Response of Spontaneous Plant Communities to Sedum mexicanum Cover and Water Availability in Green Roof Microcosms

Dean Schrieke *, Nicholas S. G. Williams and Claire Farrell

School of Agriculture, Food and Ecosystem Sciences, University of Melbourne, Melbourne, VIC 3010, Australia
* Correspondence: dean.schrieke@unimelb.edu.au

Abstract: Lack of maintenance can lead to ‘weedy’ spontaneous vegetation on green roofs. Aspects of green roof design, including substrate depth and roof height, have been shown to influence the composition of spontaneous vegetation. In drier climates, Sedum species are often planted on shallow substrate ‘extensive’ green roofs and irrigated during summer to maintain cover. However, the response of spontaneous vegetation to Sedum cover and water availability is unclear. Understanding this relationship could help minimise maintenance and maintain Sedum vegetation cover. We hypothesised that increasing Sedum (Sedum mexicanum) cover and reduced water availability would reduce the abundance, biomass, species and functional richness, and the community weighted mean specific leaf area (SLA; CWM by abundance) of spontaneous plant communities. We conducted a 10-month experiment in green roof microcosms planted with S. mexicanum (0%, 25%, 50%, 75% and 100% total cover), subjected to a well-watered or water-deficit irrigation treatment, and sown with a mix of 14 plant species that commonly occur as spontaneous on green roofs. We measured spontaneous species abundance, community biomass, and functional traits (specific leaf area, leaf dry matter content, and relative growth rate), and calculated species and functional richness. Increasing S. mexicanum cover reduced spontaneous species abundance and species and functional richness but did not affect community biomass. Species richness was affected by the interaction of S. mexicanum cover and watering treatment and was greatest in well-watered microcosms with 0% S. mexicanum cover. Increased water availability increased spontaneous plant biomass but did not affect functional richness. The SLA of spontaneous communities was affected by the interaction of S. mexicanum cover and watering and was significantly greater in well-watered treatments where S. mexicanum cover was <100%. Therefore, maximising Sedum cover and limiting water availability on green roofs will likely limit the abundance, biomass, and diversity of spontaneous vegetation. Conversely, for green roofs where substrate is left to be naturally colonised, increasing water availability could encourage establishment and increase functional richness of spontaneous vegetation.

Keywords: biodiversity; irrigation; maintenance; plant cover; spontaneous; weeds

1. Introduction

On extensive green roofs, good vegetation coverage can enhance green roof functionality and the provision of ecosystem services, such as thermal insulation [1,2], stormwater mitigation [3,4], habitat provision [5,6] and improved mental health and wellbeing [7–9]. However, fluctuating temperatures, high evaporation, wind exposure, and shallow (<20 cm) substrate depths on extensive green roofs can limit plant growth and survival [10,11]. Green roof practitioners therefore often select plant species with traits thought to improve plant survival in extreme environments (i.e., trait approach) [12,13], or that originate from natural habitats analogous to green roofs (i.e., habitat template approach) [14]. Succulent species from the genus Sedum are commonly planted on extensive green roofs due to their low-growing habit, shallow root system, high leaf succulence, water use efficiency and...
drought tolerance [15–17]. In semi-arid Mediterranean climates, research demonstrates that *Sedum* species, including *S. album*, *S. sediforme*, and *S. sexangulare*, can be successful on green roofs when planted as seedlings [18], whereas their growth capacity is diminished when they are seeded [19]. Yet, without routine maintenance, green roof plantings are often replaced by spontaneous vegetation [6,20], a process encouraged on biodiverse green roofs [21,22]. Spontaneous plants exist on green roofs without human intervention, suggesting a level of short-term adaptation to the green roof environment. However, the longevity of such adaptations under more extreme conditions remains an open question. Nonetheless, understanding how green roof conditions affect their growth and establishment could help inform green roof design, plant selection, and maintenance.

Existing green roof vegetation influences the success of spontaneously colonising species. Increasing vegetation cover and planting density limits the availability of bare substrate gaps that may otherwise provide microsites suitable for germination and establishment of spontaneous species (i.e., ‘safe sites’ [23]). However, the influence of established green roof vegetation on the coverage, species richness, and functional diversity of spontaneously colonising species, is inconsistent. For example, [24] showed greater functional diversity of existing green roof vegetation decreased spontaneous vegetation cover, whereas [25] showed a positive relationship between existing plant species richness and spontaneous species richness. Notably, [26] demonstrated that varying densities of existing plant species had a consistent negative effect on the abundance of spontaneous species. These results suggest that existing plant coverage and density may be more important than species richness in limiting spontaneous plants on green roofs.

Water availability can shift the nature of competitive/facilitative interactions between existing vegetation and spontaneous species [27,28]. Extensive green roofs can quickly become water limited, owing to freely draining substrates with low water retention [29,30] and high evaporative demand on rooftops [31]. Supplementary irrigation is often essential to establish vegetation on extensive green roofs, particularly in hot and dry climates [32,33]. Although irrigation promotes the health and survival of existing green roof vegetation, studies have also shown that irrigation during establishment increases spontaneous plant cover and richness on green roofs [34]. Greater water availability on green roofs located in higher rainfall areas in Scandinavia also increases spontaneous plant cover [35]. While lower water availability may limit spontaneous plant biomass and richness, the ‘stress-gradient’ hypothesis [36,37] suggests existing plant cover may facilitate spontaneous plant coverage under these conditions. On green roofs this was shown where *Sedum album* impeded the growth of neighbouring herbaceous perennials (*Agastache rupestris* and *Asclepias verticillate*) when water availability was high but facilitated their growth in hotter and drier conditions [38].

Competitive and facilitative effects of *Sedum* cover on green roofs are also likely to differ according to growth and resource use strategies of spontaneous plant species. Due to niche trade-offs, colonising spontaneous species that have different growth and resource use strategies to existing *Sedum* cover are likely to be more successful than those with functionally analogous traits [39]. *Sedum* species are typically stress tolerant, having high leaf succulence and CAM (Crassulacean Acid Metabolism) or facultative CAM/C3 photosynthesis [40] and static, conservative water use strategies [12,41]. Therefore, spontaneous species with resource acquisition traits such as fast growth, high specific leaf area and high water use strategies [42] should be more successful on green roofs with high *Sedum* cover. This is consistent with [43] who showed most species colonising green roof plots planted with *Sedum* mats in Malmö, Sweden, were fast-growing ‘ruderal’ [44] species such as *Erophila verna*, *Poa alpina* and *Cerastium pumilum*. However, the effects of *Sedum* cover on the traits of spontaneous green roof plant assemblages have not been investigated.

Functional traits, or features that indirectly impact growth, reproduction, and survival [45] of spontaneous green roof vegetation should reflect the green roof environment where they establish. Specific leaf area (SLA) is a key functional trait in plant ecology as it relates with plant fitness, growth, and photosynthesis [46]. Studies have shown that SLA iVs lower in less productive habitats [47] and typically increases with greater precipitation
and soil moisture [48]. Lower SLA indicates an increased investment in leaf structural tissue that helps maintain leaf turgor under drought stress [49] and has been related to greater water-use efficiency in Mediterranean vegetation [50]. For this reason, low values of SLA have been used to select non-succulent plants for Mediterranean green roofs [13]. Studies have shown positive relationships between water use and SLA [12,51–53] and higher SLA has been related to increased aboveground biomass and canopy density in experimental green roof mixtures [54].

Extensive green roofs are commonly planted with *Sedum* spp. and provided supplemental irrigation during establishment or during summer months in hot and dry climates. However, the influence of these factors on spontaneous plant species growth, abundance, traits and both species and functional richness is unclear. Understanding these factors could help predict and manage spontaneous community composition and inform green roof design, plant selection and maintenance to either minimise or enhance spontaneous green roof cover and diversity. Therefore, we conducted a green roof microcosm experiment to determine how *Sedum* (*Sedum mexicanum*) cover and water availability influence the growth, abundance, traits and species and functional richness of fourteen common spontaneous green roof plant species. We hypothesised that greater *S. mexicanum* cover and lower water availability would reduce spontaneous green roof plant community biomass, abundance and species and functional richness and reduce the community weighted mean (CWM; by abundance) SLA.

2. Materials and Methods

2.1. Species Selection and Seed Collection

We selected 14 globally cosmopolitan plant species that spontaneously occur on green roofs in Australia and across Europe (Table 1; Schrieke et al., unpublished) to maximise the geographical relevance of our study. Seeds of the fourteen species were collected from green roofs and green roof habitat analogues, to ensure species were suitably well adapted to the green roof environment, located across metropolitan Melbourne, Australia in August–September (winter–spring) 2019. Melbourne has a temperature oceanic climate (Köppen climate classification Cfb) characterised by warm summers, cool winters, and precipitation evenly distributed throughout the year. Fully mature seeds, indicated by dehiscence, brown colouration or hardness, were harvested, and stored in brown paper bags. Subsequent processing in the lab involved the removal of excess plant material, chaff, and debris to minimise the risk of disease and pests. The seeds were then stored at a consistent room temperature approximately 20–22 °C within the same brown paper bags for a period of roughly six months. This period of storage ensured that the start of our experiment matched with their typical germination season.

2.2. Seed Germination

To determine germination capacity (see Appendix A), five replicates per species, each with 25 sterilised seed (submerged in 3% active chlorine solution for 90 to 120 s then rinsed with distilled water) were placed evenly on 3 mm thick 1% agar solution (non-nutrient enriched) in sterilised Petri dishes and sealed with parafilm in June 2020. Petri dishes were placed in a growth cabinet (PGX−250, Ningbo Saifu Experimental Instrument Co., Ltd., Ningbo, China) with an alternating temperature regime of 20/10 °C (12/12 h light/dark photoperiod) and checked for germination every day for the first two weeks and then every other day for an additional three weeks. Germination was defined by emergence of the radicle through the seed coat. At the end of the germination trial, ungerminated seed was cut lengthways with a scalpel and examined under a dissecting microscope to determine viability. Seed was considered potentially viable if the embryo was intact and endosperm appeared white, turgid, and solid, and non-viable if the embryo appeared damaged, detached, discoloured and/or shrinkage was visible. No ungerminated viable seed were detected. Percentage germination was calculated as = (number of germinated seed/total number of seed sown) × 100.
Table 1. Information on the fourteen spontaneous species utilised in the green roof microcosm experiment and the country where species presence on a green roof was recorded (Schrieke et al., unpublished).

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Common Name</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epilobium parviflorum</td>
<td>Onagraceae</td>
<td>Hoary willowherb</td>
<td>Australia, United Kingdom, France, Switzerland, Belgium</td>
</tr>
<tr>
<td>Euphorbia maculata</td>
<td>Euphorbiaceae</td>
<td>Spotted spurge</td>
<td>Australia, France, Belgium</td>
</tr>
<tr>
<td>Euphorbia peplus</td>
<td>Euphorbiaceae</td>
<td>Petty spurge</td>
<td>Australia, France, Belgium, New Zealand</td>
</tr>
<tr>
<td>Helichrysum luteoalbum</td>
<td>Asteraceae</td>
<td>Jersey cudweed</td>
<td>Australia, United Kingdom, Germany</td>
</tr>
<tr>
<td>Malva neglecta</td>
<td>Malvaceae</td>
<td>Common mallow</td>
<td>Australia, Germany</td>
</tr>
<tr>
<td>Nepeta cataria</td>
<td>Lamiaceae</td>
<td>Catnip</td>
<td>Australia, Germany</td>
</tr>
<tr>
<td>Polycarpon tetraphyllum</td>
<td>Caryophyllaceae</td>
<td>Four-leaf allseed</td>
<td>Australia, France, Sweden, United Kingdom</td>
</tr>
<tr>
<td>Portulaca oleracea</td>
<td>Portulacaceae</td>
<td>Common purslane</td>
<td>Australia, France</td>
</tr>
<tr>
<td>Rumex crispus</td>
<td>Polygonaceae</td>
<td>Curly dock</td>
<td>Australia, France</td>
</tr>
<tr>
<td>Solanum nigrum</td>
<td>Solanaceae</td>
<td>Black nightshade</td>
<td>Australia, France</td>
</tr>
<tr>
<td>Sonchus oleraceus</td>
<td>Asteraceae</td>
<td>Common sowthistle</td>
<td>Australia, France, Sweden, Switzerland, New Zealand, United Kingdom</td>
</tr>
<tr>
<td>Stellaria media</td>
<td>Caryophyllaceae</td>
<td>Chickweed</td>
<td>Australia, France, United Kingdom</td>
</tr>
<tr>
<td>Taraxacum officinale</td>
<td>Asteraceae</td>
<td>Dandelion</td>
<td>Australia, Belgium, Germany, New Zealand, Switzerland, United Kingdom</td>
</tr>
<tr>
<td>Trifolium repens</td>
<td>Fabaceae</td>
<td>White clover</td>
<td>Australia, Belgium, France, Switzerland, United Kingdom</td>
</tr>
</tbody>
</table>

2.3. Relative Growth Rate

The relative growth rate (RGR) of the 14 species used in the microcosm experiment was quantified as a metric in our assessment of functional diversity within the vegetation communities. Relative growth rate (see Appendix A) was determined on individual plants sown into pots and grown in a glasshouse at the Burnley Nursery, The University of Melbourne (37°49′42.9″ S 145°01′13.8″ E), from October (spring) to December (summer) 2019. Ten seeds of each species were sown into 1.9 L (155 × 150 mm) pots (25 pots per species) filled with seed raising mix (10% washed coarse sand, 10% sieved coir peat, 80% medium pine bark) and thinned to the most central germinant. Once four ‘true leaves’ fully emerged, seedlings were blocked into numbered pairs by biomass (i.e., plants of similar size). One seedling per pair was randomly selected and harvested to determine initial shoot dry weight after oven drying whole plant mass at 70 °C for two days. Remaining seedlings were grown on until flowering (approximately three to four weeks), at which point plants were harvested to determine whole plant dry weight after oven drying at 70 °C for two days. Relative growth rate was calculated from these measurements per [55] as = (ln W2 − ln W1)/(t2 − t1), where ln W1 is the natural logarithm-transformed mean of the initial harvest whole plant dry mass (g) and ln W2 is the natural logarithm-transformed mean of the final harvest whole plant dry mass (g).

2.4. Experimental Design

The 10-month green roof microcosm experiment ran from 24 November 2020 (spring) to 20 September 2021 (spring) 2021. Sixty green roof microcosms (HDPE boxes, 55 × 35 × 20 cm) were filled with 15 cm green roof substrate (60% scoria < 8 mm, 20% 7 mm scoria, and 20% coir; [12]). This substrate has a water retention capacity of 46% and a bulk density measuring 1.26 g/cm³ [12]. Microcosms were arranged in a complete randomised block design in a fully enclosed poly-tunnel at the Burnley Nursery, the University of Melbourne (Figure 1). Other than rainfall and wind, modules were exposed to ambient conditions (see Appendix B). Controlled slow-release fertiliser (Osmocote® Pro, Everris Australia Pty Ltd., Sydney, Australia: 15 N:1.3 P:10 K) was added to the substrate surface of each microcosm (30 g m⁻²) after filling with substrate to replicate a newly established green roof. Cuttings of Sedum mexicanum were then placed evenly across the soil surface and left to establish for two months until 100% coverage was achieved. Irrigation was applied for one minute twice daily (3.30 L d⁻¹ microcosm⁻¹) with an automatic irrigation system consisting of six shrubblers® 360° adjustable flow spikes (Antelco Pty Ltd., Adelaide, Australia, 33 L h⁻¹).
flow rate) evenly spaced within each microcosm to ensure even watering. This watering regime is designed to mirror a summer ‘establishment’ watering pattern for Sedum on green roofs and promote rapid growth of S. mexicanum.

Upon reaching a full (100%) surface coverage of S. mexicanum, each microcosm was assigned to one of five coverage treatment categories: 0, 25, 50, 75, or 100% (10 microcosms per cover treatment). These treatments represent different proportions of the module covered by S. mexicanum. In the 0% coverage treatment, all S. mexicanum biomass, both above and below ground, was completely removed from the microcosm. For the 25, 50 and 75% coverage treatments, a rectangular area of S. mexicanum was removed from the centre of each microcosm while the outer edge was left with 100% S. mexicanum coverage. The 10 microcosms in each coverage treatment were then assigned one of two watering treatments: well-watered (WW) or water deficit (WD), with five replicates of each cover x watering treatment. Well-watered microcosms were irrigated twice daily for one minute (3.30 L d$^{-1}$ microcosm$^{-1}$) which was sufficient achieve field capacity (i.e., water holding capacity of 46% [12]). Whereas WD microcosms received 50% of the irrigation of WW microcosms, applied once daily (i.e., 1.65 L d$^{-1}$ microcosm$^{-1}$) which resulted in an average daily soil water content of 16% (determined gravimetrically on unplanted modules). Temperature (°C) and relative humidity (RH) within the poly-tunnel were recorded at 30 min intervals using an iButton® Hygrochron Temperature/Humidity Logger (DS1923, Maxim Integrated Products™) and daily averaged (18 °C, 68% RH; see Appendix B).
2.5. Seed Sowing in Microcosms

Sixty seed mixes (one per microcosm) of the 14 spontaneous green roof plant species were made based on each species seed mass and percent germination, so that each species had the same potential germination (i.e., species with low germination rate had a higher ratio by weight of seed in mixtures), with a final sowing rate equivalent to one germinant per 5 cm$^2$. Seed mass (see Appendix A) was measured as the weight of seed after removing all accessories and drying in an oven at 80 °C for three days [56]. Seed mixes were individually blended into 100 mL fine sand to assist spreading and sown evenly across each microcosm surface (24 November 2020; spring) leaving a 5 cm buffer unsown around the perimeter of the microcosm to limit edge effects.

2.6. Spontaneous Species Abundance, Biomass, and Trait Measures

Vegetation surveys were conducted to count and identify spontaneous species at roughly month intervals from 3 March 2021 (summer) until the end of the experiment (20 September 2021; spring). Following the final species survey, each spontaneous plant species found in microcosms was harvested and divided into leaves and stems. Leaf area was measured per [57] by randomly selecting, stripping, and weighing two fully expanded leaves from each individual plant within each microcosm, then photographed from a height of 20 cm (2nd generation Apple iPhone SE, Apple Inc., Cupertino, CA, United States of America) and imported into Image J to measure leaf area [58]. All samples were then oven dried at 70 °C until weight was constant to determine aboveground species and community biomass, and leaf mass fraction. Leaf dry matter content was calculated as leaf dry mass (g) divided by leaf fresh weight (g). Specific leaf area (SLA, m$^2$ kg$^{-1}$ leaf) was calculated as the one-sided leaf area, divided by oven dry mass.

2.7. Data Analyses

Data were checked prior to analysis to ensure univariate normality; no transformations were necessary. Species richness, functional richness, and community-level weighted means of specific leaf area (by abundance; CWM) were calculated using the FD package [59] in R 4.1.1 [60]. Functional richness, defined as the amount of functional space filled by the community, was determined by coordinating and linking trait values (specific leaf area, leaf dry matter content, and relative growth rate). We included ‘block’ as a random factor in our analyses to account for any potential variation between blocks. One-way ANOVA was used to identify significant differences in species abundance between watering treatments within each S. mexicanum cover treatment, accounting for the block effect. Two-way ANOVA was used to identify interactions between S. mexicanum cover and watering treatments for spontaneous species community biomass (total microcosm biomass), species richness and CWM SLA, with ‘block’ as a random factor. Tukey’s HSD was used for post hoc tests. We examined the residuals from our final models to ensure they meet the assumption of normality and homoscedasticity All statistical analyses were performed in R 4.1.1 [58].

3. Results

3.1. Species Abundance

Total abundance of spontaneous vegetation was significantly affected by Sedum mexicanum cover ($p < 0.001$) but there was no effect of watering ($p = 0.22$) and no interaction between treatments ($p = 0.69$; Figure 2). Microcosms with 0% S. mexicanum cover had the greatest mean total abundance (32 ± 10), followed by those with 25% (18.6 ± 7.5) and 50% S. mexicanum cover (16 ± 4.2). Microcosms with 75% (7 ± 3.6) and 100% (4.2 ± 1.8) S. mexicanum cover had significantly lower mean total abundance than microcosms with 0% cover.
Figure 2. Mean abundance of spontaneous species present in green roof microcosms at the end of the experiment. Asterisks indicate significant ($p \leq 0.05$) differences in species abundance between watering treatment within Sedum cover class (two-way ANOVA). Dissimilar letters indicate significant differences between species abundance within Sedum cover class (Tukey’s post hoc test; $p \leq 0.05$).

In the 0% S. mexicanum cover treatment with the greatest abundance of spontaneous species, Euphorbia peplus was the most abundant species (well-watered; WW 2.6 ± 0.6; water deficit; WD 4.2 ± 1.6). However, this species was absent in microcosms with 50, 75 and 100% S. mexicanum cover, regardless of watering treatment. Similarly, Euphorbia maculata was highly abundant in WW microcosms with 0% S. mexicanum cover (3.6 ± 0.6) but was absent in microcosms with 50, 75 and 100% S. mexicanum cover, regardless of watering treatment. When present, the abundance of P. tetraphyllum, R. crispus, and S. oleraceus was not significantly different in any of the S. mexicanum cover or watering treatments. Total abundance of Trifolium repens did not change significantly with S. mexicanum cover but in 75% S. mexicanum cover ($p < 0.05$) the abundance of T. repens was greater in WW microcosms. Despite having the same theoretical germination capacity, Portulaca oleracea, Stellaria media and Taraxacum officinale were absent from all microcosms. Several species, including Nepeta cataria, Malva neglecta and Epilobium parviflorum were only present at 0% S. mexicanum cover.

3.2. Biomass and Species Richness of Spontaneous Plant Communities

Overall, total biomass of the spontaneous plant community was not significantly affected by S. mexicanum cover ($p = 0.11$) but was affected by watering treatment, with increased total biomass in WW microcosms ($p < 0.001$; Figure 3). Mean total biomass of the spontaneous plant community in WW microcosms (121.41 g) was 160% greater than in WD microcosms (46.61 g). In all treatments, Trifolium repens accounted for >90% of the total biomass (see Appendix C).
Species richness of spontaneous plant communities was significantly affected by Sedum mexicanum cover \((p < 0.001)\), watering treatment \((p = 0.003)\) and the interaction between Sedum mexicanum cover and watering treatment \((p = 0.03; \text{Figure 3})\). Species richness was greatest in microcosms with 0\% Sedum mexicanum cover and within this cover class WW microcosms had 52\% greater species richness \((7.6 \pm 0.6)\) than WD microcosms \((5.0 \pm 0.5)\). Species richness in WD microcosms with 0\% Sedum mexicanum cover was greater than in microcosms with 75 and 100\% Sedum mexicanum cover, regardless of watering treatment. At greater levels of Sedum mexicanum cover \((25, 50, 75 \text{ or } 100\%)\), species richness was not significantly different between WW and WD microcosms.

3.3. Spontaneous Plant Community Leaf Traits and Functional Richness

Community weighted mean (by abundance; CWM) specific leaf area (SLA) of the spontaneous species community was significantly affected by Sedum mexicanum cover \((p < 0.001)\), watering treatment \((p < 0.003)\) and the interaction between Sedum mexicanum cover and watering treatment \((p = 0.001; \text{Figure 4})\). Community weighted mean SLA was greater in WW than WD microcosms in all Sedum mexicanum cover treatments with less than 100\% cover.
Figure 4. (a) Abundance weighted community mean (CWM) specific leaf area (m² kg⁻¹) and (b) functional richness of spontaneous vegetation in green roof microcosms at the end of the experiment. Dissimilar lowercase letters indicate significant differences between watering treatment and Sedum cover class (Tukey’s post hoc test; \( p \leq 0.05 \)). Dissimilar capital letters indicate significant (\( p < 0.001 \)) differences between Sedum cover class (two-way ANOVA).

 Functional richness was significantly affected by \( S. \) mexicanum cover (\( p < 0.001 \)) but there was no significant effect of watering treatment (\( p = 0.31 \)) and no interaction between treatments (\( p = 0.23 \)). Functional richness was greatest at 0% \( S. \) mexicanum cover, followed by 25% cover. There were no significant differences in Functional richness among 50, 75 and 100% \( S. \) mexicanum cover.

4. Discussion

We hypothesised that increasing \( S. \) mexicanum cover and lower water availability would reduce the abundance, biomass and species and functional richness of spontaneous green roof plant communities. We also hypothesised that increasing \( S. \) mexicanum cover and lower water availability would decrease the specific area (SLA; CWM by abundance) of spontaneous green roof plant communities. Increasing \( S. \) mexicanum cover reduced abundance and functional richness, whereas decreased water availability decreased biomass of spontaneous communities. However, species richness and SLA of spontaneous communities was influenced by an interaction between \( S. \) mexicanum cover and watering treatment.

4.1. Spontaneous Community Abundance and Biomass

Consistent with our hypothesis, spontaneous species abundance declined with increasing \( S. \) mexicanum cover. We suggested that \( S. \) mexicanum cover would reduce the availability
of microsites suitable for germination and establishment of spontaneous species (i.e., ‘safe sites’ [23]) and it is likely that the S. mexicanum used in our experiment physically prevented seed from reaching the substrate surface and limited light for germination. However, contrary to our hypothesis, abundance of spontaneous species was not influenced by water availability. We expected lower water availability would reduce the abundance of spontaneous species, as establishment irrigation increases spontaneous plant cover and richness on green roofs in Berlin [34] and greater water availability on Scandinavian green roofs due to higher rainfall increases spontaneous plant cover [35]. However, S. mexicanum cover may have improved water availability in the water deficit treatment by ‘mulching’ substrate and reducing evaporation. This is consistent with other pot-based green roof experiments. For example [61], showed pots planted with Sedum acre and S. reflexum held more moisture than those with more upright species including S. kamtschaticum ‘Ellacombianum’, S. scoparium, Coreopsis lanceolata or unvegetated control pots under identical watering regimes, due to greater shading at the substrate surface reducing evaporation. [62] also showed that pots planted with S. acre lost significantly less water than 13 other plant species under an ‘intermediate’ (watered to field capacity every 11 days) watering regime, due to S. acre’s conservative water use strategy and shading of the substrate surface. In addition to mulching reducing the loss of water from water-deficit microcosms, the lack of an effect of watering on species abundance may also have been due to increased competition in well-watered microcosms [63], reducing the benefit of greater water availability for some species [36,37].

Contrary to our hypothesis, biomass of the spontaneous plant communities was not significantly affected by increasing S. mexicanum cover, but was affected by water availability, with greater biomass in well-watered microcosms. In all treatments, Trifolium repens accounted for >90% of spontaneous plant biomass and likely masked differences in biomass amongst cover treatments due to its ability to grow well, regardless of S. mexicanum cover. The dominance of T. repens may have exerted competitive pressures on other spontaneous species, reducing their germination and growth. The low organic matter content of the green roof substrate used in our study [64] likely gave the nitrogen fixing T. repens [65] a competitive advantage over other species. Additionally, the leaves of T. repens have long petioles, reducing the competitive effect of S. mexicanum cover on light availability for photosynthesis. Competition between S. mexicanum and T. repens was also likely reduced by the ability of T. repens to root at nodes across the microcosm surface and its greater rooting depth (up to 20 cm) [66] than S. mexicanum (<5 cm) [67]. While T. repens growth has been shown to be limited by water availability in pot experiments with green roof substrates [42], this was not the case in our water deficit microcosms. T. repens was also shown to be limited by water availability in pot experiments with green roof substrates [42].

4.2. Spontaneous Community Species and Functional Richness

Species richness of spontaneous communities in microcosms was influenced by the interaction between S. mexicanum cover and watering, with greater species richness in well-watered microcosms with no (0%) S. mexicanum cover. It is likely that increased water availability and the lack of S. mexicanum cover maximised the availability of ‘safe sites’ in our experiment [23]. However, species richness was not significantly different between well-watered and water deficit microcosms with 25, 50, 75 and 100% S. mexicanum cover. This may reflect differences in competition and facilitation, with greater S. mexicanum cover increasing competition and limiting species richness in well-watered microcosms, whereas in water deficit microcosms greater S. mexicanum cover facilitated species richness. This is consistent with other green roof studies looking at the effects of S. mexicanum cover on other plants. For example, when water availability was high on a 13 cm deep green roof in Medford, Massachusetts, Sedum album reduced the growth of neighbouring herbaceous perennials (Agastache rupestris and Asclepias verticillata), whereas it facilitated their growth in hotter and drier conditions [40]. Species richness was also influenced by differences
in longevity and germination of the spontaneous plant species sown into microcosms. *Taraxacum officinale* did not germinate in any of the microcosms and both *Nepeta cataria* and *Malva neglecta* did not grow beyond the seedling stage in the 10-month experiment; whereas *Portulaca oleracea* and *Stellaria media* completed their life cycle and set seed prior to the end of the experiment (see Supplementary Materials).

Functional richness of the spontaneous plant community was significantly affected by *S. mexicanum* cover but not water availability in our experiment and microcosms with 0% *S. mexicanum* cover had the greatest functional richness. This may be due to ‘limiting similarity’ [68], whereby the spontaneous species which are most functionally like the *S. mexicanum* are less likely to establish and grow due to niche overlap [69,70]. For example, two species that failed to germinate and grow when *S. mexicanum* coverage was greater than 25% were *Euphorbia maculata* and *Euphorbia peplus*, both species are relatively slow growing species that exhibit conservative water use [42] and facultative CAM/C3 photosynthesis [71]. The presence of *S. mexicanum*, which has similar conservative water use and a facultative CAM/C3 photosynthetic pathway as *E. maculata* and *E. peplus*, may have impacted the germination and growth of *E. maculata* and *E. peplus* through resource limitation, increased competition, and alterations in the microenvironment [72,73]. This is likely the reason for the absence of *E. maculata* and *E. peplus* in microcosms with >25% *S. mexicanum* cover.

4.3. Spontaneous Community Specific Leaf Area

We also hypothesised that increasing *S. mexicanum* cover and lower water availability would decrease the specific area (SLA; CWM by abundance) of spontaneous green roof plant communities, as low SLA is associated with less productive habitats [74]. Spontaneous plant community SLA was lower in water deficit than well-watered microcosms with less than 100% *S. mexicanum* cover. The lower SLA for spontaneous communities in water deficit microcosms reflects greater investment in leaf structural tissue which is likely to improve leaf turgor under drought stress [50] and is consistent with research showing lower SLA in drier habitats [48,51]. Greater SLA in well-watered microcosms with 25, 50 and 75% *S. mexicanum* cover may also suggest that *S. mexicanum* cover increased soil moisture content and facilitated the spontaneous plant community [62,75,76]. At 100% *S. mexicanum* cover there was no difference in CWM SLA between well-watered and water deficit microcosms, but this likely reflects the dominance of *T. repens*, which has a relatively high SLA, in both watering treatments. Vegetation surveys of established green roofs show that fast growing species with traits such as high SLA are generally found on newly installed green roofs [25,34,77,78]; whereas slower growing, stress tolerant species, such as *E. peplus*, become increasingly abundant as green roofs age [20]. This indicates that the traits of spontaneous green roof communities are likely to change over time, which is something we could not determine in our 10-month experiment.

5. Conclusions

This research contributes valuable insights into how management of spontaneous plants and water availability on green roofs can impact their biodiversity and functionality. This study shows that active management through manipulation of *Sedum mexicanum* cover, and water availability can significantly influence spontaneous plant community characteristics. For example, spontaneous species biomass, abundance, and richness can be limited by maintaining at least 25% *S. mexicanum* cover and minimising water availability. However, our findings also indicate that some spontaneous species (e.g., *T. repens*) can become dominant on green roofs regardless of existing plant coverage (i.e., *S. mexicanum* cover) or water availability. Therefore, periodic maintenance including hand-weeding may still be necessary to avoid loss of original planted vegetation through competition. In addition to maintenance considerations, our research has implications for urban biodiversity. For example, where green roof substrates are left to be colonised by spontaneous species, such as on green roofs designed for biodiversity conservation in London [21], our results
show that spontaneous plant species abundance, richness and functional diversity will be improved with irrigation on bare substrates. However, the longer-term outcomes on green roofs require further research as our experiment was a 10-month study in controlled experimental conditions.

**Supplementary Materials:** The following supporting information can be downloaded at http://dx.doi.org/10.6084/m9.figshare.20472123, ‘Survey data’.

**Author Contributions:** Conceptualization, D.S., N.S.G.W. and C.F.; methodology, D.S., N.S.G.W. and C.F.; validation, D.S., N.S.G.W. and C.F.; formal analysis, D.S.; investigation, D.S.; resources, D.S., N.S.G.W. and C.F.; data curation, D.S., N.S.G.W. and C.F.; writing—original draft preparation, D.S.; writing—review and editing, D.S., N.S.G.W. and C.F.; visualization, D.S.; supervision, N.S.G.W. and C.F.; project administration, D.S., N.S.G.W. and C.F.; funding acquisition, D.S., N.S.G.W. and C.F. 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 openly available in FigShare at http://dx.doi.org/10.6084/m9.figshare.20472123.

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

**Appendix A**

Table A1. Mean seed weight (mg), germination (%), viability (%) and relative growth rate (RGR mg g⁻¹ day⁻¹) of spontaneous species used in this experiment.

<table>
<thead>
<tr>
<th>Species</th>
<th>Seed Weight (mg)</th>
<th>Seed Germ (%)</th>
<th>Seed Viability (%)</th>
<th>Relative Growth Rate (mg g⁻¹ day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epilobium parviflorum</td>
<td>0.4</td>
<td>92</td>
<td>96</td>
<td>121</td>
</tr>
<tr>
<td>Euphorbia maculata</td>
<td>1.3</td>
<td>0</td>
<td>95</td>
<td>106</td>
</tr>
<tr>
<td>Euphorbia peplus</td>
<td>4.8</td>
<td>80</td>
<td>92</td>
<td>90</td>
</tr>
<tr>
<td>Helichrysum luteoalbum</td>
<td>0.4</td>
<td>45</td>
<td>90</td>
<td>153</td>
</tr>
<tr>
<td>Malva neglecta</td>
<td>10</td>
<td>36</td>
<td>65</td>
<td>124</td>
</tr>
<tr>
<td>Nepeta cataria</td>
<td>5.4</td>
<td>52</td>
<td>85</td>
<td>200</td>
</tr>
<tr>
<td>Polycarpon tetraphyllium</td>
<td>0.3</td>
<td>83</td>
<td>95</td>
<td>118</td>
</tr>
<tr>
<td>Portulaca oleracea</td>
<td>4.4</td>
<td>90</td>
<td>100</td>
<td>217</td>
</tr>
<tr>
<td>Rumex crispus</td>
<td>10.5</td>
<td>96</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>Solanum nigrum</td>
<td>8.5</td>
<td>57</td>
<td>88</td>
<td>194</td>
</tr>
<tr>
<td>Sonchus oleraceus</td>
<td>1.6</td>
<td>100</td>
<td>100</td>
<td>218</td>
</tr>
<tr>
<td>Stellaria media</td>
<td>2.9</td>
<td>21</td>
<td>95</td>
<td>190</td>
</tr>
<tr>
<td>Taraxacum officinale</td>
<td>3.8</td>
<td>87</td>
<td>95</td>
<td>164</td>
</tr>
<tr>
<td>Trifolium repens</td>
<td>5.4</td>
<td>94</td>
<td>100</td>
<td>198</td>
</tr>
</tbody>
</table>
**Appendix B**

Figure A1. Daily mean relative humidity (%) and temperature (°C) in Tunnel 13 from January to November 2021.

**Appendix C**

Table A2. Mean (n = 5) biomass (g) of spontaneous vegetation in well-watered (WW) or water deficit (WD) microcosms with different Sedum cover classes (%). Bold values indicate significant differences between WW and WD treatments within each Sedum cover class (one-way ANOVA; p ≤ 0.05).

<table>
<thead>
<tr>
<th>Species</th>
<th>0%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>WD</td>
<td>WW</td>
<td>WD</td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>Epilobium parviﬂorum</td>
<td>&lt;0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Euphorbia maculata</td>
<td>0.27</td>
<td>&lt;0.01</td>
<td>-</td>
<td>0.03</td>
<td>-</td>
</tr>
<tr>
<td>Euphorbia peplus</td>
<td>0.09</td>
<td>&lt;0.01</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Helichrysum luteoalbum</td>
<td>0.02</td>
<td>0.41</td>
<td>0.03</td>
<td>0.78</td>
<td>0.15</td>
</tr>
<tr>
<td>Malva neglecta</td>
<td>&lt;0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nepeta cataria</td>
<td>&lt;0.01</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polycarpon tetraphyllum</td>
<td>1.18</td>
<td>-</td>
<td>2.15</td>
<td>2.50</td>
<td>1.24</td>
</tr>
<tr>
<td>Rumex crispus</td>
<td>0.13</td>
<td>0.06</td>
<td>0.16</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>Solanum nigrum</td>
<td>0.69</td>
<td>-</td>
<td>0.67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sonchus oleraceus</td>
<td>0.16</td>
<td>0.10</td>
<td>0.20</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>Trifolium repens</td>
<td>70.44</td>
<td>48.49</td>
<td>128.78</td>
<td>68.08</td>
<td>127.81</td>
</tr>
</tbody>
</table>

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