

Article

Effects of Saline–Alkali Composite Stress on the Growth and Soil Fixation Capacity of Four Herbaceous Plants

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Abstract: Plants play a crucial role in soil fixation and enhancement of slope stability, and saline–alkaline stress is one of the main restrictions inhibiting plant growth and development. At present, there is a lack of research on the effects of saline–alkaline composite stress on the mechanical properties of the root system and the erosion resistance of the root–soil complex. In this study, three gradients of saline–alkaline composite stress treatments and a control of saline-free treatment was set up for *Oenothera biennis*, *Perilla frutescens*, *Echinops sphaerocephalus*, and *Lychnis fulgens*. The plant salt damage rate, osmotic index, antioxidant enzyme activity and plant root morphological indicators were measured. The biomechanical characteristics were determined by stretching tests, the resistance of the plant was measured by a whole-plant vertical uprooting test, and the anti-erosion capacity of the root soil composite was measured by scrubbing test. The results showed that, at 200 mM, the salt damage index and salt damage rate of the four plants, in descending order, were as follows: *E. sphaerocephalus* < *L. fulgens* < *O. biennis* < *P. frutescens*. Among them, SOD of *Perilla frutescens* did not play an obvious protective role, and the substantial changes in CAT and POD, as well as the content of soluble sugars, soluble proteins, and proline, showed its sensitivity to saline and alkaline stresses. Root growth was also significantly suppressed in all four plants, the 100- and 200-mM concentrations of saline solution significantly reduced the average tensile strength of *O. biennis* and *P. frutescens*, while the saline–alkali solution of 200 mM significantly reduced the elongation of *E. sphaerocephalus* and *L. fulgens*, and significantly elevated the soil detachment rate of the root–soil composite for *E. sphaerocephalus*. Additionally, all three concentrations of saline treatments significantly reduced the pullout resistance of all 4 plants. There was a negative power rate relationship between tensile resistance and root diameter in four plant species, while the relationship between tensile strength and root diameter showed a negative power law only for *L. fulgens* treated with 0–50 mM saline solution. There was no significant correlation between elongation and root diameter in the four plants. *P. frutescens* had the greatest tensile resistance and strength, as well as the lowest rate of elongation, while *L. fulgens* possessed the greatest pullout resistance, and both had comparable resistance to erosion of the root–soil complex. Therefore, compared to the other three plants, *L. fulgens* is more suitable for soil reinforcement applications on saline slopes.

Keywords: highway slope; saline–alkali composite stress; plant salinity tolerance; root mechanical properties; root sequestration capacity



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1. Introduction

For the past few years, with the large-scale construction of the national highway network in China, many highway slopes have been formed. The generally steep slopes along the highways and the large amount of excavation and fill in the highway construction

process, in addition to a poor management of slope exposure, has led to a series of problems in soil and water conservation, ecological balance, and the environment [1]. Soil erosion on highway slopes mainly exists in two common forms of shallow slope damage, i.e., “shallow landslides” and “shallow erosion” [2]. Shallow erosion is usually caused by a variety of factors, including soil freeze–thaw cycles [3], snow movement, human activities and animal trampling [4], leading to small-scale damage to vegetation and topsoil. Shallow landslides, on the other hand, are caused by internal processes, where downward movement of the topsoil occurs when the gravity of the slope exceeds the mechanical resistance of the slope [5]. Throughout the process, shallow landslides play a dominant role and influence the amount of soil erosion [6]. Once the slope of a highway is destabilized, the materials on the road shift, causing traffic congestion and economic losses, even posing a threat to human safety [7]. In addition, natural restoration of slope vegetation often takes a long time, while artificial vegetation restoration not only consumes both human and financial resources, but also makes it difficult to achieve the desired results [8]. Therefore, the early prevention of slope destabilization is the best measure to effectively avoid both economic losses and environmental destruction [9], and vegetation coverage is currently considered to be the most cost-effective method to control slope soil erosion [10,11]. Additionally, Shan et al. [12] compared different methods of soil consolidation and slope protection for seasonally frozen and thawed soil slope types in Northeast China, and found that both economic and ecological benefits could be improved with the use of vegetation cover.

Vegetation, considered as organisms that conserve both soil and water, not only intercepts rainfall and slows surface runoff but also reduces soil erosion [13]. The root systems of plants have been proven to bolster soil stability and augment resistance to soil erosion. They achieve these benefits by regulating hydrological elements and mechanically reinforcing the soil [14]. The term “hydrological regulation” speaks to the capability of plant canopies and roots to intercept precipitation, thereby delaying the inception of shallow landslides. On the other hand, “mechanical reinforcement” involves the anchoring properties of thick roots and the strengthening attributes of thin roots. This dual action significantly fortifies the shear strength of the soil while enhancing its cohesive force, resulting in improved stability, particularly of shallow soil layers [15]. Studies by Li et al. [16] showed that the morphology of the root system significantly affects the structural characteristics of the soil. This influence is particularly evident when it comes to thin roots with a diameter of less than 1 mm. Given their larger surface area, these roots play a crucial role in fostering heightened surface roughness and soil cohesion, thus optimizing the soil reinforcement. Since slopes are an open system with a complex structure and function that expose plants to a variety of mechanical stresses, the improvement of slope stability depends mainly on the root characteristics of the plants on the slopes [17].

Heilongjiang Province is the main distribution area of the Songnen Plain, and the salts in the soil are dominated by the alkaline salts NaHCO_3 and Na_2CO_3 [18]. Additionally, because this is a cold region with high snowfall in winter, the use of NaCl -containing snowmelt salt exacerbates the salinization of slope soil along the highways. Soil salinization will change the physical properties of the soil, leading to soil compaction and a decline in soil fertility, jeopardizing the growth and development of plants, and causing serious impacts on the development of agriculture and forestry [19]. In an environment with high salinity stress, plants are mainly subjected to osmotic stress in their early growth stage, followed by long-term ionic toxicity [20]. The root system, which is the organ in direct contact with the soil, is the first to respond to salinity stress, and plants resist stress damage to a certain extent by remodeling the root structure in order to regulate nutrient and moisture absorption [21]. Root elongation [22] and root system structure [23] are both affected by salt stress, and the inhibition of plant root growth by saline–alkaline stress is more significant [24]. Numerous studies have shown that alkali will exacerbate the coercion of salt stress on plants [25,26]. This is due to the fact that alkali stress increases the pH value in the soil. The high pH value will cause the accumulation and precipitation of phosphorus and metal ions in the soil, damaging the plant root system and reducing root vigor, which

will in turn inhibit the uptake of Ca^{2+} and Mg^{2+} ions and impair the structure and function of the plant root membrane [27,28]. While primarily affecting the growth and development of plants, salts also have a serious impact on the plants' ability to hold soil and protect slopes. It is well known that the tolerance of plants to saline environments varies from species to species [29,30], and even by interspecies, such as *Hydrangea* [31]. Therefore, the greening of highway slopes in saline areas requires the selection of cold-resistant, drought-resistant and saline-tolerant plants, and it is of significant importance to study the effects of saline–alkaline stress on plant root growth and soil-fixing capacity.

At present, the effects of saline–alkaline stress on the root system has been studied [32], while its effects on plant roots applied to highway slopes in alpine regions have rarely been reported. Related studies have shown that herbaceous vegetation is considered to be a more suitable vegetation type for stabilizing slope soils due to its fast growth rate and high species density, which allows it to adapt to environmental changes more quickly than woody plants [2]. Meanwhile, considering the landscape effects of the slope along highways, this study uses four kinds of herbaceous plants with strong ornamental properties and potential for soil consolidation on saline–alkali highway slopes. These are *Oenothera biennis*, *Perilla frutescens*, *Echinops sphaerocephalus*, and *Lychnis fulgens*. The saline–alkaline stress was simulated with NaCl and NaHCO_3 . The objectives of this study were to study the saline tolerance of different plants under the saline–alkali environment and the biological characteristics, mechanical properties and soil-fixing capacity of the root systems of different plants in a saline–alkali environment. We hypothesized that the salinity tolerance of plants as well as their soil-fixing capacity depend on the species (Hypothesis 1); and saline stress would have an inhibitory effect on plant growth, and that the root system, as the organ in direct contact with the soil and saline stress, would be the first to suffer from salt damage and have an effect on its soil-fixing capacity (Hypothesis 2). This is not only important for understanding the plants themselves, but also for providing a theoretical basis for the selection and practical application of plants and vegetation in saline–alkali slope protection technology.

2. Materials and Methods

2.1. Plant Materials and Test Methods

The experiment was conducted in the greenhouse (temperature 25 ± 2 °C; humidity $65 \pm 5\%$; light time 12 h) of Northeast Forestry University, Harbin, Heilongjiang Province. Live seedlings of four herbaceous plants, *Oenothera biennis*, *Perilla frutescens*, *Echinops sphaerocephalus*, and *Lychnis fulgens*, were used as the test materials. Seeds were courtesy of Jiuquan Lanxiang Horticulture Seedling Co., Ltd. (Jiuquan, China). The seeds were sown in April 2021 into 50-hole hole trays, and the seedling matrix was mixed with peat, perlite, and vermiculite at 5:1:1 (properties of the growing media are shown in Table 1). At 30 d after seedling emergence, seedlings with uniform height and vigorous growth were transplanted into pots with a diameter of 150 mm and a height of 125 mm. The internal matrix of the pots was the same as the matrix ratio in the original seedling matrix. After transplanting, the plants were watered thoroughly and allowed to rest for 2 days. After that, they were watered every 3 to 5 days and cared for regularly.

Table 1. Growing media properties.

Bulk Density ($\text{g}\cdot\text{cm}^{-3}$)	Water Holding Capacity ($\text{mL}\cdot\text{kg}^{-1}$)	Total Porosity (%)	Available N ($\mu\text{g}\cdot\text{g}^{-1}$)	Available P ($\mu\text{g}\cdot\text{g}^{-1}$)	Available K ($\mu\text{g}\cdot\text{g}^{-1}$)	pH	Total Organic Carbon (%)
0.59	59.68	66.38	98.87	12.51	263.07	5.57	15.69

Sixty days after transplanting, when the seedlings were 90 d old, the plants with strong growth and consistent vigor were further selected to be used in the saline–alkali stress treatment. In the experiment, the neutral salt NaCl and the alkaline salt NaHCO_3 , which are the most abundant saline salts in the saline–alkali land in the western part of Heilongjiang

Province, were mixed at a molar ratio of 1:1 to simulate the saline–alkali stress environment. The concentrations of the solutions were set at 50, 100, and 200 mM (pH 7.98–8.24), and distilled water without salt was used as a control (pH 6.87). There were four treatments, and three replicates were set up for each treatment of each plant, with eight pots of plants in each replicate, for a total of 96 pots. Watering of the plants was reduced in frequency and volume for 5 d before stress and, in order to avoid salt shock, 100 mL of 50 mM saline solution was added for the first stress, with the concentration of the daily added solution increasing by 50 mM until the solution reached the targeted stress concentration. When the concentration of the mixed saline solution reached the target stress concentration, it was recorded as day 0 of stress, and 100 mL of saline solution was added every 5 d a total of 5 times, with experimental samples then being taken on the 30th day.

2.2. Salinity Tolerance of Four Herbaceous Plants

The plant damage symptoms were observed and counted, and the salt damage (SD) categorization criteria were referred to from Cao et al. [33]. The salt damage index and salt damage rate of each plant under different treatments were calculated according to Equations (1) and (2).

$$\text{SD index (\%)} = \frac{(\text{SD level} \times \text{Corresponding number of SD plants})}{\text{Total number of plants} \times \text{Highest SD level}} \times 100 \quad (1)$$

$$\text{SD rate (\%)} = \frac{\text{Number of plants with SD symptoms}}{\text{Total number of plants}} \times 100 \quad (2)$$

Soluble sugar content: determined by anthrone colorimetry [34]; soluble protein content: determined by Caumas Brilliant Blue method [35]; free proline content: determined by acidic ninhydrin colorimetry [36]. Superoxide dismutase (SOD) activity: measured by nitrogen blue tetrazolium method; catalase (CAT) activity: measured by ammonium molybdate method; peroxidase activity (POD): measured by guaiacol method [37]. Each treatment was repeated three times.

2.3. Determination of Root Growth

An Epson Expression 11000XL root scanner was used to scan the root system, and the scanned images were saved to the computer. Images were then analyzed for root number, root length, average diameter of the root system, total surface area of the root system, and total volume of the root system using the WinRHIZO root analysis software (https://www.regentinstrument.com/assets/winrhizo_software.html, accessed on 18 June 2024). Three intact root systems were selected for each treatment. For classification of root structure types, four species of plants were classified according to the classification of Yen [38].

2.4. Determination of Biomechanical Properties of Root Systems

A plant root system was placed into distilled water for 24 h and then placed in a plastic bag and stored in a refrigerator at 4 °C. The tensile test was completed within 3 days of storage. A root system with uniform thickness, without damage to the epidermis and without bending was selected. Root segments with a length of more than 40 mm were then selected from within the system, and their average diameters were measured and recorded by vernier calipers. Both ends of the root segment were clamped into a tensile strength fixture and, in order to prevent the root system from slipping off or being broken by the fixture, hot-melt adhesive at the appropriate temperature was used to reinforce both ends of the root system. Results were only considered successful when the root segment broke in the middle. A total of 869 root segments were tested, and 519 root segments were considered successful, with an average success rate of 59.72%. In order to determine tensile resistance, Edelberg pointer tensiometers were used to stretch the root segments. Two different tensiometers with the ranges of 0–10 N (for thin roots) and 0–50 N (for coarse roots)

were used, and the accuracies of 2 tensiometers were 0.05 N and 0.25 N, respectively. Tensile strength was calculated from the diameter of the root segment and the tensile strength by Equation (3). Elongation rate was determined from the length of the root segment and its final tensile length, using Equation (4).

$$\text{Tensile strength (MPa)} = 4F/\pi d^2 \quad (3)$$

$$\text{Elongation rate (\%)} = \Delta L/L \quad (4)$$

In the formula, F is the tensile resistance, d is the average diameter of the root segment, ΔL is the maximum elongation of the root segment before it broke, and L is the original length of the stretched root segment.

2.5. Determination of Root Pullout Resistance

The plant samples used in the test, complete with their pots, were soaked in a larger pot of tap water for 24 h to ensure that the moisture content of each test material was consistent. The test pot was removed from the water, and the above ground portion of the plant was cut off. In order to attach the root system to the testing fixture, the top 1 cm of soil was removed in order to expose a sufficient length of the root system. A three-way digital tensiometer with a range of 0–500 N and an accuracy of 0.05 N was used to measure the pullout resistance of the root system. At least three samples of each plant under each of the different stresses were selected for testing.

2.6. Determination of Absolute Soil Stripping Rate of Root–Soil Complexes

The above-ground portions of the plants were cut off and soaked in water for 24 h. The soil containing the root system of the plant was removed intact from the pots and placed in a scouring device with a slope set at 15° , and each specimen was scoured at a flow rate of 0.8 m/s for 5 min. The washed-out sediment was collected and placed into an aluminum box, dried in an oven until constant weight, weighed, and the absolute soil stripping rate was calculated using Equation (5). At least three samples of each plant under each of the different stresses were selected for testing.

$$\text{Absolute soil detachment rate ASD (\%)} = W/TA \quad (5)$$

In the formula, W is the dry weight of the amount of sediment lost by washout (g), T is the washout time (s), and A is the exposed surface area of the soil sample (m^2).

2.7. Data Analysis

The experimental data were organized and calculated by Excel 2016 software, and one-way ANOVA and non-linear regression analyses were performed using SPSS 25.0 software (version 25.0, IBM, Armonk, NY, USA). Duncan's method ($p < 0.05$) was used to test for significant differences, and graphs were made with Origin software (version 2021, Origin Lab, <https://www.originlab.com/2021>, accessed on 6 July 2024).

3. Results

3.1. Salinity Tolerance of Four Herbaceous Plants

As shown in Figure 1, the salt damage symptoms of all four plants increased with the concentration of the mixed saline solution. *O. biennis* showed small yellow spots on the leaves at 50 mM concentration and, at 100- and 200-mM concentrations, the yellow spots gradually increased, and obvious scorching could be seen on the leaf tips. At 50 mM concentration, the leaf margins of *P. frutescens* already started to yellow, at 100 mM concentration, the yellowing of the leaf blades increased and the leaf blades appeared to be curled and scorched, and at 200 mM concentration, the leaf tips were obviously scorched, the leaf blades fell off, and some of the plants died. *E. sphaerocephalus* had no salt damage symptoms on the leaves at 50 mM concentration, the leaf area decreased at 100 mM concentration, the

leaf margins started to appear curling and, at 200 mM concentration, the curling of the leaf margins was aggravated and there was scorching. *L. fulgens* showed yellowing at the tips of some plant leaves at a concentration of 50 mM, the yellowing area of the leaves increased at 100 mM, and the yellowing of the leaf margins was further aggravated at a concentration of 200 mM, but the yellowed leaves did not exceed half of the leaves of the whole plant. The salt damage index and salt damage rate of the four plants gradually increased as the concentration of the mixed saline solution increased (Table 2). At 50 mM concentration, the salt damage grade of all four plants reached grade 1, with the lowest salt damage index and salt damage rate for *E. sphaerocephalus*, followed by *L. fulgens* and *O. biennis*, and the highest salt damage index and salt damage rate for *P. frutescens*. At 100 mM, the salt damage index and salt damage rate were the same and the lowest among the four plants, the salt damage rate of *P. frutescens* was the highest, with 100%. 200 mM, and the salt damage index and salt damage rate of the four plants were as follows in descending order: *E. sphaerocephalus* < *L. fulgens* < *O. biennis* < *P. frutescens*.

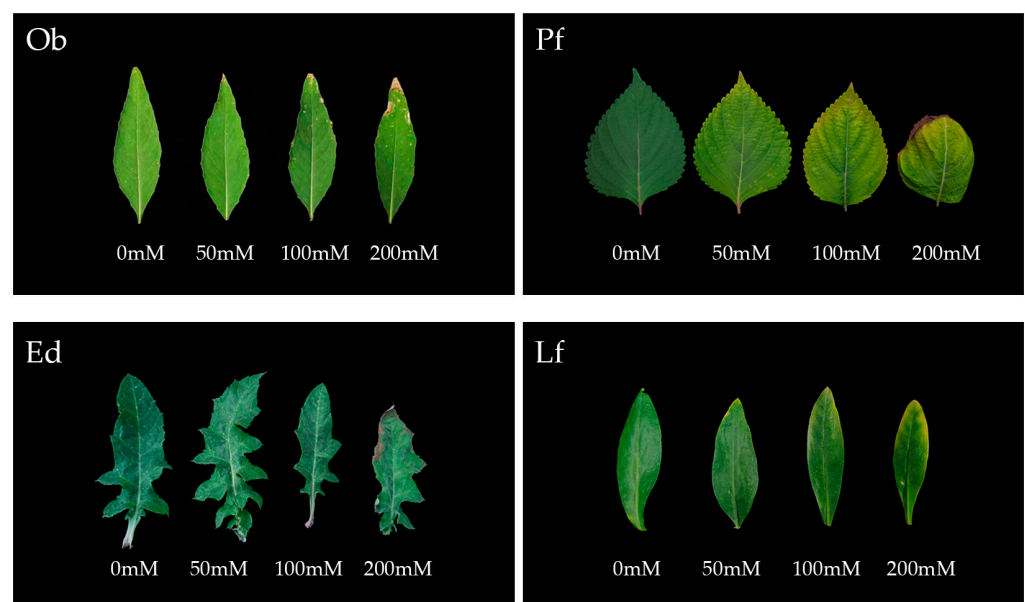


Figure 1. Leaf morphology of 4 herbaceous plants under complex saline–alkali stress. *Oenothera biennis* (Ob), *Perilla frutescens* (Pf), *Echinops sphaerocephalus* (Es), and *Lychnis fulgens* (Lf).

Table 2. Salt damage rate and salt damage index of 4 herbaceous plants under complex saline–alkali stress.

Index	Solution Concentration (mM)	Plant			
		<i>O. biennis</i>	<i>P. frutescens</i>	<i>E. sphaerocephalus</i>	<i>L. fulgens</i>
SD index	0	0.00 ± 0.000 d	0.00 ± 0.000 d	0.00 ± 0.000 d	0.00 ± 0.000 d
	50	16.67 ± 0.304 c	58.33 ± 0.936 c	8.33 ± 0.403 c	12.50 ± 1.047 c
	100	27.08 ± 0.347 b	77.08 ± 1.699 b	25.00 ± 0.851 b	25.00 ± 0.487 b
	200	58.33 ± 1.433 a	80.21 ± 0.972 a	52.08 ± 0.420 a	58.33 ± 0.790 a
SD rate	0	0.00 ± 0.000 d	0.00 ± 0.000 c	0.00 ± 0.000 d	0.00 ± 0.000 d
	50	16.67 ± 0.267 c	58.33 ± 1.175 b	8.33 ± 0.263 c	12.50 ± 0.482 c
	100	45.83 ± 0.906 b	100.00 ± 0.000 a	41.67 ± 0.634 b	41.67 ± 0.866 b
	200	83.33 ± 0.839 a	100.00 ± 0.000 a	75.00 ± 0.644 a	79.17 ± 0.509 a

The values in the table are the mean ± standard deviation ($n = 3$), and different lowercase letters indicate significant differences between treatments ($p < 0.05$).

The soluble sugar content of the four plants showed different trends (Figure 2a), in which *O. biennis* showed a decreasing and then increasing trend with increasing solution

concentration, and decreased to the lowest at 50 mM, which was significantly lower than the control by 16.4% ($p < 0.05$), and then increased to 17.1 mg/g at the concentration of 200 mM, which was significantly different from control ($p < 0.05$). The soluble sugar content of *P. frutescens* and *E. sphaerocephalus* gradually increased with the increase in solution concentration, and *P. frutescens* was significantly increased by 17.5% to 287.39% under saline stress from 50 to 200 mM compared with control ($p < 0.05$); *E. sphaerocephalus* did not differ significantly from control at 50 mM concentration and was significantly higher than control at 100- and 200-mM concentrations ($p < 0.05$). The soluble protein content of all four plants showed a decreasing trend with increasing solution concentration under saline stress (Figure 2b), among which *O. biennis* and *E. sphaerocephalus* showed significant differences among concentrations, with a significant decrease of 83.44% and 77.22%, respectively, compared with the control at 200 mM ($p < 0.05$). Salinity stress significantly reduced the soluble protein of *P. frutescens* by 49.53% to 79.57% ($p < 0.05$) compared with the control, but the differences were not significant between 100- and 200-mM treatments. The difference between *L. fulgens* and control was not significant at 50 mM and decreased to 51.24% of control at 200 mM and was significant ($p < 0.05$). Proline content (Figure 2c) of all four plants showed an increasing trend with increasing concentration, and the proline content of *O. biennis*, *P. frutescens*, *E. sphaerocephalus* and *L. fulgens* increased by 110.55% at 200 mM compared with control, respectively, and 1252.65%, 304.59% and 203.64%, respectively, at 200 mM, the differences being significant ($p < 0.05$).

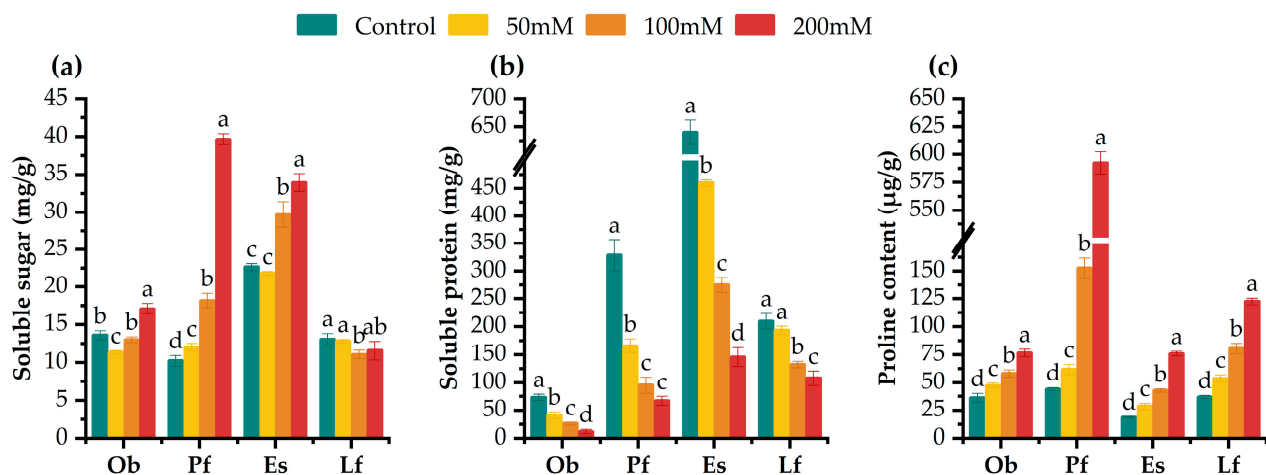


Figure 2. Effect of saline–alkaline composite stress on the content of osmoregulatory substances in four herbaceous plants. Soluble sugar (a), Soluble protein (b), Proline content (c). *Oenothera biennis* (Ob), *Perilla frutescens* (Pf), *Echinops sphaerocephalus* (Es), and *Lychnis fulgens* (Lf). Error lines are standard deviations from the mean ($n = 3$) and different lowercase letters indicate significant differences ($p < 0.05$).

As shown in Figure 3a, the SOD activities of the four plants showed different trends, with *O. biennis* and *L. fulgens* showing an increasing and then decreasing trend with the increase in solution concentration, and both of them rose to the maximum at a concentration of 50 mM, in which *O. biennis* was significantly increased by 4.05% compared with control ($p < 0.05$), while the difference between *L. fulgens* and control was not significant. When the concentration reached 200 mM, the SOD activity of both plants decreased significantly ($p < 0.05$) compared to control, while the SOD activity of *P. frutescens* showed an overall trend of gradual decrease with increasing concentration, and both of them were significantly lower than control ($p < 0.05$). The SOD activities of *E. sphaerocephalus* increased with increasing concentration and were significantly higher than control at 50–200 mM ($p < 0.05$). The CAT activities of the four plants showed an increasing and then decreasing tendency with increasing concentration (Figure 3b), among which the highest values of *O. biennis* and *E. sphaerocephalus* were reached at 50 mM, which were significantly increased by 42.11%

and 66.42%, respectively, compared with control ($p < 0.05$); at 200 mM concentration, there was no significant difference between *O. biennis* and control, while *E. sphaerocephalus* significantly decreased by 20.63% compared with control ($p < 0.05$). *P. frutescens* and *L. fulgens* showed a peak at 100 mM treatment and reached a minimum at 200 mM, and both of them were significantly ($p < 0.05$) different from control. Under saline stress, the POD (Figure 3c) activities of *O. biennis*, *E. sphaerocephalus* and *L. fulgens* showed an increasing and then decreasing tendency with increasing concentration, and all of them reached a peak at 50 mM, for which the differences between *O. biennis* and control were not significant. In contrast, the POD activity of *P. frutescens* decreased continuously with the increase in the degree of stress. This was significantly reduced by 30.48% and 57.45% at 100- and 200-mM concentrations, respectively, compared with control ($p < 0.05$).

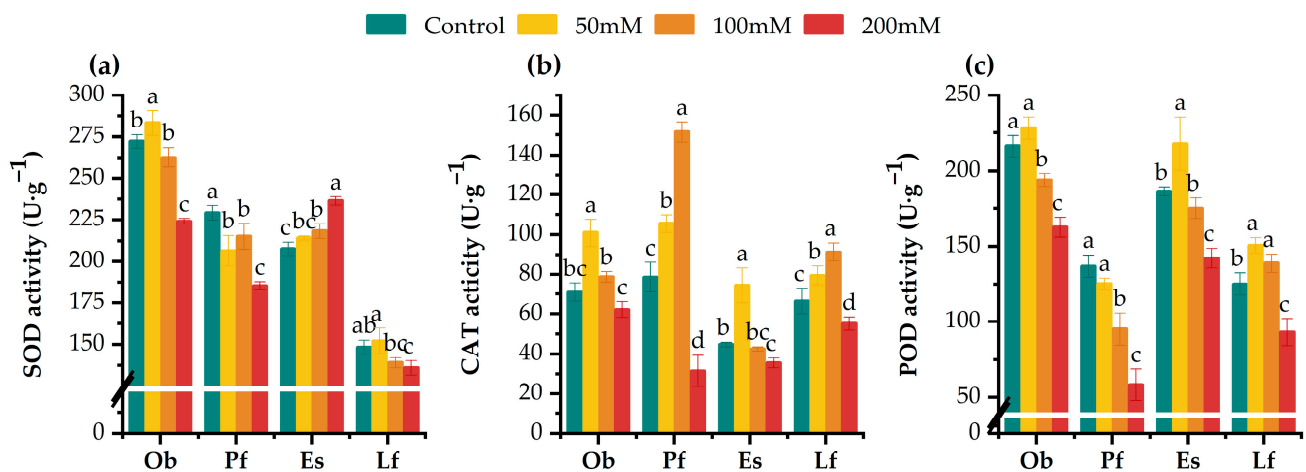


Figure 3. Effect of saline–alkaline composite stress on antioxidant enzyme activities of four herbaceous plants. SOD activity (a), CAT activity (b), POD activity (c). *Oenothera biennis* (Ob), *Perilla frutescens* (Pf), *Echinops sphaerocephalus* (Es), and *Lychnis fulgens* (Lf). Error lines are standard deviations from the mean ($n = 3$) and different lowercase letters indicate significant differences ($p < 0.05$).

3.2. Characterization of Root Conformation

The ratios of total root number, total root length, total root surface area, and total root volume of the four plants to control showed a gradual decreasing trend with increasing solution concentration under mixed salinity stress (Figure 4). Under three different concentrations of saline stress, total root number and total root length of *L. fulgens* and *E. sphaerocephalus* had the greatest ratios to control and were significantly higher than those of *P. frutescens* and *O. biennis* ($p < 0.05$). At concentrations ranging from 100 to 200 mM, the ratio of mean diameter of *P. frutescens* to control decreased significantly to 63.02% ($p < 0.05$). The ratios of total root surface area and total root volume of *O. biennis* to control were significantly lower ($p < 0.05$) than those of the other three plants, and the ratios of total root surface area and total root volume to control were greatest for *L. fulgens* and *E. sphaerocephalus* at a concentration of 200 mM, but the differences were not significant compared with those of the remaining two plants ($p > 0.05$).

The classifications of the root structure types of the four herbaceous plants are shown in Figure 5 and Table 3. The root systems of *O. biennis* and *E. sphaerocephalus* belong to the R-type, with thick, obvious main roots and abundant inclined roots; the root systems of *P. frutescens* and *L. fulgens* 's purse belong to the M-type, with an inconspicuous main root, a root system that is distributed in all directions, with many and dense branches, and an abundance of fibrous roots.

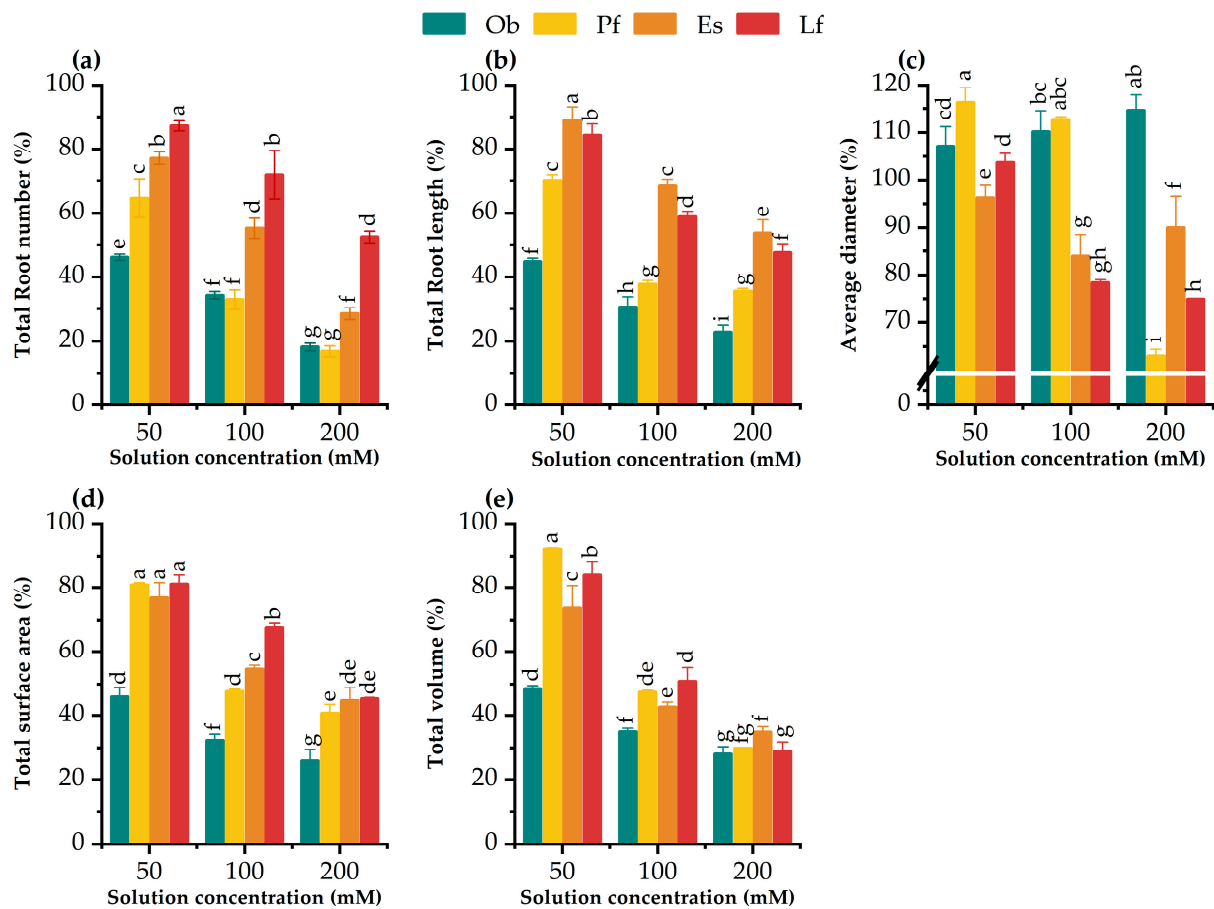


Figure 4. Percentage of plant root growth indexes under different treatments compared to control. Total root number (a), total root length (b), average diameter (c), total surface area (d), and total volume (e) of the four plants under saline–alkali composite stresses. *Oenothera biennis* (Ob), *Perilla frutescens* (Pf), *Echinops sphaerocephalus* (Es), and *Lychnis fulgens* (Lf). Error lines are standard deviations from the mean ($n = 3$) and different lowercase letters indicate significant differences ($p < 0.05$).

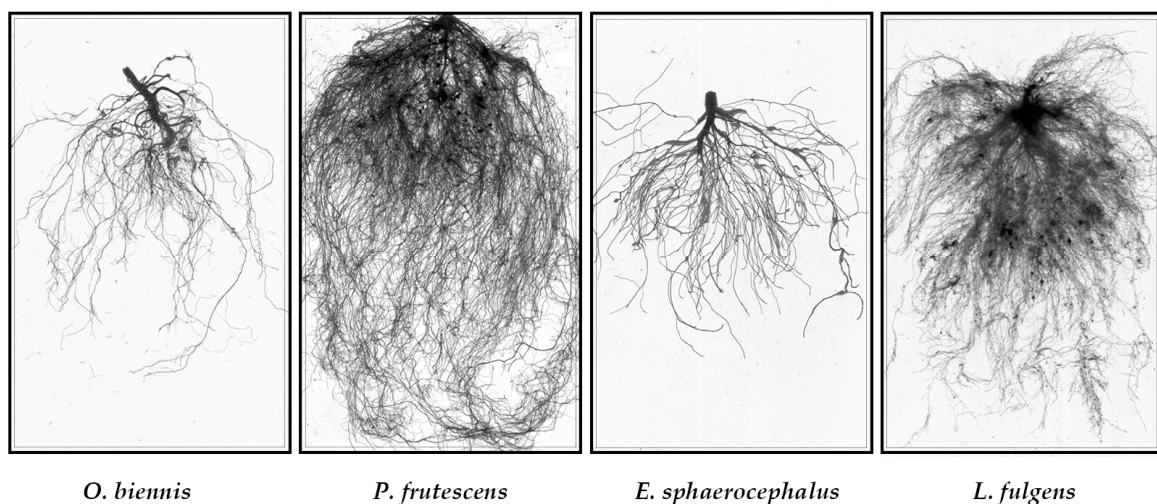


Figure 5. Root morphology of four plant species.

Table 3. Types and characteristics of root structure in four herbaceous plants.

Root Structure Type	Plant	Root Description
R-type	<i>O. biennis</i> <i>E. sphaerocephalus</i>	The main root is obvious and contains a large number of inclined roots
M-type	<i>P. frutescens</i> <i>L. fulgens</i>	Abundant fibrous roots, dense root system in shallow soils

3.3. Mechanical Properties of Root System

The impact of different degrees of saline–alkali stress on the average tensile resistance of the four plants was not significant (Table 4), whereas the tensile resistance of *P. frutescens* was significantly higher than that of the remaining three plants under the same stress conditions ($p < 0.05$). As the concentration of the mixed salt stress solution increased, the average tensile strength of *O. biennis* and *E. sphaerocephalus* gradually decreased, while that of *P. frutescens* and *L. fulgens* showed an increasing and then a decreasing trend, in which *O. biennis* and *P. frutescens* significantly decreased under both 100 and 200mM treatments compared to control ($p < 0.05$). With the increase in stress, the average elongation of the four plants roughly decreased, but the changes of *O. biennis* and *P. frutescens* were not significant compared to the control. At the same time, *E. sphaerocephalus* and *L. fulgens* were reduced by 29.71% and 42.5%, respectively, under 200 mM treatment compared with the control, which was significant ($p < 0.05$). Under the same conditions, the average tensile resistance and average tensile strength of the four plants ordered from largest to smallest are: *P. frutescens*, *O. biennis*, *L. fulgens*, and *E. sphaerocephalus*. The average elongation rate from largest to smallest was *L. fulgens*, *E. sphaerocephalus*, *O. biennis*, *P. frutescens*.

Table 4. Mechanical properties of root systems of the four species under saline–alkali composite stresses.

Index	Solution Concentration (mM)	Plant			
		<i>O. biennis</i>	<i>P. frutescens</i>	<i>E. sphaerocephalus</i>	<i>L. fulgens</i>
Average tensile resistance (N)	0	5.95 ± 4.13 abcde	14.30 ± 10.14 ab	1.88 ± 1.31 cde	2.77 ± 1.65 cde
	50	5.41 ± 3.92 bcde	15.00 ± 8.98 a	1.29 ± 1.05 e	2.68 ± 1.61 cde
	100	3.10 ± 2.86 cde	11.54 ± 8.69 abc	1.23 ± 1.01 e	1.98 ± 0.90 cde
	200	2.43 ± 2.77 cde	11.15 ± 9.41 abcd	1.01 ± 0.80 e	1.48 ± 1.02 de
Average tensile strength (MPa)	0	26.15 ± 5.73 c	73.48 ± 2.32 a	8.13 ± 1.20 e	13.45 ± 2.79 cde
	50	22.25 ± 5.76 cd	77.82 ± 15.25 a	5.52 ± 0.60 e	13.85 ± 2.88 cde
	100	12.36 ± 3.98 de	52.88 ± 9.47 b	5.15 ± 1.66 e	11.20 ± 2.58 de
	200	8.55 ± 4.90 e	44.65 ± 18.33 b	4.45 ± 0.71 e	6.42 ± 0.84 e
Average elongation rate (%)	0	11.95 ± 2.56 cdef	10.29 ± 5.85 defg	17.77 ± 3.10 b	22.47 ± 3.04 a
	50	10.42 ± 2.67 defg	9.68 ± 3.16 defg	16.25 ± 1.74 bc	23.46 ± 1.79 a
	100	9.15 ± 2.43 defg	8.53 ± 1.42 efg	14.08 ± 1.31 bcd	18.82 ± 1.93 ab
	200	7.63 ± 3.02 fg	5.56 ± 2.14 g	12.49 ± 2.00 cdef	12.92 ± 0.70 cde

The values in the table are the mean ± standard deviation ($n = 3$), and different lowercase letters indicate significant differences between treatments ($p < 0.05$).

The tensile resistance of all four plants increased as a power function of root diameter (Figure 6). The index of the power function in the fitting equation can indicate the rate of increase in the tensile resistance as the root diameter is increased and, the larger its value, the higher the rate of increase in tensile resistance with root diameter. As the degree of saline stress increased, the β values of *O. biennis*, *P. frutescens* and *L. fulgens* all showed a gradually increasing trend. Under the three treatments at 0, 50 and 100 mM, among the four plants, a negative power rate relationship between tensile strength and root diameter was seen in *L. fulgens* only (Figure 7), and the exponent in the function varied with the degree of stress in accordance with the tensile resistance. There was no significant correlation between elongation and the root diameter for any of the four species (Figure 8).

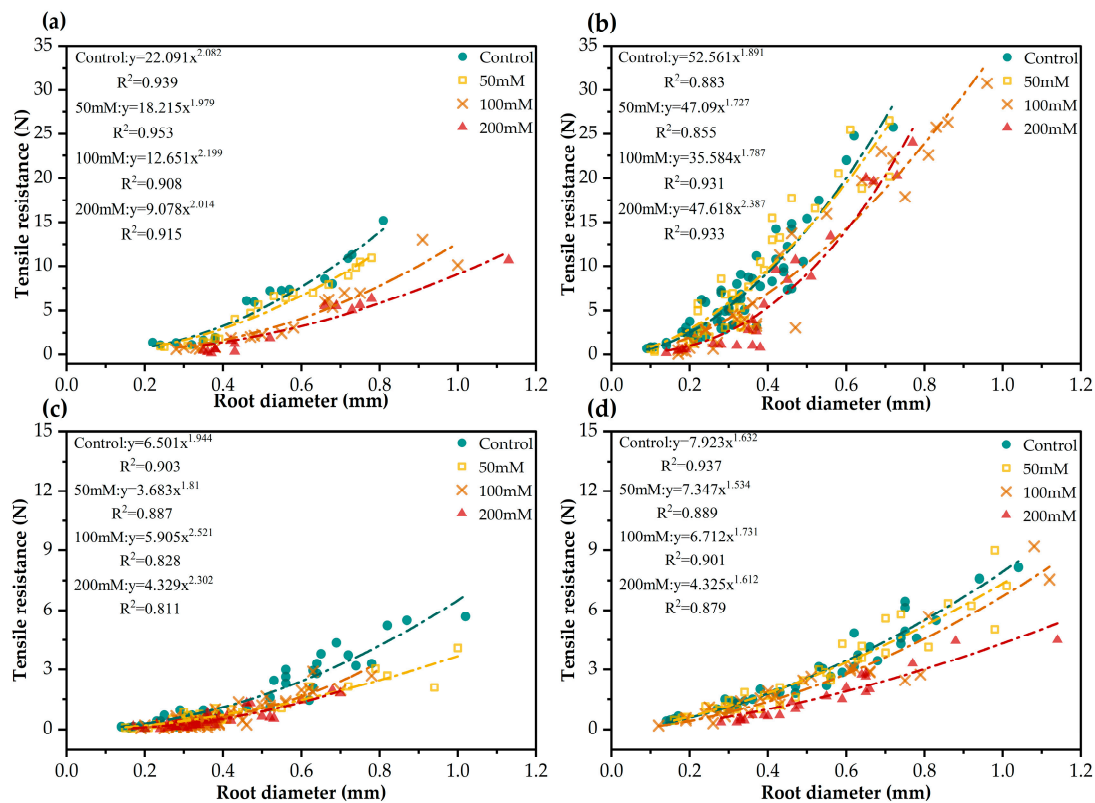


Figure 6. Tensile resistance–diameter correlation of *O. biennis* (a), *P. frutescens* (b), *E. spherocephalus* (c), and *L. fulgens* (d) under different saline–alkali composite stresses.

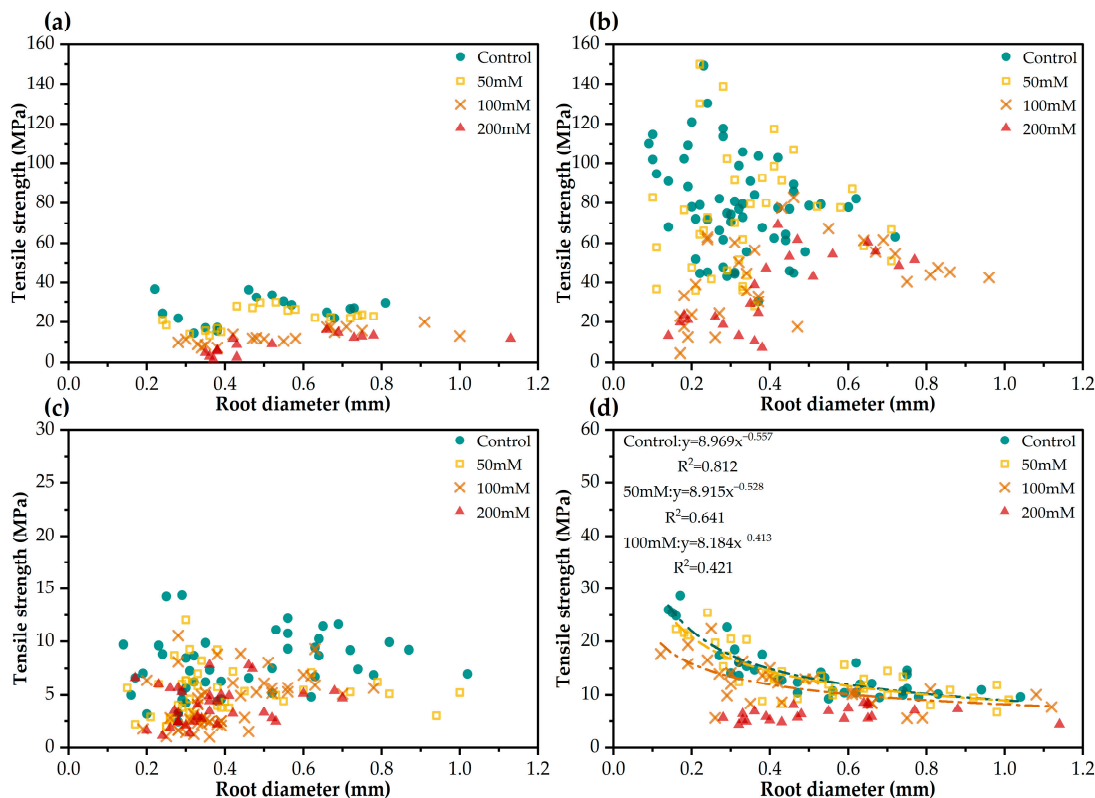


Figure 7. Tensile strength–diameter correlation of *O. biennis* (a), *P. frutescens* (b), *E. spherocephalus* (c), and *L. fulgens* (d) under different saline–alkali composite stresses.

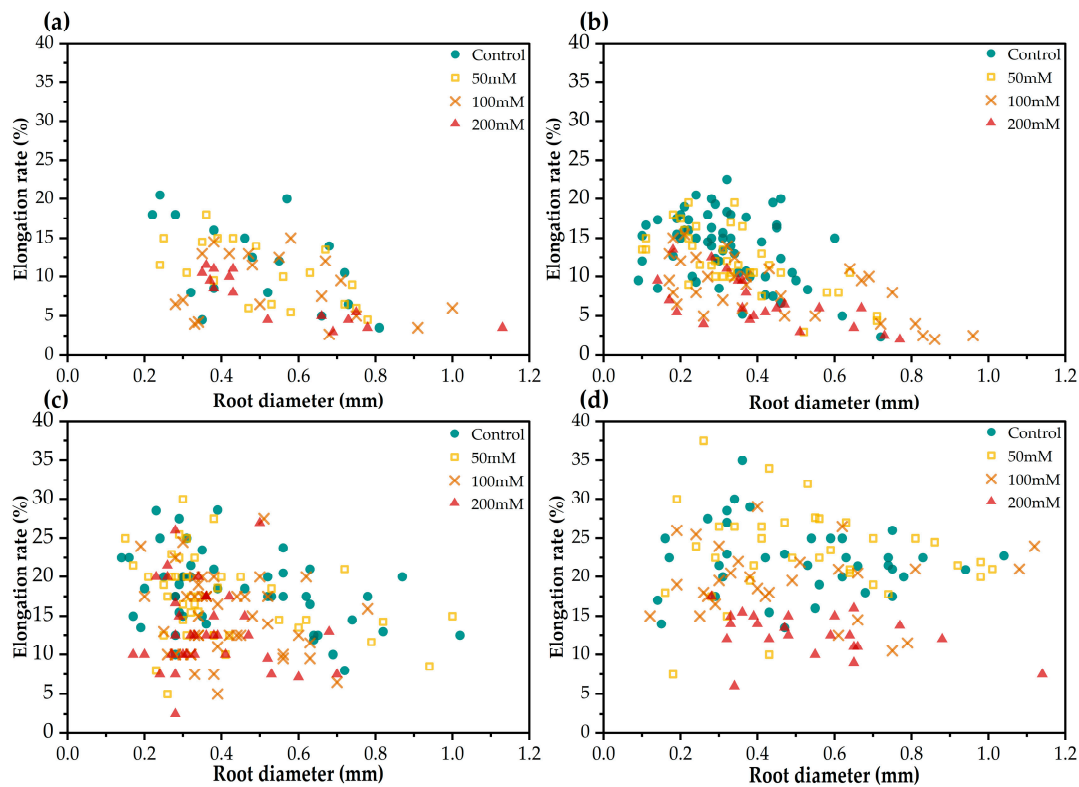


Figure 8. Elongation rate–diameter correlation of *O. biennis* (a), *P. frutescens* (b), *E. sphaerocephalus* (c), and *L. fulgens* (d) under different saline–alkali composite stresses.

3.4. Root Pullout Resistance

As shown in Figure 9, the pullout resistance of all four plants decreased gradually with the increase in the concentration of mixed saline solution and, under 50 to 200 mM treatments, *O. biennis*, *P. frutescens*, *E. sphaerocephalus*, *L. fulgens* decreased by 30.31% to 52.23%, 18.98% to 79.72%, 27.61% to 73.23% and 37.82% to 65.26%, respectively, compared with control, this difference being significant ($p < 0.05$). Under the four different treatments, the pullout resistance of all four plants in descending order was: *L. fulgens* > *P. frutescens* > *O. biennis* > *E. sphaerocephalus*, and the pullout resistance of *L. fulgens* and *P. frutescens* was significantly higher than that of the remaining two plants, with an additional significant difference between *L. fulgens* and *P. frutescens* ($p < 0.05$).

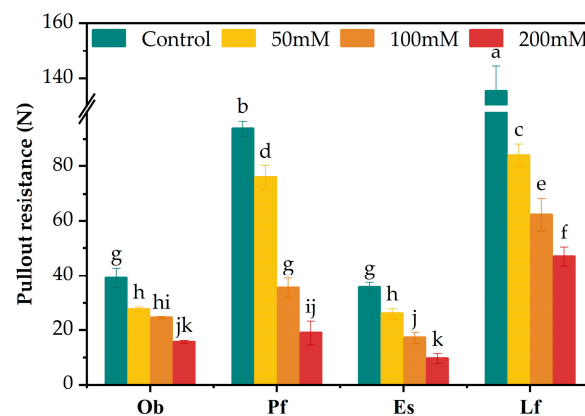


Figure 9. Pullout resistance of four species of plants under different saline–alkaline composite stresses. *Oenothera biennis* (Ob), *Perilla frutescens* (Pf), *Echinops sphaerocephalus* (Es), and *Lychnis fulgens* (Lf). Error lines are standard deviations from the mean ($n = 3$) and different lowercase letters indicate significant differences ($p < 0.05$).

3.5. Impact Resistance Index of Root–Soil Complexes

As shown in Figure 10, the ASD of the root–soil complex of the four plants increased with the degree of saline–alkali composite stress. Under 50 to 200 mM treatments, the absolute soil detachment rate of the root–soil complex of *E. sphaerocephalus* was elevated by 68.24%, 150.42% and 242.44%, respectively, compared to control, and the differences were significant ($p < 0.05$). In contrast, the ASD of the root–soil complex of *O. biennis*, *P. frutescens* and *L. fulgens* was not significantly elevated compared to the control. Under the same treatment, the ASD of the root–soil complex of *O. biennis*, *P. frutescens* and *L. fulgens* were not significantly different, and all three were significantly lower than that of *E. sphaerocephalus*. Under the 200 mM treatment, the ASD of the root–soil complex of *E. sphaerocephalus* reached 5.51, 6.28 and 5.65 times higher than that of *O. biennis*, *P. frutescens* and *L. fulgens*.

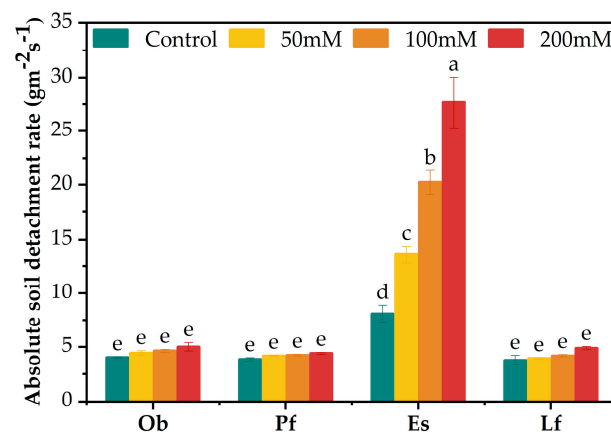


Figure 10. Absolute soil detachment rate of the root–soil complex of four plants under different salinity complex stresses. *Oenothera biennis* (Ob), *Perilla frutescens* (Pf), *Echinops sphaerocephalus* (Es), and *Lychnis fulgens* (Lf). Error lines are standard deviations from the mean ($n = 3$) and different lowercase letters indicate significant differences ($p < 0.05$).

4. Discussion

The growth condition of plants can visually reflect their salinity tolerance [39]. In this study, under different concentrations of saline stress, the salt damage index and salt damage rate of the four plants increased with increasing solution concentration, with *P. frutescens* being the most severely damaged. Saline environments can cause osmotic stress and oxidative stress to plants, but plants can defend themselves against water uptake difficulties due to osmotic stress by accumulating osmoregulatory substances. In this study, it was found that the soluble sugar content of *O. biennis*, *P. frutescens* and *E. sphaerocephalus* increased gradually with increasing concentration, which is in agreement with the findings of Jia et al. [40], whereas *L. fulgens* were not significantly different from control in any of the other treatments, except for a decrease in the concentration of 100 mM as compared to control, which suggests that soluble sugar has little role to play in osmoregulation in *L. fulgens*. Jabeen et al. [41] subjected *Helianthus annuus* L. and *Carthamus tinctorius* L. to salt stress and found that their soluble protein content gradually decreased with increasing solution concentration, which is in agreement with the results of the present experiment, and the lowest decrease was observed in *L. fulgens* among the four species, which indicates that it is also the most resistant to salinity. Like *Juglans microcarpa* [42], the proline content of all four plants in this study increased significantly with increasing solution concentration, indicating that proline plays a key role in osmoregulation in plants. Antioxidant enzymes in plants can protect plants from toxicity by eliminating excess reactive oxygen radicals [43] produced in plants under adverse conditions. Wang et al. [44] found that *Melilotus albus* showed low concentration promotion and high concentration inhibition of SOD, POD and CAT under saline stress. In this experiment, CAT activity of all four plants under

saline–alkali composite stress showed low concentration promotion and high concentration inhibition. SOD and POD activities changed inconsistently, with SOD and POD activities of *O. biennis* and *L. fulgens* showing low promotion and high inhibition; SOD and POD activities of *P. frutescens* decreased with the increase of the stress level; and SOD activity of *E. sphaerocephalus* increased with the increase of the concentration, while POD activity first increased and then decreased. This suggests that SOD and CAT play a predominantly protective role in the scavenging of reactive oxygen species under saline stress, and that enzyme activities begin to decrease when the level of stress is too high and the enzyme synthesis pathway is blocked. Of the four plants, *P. frutescens* had the greatest decrease in enzyme activity and its salinity tolerance was also the lowest.

Salt stress regulates the distribution of root growth hormone [45], and reduces plant cell division and cell growth, thereby inhibiting the growth of the primary and lateral roots [46]. This results in changes in the morphological structure of the root system. Compared with neutral salts, alkaline salts have a stronger inhibitory effect on plant growth [47], and saline–alkaline combined stress will further amplify the inhibitory effect on plant root growth [48], severely damaging the cell membrane system, resulting in reduced root vigor and even rotting [49]. In this study, the total root number, total root length, total root surface area and total root volume of the four plants were significantly reduced. This shows that the composite saline–alkali stress inhibited the root growth, which is in line with previous studies [50–52]. Among them, *O. biennis* and *P. frutescens* had smaller ratios of total root number, total root length, and total root surface area to control at 50 to 200 mM than the other two plants, which may be due to their weaker salinity tolerance and greater sensitivity to saline and alkaline stresses. The average diameter of the root systems of the four plants showed different trends with increasing solution concentration. Among them, the ratio of the average diameter of *O. biennis* to control gradually increased, while the *E. sphaerocephalus* showed a decreasing and then increasing trend, which may be due to the fact that the fine roots of *O. biennis* and *E. sphaerocephalus* decayed excessively under saline stress, while the thicker roots were less affected. The decrease in the ratio of average diameters of *P. frutescens* to control at concentrations of 50–100 mM was small, whereas it decreased sharply from 100 to 200 mM. This may be due to the fact that the 50 mM treatment promoted the thickening of the diameters of the root system through the greater inhibitory effect on the fine roots at low levels of saline stress when compared to the coarser roots but, when the concentration of the solution continued to increase, the growth of both the coarse and the fine roots was significantly inhibited. The root system's diameter in *L. fulgens* exhibited a gradual decrease with increasing solution concentration, highlighting a significant inhibitory effect of saline stress on root diameter growth. Previous research has indicated a positive correlation between total root surface area and the root system's anchoring capacity [53]. In addition, because the root system of herbaceous plants is generally distributed in shallow soil, they mainly play a reinforcing role in soil consolidation and slope protection [2]. According to the structural type of the root system, *P. frutescens* and *L. fulgens* belong to the M-type, with abundant fibrous roots, complex and dense distribution in the soil, and strong reinforcing capacity. *O. biennis* and *E. sphaerocephalus* belong to the R-type root system, and have more inclined roots and a smaller proportion of fine roots than *P. frutescens* and *L. fulgens*, therefore their reinforcing capacity is poor compared with *P. frutescens* and *L. fulgens*. Among the four herbaceous plants, the root morphology indexes of *P. frutescens* and *L. fulgens* were significantly higher than those of the remaining two plants under all saline–alkali stress, which indicates that their root systems are more developed, with stronger anchoring capacities, and their specialized root system configuration exerts a stronger reinforcing effect. In contrast, the root morphology of *L. fulgens* and *E. sphaerocephalus* changed more gently under saline stress than the remaining two plants.

The results of recent studies have shown that slope [54], fungal inoculation [55], the use of herbicides [56] and flooding [57] will affect the mechanical characteristics of plant roots, but studies related to the effects of saline–alkali stress on the mechanical

characteristics of plant roots are relatively lacking. The root system's ability to anchor plants is predominantly influenced by its tensile strength [58], and a higher tensile strength in the root system corresponds to an increased level of soil stability [59]. Research has demonstrated a positive correlation between the tensile strength of roots and the presence of cellulose and lignin [60,61], and salt stress can cause a decrease in plant cellulose and lignin content [62,63]. Theoretically, then, saline–alkali composite stress may reduce the tensile strength and affect the mechanical properties of the root system. The experimental findings indicated that the mechanical properties of the root system across the four plant species were indeed influenced by saline–alkali composite stress. The average tensile strength of *P. frutescens* and *O. biennis* was significantly reduced under 200 mM treatment, whereas the decreases in the tensile strengths of *E. sphaerocephalus* and *L. fulgens* did not reach a significant level, suggesting that the tensile strengths of the root systems of the different species of plants respond differently to saline–alkali composite stress. Previous studies have found that, the higher the tensile strength and the smaller the elongation of the plant root system under tensile fracture, the greater the role it plays in improving soil stability [64,65]. In this experiment, the tensile resistance and elongation of the four plants also decreased gradually under the combined saline and alkaline stresses, but this did not reach significant levels. The average tensile resistance and the average tensile strength of *P. frutescens* surpassed the corresponding attributes in the other three plants. The root elongation rate of *P. frutescens* was the smallest among the four plants, significantly lower than that of *E. sphaerocephalus* and *L. fulgens* in all the treatments used, which suggests that *P. frutescens* had the greatest enhancement effect on soil stability. Numerous studies have been conducted to show that both the tensile resistance and the tensile strength of the root system correlate with root diameter [60,66,67]. In this experiment, the tensile resistance of the four plants increased as a power function of the root diameter. As for the tensile strength, only *L. fulgens* showed a negative power law relationship between the tensile strength and the diameter under the control, 50- and 100-mM treatments, which seems to be caused by the differences between species. In addition, the experimental materials used in this test were all herbaceous plants with thin root systems, whose mechanical integrity can be compromised by material defects present in the microstructure of the cell walls [68], resulting in highly variable strengths and stiffnesses, and therefore prone to extreme values, which can ultimately result in errors [43]. It should be noted that the exponents in the power functions of tensile resistance–diameter obtained from the regression analysis for the root systems of the four plant species tended to increase with the degree of saline–alkali composite stress, which suggests that the tensile force of the fine roots is more susceptible to stress than that of the thicker roots. Findings from tensile tests carried out by Wu et al. [69] on *Vetiveria zizanioides*, conducted both in controlled pots and field conditions, revealed no notable correlation between the root system's elongation rate and root diameter in potted *V. zizanioides*, aligning with the current study. Yet, due to the limited number of studies examining root system elongation rates, further comparative analysis could not be conducted.

The plant root system can increase the soil strength through root–soil friction, improve the stability of the slope, and prevent plants from harm due to shear stress on the slope or being uprooted by the wind [70], so the resistance to uprooting is an important index to measure the ability of plants to stabilize soil and protect slopes. In the study by Yang et al. [71], it was found that the pullout resistance of plants exhibited a positive correlation with the root length and the root surface area, highlighting a strong relationship between root surface area and pullout resistance. In addition, the growth stage of the plant and the type of root structure is also a factor that affects the tensile resistance of the plant [72–74]. Environmental factors, such as soil water content [70] and wind stress [75], have been shown to have a notable impact on plant pullout resistance. (The findings of this study demonstrated a substantial decrease in the pullout resistance of *O. biennis*, *P. frutescens*, *E. sphaerocephalus*, and *L. fulgens* under saline–alkali composite stress. This reduction can be attributed to the inhibition of plant root growth under saline–alkali compound stress,

with significant reduction in root length, root surface area and root volume. Among the four plants in this experiment, *L. fulgens* had the greatest pullout resistance, followed by *P. frutescens*. This is attributed to the root morphology indexes of these two plants, which are significantly higher than those of *O. biennis* and *E. sphaerocephalus*, and have more developed root systems in comparison, showing stronger soil consolidation and slope protection ability.

Numerous studies have demonstrated the ability of plant root systems to reduce the erosive capacity of water flow on soil [58,76–78]. In this experiment, the saline–alkaline composite stress caused an increase in the soil stripping rate of the root–soil complex of *E. sphaerocephalus*, indicating that the damage inflicted on the plant root system by the compound stress diminishes the complex’s ability to resist erosion caused by water flow. This is because the root system is able to bind directly to the soil [79] and adhere to soil particles through root secretions [80], thus improving the stability of soil aggregates. Fine roots play a major role in reducing the rate of soil erosion [76,81]. Conversely, the stress from salinity complexes hindered the growth of primary and lateral plant roots, resulting in insufficient fine roots to maintain soil stability. It is noteworthy that, although the root morphology indicators of *O. biennis*, *P. frutescens* and *L. fulgens* were significantly affected by the saline–alkali composite stress, the soil stripping rate of their root–soil complexes did not change significantly. This is probably because the root systems of these three plants were more developed on their own, and their root–soil complexes were still able to maintain their stability under the water flow in this experiment, even under the 200 mM treatment. The values of total root number, total root length, total root surface area, and total root volume of *O. biennis*, *P. frutescens* and *L. fulgens* under the 200 mM treatment were higher than those of *E. sphaerocephalus* under the control treatment, proving the previous conjecture to a certain extent.

5. Conclusions

The results of this study showed the plant salinity tolerance in order from strongest to weakest: *E. sphaerocephalus* > *L. fulgens* > *O. biennis* > *P. frutescens*. The root structure types of *P. frutescens* and *L. fulgens* performed better in reinforcing the soil and stabilizing the slopes compared to the other two types, and the root morphology of *L. fulgens* and *E. sphaerocephalus* showed higher saline tolerance in response to saline stresses. The saline–alkali compound stress significantly inhibited the average tensile only of *O. biennis* and *P. frutescens*. In terms of the average elongation, the combined saline and alkaline stresses produced significant inhibition only in *E. sphaerocephalus* and *L. fulgens*. Among the four species, the root system of *P. frutescens* had the largest average tensile resistance and average tensile strength, and the average elongation was the smallest, resulting in it having the greatest enhancement effect on soil stability. Negative power rate relationship between tensile resistance and diameter for four species, and the exponents of the power functions all increased with increasing levels of saline–alkali stress; under the control, 50- and 100-mM treatments, a negative power rate relationship between tensile strength and the diameter of the root system was seen in *L. fulgens* only. The exponent of the power function also increased with increasing stress and the elongation rates of the four plants did not have any significant correlation with their diameters. The saline and alkaline composite stresses significantly reduced the pullout resistance of the four plants. Under the same treatment, *L. fulgens* had the greatest pullout resistance, followed by *P. frutescens*. The soil stripping rate of the root–soil complex of *E. sphaerocephalus* was significantly higher under saline–alkali composite stress, while the changes were not significant in the remaining three species. The resistance to erosion of the root–soil complex between *O. biennis*, *P. frutescens*, and *L. fulgens* had a significantly higher resistance to erosion than *E. sphaerocephalus*.

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