# PLANT-SOIL FEEDBACKS IN A GRASSLAND ECOSYSTEM: EFFECTS OF PLANT FUNCTIONAL GROUPS AND SOIL FERTILITY

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Kobe N. Luu

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PLANT-SOIL FEEDBACKS IN A GRASSLAND ECOSYSTEM: EFFECTS OF PLANT FUNCTIONAL GROUPS AND SOIL FERTILITY

The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science, with a major in Biological Sciences.

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Gregory Houseman, Committee Chair

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Leland Russell, Committee Member

_____
Andrew Swindle, Committee Member

# DEDICATION

To my parents, my sister, my brother, and my dear friends

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# ABSTRACT

Plant-soil feedbacks (PSFs) occur when plants alter soil conditions, subsequently affecting plant success. This process may play a key role in maintaining biodiversity and ecosystem functioning. Many PSF experiments have reported species-specific responses and there is growing interest in determining whether the response of plant functional groups (PFGs), in which species are grouped by similar plant functional traits, can be used to predict the likelihood of PSFs. One reason for the variable responses reported is that PSFs can be dependent on soil fertility, which can serve as a general indicator of succession and ecosystem development. To test how plant functional groups and soil fertility relate to PSF, we grew 19 grassland species from 3 functional groups (graminoids, forbs, legumes) in 3 levels of soil fertility for 4 years. We used this field conditioned soil to conduct greenhouse assays of plant growth rates for species representing each PFG. We found that on average, forbs exhibited positive PSF in the most-developed soil, while the graminoids and legumes exhibited negative PSFs regardless of soil fertility. Despite these trends, we found strong species-specific and soil fertility effects on PSF. These results most likely emerged due to the species-specific soil biota that accumulates over time during the conditioning phase, which includes both harmful and beneficial biota, with the net PSF effect determined by the dominant influence. Generally, we found that PSFs became more positive/less negative as soil fertility increased, most likely due to the increased nutrient concentrations and beneficial soil biota outweighing the effects of the harmful soil biota and lack of soil nutrients. These findings help to understand that different species have unique roles in plant community dynamics, and that their roles within the community are going to change over time, as is total ecosystem productivity.

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# CHAPTER 1

## INTRODUCTION

Plant-soil feedbacks (PSFs) occur when a plant alters biotic and abiotic soil conditions that subsequently affect the survival or growth of plant neighbors or descendants (Martorell et al. 2021). This process has gained interest as a mechanism that helps explain plant community dynamics such as succession, species invasions, species richness and diversity, and ecosystem productivity and functioning (Van der Putten et al. 2013). PSF effects can be either positive or negative and are determined by comparing plant performance between different soil conditioning treatments (Crawford et al. 2019). The direction and magnitude of PSF provides implications for a given species' abundance within a community, which ultimately affects community productivity and ecosystem services (Pernilla Brinkman et al. 2010). Negative PSFs may promote species coexistence and maintain ecosystem diversity by preventing the dominance of a particular species (Petermann et al. 2008), while positive PSFs increase the likelihood that a species will dominate a plant community (Van der Putten et al. 2013). The overall PSF effect is primarily driven by the cumulative effects of the soil community that accumulates and changes as plants grow in soil.

Prior studies have shown that the direction and magnitude of PSF effects are heavily driven by the soil community, as plants accumulate beneficial soil mutualists that aid growth and soil pathogens that hinder growth, with these microbes persisting after the plant dies and affects the growth of the succeeding plant (Bennett et al. 2017, Facelli et al. 2018, Miller et al. 2019). In addition, meta-analyses have shown that PSF effects driven by the soil microbial community are also likely to differ based on a plant's functional group (Kulmatiski et al. 2008, Meisner et al. 2014). Plant functional groups serve as a way of organizing species based on similar plant

functional traits. All plant species have their unique set of plant functional traits, which impact a plant's ability to survive and reproduce, including many traits such as root length, specific leaf area, growth rates, plant height, and shade tolerance. Several studies have shown that functional traits strongly influence plant-soil feedback effects (Uriarte et al. 2015, Cortois et al. 2016, Kuťáková et al. 2018, Wilschut et al. 2019) and that functional traits respond significantly to changes in the soil microbial community (Hahn et al. 2018, Saar et al. 2018). However, there are comparatively fewer studies that have tested whether PSF effects can be generalized by plant functional groups.

Plant species can be sorted into various functional groups according to similar plant functional traits and ecosystem niche. For example, grassland functional groups include legumes, forbs, and graminoids. Between species of the same functional group, their shared functional traits result in similar resource allocation and nutrient acquisition strategies, so these plants should have similar effects on soil characteristics, and thus, on PSFs (Faucon et al. 2017). Graminoids refer to herbaceous plants that consist of elongated culms with long, blade-like leaves. Graminoids tend to have higher N:P ratios than other species, which is negatively correlated with growth rate. (Güsewell 2004). The growth rate hypothesis theorizes that plant growth rate is negatively correlated with constitutive resistance, induced defense, and nutrient-use efficiency (Van Zandt 2007). Therefore, graminoids are more resilient than other grassland species at the cost of leaf litter quality, which makes the leaf litter more difficult to decompose, decreasing the return of nutrients to the soil, and thus decreasing future plants' growth (Yahdjian et al. 2017). In contrast, forbs and legumes refer to herbaceous plants that are not grass-like. They often allocate more resources towards forming symbiotic relationships with arbuscular mycorrhizal fungi (AMF) to increase nutrient uptake, allowing for more efficient growth, and a

meta-analysis showed that forbs had higher AMF colonization levels (Bunn et al. 2015). In addition, legumes form the majority of the plants that have evolved the ability to form root nodules, helping them fix atmospheric nitrogen in symbiosis with nitrogen-fixing bacteria called rhizobia, to a form more readily absorbed into plant roots and less prone to leaching (Graham and Vance 2003). As a result, forbs and legumes have higher relative growth compared to graminoids and produce litter with lower C:N ratios. This litter decomposes faster, increasing soil nutrient content (Duchene et al. 2017). However, their allocation of resources towards growth rather than defense makes them more susceptible to pathogens (Karasov et al. 2017). Species from these plant functional groups have been shown to develop soil bacterial communities that differ in both composition and diversity depending on the richness and identity of the conditioning plant(s) (Dassen et al. 2017, Emilia Hannula et al. 2019, Schmid et al. 2019, Šmilauer et al. 2020). Several studies have shown that soil biota strongly influence plant-soil feedbacks (Bennett et al. 2017, Facelli et al. 2018, Miller et al. 2019, Bennett et al. 2020). However, while PSFs have been studied in relation to soil biota and plant functional traits, it is unknown if they can be generalized by the commonalities between species.

Plants alter soil characteristics as they grow through the accumulation of soil biota and altering nutrient concentrations, but it is relatively unknown how these conditioning effects vary by soil fertility. Soil fertility is often correlated with ecological succession, as plants alter soil characteristics to become more favorable to the succeeding plants. PSFs are expected to vary as succession occurs, as positive feedback occurs initially as plants add more nutrients and accumulate soil biota, eventually becoming more negative as the altered soil characteristics facilitate later-successional species to displace them through the accumulation of species-specific pathogens and outcompeting earlier-successional species for nutrients (Van der Putten et al.

2013). Understanding how PSFs vary along soil fertility gradient may provide insight on one of the mechanisms that drive succession; however, there have only been a limited number of studies that studied these effects (Kardol et al. 2007, Castle et al. 2016), even though this could largely influence the composition of succeeding plant communities.

In this study, we examine how several native grassland species of three functional groups (graminoids, forbs, legumes) and soil types of varying fertility affect the net plant-soil feedback. This will be examined by comparing the growth of a given plant species in soil conditioned by its own species and its growth in soil conditioned by all species. The following questions will be addressed: 1) How do plant-soil feedback effects differ between plant functional groups? 2) How does soil fertility alter plant-soil feedbacks?

To test these questions, we conducted a plant-soil feedback experiment where we grew monocultures for nineteen species in large pots out in the field, to simulate realistic field conditions. These species were chosen based on relative abundance out in the field and successful growth during the conditioning phase and included species from common grassland functional groups, including graminoids, forbs, and legumes. At the field site, we collected soil from different depths along a soil horizon, assuming that they represent differences in soil development and fertility over time. After several years of growth, the conditioned soil was collected and used in a greenhouse assay where we grew species in soil conditioned by their own species and in a soil mixture containing soil conditioned by each species. Following this, biomass was harvested and growth was compared between the soil conditioning and the soil fertility treatments, to compare PSFs among functional groups and different levels of soil fertility.

We expect graminoids to have more negative plant-soil feedback effects because their greater specific root length is associated with lower AMF colonization (Šmilauer et al. 2020) and a larger accumulation of soil pathogens compared to other functional groups (Cortois et al. 2016). We expect that forbs will have intermediate feedback compared to graminoids and legumes because their roots are associated with higher AMF colonization, which aids in acquiring limiting nutrients (Šmilauer et al. 2020). We expect legumes to have more positive plant-soil feedback effects because in addition to higher AMF colonization, they also form beneficial associations with nitrogen-fixing bacteria by forming root nodules, resulting in increased nitrogen uptake and soil nitrogen content (Graham and Vance 2003). The increased growth will create more diverse soil microbiomes that may reduce the harmful effects of soil pathogens (Zhang et al. 2020). We expect that conditioning soil collected closer to the surface will have more positive plant-soil feedbacks, as this more developed soil generally contains more beneficial soil biota and higher nutrient concentrations from the decomposed plant matter, which will improve plant growth (Hobbie 2015).

# CHAPTER 2

# METHODS

## 2.1 Study System and Experimental Species

The plant species used for the plant-soil feedback experiment were selected based on their success in the conditioning phase and their relative abundance in natural systems. A two-phase PSF experiment was carried out where we tested the growth of nineteen species in two soil treatments. The first was soil fertility, with three soils collected from different depths. The second was soil conditioning with two levels: self-conditioned soil, where a plant species was grown in soil conditioned by its own species, representing conspecific PSF; and mixed-conditioned, where a plant species was grown in soil that was homogenized from soil conditioned by each species, representing heterospecific PSF. The conditioning phase took place at the Wichita State University Ninnescah Reserve while the feedback phase occurred in a greenhouse on the Wichita State University campus.

We obtained three different soils that reflected differences in soil development and fertility by extracting soil from three vertical strata at the experimental site. We sequentially removed each layer in 15 cm intervals resulting in an upper, middle, and lower soil layer using a tractor bucket. We labeled these strata as soil 1, 2, and 3, respectively so that the depth names would not be confused when interpreting the original position in the field with the position of soil in the pots. Each soil layer was placed separately in a large, wooden container and then homogenized to the extent possible with a rototiller. We then filled 68 L pots with one of the three soil types. Once all the buckets were filled with the appropriate soil type, we moved each

to an assigned position in the area where the soils had been extracted. Pots were arranged in two blocks using a complete randomized block design. Pots were spaced approximately 20 cm apart. Covers were placed over the pots and remaining soils were used to backfill around the pots. When completed, the pots were buried in the field at a level that approximated the surrounding areas.

We then sowed seeds for nineteen plant species representing three functional groups according to the treatment combinations. We placed a germination blanket on the soil within each pot to improve germination rates, reduced seed loss, and minimized weed establishment. We then periodically weeded each of these pots over four growing seasons. The pots experienced field conditions over this time. In some cases, the field was temporarily inundated with rain leading to flooding of pots. Because the lip of the pots was higher than surrounding soil, some pots retained water for longer than surrounding plant communities. To reduce this effect, we drilled additional holes as necessary using a long drill bit to expedite drainage.

### 2.2 Soil Conditioning Phase

At the beginning of May 2021, seeds from all nineteen species were placed into zipper bags half-filled with water. After pushing out the air and zipping the bags shut, the seed bags were placed into a freezer for several weeks prior to seeding. Over the next few weeks, these bags were brought in and out of the freezer several times. Its purpose was to simulate freeze-thaw cycles that occur naturally in the field, to try to increase germination.

### 2.3 Feedback Phase

At the end of May 2021, the monoculture pots were extracted from the field. The top fifteen cm of soil was extracted from the pot. After removing the belowground biomass, the soil was thoroughly homogenized. The soil was then sieved through a 4 mm mesh and further homogenized. Afterwards, all pots with the self-conditioned (SC) soil conditioning treatment were filled with soil conditioned by the monocultures. Following this, each soil conditioned by the nineteen species were mixed thoroughly to create the soil for the mixed-conditioned (MC) soil conditioning treatment, and the mixed soil was poured into the pots. After the pots were filled with soil, each pot was watered extensively so that the water percolated to the bottom of the pot, ensuring that the soil remained saturated throughout the soil depth.

Species from each functional group were split into 3 batches, to be sown two weeks apart from each other. Species from batch one include: *Ratibida pinnata*, *Liatris pycnostachya*, *Dalea purpurea*, *Baptisia australis*, *Panicum virgatum*, and *Schizachyrium scoparium*. Species from batch two include *Helianthus maximiliani*, *Achillea millefolium*, *Symphyotrichum oblongifolium*, *Desmodium canadense*, *Desmanthus illinoensis*, *Andropogon gerardii*, and *Bouteloua curtipendula*. Species from batch three include *Salvia azurea*, *Solidago rigida*, *Lespedeza capitata*, *Mimosa quadrivalvis*, *Sorghastrum nutans*, and *Sporobolus compositus*. The pots used during the feedback experiment were conical in shape and had a volume of 655.5 $cm^3$. Pots were placed in every other position on the rack to reduce contamination. About 5 – 10 seeds of each species were sown into the pots, approximately three centimeters below the soil surface. Immediately following sowing and for the first two weeks following germination, pots were watered extensively to promote growth. Afterwards, pots were watered extensively every other day. The number of successful germinated pots was recorded in Table 1. Pots were thinned to ensure that only one individual of each species was growing within a pot. The pot racks on the

greenhouse tables were rotated in a serpentine pattern three times a week to account for differences in light availability in the greenhouse. As the experiment progressed into the fall, light was augmented using LED lighting system to ensure that light intensity and duration were representative of field conditions. The duration of augmented lighting increased throughout the fall varying from 0-11 hours per day.

After a growth period of twelve weeks, plants were harvested and dried. The aboveground biomass was clipped from the soil surface. Roots were separated from soil by placing the soil in a 4 mm soil sieve suspended in water. We gently agitated the roots to separate them from the soil and then patted the roots dry with paper towel. The root or shoot biomass was placed into labeled envelopes and dried for at least forty-eight hours in an oven at 60°C. Aboveground and belowground biomass were weighed and recorded. We used total biomass per pot to calculate PSF as log (biomass when grown on home soil) / (biomass when grown on mixed soil) following (Pernilla Brinkman et al. 2010).

### 2.4 Statistical Analysis

We used model fitting to analyze the effects of our variables on biomass and PSF because model fitting could serve as the most flexible and reliable method of dealing with uncertainty in data (Dudenhöffer et al. 2018, McGinn et al. 2018). The estimation of uncertainty is altered by small sample sizes and unbalanced experimental designs, and model fitting is probably more robust at dealing with these issues compared to other approaches (Hesterberg 2014).

For biomass data analysis, R version 4.1.2 was used to test for effects of soil type and soil source on plant biomass. For each species, a linear regression model and a general linear mixed effect model (GLMM) were created with soil type and soil source as fixed effects and block as a

random effect. To select the optimal model, we compared the Akaike Information Criterion (AIC) values and selected the model with the lower AIC value. If a linear regression model was selected, we also transformed the data with both a log transformation and a square-root transformation and created linear regression models using the transformed data. If either of these models had a lower AIC score than the non-transformed data model, the model with the lowest score was used for analysis. A 3-way Analysis of Variance was fitted to the linear regression models while a 3-way Analysis of Deviance was fitted to the GLMM to test for treatment effects. Pairwise results for any significant differences were calculated using the emmeans package in R. The models' residual diagnostic plots were examined using the ggResidpanel package in R.

A similar analysis was employed to test for the effects of plant functional group and soil type on the PSF metric. Within each treatment combination of species, soil type, and block, total biomass for both SC and MC soil were sorted in descending order. Net PSF was calculated as log (total dry biomass in SC soil/total dry biomass in MC soil). This metric is widely used in PSF studies because it is easy to interpret (positive and negative values equate to positive and negative feedback, respectively) and is dimensionless, which allows more robust comparisons between species (Bates et al. 2020) Positive values indicated that a given species produced more biomass when grown on SC soil compared to MC soil. Negative values indicated the reverse, while a value of zero indicated neutral feedback. A one-sample t-test was used to determine if average net PSF for each species and soil type combination significantly differed from zero. Using the calculated PSF metric, the same model selection process was performed for each species with soil type as a fixed effect for each species. A 3-way Analysis of Variance was fitted to the linear regression models while a 3-way Analysis of Deviance was fitted to the GLMM to test for the treatment effect. Pairwise results for any significant differences were calculated using

the emmeans package in R. The models' residual diagnostic plots were examined using the ggResidpanel package in R.

# CHAPTER 3

# RESULTS

## 3.1 Effect of Soil Conditioning on Biomass

Of the 19 species grown during this experiment, 3 species were independently affected by both the soil fertility and the soil conditioning treatment (Table 2, Figures 1-3). For *L. capitata* and *D. illinoensis*, the soil conditioning effect was quite clear with the biomass higher in MC compared with SC soil ($P < 0.0012$, Table 3). A similar response was exhibited by *P. virgatum*, but this effect was marginally significant ($P = 0.059$, Table 3). Additionally, all three species were significantly affected by soil fertility with the biomass of each decreasing as soil fertility decreased, from soil type 1 to 3 ($P < 0.0001$, Table 3).

## 3.2 Effect of the Soil Conditioning and Fertility Interaction on Biomass

There were clear interactive effects between soil conditioning and soil fertility on plant biomass for 8 species including *B. curtipendula*, *D. canadense*, *A. millefolium*, *B. australis*, *S. azurea*, *S. compositus*, *R. pinnata*, and S. *oblongifolium* and marginal interactive effects for three species including *D. illinoensis*, *H. maximiliani*, and *S. scoparium* (Table 2). Of these eleven species, plant biomass was higher for eight of the species when grown in MC soil for certain soil types (Table 3). For *B. australis*, biomass was higher in MC soil for soil types 1 and 3 (Figure 4). A similar response was exhibited by *D. purpurea*, but the effect was marginal and was only present in soil type 1 ($P = 0.0603$, Figure 5). For *S. compositus* and *R. pinnata*, biomass was higher in MC soil for soil type 2 (Figures 6-7). For *B. curtipendula* and *A. millefolium*, biomass was higher in MC soil for soil types 2 and 3 (Figures 8-9). However, the conclusions for *A.*

*millefolium* were based on pooling soil types 2 and 3 due to the low number of successful replicates. For *D. canadense*, biomass was higher in MC soil for soil type 3 (Figure 10). A similar response was exhibited by *S. scoparium*, but the effect was marginal ($P = 0.0731$, Figure 11).

There were three species where plant biomass was higher when grown in SC soil for certain soil types (Table 3). For *S. oblongifolium*, biomass was higher in SC soil for soil type 1 (Figure 12). A similar response was exhibited by *S. azurea*, but for both soil types 1 and 2 (Figure 13). For *H. maximiliani*, biomass was clearly higher in SC soil for all soil types, with the marginally significant interaction being driven by differences in the magnitude between SC and MC among the three soil types ($P = 0.0715$, Figure 14).

### 3.3 Effect of Soil Type on Biomass

Of the species grown in this experiment, two species responded to differences in soil type without any evidence of sensitivity to soil conditioning. These species were the graminoids *A. gerardii* and *S. nutans* (Table 2). Compared to soil type 1, *A. gerardii* had a 37.2% reduction in biomass when grown in soil type 2 and a 63.1% reduction in biomass when grown in soil type 3 (Table 6, Figure 15). Compared to soil type 1, *S. nutans* had a 18.6% reduction in biomass when grown in soil type 2 and a 49.3% reduction in biomass when grown in soil type 3 (Table 6, Figure 16).

### 3.4 Non-detectable Effects on Biomass

Of the species grown in this experiment, *L. pycnostachya*, *S. rigida*, and *M. quadrivalvis* exhibited no detectable biomass response to soil conditioning, soil type or the interaction between the two factors (Table 2, Figures 17-19).

### 3.5 Effect of Soil Type and Plant Species on PSF

Of the 19 species grown in this experiment, 14 species demonstrated at least one PSF response that significantly differed from zero, while 2 species demonstrated a PSF response that marginally differed from zero (Table 7). Of them, five species exhibited no detectable difference in average net PSF among soil types (Table 8). For *D. purpurea*, PSF was negative for soil types 1 and 2 but neutral for soil type 3 (Figure 22). For *S. rigida*, PSF was neutral in soil types 1 and 3 but marginally negative in soil type 2 ($P = 0.0585$, Figure 21). For *R. pinnata*, PSF was neutral in soil type 1 but became negative in soil type 2; only one PSF metric was calculated for soil type 3, so the one-sample t-test could not be conducted (Figure 21). For *L. capitata* and *D. illinoensis*, PSF was negative regardless of soil fertility (Figure 22).

Of the 19 species grown in this experiment, 11 species demonstrated a significant PSF response to soil fertility. These species were the graminoids *B. curtipendula*, *S. scoparium*, *S. nutans*, *S. compositus*, the forbs *A. millefolium*, *S. oblongifolium*, *S. azurea*, and *H. maximiliani*, and the legumes *D. canadense*, *B. australis*, and *M. quadrivalvis* (Table 8).

Seven species exhibited positive or neutral PSF in more fertile soil and became negative as soil fertility decreased (Table 9). For example, *B. curtipendula* had positive PSF in soil type 1 that became progressively negative as soil fertility decreased (Figure 20). Likewise, *S. azurea* and *D. canadense* had positive PSF in soil types 1-2 that became negative in soil type 3 (Figures 21-22). Similarly, *S. compositus* and *S. oblongifolium* had positive PSF in soil type 1 (Figures 20-21), but PSF became negative in soil types 2 (for both species) and 3 (*S. compositus* only). Note that for *S. oblongifolium*, there were no successful MC replicates for soil type 3, so PSF could not be calculated. For *A. millefolium*, PSF was neutral in soil type 1 but became marginally negative in soil type 2 ($P = 0.0543$, Figure 21); as with biomass, PSF data from soil types 2 and 3

were pooled. Likewise, *S. scoparium* had neutral PSF in soil types 1-2, but became negative in soil type 3 (Figure 20).

Two species exhibited negative PSF in more fertile soil and became neutral or positive as soil became less fertile (Table 9). For example, *S. nutans* had negative PSF in soil type 1 but became neutral in soil types 2 and 3, respectively (Figure 20). Likewise, *M. quadrivalvis* had negative PSF in soil type 1 but became positive in soil type 3 (Figure 22); only one PSF metric was calculated for soil type 2, so the one-sample t-test could not be conducted.

Two species exhibited the most positive PSF in soil of intermediate fertility (Table 9). For example, *H. maximiliani* had positive PSF regardless of soil fertility, but the highest positive PSF was exhibited in soil type 2 (Figure 21). Likewise, *B. australis* had neutral PSF in soil type 2 and negative PSF in soil types 1 and 3 (Figure 22).

Of the species grown in this experiment, *P. virgatum*, *A. gerardii*, and *L. pycnostachya* exhibited non-significant PSF, regardless of soil fertility, and no significant differences in PSF among soil types (Tables 8-9, Figures 20-21).

# CHAPTER 4

# DISCUSSION

We hypothesized that graminoids would generate the most negative PSF, which was supported by our results that showed that the average net PSF for graminoids varied from neutral to strongly negative as soil fertility decreased (Figures 20, 23). This result suggests that while graminoids produce less biomass in SC soil compared to MC soil regardless of soil fertility, the proportional decrease in biomass was higher for SC soil compared to MC soil. The larger decrease in biomass suggests that even though the number of soil pathogens were likely higher in the more developed soil, the increased nutrient content and accumulation of mutualists may ameliorate these effects, though not reverse them completely. Other studies that examine graminoids' conditioning effects on soils support this interpretation, as graminoids have been shown to both increase the accumulation of soil bacteria that can antagonize soil pathogens (Maharning et al. 2009, Latz et al. 2012, Chen et al. 2016) and increase the accumulation of AMF (De Deyn et al. 2011, De Deyn and Kooistra 2021). Graminoids have been suggested to exhibit the most negative PSF effects compared to forbs and legumes due to their more extensive root systems increasing their exposure and accumulation to harmful soil biota (Kulmatiski et al. 2008). Studies have shown that graminoids generated the most negative PSF in comparison to other functional groups through the accumulation of soil pathogens (Cortois et al. 2016, Forero et al. 2021), which is in general agreement with our results.

We hypothesized that forbs would generate intermediate feedback compared to graminoids and legumes; however, the average net PSF for forbs in soil type 1 was positive, in contrast to the negative feedback for the decreased soil fertility levels (Figures 21, 23). Similar results were reported in Cortois et al. 2016, though their study separated their experimental

species of forbs into ‘tall herbs’ and ‘short herbs’, with tall herbs generating positive PSF. Their findings resulted from their tall herbs responding more positively to their own microbes than those from other species. In comparison to graminoids, forbs have been shown to have higher diversity in AMF that colonize their roots, and that the presence of forbs in mixed communities induced higher mycorrhizal colonization levels for both forbs and graminoids (Šmilauer et al. 2020). Indeed, another study has shown that graminoids produced more biomass when grown in soil conditioned by forbs compared to other graminoids (Bezemer et al. 2006). In general, it appeared that the positive feedback exhibited in the most fertile soil may have been due to strong effects of beneficial, species-specific soil biota than harmful biota, especially in comparison to legumes and graminoids.

We also hypothesized that legumes would generate the most positive feedback; however, we found that the average net PSF for legumes varied from strongly negative to neutral as soil fertility decreased (Figures 22-23). We predicted that the accumulation of beneficial soil microbes and nitrogen in soil would improve the growth of legumes in SC soil (Graham and Vance 2003), as legumes increase soil nitrogen content regardless of plant diversity and competition (Furey and Tilman 2021). However, nitrogen-fixing plants are likely to be outcompeted as soil nitrogen increases because fixing atmospheric nitrogen is more energetically expensive under nitrogen-rich conditions (Zheng et al. 2020). Because of this, we expect legumes to exhibit more negative PSF over time through the combined effects of the depletion of soil nutrients needed for nitrogen-fixation and the accumulation of species-specific pathogens (Davis et al. 2021). Our results agreed with this, as PSF became neutral as soil fertility lessened. Other studies have shown that legumes exhibit more neutral PSF (Cortois et al. 2016, Forero et al. 2021), while another study (Ma et al. 2017) had similar results in that legumes exhibit mostly

negative PSF. In contrast to our study, these studies used different soil inoculum (Ma et al. 2017) or sterilization (Cortois et al. 2016) treatments to simulate different stages of soil development.

The most positive PSF was generated from the most well-developed soil, which agrees with our second hypothesis, but these effects were inconsistent among plant functional groups and species. We assumed that the soil type treatment in this experiment represented a soil fertility gradient, where soil collected closer to the surface were more fertile due to higher accumulation of soil microbes, organic matter, and nutrients. Soil depth has been shown to influence soil characteristics such as the structure of microbial communities, decreasing abundance and diversity as depth increases (Eilers et al. 2012, Hao et al. 2021, Xu et al. 2021) as well as nutrient content (Li et al. 2022). Based on these prior studies, we can infer that more species generated the most positive/least negative PSF in the most fertile soil, and where the harmful effects of soil pathogen accumulation were outweighed by the accumulation of beneficial soil biota and soil nutrients (Smith-Ramesh and Reynolds 2017). A similar outcome was reported for a tropical system, where the most positive feedback effects were generated by the most fertile soil, and these effects were heavily driven by soil microbes and their response to nutrient availability (McCarthy-Neumann and Kobe 2019). However, such positive responses were variable in our experiment suggesting that individual plant species respond differently to the soil fertility gradient, ultimately affecting how they condition the soil. In summary, our results suggest that plant conditioning effects are dependent on how well-developed the soil is, and how these conditioning effects change as soil conditions change is unique to each species.

Our results suggest that strong species-specific effects were present with relatively high variability among species and soil types. Notably, *H. maximiliani* was the only species to exhibit positive PSF regardless of soil fertility, which suggests that they benefit from species-specific

mutualists far more than they are harmed by species-specific pathogens. In addition, comparing species within each functional group showed that they had different responses in terms of PSF. Regarding other species, 1 graminoid and 2 legumes exhibited negative PSF in the most fertile soil that became neutral/positive as soil fertility lessened, in contrast to the 3 graminoids, 5 forbs, and 1 legume that exhibited positive/neutral feedback in the most fertile soil that became neutral/negative as soil fertility lessened. For the remaining species, 2 legumes exhibited negative PSF regardless of soil fertility while 1 forb and 2 graminoids exhibited neutral PSF regardless of soil fertility. *B. australis* was unique in that negative PSF was exhibited by the contrasting ends of soil fertility, while neutral PSF was exhibited by the intermediate soil. While the underlying mechanisms that drove these effects are unknown, we assume that they were driven by each species responding to how soil fertility in species-specific ways, ultimately affecting the accumulation of soil biota and changing the net conditioning effect, based on the results from other PSF studies and how they determined what drove PSFs.

In general, our results were similar to other studies. Specifically, plant functional groups serve as weak predictors of net PSF, with much variation among species in a functional group. However, the weak trends in our results were similar to other studies that showed neutral to negative average PSF when comparing PSF among plant functional groups and species, though the PSF indexes slightly differed from ours (Harrison and Bardgett 2010, Bukowski et al. 2018, Forero et al. 2021). Likewise, Martorell et al. (2021) found more instances of positive PSF, but the instances of negative PSF were much stronger, resulting in negative net average PSF. PSF studies can vary in experimental design, with this study contrasting with ours slightly by having graminoids generating the most positive feedback while legumes generating the most negative feedback (Ma et al. 2017). Strong species-specific effects were also shown in Bezemer et al.

(2006), where two graminoid species had significant differences in growth in SC soil compared to soil conditioned by other species. However, these studies are often limited by the number of species from each functional group tested (Harrison and Bardgett 2010, Bukowski et al. 2018, McKenna et al. 2018) or study a limited number of functional groups because of species variation between communities (Bezemer et al. 2006, Šmilauerová and Šmilauer 2016, Martorell et al. 2021). In many studies that examine PSF, including those not listed here, PSF effects are largely context-dependent, with contrasting results based on experimental species and testing for various soil characteristics, much like our study.

With our results indicating that PSFs are influenced more at the species level rather than at the functional group level, future research should examine how PSFs are affected by functional traits. Though plant functional groups are sorted based on similar plant functional traits, functional trait variation among species in same plant functional group is likely to create large differences in PSFs, through the effects on both plant growth and on the development of the soil community. For grassland communities in particular, plant functional groups are more closely associated with plant family than specific plant functional traits, so the trait variation within a functional group is expected to be even larger. Plant species identity and diversity in a community has been shown to influence soil characteristics such as nutrient content (Zhang et al. 2018, Fischer et al. 2019, Steinauer et al. 2020, Teixeira et al. 2020, Zhang et al. 2020), microbial and fungal community composition and abundance (Dassen et al. 2017, Schmid et al. 2019, Šmilauer et al. 2020, Steinauer et al. 2020), and pH (Teixeira et al. 2020). Several studies have also shown that functional trait variation influences PSFs, for both aboveground traits (Cortois et al. 2016, Kuťáková et al. 2018) and belowground traits (Cortois et al. 2016, Wilschut et al. 2019). However, our results also indicate that PSFs are likely to be affected by soil fertility,

which have been poorly studied (Castle et al. 2016). Undoubtedly these functional trait-soil fertility dynamics influence PSFs, and future research should examine these interactions as drivers of PSFs due to the important implications this knowledge has for understanding succession and ecosystem functioning.

There were several limitations in our experimental design and analysis that could have prevented us from accurately determining what drove PSF effects in our study. We had a limited number of pots in the field due to the difficulty in creating and maintaining them, which could have created the higher variability than in a highly replicated experiment. We attempted to account for this variability by using several species from each functional group to sufficiently test at the functional group level and allowing the duration of culturing phase in the field to be much longer than most studies. Our results indicated that a species' functional group cannot reliably predict PSF and that net PSF is strongly influenced by individual species, so decreasing the number of study species while increasing the number of replicates per treatment combination should increase power enough to narrow down the net PSF of a given species. Ideally, we would have analyzed the soil microbial community of conditioned soil before and after the feedback phase, as doing so would allow us to infer drivers of PSF for individual species.

In conclusion, our results suggest plant functional groups alone are unlikely to predict PSF response because of species-specific responses and the sensitivity to soil conditions. Nevertheless, our results were similar to other PSF studies in that plant functional groups were weak predictors of PSF, and future research should investigate PSF effects at the species level to understand what drives differences between species and make PSF effects more predictable. Altogether, these findings suggest that species contribute differently to plant community dynamics over time through their unique conditioning effects on soil, which has important

implications for maintaining biodiversity and promoting terrestrial ecosystem productivity and functioning.

## TABLES

Table 1. Table showing the number of replicates per species that successfully grew for the 12-week duration of the experiment, for all treatment combinations. Species are sorted in alphabetical order by plant functional group,

| | | Home | | | Mixed | | |
|---|---|---|---|---|---|---|---|
| **Species** | **Functional Group** | **1** | **2** | **3** | **1** | **2** | **3** |
| *Andropogon gerardii* | Graminoid | 12 | 12 | 12 | 12 | 12 | 10 |
| *Bouteloua curtipendula* | Graminoid | 12 | 12 | 12 | 12 | 12 | 12 |
| *Panicum virgatum* | Graminoid | 3 | 11 | 12 | 9 | 7 | 8 |
| *Schizachyrium scoparium* | Graminoid | 11 | 10 | 7 | 10 | 10 | 7 |
| *Sorghastrum nutans* | Graminoid | 8 | 11 | 12 | 11 | 10 | 9 |
| *Sporobolus compositus* | Graminoid | 12 | 12 | 12 | 12 | 11 | 12 |
| *Achillea millefolium* | Forb | 8 | 1 | 3 | 10 | 2 | 3 |
| *Helianthus maximiliani* | Forb | 12 | 12 | 9 | 12 | 11 | 10 |
| *Liatris pycnostachya* | Forb | 7 | 7 | 6 | 7 | 8 | 5 |
| *Ratibida pinnata* | Forb | 12 | 11 | 6 | 6 | 4 | 2 |
| *Salvia azurea* | Forb | 5 | 8 | 7 | 10 | 11 | 11 |
| *Solidago rigida* | Forb | 4 | 3 | 2 | 5 | 2 | 2 |
| *Symphyotrichum oblongifolium* | Forb | 10 | 7 | 11 | 7 | 6 | 0 |
| *Baptisia australis* | Legume | 12 | 12 | 10 | 12 | 12 | 12 |
| *Dalea purpurea* | Legume | 12 | 11 | 12 | 11 | 11 | 12 |

Table 1 (continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Desmanthus illinoensis* | Legume | 11 | 12 | 12 | 11 | 12 | 12 |
| *Desmodium canadense* | Legume | 12 | 12 | 12 | 12 | 10 | 12 |
| *Lespedeza capitata* | Legume | 12 | 12 | 12 | 12 | 12 | 12 |
| *Mimosa quadrivalvis* | Legume | 2 | 1 | 6 | 4 | 2 | 3 |

Table 2. Linear regression results for the linear mixed-effects models/generalized linear mixed models for each species, showing the effects of soil fertility (SOIL TYPE), soil conditioning (SOIL CND), and the interaction effect (ST * SC) on biomass. Final model selection was based on Akaike Information Criterion scores.

| **SPECIES** | **SOIL TYPE** | **SOIL CND** | **ST * SC** |
|---|---|---|---|
| *L. capitata* | <0.0001 *** | 0.0012 ** | 0.4018 |
| *D. illinoensis* | <0.0001 *** | 0.0012 ** | 0.9383 |
| *P. virgatum* | <0.0001 *** | 0.0590 . | 0.8659 |
| *B. curtipendula* | <0.0001 *** | 0.2138 | <0.0001 *** |
| *D. canadense* | <0.0001 *** | 0.1178 | 0.0002 *** |
| *A. millefolium* | <0.0001 *** | 0.9808 | 0.0014 ** |
| *B. australis* | <0.0001 *** | <0.0001 *** | 0.0028 ** |
| *S. azurea* | <0.0001 *** | 0.0007 *** | 0.0258 * |
| *S. compositus* | <0.0001 *** | 0.2053 | 0.0265 * |
| *R. pinnata* | <0.0001 *** | 0.3276 | 0.0362 * |
| *S. oblongifolium* | <0.0001 *** | 0.2340 | 0.0373 * |
| *D. purpurea* | 0.0012 ** | 0.0155 * | 0.0603 . |
| *H. maximiliani* | <0.0001 *** | <0.0001 *** | 0.0715 . |
| *S. scoparium* | <0.0001 *** | 0.0707 . | 0.0731 . |
| *A. gerardii* | <0.0001 *** | 0.4660 | 0.4193 |
| *S. nutans* | 0.0002 *** | 0.2105 | 0.9058 |
| *L. pycnostachya* | 0.1274 | 0.7209 | 0.8979 |
| *S. rigida* | 0.5625 | 0.6515 | 0.4899 |

Table 2 (continued)

| | | | |
|---|---|---|---|
| *M. quadrivalvis* | 0.8603 | 0.7210 | 0.1820 |

Table 3. Post-hoc comparisons for biomass models between self-conditioned soil and mixed-conditioned soil for each species and soil type. Soil type represents soil fertility along depth, decreasing in soil fertility as soil depth increases from soil types 1 to 3.

| **Species** | **1** | **2** | **3** |
|---|---|---|---|
| *L. capitata* | 0.0012 ** | 0.0069 ** | 0.1701 |
| *D. illinoensis* | 0.0473 * | 0.0344 * | 0.0938 . |
| *P. virgatum* | 0.4433 | 0.1043 | 0.3328 |
| *B. curtipendula* | 0.2138 | 0.0023 ** | <0.0001 *** |
| *D. canadense* | 0.1178 | 0.3603 | 0.0001 *** |
| *A. millefolium* | 0.9808 | 0.0012 ** | N/A |
| *B. australis* | 0.0001 *** | 0.3963 | 0.0282 * |
| *S. azurea* | 0.0007 *** | 0.0001 *** | 0.7377 |
| *S. compositus* | 0.1424 | 0.0300 * | 0.1526 |
| *R. pinnata* | 0.3276 | 0.0191 * | 0.6718 |
| *S. oblongifolium* | 0.0771 . | 0.2007 | N/A |
| *D. purpurea* | 0.0042 ** | 0.1024 | 0.6993 |
| *H. maximiliani* | <0.0001 *** | <0.0001 *** | 0.0013 ** |
| *S. scoparium* | 0.8515 | 0.7665 | 0.0086 ** |
| *A. gerardii* | 0.6183 | 0.1735 | 0.6938 |
| *S. nutans* | 0.4113 | 0.6998 | 0.3386 |
| *L. pycnostachya* | 0.7209 | 0.9448 | 0.6141 |
| *S. rigida* | 0.6515 | 0.3304 | 0.4934 |
| *M. quadrivalvis* | 0.7318 | 0.9440 | 0.1041 |

Table 4. Post-hoc comparisons for biomass models among soil types for self-conditioned soil for each species. Soil type represents soil fertility along depth, decreasing in soil fertility as soil depth increases from soil types 1 to 3.

| **Species** | **1 vs 2** | **1 vs 3** | **2 vs 3** |
|---|---|---|---|
| *L. capitata* | <0.0001 *** | 0.1396 | 0.0061 ** |
| *D. illinoensis* | 0.0003 *** | 0.0020 ** | 0.8274 |
| *P. virgatum* | 0.1783 | 0.0139 * | 0.1619 |
| *B. curtipendula* | <0.0001 *** | <0.0001 *** | <0.0001 *** |
| *D. canadense* | <0.0001 *** | <0.0001 *** | <0.0001 *** |
| *A. millefolium* | <0.0001 *** | N/A | N/A |
| *B. australis* | 0.8202 | 0.0002 *** | <0.0001 *** |
| *S. azurea* | 0.0001 *** | <0.0001 *** | 0.0039 ** |
| *S. compositus* | <0.0001 *** | <0.0001 *** | 0.2743 |
| *R. pinnata* | 0.0001 *** | 0.0066 ** | 0.8503 |
| *S. oblongifolium* | 0.0188 * | 0.0001 *** | 0.0270 * |
| *D. purpurea* | 0.7058 | 0.5869 | 0.9844 |
| *H. maximiliani* | 0.0326 * | <0.0001 *** | 0.0700 . |
| *S. scoparium* | 0.0001 *** | <0.0001 *** | 0.0002 *** |
| *A. gerardii* | <0.0001 *** | <0.0001 *** | 0.0464 * |
| *S. nutans* | 0.8307 | 0.1016 | 0.1940 |
| *L. pycnostachya* | 0.4312 | 0.1792 | 0.6482 |
| *S. rigida* | 0.7944 | 0.6072 | 0.8741 |
| *M. quadrivalvis* | 0.8892 | 0.8883 | 0.9765 |

Table 5. Post-hoc comparisons for biomass models among soil types for mixed-conditioned soil for each species. Soil type represents soil fertility along depth, decreasing in soil fertility as soil depth increases from soil types 1 to 3.

| **Species** | **1 vs 2** | **1 vs 3** | **2 vs 3** |
|---|---|---|---|
| *L. capitata* | <0.0001 *** | 0.0006 *** | 0.1857 |
| *D. illinoensis* | 0.0004 *** | 0.0005 *** | 0.9912 |
| *P. virgatum* | 0.0894 . | 0.0001 *** | 0.0851 . |
| *B. curtipendula* | <0.0001 *** | <0.0001 *** | 0.8726 |
| *D. canadense* | 0.0001 *** | <0.0001 *** | 0.5753 |
| *A. millefolium* | <0.0001 *** | N/A | N/A |
| *B. australis* | 0.0001 *** | <0.0001 *** | 0.1560 |
| *S. azurea* | <0.0001 *** | <0.0001 *** | 0.8405 |
| *S. compositus* | <0.0001 *** | <0.0001 *** | 0.0600 . |
| *R. pinnata* | 0.8076 | 0.1124 | 0.1824 |
| *S. oblongifolium* | 0.9819 | N/A | N/A |
| *D. purpurea* | 0.2037 | 0.0002 *** | 0.3901 |
| *H. maximiliani* | <0.0001 *** | 0.0003 *** | 0.8043 |
| *S. scoparium* | 0.0004 *** | <0.0001 *** | 0.1877 |
| *A. gerardii* | 0.0052 ** | <0.0001 *** | 0.0056 ** |
| *S. nutans* | 0.5115 | 0.1511 | 0.4259 |
| *L. pycnostachya* | 0.2272 | 0.2776 | 0.9485 |
| *S. rigida* | 0.6562 | 0.9994 | 0.8020 |
| *M. quadrivalvis* | 0.5659 | 0.0966 . | 0.4461 |

Table 6. Table showing percent reduction of biomass across reduced soil fertility for two species

| Species | Mean Biomass (g) Soil Type 1 | Mean Biomass (g) Soil Type 2 | Mean Biomass (g) Soil Type 3 | Percent Reduction Soil Type 1 – Soil Type 2 | Percent Reduction Soil Type 1 – Soil Type 3 |
|---|---|---|---|---|---|
| *A. gerardii* | 3.4520 | 2.1671 | 1.2751 | 37.22 | 63.06 |
| *S. nutans* | 3.9372 | 3.2049 | 1.9962 | 18.60 | 49.30 |

Table 7. Results of the one-sample t-test, showing significance values for each species and soil type to test whether average net plant-soil feedback was significantly different from zero. Soil type represents soil fertility along depth, decreasing in soil fertility as soil depth increases from soil types 1 to 3.

| **Species** | **1** | **2** | **3** |
|---|---|---|---|
| *B. curtipendula* | 0.0138 * | <0.0001 *** | <0.0001 *** |
| *H. maximiliani* | <0.0001 *** | <0.0001 *** | 0.0150 * |
| *D. canadense* | 0.0019 ** | 0.0036 ** | <0.0001 *** |
| *L. capitata* | 0.0454 * | 0.0043 ** | 0.0013 ** |
| *S. compositus* | 0.0617 . | 0.0009 *** | 0.0228 * |
| *D. illinoensis* | 0.0017 ** | 0.0211 * | 0.0841 . |
| *S. azurea* | 0.0443 * | 0.0508 . | 0.0289 * |
| *B. australis* | 0.0003 *** | 0.6269 | <0.0001 *** |
| *D. purpurea* | 0.0004 *** | 0.0464 * | 0.851 |
| *S. oblongifolium* | 0.0005 *** | 0.0026 ** | N/A |
| *S. nutans* | <0.0001 *** | 0.9143 | 0.2611 |
| *S. scoparium* | 0.2798 | 0.7421 | 0.0003 *** |
| *R. pinnata* | 0.0208 * | 0.8422 | N/A |
| *M. quadrivalvis* | 0.3429 | N/A | 0.0230 * |
| *A. millefolium* | 0.5148 | 0.0543 . | N/A |
| *S. rigida* | 0.4153 | 0.0585 . | 0.2717 |
| *L. pycnostachya* | 0.5638 | 0.1923 | 0.1021 |
| *P. virgatum* | 0.3921 | 0.2029 | 0.211 |

Table 7 (continued)

| | | | |
|---|---|---|---|
| *A. gerardii* | 0.5482 | 0.6122 | 0.7958 |

Table 8. Linear regression results for each species, showing the effects of soil fertility (Soil Type) on a calculated plant-soil feedback metric, calculated as log (biomass when grown on self-conditioned soil/biomass when grown on mixed-conditioned soil). Final model selection was based on Akaike Information Criterion scores.

| **Species** | **Soil Type** |
|---|---|
| *B. curtipendula* | <0.0001 *** |
| *D. canadense* | <0.0001 *** |
| *A. millefolium* | <0.0001 *** |
| *B. australis* | <0.0001 *** |
| *S. oblongifolium* | <0.0001 *** |
| *S. scoparium* | <0.0001 *** |
| *S. nutans* | <0.0001 *** |
| *S. compositus* | 0.0029 ** |
| *S. azurea* | 0.0037 ** |
| *H. maximiliani* | 0.0208 * |
| *M. quadrivalvis* | 0.0295 * |
| *D. purpurea* | 0.1337 |
| *L. pycnostachya* | 0.2862 |
| *S. rigida* | 0.3162 |
| *P. virgatum* | 0.3503 |
| *R. pinnata* | 0.3885 |
| *L. capitata* | 0.4079 |
| *A. gerardii* | 0.7420 |

Table 8 (continued)

| | |
|---|---|
| *D. illinoensis* | 0.7898 |

Table 9. Post-hoc comparisons for plant-soil feedback models among soil types for each species. Soil type represents soil fertility along depth, decreasing in soil fertility as soil depth increases from soil types 1 to 3.

| **Species** | **1 vs 2** | **1 vs 3** | **2 vs 3** |
|---|---|---|---|
| *B. curtipendula* | <0.0001 *** | <0.0001 *** | <0.0001 *** |
| *D. canadense* | 0.9381 | <0.0001 *** | <0.0001 *** |
| *A. millefolium* | 0.0031 ** | N/A | N/A |
| *B. australis* | 0.0006 *** | 0.5347 | 0.0177 * |
| *S. oblongifolium* | <0.0001 *** | N/A | N/A |
| *S. scoparium* | 0.6664 | <0.0001 *** | <0.0001 *** |
| *S. nutans* | 0.0004 *** | 0.0001 *** | 0.6889 |
| *S. compositus* | 0.0947 . | 0.0020 ** | 0.3021 |
| *S. azurea* | 0.5155 | 0.1779 | 0.0109 * |
| *H. maximiliani* | 0.1235 | 0.5654 | 0.0191 * |
| *M. quadrivalvis* | 0.9999 | 0.0352 * | 0.0654 . |
| *D. purpurea* | 0.9995 | 0.2061 | 0.2342 |
| *L. pycnostachya* | 0.3204 | 0.6368 | 0.9136 |
| *S. rigida* | 0.5520 | 0.5047 | 0.9951 |
| *P. virgatum* | 0.7379 | 0.3783 | 0.6902 |
| *R. pinnata* | 0.4212 | 0.6481 | 0.9926 |
| *L. capitata* | 0.9985 | 0.4916 | 0.4611 |
| *A. gerardii* | 0.7943 | 0.9977 | 0.7745 |
| *D. illinoensis* | 0.9688 | 0.7809 | 0.8931 |

**FIGURES**

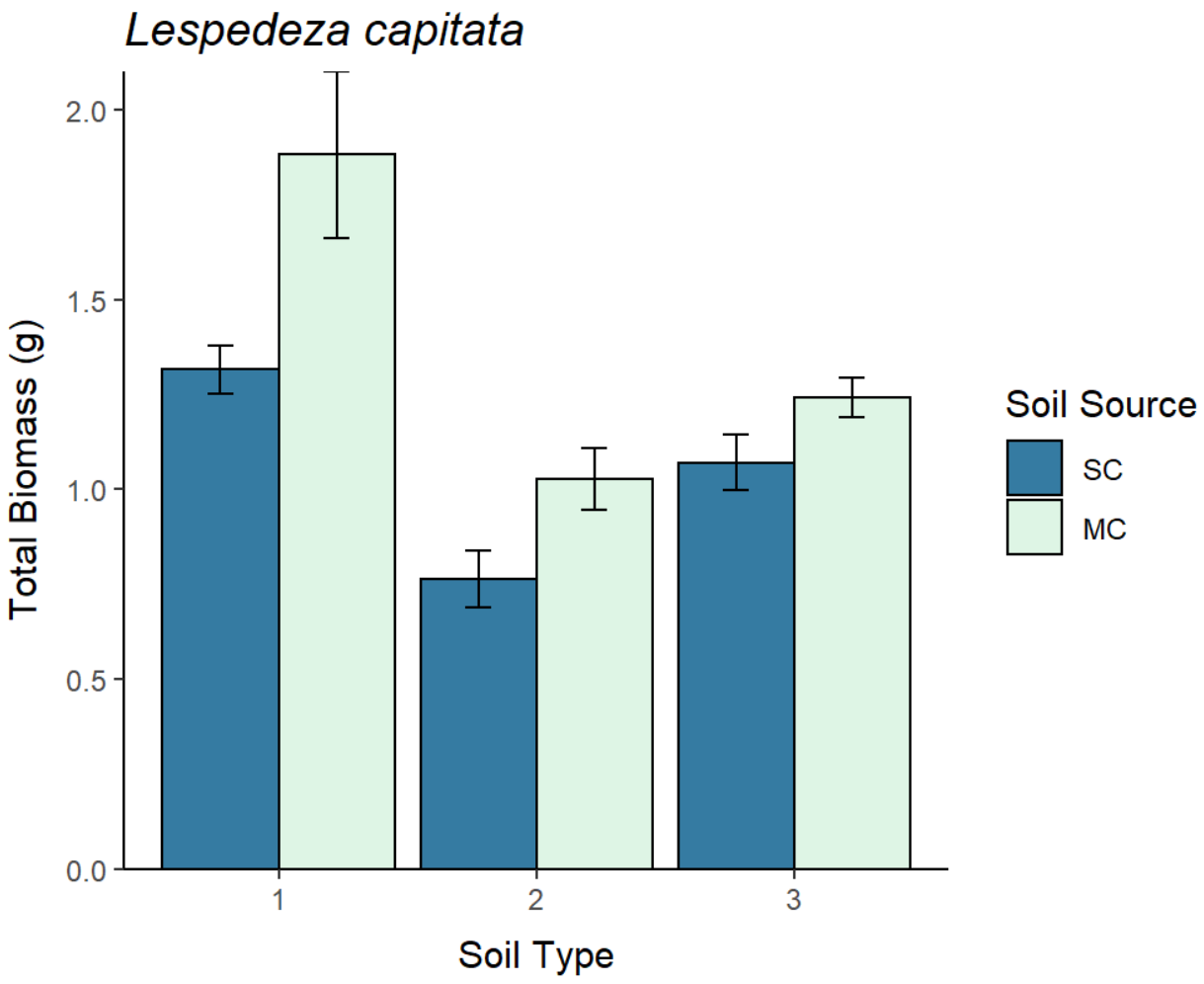


Figure 1. Average total biomass (mean ± 1 SE) of *L. capitata* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

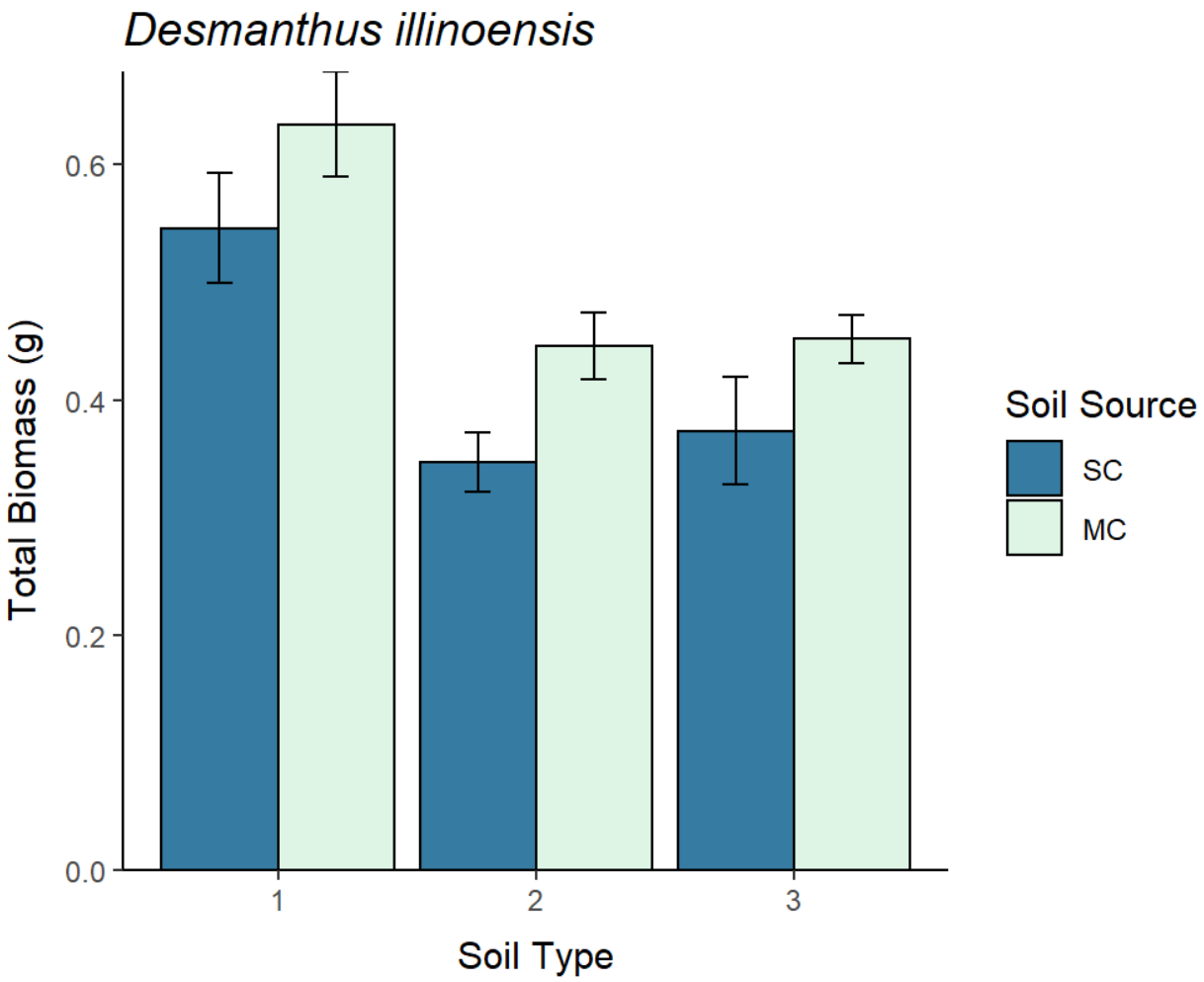


Figure 2. Average total biomass (mean ± 1 SE) of *D. illinoensis* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

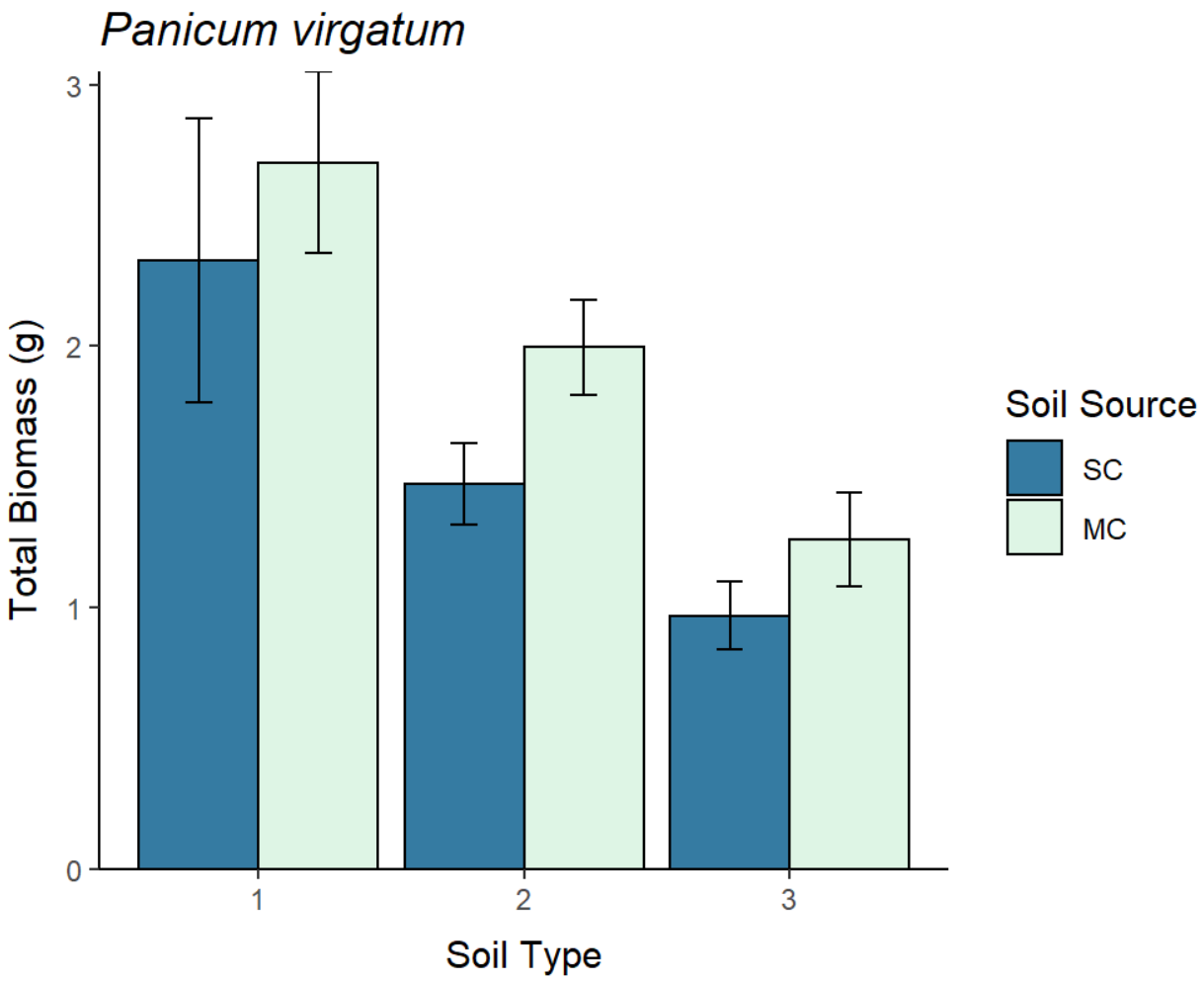


Figure 3. Average total biomass (mean ± 1 SE) of *P. virgatum* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

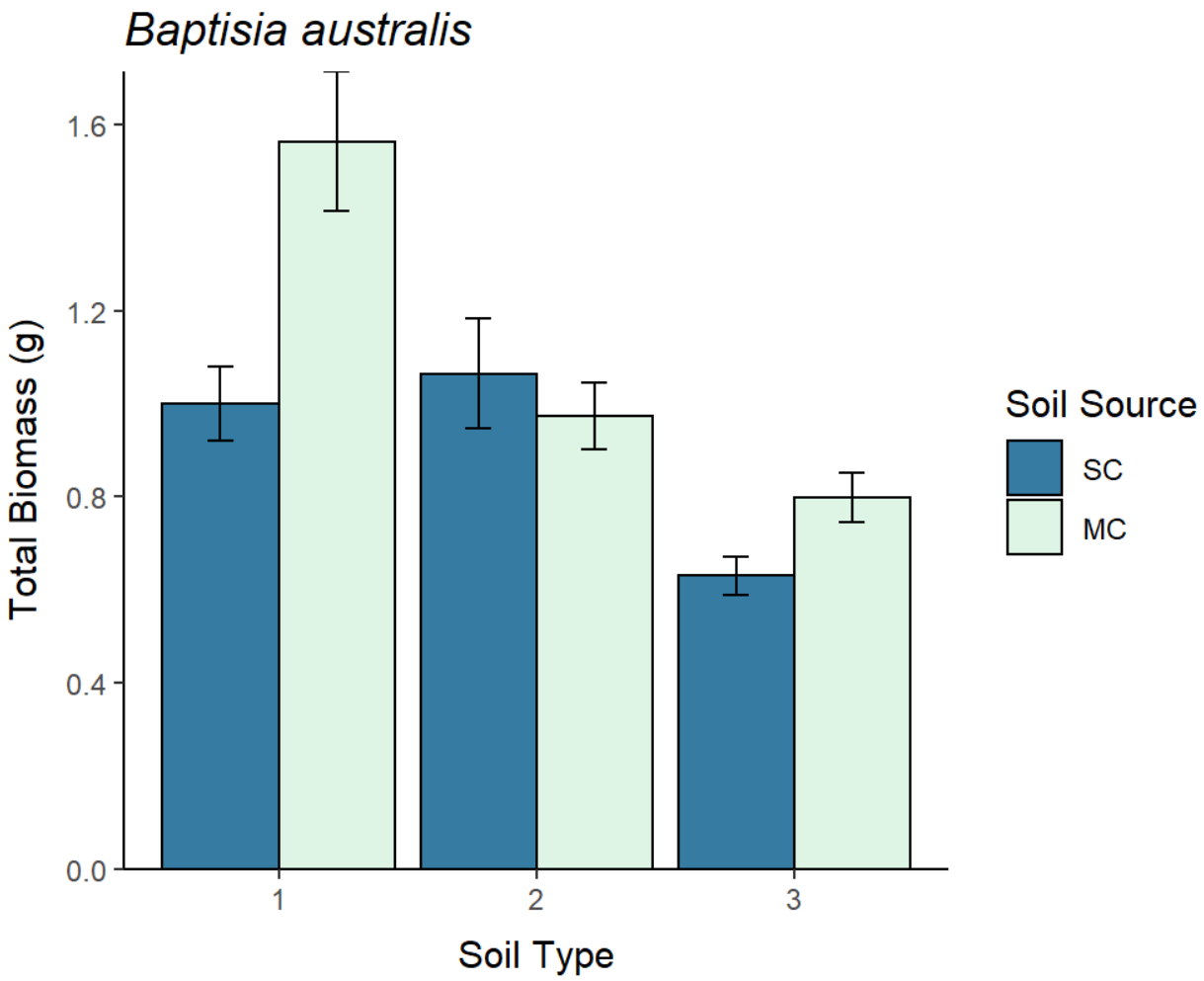


Figure 4. Average total biomass (mean ± 1 SE) of *B. australis* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

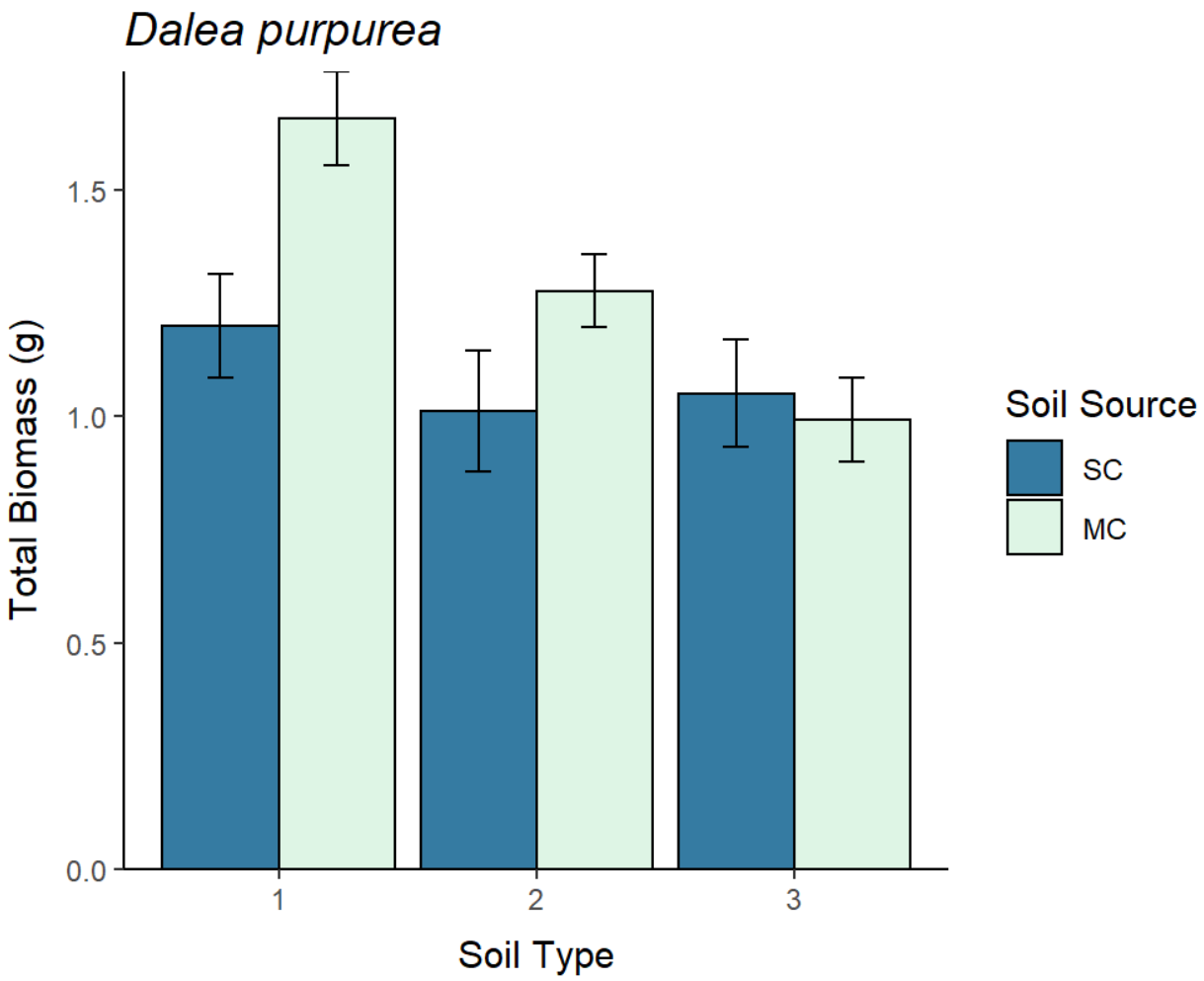


Figure 5. Average total biomass (mean ± 1 SE) of *D. purpurea* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

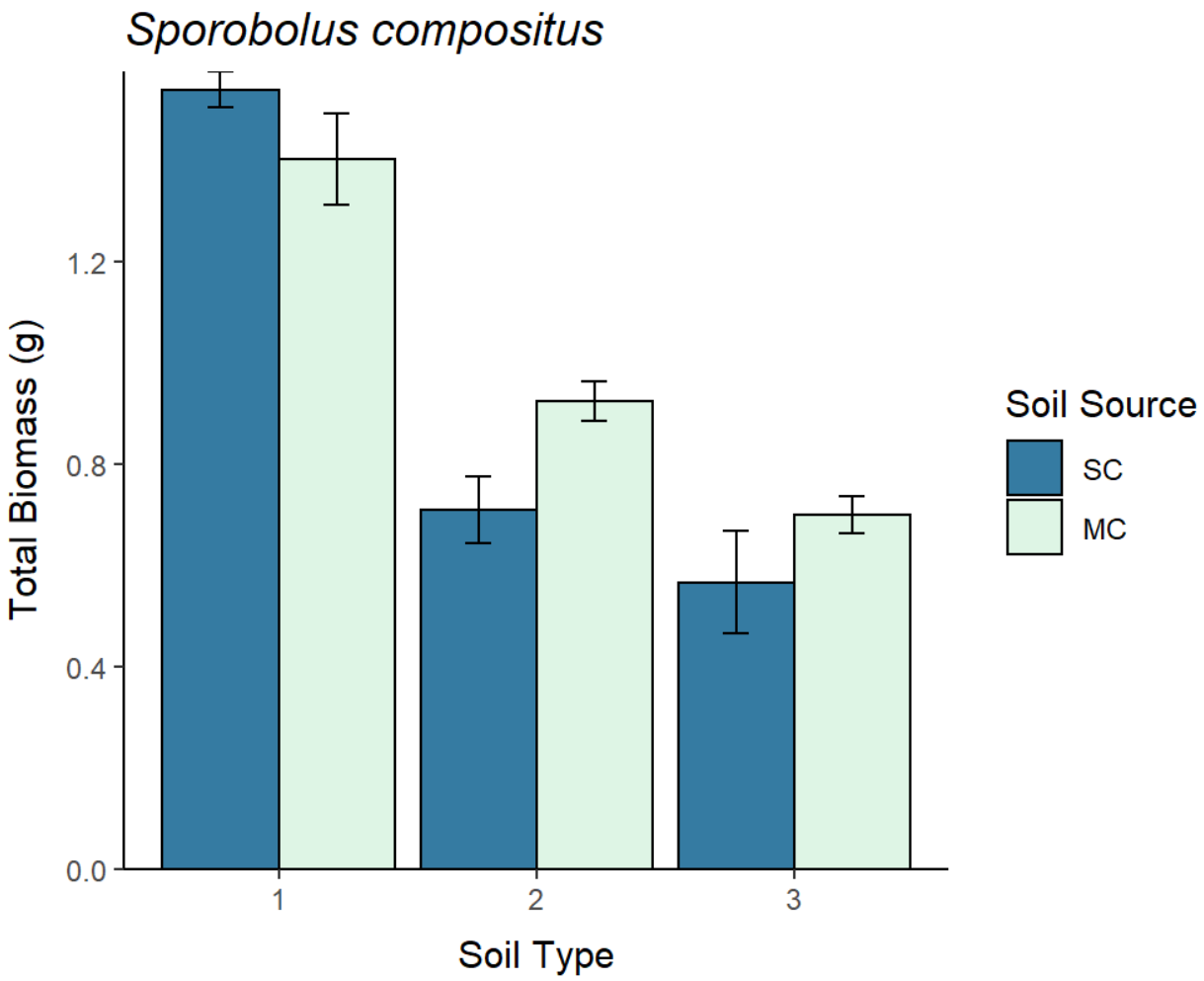


Figure 6. Average total biomass (mean ± 1 SE) of *S. compositus* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

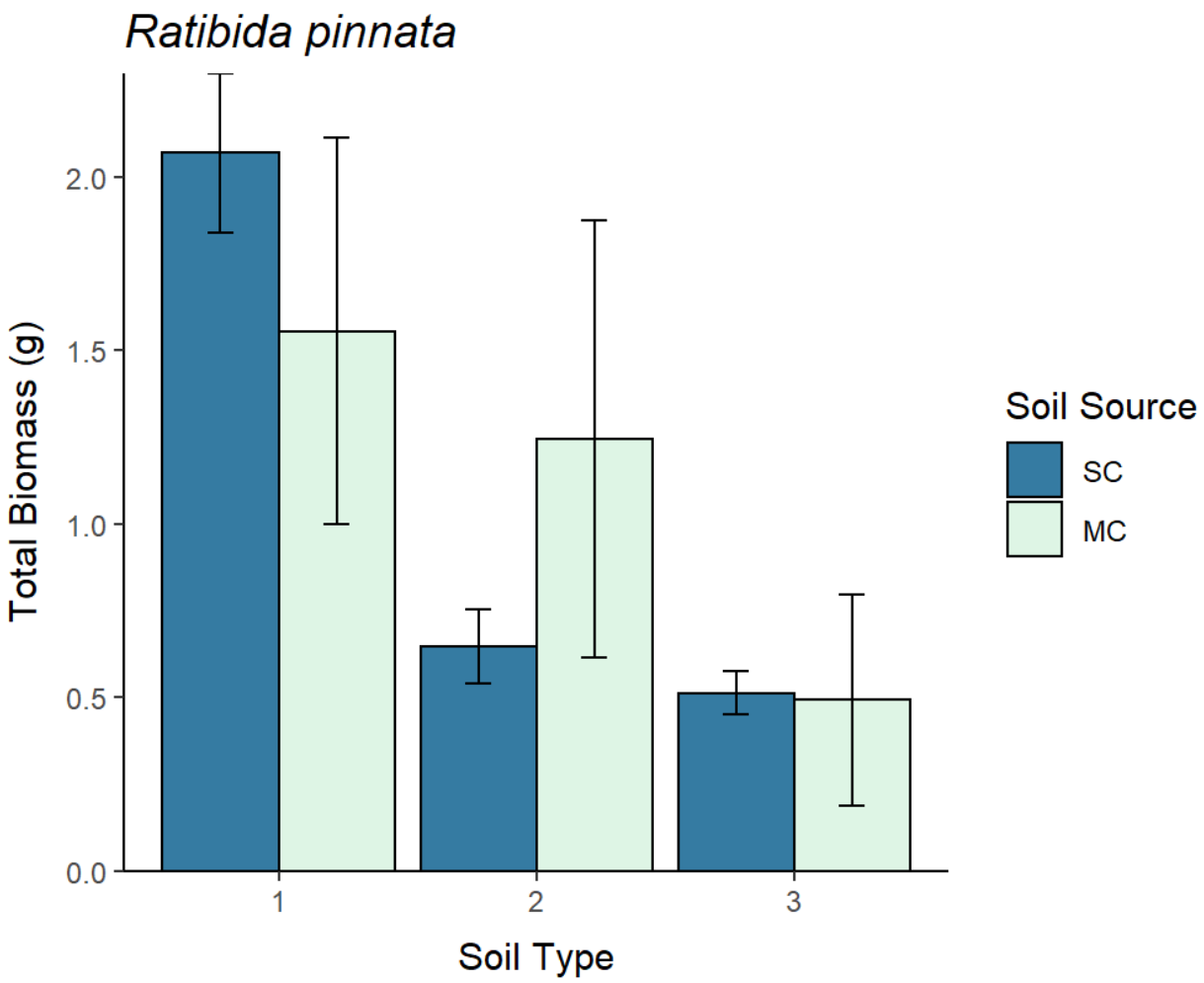


Figure 7. Average total biomass (mean ± 1 SE) of *R. pinnata* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

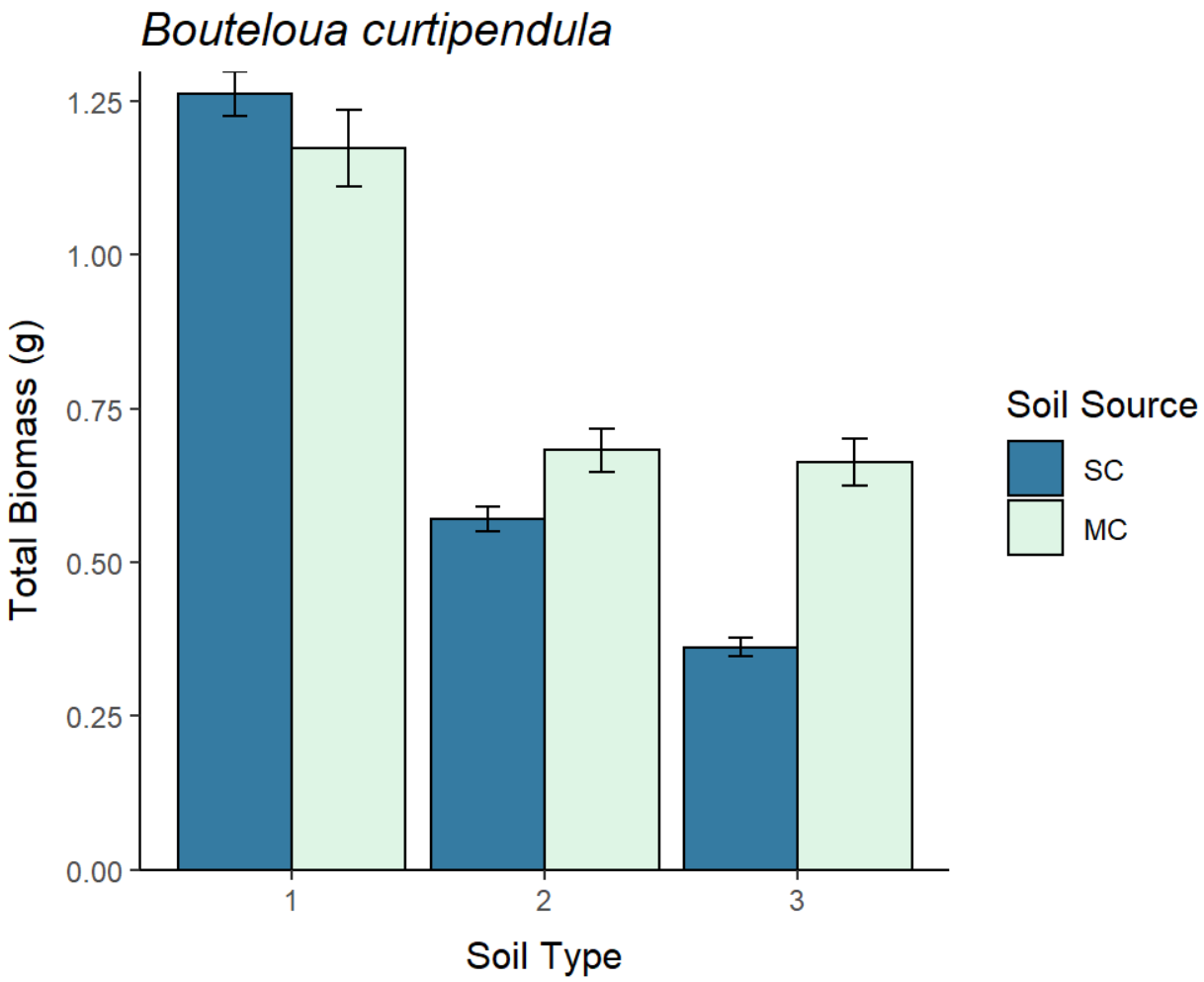


Figure 8. Average total biomass (mean ± 1 SE) of *B. curtipendula* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

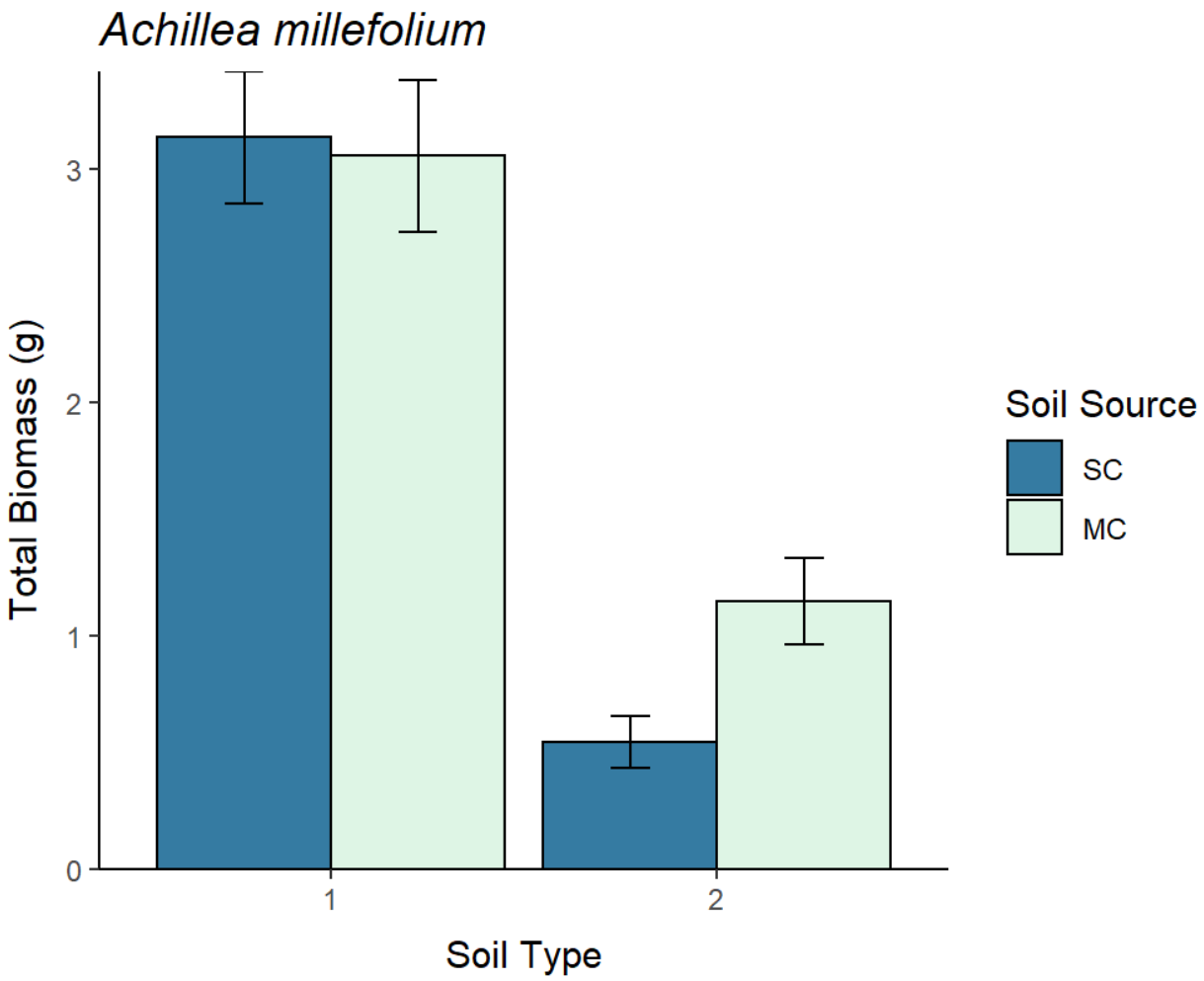


Figure 9. Average total biomass (mean ± 1 SE) of *A. millefolium* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the two levels of soil fertility. Because of the low number of successful replicates, biomass data from soil fertility levels 2 and 3 were pooled.

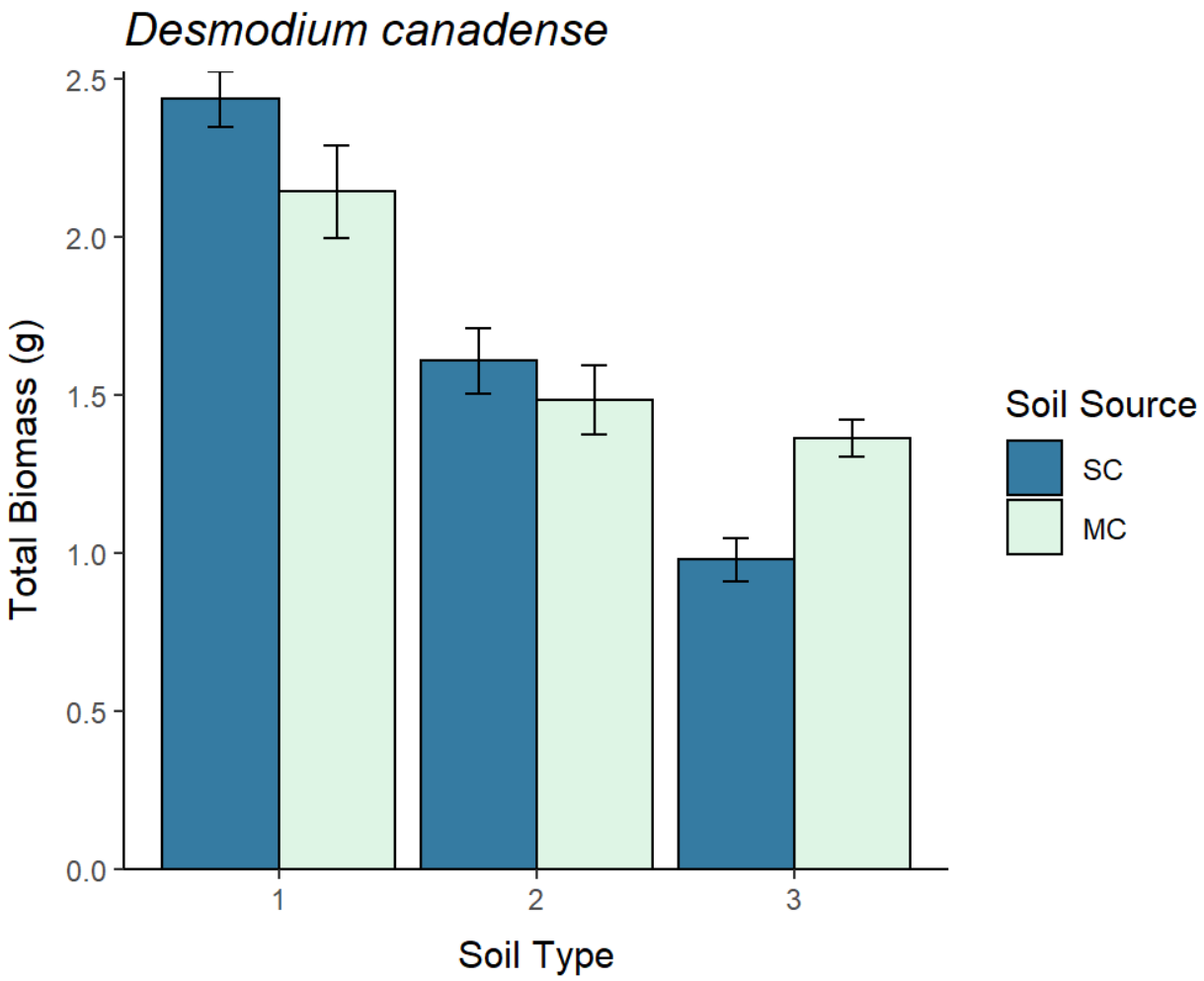


Figure 10. Average total biomass (mean ± 1 SE) of *D. canadense* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

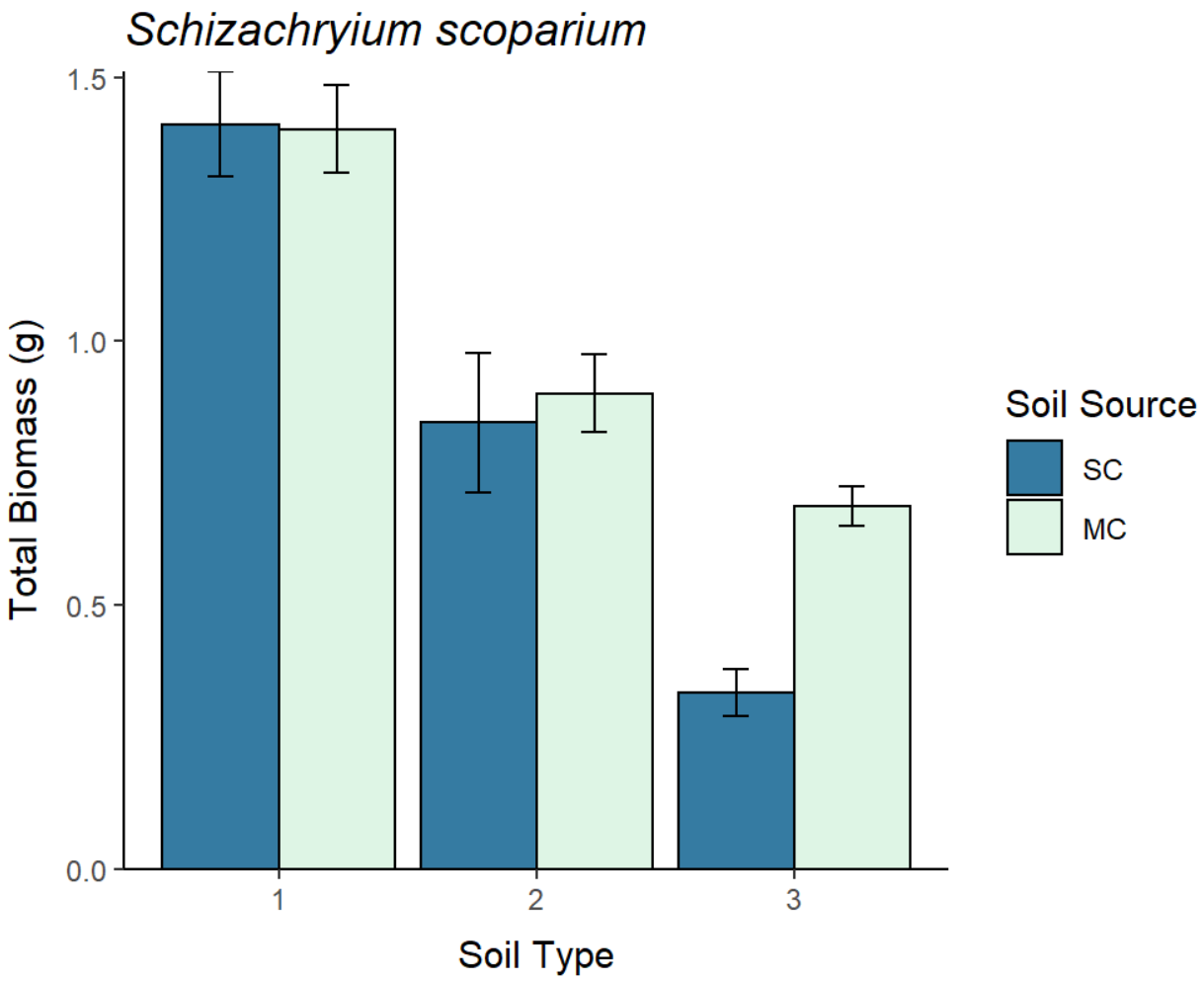


Figure 11. Average total biomass (mean ± 1 SE) of *S. scoparium* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

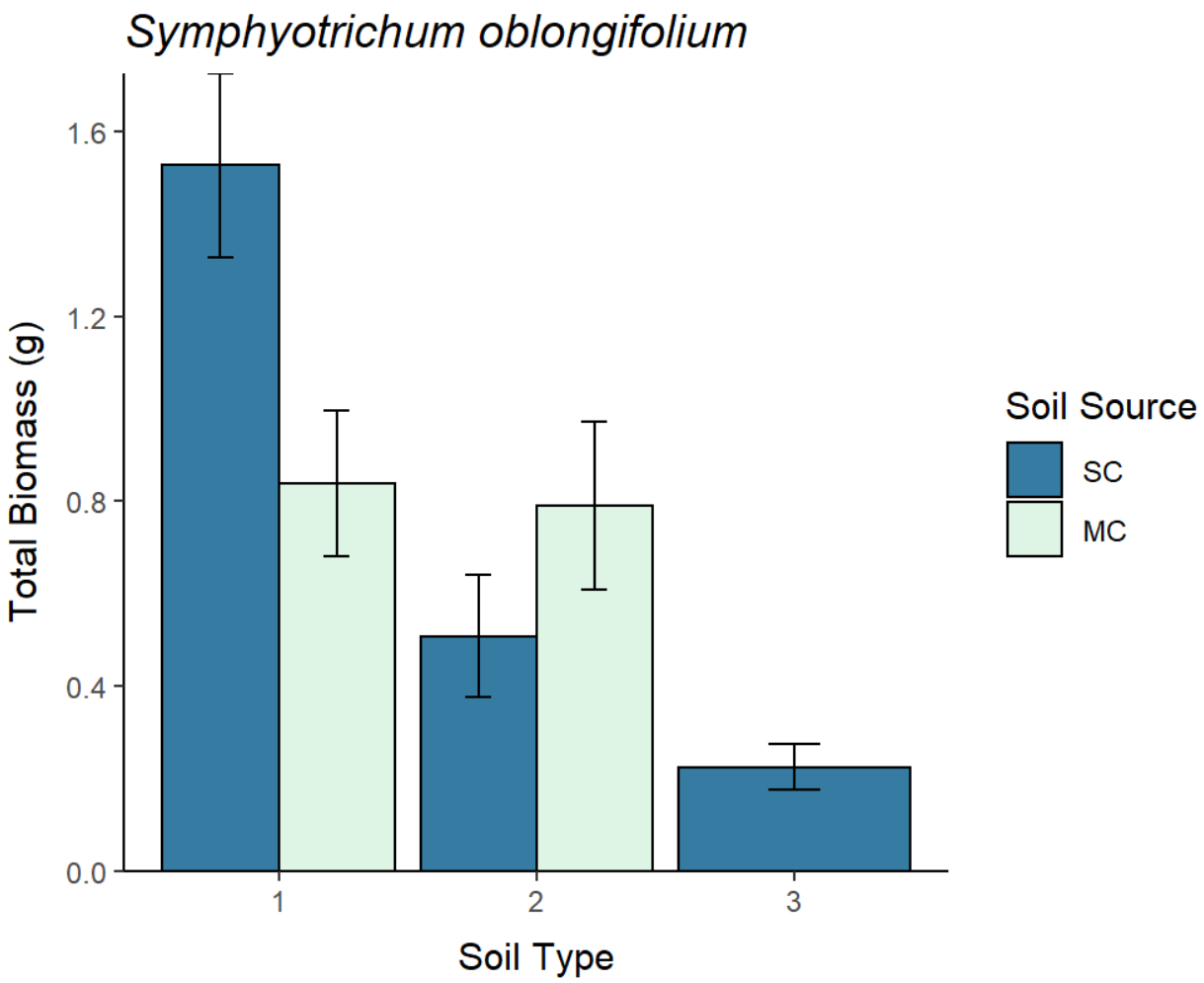


Figure 12. Average total biomass (mean ± 1 SE) of *S. oblongifolium* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil. There were no successful replicates in MC soil for soil fertility level 3.

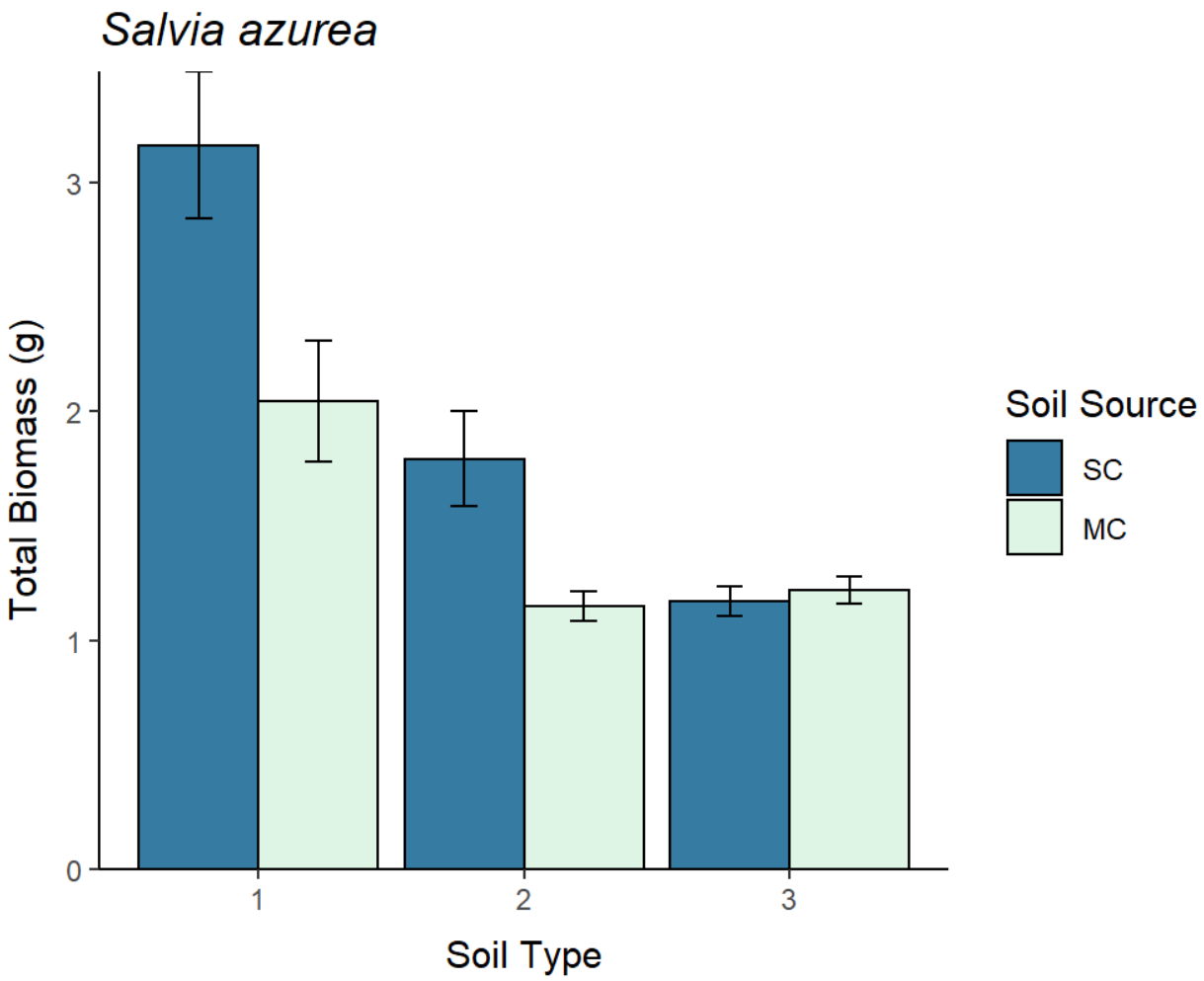


Figure 13. Average total biomass (mean ± 1 SE) of *S. azurea* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

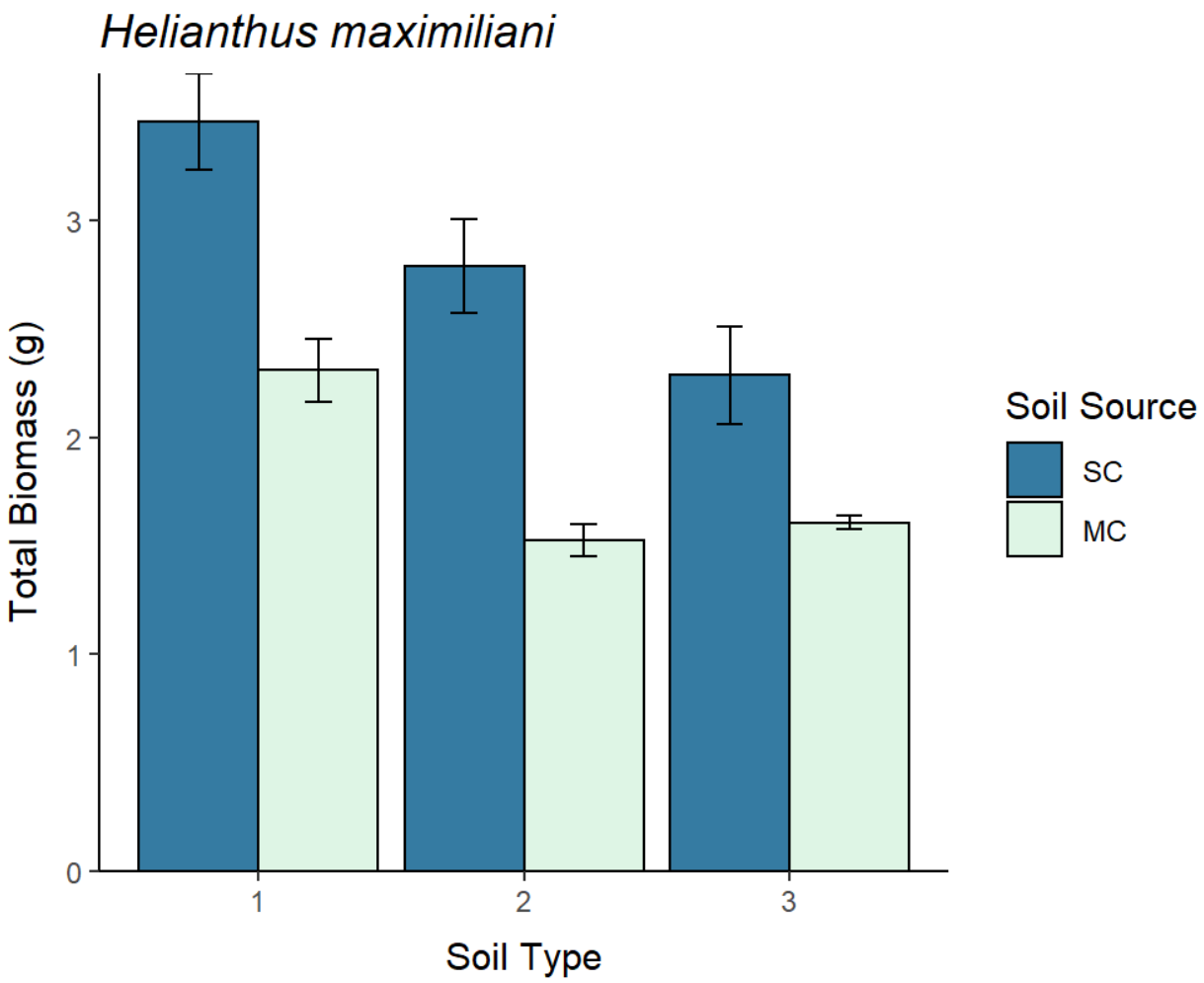


Figure 14. Average total biomass (mean ± 1 SE) of *H. maximiliani* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

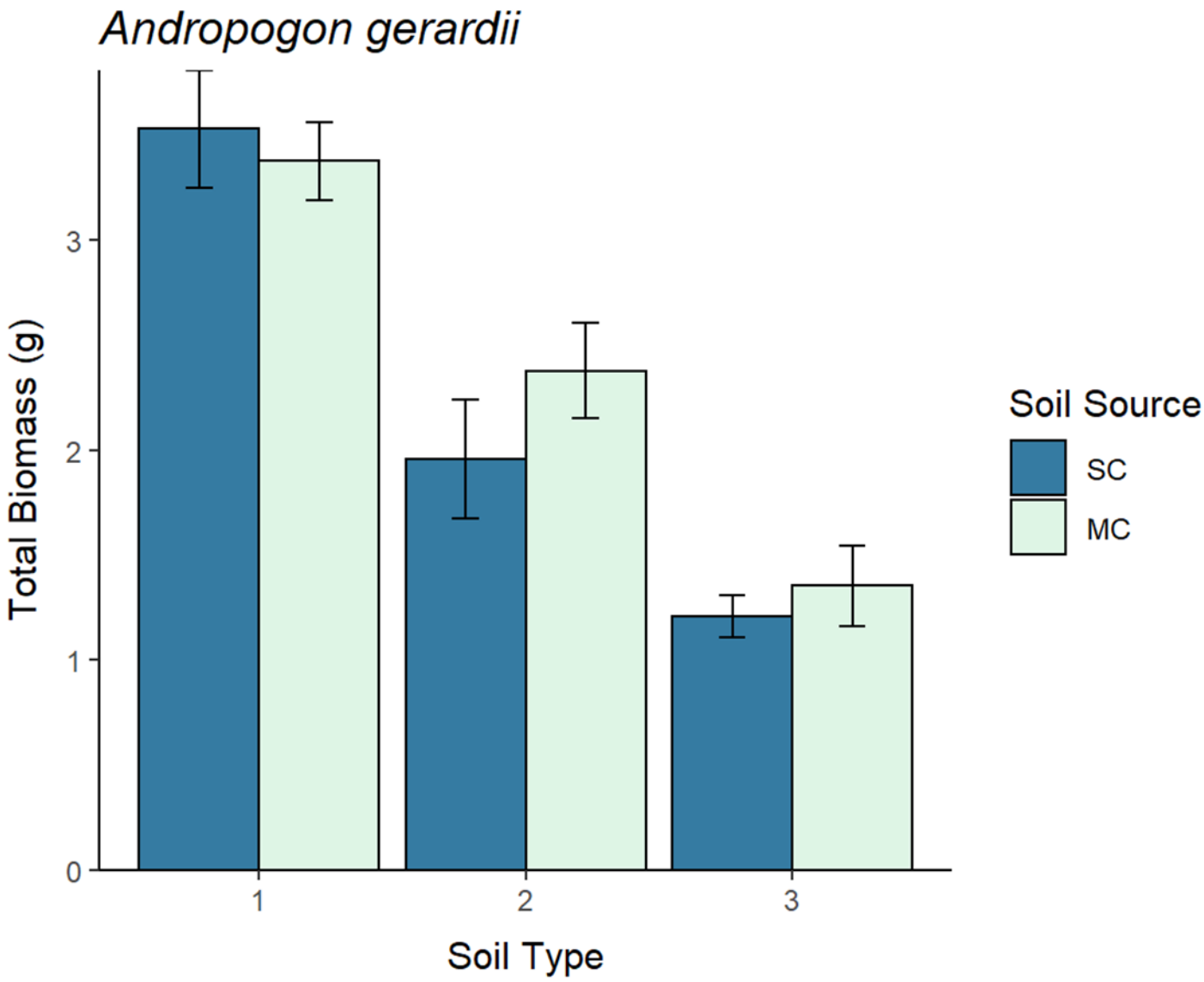


Figure 15. Average total biomass (mean ± 1 SE) of *A. gerardii* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

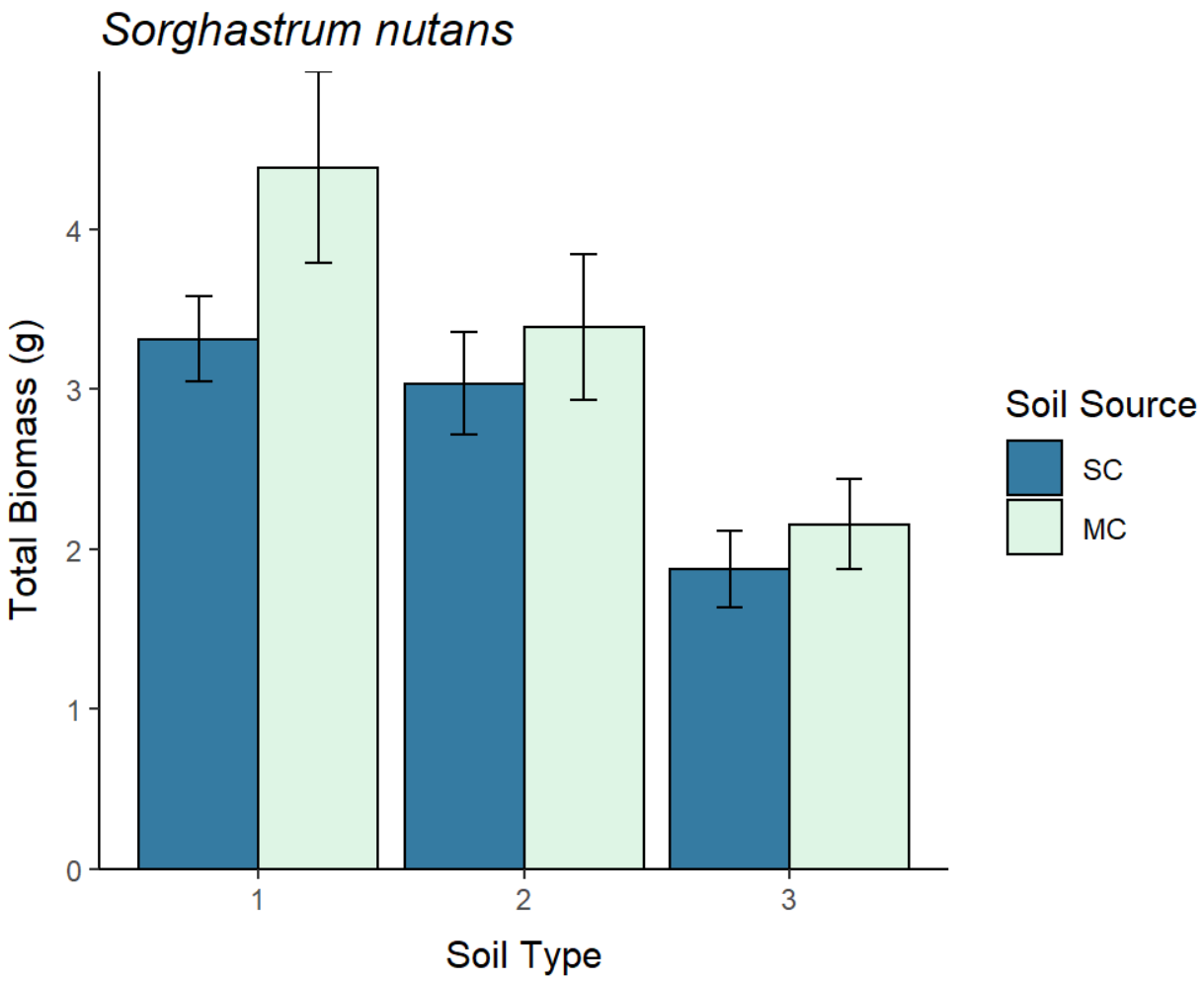


Figure 16. Average total biomass (mean ± 1 SE) of *S. nutans* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

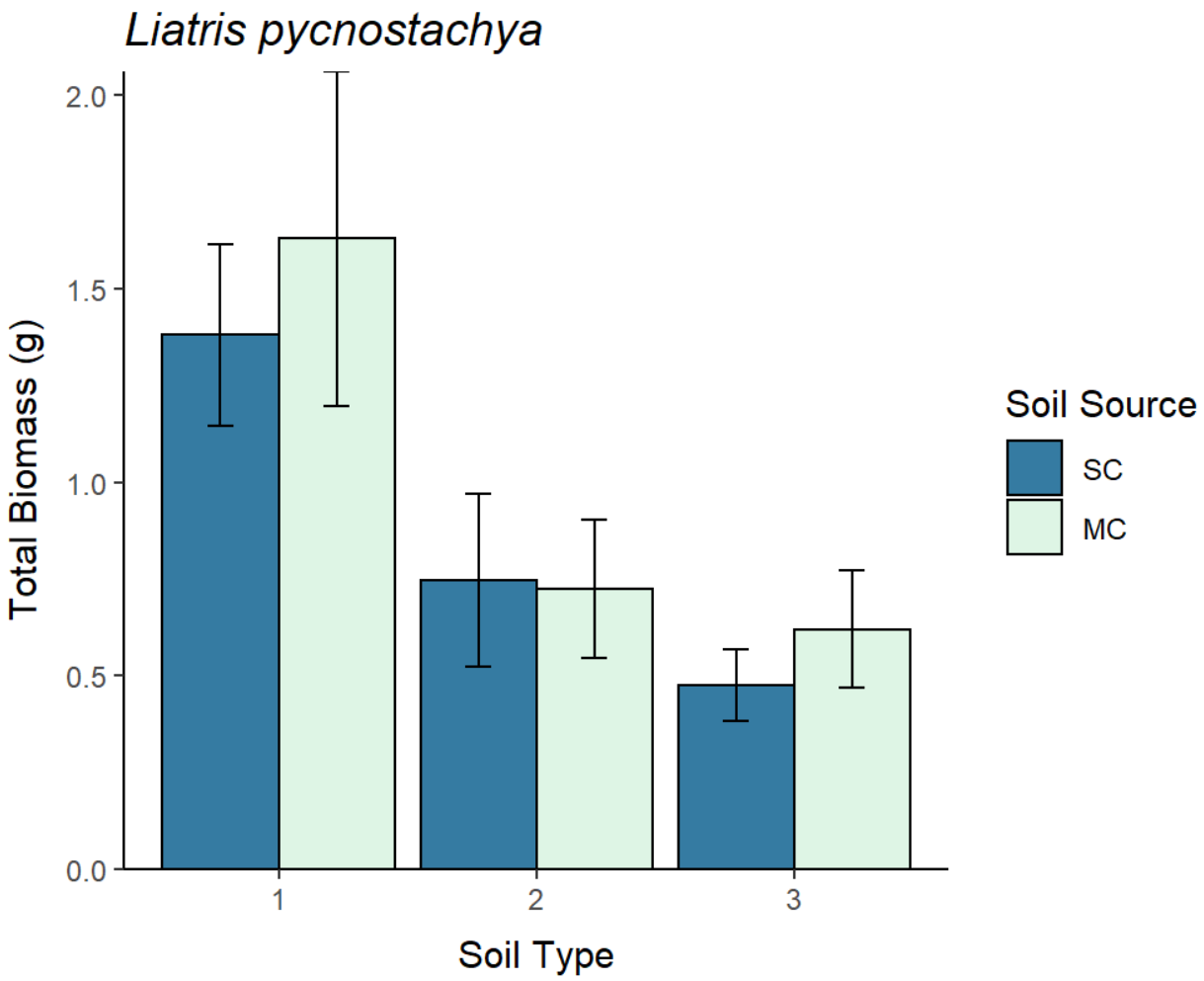


Figure 17. Average total biomass (mean ± 1 SE) of *L. pycnostachya* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three levels of soil fertility. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

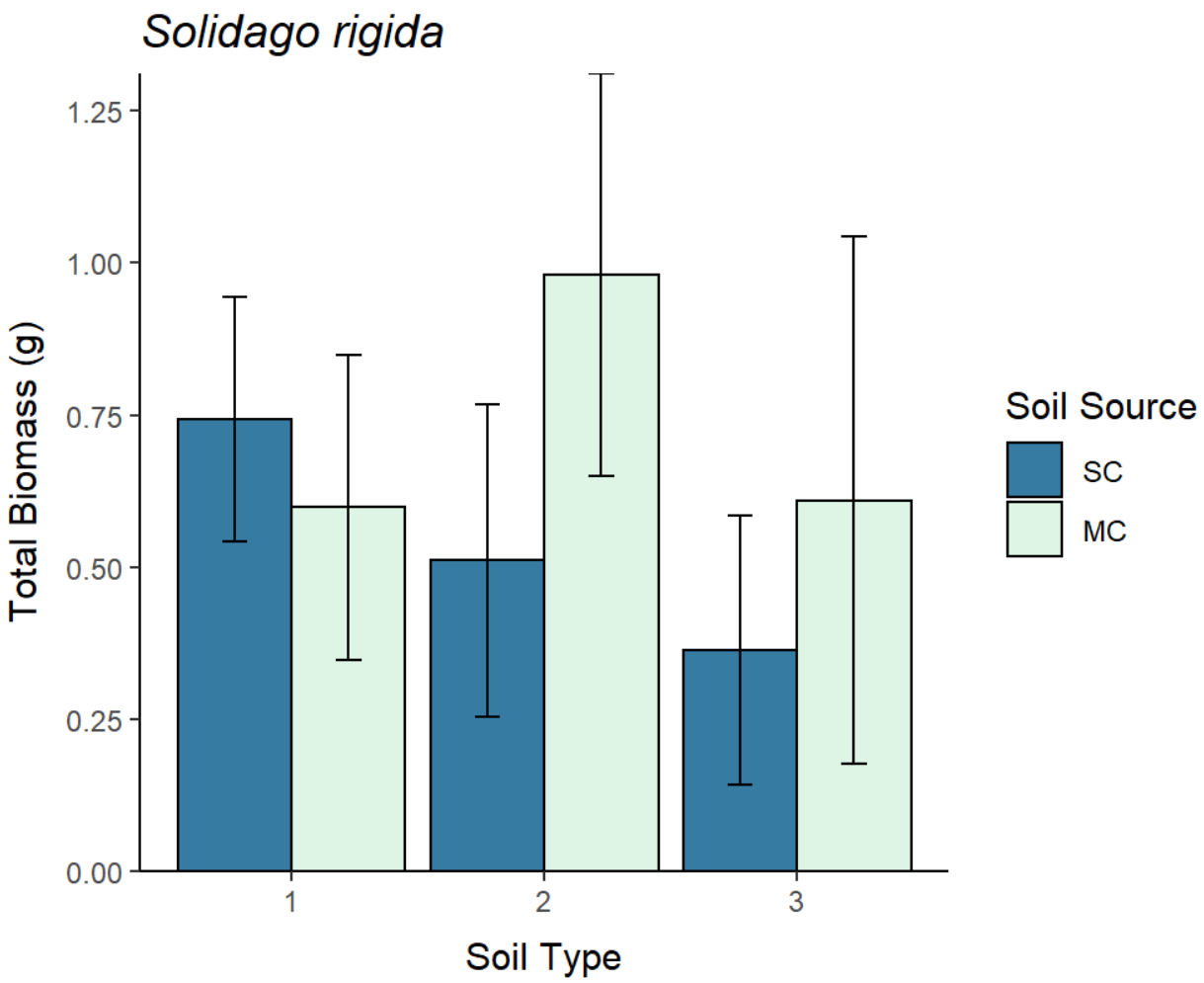


Figure 18. Average total biomass (mean ± 1 SE) of *S. rigida* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three levels of soil fertility. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

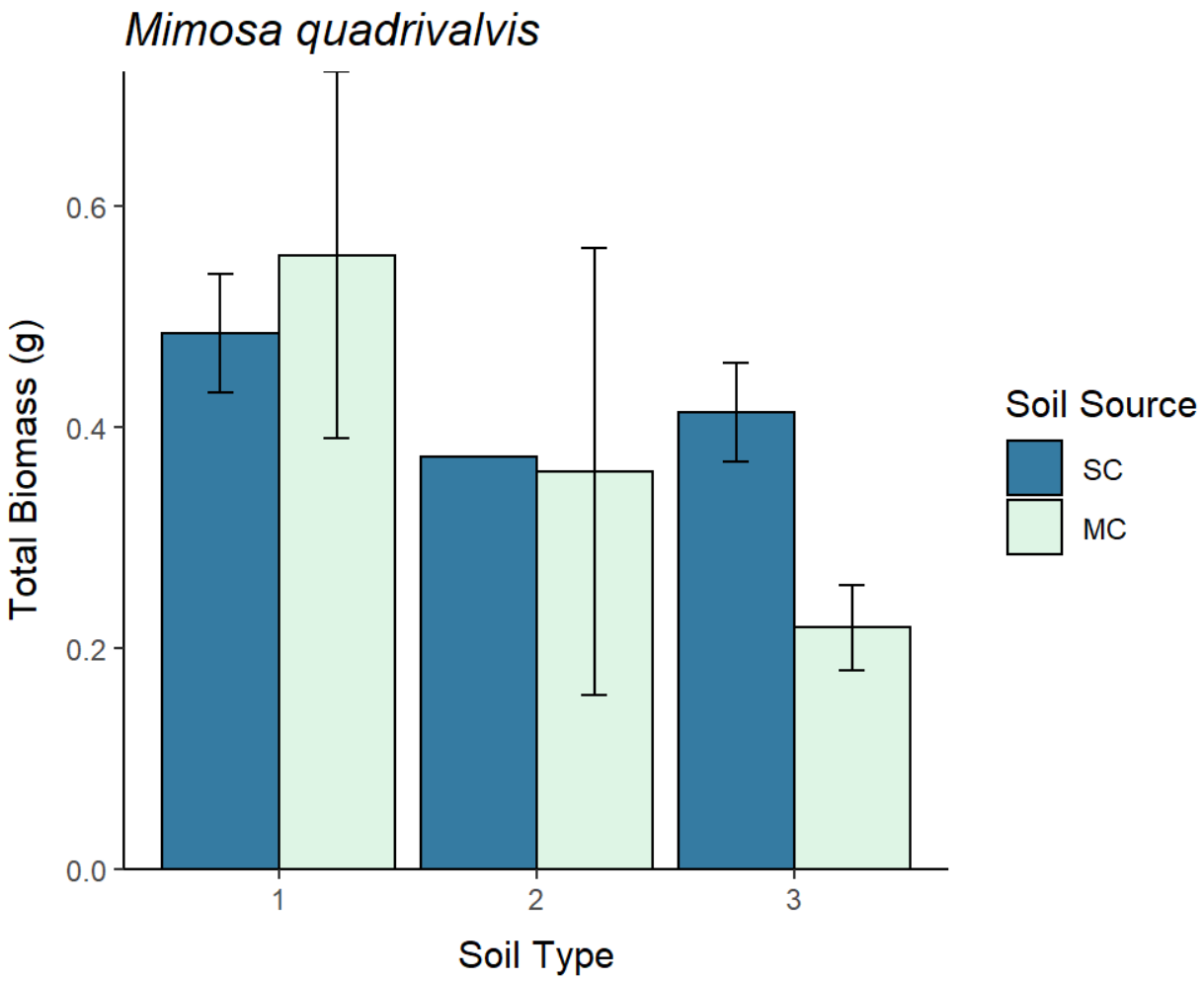


Figure 19. Average total biomass (mean ± 1 SE) of *M. quadrivalvis* grown in self-conditioned (SC) soil or in mixed-conditioned (MC) in relation to the three levels of soil fertility. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil.

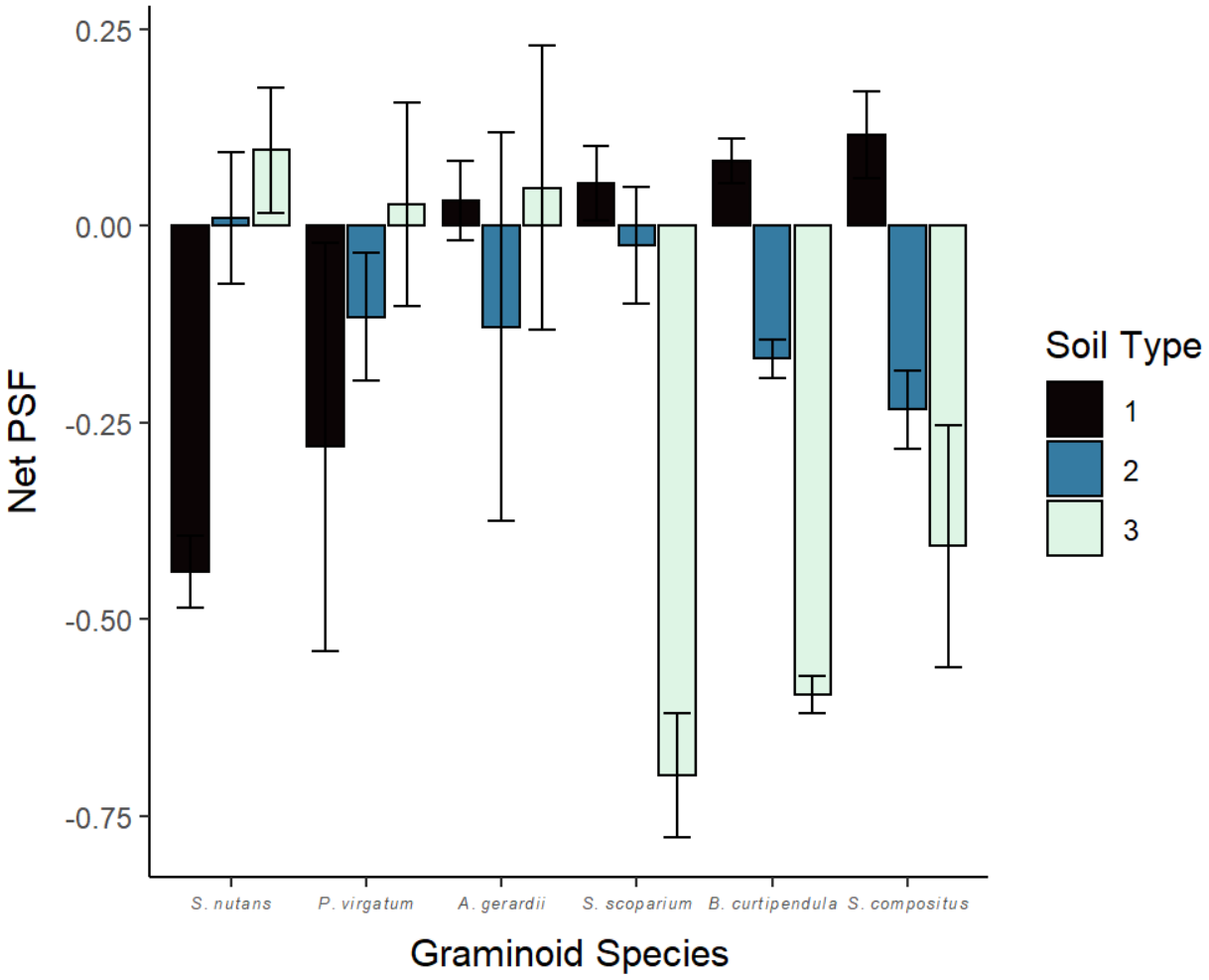


Figure 20. Average net plant-soil feedback (1 ± SE) of the graminoid species in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil. Net plant-soil feedback was calculated as log (biomass when grown in self-conditioned soil / biomass when grown in mixed-conditioned soil). A positive feedback value indicates that plants produced more biomass when grown in self-conditioned soil compared to mixed-conditioned soil, with a negative feedback value indicating the reverse. A feedback value of zero indicates that no difference in biomass was produced between the two soil conditioning treatments.

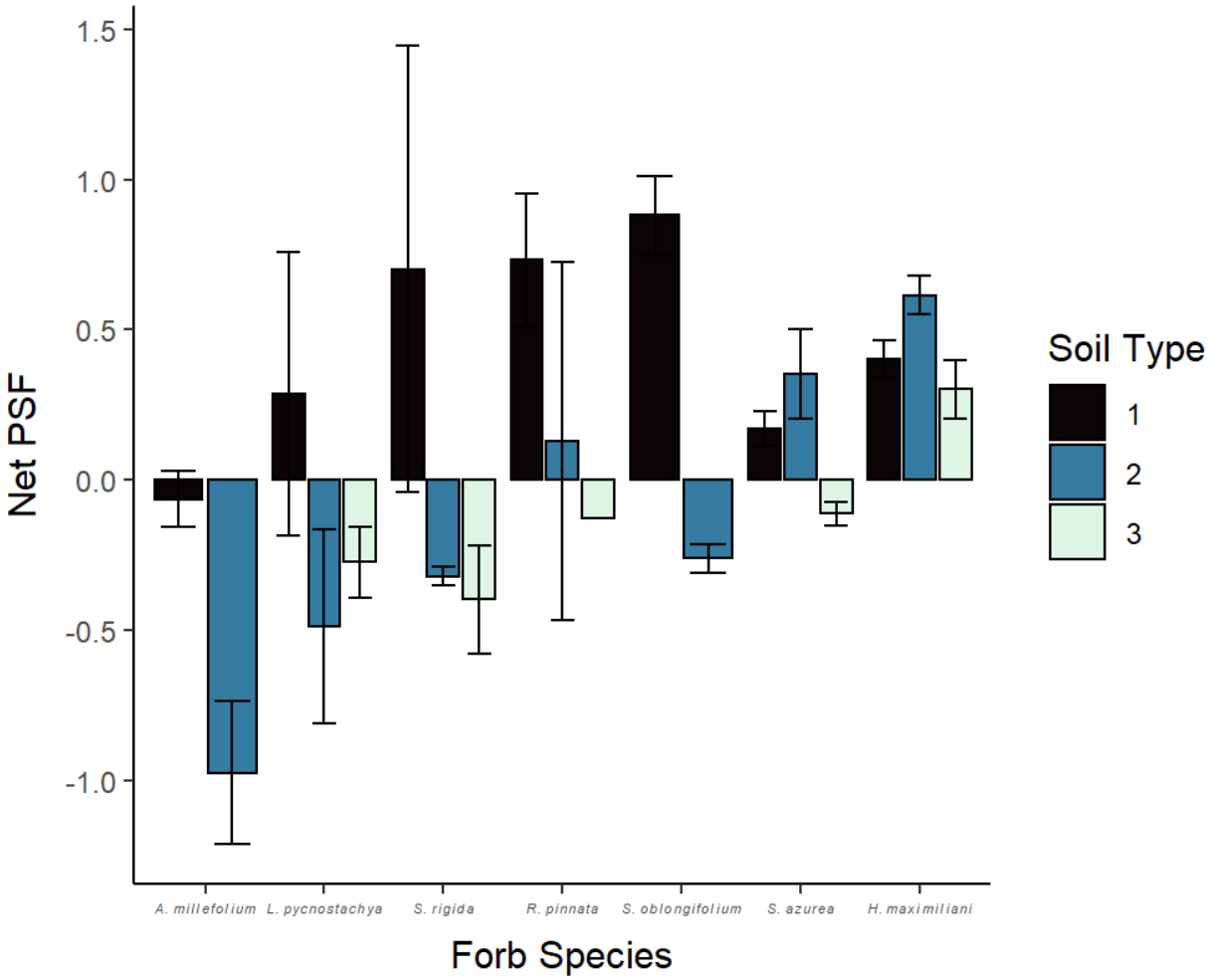


Figure 21. Average net plant-soil feedback (1 ± SE) of the forb species in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil. Net plant-soil feedback was calculated as log (biomass when grown in self-conditioned soil / biomass when grown in mixed-conditioned soil). Because of the low number of successful replicates for *A. millefolium*, biomass data from soil fertility levels 2 and 3 were pooled. Only one plant-soil feedback metric could be calculated by *R. pinnata* for soil type 3, and no plant-soil feedback metrics could be calculated for *S. oblongifolium* for soil type 3. A positive feedback value indicates that plants produced more biomass when grown in self-conditioned soil compared to mixed-conditioned soil, with a

negative feedback value indicating the reverse. A feedback value of zero indicates that no difference in biomass was produced between the two soil conditioning treatments.

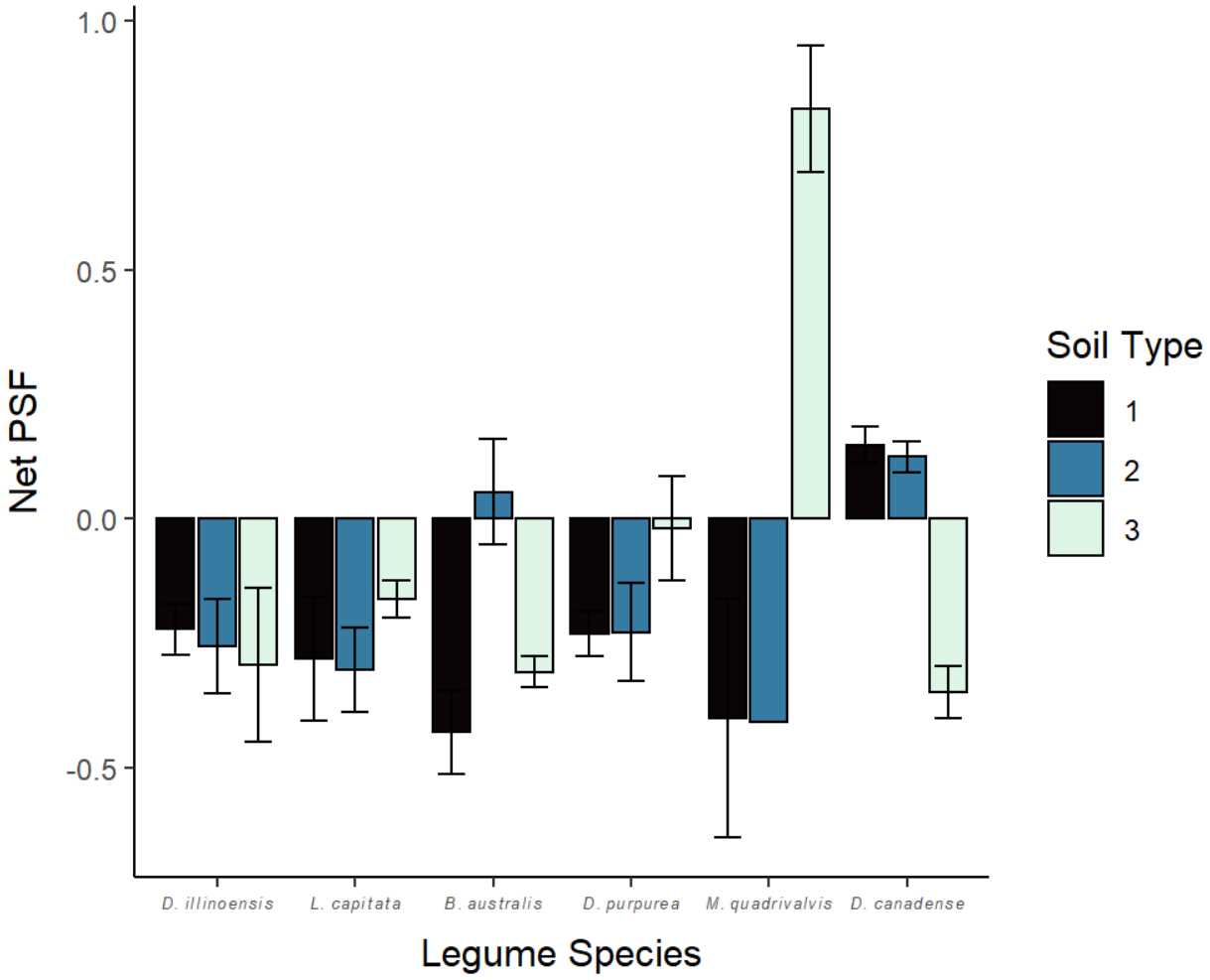


Figure 22. Average net plant-soil feedback (1 ± SE) of the legume species in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil. Net plant-soil feedback was calculated as log (biomass when grown in self-conditioned soil / biomass when grown in mixed-conditioned soil). Only one plant-soil feedback metric could be calculated by *M. quadrivalvis* for soil type 2. A positive feedback value indicates that plants produced more biomass when grown in self-conditioned soil compared to mixed-conditioned soil, with a negative feedback value indicating the reverse. A feedback value of zero indicates that no difference in biomass was produced between the two soil conditioning treatments.

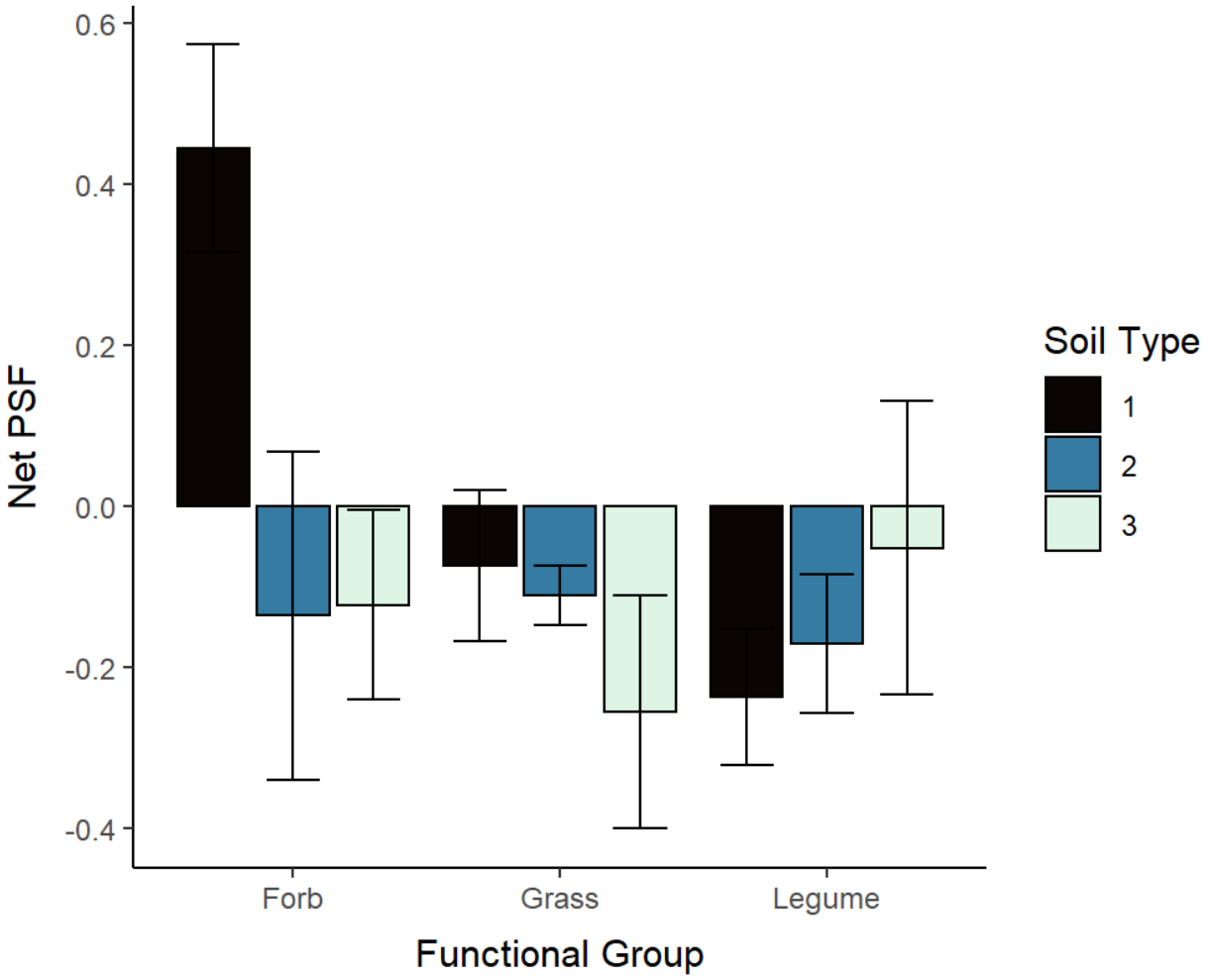


Figure 23. Average net PSF (1 ± SE) of all species in the plant functional groups in relation to the three soil types. Soil type represents fertility along depth, with soil type 1 representing the most fertile soil and soil type 3 representing the least fertile soil. Net plant-soil feedback was calculated as log (biomass when grown in self-conditioned soil / biomass when grown in mixed-conditioned soil). A positive feedback value indicates that plants produced more biomass when grown in self-conditioned soil compared to mixed-conditioned soil, with a negative feedback value indicating the reverse. A feedback value of zero indicates that no difference in biomass was produced between the two soil conditioning treatments.

# REFERENCES

# REFERENCES


Bates, S. E., E. M. Wandrag, and R. P. Duncan. 2020. Calculating the uncertainty associated with log response ratios in plant–soil feedback studies. Plant Ecology **221**:829-836.

Bennett, J. A., H. Maherali, K. O. Reinhart, Y. Lekberg, M. M. Hart, and J. Klironomos. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science **355**.

Bennett, S. I., C. Howard, R. Albrecht, L. M. Smith-Ramesh, and H. Reynolds. 2020. Simulated Herbivory Weakens Plant-Soil Feedbacks in Competitive Mixtures of Native and Invasive Woodland Plants. Frontiers in Ecology and Evolution **7**.

Bezemer, T. M., C. S. Lawson, K. Hedlund, A. R. Edwards, A. J. Brook, J. M. Igual, S. R. Mortimer, and W. H. Van Der Putten. 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. Journal of Ecology **94**:893-904.

Bukowski, A. R., C. Schittko, and J. S. Petermann. 2018. The strength of negative plant–soil feedback increases from the intraspecific to the interspecific and the functional group level. Ecology and Evolution **8**:2280-2289.

Bunn, R. A., P. W. Ramsey, and Y. Lekberg. 2015. Do native and invasive plants differ in their interactions with arbuscular mycorrhizal fungi? A meta-analysis. Journal of Ecology **103**:1547-1556.

Castle, S. C., Y. Lekberg, D. Affleck, and C. C. Cleveland. 2016. Soil abiotic and biotic controls on plant performance during primary succession in a glacial landscape. Journal of Ecology **104**:1555-1565.

Chen, D., Q. Pan, Y. Bai, S. Hu, J. Huang, Q. Wang, S. Naeem, J. J. Elser, J. Wu, and X. Han. 2016. Effects of plant functional group loss on soil biota and net ecosystem exchange: A plant removal experiment in the Mongolian grassland. Journal of Ecology **104**:734-743.

Cortois, R., T. Schröder-Georgi, A. Weigelt, W. H. van der Putten, and G. B. De Deyn. 2016. Plant–soil feedbacks: role of plant functional group and plant traits. Journal of Ecology **104**:1608-1617.

Crawford, K. M., J. T. Bauer, L. S. Comita, M. B. Eppinga, D. J. Johnson, S. A. Mangan, S. A. Queenborough, A. E. Strand, K. N. Suding, J. Umbanhowar, and J. D. Bever. 2019. When and where plant-soil feedback may promote plant coexistence: a meta-analysis. Ecology Letters **22**:1274-1284.

Dassen, S., R. Cortois, H. Martens, M. de Hollander, G. A. Kowalchuk, W. H. van der Putten, and G. B. De Deyn. 2017. Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. Molecular Ecology **26**:4085-4098.

Davis, E. J., S. Grove, K. A. Haubensak, and I. M. Parker. 2021. A widespread nitrogen-fixing invader experiences negative soil feedbacks despite enhancing the abundance of beneficial soil microbes. Plant and Soil **462**:257-271.

De Deyn, G. B., and L. Kooistra. 2021. The role of soils in habitat creation, maintenance and restoration. Philosophical Transactions of the Royal Society B: Biological Sciences **376**.

De Deyn, G. B., H. Quirk, and R. D. Bardgett. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters **7**:75-78.

Duchene, O., J. F. Vian, and F. Celette. 2017. Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. Agriculture, Ecosystems and Environment **240**:148-161.

Dudenhöffer, J. H., A. Ebeling, A. M. Klein, and C. Wagg. 2018. Beyond biomass: Soil feedbacks are transient over plant life stages and alter fitness. Journal of Ecology **106**:230-241.

Eilers, K. G., S. Debenport, S. Anderson, and N. Fierer. 2012. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry **50**:58-65.

Emilia Hannula, S., A. M. Kielak, K. Steinauer, M. Huberty, R. Jongen, J. R. De Long, R. Heinen, and T. M. Bezemer. 2019. Time after time: Temporal variation in the effects of grass and forb species on soil bacterial and fungal communities. mBio **10**.

Facelli, E., S. F. McKay, J. M. Facelli, and E. S. Scott. 2018. A soil-borne generalist pathogen regulates complex plant interactions. Plant and Soil **433**:101-109.

Faucon, M. P., D. Houben, and H. Lambers. 2017. Plant Functional Traits: Soil and Ecosystem Services. Trends in Plant Science **22**:385-394.

Fischer, C., S. Leimer, C. Roscher, J. Ravenek, H. de Kroon, Y. Kreutziger, J. Baade, H. Beßler, N. Eisenhauer, A. Weigelt, L. Mommer, M. Lange, G. Gleixner, W. Wilcke, B. Schröder, and A. Hildebrandt. 2019. Plant species richness and functional groups have different effects on soil water content in a decade-long grassland experiment. Journal of Ecology **107**:127-141.

Forero, L. E., A. Kulmatiski, J. Grenzer, and J. M. Norton. 2021. Plant-soil feedbacks help explain biodiversity-productivity relationships. Communications biology **4**:789.

Furey, G. N., and D. Tilman. 2021. Plant biodiversity and the regeneration of soil fertility. Proceedings of the National Academy of Sciences of the United States of America **118**.

Graham, P. H., and C. P. Vance. 2003. Legumes: Importance and constraints to greater use. Plant Physiology **131**:872-877.

Güsewell, S. 2004. N:P ratios in terrestrial plants: Variation and functional significance. New Phytologist **164**:243-266.

Hahn, P. G., L. Bullington, B. Larkin, K. LaFlamme, J. L. Maron, and Y. Lekberg. 2018. Effects of short- And long-term variation in resource conditions on soil fungal communities and plant responses to soil biota. Frontiers in Plant Science **871**.

Hao, J., Y. N. Chai, L. D. Lopes, R. A. Ordóñez, E. E. Wright, S. Archontoulis, and D. P. Schachtman. 2021. The Effects of Soil Depth on the Structure of Microbial Communities in Agricultural Soils in Iowa (United States). Applied and Environmental Microbiology **87**.

Harrison, K. A., and R. D. Bardgett. 2010. Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. Journal of Ecology **98**:384-395.

Hesterberg, T. 2014. Bootstrap. Pages 62-101 Methods and Applications of Statistics in Clinical Trials. Wiley Blackwell.

Hobbie, S. E. 2015. Plant species effects on nutrient cycling: revisiting litter feedbacks. Trends in Ecology and Evolution **30**:357-363.

Karasov, T. L., E. Chae, J. J. Herman, and J. Bergelson. 2017. Mechanisms to mitigate the trade-off between growth and defense. Plant Cell **29**:666-680.

Kardol, P., N. J. Cornips, M. M. L. Van Kempen, J. M. T. Bakx-Schotman, and W. H. Van Der Putten. 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecological Monographs **77**:147-162.

Kulmatiski, A., K. H. Beard, J. R. Stevens, and S. M. Cobbold. 2008. Plant-soil feedbacks: A meta-analytical review. Ecology Letters **11**:980-992.

Kuťáková, E., T. Herben, and Z. Münzbergová. 2018. Heterospecific plant–soil feedback and its relationship to plant traits, species relatedness, and co-occurrence in natural communities. Oecologia **187**:679-688.

Latz, E., N. Eisenhauer, B. C. Rall, E. Allan, C. Roscher, S. Scheu, and A. Jousset. 2012. Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. Journal of Ecology **100**:597-604.

Li, Y., J. Gong, J. Liu, W. Hou, I. Moroenyane, Y. Liu, J. Jin, J. Liu, H. Xiong, C. Cheng, K. Malik, J. Wang, and Y. Yi. 2022. Effects of Different Land Use Types and Soil Depth on Soil Nutrients and Soil Bacterial Communities in a Karst Area, Southwest China. Soil Systems **6**.

Ma, H. K., A. Pineda, A. W. G. Van Der Wurff, C. Raaijmakers, and T. M. Bezemer. 2017. Plant–soil feedback effects on growth, defense and susceptibility to a soil-borne disease in a cut flower crop: Species and functional group effects. Frontiers in Plant Science **8**.

Maharning, A. R., A. A. S. Mills, and S. M. Adl. 2009. Soil community changes during secondary succession to naturalized grasslands. Applied Soil Ecology **41**:137-147.

Martorell, C., A. MartÍnez-Blancas, and D. García-Meza. 2021. Plant–soil feedbacks depend on drought stress, functional group, and evolutionary relatedness in a semiarid grassland. Ecology **102**.

McCarthy-Neumann, S., and R. K. Kobe. 2019. Site Soil-Fertility and Light Availability Influence Plant-Soil Feedback. Frontiers in Ecology and Evolution **7**.

McGinn, K. J., W. H. van der Putten, P. E. Hulme, N. Shelby, C. Weser, and R. P. Duncan. 2018. The influence of residence time and geographic extent on the strength of plant–soil feedbacks for naturalised Trifolium. Journal of Ecology **106**:207-217.

McKenna, T. P., B. J. Darby, and K. A. Yurkonis. 2018. Effects of monoculture-conditioned soils on common tallgrass prairie species productivity. Journal of Plant Ecology **12**:474-484.

Meisner, A., W. H. Gera Hol, W. de Boer, J. A. Krumins, D. A. Wardle, and W. H. van der Putten. 2014. Plant–soil feedbacks of exotic plant species across life forms: A meta-analysis. Biological Invasions **16**:2551-2561.

Miller, E. C., G. G. Perron, and C. D. Collins. 2019. Plant-driven changes in soil microbial communities influence seed germination through negative feedbacks. Ecology and Evolution **9**:9298-9311.

Pernilla Brinkman, E., W. H. Van der Putten, E. J. Bakker, and K. J. Verhoeven. 2010. Plant-soil feedback: Experimental approaches, statistical analyses and ecological interpretations. Journal of Ecology **98**:1063-1073.

Petermann, J. S., A. J. F. Fergus, L. A. Turnbull, and B. Schmid. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. Ecology **89**:2399-2406.

Saar, S., M. Semchenko, J. M. Barel, and G. B. De Deyn. 2018. Spatial heterogeneity in root litter and soil legacies differentially affect legume root traits. Plant and Soil **428**:253-264.

Schmid, M. W., T. Hahl, S. J. van Moorsel, C. Wagg, G. B. De Deyn, and B. Schmid. 2019. Feedbacks of plant identity and diversity on the diversity and community composition of rhizosphere microbiomes from a long-term biodiversity experiment. Molecular Ecology **28**:863-878.

Šmilauer, P., J. Košnar, M. Kotilínek, and M. Šmilauerová. 2020. Contrasting effects of host identity, plant community, and local species pool on the composition and colonization levels of arbuscular mycorrhizal fungal community in a temperate grassland. New Phytologist **225**:461-473.

Šmilauerová, M., and P. Šmilauer. 2016. Functional groups affect seedling survival both through a negative soil feedback and changes in abiotic conditions. Preslia **88**:347-368.

Smith-Ramesh, L. M., and H. L. Reynolds. 2017. The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. Journal of Vegetation Science **28**:484-494.

Steinauer, K., R. Heinen, S. E. Hannula, J. R. De Long, M. Huberty, R. Jongen, M. Wang, and T. M. Bezemer. 2020. Above-belowground linkages of functionally dissimilar plant communities and soil properties in a grassland experiment. Ecosphere **11**.

Teixeira, L. H., F. A. Yannelli, G. Ganade, and J. Kollmann. 2020. Functional diversity and invasive species influence soil fertility in experimental grasslands. Plants **9**.

Uriarte, M., B. L. Turner, J. Thompson, and J. K. Zimmerman. 2015. Linking spatial patterns of leaf litterfall and soil nutrients in a tropical forest: A neighborhood approach. Ecological Applications **25**:2022-2034.

Van der Putten, W. H., R. D. Bardgett, J. D. Bever, T. M. Bezemer, B. B. Casper, T. Fukami, P. Kardol, J. N. Klironomos, A. Kulmatiski, J. A. Schweitzer, K. N. Suding, T. F. J. Van de Voorde, and D. A. Wardle. 2013. Plant-soil feedbacks: The past, the present and future challenges. Journal of Ecology **101**:265-276.

Van Zandt. 2007. Plant defense, growth rate, and habitat specialization: A comparative assessment of constitutive and induced resistance. Ecology **88**: 1984–1993.

Wilschut, R. A., W. H. van der Putten, P. Garbeva, P. Harkes, W. Konings, P. Kulkarni, H. Martens, and S. Geisen. 2019. Root traits and belowground herbivores relate to plant–soil feedback variation among congeners. Nature Communications **10**.

Xu, T., X. Chen, Y. Hou, and B. Zhu. 2021. Changes in microbial biomass, community composition and diversity, and functioning with soil depth in two alpine ecosystems on the Tibetan plateau. Plant and Soil **459**:137-153.

Yahdjian, L., P. M. Tognetti, and E. J. Chaneton. 2017. Plant functional composition affects soil processes in novel successional grasslands. Functional Ecology **31**:1813-1823.

Zhang, W., C. Ren, J. Deng, F. Zhao, G. Yang, X. Han, X. Tong, and Y. Feng. 2018. Plant functional composition and species diversity affect soil C, N, and P during secondary succession of abandoned farmland on the Loess Plateau. Ecological Engineering **122**:91-99.

Zhang, Y., L. Hou, Z. Li, D. Zhao, L. Song, G. Shao, J. Ai, and Q. Sun. 2020. Leguminous supplementation increases the resilience of soil microbial community and nutrients in Chinese fir plantations. Science of the Total Environment **703**.

Zheng, M., H. Chen, D. Li, Y. Luo, and J. Mo. 2020. Substrate stoichiometry determines nitrogen fixation throughout succession in southern Chinese forests. Ecology Letters **23**:336-347.