Understanding the shifts of Plant-Soil Feedback in response to drought

Research question: How does drought legacy influence PSF shifts of forb and grass species in grasslands?

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Abstract: Increasing droughts due to climate change are affecting the interactions between plants and their soil, with possible consequences for plant species composition and ecosystem functioning. Shifts in plant-soil feedbacks, the effect a plant species has on its own growth, have previously been detected in response to drought. In a greenhouse experiment, I will compare the growth of three plant species, with half the plants exposed to a soil that has endured 21 day drought period with the rest of the plants being control. Aboveground and belowground biomass, height and leave production of the grasses *Anthoxanthum odoratum, Dactylis glomerata* and the forb *Rumex Acetosa* are determined after a growth period of 16 weeks. This data will be analysed to provide the Plant-soil Feedback (PSF) values for the plants which through statistical analysis will compare the different soil treatments. The results will allow us to better understand the interactions happening between plant and soil biota in the case of droughts, which can help in our approaches to manage ecosystems under drought to ensure their stability and functioning under these disturbances.

List of abbreviations: PSF (Plant-soil Feedback) Ao (*Anthoxanthum odoratum)* Dg *(Dactylis glomerata)* Ra *(Rumex Acetosa)* AMF *(Arbuscular mycorrhizal fungi)* WHC (Water holding capacity)

Keywords: drought legacy, Plant-soil Feedback, grass, biomass, rhizosphere

Introduction

Since water is an essential element to the growth of plants, it is without a doubt that droughts are the most damaging stress to a plant's productivity (Williams & de Vries, 2020). Droughts have increased in frequency and intensity worldwide, with climate change being the main factor of this shift. This has led to severe damage to plant ecosystems and the overall ecosystem's stability (Kaisermann et al., 2017). There is a growing consensus among scientists that plants are intrinsically linked with the rhizosphere and organisms that live in the soil beneath (Philipot et al., 2013; Fry et al., 2018; Hassan et al., 2021; De long et al. 2019b). These interactions between plants and the soil microbial community on and around their roots are central to understanding the complex nature of how plants combat abiotic stresses such as droughts. The majority of research has focused on the aboveground features of plants, but not much research has been done on the response of roots (Williams & de Vries, 2020).

Plant-plant interactions are well documented in the scientific field (Kaisermann et al., 2017) as their impacts on the community and ecosystem dynamics are well known. Nonetheless, Plant-soil interactions is a topic that is less reported about. More precisely plant-soil feedback as they are a recent tool in measuring the impacts of a specifically conditioned soil on a certain plant species (Fitzpatrick et al., 2018; Wilschut & van Kleunen, 2021; Hassan et al., 2021). Plant-Soil Feedback (PSF) is an index representing the relative growth of a plant in its own conspecific soil (same species grown previously) compared to a plant grown in a heterospecific soil (different species grown previously) (Kaisermann et al., 2017). PSF is measured when plants alter biotic and abiotic soil properties in the rhizosphere, resulting in positive, negative, or neutral feedback back to the plant, creating a feedback loop (van der Putten et al., 2013). This positive or negative feedback can promote or reduce respectively the growth of the plant (Hassan et al., 2021). This shift can be due to the presence of pathogens, Arbuscular mycorrhizal fungi (AMF), or nutrient depletion. The feedback from the plant-soil interaction is also reciprocal with the plant interacting with the soil's biota and changing the soil's microbial composition (Cortois et al., 2016). Furthermore, the legacy effect of a soil's conditioning history can have a significant impact on plant growth (Kaisermann et al., 2017). The legacy effect is the impact a species or condition (e.g. drought or flood) has on the soil after the plant's growth cycle. Multiple studies have indicated a long-term legacy effect on plants after the drought period (van der Putten et al., 2013 & Kaisermann et al., 2017). Plant-Soil feedback also has implications for ecosystem development, not just on the individual plant but for the species as a whole in its ecosystem (fig.1, van der Putten et al., 2013). PSF is also useful to predict how the ecosystem will develop which is needed when looking at drought in grasslands (van der Putten et al., 2013).

Regarding the context of this field, many researchers have studied the impacts of droughts on plants, and there has been growing interest in Plant-Soil feedback experiments that look at droughts (De Long et al., 2019(a)). There is a growing consensus from the scientific community that a shift in PSF values can be experienced when an environment is exposed to stress such as droughts. However, this is also criticised as PSF values can vary and are unpredictable in field conditions (De Long et al., 2019(b)). In an experiment conducted by Cortois et al. (2016), the authors identified that certain slow-growing and more defensive plants suffer less from negative

PSF than fast-growing plant species, that are more vulnerable to stress and disturbance. This same study also showed how PSF values vary depending on the previous species of plant in that soil, which indicates how previous plants have a conditioning impact on the soil which can vary the PSF values. When comparing PSF values in the case of droughts, an experiment by Kaisermann et al. (2017) indicated that grass species that were grown in conspecific soil showed lower overall biomass than plants in heterospecific soil. The PSF was more negative than under controlled conditions. A similar study by Wilschut & van Kleunen (2021) compares the effect of drought on PSF of seven Geranium species grown in conspecific soil, unplanted soil or soil conditioned by a grass. The results showed only small shifts in PSF, that are connected to biomass allocation which is a contrast to Kaisermann et al.'s (2017) study. The diverse effects of plant growth in conspecific or heterospecific soil relate to plant community diversity mentioned by Philippot et al. (2013). It was observed that the more diverse the plant community is, the more diverse the composition of plant residues and rhizodeposits, and consequently microbial diversity (Philippot et al., 2013). This relates to the PSF as the rhizosphere's microbial community impacts how the plant will develop.

Until now, there is a limited amount of research on PSF under drought legacy conditions (Wilschut & van Kleunen, 2021). PSF is mainly used to study ecosystem developments without including environmental disturbances. Furthermore, few studies have differentiated between the PSF of forbs and grasses in response to drought. Grass and forbs are both herbaceous species which means they don't produce woody tissues and die at the end of the growing season. Forbs have broad leaves which is what differentiates them from grass. Both groups are present in grasslands across the Netherlands, which is important to study as they are often in the same ecosystems. This leads us to question how does the drought legacy effect influence the PSF and plant growth in grasslands. How does the drought legacy effect differ between forb and grass species?

Methodology

Experimental design

The experiment took place in two phases, the first phase conditioned the soils with a specific plant species and about half of the plants experienced a drought period. The second phase used the conditioned soil to grow the final plants, they did not experience any drought period, this allowed us to study the legacy effect on the soil and calculate the PSF values for each species.

The experiment took place in greenhouse conditions at Science Park to control the plant species' environment. Based on multiple factors, three plant species were chosen for the study : *Anthoxanthum odoratum, Dactylis glomerata* (which are grasses) and *Rumex acetosa* (which is a forb). Firstly, they are broadly distributed worldwide and common in the Netherlands which will make our research more impactful as it will be used to assess drought in Dutch grasslands. Secondly, they differ in their growth strategy with *Anthoxanthum odoratum* being a slow-growing grass species, *Dactylis glomerata* being a fast-growing grass species and Rumex Acetosa being a fast-growing forb. This growth-rate difference gives a contrast to the plant's drought response patterns. Furthermore, these two species have been used frequently in previous research, which

provides much knowledge about their traits and their drought response which we can compare in our study (De Long et al., 2019; Fry et al., 2018; Williams & De Vries, 2019 & Kaisermann et al., 2017). The experiment includes a total of 72 individual plants grown in pots, with 24 of each of the three species of plants.

The first phase of the experiment took 16 weeks and is called the conditioning phase. Half of the samples from the three grass species will be subjected to three weeks of drought stress at 20% water-holding capacity (WHC) while the other half will be watered at 60 % WHC. This period of drought will be followed by a one week recovery period in which plants will be brought back to 60% water holding capacity. This recovery period which was done in multiple studies allows the plant to refill its cells with water in order to not wilt (Hassan et al., 2021; Kaisermann et al., 2017). The soil from this first phase will then be recovered in order to be used in the second phase of the experiment which allows us to measure the biomass and PSF values.

The second phase of the experiment also lasted 16 weeks and took place in the same environmental greenhouse conditions as phase one, this time all 72 plants were given water at 60 % WHC throughout their entire growth period (fig.1). A third of the *Anthoxanthum odoratum* plants were grown in conspecific soil while the other third was grown in the soil conditioned by *Dactylis glomerata* in phase one and the last third was grown in the soil conditioned by the *Rumex Acetosa* in phase one. Similarly, the other two species (*Dactylis glomerata* & *Rumex Acetosa*) were also grown in conspecific and heterospecific soil. For scientific and statistical validity, the experience which is done on 18 plants is replicated four times which amounts to a total of 72 plants.

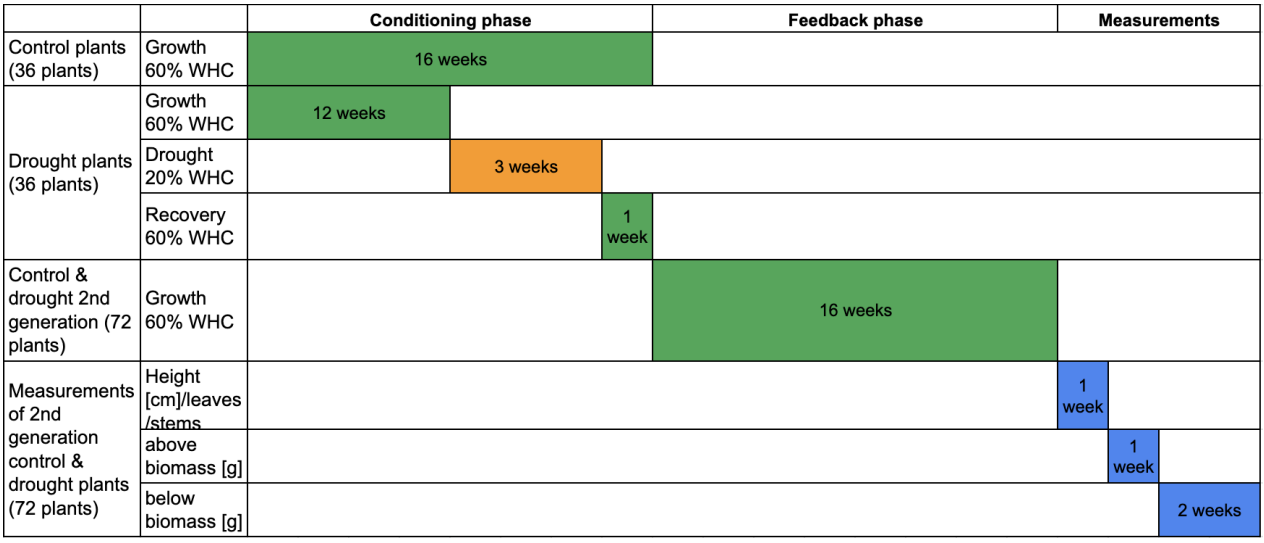

Figure 1: *Timetable of the steps and duration of the study.*

Data collection & calculations

Once the end of the second phase was completed, multiple factors were measured. From the aboveground part of the grasses: the number of leaves, shoots and height were measured and collected (Table S1). The height was measured with a measuring tape while the other two features were counted manually (Table S1). This step took a week to replicate on all samples (fig.1). The aboveground and belowground dry biomass were also measured (Table S1). The aboveground biomass is harvested by cutting the grass at its origin point and drying it in an oven at 70 degrees Celsius for 48 hours to get rid of all water content (Hassan et al., 2021). These aboveground measurements took a week to complete the data collection. For the belowground biomass, the measurement is done through a process of root washing where each pot of grass containing the roots is soaked and rinsed in order to eliminate all material that is not root (soil and rocks). The roots are then dried in the same oven as the shoots. This process took just over two weeks (fig.1).

Once all the data was collected (Table S1), we calculated the PSF values and analysed them and the biomass data through boxplot diagrams on the software RStudio 1.3.1093. The PSF values were calculated by the natural logarithm of the biomass values from a plant grown in conspecific (same species) soil divided by the same species grown in a heterospecific (different species) soil (Equation.1) (Fitzpatrick et al., 2018; Wilschut & van Kleunen, 2021; Hassan et al., 2021). In our experience, we would get 48 PSF values in total as 1/3 of the plants are grown in conspecific soil which have PSF values of 0 and are therefore not accounted for (Table S1). Out of those 48 PSF values, there are 16 values for each species and 8 of each in either drought or control conditioning.

PSF index = ln(biomass produced in conspecific soil/biomass produced in heterospecific soil)

Equation 1: PSF Index equation

Statistical analysis

Using Rstudio 1.3.1093, A linear mixed-effect model was used to test the effect of drought and soil conditioning on plant biomass and PSF. The dependent variables that were tested are the above, below and total of both biomass and PSF values, while the independent variables were drought and the plant species. Three ANOVA (analysis of variance) assumptions were tested to make sure our data is normally distributed and homogenous (Xi et al., 2018): the Shapiro-Wilk Normality test and a ggdensity plot to test for normal distribution of the samples and the Levene's test to test for homogeneity of variance (Fitzpatrick et al., 2018). For the models that met these three assumptions, we used an ANOVA. In the scenario that the assumption tests are not all met for specific datasets the data was transformed, or a non-parametric test such as the Kruskal-Wallis H test was performed. This was done for both below and total PSF datasets. For the shoot and the root biomass datasets, we transformed the data through square root and logarithm of the

biomass values respectively in order to meet the three assumptions. The datasets of the total biomass and the aboveground PSF values met all three assumptions and therefore did not need to be transformed and could be tested with the ANOVA directly.

Results

Biomass

In all three diagrams, we have the controlled and drought biomass for all three species of the experiment (fig.2). The plants grown in soil that have experienced drought showed lower biomass results than the plants grown in soil that was not droughted. This is valid for all three species grown. When looking at the total biomass of both shoot and root combined (the right panel in Figure 2) we notice significant differences between the medians of the droughted and control plants. This difference in biomass for each species is specific to the drought and not to the species as results are significant (Table 2) in the drought variable but not in the species:drought variable.

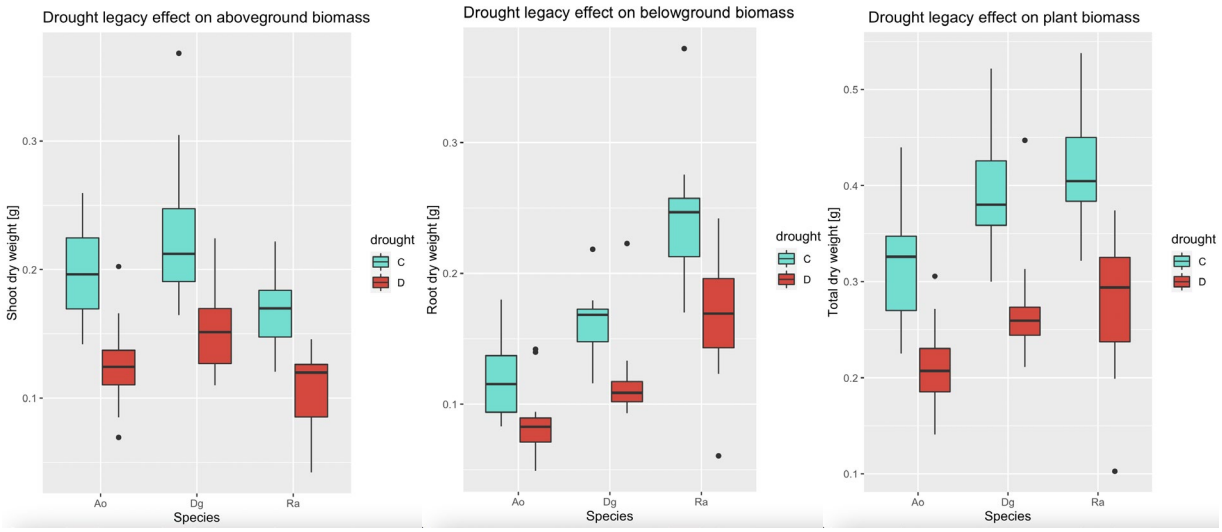

Fig.2: *Box plot diagram of Drought legacy effect on Shoot, Root & Total biomass of plant species. (Ao: Anthoxanthum odoratum; Dg: Dactylis glomerata ; Ra: Rumex Acetosa ; DW: Dry Weight ; C: Control & D: Drought) (the y-axis is the biomass in grams). Each boxplot contains the data of 12 samples. A boxplot is a standardized way of showing the distribution of data. There are five key elements in a boxplot, starting from the bottom towards the top: The "minimum" is the lowest value of the dataset represented by the tip of the vertical line, Q1 is the first Quartile and the lowest horizontal bar of the box plot, the median is the horizontal line separating the box in two, Q3 is the third quartile and the highest bar of the box plot and the upper tip of the vertical line is the maximum values. The dots outside of the box diagram are the outliers which represent the extreme 0.7% of the data set.*

Table 2: *Results of Analysis of Variance for Biomass data of the three species giving us the respective p-values in the last column for each variable tested.*

Shoot DW					
ANOVA	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	2	0.0539	0.02696	12.152	3.2E-05
drought	1	0.1351	0.13514	60.912	5.9E-11
Species : drought	2	0.0002	0.00010	0.044	0.96
Residuals	66	0.1464	0.00222	*NA*	*NA*

Root DW					
ANOVA	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	2	5.8956	2.94782	42.984	1.1E-12
drought	1	2.3683	2.36835	34.534	1.5E-07
Species : drought	2	0.0196	0.00982	0.143	0.87
Residuals	66	4.5263	0.06858	*NA*	

Total DW					
ANOVA	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	2	0.0916	0.04578	11.597	4.8E-05
drought	1	0.2600	0.25999	65.857	1.6E-11
Species : drought	2	0.0031	0.00154	0.390	0.68
Residuals	66	0.2606	0.00395	*NA*	

Similarly, the species variable also has significant p-values, the *Anthoxanthum odoratum's* median value while being grown in controlled soil has a total biomass of 0.33 grams while in droughted soil the median value is 0.21 grams. *Anthoxanthum odoratum*, therefore, has an average loss of 37.12% of total biomass when grown in soil that has a drought legacy. Similarly, *Dactylis glomerata* and *Rumex Acetosa* in droughted soil have an average total biomass loss of 31.7% and 29.26% respectively. Therefore the biomass loss ranges from 29 to 37% for all three species with *Anthoxanthum odoratum* showing the biggest loss in biomass when grown in droughted soil. This can be expected as *Anthoxanthum odoratum* is the only of the three species classified as slow-growing. The last variable of the analysis being the interaction of species and droughts has high p-values. These high values can be observed on the boxplot (fig.1) as the drought legacy effect has a similarly strong effect on all 3 species with no significant difference.

Height/Leaves/Stems

The results for the height of the plants, the number of leaves and the number of stems for each species are displayed in Figure 3. In the height boxplot, we can observe that the values for the controlled plant are always higher than the values of the plant in droughted soil (fig.3). The differences are more noticeable for species *Dactylis glomerata* than *Anthoxanthum odoratum* and *Rumex Acetosa*, although this could be due to the taller nature of the *Dactylis glomerata* and therefore have slightly bigger height differences. Similarly with the boxplot on the number of leaves and stems per plant (fig.3). In nearly all cases the plant grown in control soil has a higher count of leaves or stem than the one in drought legacy soil. The exception is for the number of stems for *Rumex Acetosa* but that is due to the species having a singular stem (fig.3). These boxplots provide a insightful observation of the impact of drought legacy on specific features of the plant's growth as height, number of leaves and stems indicate how much plant material has been produced during its growth period. These results are however not as relevant as our biomass results as there are more variations due to the species having different height/ leaves and stem features per species, while biomass variation is more representative of the overall plant's growth.

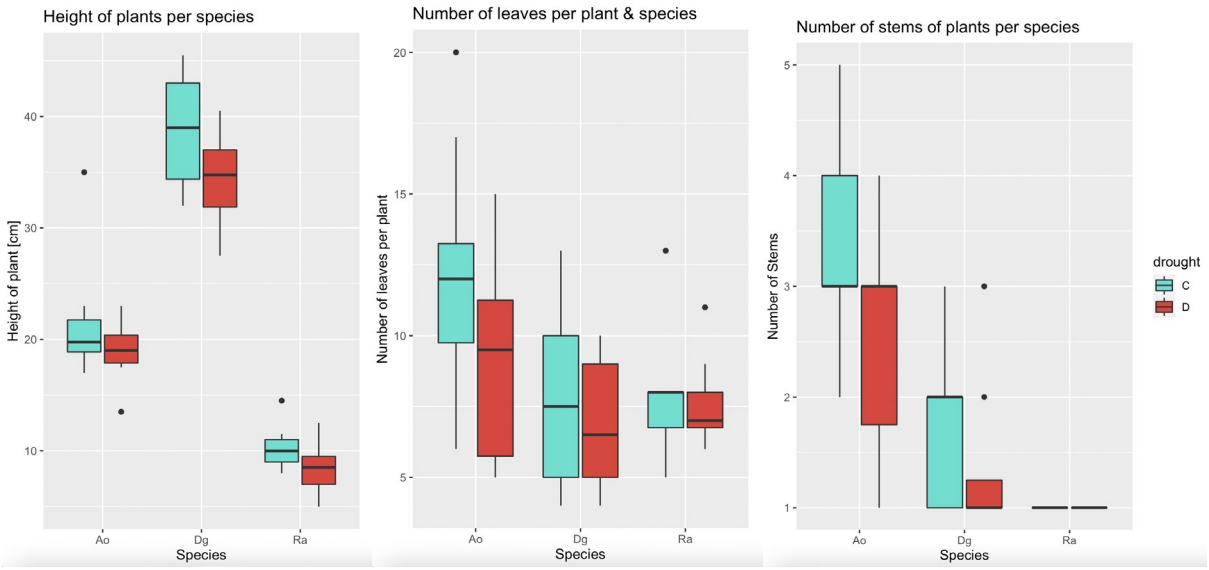

Fig 3: *Boxplot diagram of the height (in cm), number of leaves and number of stems of three plant species in control and drought legacy conditions. (Ao: Anthoxanthum odoratum; Dg: Dactylis glomerata ; Ra: Rumex Acetosa ; DW: Dry Weight ; C: Control & D: Drought) The y-axis for the most left diagram is the height of the plants in centimetres. The y-axis of the middle and most right diagrams is the count of leaves and stems per plant respectively. The red boxplots are the plants grown in drought legacy soil while the blue box plots are the plants grown in control soil.*

Height (cm)			
Kruskal Test	**chi-squared**	**Df**	**p-value**
Species	62.007	2	3.43E-14
drought	1.3212	1	0.2504
Species : drought	63.397	5	2.41E-12

Nbr Leaves			
Kruskal Test	**chi-squared**	**Df**	**p-value**
Species	13.258	2	0.001321
drought	2.8739	1	0.09003
Species : drought	17.52	5	0.003612

Nbr Stems			
Kruskal Test	**chi-squared**	**Df**	**p-value**
Species	40.401	2	1.69E-09
drought	1.9833	1	0.159
Species : drought	43.41	5	3.05E-08

Table 3: *Kruskal Wallis H test results for Height, Number of Leaves and Stems of all three species.*

Plant-Soil Feedback

The results for the Plant-Soil feedback values for above, below and total biomass are displayed through a boxplot diagram in Figure 4. Regarding the variables calculated in the ANOVA and Kruskal tests we notice that the species variable is significantly different (Table 4). These significant p-values can be seen in all above, below and total PSF diagrams where *Anthoxanthum odoratum* has the highest PSF values of the three species and *Rumex Acetosa* the lowest (fig.4). On the total PSF boxplot, *Rumex Acetosa*'s average PSF value is around 0.25 less than the PSF value of *Anthoxanthum odoratum.* Furthermore, *Rumex Acetosa* in all three graphs has a negative PSF value regardless of drought or control soil, meaning that it grows worse in its conspecific soil.

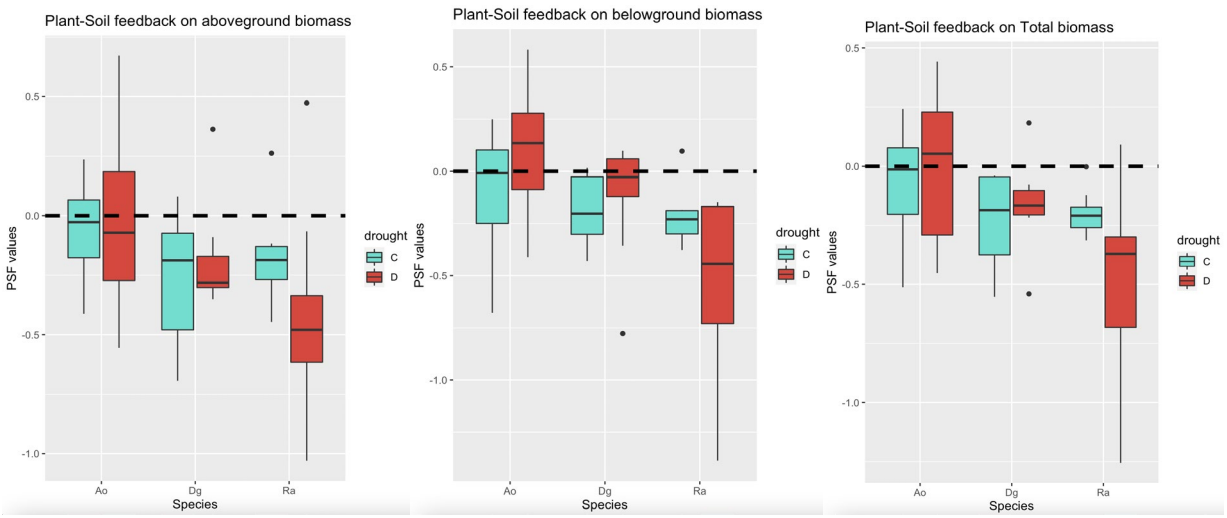

Fig.4: *Box plot diagram of the Plant-Soil Feedback (PSF) values for aboveground, belowground and total biomass of three plant species in drought legacy soil and controlled soil. (Ao: Anthoxanthum odoratum; Dg: Dactylis glomerata ; Ra: Rumex Acetosa ; DW: Dry Weight ; C: Control & D: Drought) The y axis is an index value of the PSF, it is therefore arbitrary. The dashed black line at y=0 indicates the separation between positive and negative PSF values. The blue box plots represent the PSF values from controlled soil while the red box plots are from the soil with a drought legacy.*

PSF Above					
ANOVA	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Species	2	0.5738	0.28692	2.740	0.08
drought	1	0.0278	0.02783	0.266	0.61
Species : drought	2	0.2648	0.13241	1.264	0.29
Residuals	42	4.3980	0.10472	*NA*	

PSF Below			
Kruskal Test	**chi-squared**	**Df**	**p-value**
Species	8.979	2	0.01123
drought	0.12287	1	0.7259
Species : drought	11.407	5	0.04388

PSF Total			
Kruskal Test	**chi-squared**	**Df**	**p-value**
Species	7.6856	2	0.02143
drought	0.13776	1	0.7105
Species : drought	10.598	5	0.05995

Table 4: *Statistical test (Kruskal Wallis H test & ANOVA test) results for PSF values in all three species. The ANOVA test was done for the Above PSF while the Kruskal Wallis H test was done for both Below and Total PSF.*

In regards to the PSF values of both *Dactylis glomerata* and *Rumex Acetosa*, we can also observe that on average the PSF values are negative meaning that these species growing in conspecific soil are affecting themselves more negatively than if grown in a heterospecific soil. On the other hand, for *Anthoxanthum odoratum,* the PSF values are on average near 0 meaning that there is no significant effect of being grown in conspecific or heterospecific soil (fig.4). When comparing the PSF values of aboveground and belowground we can also observe differences mainly for species *Dactylis glomerata* where the PSF values are much more negative in the above than the belowground values (fig.4).

When observing the results from the drought variable, we can notice that the p-values are not very significant (Table 4). These results in the boxplots show no clear or significant difference between the control and drought variables (fig.4). Therefore, this indicates how drought in itself has no significant impact on the PSF values.

Finally, when observing the variable of species and droughts interacting together, there is a somewhat significant difference as can be seen by the p-values (Table 4). The aboveground PSF p-value is not very significant but the below and total PSF p-values are in the range of being significantly different. When looking at the diagrams (fig.4), the forb *Rumex Acetosa* in all three boxplots has a lower PSF value in drought than control. The difference is clearer in aboveground PSF than in the other PSF plots. Regarding *Anthoxanthum odoratum* and *Dactylis glomerata* the two grass species, there seems to be no drought legacy effect on the PSF values in both above and belowground biomass, as there is some overlap in PSF values for drought and control (fig.4). Another point to observe from the Species:drought variable is that both *Rumex Acetosa* and *Dactylis glomerata* have similar PSF values in control but are lower for *Rumex Acetosa* in droughts than *Dactylis glomerata* (fig.4). This indicates how the drought impacts more negatively *Rumex Acetosa* than *Dactylis glomerata* in regard to the plant-soil interactions.

Discussion

Biomass

Our results indicate that the drought legacy effect does negatively impact the plant's biomass production and therefore their growth across all three species (fig.2). This comes in line with previous studies and evidence on how drought legacy impacts plant growth (Kaisermann et al., 2017; Fry et al., 2018; Wilschut & van Kleunen, 2021). Our results of drought legacy on biomass are significant with a loss of up to 37%. All plants from the drought legacy soil and controlled soil were given the same amount of water. Lower soil moisture content can have a negative impact on plant growth as mentioned in Wilschut & Kleunen ’s study (2021). However, in our experiment, the focus of the impact is specifically on the drought legacy effect rather than the drought effect. It is remarkable that drought has a remaining effect on plant growth even after it has been rewetted and also for plants that have never experienced drought.

In addition, when looking at the biomass results there is no significant difference between species in terms of biomass loss under drought legacy (fig.2). The study by Hassan et al. (2021) indicates that there are some differences in biomass between the two groups yet there is nothing significantly conclusive about the differences in specific species. In his study, some species of forbs, C3 and C4 (two different methods that plants do photosynthesis) grasses had higher biomass values when experiencing drought legacy than control while some other species indicated opposite results (fig.1, Hassan et al., 2021). These results are not significant when looking at forbs and grass separately but Hassan’s study does confirm that a prolonged drought legacy does reduce the biomass outputs for both groups (Hassan et al., 2021). We can therefore look at grass and forbs together under the category plants rather than separately.

A possible explanation for the decrease in biomass in drought legacy soil can be from nutrients available in the soil. Nutrients are key for a plant’s biomass production, microbes are also important and are more significant in Plant-Soil interactions as they are key to the communication between plant and the rhizosphere (Xi et al., 2018). Droughts reduce the microbial ability that

helps nutrient breakdown processes such as nitrification which converts Nitrogen in the soil to nitrate which is accessible for plants to uptake (Schimel, 2018). Further results from the same experiment indicate that the levels of available Nitrogen and Phosphate in the soil are higher in the drought legacy soil than in the control soil (Enderle, 2022, personal communication). The amount of Nitrogen available in the soil was on average 10% higher in drought legacy soil than control soil. The difference is even more noticeable with Phosphate as the soil had on average 28% more phosphate in drought legacy soil than control soil (Enderle, 2022, personal communication). The higher content of nutrients in drought legacy soil resulted in a lower biomass than the control soil, this indicates that the plants had trouble uptaking these nutrients for their growth. On the other hand, the controlled plants had less nutrients in the soil with higher biomass results as more of them had been uptaken. This rules out the possibility of higher nutrient availability being responsible for our reduced biomass results (fig.2) and leads us to question the role of microbes in these results.

The final explanation for this decrease in biomass would then be the variation of microbial composition and activity of the soil after the drought period, this happened in Phase 1 when the drought period killed a certain amount of soil microbes (Fitzpatrick et al., 2018). Microbes are key components in the soil to break down nutrients for the plants to take up (Philippot et al., 2013). Therefore, with a lack of soil microbes, the plant will take longer to assimilate similar levels of biomass as a healthy soil with sufficient soil microbial activity. This has been measured in further results of the same experiment, where the microbial Carbon and Nitrogen are higher in the control soil than the drought legacy soil (Enderle, 2022, personal communication). The microbial Carbon content is on average 12% higher in control than in drought, similarly, the microbial Nitrogen content for control soil is on average 10% higher than in drought legacy soil (Enderle, 2022, personal communication). Lower numbers of microbes in the soil are therefore an explanation for the decrease in plant growth which is seen in all three plant species.

Plant-Soil Feedback

Our results in regards to the PSF values in plants grown in drought legacy soil have more variation and complexity than the biomass results. One key finding is that our forb species (*Rumex acetosa*) has a significantly lower PSF value than the other two grass species (fig.4). In Cortois' study (2016) contrasting results to ours were found for both *Dactylis glomerata* and *Anthoxanthum odoratum* having negative PSF values and *Rumex acetosa* having slightly positive PSF values (fig.2, Cortois et al., 2016). Cortois's study looks specifically at the PSF values in control conditions and not drought, these control results are therefore different to mine. In Hassan's study (2021), the PSF results for both forbs and grass species are different from mine. The PSF values for forbs are positive in controlled soil and the PSF for grasses vary from negative to positive depending on if the plant is a C3 or C4 plant respectively (fig.3, Hassan et al., 2021). The forb results differ from our results as the PSF for *Rumex Acetosa* is negative in both drought and control (fig.4). This difference can be from *Rumex Acetosa* being a single species of forb compared to the combined results of the three forbs chosen by Hassan's study (2021).

Another reason why our results for *Rumex Acetosa* might be different from other studies could be due to different soil conditioning times. A study by Ke et al.(2021) indicated that the conditioning time can impact the strength of plant-soil microbe interactions. Longer conditioning times leads to decreased strength in the interactions, except in the case of legumes which are not part of our experiment (Ke et al., 2021). In both our study and Cortois' study (2016) we had a conditioning phase of 16 weeks (Cortois et al., 2016). In the case of Hassan's study, the conditioning phase was of 12 weeks which is 25% less time than our study, this can be an explanation to the PSF results being slightly different as mentioned previously (Hassan et al., 2021).

An important element in a plant's growth which could be a possible explanation for our results is the role microbial pathogens and of beneficial microbes which include arbuscular mycorrhizal fungi (AMF). Together these components impact the growth performance of plants and also the following generation of plants through the legacy process of Plant-Soil feedback (van der Putten et al., 2013; Cortois et al., 2016 & Wilschut et al., 2021). AMF allows for symbiosis between plant and soil elements to occur, facilitating beneficial exchanges of carbon and nutrients between the two. On the other hand, microbial pathogens can have a negative impact on plant growth as they cause diseases to the plant and often spread more when in the presence of a conspecific plant species (Cortois et al., 2016). The content of AMF and pathogens in the soil can affect the PSF value, additionally, droughts can impact the soil content of both AMF and soil pathogens (Wilschut et al, 2021). For instance, droughts can decrease the amount of AMF in the soil, while they can also decrease the suppression rate of pathogens, leading to more pathogens remaining in the soil (Wilschut et al, 2021 & de Vries et al., 2018).

In our results, we can observe how *Rumex Acetosa* has a much lower PSF value than the other two types of grass. A study by Cortois et al. (2016) indicates a difference in the percentage of AMF in the soil for 48 species including the three species of our study. The results indicate that *Rumex Acetosa* has a lower percentage of both AMF in conspecific and heterospecific soil than the two types of grass (Ao & Dg), this result includes other variables such as specific leaf area and specific root length which we will not assess (fig.1, Cortois et al., 2016). These results can be an indication that *Rumex Acetosa*'s lower PSF values in all above, below and total biomass compared to the other two grasses is due to lower AMF in the soil and therefore fewer symbiotic connections between the rhizosphere and the plant.

Another explanation for *Rumex Acetosa* having lower PSF values can be linked to the higher rates of pathogens present in the soil in the case of droughts. A second argument is that plants grown in conspecific soil are more likely to develop more pathogens as they grow familiar to the specific species and are able to spread more easily (Wilschut et al., 2021). Similarly with the effect of drought on pathogens and PSF values, there is a correlation between droughts increasing the presence of pathogens and therefore lowering plant-soil feedback. To counter the damage from pathogens, a grass species grown in a diverse soil in the presence of other plant species has a stronger resistance to pathogens (Fitzpatrick et al., 2018).

I acknowledge that I have studied a limited amount of plant variables in order to have more in-depth results of PSF shifts in droughts. *Rumex Acetosa* was our only forb representative species

in the experiment while we had two grass species, having an additional forb and legume species would have been more representative of the whole group of herbaceous plants. Other elements that need to be analyzed in future studies are the AMF and pathogen abundance in the soil during the experiment. This will help with determining more precisely the causes of PSF shifts. Most studies agree that a more diverse soil provides better protection in the case of droughts but we are unable to assess how much does heterospecific soil protect plant-soil interactions (de Vries et al., 2018; Fitzpatrick et al., 2018 & P. Schimel., 2018). This can be implemented in future studies by growing the three species in same pots for a greenhouse experimentor or by growing them together in a field experiment. This would help the understanding of the relations between these three species altogether as they are often in the same ecosystem.

Conclusion

In conclusion, our findings on plant biomass under drought legacy effect follows the results of similar studies that drought legacy has a negative effect on plant growth regardless of the plant species or functional group. This could be due to a change in soil microbial abundance, composition or activity rather than nutrient availability, which could result in reduced nutrient uptake by plants in previously droughted soil. Regarding PSF findings there seems to be a more negative impact on forb than grass species but this claim needs further research to be confirmed. A key conclusive finding is that droughts leave a negative legacy in the soil meaning that even if plants recover they will grow worst in that same soil independent of that plant's species. These results lead us to a broader point on the importance of a diverse rhizosphere and ecosystem for grassland plant species to grow in. If the three species from our experiment were growing together in a natural environment in the presence of drought, we would most likely see the grass species be more dominant over the forb as their PSF values are more positive. Rumex Acetosa would also struggle in a monoculture, but would grow better in a diverse grass ecosystem as it would help reduce plant-specific pathogens. This applies also to *Dactylis glomerata* and *Anthoxanthum odoratum* as they also have negative PSF values. In regards to the increasing amount of droughts across ecosystems due to climate change, there is a pressing need to diversify grasslands as most grasslands are monocultures and are therefore prone to higher risks of decreased growth. This decreased growth would impact not only the grass species but also the entire rhizosphere and soil health in general.

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Appendix

Table S1: Table of all data collected per sample.

Nr replicate	Condition species	Control/ Drought	Species	Nr stems	Nr leaves	height [cm]	Shoot DW [g]	Root DW [g]	Total DW [g]	PSF above	PSF below	PSF Total
1	Ao	C	Ao	4	13	20	0.192	0.155	0.347	0.000	0.000	0.000
1	Dg	C	Ao	3	13	19	0.152	0.121	0.272	0.236	0.249	0.242
1	Ra	C	Ao	3	11	19	0.201	0.141	0.341	-0.046	0.098	0.016
1	Ao	C	Dg	3	10	34	0.193	0.169	0.362	-0.083	0.009	-0.039
1	Dg	C	Dg	2	10	32	0.178	0.171	0.348	0.000	0.000	0.000
1	Ra	C	Dg	1	6	39	0.211	0.168	0.379	-0.174	0.017	-0.085
1	Ao	C	Ra	1	8	14.5	0.174	0.252	0.425	-0.367	-0.223	-0.279
1	Dg	C	Ra	1	8	11	0.150	0.242	0.392	-0.216	-0.185	-0.197
1	Ra	C	Ra	1	6	9.5	0.120	0.201	0.322	0.000	0.000	0.000
1	Ao	D	Ao	3	8	19	0.100	0.094	0.194	0.000	0.000	0.000
1	Dg	D	Ao	3	13	18	0.166	0.140	0.306	-0.504	-0.395	-0.453
1	Ra	D	Ao	1	5	19	0.122	0.142	0.264	-0.194	-0.411	-0.305
1	Ao	D	Dg	3	10	40.5	0.196	0.111	0.306	-0.298	0.055	-0.155
1	Dg	D	Dg	2	9	38	0.145	0.117	0.262	0.000	0.000	0.000
1	Ra	D	Dg	1	7	32	0.194	0.119	0.313	-0.289	-0.018	-0.177
1	Ao	D	Ra	1	6	12	0.146	0.172	0.318	-0.504	-0.173	-0.311
1	Dg	D	Ra	1	7	9.5	0.135	0.168	0.303	-0.426	-0.149	-0.263
1	Ra	D	Ra	1	6	8.5	0.088	0.145	0.233	0.000	0.000	0.000
2	Ao	C	Ao	5	17	17	0.163	0.092	0.254	0.000	0.000	0.000
2	Dg	C	Ao	3	12	18.5	0.185	0.112	0.297	-0.129	-0.201	-0.156
2	Ra	C	Ao	4	20	18.5	0.224	0.136	0.360	-0.319	-0.395	-0.347
2	Ao	C	Dg	1	5	43.5	0.213	0.169	0.382	0.080	-0.231	-0.046
2	Dg	C	Dg	1	5	40.5	0.231	0.134	0.365	0.000	0.000	0.000
2	Ra	C	Dg	2	12	34.5	0.242	0.139	0.381	-0.047	-0.039	-0.044
2	Ao	C	Ra	1	7	10	0.175	0.216	0.391	-0.133	-0.239	-0.190
2	Dg	C	Ra	1	13	10	0.194	0.248	0.443	-0.236	-0.378	-0.313
2	Ra	C	Ra	1	6	11	0.153	0.170	0.324	0.000	0.000	0.000
2	Ao	D	Ao	3	11	17.5	0.136	0.084	0.219	0.000	0.000	0.000
2	Dg	D	Ao	1	6	17.5	0.069	0.072	0.141	0.672	0.154	0.442
2	Ra	D	Ao	4	10	20	0.129	0.082	0.212	0.051	0.013	0.036
2	Ao	D	Dg	1	4	27.5	0.146	0.103	0.249	-0.198	0.070	-0.078
2	Dg	D	Dg	1	5	31.5	0.120	0.111	0.230	0.000	0.000	0.000
2	Ra	D	Dg	1	4	37	0.157	0.100	0.258	-0.274	0.099	-0.112
2	Ao	D	Ra	1	7	8.5	0.118	0.242	0.360	-1.030	-1.387	-1.255
2	Dg	D	Ra	1	8	9	0.109	0.187	0.295	-0.949	-1.126	-1.057
2	Ra	D	Ra	1	6	7	0.042	0.061	0.103	0.000	0.000	0.000
3	Ao	C	Ao	2	6	35	0.228	0.106	0.334	0.000	0.000	0.000
3	Dg	C	Ao	4	10	19.5	0.223	0.095	0.318	0.023	0.115	0.052

3	Ra	C	Ao	3	12	20.5	0.230	0.119	0.349	-0.009	-0.112	-0.043
3	Ao	C	Dg	2	8	43	0.305	0.178	0.483	-0.505	-0.430	-0.477
3	Dg	C	Dg	1	5	45.5	0.184	0.116	0.300	0.000	0.000	0.000
3	Ra	C	Dg	2	8	34	0.368	0.153	0.522	-0.694	-0.279	-0.553
3	Ao	C	Ra	1	8	9	0.203	0.275	0.478	-0.118	-0.305	-0.221
3	Dg	C	Ra	1	5	11.5	0.139	0.245	0.384	0.262	-0.190	-0.002
3	Ra	C	Ra	1	7	9	0.180	0.203	0.383	0.000	0.000	0.000
3	Ao	D	Ao	1	5	23	0.116	0.088	0.204	0.000	0.000	0.000
3	Dg	D	Ao	3	12	13.5	0.141	0.049	0.190	-0.196	0.582	0.070
3	Ra	D	Ao	4	15	21.5	0.202	0.069	0.272	-0.556	0.241	-0.286
3	Ao	D	Dg	1	9	33.5	0.162	0.097	0.259	-0.314	-0.039	-0.202
3	Dg	D	Dg	1	6	36	0.118	0.093	0.211	0.000	0.000	0.000
3	Ra	D	Dg	1	5	31	0.129	0.133	0.262	-0.091	-0.357	-0.217
3	Ao	D	Ra	1	7	12.5	0.133	0.242	0.374	-0.066	-0.562	-0.357
3	Dg	D	Ra	1	7	6	0.077	0.162	0.239	0.473	-0.160	0.091
3	Ra	D	Ra	1	8	9.5	0.124	0.138	0.262	0.000	0.000	0.000
4	Ao	C	Ao	2	8	21.5	0.172	0.091	0.263	0.000	0.000	0.000
4	Dg	C	Ao	3	14	22.5	0.260	0.180	0.440	-0.413	-0.678	-0.513
4	Ra	C	Ao	3	9	23	0.142	0.083	0.225	0.191	0.095	0.156
4	Ao	C	Dg	1	4	38.5	0.201	0.218	0.420	-0.201	-0.372	-0.287
4	Dg	C	Dg	2	7	43	0.165	0.151	0.315	0.000	0.000	0.000
4	Ra	C	Dg	3	13	39	0.264	0.179	0.443	-0.471	-0.176	-0.341
4	Ao	C	Ra	1	8	10	0.222	0.250	0.472	-0.446	0.097	-0.122
4	Dg	C	Ra	1	8	9	0.166	0.372	0.538	-0.156	-0.299	-0.253
4	Ra	C	Ra	1	8	8	0.142	0.276	0.418	0.000	0.000	0.000
4	Ao	D	Ao	3	10	19	0.127	0.083	0.210	0.000	0.000	0.000
4	Dg	D	Ao	3	9	19	0.114	0.056	0.170	0.112	0.388	0.212
4	Ra	D	Ao	2	5	22.5	0.085	0.074	0.159	0.401	0.115	0.278
4	Ao	D	Dg	1	5	35.5	0.110	0.107	0.217	0.362	-0.043	0.183
4	Dg	D	Dg	3	9	37	0.158	0.102	0.260	0.000	0.000	0.000
4	Ra	D	Dg	1	7	34	0.224	0.223	0.447	-0.351	-0.777	-0.541
4	Ao	D	Ra	1	7	8	0.124	0.224	0.348	-0.487	-0.599	-0.558
4	Dg	D	Ra	1	9	7	0.122	0.171	0.292	-0.472	-0.326	-0.384
4	Ra	D	Ra	1	11	5	0.076	0.123	0.199	0.000	0.000	0.000