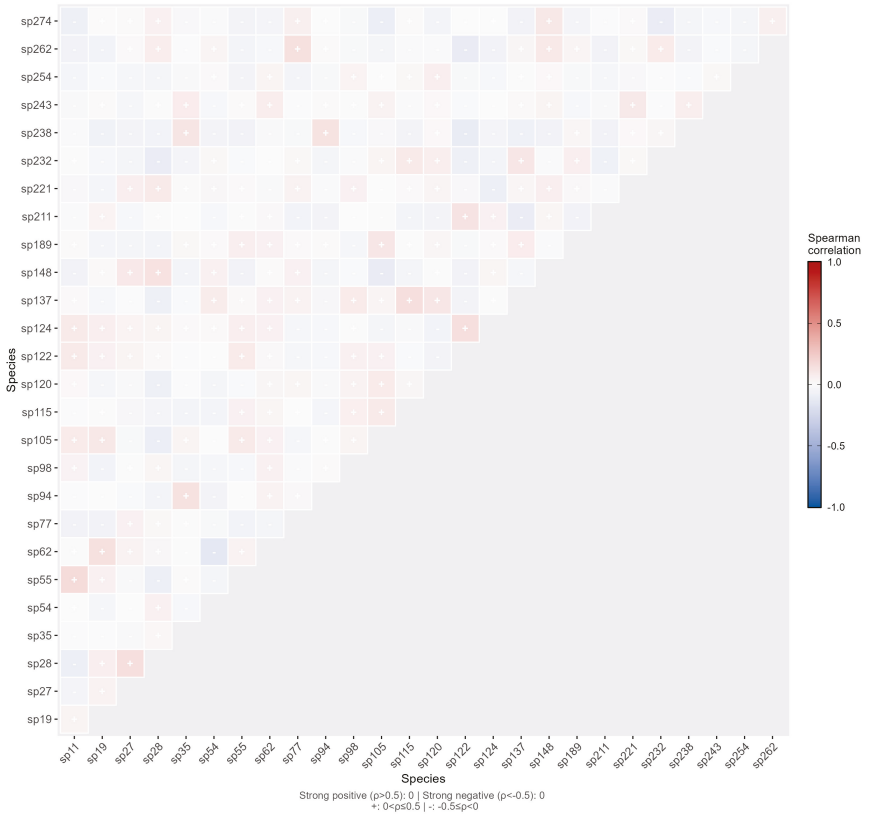

Figure 11. Semi-matrix Plot of Spearman's Rank Correlation Test for Interspecific Association of Dominant Spontaneous Plant Species.

2.4. Interaction Relationships of Diversity Between Exotic Spontaneous Plants and Native Spontaneous Plants

This study systematically analyzed the relationship between spontaneous native and alien plant diversity across 1267 quadrats distributed in five habitat types using multimodel inference (based on AICc) and Bayesian regression. All four diversity metrics—Shannon index, Simpson index, species richness, and Pielou's evenness—revealed a significant positive correlation between native and alien diversity (Pearson's r range: 0.112–0.155, all $p < 0.001$), albeit with a weak correlation strength.

Model comparison analysis indicated that the model structure with the highest explanatory power varied with diversity metrics. For Shannon and Simpson diversity, the additive model (native diversity + habitat type) received the strongest support, with Akaike weights of 76.4% and 81.5% (Figure 12), respectively. Under this model, native diversity exerted a consistent positive effect on alien diversity (standardized coefficient $\beta = 0.132$ and 0.112, respectively, both $p < 0.001$). For species richness, the interaction model (native diversity × habitat type) was the top-supported model (weight = 66.8%), yet it competed with the additive model (weight = 33.2%) with a ΔAICc value of 1.40. This suggests that the relationship might vary across habitats, though the evidence remains inconclusive. The best-fit model for Pielou's evenness was also the additive model (weight = 49.6%), but its support was very close to that of the interaction model (weight = 42.0%), indicating high model uncertainty.

The results of model averaging showed that, after accounting for the uncertainty of all candidate models, native diversity (richness_native, shannon_native, simpson_native, pielou_native) exerted a stable positive effect on all four alien diversity metrics (all full-model average coefficients were positive). Meanwhile, the effect of habitat type (with "HBL" as the reference category) was also evident; for instance, in the Shannon diversity model, the "LDB" habitat exhibited a significant positive effect. Collectively, these findings

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demonstrate that an increase in native plant diversity tends to be accompanied by a rise in alien plant diversity, and this relationship is prevalent across different habitats. However, the specific pattern (i.e., the presence or absence of interaction effects) differs across various dimensions of diversity.

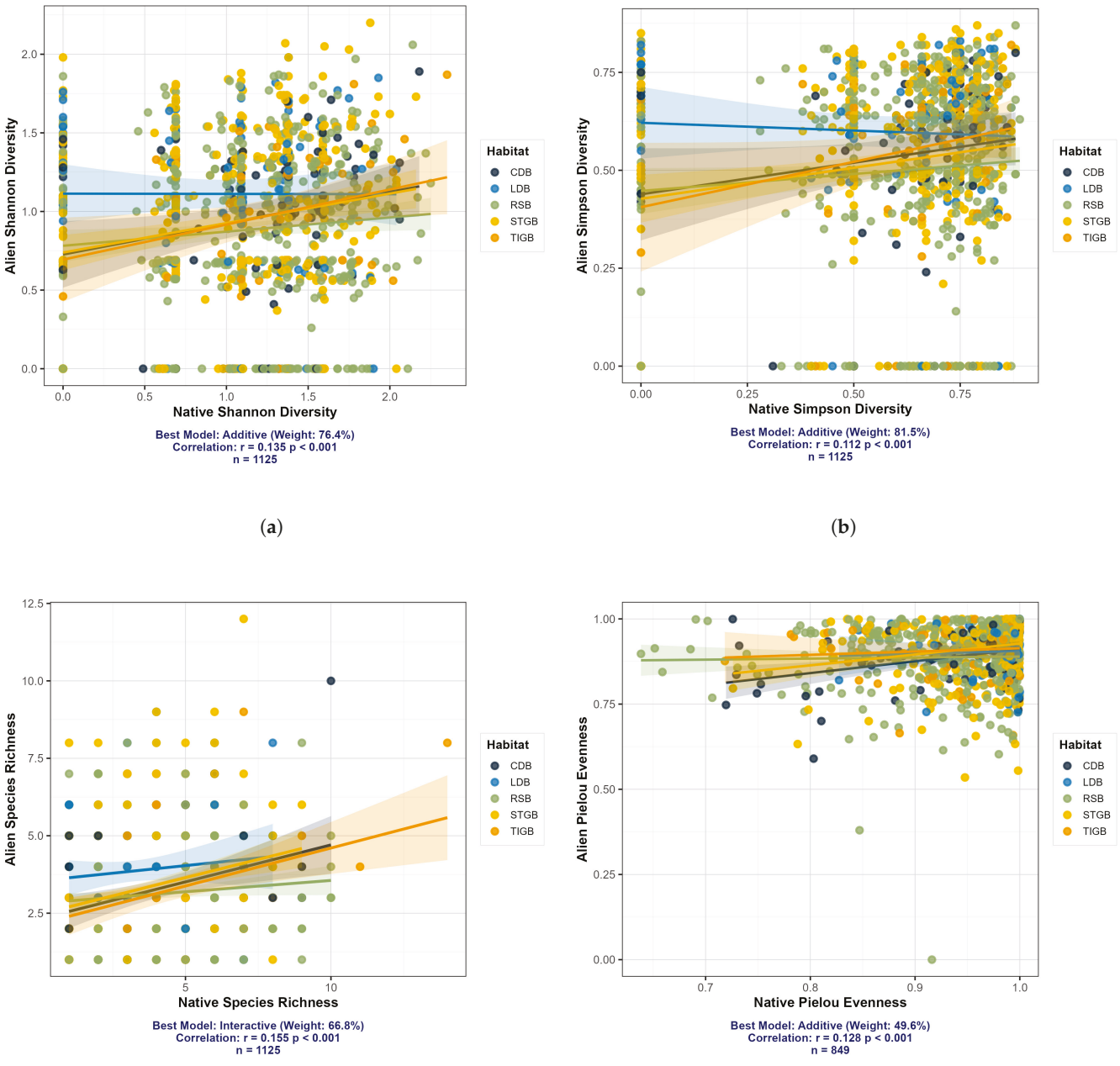

Figure 12. Multi-model Inference Analysis. (**a**) Shannon–Wiener Index. (**b**) Simpson's Diversity Index. (**c**) Species Richness. (**d**) Pielou's Evenness Index.

3. Discussion

3.1. Composition Characteristics of Species Diversity of Exotic Spontaneous Plants in Road Green Spaces

Urbanization has profoundly shaped the family and genus composition of exotic spontaneous plants in road green spaces. This is manifested in the community being dominated

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by a few dominant families, while species richness is mainly contributed by a large number of oligotypic families and monotypic families. Although the number of genera is very rich, the contribution of dominant genera to the total number of species is insufficient, indicating a high degree of plant species differentiation and complex composition in this area. This phenomenon is also a direct reflection of the heterogeneity of the urban environment, which is consistent with the research results of Tian Zhihui et al. [39,40]. Asteraceae is an important source of exotic spontaneous plants, which is related to their strong adaptability, large number of species, and better ability to adapt to harsh environments [41]. In terms of species origin, a quarter of the exotic spontaneous plants in road green spaces are escaped species, and most of them are tropical species, indicating that human preferences have an important impact on the composition of exotic spontaneous plants [42]. In the statistics of geographical component composition, it is found that the exotic spontaneous plants in road green spaces are dominated by cosmopolitan species, which is significantly different from the pattern where tropical component families are dominant in southern cities [22]. This phenomenon occurs because, restricted by the geographical conditions of cold regions, the low-temperature environment screens out species with strong adaptability and prominent diffusion ability, and a large number of random immigrations lead to cosmopolitan species occupying an obvious dominant position.

There are significant differences in niche structure between spontaneous native and exotic plant communities. The spontaneous exotic community is strongly dominated by a single dominant species, Setaria viridis, and approximately 17.2% of spontaneous exotic species exhibit niche breadth exceeding random expectations, indicating that exotic plants may possess broader resource utilization capabilities or stronger environmental adaptability. This pattern may be related to the niche release of spontaneous exotic plants under frequent human disturbances, enabling them to gain an advantage in resource competition. The dominant exotic species show high niche overlap, suggesting that these species may have highly similar resource requirements or microhabitat preferences, and may even co-invade through interspecific facilitation. In contrast, the niche overlap network of native species is more dispersed, reflecting niche differentiation formed during long-term coexistence, which may contribute to maintaining the stability of spontaneous native communities. The above results support the hypothesis that "spontaneous exotic species can establish dominance through niche expansion and high-intensity niche overlap". This is highly consistent with the findings of Zhang et al. that Erigeron annuus, an invasive plant species, exhibits the widest niche breadth, dominates the community, and mostly presents a competitive relationship with its main associated plant species [43]. Future studies can combine functional traits and long-term dynamic data to further reveal the causal relationship between niche structure and invasion success.

3.2. Adaptation Strategies of Exotic Spontaneous Plants Across Different Habitat Types and Urbanization Levels

There were no significant differences in the α-diversity of spontaneous exotic plants across different habitats [44,45], whereas the β-diversity was extremely high with a very low explanatory power, and this variation was almost entirely contributed by species turnover. This indicates that, although various habitats sustain a similar level of species richness of spontaneous exotic plants, their community compositions are heterogeneous, presenting a pattern of 'similar diversity level yet distinct species composition'.

The lack of significant differences in α-diversity across habitats may reflect that spontaneous exotic plants are able to find exploitable niches or resource opportunities in all the habitats defined in this study, or indicate that the colonization of exotic plants in the study area has reached a certain state of 'saturation'. In addition, anthropogenic disturbance, habitat fragmentation, or the reduction in dispersal limitation may have facilitated the

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establishment of spontaneous exotic species populations across different habitats, thereby homogenizing the diversity levels. The combined results of PERMANOVA and β-diversity partitioning analysis confirm that the differences in species composition across habitats, albeit limited, are statistically significant, and these differences are almost entirely derived from species turnover [46]. The assembly of spontaneous exotic plant communities may thus be more dependent on habitat-specific species replacement rather than the overall increase or decrease in species number. This implies that even if different habitats harbor a similar number of species, the differences in their species composition may still lead to variations in ecosystem functions [42].

Habitats such as road verges, median strips, and traffic islands exhibit differences in terms of width, soil volume, moisture conditions, intensity of anthropogenic trampling and mowing, and the impacts of vehicle exhaust and snow-melting agents. The heterogeneity of these physical and disturbance conditions constitutes an "environmental filter" [47] that selects for species with distinct tolerances and ecological strategies. For instance, drought-tolerant and compaction-resistant species may dominate the central median strips, whereas shade-tolerant species or those capable of withstanding intermittent waterlogging tend to concentrate in road verges or street tree green belts. This habitat-specific filtering mechanism may account for the high rate of species turnover alongside the convergence of overall species richness.

At the urban gradient scale, although a species composition pattern with high turnover was maintained among different habitat types, the variation in plant communities along the macro-spatial gradient from urban center to outer suburbs was extremely limited. Specifically, α-diversity only exhibited a weak statistical gradient with limited practical significance, while β-diversity failed to form a significant spatial differentiation pattern. This contrast may indicate that the filtering effect of local habitat characteristics has a stronger influence than the macro-scale urbanization gradient effect.

Principal Coordinates Analysis (PCoA) revealed that community composition did not exhibit differentiation along the urban gradient. This finding stands in contrast to the high species turnover rate observed among different habitat types (significant in PERMANOVA). This discrepancy may be attributed to the dominance of local habitat filtering over the urban gradient effect: specifically, the plant community in a traffic island in the urban center may differ substantially from that of the adjacent roadside community yet be quite similar to the plant community in a traffic island in the outer suburbs.

The conclusions of this study are based on a specific city, and the length and intensity of its urban gradient (e.g., the historical development of the urban center, suburban development modes) may affect the generalizability of the results.The low explanatory power of the "region type" indicator suggests that future studies should quantitatively identify specific local driving factors (e.g., soil physicochemical properties, precise disturbance frequency, surrounding land use types) [48,49] instead of relying on macro-scale classification schemes. Future research can combine functional trait and phylogenetic analyses to further test the hypothesis that habitats of the same type across different regions filter out plant communities with similar functional characteristics.

3.3. Diversity and Interspecific Relationships Between Exotic and Native Spontaneous Plants in Road Green Spaces

This study reveals that the interspecific associations among dominant spontaneous plant species in the road green spaces of Changchun exhibit a pattern of 'overall significant positive correlation, but predominantly weak pairwise interactions' [50,51]. The overall variance ratio indicates a positive correlation, suggesting a tendency for species coexistence at the landscape scale. However, correlation analyses of specific species pairs show that up to 100% of species pairs exhibit weak correlations ($|r| \leq 0.3$), with ecologically significant

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associations being extremely rare. This widespread pattern of weak correlations is distinctly different from the characteristics of natural ecosystems and represents a typical outcome of urban artificial habitats [52]. The formation of this pattern can be attributed to frequent and intense anthropogenic disturbances: routine management practices such as mowing, weeding, and trampling continually reset community succession processes, hindering the establishment and development of stable long-term interspecific relationships. It is also related to the significant and unstable heterogeneity of road habitats in terms of soil properties, moisture conditions, and pollution levels. In such environments, species distribution is more constrained by short-term tolerance and responses to heterogeneous habitats rather than fixed biotic interactions. Among these species, those capable of natural colonization and becoming dominant typically possess strong dispersal abilities, fast growth rates, and short life cycles, characteristics associated with broad ecological niches and an opportunistic survival strategy.

Combined with previously observed high species turnover rates among habitats and weak urban macro-gradient effects, the weak biotic interactions can be integrated into a continuous framework of "environmental filtering → random assembly → loose co-existence." Within this framework, environmental filtering primarily operates between different habitat types, while random processes and weak biotic interactions dominate the specific species combinations within each habitat type. The current study deduces inter-specific associations from coexistence data; future research should incorporate controlled experiments to verify the nature and strength of these interactions. Additionally, further analysis of the distribution of species functional traits is needed to clarify whether this "weak association" corresponds to high functional similarity (niche overlap) or significant trait differences (niche differentiation) among species.

Furthermore, by integrating multi-model inference and Bayesian analysis, a widespread but weak positive correlation was found between the diversity of spontaneous native and exotic plants in urban road green spaces. This finding contradicts the traditional view of the diversity-invasibility hypothesis—which posits that highly diverse communities tend to inhibit the natural colonization of exotic species—and instead supports the co-variation hypothesis or habitat filtering mechanism. That is, specific environmental factors such as resource enrichment and disturbance can simultaneously promote the diversity of both native and spontaneous exotic plants. This conclusion aligns with the findings of indicating that under different environmental conditions, mutualistic interactions among species can determine the success or failure of biological invasions.

The weak positive correlation between spontaneous native and exotic plant diversity likely stems from their coordinated response to habitat heterogeneity. As mentioned earlier, exotic spontaneous herbaceous plants exhibit high turnover rates across different habitat types (e.g., roadside verges, central medians). Habitat patches with complex microenvironments, diverse resource types, or moderate disturbance intensities can provide opportunities for the colonization and coexistence of both spontaneous native and exotic plants. Therefore, the observed positive correlation is not a result of direct causal interactions between species but rather a parallel response of both groups to highly suitable or high-capacity habitats [53].

The widespread pattern of weak interspecific associations identified in this study provides crucial ecological context for the aforementioned positive correlation. In habitats dominated by high environmental stress (e.g., anthropogenic disturbance, soil constraints) and random dispersal, intense competitive exclusion among successfully established species is diminished. This loose community structure reduces the biotic resistance faced by new colonizers (including exotic species), allowing native and spontaneous exotic plants to share niche spaces shaped by environmental heterogeneity without strong mutual exclusion.

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Analysis based on the Shannon and Simpson indices (reflecting species richness and evenness) shows that additive models dominate, indicating that the positive effect of spontaneous native plant diversity is relatively consistent across different habitats. This implies that community structures or resource states capable of maintaining high richness and evenness of spontaneous native plants inherently provide invasion opportunities or utilizable resource spectra for exotic species. In the analysis of species richness, interactive models received some support, suggesting that the slope of the spontaneous native–exotic species richness correlation may vary with habitat type. For example, in habitats with high resource variability and intense disturbance (e.g., central medians), spontaneous native plants may struggle to form effective competitive barriers, resulting in a neutral or positive correlation between spontaneous native and exotic species richness. Conversely, in habitats with relatively stable resources and complex structures (e.g., wide road green belts), native communities may exert a weak inhibitory effect on exotic species, flattening the slope of the positive correlation.

All core findings of this study can be integrated into a unified conceptual model describing the plant community assembly process in urban road ecosystems: At the landscape scale, habitat filtering dominates. At the patch scale, habitat heterogeneity and random dispersal jointly shape diversity levels. Within specific habitats, local environmental heterogeneity and stochastic seed dispersal events collectively determine the total number of species a patch can accommodate (α-diversity). Highly heterogeneous patches can simultaneously support more native and exotic spontaneous species, resulting in a weak positive correlation between their diversity.

Within communities, loose coexistence and weak interactions are characteristic. Under high-frequency disturbances and environmental stress, successfully coexisting species form a widespread pattern of weak associations, with intense competition or mutualism being extremely rare. Community structure exhibits a high degree of stochasticity and plasticity.

It should be noted that the causal mechanisms underlying the positive correlations revealed by this observational study require validation through controlled experiments such as seed addition or species removal. Future research should directly quantify key environmental factors like soil nutrients and physical stress intensity, and integrate species functional trait spectra to more precisely elucidate the mechanism chain of environmental filtering $\rightarrow$ species coexistence.

4. Materials and Methods

4.1. Overview of the Study Area

Changchun City (43°10′–45°15′ N) is situated in Northeast China and experiences a temperate continental monsoon climate. Based on the 1991–2020 climate normals, the city has an annual mean temperature of 6.6 °C and receives an average annual precipitation of 592.7 mm. Seasonal contrasts are pronounced: the warmest month (July) averages 21.9 °C, while the coldest month (January) averages –12.0 °C. Spring is characterized by strong winds, with maximum speeds occasionally reaching 30 m/s, whereas autumn typically sees milder wind conditions.

In the regional context, families such as Asteraceae, Amaranthaceae, Poaceae, Fabaceae, and Solanaceae are reported to be among the main families of exotic invasive plants in Jilin Province. At the urban scale, according to the Changchun Statistical Yearbook 2022, roads occupy approximately 12% of the built-up area, with both road-green coverage and road density having shown increasing trends in recent years.

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4.2. Research Methods

4.2.1. Plot Setup

In ArcGIS, a cross-shaped transect with a width of 5 km was drawn with Changchun's city center (People's Square) as the origin, ensuring coverage of most road distribution areas within the built-up area (Figure 13). The species names of street trees within the transect were identified via Baidu Maps Street View and field surveys. Dominant tree species were selected to set up research sampling points, with the number of quadrats for different dominant tree species kept approximately equal. During the survey, centered on each selected sampling point, one 20 m × 20 m arbor quadrat, four 5 m × 5 m shrub quadrats, and five 1 m × 1 m herb quadrat were laid out. The length and width of the quadrats were set according to the crown width of the arbor trees, with the requirement that crown width × length = 400 m². Information was recorded for spontaneous plants in 1268 quadrats across 269 sampling sites, including their names, cultivation types, height, coverage, diffusion types, as well as the management status, health conditions of street trees, and road-related information. Clear photographs were taken of the habitats, surrounding environments, overall road scenes, and each layer of the quadrats.

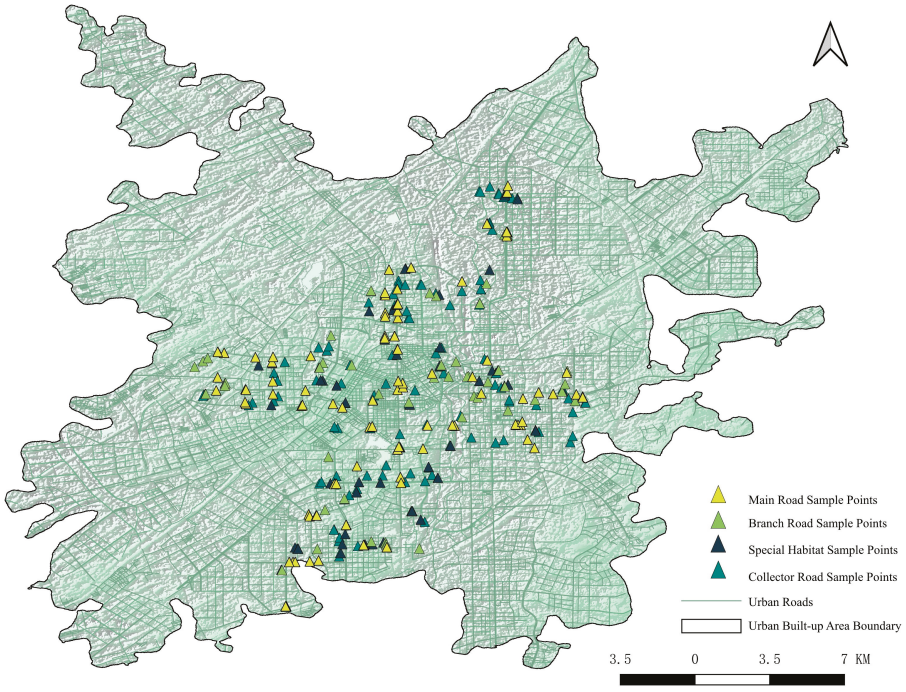

Figure 13. Distribution Map of Surveyed Sampling Sites.

4.2.2. Habitat Type Classification

In accordance with the Standard for Classification of Urban Green Space (CJJT 85-2017), the urban road green spaces in Changchun City were divided into the following 5 categories based on the different positions of green belts within the road cross-section (Figure 14).

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Habitat Type	Habitat Code	Habitat Description	Habitat Photo
Sidewalk Planting Strip	STGB	A green belt located between sidewalks and non-motorized vehicle lanes, or between sidewalks and motorized vehicle lanes, which is dominated by street tree planting.	
Roadside Planting Strip	RSB	A green belt located between the outer edge of the sidewalk and the road red line on the same side.	
Lateral Divider Green Belt	LDB	A divider green belt located between motorized vehicle lanes and non-motorized vehicle lanes, or between motorized vehicle lanes traveling in the same direction.	
Central Divider Green Belt	CDB	A divider green belt located between the upward and downward motorized vehicle lanes	
Traffic Island Green Space	TIGB	A vegetation-dominated green area located within traffic islands established at urban road intersections, roundabouts, or the mid-sections of road segments.	

Figure 14. Habitat Classification Standards.

4.2.3. Classification of Urbanization Level and Disturbance Intensity

Based on the 10 m resolution Landsat series satellite images from 2023, this study used ENVI 5.6 and ArcGIS 10.7 to interpret the land cover of the study area into five types: urban construction land, water bodies, vegetation, cultivated land, and bare land. The maximum distance from each sampling point to the urban boundary was calculated to characterize the urbanization level, and the equal interval method was used to divide the study area into three urbanization level gradients: outer suburbs, inner suburbs, and city center.

4.3. Data Processing

(1) This study quantified and compared the ecological strategies of native and exotic plant communities using three complementary niche metrics. First, we calculated the overall importance value for each species by integrating its relative frequency and abundance across all plots. Second, the niche breadth of each species was assessed using Levin's standardized index, which measures the uniformity of a species' distribution across environmental gradients (ranging from 0, specialist, to 1, generalist). Third, pairwise niche overlap among species was quantified using Pianka's index, ranging from 0 (no overlap) to 1 (complete overlap). To control for the influence of dominant species and reduce computational complexity, analyses of niche breadth and overlap were focused on the top 30 species ranked by overall importance. To determine whether the observed niche patterns differed from random expectations, we performed null model analyses. We used a randomization algorithm that shuffled species occurrence and abundance data among plots while preserving the matrix structure. Significance was tested with 999 permutations,

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and resulting p-values were adjusted using the False Discovery Rate (FDR) correction. All statistical analyses and visualizations were performed in R version 4.5.2. The average value of the experimental data is calculated using the following formula:

$$\mathrm{IV}_i = \frac{\mathrm{RF}_i + \mathrm{RC}_i}{2} \tag{1}$$

In the formula, IV_i represents the importance value of the *i*-th species; RF_i represents the relative frequency of the *i*-th species, and RC_i represents the relative coverage of the *i*-th species.

- Relative Frequency (RF) = Frequency of a certain species in the quadrat/Sum of frequencies of all species
- Relative Coverage (RC) = Coverage of a certain species in the quadrat/Sum of coverages of all species

(2) Calculation of α-diversity, including the Richness index (R), Shannon–Weiner diversity index (H'), Pielou evenness index (J), and Simpson dominance index (D). The calculation formulas are as follows:

$$R = S \tag{2}$$

In the formula, S denotes the number of species in the quadrat.

$$H' = -\sum_{i=1}^{S} p_i \ln p_i \tag{3}$$

where:

- H': Shannon-Weiner diversity index
- S: total number of species
- p_i: relative abundance of the *i*-th species, $p_i = \frac{n_i}{N}$
- n_i: number of individuals of species *i*
- N: total number of individuals, $N = \sum_{i=1}^{S} n_i$

The Pielou evenness index (J) is calculated as:

$$J = \frac{H'}{H_{\max}} = \frac{H'}{\ln S} \tag{4}$$

where:

- J: Pielou evenness index (ranges from 0 to 1)
- H': observed Shannon-Weiner diversity index
- $H_{\max}$: maximum possible diversity, $H_{\max} = \ln S$
- S: total number of species

The Simpson dominance index (D) is calculated as:

$$D = \sum_{i=1}^{S} p_i^2 \tag{5}$$

where:

- D: Simpson dominance index (ranges from 0 to 1)
- p_i: relative abundance of the *i*-th species, $p_i = \frac{n_i}{N}$
- S: total number of species

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(3) The probability of species presence across habitat types was modeled using generalized linear models with a logit link function:

$$\log\left(\frac{p_{ij}}{1-p_{ij}}\right) = \beta_0 + \beta_1 \cdot \text{CityCenter}_j + \beta_2 \cdot \text{MiddleSuburbs}_j + \beta_3 \cdot \text{OuterSuburbs}_j + \epsilon_{ij} \quad (6)$$

where:

- p_{ij} is the probability of presence (either alien or native species) in plot i within habitat type j
- β_0 is the intercept (baseline log-odds when all predictors are 0)
- β_1, β_2, β_3 are coefficients for habitat types (with reference level encoded in the model matrix)
- CityCenter_j, MiddleSuburbs_j, OuterSuburbs_j are indicator variables for the three habitat types
- ϵ_{ij} is the error term

Separate models were fitted for alien and native species presence.

4.4. α-Diversity Differences Among Habitats (Linear Models)

To compare diversity indices among habitat types, linear models were applied after appropriate transformations to meet normality assumptions:

$$Y'_{ijk} = \mu + \alpha_j + \epsilon_{ijk} \quad (7)$$

$$\epsilon_{ijk} \sim \mathcal{N}(0, \sigma^2) \quad (8)$$

where:

- Y'_{ijk} is the transformed diversity index value for:
  - Species richness: $Y' = \log(Y + 1)$
  - Shannon index: $Y' = \sqrt{Y + 0.01}$
  - Pielou's evenness: $Y' = Y$ (no transformation, range 0–1)
  - Simpson's index: $Y' = Y$ (no transformation, range 0–1)
- μ is the overall mean
- α_j is the fixed effect of habitat type j ($j = 1, 2, 3$)
- ϵ_{ijk} is the residual error, assumed normally distributed with mean 0 and variance σ^2

When the overall F-test from Type III ANOVA was significant ($p < 0.05$), **Tukey's Honestly Significant Difference (HSD)** post hoc test was conducted for all pairwise comparisons between habitat types.

4.5. Diversity Trends Along Urbanization Gradient (Linear Regression)

To test for linear trends in diversity indices along the urbanization gradient, simple linear regression models were fitted:

$$Y'_{ik} = \beta_0 + \beta_1 \cdot X + \epsilon_{ik} \quad (9)$$

where:

- Y'_{ik} is the transformed diversity index (same transformations as above)
- X is the numerical urbanization gradient: City Center = 1, Middle Suburbs = 2, Outer Suburbs = 3
- β_0 is the intercept

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- β_1 is the slope coefficient representing the linear trend
- ϵ_{ik} is the error term

5. Conclusions

This study clarifies the diversity distribution patterns and adaptive mechanisms of spontaneous plant communities in road greenbelts of Changchun City; the key conclusions are as follows. At the family and genus levels, exotic spontaneous plants are characterized by a composition dominated by dominant families and enriched with oligotypic families, and their niche structure features the predominance of single dominant species, broad niche breadth, and high niche overlap, which reflects their adaptive expansion strategies under frequent anthropogenic disturbances. There are no significant differences in the α-diversity of exotic spontaneous plants across different habitats, whereas extremely high species turnover rates are observed among habitat types, indicating that the filtering effect of local habitats plays a dominant role, while the impact of macro-urbanization gradients is negligible. In addition, a weak positive correlation exists between the diversity of exotic and native spontaneous plants, and a prevalent pattern of weak interspecific associations is exhibited throughout the entire community. Collectively, these findings support a unified conceptual model of **"environmental filtering → stochastic assembly → loose coexistence"**; specifically, at the landscape scale, species are filtered by habitat heterogeneity, within habitat patches, community assembly is dominated by stochastic dispersal and weak biotic interactions, and ultimately, this process gives rise to urban artificial plant communities with a loose structure and high plasticity.

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Appendix A

The information about native plants and their codes involved in the interspecific association analysis in the article is shown in the table below.

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Table A1. Table of Interspecific Association Species.

Species Code	Latin Name
sp11	Echinochloa crus-galli
sp19	Polygonum aviculare
sp27	Potentilla supina
sp28	Plantago asiatica
sp35	Cirsium arvense var. integrifolium
sp54	Stellaria media
sp55	Amaranthus retroflexus
sp62	Setaria viridis
sp77	Youngia japonica
sp94	Sonchus wightianus
sp98	Sonchus oleraceus
sp105	Chenopodium album
sp115	Solanum nigrum
sp120	Cynanchum rostellatum
sp122	Portulaca oleracea
sp124	Digitaria sanguinalis
sp137	Galinsoga parviflora
sp148	Taraxacum mongolicum
sp189	Acalypha australis
sp211	Polygonum plebeium
sp221	Erigeron canadensis
sp232	Commelina communis
sp238	Glycine soja
sp243	Lactuca serriola
sp254	Ulmus pumila
sp262	Viola prionantha
sp274	Ixeris chinensis

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