The effect of plant diversity on plant performance under a prolonged summer drought.

Anne Jansma
920404394130

August 2015

The effect of plant diversity on plant performance under a prolonged summer drought

Anne P. Jansma
920404394130
Master Forest and Nature Conservation
August 2015

NCP-80436

Supervisors:
Dr. J. van Ruijven
MSc L. Bakker
Nature Conservation and Plant ecology group

Abstract

It is established that one of the effects of climate change is an increase in the frequency and intensity of prolonged summer droughts. In the long term this could lead to changes in local species composition and a decrease in plant diversity in grassland communities. This can have consequences for both community and species performance. Several studies found that a higher diversity results in a higher productivity and a higher resistance to perturbations, indicating that diversity is an important ecosystem property. In this research the effect of species diversity and plant traits on individual plant performance under a prolonged summer drought was investigated. In total 16 different species were planted in monocultures, 4 species mixtures and 16 species mixtures in 198 plots of 0.5 m^2. For most species there was no effect of diversity and drought on species performance, which was expressed in plant height, number of stems, rosette width and the number of flowering stems. *Leucanthemum* showed a drought and diversity effect for plant height, number of stems and rosette width. Although not all species showed a drought or diversity effect, species performance in mixtures was influenced by interspecific interactions. This study indicates that both rooting depth and Ellenberg moisture value could be used to explain a species' tolerance to drought. For several species a species richness effect was observed. However, diversity didn't contribute to a better performance of moist loving species under drought in this experiment. As it is expected that the frequency and intensity of prolonged summer droughts will increase, it is assumable that the species composition of Western European grassland communities will change and that overall diversity will decrease in future.

Table of contents

Introduction

It is now well established that climate change will result in changes in global and local weather conditions. Recent predictions suggest that the mean annual temperature and the frequency of extreme weather events will increase, and that precipitation patterns will change (IPCC, 2007). In Western Europe winters will become wetter and summers will become drier (IPCC, 2007). One of the consequences of this decreased precipitation in summer is that the frequency and intensity of prolonged summer droughts will increase. Drought can have a large impact on plant performance. Reichstein et al. (2007) investigated the effect of the severe summer drought of 2003 on the European biosphere's production. The severe drought led to lower respiration rates and a drop in productivity. Tilman & Haddi (1992) also found a decrease in productivity. During a severe summer drought in Minnesota in 1988 biomass production decreased by 50% compared to the previous year. In the long term drought can also lead to changes in local species composition and reductions in species diversity (Chaves & Oliveira, 2004; Grime et al., 2000). This in turn can have important consequences for the functioning of ecosystems, like grasslands. It is well established that loss of plant diversity leads to a decline in community productivity (e.g. Hector et al. 1999; Tilman et al., 2001; Van Ruijven and Berendse, 2005; Marquard et al., 2009). In addition, several studies have shown that a decline in plant diversity leads to a decreased temporal stability of grasslands (e.g. Tilman et al., 2006; Van Ruijven & Berendse, 2007). The effect of a drought on the temporal stability of an ecosystem is determined by its two components resistance and resilience. Resistance is the ability of a plant community or plant individual to withstand a drought and is measured directly after the drought period. Resilience includes both resistance and the recovery in the period after a drought and is often measured as the ratio of post- to pre-drought biomass (Van Ruijven & Berendse, 2010). Several studies showed that the resistance and resilience after a drought is higher in systems with a high plant species diversity (e.g. Tilman & Downing, 1994; MacGillivray et al., 1995), while others found varying results. Frank & McNaughton (1991) and Vogel et al. (2012) only found a higher resistance with increased diversity. Van Ruijven & Berendse (2010) found a higher resilience but a lower resistance, whereas Pfisterer & Schmid (2002) found both a lower resistance and resilience in more diverse plant communities. Others found no effect of species richness on the resistance and/or resilience of grassland communities (Wang et al., 2007; Kahmen et al., 2005). In these two studies there was also no effect of species richness on productivity. These studies suggest that resistance depends on pre-perturbation biomass rather than species richness per se (Vogel et al., 2012). High biomass systems have a higher reduction in growth compared to low biomass system, possibly resulting in a lower resistance (Wang et al., 2007). Despite the variety in research outcomes of the effect of species diversity on resistance and resilience in grassland systems, most studies indicate that species diversity can have an important contribution to the temporal stability of grassland systems.

A higher resistance and resilience in species rich systems is often explained by two mechanisms the complementarity effect and the insurance hypothesis (Van Ruijven & Berendse, 2005). Complementarity or niche complementarity means that plant communities with higher species numbers can utilize the available resource niches more completely in space and time, which results in complementation (Hooper & Vitousek, 1997). A high diversity in rooting systems results in an increase in the root density in the topsoil, thereby increasing the water holding capacity. This could result in a higher water use efficiency on community level, resulting in a higher drought resistance. A second important mechanism is the insurance hypothesis, which states that species diversity influences the temporal stability of ecosystems to perturbations like drought. A higher diversity increases the likelihood of having an important functional or environmentally well adapted species in the plant community, for example a high productive or drought tolerant species (Yachi & Loreau, 1999; Kahmen et al., 2005). The negative effect of drought on the performance of non adapted plant species can be compensated for by drought resistant and resilient species present in the plant community. The insurance hypothesis therefore predicts that drought resistance and resilience should increase with species richness (Yachi & Loreau, 1999; Van Ruijven & Berendse, 2010).

Another mechanism that is used in further elaborating the diversity-productivity relationship is the concept of functional traits (e.g. Grime, 1997; Hooper & Vitousek., 1997). According to Naeem & Wright (2003) a functional trait is “a specific character or phenotypic trait of a species that is associated with a biochemical process or ecosystem property”. Multiple studies found that functional trait diversity is an important factor in explaining the higher productivity in ecosystems with a high plant diversity (Mokany et al. 2008; Schumacher & Roscher, 2009; Roscher et al. 2012). However, in explaining the resistance and resilience of vegetation communities to drought the concept of functional traits is rarely used. Yet it is very likely that plant traits determine the resilience and resistance to drought, not plant species richness per se (MacGillivray et al., 1995; Naeem & Wright, 2003). Plant traits like rooting depth, root diameter, specific leaf area (SLA) and stomatal conductance are important traits in determining if a plant can deal with low moisture levels (Chaves et al., 2003). A plant community with a high diversity in functional traits is therefore likely to have a higher resistance to perturbations like drought.

A lot of studies indicate that plant diversity matters, not only in species but also in their traits. Current research at diversity-productivity relationships often focuses on community performance and not specifically on species performance. Besides, not much research is done at the effect of perturbations like drought in combination with plant functional traits on the diversity-productivity relationship. Research at this discipline is relevant because it can demonstrate the importance of diversity in grassland systems under future climatic changes. In this study the effect of plant species diversity on plant performance under a prolonged summer drought will be investigated. The main focus will be on individual species performance. This will give insight in the resistance and performance of species under drought in mixtures compared to monocultures. This objective resulted in the following research questions:

- What is the effect of plant diversity on species performance under a prolonged summer drought?
    - Do species perform better in mixtures compared to monocultures under a prolonged summer drought?
    - Can functional traits like rooting depth explain a species’ susceptibility to drought?

Based on previous research it could be expected that individuals will perform better in more diverse plant mixtures under a prolonged summer drought. Community biomass production in mixtures is often found to be higher. However, individual performance depends on interspecific interactions between neighboring species, like competition and facilitation. In a monocultures the response to drought could be regarded as a species character, whereas in mixtures interactions with other species also determine the drought response. It could therefore be expected that the competitive ability of species plays an important role in the performance of individuals during drought. Besides, the presence of certain functional plant traits in the community could positively increase the performance of individuals under drought. A shallow rooting system with a high root density can increase the water holding capacity, thereby increasing drought tolerance of the plant community (Van Eekeren et al., 2011; Van Dijk & Van Miltenburg, 2013). In addition, deep rooting species can increase the hydraulic lift, transporting water upwards from deeper soil layers, thereby facilitating the water uptake of shallow rooting species (Caldwell & Richards, 1989). Deep rooting species could therefore provide an important function during prolonged summer droughts. It could be expected that drought resistance is higher if plant communities contain deep rooting species.

Methods

Study site and data collection

In 2014 a large drought biodiversity experiment was established on an experimental field located in Wageningen. Average annual precipitation in Wageningen is 825 mm, with a precipitation surplus in winter and a deficit in summer (KNMI, 2011). The average daily temperature is 10 °C, ranging from 3 °C in January to 17.5 °C in July (KNMI, 2011). In total 16 different species, 8 grasses and 8 herbs differing in traits were used in this drought experiment. Before planting, the upper 80 cm nutrient rich soil was removed, until the sandy mineral layer was reached. Plots were filled with a homogeneous soil mixture, consisting of river sand (3/4) and potting soil (1/4), with a pH (H_2O) of 7.08 and an OM content of 1.4%. The species were seeded in the greenhouse and planted on the experimental field in plots of 0.5 m^2 after 5 weeks. In total 198 plots were established in three blocks of 66 (22*3) plots, each plot contained 64 (8*8) individuals. Species were planted in monocultures, 4 species mixtures and 16 species mixtures to investigate the effect of plant diversity on drought resistance. Drought was simulated by placing a transparent roof over all the plots. The duration of the drought was 4 weeks, running from the 6th of June till the 7th of July. The roof has been above the plots from the 10th of June till the 14thof July. Control plots were watered every 2 or 3 days in order to investigate the development of the mixtures and monocultures without drought. In total 68.4 mm was given to the control plots, every time control plots received 5 till 7.5 mm. This is equal to the amount of rainfall that on average falls during this time period (KNMI, 2014).

Table 1. The plant species that are used in the drought-biodiversity experiment with their rooting depth and Ellenberg moisture value. The species in bolt are used in this research at plant performance.

Grasses		**Rooting depth**	**Ellenberg moisture value**
Meadow fescue	***Festuca pratensis***	**Deep**	**6.4 (Wet)**
Tall oat-grass	***Arrhenatherum elatius***	**Deep**	**5.5 (Moderate)**
Timothy	*Phleum pratense*	Deep	5.7 (Moderate)
Yellow-oat grass	*Trisetum flavescens*	Deep	4.9 (Dry)
Sweet meadow grass	***Anthoxanthum odoratum***	**Shallow**	**6.3 (Wet)**
Creeping bentgrass	*Agrostis stolonifera*	Shallow	7 (Wet)
Red fescue	*Festuca rubra*	Shallow	5.6 (Moderate)
Queking grass	*Briza media*	Shallow	5.6 (Moderate)
Herbs			
Great burnet	*Sanguisorba officinalis*	Deep	7.2 (Wet)
Oxeye daisy	***Leucanthemum vulgare***	**Deep**	**5.1 (Moderate)**
Yarrow	*Achillea millefolium*	Deep	5.1 (Moderate)
Brown knapweed	*Centaurea jacea*	Deep	5.2 (Moderate)
Hedge bedstraw	*Gallium molugo*	Deep	4.8 (Dry)
Creeping buttercup	*Ranunculus repens*	Shallow	6.4 (Wet)
Common selfheal	***Prunella vulgaris***	**Shallow**	**6.3 (Wet)**
Autumnal hawkbit	*Leontodon autumnalis*	Shallow	4.9 (Dry)
Rough hawkbit	*Leontodon hispidus*	Shallow	4.7 (Dry)

In this study the performance of individuals of five of the sixteen species was monitored before, during and after the drought in monocultures, 4 species mixtures and 16 species mixtures. The five selected species comprise two functional groups, 3 grasses and 2 herbs (Table 1). Within each functional group species were selected based on their traits, although practical considerations like the formation of rhizomes were also taken into account. If a plant species develops rhizomes (e.g. yarrow, creeping bentgrass) following individual plants over time is difficult, as it is difficult to determine if adjacent stems belong to the same individual. Within functional groups species were selected based on their rooting depth and Ellenberg moisture value (Table 1). Ellenberg indicator values indicate the moisture, nitrogen and pH values of the soil by averaging the indicator values of the different plant species present in the vegetation community (Ellenberg et al. 1992). Some plant species are typical for moist soils, whereas others are typical for dry soils. These species have respectively a high and low Ellenberg moisture value. It can be expected that species of dry growing conditions are less vulnerable to drought, and that species with a deep rooting systems are able to obtain water from deeper soil layers (Geerts et al., 2014).

The individuals that were followed were randomly selected for each plot. Individuals at the edge will not be selected to exclude potential edge effects. For each species, 86-90 individuals will be monitored (438 individuals in total). Each species is present in six monoculture plots, twenty-two or twenty-four 4-species mixtures and in twelve 16-species mixtures. Half of these plots are control plots, the other identical half are subjected to drought. In the 4- and 16-species mixtures two individuals and in the monoculture three individuals were randomly selected.

Individual plant performance measurements took place at the 8th of April, 5th of May and the 8th of June (before drought), the 24th of June (during the drought) and at the 3rd of July (after the drought). Plant performance was measured by measuring stem height, rosette width of herbs and the tuft width of grasses and by counting the number of stems and flowering stems. To determine the moisture content of the plots TDR (Time Domain Reflectometry) measurements were done several times with an Imko hygrometer type HD2 at a depth of 15 cm.

Data analysis

Data was analyzed in IBM SPSS statistics 2.0. The performance of every species was analyzed separately, as the aim was to look at the development of species individuals over time. For all species the data followed a normal distribution. All relationships were determined with a linear mixed model with the plant performance proxies as dependent variable, measure date, drought (drought/control) and diversity (1, 4 and 16) as factor and neighboring species as covariate. Estimated marginal means were used to check if there were factorial effects and interactions. In the case of significant effects an LSD test was carried out to look for differences within factors.

Results

Conditions

From 9 June till 6 July all plots were covered with a transparent roof. During the drought period the average temperature under the roofs was 2.7 °C higher compared to the outside temperature. In the third and fourth week of the drought period high temperatures were achieved. The maximum temperature measured outside was 38 °C and under the roofs even values of 48 °C were achieved. Relative humidity was on average 6.5% lower under the roof.

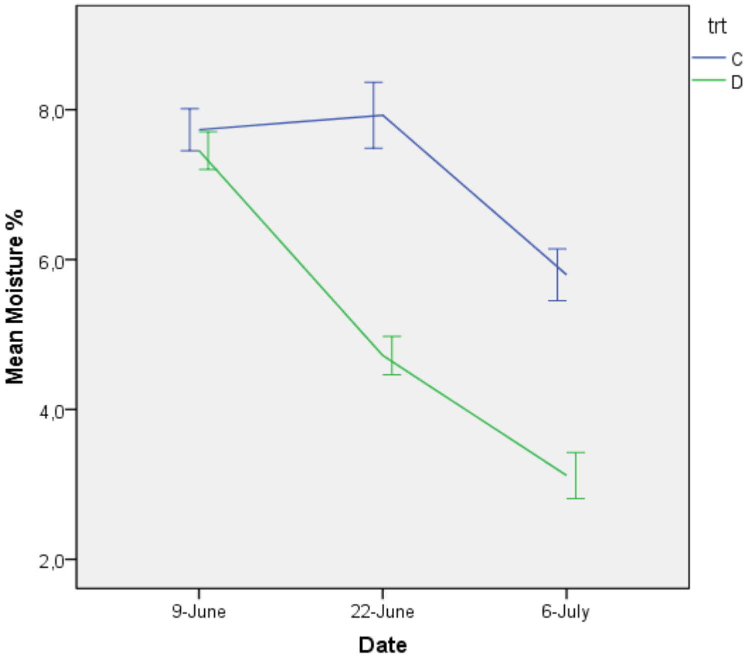

Figure 1. The average moisture content (%) in control and drought plots on 9 June, 22 June and 6 July. Error bars show mean +- 2 SE.

At the start of the drought at 9 June the average moisture content in the control and drought plots was almost the same. In the drought plots the moisture content was 7.5% compared to 7.7% in the control plots. In the first two weeks of the drought period the moisture content in the control plots slightly increased to 7.9%. At the end of the drought period the moisture content in the control plots was decreased to 5.8%, whereas the moisture content in the drought plot was 3.4% at the end of the drought (Fig. 1). Presence of *Leucanthemum* resulted in a lower moisture content of the plots ($F_{1,19}$=24.6, P<0.001).

Species performance

Table 2. The effect (P- and F-values) of Drought (D), Species Richness (SR), Date and Drought*Species Richness (Dr*SR) on plant height, number of stems, rosette width and the number of flowering stems for the species Prunella, Leucanthemum, Arrhenatherum, Anthoxanthum and Festuca p. Significant effects are displayed in bolt.

		Prunella		*Leucanthemum*		*Arrhenatherum*		*Anthoxanthum*		*Festuca p.*	
		P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value
Plant height	D	0.782	$F_{1,29}$=0.1	**0.003**	$F_{1,21}$=9.0	0.538	$F_{1,24}$=0.4	0.454	$F_{1,23}$=0.6	0.531	$F_{1,25}$=0.4
	SR	0.930	$F_{2,28}$=0.1	**0.003**	$F_{2,20}$=6.1	0.237	$F_{2,23}$=1.5	0.428	$F_{1,22}$=0.9	0.101	$F_{2,24}$=2.3
	Date	**0.000**	$F_{4,26}$=1261	**0.000**	$F_{4,18}$=2171.6	**0.000**	$F_{4,21}$=229.4	**0.000**	$F_{4,20}$=777.9	**0.000**	$F_{4,22}$=150.3
	Dr*SR	0.832	$F_{2,28}$=0.2	0.395	$F_{2,20}$=0.94	**0.000**	$F_{2,23}$=9.0	0.141	$F_{2,24}$=2.0	0.126	$F_{2,24}$=0.1
# of stems	D	0.939	$F_{1,27}$=0.0	**0.018**	$F_{1,27}$=5.6	0.116	$F_{1,25}$=2.5	**0.000**	$F_{1,28}$=20.0	0.743	$F_{1,27}$=0.1
	SR	0.711	$F_{2,26}$=0.1	**0.000**	$F_{2,26}$=72.7	0.497	$F_{2,24}$=0.7	**0.000**	$F_{2,27}$=11.9	0.084	$F_{2,26}$=2.5
	Date	**0.000**	$F_{4,24}$=592.4	**0.000**	$F_{4,24}$= 5.5	**0.000**	$F_{4,22}$=35.0	**0.045**	$F_{4,25}$=2.5	**0.000**	$F_{4,24}$=7.6
	Dr*SR	0.501	$F_{2,26}$=0.7	**0.000**	$F_{2,26}$=8.3	0.064	$F_{2,24}$=2.8	0.084	$F_{2,27}$=2.5	0.252	$F_{2,26}$=0.3
Rosette width	D	0.981	$F_{1,24}$=0.0	**0.007**	$F_{1,26}$=7.5	0.165	$F_{1,27}$=1.9	**0.013**	$F_{1,24}$=6.2	0.595	$F_{1,25}$=0.3
	SR	0.193	$F_{2,23}$=1.7	**0.000**	$F_{2,25}$=75.0	**0.000**	$F_{2,26}$=7.9	0.594	$F_{2,23}$=0.5	**0.000**	$F_{2,24}$=10.0
	Date	**0.000**	$F_{4,21}$=197.4	**0.000**	$F_{4,23}$=37.9	**0.000**	$F_{4,24}$=24.9	**0.000**	$F_{4,21}$=22.5	**0.000**	$F_{4,22}$=24.8
	Dr*SR	0.327	$F_{2,23}$=0.3	**0.003**	$F_{2,25}$=5.9	**0.003**	$F_{2,26}$=5.8	0.260	$F_{2,23}$=1.4	0.964	$F_{2,24}$=0.04
# of flowering stems	D	1.000	$F_{1,20}$=0.0	0.405	$F_{1,29}$=0.7	0.942	$F_{1,22}$=0.01	**0.003**	$F_{1,29}$=8.9	0.857	$F_{1,24}$=0.03
	SR	1.000	$F_{2,19}$=0.0	**0.000**	$F_{2,28}$=21.2	0.956	$F_{2,21}$=0.05	**0.000**	$F_{2,28}$=14.8	0.822	$F_{2,23}$=0.2
	Date	**0.000**	$F_{3,18}$=54.5	**0.000**	$F_{4,26}$=655.7	**0.000**	$F_{4,19}$=32.9	**0.000**	$F_{4,26}$=14.0	**0.000**	$F_{3,22}$=46.9
	Dr*SR	1.000	$F_{2,19}$=0.0	**0.001**	$F_{2,28}$=7.6	0.887	$F_{2,21}$=0.02	0.085	$F_{2,28}$=2.5	**0.045**	$F_{2,23}$=3.3

Plant height

There was no relationship between diversity and the development of plant height over time, except for *Leucanthemum* which showed a significant difference between the monoculture and 16 species mixture (Table 2; Fig. 2). For most species there was also no difference between the drought and control treatment, indicating that there was no effect of drought on plant height. Only *Leucanthemum* showed an (positive) effect of drought on plant height (Table 2). For *Arrhenatherum* there was a significant interaction between drought and species number (Table 2), indicating that the drought effect differs per diversity level. However, in the 16 species mixture the drought effect was adverse from what was expected, plant height was on average higher in the drought treatment.

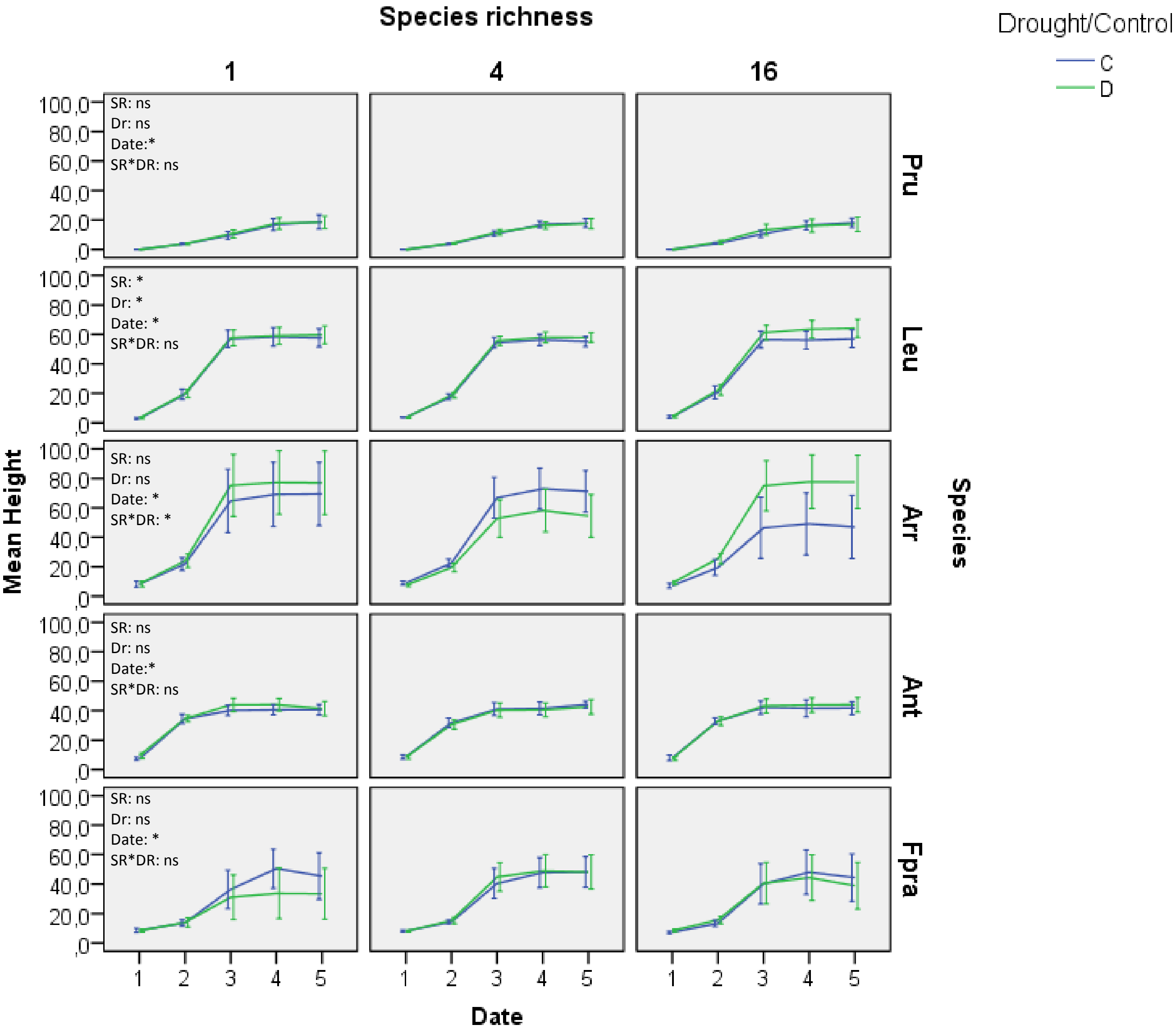

Figure 2. The growth in plant height (cm) in 1, 4 and 16 species plots and drought and control plots over time of the species *Prunella vulgaris, Leucanthemum vulgare, Arrhenatherum elatius, Anthoxanthum odoratum* and *Festuca pratensis*. Data shows means +- 2 SE.

Number of stems

For *Anthoxanthum* there was a difference in the number of stems between the drought and the control plots (Table 2). The mean number of stems was higher in the control plots of the 1 and 4 species mixtures (Fig. 3). There was also an effect of species richness (Table 2), the number of stems was higher in the monocultures and 4 species mixture compared to the 16 species mixture. Besides, *Anthoxanthum* showed an increase in the number of stems in the control plots during the drought period. As *Anthoxanthum* is an early flowering grass it already started regenerating in June before harvest. Regeneration was higher in control plots, likely due to the higher moisture content. For *Arrhenatherum* this effect is in lesser extent also visible in the control plots in the 1 and 4 species mixtures (Fig. 3). For *Leucanthemum* the number of stems was lowest in the monoculture and 4 species mixture and highest in the 16 species mixture (Table 2). The mean number of stems was higher in the drought plots. In this case there was also an interaction between the two factors (Table 2). The difference in the number of stems between the drought and control treatments was highest in the 16 species mixtures, having a higher number of stems in the drought treatment. For the other species there was no significant effect of drought or species richness on the number of stems. In this experiment the number of flowering stems was also measured. The outcomes were comparable with the number of stems and are therefore only shown in Table 2.

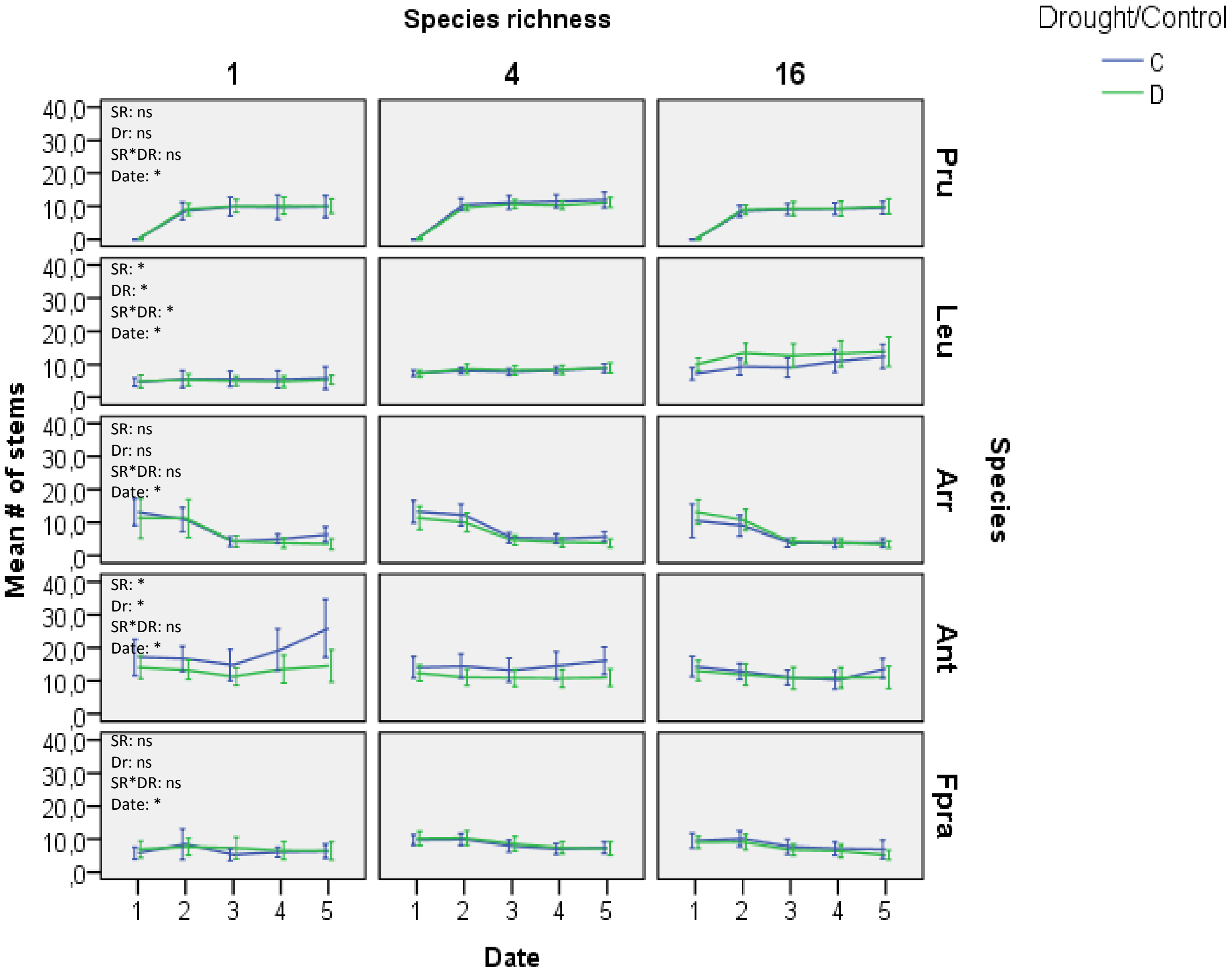

Figure 3. The number of stems in the 1, 4 and 16 species mixtures divided in drought and control plots of the species *Prunella vulgaris*, *Leucanthemum vulgare*, *Arrhenatherum elatius*, *Anthoxanthum odoratum* and *Festuca pratensis*. Data shows means +- 2 SE.

Rosette width

For *Leucanthemum* there was a drought and species richness effect (Table 2). *Leucanthemum* had a larger rosette width in the drought plots. In addition, average rosette width increased with species richness, having the highest rosette width in the 16 species mixtures. For *Leucanthemum* there was also a significant interaction between the two factors (Table 2). For *Arrhenatherum* a species richness effect was observed, having a larger rosette width in 16 species mixtures than in the monocultures and 4 species mixtures. *Anthoxanthum* had besides a higher number of stems and flowering stems also a higher rosette width in the control plots (Table 2). For *Festuca p*. a species richness effect was observed, rosette width was highest in 16 species mixtures (Table 2). However, average rosette width was higher in monocultures compared to 4 species mixtures. From figure 4 it can be seen that for all species the rosette width decreased over time. For the grasses *Arrhenatherum* and *Festuca p*. this is partly caused by the dying off of stems in an early stage (Fig. 3). However, by most species this is mainly caused by stretching of stems during height growth. In the beginning rosette width was therefore hard to measure as stems were growing vertically.

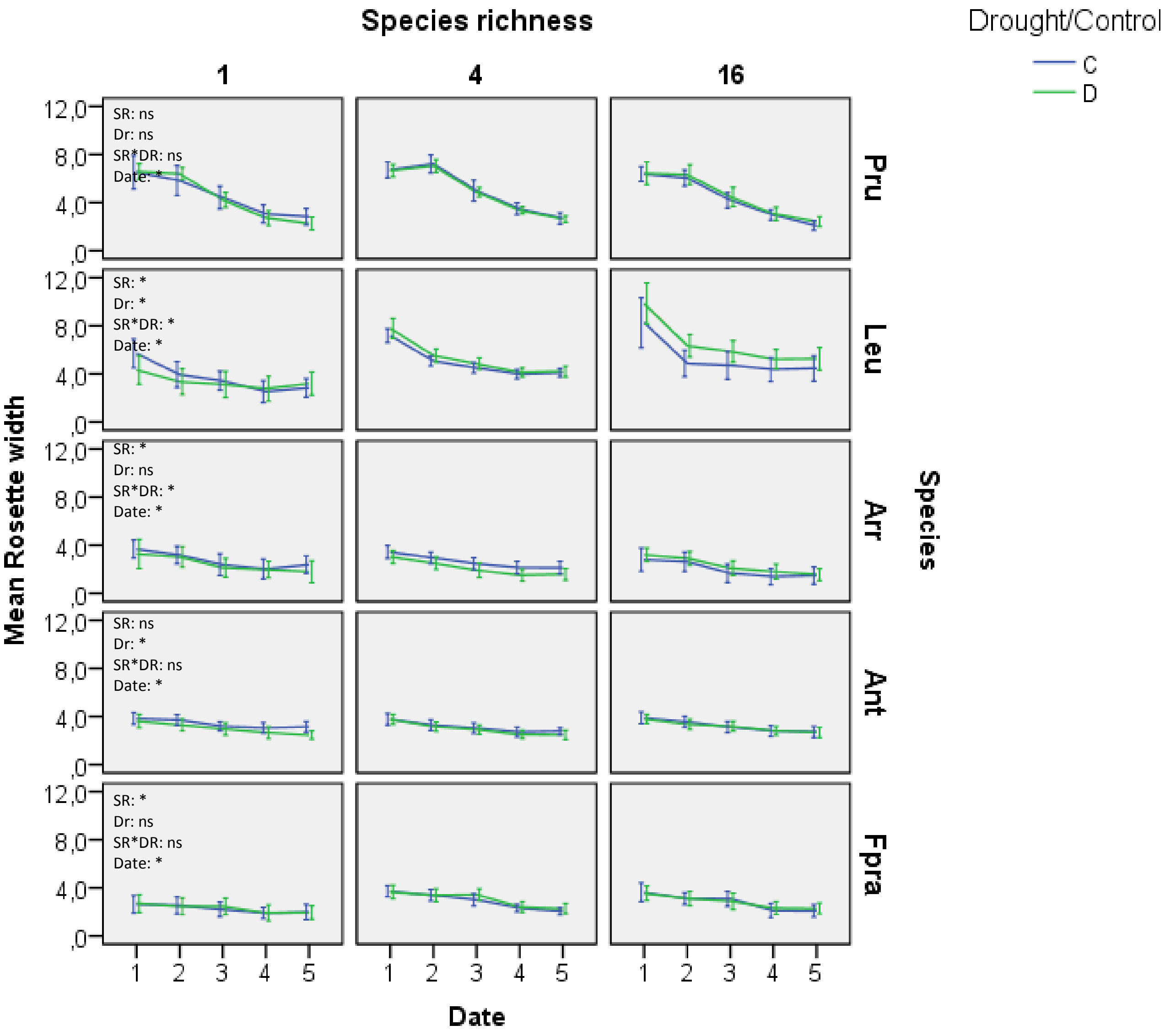

Figure 4. The average rosette/tuft width in the 1, 4 and 16 species mixtures divided in drought and control plots of the species *Prunella vulgaris*, *Leucanthemum vulgare*, *Arrhenatherum elatius*, *Anthoxanthum odoratum* and *Festuca pratensis*. Data shows means +- 2 SE

Neighboring interactions

Performance of individuals can also be influenced by neighboring species in the mixtures. Individuals of other species can have a positive, negative or neutral effect on individual plant performance. High competition between neighboring individuals could result in a lower performance during drought. From Table 3 it can be seen that the height of *Prunella* and *Leucanthemum* is not influenced by neighboring plant species. The number of stems and rosette width of Leucanthenum however was higher when *Centaurea, Ranunculus, Gallium, Arrhenatherum* and *Festuca p.* were present in the mixture. Co-occurrence with these species resulted in larger individuals of *Leucanthemum*. In general *Leucanthemum*, *Centaurea* and *Achillea* had a negative influence on the performance of grasses, indicating that these species have a high competitive ability. In contrast to the two herbs, *Leucanthemum* and *Prunella,* height of grasses is much more affected by neighboring species (Table 3).The plant height of *Arrhenatherum* and *Festuca p.* was negatively influenced by *Leucanthemum*, whereas the height of *Arrhenatherum* and *Anthoxanthum* was negatively influenced by respectively *Achillea* and *Centaurea*. *Arrhenatherum* negatively influenced the performance of *Anthoxanthum* and *Festuca p.*, whereas the grasses *Trisetum* and *Briza* had a positive effect on the performance of *Arrhenatherum* and *Festuca p.*. In addition, the height of *Anthoxanthum* was positively correlated with the presence of *Trisetum* in the mixture (Table 3).

Table 3. The effect of neighboring species on the plant performance (height, # of stems and rosette width) of Prunella, Leucanthenum, Arrhenatherum, Anthoxanthum and Festuca p.. Where 0 is no effect, - a negative effect and + a positive effect (p<0.05). Species in *italic are deep rooting.**

Height	Pru	Leu*	Ach*	Cen*	Leo	Ran	Gal*	San*	Arr*	Ant	Fpra.*	Tri	Bri	Frub	Agr	Phl*
Pru	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arr	0	-	-	0	0	0	0	0	0	0	0	0	+	0	0	0
Ant	0	0	0	-	0	0	0	0	0	0	0	+	0	0	0	0
Fpra	0	-	0	0	0	0	0	0	0	0	0	+	+	0	-	0

Stems	Pru	Leu*	Ach*	Cen*	Leo	Ran	Gal*	San*	Arr*	Ant	Fpra*	Tri	Bri	Frub	Agr	Phl*
Pru	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	0	0	0	+	0	+	+	-	+	0	0	0	0	+	0	0
Arr	0	-	0	0	0	0	0	+	0	0	0	+	+	0	0	0
Ant	0	-	+	-	-	0	0	0	-	0	0	0	0	0	+	-
Fpra	+	0	0	-	0	0	+	0	-	0	0	+	+	0	0	0

Rosette	Pru	Leu*	Ach*	Cen*	Leo	Ran	Gal*	San*	Arr*	Ant	Fpra*	Tri	Bri	Frub	Agr	Phl*
Pru	0	0	0	0	0	0	+	+	+	0	0	0	0	0	0	0
Leu	0	0	0	+	0	+	+	0	+	0	0	0	0	+	0	0
Arr	0	-	-	0	0	+	-	0	0	0	0	+	+	0	0	0
Ant	0	0	0	-	0	0	0	0	0	0	-	0	-	0	0	0
Fpra	+	0	+	0	0	0	0	+	-	0	0	+	0	0	0	0

In a plant community competition takes place at different levels. Belowground root competition for nutrients and water takes place. To optimize the uptake of resources species have developed different rooting strategies. In general plant rooting systems can roughly be divided in deep rooting and shallow rooting systems. These different rooting systems can influence the competition for resources between species. From Table 3 it can be seen that the performance of *Arrhenatherum*, which is a deep rooting species, is negatively influenced by the deep rooting species *Leucanthemum* and *Achillea*. For *Festuca p.* this could also be observed for plant height and the number of stems. Table 3 also shows that the shallow rooting species *Ranunculus*, *Trisetum* and *Briza* had a positive effect on species performance. This could imply that for deep rooting species competition for water and nutrients is stronger, whereas competition is lower between deep rooting and shallow rooting species.

Discussion

In this experiment there was for most species no effect of diversity and drought on species performance, which was expressed in plant height, number of stems and rosette width. Only *Leucanthemum* showed a drought and diversity effect for height, the number of stems and rosette width. In all cases *Leucanthemum* performed better in drought plots compared to control plots. *Leucanthemum* has from the five selected species the lowest Ellenberg moisture value, which indicates that it can grow quite well in moderate to dry circumstances (Table 1). The TDR measurements also showed that the moisture content was lower in plots where *Leucanthemum* was present. *Leucanthemum* had a high performance compared to other species (Fig. 1 and 2), a high biomass and transpiration probably have resulted in a higher water and nutrient use, thereby reducing the resource availability of other species especially under dry conditions. As showed in table 3 this resulted in a lower performance of other species. This is in line with the expectation that interspecific interactions play important role in mixtures during drought. Besides, this indicates that species with a low Ellenberg moisture value have a higher competitive advantage during drought, probably because they are better adapted to dry conditions. This could also explain the higher height of *Arrhenatherum* in the drought plots of 16 species mixtures. *Arrhenatherum* also has a relatively low Ellenberg moisture value (Table 1), indicating that the Ellenberg moisture value can be used to explain a species' tolerance to drought.

The higher performance of Leucanthenum in drought plots could indicate that deep rooting species with a low Ellenberg moisture value have a higher drought resistance. The high competitive ability of the deep rooting species *Achillea* and *Centaurea* could confirm this indication (Table 3). However, it also seems that competition for resources between deep rooting species is stronger than competition between shallow and deep rooting species (Table 3). This could possibly be explained by the fact that shallow rooting species are dependent on rainfall, whereas deep rooting species also obtain water from deeper soil layers. A high rooting density in the top soil increases the water holding capacity (Van Dijk & Van Miltenburg, 2013). Shallow rooting species could therefore facilitate other species in the uptake of water. For deep rooting species competition could be higher, as less rain water will reach the deeper soil layers. This is especially the case on sandy soils were the capillary rise is low, resulting in a lower uptake capacity. The presence of deep rooting species also didn't lead to a better performance of the shallow rooting species *Prunella* and *Anthoxanthum*, indicating that there was no strong facilitative effect of hydraulic lift.

In this experiment only five species were followed over time. Only a few species and their traits were included in the experiment, this makes it difficult to observe and draw hard conclusions. It should therefore be better to correlate the performance with the Ellenberg moisture value and the actual rooting depth of all 16 species. This could give a good indication of which trait is important in determining a species' susceptibility to drought. Due to the limited amount of time actual rooting depth and biomass measurements could not be included in the analysis. In this study plant height, rosette width and the number of stems were used as a proxy for plant performance. However, it is likely that biomass is a more direct indicator of performance, possibly resulting in more clear patterns.

The reason that there was for most species no effect of drought on species performance could be caused by the time of the drought. Drought started at the beginning of June, some species however already reached maturity in May. This can be seen from Figure 1, after the second and third measure date the plant height is stable for *Leucanthemum* and *Anthoxanthum*. It is likely that this is the reason that for *Anthoxanthum* there was no effect of drought on plant height, while there was an effect of drought on the number of stems and rosette width. Wang et al. (2007) mentioned that pre-perturbation biomass determines a species' resistance to drought. However, it could also be that pre-perturbation performance in general determines the susceptibility of a species to drought. If a species or community has a high performance and develops fast in spring, it will have a higher resistance to a summer drought as it already reached maturity and ceased blossoming in an early stage.

Another important reason is that the moisture levels of the control treatment were also low at the end of the drought period (Fig. 1), it is therefore likely that plants in the control plots were also having drought stress. Besides, the spring and start of the summer in the Netherlands were dry. At the start of the drought in the beginning of June the precipitation deficit was already 90 mm compared to 50 mm on average (KNMI, 2015). The soil used in the experiment is a sandy soil with a low OM content, which has a low water holding capacity. This makes that the soil is very drought sensitive. Despite the fact that the plots were artificially watered several times before the drought period, the average moisture content was already quite low at the start of the drought period (Fig. 1). It is therefore likely that there was already some drought stress in the period before the simulated drought. In addition, temperatures were high in the third and fourth week of the drought period, the temperature under the roof achieved even values above 40 degrees Celsius. These high temperatures probably have resulted in heat stress, affecting plant performance in both drought and control plots.

In this experiment most species performed equally in drought and control plots. Only *Leucanthenum* had a higher performance in drought plots, indicating a higher drought resistance for this species. According to Ingram & Bartels (1997) most temperate herbaceous species are resilient rather than resistant. It therefore could be that resilience is a much more important plant character for indicating how well plant species can withstand drought. In this research it was not possible to look at resilience, as for determining the resilience the biomass in the next year should be measured.

Several studies found an increasing diversity-productivity relationship over time (e.g. Tilman et al., 2001; Van Ruijven & Berendse, 2005). In this study several species already showed a species richness effect in the second year. If a species performs better in monocultures or mixtures depends on interspecific interactions. *Anthoxanthum* had a higher performance in monocultures, indicating that it has a low competitive ability, whereas *Leucanthemum* and *Arrhenatherum* had a higher performance in mixtures, indicating that these species have a strong competitive ability. The performance of *Prunella* and *Anthoxanthum*, both species of wet growing conditions, didn't increase with species richness in drought plots. This implies that diversity didn't contribute to a better performance of moist loving species under drought.

Conclusion

In this experiment interspecific interactions had an effect on the performance of individuals in mixtures during drought. *Leucanthemum* had a high competitive ability, thereby having a negative influence on the performance of individuals of *Arrhenatherum*, *Anthoxanthum* and *Festuca p*.. Although it is only the second year of the experiment for several species an effect of species richness on individual species performance was observed. Although the results should be interpreted with care, as only a few species and thereby traits were included, there are indications that both rooting depth and Ellenberg moisture value could be used to explain a species' susceptibility to drought. According to the IPCC it is expected that the frequency and intensity of prolonged summer droughts will increase. This development seems to be in favor of species adapted to dry circumstances. A higher species richness didn't contribute to a better performance of moist loving species under drought in this experiment. It therefore seems assumable that the species composition of grassland communities in Western Europe will change and that overall diversity will decrease in future. However, this could still have an impact on the performance of plant communities, as the temporal stability to other perturbations like insect infestations is often found to be higher in communities with a high plant diversity.

References

Caldwell, M.M. & Richards, J.H. 1989. Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. Oecologia 79, 1-5.

Chaves, M.M., Maroco, J.P. & Pereira, J.S. 2003. Understanding plant responses to drought-from genes to the whole plant. Functional Plant Biology 30, 239-264.

Chaves, M.M. & Oliveira, M.M. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. Journal of Experimental Botany 55: 2365–2384.

Ellenberg, H., Weber, H.E., Dill, R., Wirth, V., Werner, W. & PauliBen, D. 1992. Zeigerwerte von Pflanzen in Mitteleuropa, 2nd ed. Scr. Geobot. 18: 1-258.

Geerts, R., Korevaar, H. & Timmerman, A. 2014. Kruidenrijk grasland, Meerwaarde voor vee, bedrijf en weidevogels. Plant Research International, Wageningen UR.

Grime, J.P. 1997. Biodiversity and ecosystem function: the debate deepens. Science 277, 1260-1261.

Grime, J.P., Brown, V.K., Thompson, K., Masters, G.J., Hillier, S.H., Clarke, I.P., Askew, A.P., Corker D, & Kielty J.P. 2000. The response of two contrasting limestone grasslands to simulated climate change. Science 298: 762–765.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G. et al. 1999. Plant diversity and productivity experiments in European grasslands. Science, 286, 1123–1127

Hooper, D. U. & Vitousek, P.M. 1997. The effects of plant composition and diversity on ecosystem processes. Science 277, 1302-1305.

Ingram J. & Bartels D. 1996. The molecular basis of dehydration tolerance in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47, 377–403.

IPCC: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, edited by Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Kahmen, A., Perner, J. & Buchmann, N. 2005. Diversity-dependent productivity in semi-natural grasslands following climate perturbations. Functional Ecology, 19, 594–601.

KNMI, 2011. De Bosatlas van het klimaat; Langjarige gemiddelden 1981-2010. Noordhoff uitgevers/KNMI.

KNMI, 2014. Maandoverzicht van het weer in Nederland, juni 2014. Koninklijk Nederlands Metereologisch Instituut (KNMI). http://www.knmi.nl/klimatologie/mow/pdf/mow_201406.pdf

KNMI, 2015. Neerslag tekort in Nederland in 2015. Visited on July 30. http://www.knmi.nl/klimatologie/geografische_overzichten/neerslagoverschot_tijdgrafiek.html

MacGillivray, C.W., Grime, J.P. & Team, T.I.S.P. 1995. Testing predictions of the resistance and resilience of vegetation subjected to extreme events. Functional Ecology, 9, 640–649.

Marquard, E., Weigelt, A., Temperton, V.A., Roscher, C., Schumacher J., Buchmann, N., Fischer, M., Weisser, W.W. & Schmid, B. 2009. Plant species richness and functional composition drive over yielding in a six-year grassland experiment. Ecology 90 (12), 3290-3302.

Mokany, K., Ash, J. & Roxburgh, S. 2008. Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. J Ecol 96: 884–893.

Naeem, S. & Wright, J. P. 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. Ecol. Lett. 6, 567-579.

Pfisterer, A.B. & Schmid, B. 2002. Diversity-dependent production can decrease the stability of ecosystem functioning. Nature 416, 84–86.

Reichstein, M., Ciais, P., Papale, D., Valentini, R. et al. 2007. Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modeling analysis. Global Change Biology 13, 634-651.

Roscher, C., Schumacher, J., Gubsch, M., Lipowsky, A., Weigelt, A., Buchmann, N., Schmid, B. & Schulze, E. 2012. Using plant functional traits to explain diversity-productivity relationships. PLoS ONE 7(5), e36760.

Schumacher, J. & Roscher, C. 2009. Differential effects of functional traits on aboveground biomass in semi-natural grasslands. Oikos 118; 1659-1668.

Tilman, D. & El Haddi, A. 1992. Drought and biodiversity in grasslands. Oecologia 89, 257-264

Tilman, D. & Downing, J.A. 1994. Biodiversity and stability in grasslands. Nature, 367, 363–365.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T. & Lehman, C. 2001. Diversity and productivity in a long-term grassland experiment. Science, 294, 843-845

Tilman, D., Reich, P.B. & Knops, J.M.H. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441, 629–632.

Van Dijk, M. & Van Miltenburg, S. 2013. De invloed van bodemstructuur op het watersysteem; Een verkenning. Stichting Toegepast Onderzoek Waterbeheer (STOWA). Rapportnummer 13A.

Van Eekeren, E., Deru, J., de Boer, H. & Philipsen, B. 2011. Terug naar de graswortel; Een betere nutriëntenbenutting door een intensievere en diepere beworteling. Louis Bolk Instituut.

Van Ruijven, J. & Berendse, F. 2005. Diversity-productivity relationships: initial effects, long-term patterns, and underlying mechanisms. Proceedings of the National Academy of ScienceUSA 102, 695–700.

Van Ruijven, J. & Berendse, F. 2007. Contrasting effects of diversity on the temporal stability of plant populations. Oikos 116, 1323–1330.

Van Ruijven, J. & Berendse, F. 2010. Diversity enhances community recovery, but not resistance, after drought. Journal of Ecology 98, 81-86.

Vogel, A., Scherer-Lorenzen, M. & Weigelt, A. 2012. Grassland resistance and resilience after drought depends on management intensity and species richness. PLoS ONE 7 (5), e36992.

Wang, Y.F., Yu, S.X. & Wang, J. 2007. Biomass-dependent susceptibility to drought in experimental grassland communities. Ecology Letters 10, 401–410.

Yachi, S. & Loreau, M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. Proceedings of the National Academy of Science USA, 96, 1463–1468.