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Do interactions with neighbours modify the above-ground productivity response to drought? A test with two grassland species

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Abstract

Natural systems are predicted to be exposed to more frequent and more intense drought events in the near future. Plant-plant interactions form an important part of the whole of mechanisms that govern the responses of plant species and communities to drought. The accuracy of predictions on ecosystem functioning would therefore be improved by determining when plant interactions need to be considered and how these interactions can drive species responses. In this study, we assessed the effect of neighbour plants on the drought response of a target plant. Two grassland species (*Plantago lanceolata* and *Lolium perenne*) were grown in the presence of either six conspecific or three conspecific and three heterospecific neighbours in sunlit growth chambers. They were subjected to drought by withholding water for 20 days. Regardless of the identity of the target plant, having *P. lanceolata* as a neighbour increased the susceptibility to negative drought effects on biomass production while *L. perenne* neighbours buffered the target's drought response. Our results therefore suggest that drought responses depended largely on the identity and the traits of the neighbours. Such findings demonstrate that current models to predict ecosystem functioning may be misleading by not sufficiently taking plant-plant interactions into account.

Keywords:

drought, plant interactions, biomass production, conspecific neighbours, heterospecific neighbours

Introduction

Natural systems are increasingly subjected to environmental change. Low soil water availability caused by drought is one of the major limitations for plant growth (Chaves and Oliveira, 2004; Schulze et al., 1987), while the probability and severity of drought episodes is projected to further increase (IPCC, 2013). Drought studies have therefore been carried out in considerable numbers, typically focusing on the morphological, physiological and biochemical changes of individual plants (e.g. Chaves et al., 2002; Flexas et al., 2004; Lawlor and Tezara, 2009). Decreased water availability limits plant productivity mainly by stomatal closure, down-regulation of photosynthesis, slower cell expansion and/or by carbon allocation to the roots and to protective molecules (Benjamin and Nielsen, 2006; Chaves et al., 2002; Praba et al., 2009). However, how individual plants in a community respond to water deprivation is not only determined by species-specific characteristics but also by the nature of interactions with neighbouring plants.

Differences between species in the ability to sequester resources and to produce biomass lead to diverse plant-plant interactions involving competition and partitioning of resources in varying degrees (Hooper, 1998; Hooper et al., 2005; Verheyen et al., 2008). These interactions are obviously critical in determining the outcome of the response to resource depletion. For instance, if competition between species for water is low, a species will likely respond less to water deprivation when surrounded by neighbours from the other species (= heterospecific) than when surrounded by neighbours from the same species (= conspecific) (if the water content before the onset the drought is similar, see Van Peer et al. 2004). Spatial complementarity in water use occurs when species differ in root distribution, so that heterospecific neighbours at least partially extract water from different soil zones (Nippert and Knapp, 2007; Verheyen et al., 2008). On the other hand, differences in water use between species can cause an altered timing and intensity of soil drying, which can favour or

disadvantage the target's susceptibility to water deprivation. If a species is surrounded by neighbours with a high water consumption, it will likely suffer from water deprivation earlier than if it were surrounded by water saving species, irrespective of whether its neighbours are con- or heterospecific.

In this study, two target species were subjected to drought by withholding water for 20 days. To investigate the importance of neighbours on the drought response, the targets were surrounded either by six conspecific or by an equal mix of conspecific and heterospecific neighbours. We hypothesize that, if complementarity is limited, such as is presumably the case for the two species we selected (Weeve 1975), neighbours can favour or disadvantage target plants, by altering the water availability for the targets, depending on the rate of water use of the neighbours. To detect these effects, we determined above- and below-ground biomass, soil water status, stomatal conductance and photosynthesis.

Materials and Methods

Experimental set-up

For this study, two common and co-occurring grassland species with comparable height and rooting depth (Weeve, 1975) (*Lolium perenne* L. and *Plantago lanceolata* L.) were each grown as target plant surrounded by six neighbour plants at equal distances. The six neighbours were either conspecific to the target (further referred to as 'conspecific neighbours') or composed of three conspecific and three heterospecific plants (further referred to as 'heterospecific neighbours') (Fig. 1). This yields four designs with different combinations of targets and neighbours: *L. perenne* or *P. lanceolata* with conspecific or heterospecific neighbours (further referred to as L_{con}, L_{het}, P_{con} and P_{het}, respectively) (Fig. 1). The species were sown end of March 2010, with a time lag to prevent differences in size at the start of the experiment (Cotrufo and Gorissen, 1997) due to differences in germination rate

(*P. lanceolata* day of year (DOY) 76, *L. perenne* DOY 88). The seedlings were transplanted end of April (DOY 116 - 118) in PVC containers (19 cm inner diameter, 40 cm height, 11.33 L), filled with sandy soil (93.2% sand, 4.6% silt, 2.2% clay; field capacity 0.13 $m^3\ m^{-3}$; pH 7.6; total Kjeldahl-N 0.42 g kg^{-1}; 1% C in humus). The seven plants were placed in a hexagonal grid at 5 cm interspace with the target species positioned at the centre of the grid. All communities were fertilised with 10 g m^{-2} NH_4NO_3, 5 g m^{-2} P_2O_5, 10 g m^{-2} K_2O and micro-elements (Fe, Mn, Zn, Cu, B, Mo). The fertiliser was given dissolved in water in two equal amounts at DOY 140 and 180.

Three replicates per design (yielding 12 containers) were randomly placed in each of eight sunlit, climate-controlled chambers facing south. The study was conducted in climate controlled greenhouses so that water deprivation was the only environmental variable. The chambers followed fluctuating air temperatures mimicking an average daily air temperature course, calculated over the period 1996 - 2005. The distances between the chambers were maximised to avoid mutual shading. The interior surface area was 1.5 x 1.5 m, the height at the north side 1.5 m and at the south side 1.2 m. The top of the chambers consisted of a colourless polycarbonate plate (4 mm thick), whereas the sides were made of polyethylene film (200 μm thick), both UV transparent. The experimental set-up is located at the Drie Eiken Campus, University of Antwerp, Wilrijk, Belgium (51º 09′ N, 04º 24′ E), where average annual precipitation is 776 mm (evenly distributed throughout the year) and average annual air temperature 10.6 ºC. Every half hour, the air temperature was monitored with a temperature sensor (Siemens, type QFA66, Erlangen, Germany). During the experiment (28 April – 5 August 2010, DOY 118 - 217), monthly average air temperature was 12.7, 18.4 and 20.9 °C in May, June and July, respectively.

Irrigation was calculated from the monthly rainfall over the period 1995-2005 and corrected for differences in evapotranspiration (ET) inside and outside the chambers. To this end, De

Boeck et al. (2006) calculated ET inside the chambers from changes in soil water content (SWC) and the amount of administered water, and the outside ET with Hamon's equation (Haith and Shoemaker, 1987) based on day length, vapour pressure and air temperature. The containers were watered every two days according to the 10 year average of 14 to 15 raining days per month during the growing season. Total monthly irrigation matched 61.5, 64.4 and 85.1 mm in May, June and July, respectively. Water could freely drain from the containers while capillary rise of ground water towards the containers was prevented by a drainage system placed below the chambers.

Imposed drought

Each design was subjected to two treatments: a drought treatment and a control treatment (Fig. 1). The treatments were given at chamber level. Four of the eight chambers were subjected to a period of water deprivation by withholding water for 20 days (DOY 197 - 217) (referred to as 'imposed drought' or 'drought'). In the other four chambers, the watering regime was not interrupted (control treatment). The length of the imposed drought was chosen to be severe but not extreme, based on a previous experiment in the same climate chambers and on the same soil, in which mesocosm communities were exposed to differing lengths of imposed drought periods (Naudts et al., 2011). In each chamber, one container per design contained a profile probe tube for the PR2 soil moisture sensor (Delta-T Devices Ltd., UK). The probe was placed next to the centre of the container. Soil water content (SWC) was measured once a week before the imposed drought (DOY 130 - 193) and twice a week during drought (Table 1). For each container SWC was measured at three different depths (0, 20 and 40 cm) from which the mean was calculated.

Biomass harvest

The total leaf area of the communities (targets + neighbours) was determined with a portable area meter (LI-3000A, Li-COR, NE, USA) just before the imposed drought (DOY 197) in one replicate per chamber (drought treated and control communities were still identical at this moment). Above-ground biomass of the targets was harvested immediately after the imposed drought (DOY 217) in the two remaining replicates per design per chamber, in control chambers as well as in drought treated chambers. Root biomass of the communities was determined in one of these replicates. Root samples were washed until they were free of soil. All plant material was dried at 70 °C for 48 h and then weighed. See Table 1 for details on replicates and statistical analysis.

Gas exchange

All gas exchange measurements were conducted between 9 and 10 am on clear days to assure comparable light conditions. Leaf stomatal conductance (g_s) of the targets was measured five times during the imposed drought (DOY 197, 207, 210, 214 and 217), on the most recently matured leaf with a porometer (AP4, Delta-T Devices Ltd., UK), taking into account abaxial and adaxial sides of the leaves. Measurements were performed at the environmental conditions in the chambers which is most relevant to detect the drought impact on stomatal conductance. Before the start of the imposed drought (DOY 197) the pre-drought levels were measured on one replicate per design in each of the eight chambers. During and at the end of the drought two replicates per design were measured in each of the four chambers per treatment, yielding eight measurements per design and treatment combination (see Table 1). Light-saturated net CO_2 assimilation rate (A_{sat}) of the targets was determined on the most recently matured leaf with a portable gas exchange system (LI-6400, Li-COR, NE, USA) at the end of the imposed drought (DOY 217), in each of the four chambers per treatment (Table

1). Leaf chamber conditions were controlled at 380 ppm CO_2 and 23.5 °C (block temperature) at saturating photon flux density (1500 µmol m^{-2} s^{-1}) and ambient relative humidity.

Data analysis

The experiment had a split-split plot design with 'treatment' (control vs. drought) as the main plot factor, 'target species' (*L. perenne* vs. *P. lanceolata*) as the subplot factor and 'neighbours' (conspecific vs. heterospecific) as the sub-subplot factor (Fig. 1). Measurements were conducted on the targets or on the whole community. They were conducted once or repeated over time. All measurements were analysed with analyses of variance (ANOVA) (see Table 1 for an overview). Chamber was always included in the model as a random factor nested within treatment to test for possible chamber effects. The factor chamber was never significant so was always excluded from the model. Non-significant interaction terms were excluded from the model, while in case of significant interactions, the means were compared a posteriori with Tukey corrections for multiple comparisons (see Table 2 and 3). Analyses were performed in SAS (version 9.1, SAS Institute Inc., Cary, NC) using the mixed procedure (Littell et al., 1996).

Above-ground biomass and A_{sat} of the targets were analysed separately for each target species with treatment and neighbours as fixed factors. The course of g_s was measured only in the drought treated targets and was analysed separately for each target species by repeated measures (RM) ANOVA with DOY and neighbours as fixed factors. Additionally, g_s just before the drought period (DOY 197), was compared between the two target species with target species and neighbours as fixed factors and (Fig**.** 1).

Root biomass was measured at community level and analysed separately per target species with treatment and neighbours as fixed factors. To compare total leaf area before the imposed drought between all four designs the ANOVA was performed over both species, with design

as fixed factor. Mean SWC before and during drought was analysed with RM-ANOVA with design and DOY as fixed factors. For the analysis of SWC during drought over the three different depths, the fixed factor depth was added to the model.

Results

Biomass

The effect of an imposed drought on the above-ground biomass production of the target species differed whether the targets were grown with conspecific or with heterospecific neighbours. Moreover, the effect of neighbour type was opposite for the two target species (Fig. 2). *Lolium perenne* targets grew as well under drought as under control conditions when surrounded by conspecific neighbours, while biomass production was reduced by the imposed drought when surrounded by heterospecifics (treatment × neighbour significant, Table 2). For *P. lanceolata* it was the other way around: the targets grew as well under drought as under control conditions when surrounded by heterospecific neighbours, while biomass production was reduced by an imposed drought when surrounded by conspecifics (treatment × neighbour significant, Table 2). Hence in both cases having *P. lanceolata* as a neighbour, either conspecific or heterospecific, resulted in a more negative response to drought. The impact of water deprivation on root biomass did not differ between the designs for *L. perenne* nor for *P. lanceolata* (no treatment × neighbour interaction, Table 2). Root biomass decreased with drought for *P. lanceolata* (from 15.72 ± 0.88 (SE) to 11.99 ± 0.88 g m^{-2}) while it did not decrease for *L. perenne* (15. 41 ± 1.10 and 12.88 ± 1.10 g m^{-2} in drought and control treatment, respectively).

Soil water status

Before the imposed drought, SWC was on average 0.12 ± 0.01 $m^3 m^{-3}$ (which is close to field capacity) and no significant differences were observed between the different designs (P_{con}, L_{con} or heterospecific) (Table 3). From the onset of drought, SWC (mean over three different depths) decreased in the drought treatments, with the decrease being faster in P_{con} than in L_{con} or the heterospecific designs (significant DOY × design interaction, Table 3; a posteriori comparison, Fig. 3). As a result, SWC in P_{con} designs reached its lowest point (wilting point) earlier than in the other designs. In addition, SWC was lower in P_{con} designs than in the heterospecific designs during half of the drought period (DOY 203, 208 and 211) and lower than L_{con} on DOY 208 and 211. These results demonstrate that containers with only *P. lanceolata* consumed more water than containers with *L. perenne* or with both species. The same conclusion is drawn when analysing SWC at the different depths. Although all designs dried faster at the surface than in the deeper layers, the faster decline of SWC in P_{con} designs applied at each of the three depths (significant DOY × design × depth interaction, Table 3; a posteriori comparison, Fig. S1 and Table S1). Furthermore, SWC of P_{con} being lower than the heterospecific designs during half of the drought period applied to depth = 0 and 20 cm and for depth =40 cm SWC was lower only on DOY 208 and 211; this because the soil dries slower at the bottom of the containers.

Gas exchange: g_s and A_{sat}

At the start of the imposed drought (DOY 197), g_s was significantly higher in *P. lanceolata* than in *L. perenne*, irrespective of neighbour type (Table 3, Fig. 4). To be able to compare the potential water consumption among the different designs at the start of the imposed drought, both g_s and total leaf area need to be considered. Total leaf area at the start of drought did not differ between the designs (0.15 ± 0.01 m^2 (SE), Table 3). Since *P. lanceolata* had the highest g_s, water consumption was expected to increase with an increasing abundance of *P.*

lanceolata. Given that P_{con} systems contained the most individuals of *P. lanceolata*, these communities were expected to consume the most water while L_{con} systems contained no *P. lanceolata* individuals and therefore were expected to consume the least amounts of water. This was confirmed by SWC data, showing both a lower soil water content in P_{con} systems during half of the drought period and a faster decline of SWC in P_{con} (Table 3, Fig. 3). During the imposed drought we observed a decrease of g_s for both species, which was unaffected by neighbour identity at any time (Table 3, Fig. 4). The drier soils found in P_{con} did therefore not cause the stomata of the P_{con} targets to close earlier.

At the end of the imposed drought, A_{sat} of *P. lanceolata* was lower in the drought treatment than in the control treatment, irrespective of neighbour type (no treatment × neighbour interaction; Table 2, Fig. 5). For *L. perenne*, on the other hand, the response of A_{sat} depended on neighbour type (treatment × neighbour interaction), with a significant decrease of A_{sat} observed for L_{het} during drought, while no significant effects of drought on maximum photosynthesis were found for L_{con} (Table 2, Fig. 5).

Discussion

Our results demonstrate that a plant's response to abiotic stress can be affected not only by its own inherent traits, but also to an important extent by the nature of its neighbours. Indeed, both *Plantago lanceolata* and *Lolium perenne* suffered more from drought in terms of a lower productivity if surrounded by more *Plantago* individuals or, from another point of view, by less *Lolium* neighbours. We propose that several mechanisms potentially contribute to this differential neighbour effect. First, the higher water consumption of *Plantago* compared to *Lolium*, which caused a faster decrease of SWC in P_{con} than in P_{het}, could have been an important driver. Although both species responded to soil drying by decreasing their stomatal conductance, the faster decreasing SWC in P_{con} designs did not cause the stomata of those

targets to close earlier than P_{het} targets. This raises the question why aboveground biomass was nevertheless more severely affected by the drought in P_{con} than P_{het}? The fact that the biomass of P_{het} targets did not decrease to a significant extent upon three weeks of drought, may reflect that g_s only reached low levels (< 200 mmol m^{-2} s^{-1}) in the last 10 days of drought. For P_{con} targets, the lower soil water availability presumably suppressed photosynthesis by non-stomatal limitations such as inhibition of the internal CO_2 transport (Flexas et al., 2004; Lawlor and Tezara, 2009) or down-regulation of the electron transport (Damour et al., 2009; Llorens et al., 2003).

A second mechanism for the effect of neighbour composition on the drought response of the *Plantago* targets may be found in the competitive behaviour of *Lolium*. This species has been reported to suppress root production of several herbaceous species mainly in the top soil and less in the deeper layers (Wardle and Peltzer, 2003). Although in our study the total root production was not altered by neighbour composition, a higher presence of *Lolium* neighbours may have displaced root production of the *Plantago* targets towards the deeper layers. As observed in the analysis of SWC at three different depths, the soil dries faster at the top than at the bottom. If *Plantago* targets indeed root deeper when surrounded by more *Lolium* neighbours (as in P_{het}), prolonged access to soil water could ensue. Apart from a negative impact of *Plantago* on the available soil water because of its greater water use, the differential effect of the two species on their neighbours may therefore also have been caused by an indirect positive effect of *Lolium* on water extraction. In a study on two desert grassland species under drought conditions by Novoplansky and Goldberg (2001), one species as a neighbour caused an increase in the root/shoot ratio of both target species compared to when the other species was the neighbour. This is reminiscent of our suggestion that *Lolium* may have displaced the roots of its neighbours.

Finally, for *Lolium*, SWC did not decrease significantly faster when growing together with *Plantago*, although it too suffered from a lower productivity under drought (and a lower maximum photosynthesis) in the presence of *Plantago*. We speculate that local horizontal differences in SWC may have been involved. In a study on grass-shrub interactions, neighbour species have been found to locally increase water availability through shading, reducing evaporation (Maestre et al., 2003). Structural differences between species, here between the more pending leaves of *Lolium* and the more erect *Plantago* leaves, may have locally buffered soil drying in the L_{con} target positions.

One might expect that an individual would suffer less from drought when surrounded by heterospecific neighbours than when surrounded by conspecific neighbours due to complementarity. Obviously this not necessarily the case as the intrinsic absolute water consumption of the neighbour might overrule any benefit from complementarity. Conversely, having a high water consumer as a neighbour could be less negative than expected when its roots extract water predominantly from a different soil zone than its heterospecific neighbours and as consequence more of the total amount of soil water is used (Nippert and Knapp, 2007; Verheyen et al., 2008). In this way, complementarity may diminish a negative effect (or amplify a positive effect). As a consequence, selecting a different species pair with other traits may yield different results.

Comparing the current study with others is difficult since most related experiments and conceptual models focus on how gradients of resource availability modify plant-plant interactions (Gaucherand et al., 2006; Goldberg and Novoplansky, 1997; Grime, 1979; Tilman, 1988).When looking at the current study from this point of view one could conclude that under control conditions neighbours determine the aboveground biomass of the targets while under drought conditions neighbours have no impact (Fig. 2). However, no statistical differences were detected between targets surrounded by conspecific or heterospecific

neighbours under control conditions for both species. Furthermore, it was our choice for the current study to focus on the effect of plant-plant interactions on plant responses to differences in resource availability, rather than on the opposite (the effect of resource availability on interactions). In this way we concur with Brooker (2006), who stated that a major challenge is to understand the role of plant-plant interactions in mediating the response of natural systems to environmental changes. Disentangling the role of plant interactions can help to clear the controversies on the role that plant diversity plays in the resistance to stress, i.e. does greater plant diversity improve (Frank and McNaughton, 1991; Tilman, 1996; Tilman and Downing, 1994) or reduce (Pfisterer and Schmid, 2002; Van Peer et al., 2004) the system's resistance to stress? Recent studies have suggested that community composition may be more important than plant diversity per se, but general mechanisms have not been found (Baggs et al., 2003; Lawlor and Tezara, 2009; Prieto et al., 2009; Wilson, 2007). Studies like this can be a catalyst in the search as to when diversity effects like complementarity are overridden by species-specific effects. Future research efforts should encompass a wider array of species to unravel the mechanistic basis needed for further upscaling and generalisation. This is for example of relevance if plant-plant interactions are to be included in models that predict ecosystem functioning in a future climate. Currently, bioclimatic or climate envelope models relate species current geographic distributions to a set of current climatic factors, using predicted future climate variables as inputs for these models to predict future distributions. Unfortunately, these models largely ignore biotic relationships, in which the distribution of one species is affected by the distribution of one or more other species (Lawler et al., 2006; Pearson and Dawson, 2003).

Conclusion

This study demonstrates that drought responses of two target species may depend largely on the identity and the traits (such as water consumption, rooting distribution) of the neighbours. This finding illustrates that models to predict ecosystem functioning should take plant-plant interactions into account. Then again, plant density could strengthen or weaken interactions, so future experiments should also include this factor. Our study, together with similar studies that explore traits such as plant fitness and seed production in the long term, can help to elucidate how plant-plant interactions interact with changes in abiotic conditions in order to more accurately project the structure and distribution of plant communities in a future climate.

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Table 1 Details on measured parameters; above-ground (AG) biomass, root biomass, leaf area, stomatal conductance (g_s), light saturated net CO_2 assimilation rate (A_{sat}), volumetric soil water content (SWC); and details on the analyses of variance (ANOVA). Parameters were measured on the targets or on the whole community (target + neighbours; referred to as 'total'). Day of year (DOY), number of chambers, number of replicates (rep) and number of repetitions (if applicable) are presented.

		start of drought DOY 197	during drought	end of drought DOY 217	ANOVA	
		8 chambers identical	4 chambers drought 4 chambers control		compare between…	fixed factors
AG biomass	target	/	/	2 reps/chamber	2 designs/species	treatment, neighbours
leaf area	total	1 rep/chamber	/	/	all 4 designs	design
root biomass	total	/	/	1 rep/chamber	2 designs/species	treatment, neighbours
g_s (start drought)	target	1 rep/chamber	/	/	two target species	target species, neighbours
g_s (during)	target	/	2 reps/chamber DOY 207, 210, 214	2 reps/chamber	2 designs/species	DOY, neighbours
A_{sat}	target	/	/	2 reps/chamber	2 designs/species	treatment, neighbours
		before drought DOY 130-197	during drought	end of drought DOY 217		
SWC	total	1 rep/chamber 1/week	1 rep/chamber 2/week		all 4 designs	DOY, design

Table 2 Analysis of variance (ANOVA) of above-ground (AG) biomass of targets, root biomass of the communities and light-saturated net CO_2 assimilation rate (A_{sat}) of targets. The designs consist of a target species (*Lolium perenne* or *Plantago lanceolata*) either surrounded by six conspecific neighbours (con) or by three conspecific and three heterospecific neighbours (het). They were subjected to an imposed drought and a control treatment. Degrees of freedom (DF), F values and significance levels (p); p-values are presented in bold when significant (< 0.05). In case of a significant interaction the means of the treatments (drought vs. control) were compared between the two designs per target species with Tukey corrections for multiple comparisons; in case of non-significance of the interaction, the effect of treatment, irrespective of neighbour type, is presented.

	treatment × neighbour			a post. comparison of treatment		treatment		
	DF	F	p	con (p)	het (p)	DF	F	p
AG biomass target								
L. perenne designs	1,28	5.62	**0.025**	0.980	**0.011**	/	/	/
P. lanceolata designs	1,28	4.53	**0.042**	**0.009**	0.973	/	/	/
Root biomass								
L. perenne designs	1,8	0.58	0.467	/	/	1,9	2.78	0.130
P. lanceolata designs	1,8	0.01	0.975	/	/	1,9	9.93	**0.012**
A_{sat} target								
L. perenne designs	1,28	5.38	**0.026**	0.647	**0.007**	/	/	/
P. lanceolata designs	1,28	2.12	0.153	/	/	1,6	13.55	**0.010**

Table 3 Analysis of variance (ANOVA) of volumetric soil water content (SWC), leaf area and stomatal conductance (g_s). The experimental set-up consisted of 4 designs with a target species (*Lolium perenne (Lp)* or *Plantago lanceolata (Pl)*) either surrounded by six conspecific neighbours or by three conspecific and three heterospecific neighbours. The designs were subjected to an imposed drought by withholding water for 20 days (day of year (DOY) 197 - 217) and a control treatment. SWC was calculated as the mean at three different depths (0, 20 and 4 cm). The course of SWC before and during the imposed drought was compared between the four designs and between the measurement days, and if applicable, between the different depths; leaf area was compared between the four designs; g_s at the start of drought was compared between the two target species surrounded either by conspecific or heterospecific neighbours; the course of g_s during drought was analysed for each target and compared between neighbour composition and measurement days. Degrees of freedom (DF), F values and significance levels (p); p-values are presented in bold when significant (< 0.05).

	DF	F	p	DF	F	p	DF	F	p
SWC before drought: mean ≠ depths	DOY × design			DOY			design		
all 4 designs	27,917	0.56	0.965	9,917	0.57	0.555	3,917	0.64	0.589
SWC during drought	DOY × design								
all 4 designs	15,148	3.93	**< 0.001**	See Fig. 3 for a posteriori analyses					
SWC during drought: ≠ depths	DOY × design × depth								
all 4 designs	30,450	1.83	0.005	See Table S1 for a posteriori analyses					
leaf area at start drought	design								
all 4 designs	3,12	1.33	0.311						
g_s at start drought	species × neighbours			species			neighbours		
compare Pl targets with Lp targets	1,49	0.18	0.671	1,50	11.23	**0.001**	1,50	0.40	0.528
g_s during drought	DOY × neighbours			DOY			neighbours		
L. perenne targets	4,169	0.94	0.444	4,173	18.88	**< 0.001**	1,173	1.83	0.177
P. lanceolata targets	4,170	0.68	0.606	4,174	12.91	**< 0.001**	1,174	0.01	0.919

Figure 1 Schematic presentation of the target-neighbour designs with control (C) and drought (D) treatments. The drought treatment was obtained by withholding water for 20 days (day of year (DOY) 197 - 217), whereas in the control treatment the watering regime was not interrupted. White circles represent *Lolium perenne* individuals, grey circles represent *Plantago lanceolata* individuals. The inner diameter of the containers is 19 cm and plants are positioned at 5 cm interspace.

Figure 2 Above-ground biomass of *Lolium perenne* (L) or *Plantago lanceolata* (P) target plants subjected to an imposed drought and a control treatment. The targets were surrounded by either six conspecific neighbours (con) or three conspecific and three heterospecific neighbours (het). The drought treatment was obtained by withholding water for 20 days (day of year (DOY) 197 - 217), whereas in the control treatment the watering regime was not interrupted. Means ± SE (n = 8). In each panel, bars that do not have a common letter differ statistically ($p < 0.05$).

Figure 3 Volumetric soil water content (SWC) of target-neighbour designs subjected to an imposed drought by withholding water for 20 days (day of year (DOY) 197 – 217). The designs consist of a target species (*Lolium perenne* (L) or *Plantago lanceolata* (P)) either surrounded by six conspecific neighbours (con) or by three conspecific and three heterospecific neighbours (het). SWC was calculated as the mean at three different depths (0, 20 and 40 cm; see Figure S1 and Table S1 for data and analyses). Means ± SE (n = 8). A posteriori analyses of the DOY × design interaction show that SWC decreased fastest in P_{con} and that SWC was lower in P_{con} than in the other designs on several days ($p < 0.05$ for each comparison).

Figure 4 Stomatal conductance (g_s) of *Lolium perenne* (L) or *Plantago lanceolata* (P) target plants subjected to an imposed drought. The targets were surrounded by either six conspecific neighbours (con) or three conspecific and three heterospecific neighbours (het). The drought treatment was obtained by withholding water for 20 days (day of year (DOY) 197 - 217).

Figure 5 Light saturated net CO_2 assimilation rate (A_{sat}) at the end of an imposed drought (day of year 217), of *Lolium perenne* (L) or *Plantago lanceolata* (P) target plants. The targets were surrounded by either six conspecific neighbours (con) or three conspecific and three heterospecific neighbours (het). The drought treatment was obtained by withholding water for 20 days (DOY 197 - 217), whereas in the control treatment the watering regime was not interrupted. Means ± SE (n = 8). In each panel, bars that do not have a common letter differ statistically ($p < 0.05$).

Figure 1

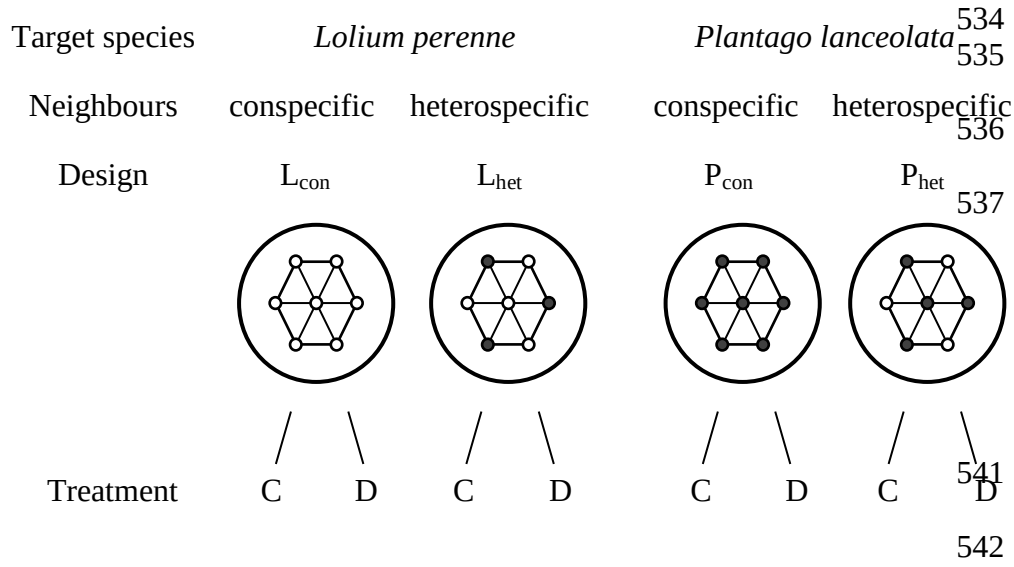
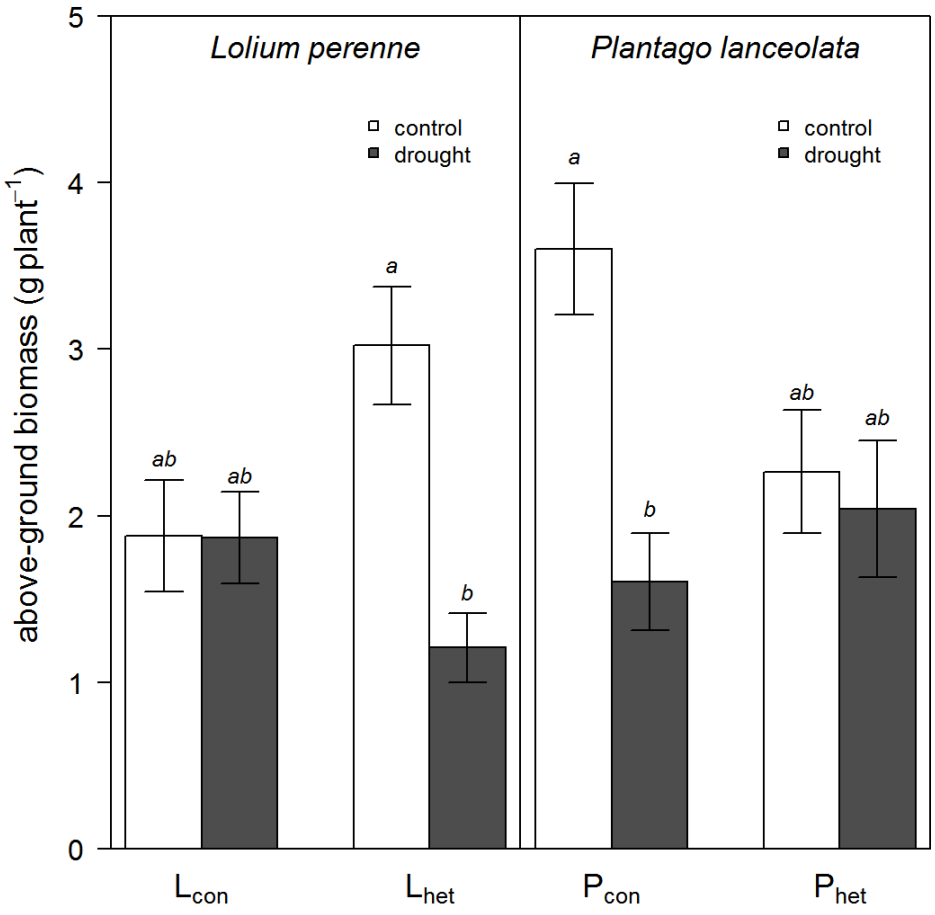
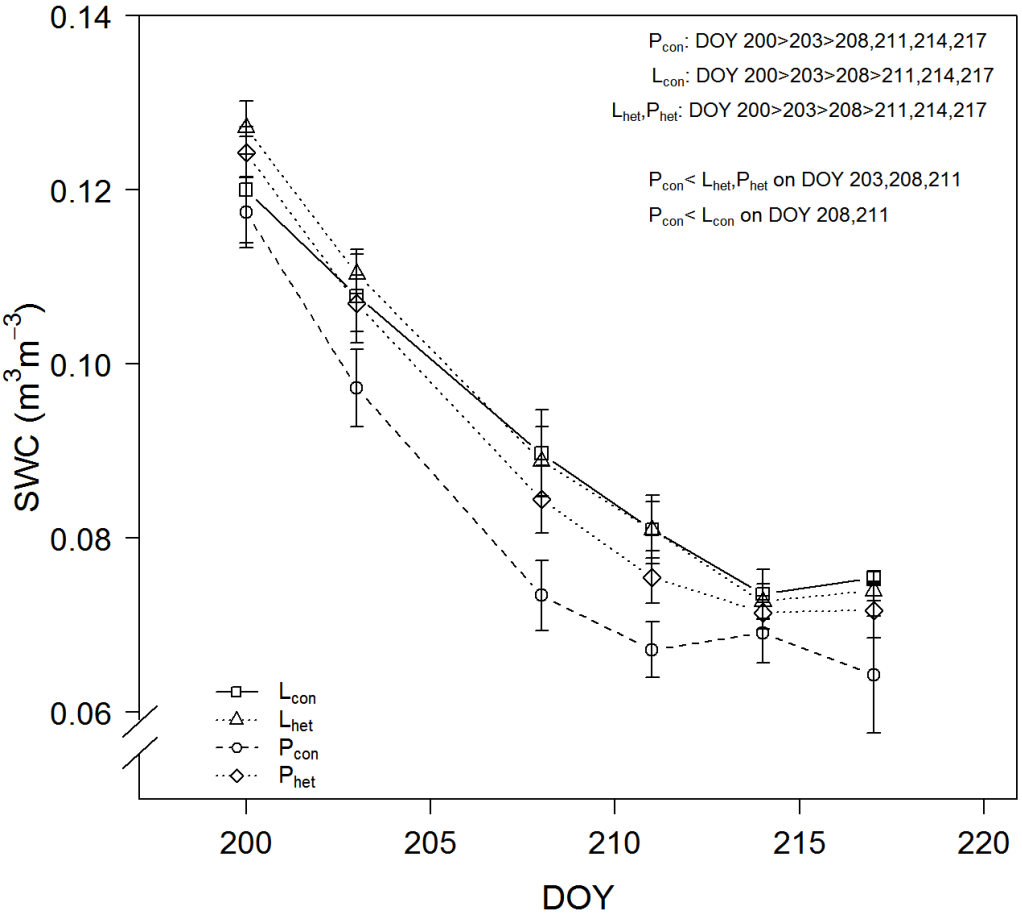

Target species
Lolium perenne
Plantago lanceolata
Neighbours
conspecific
heterospecific
conspecific
heterospecific
Design
Lcon
Lhet
Pcon
Phet
Treatment
C
D
C
D
C
D
C
D

Figure 2


Lolium perenne
Plantago lanceolata
control
drought
control
drought
above-ground biomass (g plant^{-1})
0
1
2
3
4
5
ab
ab
a
b
a
b
ab
ab
L_{con}
L_{het}
P_{con}
P_{het}

549 **Figure 3**

550



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554 **Figure 4**

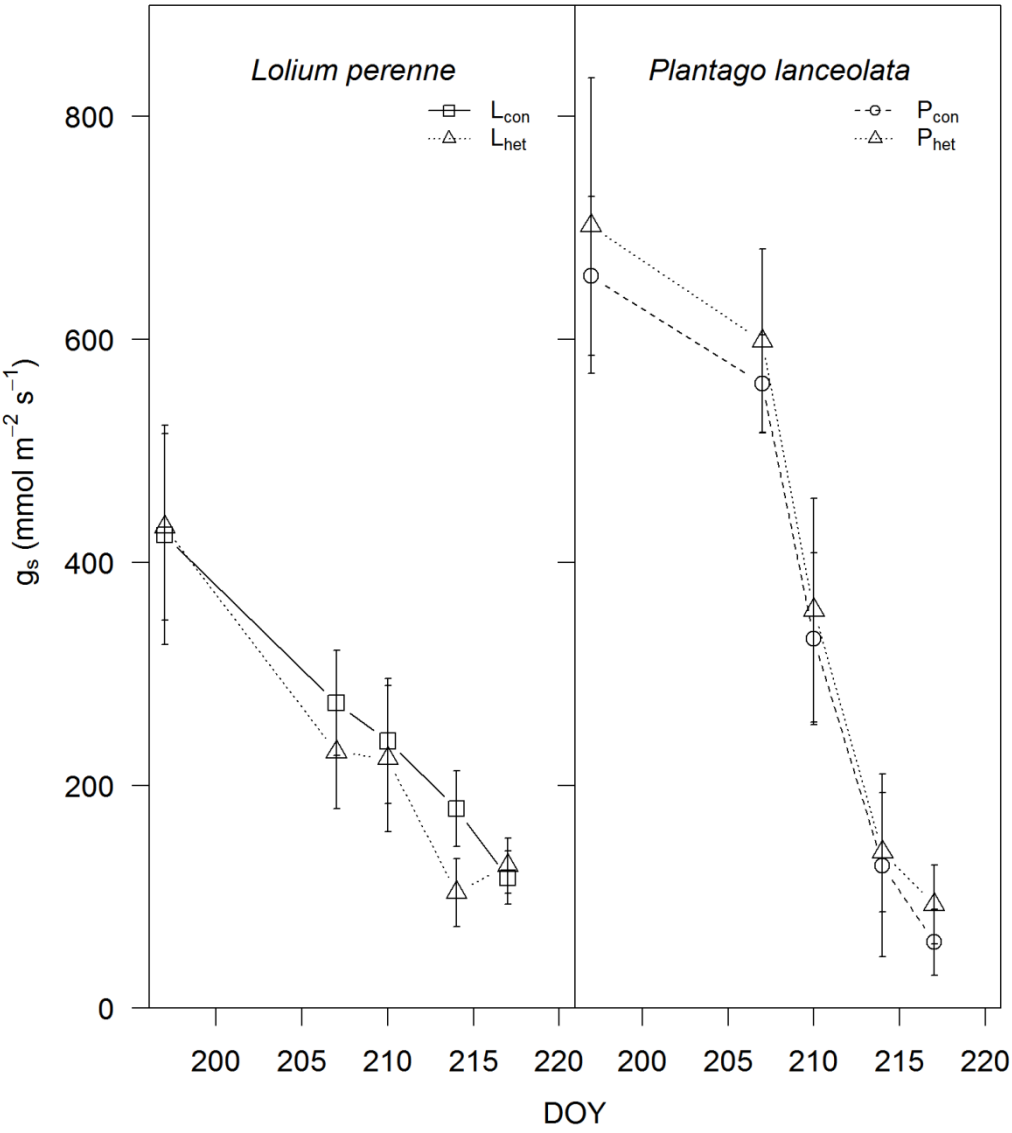

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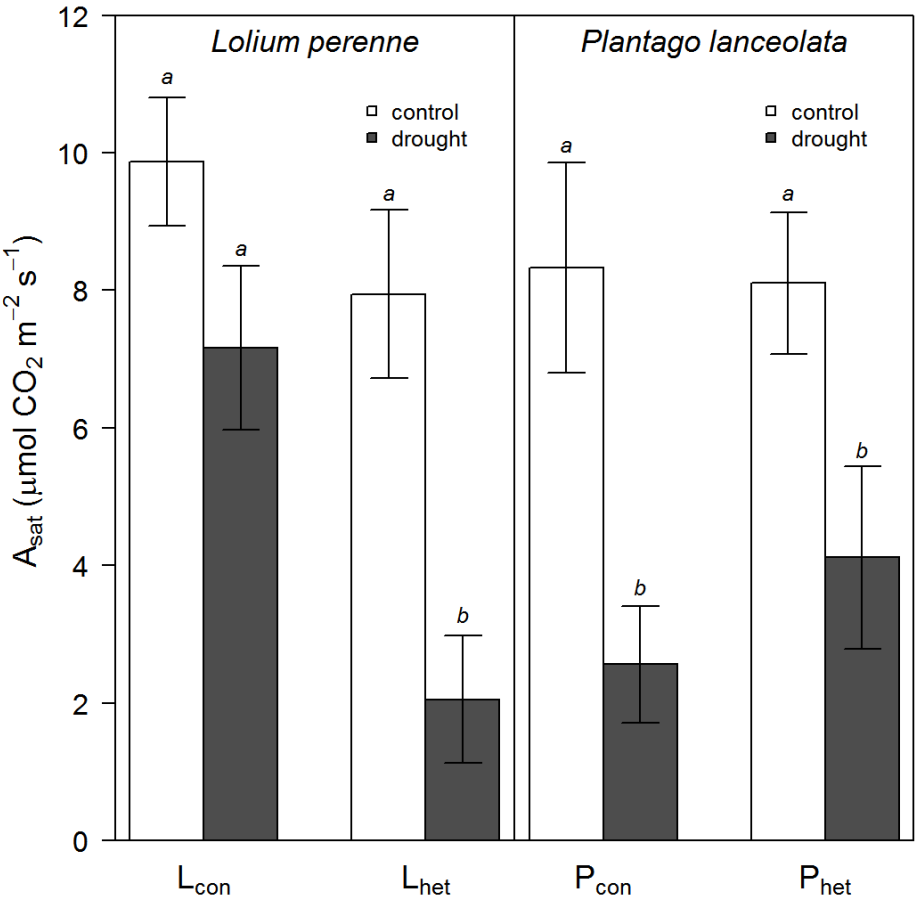
Figure 5


Lolium perenne
Plantago lanceolata
control
drought
control
drought
A_{sat} (µmol CO_2 m^{-2} s^{-1})
12
10
8
6
4
2
0
a
a
a
b
a
b
a
b
L_{con}
L_{het}
P_{con}
P_{het}