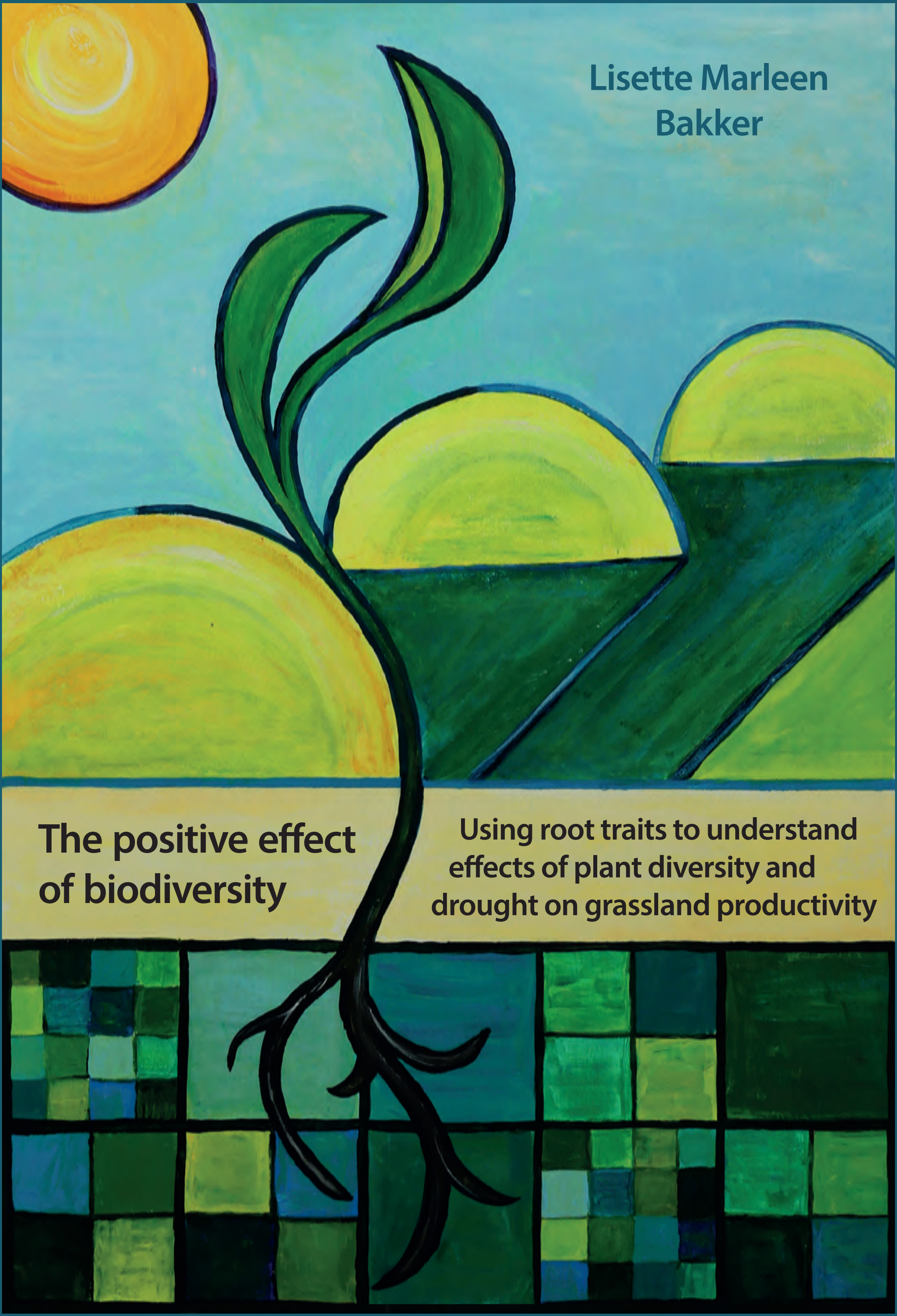

Lisette Marleen
Bakker

The positive effect of biodiversity

Using root traits to understand effects of plant diversity and drought on grassland productivity

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The positive effect of biodiversity

Using root traits to understand effects of plant diversity and drought on grassland productivity

Lisette Marleen Bakker

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Chapter 1

General introduction

1.1 Biodiversity loss and drought

The earth is home to an enormous diversity of plants, the primary producers of our ecosystems. These plant communities are essential for humans, providing food, purifying water supplies, generating oxygen, and supplying building materials and other products (Daily, 1997). Despite the importance of plants for ecosystem functioning and human livelihood, plant diversity is threatened by intensifying human pressure on natural ecosystems (Millennium Ecosystem Assessment, 2005, Pereira *et al.*, 2010, Newbold *et al.*, 2015). Due to habitat conversion, degradation, and fragmentation, invasive species, overexploitation, pollution and climate change, biodiversity has declined rapidly (Millennium Ecosystem Assessment, 2005, Butchart *et al.*, 2010). Current extinction rates well exceed those in the geological past (Secretariat of the Convention on Biological Diversity, 2010, Pimm *et al.*, 2014) and are predicted to increase even further in the future (Millennium Ecosystem Assessment, 2005, Pereira *et al.*, 2010, Secretariat of the Convention on Biological Diversity, 2010).

Climate change is one of the most important factors that can reduce biodiversity (Thomas *et al.*, 2004, Pereira *et al.*, 2010). This occurs mainly through gradual climate warming, affecting the suitability of species' habitats, which can result in major species extinctions (Thomas *et al.*, 2004). In addition to gradual climate warming, climate change may also threaten functioning of plant communities and ecosystems through climate extremes (Jentsch and Beierkuhnlein, 2008). Global climate models predict that the frequency and occurrence of extreme events, such as prolonged summer droughts, will increase in the future (Christensen *et al.*, 2011, Stocker *et al.*, 2013). A (meteorological) drought, i.e. a period with below average precipitation, often accompanied by high atmospheric vapour pressure deficits (Dai, 2011), typically results in low water availability in the soil, limiting plant growth and affecting plant interactions and vegetation structure (Morecroft *et al.*, 2004, Debouk *et al.*, 2015). Previous summer droughts have been shown to reduce ecosystem productivity throughout Europe (Ciais *et al.*, 2005) and they are predicted to increase in Central and Southern Europe in the next decades, with up to 40% less precipitation in summer in 2071-2100 (Dankers and Hiederer, 2008) (Fig. 1.1). More severe droughts are also expected in many other regions in the world (Dai,

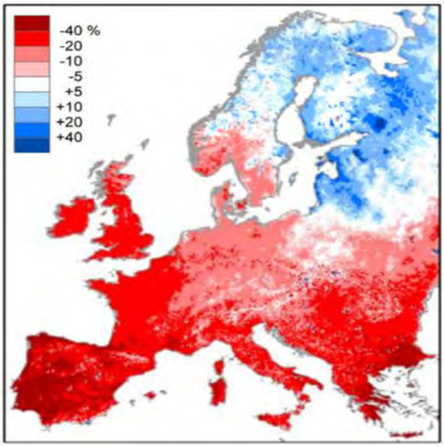

Figure 1.1 Precipitation in Europe is expected to become more extreme: relative change in the seasonal precipitation amounts in the scenario period (2071-2100) in summer (June to August). Figure modified from Dankers and Hiederer (2008).

2011), threatening ecosystem functioning and productivity. Thus in the future, plant communities will have to cope with more frequent and prolonged drought periods, while simultaneously facing loss of biodiversity. *In this thesis, I study the interactive effect of biodiversity and drought on grassland communities.*

1.2 Biodiversity and ecosystem functioning

The rapid loss of biodiversity raised concerns about the ecological consequences of the loss of species for ecosystem functioning during the 1980s. From the 1990s onwards, these concerns subsequently led to numerous biodiversity experiments with grassland plant communities, examining the relationship between plant species richness and ecosystem functioning (Tilman *et al.*, 1996, Tilman, 1999a, Cardinale *et al.*, 2012). Twenty-five years later, there is consensus that species richness has indeed strong effects on many ecosystem functions, including a positive effect of plant species richness on ecosystem stability and productivity (Hooper *et al.*, 2005, Isbell *et al.*, 2011, Cardinale *et al.*, 2012, Tilman *et al.*, 2014). This increased yield in mixtures compared to monocultures is often referred to as "overyielding".

The positive relationship between species richness and productivity has been shown to not only depend on plant species richness *per se*, since other biodiversity components, such as functional group richness (Tilman *et al.*, 1997a, Hooper and Dukes, 2004, Marquard *et al.*, 2009), species composition (Hooper and Vitousek, 1997, Tilman *et al.*, 1997a, Hooper, 1998, Hector *et al.*, 2011, Avolio *et al.*, 2014), or the presence of particular key species, can also affect plant community productivity. Soon after the first papers reported positive effects of species richness on ecosystem functioning, the question was raised whether these effects were just "sampling effects", i.e. due to the increased chance to include highly productive, competitive species with increasing species richness (Aarssen, 1997, Huston, 1997, Tilman, 1999b). With the development of the "additive partitioning method" of Loreau and Hector (2001), it became possible to distinguish between the effects due to the inclusion and dominance of a few productive species (calculated as selection effect) and those due to positive biotic interactions in species mixtures, such as resource partitioning and facilitation (calculated as 'complementarity effects'; see Box 1.1). Application of this method revealed that although the inclusion of dominant productive species does contribute to increased biomass in species mixtures in many studies, the biomass increase is mainly attributed to complementarity effects (Loreau and Hector, 2001, Cardinale *et al.*, 2007). Moreover, these complementarity effects have been shown to increase over time (Cardinale *et al.*, 2007, Fargione *et al.*, 2007, Meyer *et al.*, 2016). However, the exact biological mechanisms that drive these complementarity effects remain debated. *In this thesis, I will further investigate the biological mechanisms that drive these positive complementarity effects.*

In addition to the positive effect of species richness on productivity, a positive relationship between species richness and community stability has been found (McNaughton, 1977, Naeem and Li, 1997, Hector *et al.*, 2010, Gross *et al.*, 2014). This means that species-rich plant communities show less variation in community biomass over time than species-poor communities, maintaining important community functions over time. This increased stability suggests that species richness may play a role in maintaining productivity during climate disturbances or extreme weather events such as prolonged summer droughts. Indeed, a recent meta-analysis has shown that resistance to drought (and wet) events – the ability to withstand a decrease in biomass productivity *during* such an event – increases with the number of species (Isbell *et al.*, 2015). However, the variability in species richness effects on drought resistance was high among the studies included. Other experimental studies have also found neutral and negative effects (Pfisterer and Schmid, 2002, Kahmen *et al.*, 2005, De Boeck *et al.*, 2008, Vogel *et al.*, 2012) of species richness on drought resistance. Moreover, like the increase in productivity in mixtures, it is not entirely clear *how* species richness can increase drought resistance. Different mechansisms

Box 1.1 Biodiversity effects

The additive partitioning method of Loreau and Hector (2001) allows us to calculate the change in yield (biomass) in plant community mixtures compared to plant monocultures. This change in yield in mixture, compared to the average monoculture yield, is called the net effect (NE) of biodiversity (Figure 1.2A). Subsequently, NE can be partitioned into selection effects (SE) and complementarity effects (CE): NE = CE+SE. The SE represents the biomass change in mixture as a result of the link between species' performance in mixture (ΔRY) and its productivity in monoculture. The selection effect is positive if the most productive species also have highest yield increases in mixture (Figure 1.2B, *left*), but negative if the least productive species in monoculture increase their performance in mixtures most (*right*). Note that if there is no link between performance in mixture and monoculture among species, then SE will approximate zero. CE represents the biomass change in mixtures compared to monocultures as a result of biotic interactions. Positive CE occurs when the average species relative performance in mixture $(\overline{\Delta RY})$ is greater than zero.

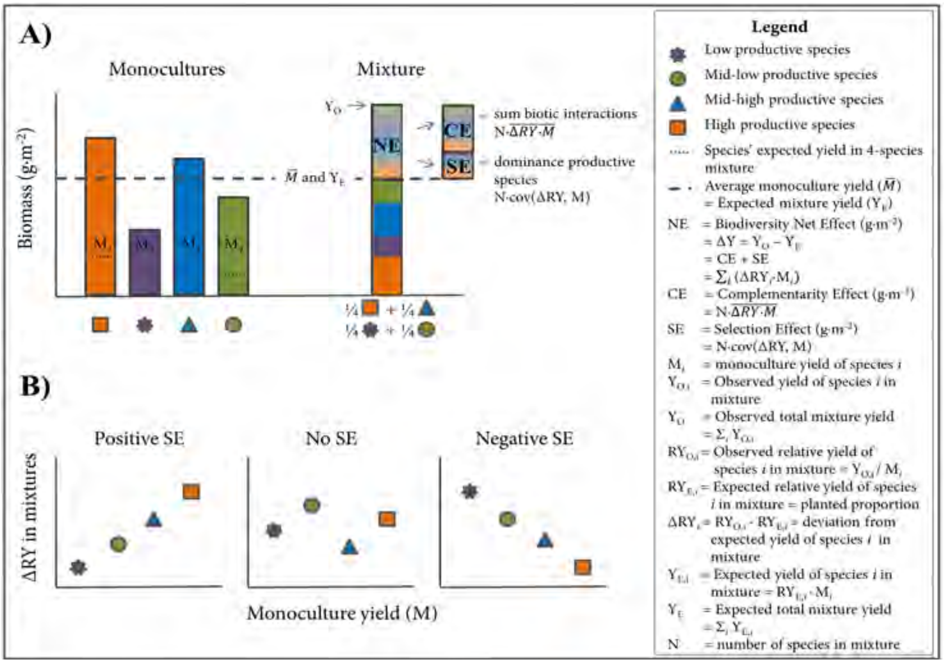

Figure 1.2 Graphical representation of A) the additive partitioning method of Loreau and Hector (2001) and (B) hypothetical relationships between species relative performance in mixtures (ΔRY) and in monoculture (M) associated with positive, no and negative selection effects, respectively.

Box 1.1 Biodiversity effects *(continued)*

Relative biodiversity effects

Biodiversity effects (NE, CE, SE) represent the change in yield in mixture and are expressed in grams per m^2. As can be seen in the equations above, they depend on the biomass in monocultures (M). Therefore, a species mixture that contains very productive species (high M) will have higher biodiversity effects than a community that contains low productive species (low M), even when both communities have a similar increase in relative performance of species (e.g. a ΔRY of 0.25). This makes it difficult to compare biodiversity effects between plant communities consisting of species that differ in monoculture biomass. To account for these differences in monoculture yield and focus on the relative yield changes, relative NE, CE and SE (rNE, rCE, rSE) can be used. This is done by dividing communities' biodiversity effects by their average monoculture yield (Craven *et al.*, 2016). These relative biodiversity effects are independent of community biomass. *In this thesis, I will use relative biodiversity effects, with a focus on rCE, to elucidate the mechanisms underlying these relative changes in plant mixtures.*

(see next paragraphs) may drive the interaction between plant diversity and drought resistance (Yachi and Loreau, 1999, Caldeira *et al.*, 2001, Van Peer *et al.*, 2004, Leimer *et al.*, 2014, Wright *et al.*, 2017), and the relative importance of these mechanisms may differ between studies and environmental conditions (Wardle *et al.*, 2004, Tylianakis *et al.*, 2008, Hiddink *et al.*, 2009). *In this thesis, I will examine different mechanisms through which plant diversity may affect drought resistance.*

1.3 Mechanisms underlying the positive effects of plant diversity on productivity and drought resistance

1.3.1 Resource partitioning

Since the beginning of the biodiversity-ecosystem functioning research, it has been hypothesized that the positive biodiversity-productivity relationship is the result of resource partitioning among species (Tilman *et al.*, 1997b, Hooper *et al.*, 2005, Cardinale *et al.*, 2011). This hypothesis is based on the assumption that species differ in resource acquisition strategies in space, chemical form or time, and states that total exploitation of available resources is increased when different species grow together. This increase in resource uptake in species mixtures leads to increased biomass production compared to monocultures (Tilman *et al.*, 1997b, Dimitrakopoulos and Schmid, 2004).

Resource partitioning may be especially important *belowground*, since several studies have suggested that belowground processes underlie the positive biodiversity-productivity relationship (van Ruijven and Berendse, 2005, de Kroon *et al.*, 2012, Bardgett *et al.*, 2014). A classic example of belowground resource partitioning is via vertical root distribution: a plant community with both shallow and deep-rooting species is expected to explore a larger soil volume for nutrients and water than each does separately in monoculture, enhancing total resource uptake and community biomass. Research suggest that species indeed differ in their vertical root distributions (Parrish and Bazzaz, 1976, Yeaton *et al.*, 1977, Berendse, 1979, Berendse, 1982, Jackson *et al.*, 1996, Fargione and Tilman, 2005), nutrient (depth) uptake (Fitter, 1986, McKane *et al.*, 1990, Mamolos *et al.*, 1995, Mamolos and Veresoglou, 2000, von Felten *et al.*, 2012), or water uptake (Nippert and Knapp, 2007b, Silvertown *et al.*, 2015). In the theoretical study of Postma and Lynch (2012) and experimental study of Zhang *et al.* (2014), differences in root architecture between three crop species were indeed coupled to increased total nutrient uptake and biomass.

However, in grassland biodiversity experiments, the evidence for an important role of resource partitioning in biodiversity effects is limited, and results are mixed. Several studies found positive biodiversity effects, but no experimental evidence for differentiation in root distribution and nitrogen uptake in species mixtures (Mommer *et al.*, 2010, Schultz *et al.*, 2012, Ravenek *et al.*, 2014). This would suggest that differences in root distribution or N uptake between species may not be the main driver of positive biodiversity effects. In addition, several tracer studies have tried to examine the importance of resource partitioning via root distribution by injecting stable isotopes (^{15}N, rubidium, ^{18}O) into different soil layers. They did not find evidence for increased community nitrogen and water uptake with increasing species richness, despite significant differences in resource uptake among species (Kahmen *et al.*, 2006, von Felten *et al.*, 2009, Bachmann *et al.*, 2015, Hoekstra *et al.*, 2015). In contrast, other experimental studies have demonstrated that, together with aboveground biomass, community water use (Caldeira *et al.*, 2001, Guderle *et al.*, 2018) and total community nutrient uptake (Tilman *et al.*, 1996, Tilman *et al.*, 1997a, van Ruijven and Berendse, 2005, Schultz *et al.*, 2012, Zhang *et al.*, 2014) increased with species richness. However, these studies did not show that the increase in resource uptake at the community level were due to resource partitioning among species.

Resource partitioning, or more specifically, water partitioning, may be particularly important during periods with decreased water availability (Nippert and Knapp, 2007b). Therefore, water partitioning may be an important mechanism through which species richness positively affect the drought resistance of a community. For example, in monocultures, neighbouring plants may compete for water during a drought in particular soil layers, since all plants have similar water uptake strategies (root distributions). In species mixtures, differences in rooting distribution among the neighbour plants may decrease competition for water in particular soil layers. Total available water is thus likely higher in plant mixtures, increasing drought resistance of the community compared to monoculture communities. In agreement with this hypothesis, Nippert and Knapp (2007a) reported that during dry periods, C3 grasses shifted their water uptake to deeper soil layers, while C4 grasses used water from the upper soil layers. In addition, less water stress (Caldeira *et al.*, 2001) and increased water uptake or transpiration have been found in species-rich communities (Van Peer *et al.*, 2004, Verheyen *et al.*, 2008, Milcu *et al.*, 2016, Guderle *et al.*, 2018). However, other studies could not find evidence that species differences in water uptake or root distribution contribute to productivity under drought (Hoekstra *et al.*, 2014, Barkaoui *et al.*, 2016).

1.3.2 Facilitation

Another important mechanism that may drive positive biodiversity effects in species mixtures is facilitation (Wright *et al.*, 2017). With facilitation, individual plants may positively affect neighbouring plants by affecting the environmental conditions, for example by increasing resource availability, by affecting soil biota, or by protecting from herbivory or harsh environmental conditions (Callaway, 1995, Wright *et al.*, 2017). These faciliatating interactions have been hypothesized to increase under stress conditions (stress gradient hypothesis; Callaway and Walker, 1997, Brooker *et al.*, 2008), and could therefore play a major role in the performance of plant communities under dry conditions and the biodiversity-drought interaction. For example, increased leaf area (LAI) (Hector *et al.*, 1999, Weisser *et al.*, 2017, Guderle *et al.*, 2018) and transpiration (Verheyen *et al.*, 2008, Milcu *et al.*, 2016, Guderle *et al.*, 2018) in species-rich communities can result in an increase in relative air humidity and decrease in temperature and vapour pressure deficit in the canopy, enhancing plant performance (Wright *et al.*, 2014, Wright *et al.*, 2017). In addition, increased LAI can reduce soil evaporation by covering the soil surface, increasing soil moisture in the upper soil layers (Rosenkranz *et al.*, 2012, Wright *et al.*, 2014). Note, however, that increased LAI and transpiration in species-rich communities may also decrease water availability during a prolonged drought period (De Boeck *et al.*, 2006, Mokany *et al.*, 2008, Leimer *et al.*, 2014), and thus potentially decreases community drought resistance instead of increase resistance. Last, facilitation may also occur via hydraulic lift, when water uptake by deep-rooting species increases soil water availability in the shallow layers (Caldwell and Richards, 1989, Caldwell *et al.*, 1998, Neumann and Cardon, 2012, Prieto *et al.*, 2012). Hence, shallow rooting species may profit from standing to a deep rooting neighbour.

1.3.3 Insurance hypothesis

Last, a different mechanism that can play a role in the enhanced drought resistance in mixture is based on the 'insurance hypothesis' (Yachi and Loreau, 1999). This hypothesis states that more diverse communities have a greater probability of containing species that can maintain functioning during environmental fluctuations. In case of a drought, this hypothesis predicts that diverse communities have a higher probability to contain (productive) drought tolerant species that can maintain community biomass by compensating for the drought-induced growth reductions of other species.

1.3.4 Biodiversity effects and drought

The importance of the mechanisms discussed above may be reflected in the size of biodiversity effects (sensu Loreau and Hector, 2001; Box 1.1) under drought. Complementarity effects can capture positive interactions like water use partitioning and facilitation. If water use partitioning and facilitation are the most important mechanisms enhancing drought resistance of mixtures compared to monocultures, then complementarity effects are expected to increase during a prolonged drought. In contrast, if drought tolerant species increase in dominance and maintain biomass production in mixtures, then we would expect to see an increase in selection effects during a drought. *In this thesis, I will investigate if the three mechanisms discussed above play a role in the effect of plant diversity on productivity or drought resistance.*

1.4 The trait approach

1.4.1 Linking traits to function

In the last decades, ecosystem functions are increasingly predicted by the inherent characteristics or "traits" of species instead of taxonomic identity (Weiher *et al.*, 1999, Lavorel and Garnier, 2002, Violle *et al.*, 2007). Plant functional traits can be defined as "morpho-physio-phenological traits which impact fitness indirectly via their effects on growth, reproduction and survival, the three components of individual performance" (Violle *et al.*, 2007). These traits are for example plant maximum height, specific leaf area, or rooting depth (Cornelissen *et al.*, 2003). Indeed, such traits have been successfully coupled to many ecosystem functions (Diaz *et al.*, 1998, Lavorel and Garnier, 2002, Chapin, 2003, de Bello *et al.*, 2010, Bardgett *et al.*, 2014, Faucon *et al.*, 2017), including productivity of species (Schroeder-Georgi *et al.*, 2016, Herz *et al.*, 2017) and communities (Mokany *et al.*, 2008, Schumacher and Roscher, 2009, Roscher *et al.*, 2012). Also biodiversity research started to use traits to understand the role of "functional diversity" and community composition instead of species richness *per se* (Diaz and Cabido, 2001). Interestingly, the use of traits could disentangle the importance of functional identity and functional diversity for biodiversity effects, by both the average community trait values, i.e. "community weighted means" (CWMs) and the diversity in these trait values within a plant community (Schumacher and Roscher, 2009, Mason *et al.*, 2013). So far, most biodiversity studies found that both trait CWMs and trait diversity could explain variation in biomass (Mokany *et al.*, 2008, Finegan *et al.*, 2015) or drought resilience (Barkaoui *et al.*, 2016), but found a larger importance of CWMs. Further, Roscher *et al.* (2012) reported that trait CWMs were more important for productivity than trait diversity, but that CWMs and trait diversity contributed equally to complementarity and selection effects (sensu Loreau and Hector, 2001).

1.4.2 Belowground traits and biodiversity research

Until recently, most research used aboveground (leaf) traits to predict ecosystem functions (e.g.Wilson *et al.*, 1999, Wright *et al.*, 2004). However, the increased attention to the importance of belowground processes for plant performance (Bardgett *et al.*, 2014, Bardgett, 2017) and plants large investments in root biomass – more than 70% of total biomass in grasslands (Poorter *et al.*, 2012) – have led to an increased focus on root traits (Iversen *et al.*, 2017). Root traits – such as rooting depth, specific root length, and root length density – have been shown to be important predictors of nutrient and water uptake (Lynch, 1995, Hernández *et al.*, 2010, Fort *et al.*, 2017), competitive ability (Casper and Jackson, 1997, Semchenko *et al.*, 2017), and monoculture biomass (Schroeder-Georgi *et al.*, 2016). Also under drought conditions, root traits have been good predictors of species or community biomass (Morecroft *et al.*, 2004, Skinner *et al.*, 2006, Comas *et al.*, 2013, Barkaoui *et al.*, 2016, Zeiter *et al.*, 2016). Hence, diversity in morphological (e.g. specific root length and root tissue density) or architectural root traits (e.g. rooting depth and root length density; Bardgett *et al.*, 2014), could play a role in belowground resource partitioning, and therefore be coupled to positive complementarity effects. Knowing which traits are involved in the increased productivity in mixtures could shed light on the underlying mechanisms. Although (root) traits have been successfully coupled to *productivity* (Mokany *et al.*, 2008, Barkaoui *et al.*, 2016, Schroeder-Georgi *et al.*, 2016), the link between root traits and *biodiversity effects* has rarely been studied. To my knowledge, Roscher *et al.* (2012) was one of the first to link trait means and diversity to biodiversity effects, but focused primarily on aboveground traits. However, they did find that including rooting depth and rooting type, obtained from the literature, could explain additional variation in net biodiversity effects. Further, in forest stands, diversity in specific root length (SRL) could explain positive complementarity effects (Bu *et al.*, 2017). This suggests that root traits are potentially important predictors of biodiversity effects. *In this thesis, I will establish the relationships between root traits and biodiversity effects to investigate the potential role of resource partitioning, and the importance of (diversity in) rooting depth for drought resistance. I specifically couple trait CWMs and trait diversity to complementarity and selection effects.*

1.4.3 Increasing resolution: from plot to plant individuals

It is important to note that biodiversity-trait studies have mostly focussed on the community (plot) level, linking total community biomass to community trait values. By using community values, the focus is on the net result of multiple species interactions within a community, averaged over the area of a plot (typically one to several m^2). This may obscure the outcomes of specific interactions between plant individuals that typically take place at smaller scales in grasslands. Consequently, trait diversity-performance relationships studied at the plot scale may be weak, especially when plot size is large and species respond differently to different neighbouring species (with different traits). Therefore, an alternative method to specifically examine if trait differences among species within a community contribute to increased growth, is to scale down to the species level and examine how plant traits of the local neighbourhood affect the performance of individual plants of different species in mixtures. This may provide more insight in species responses to trait differences, thereby elucidating the interactions that contribute to enhanced biomass production in diverse communities. *In this thesis, I will examine whether the performance of individual plants depends on their own rooting depth and the rooting depth of their neighbouring plants.*

1.4.4 Increasing resolution: changes in biodiversity effects over time

Further, to improve our biodiversity predictions using traits, it may be important to take into account that both environmental condition and biodiversity effects may change over time (Cardinale *et al.*, 2007). For example, during a dry period, deep roots and diversity in rooting depth among species may be more important than during a wet period (Morecroft *et al.*, 2004, Skinner *et al.*, 2006, Comas *et al.*, 2013, Barkaoui *et al.*, 2016, Zeiter *et al.*, 2016). One way to deal with this is to specifically look at the biodiversity effect changes during such a specific period, and to couple the traits to changes in biodiversity effects over time. To my knowledge, this has never been done before, but it may strengthen our trait-biodiversity effect relationships. *In this thesis, I will use the change in biodiversity effects during a dry period to examine the role of root trait diversity and root trait means.*

1.5 The scope and outline of this thesis

The positive relationship between plant diversity and productivity is well established, and plant diversity may also positively affect the maintenance of productivity during a prolonged drought (drought resistance). However, the underlying biological mechanisms are still debated. In this thesis I will further investigate these mechanisms in two research lines. In the first research line, the aim is to get more insight into the role of resource partitioning in overyielding using a belowground trait approach: can we explain positive biodiversity effects in grassland mixtures using (diversity in) root traits? This question is addressed in chapter 2, 3, and 4, using a new established grassland biodiversity experiment (see below; Fig.1.3). Important morphological or architectural traits, associated with resource uptake, are used, with a focus on rooting depth. In the second research line, I focus on the interaction between plant diversity and drought: does drought affect the resistance of grassland monocultures and mixtures differently? And how does drought affect the biodiversity effects (sensu the partitioning method, Box 1.1)?

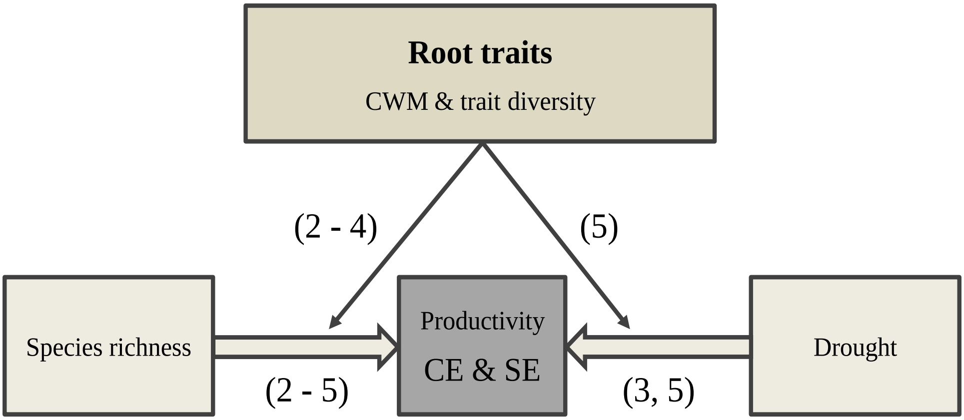

Figure 1.3 Schematic diagram of this thesis. The central question is how plant species richness and drought affect plant productivity, and biodiversity effects – complementarity effects (CE) and selection effects (SE) – in particular. To answer this question, variation in root traits is related to the response of plant communities (chapters 2, 3 & 5) and individual plants (chapter 4). The traits used were taken from the literature or measured in a pot experiment (chapter 2) or measured in monocultures in the biodiversity experiment (chapter 3-5). The numbers between the brackets represents the number of the chapter(s) in which a particular relationship is investigated.

In the first research line, I first combined the additive partitioning method with a functional trait approach by linking the diversity of several root traits to complementarity effects: can we predict initial complementarity effects using root traits that were obtained from a separate pot experiment and literature **(chapter 2)**? Second, I investigated the relationships between trait community weighted means (CWMs) and trait diversity, and complementarity effects and selection effects. Species traits were obtained from the monocultures. As a new approach, I used the change in complementarity effects and selection effects from the first to the second year to specifically investigate biodiversity effect changes during a dry period: can we predict changes in complementarity effects and selection effects over time, after a dry period, using monoculture root traits **(chapter 3)**? Third, I focused on the importance of species' differences in rooting depth for overyielding at a more local scale than a whole community to increase resolution. We measured the aboveground biomass of almost 1700 plants of 16 species, over three growing seasons to answer the research question: can we explain performance of individual plants in mixtures using species own rooting depth and the rooting depth of the neighbouring plants **(chapter 4)**? For the second research line, presented in **chapter 5**, the communities in the biodiversity experiment were exposed to an experimental drought, to investigate the effect of drought on biomass production and biodiversity effects. I also investigated whether species and community rooting depth, community cover and soil moisture play a role in potential differences in drought resistance between mixtures and monocultures. Investigating the effect of drought on biodiversity effects may provide new insights into the difference in drought resistance between monocultures and mixtures. Moreover, it may provide insight regarding the biological mechanisms underlying the positive biodiversity effects under changing environmental conditions. These insights are important to predict ecosystem functioning in a changing world in which biodiversity decreases rapidly. The findings of the research chapters will be summarized, combined, and discussed in a broader context in **chapter 6**.

1.6 The biodiversity experiment

To investigate the relationship between biodiversity and productivity, several multi-year biodiversity field experiments have been established in the past two decades. In these experiments, a field from which the previous vegetation has been removed is divided into 50 to more than 150 plots of one to several m^2. These plots are then sown or planted with one (monocultures) or more different grassland plant species and allowed to grow for several years. Each year, aboveground biomass is removed to measure annual plant productivity. Famous biodiversity experiments include the long-term Cedar Creek Biodiversity experiment (Tilman *et al.*, 1997a), the European multi-site BIODEPTH experiment (Hector *et al.*, 1999), and the Jena biodiversity experiment (Roscher *et al.*, 2004).

To answer the research questions of this thesis, a new multi-year biodiversity experiment was established in Wageningen, the Netherlands, in April 2014. In total, I established 198 plots of 0.5 m^2 (see Fig. 1.4) with monocultures (six per species), 4-species mixtures (45 different species compositions) and 16-species mixtures (six different planting positions). Thus, the experiment includes three species richness levels. However, this experiment is particularly focussed on the effect of plant diversity via community (root) composition, and thus on the variation in performance among the 4-species mixtures compared to monocultures.

For the species pool, we chose perennial grassland species that co-occur in Dutch hay meadows (Schaminée *et al.*, 1996; Table 1.1 and Fig. 1.5). Additional selection criteria included functional group (I selected eight grasses and eight

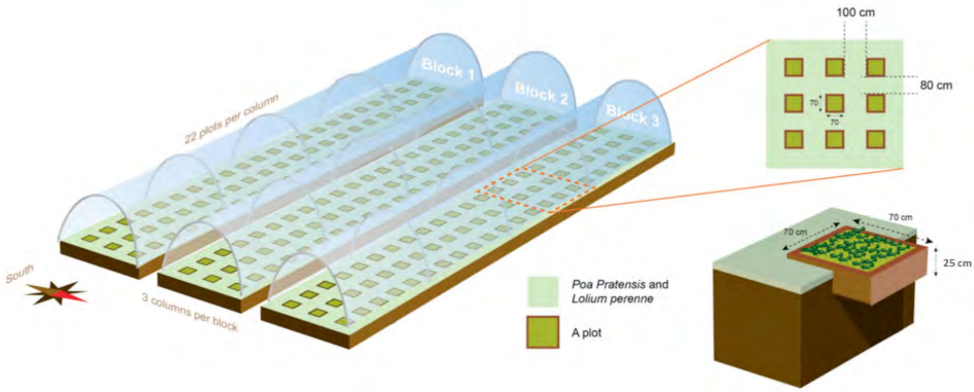

Figure 1.4 Experimental setup of the biodiversity experiment. Adapted from a drawing made by Kristle Kranenburg and Jeroen Stolwijk.

forbs) and species' maximum rooting depth (literature values from Van Duuren *et al.*, 2003). For the latter, species were selected in such a way that half of the species were 'shallow'-rooted (maximum rooting depth < 0.35 m) and half 'deep'-rooted (maximum rooting depth > 0.35m). By combining shallow and deep rooting species in the 4-species mixtures, a gradient in rooting depth was created: 4-species mixtures contained zero to four deep rooting species. This way, I tried to create variation in rooting depth CWM and rooting depth diversity, independent of species richness. Further, to specifically investigate the combined effect of plant diversity and drought on aboveground productivity, all plots were covered with rainout shelters for several weeks to simulate a prolonged drought (see Fig. 1.4, 1.6 and 1.7). During this period, half of the plots were watered, while the other half with similar species compositions did not receive any water. In this way, each species composition includes a drought plot and a control plot, which can be compared.

Table 1.1 Species that are used in the biodiversity experiment and their functional group (FG).

Latin name	Dutch name	English name	Species code	FG	Rooting depth class
Achillea millefolium (L.)	duizendblad	yarrow	Ach	Forb	deep
Agrostis stolonifera (L.)	fioringras	creeping bentgrass	Agr	Grass	shallow
Anthoxanthum odoratum (L.)	gewoon reukgras	sweet vernalgrass	Ant	Grass	shallow
Arrhenatherum elatius (L.)	glanshaver	false oat-grass	Arr	Grass	deep
Briza media (L.)	bevertjes	quaking grass	Bri	Grass	shallow
Centaurea jacea (L.)	knoopkruid	brown knapweed	Cen	Forb	deep
Festuca pratensis (Huds.)	beemdlangbloem	meadow fescue	Fpra	Grass	deep
Festuca rubra (L.)	rood zwenkgras	red fescue	Frub	Grass	shallow
Galium mollugo (L.)	glad walstro	hedge bedstraw	Gal	Forb	deep
Leontodon hispidus (L.)	ruige leeuwentand	rough hawkbit	Leo	Forb	shallow
Leucanthemum vulgare (L.)	gewone margriet	oxeye daisy	Leu	Forb	deep
Phleum pratense (L.)	timoteegras	timothy grass	Phle	Grass	deep
Prunella vulgaris (L.)	gewone brunel	selfheal	Pru	Forb	shallow
Ranunculus repens (L.)	kruipende boterbloem	creeping buttercup	Ran	Forb	shallow
Sanguisorba officinalis (L.)	grote pimpernel	great burnet	San	Forb	deep
Trisetum flavescens (L.)	goudhaver	yellow oat-grass	Tri	Grass	shallow

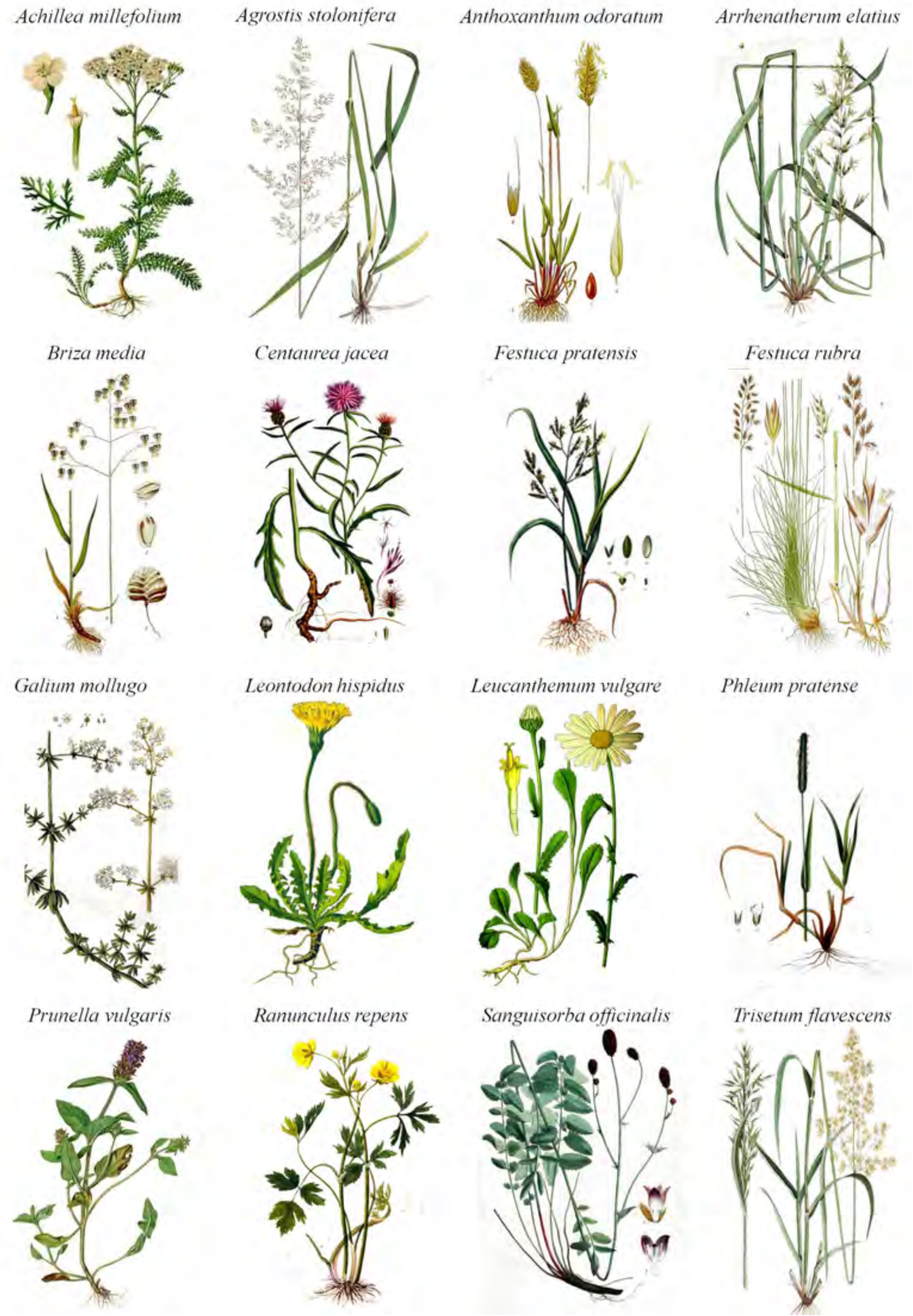

Figure 1.5 Species used in the biodiversity experiment.

Figure 1.6 Picture of the biodiversity-drought experiment, placing the rain shelters.

Figure 1.7 Picture of the biodiversity-drought experiment during the experimental drought.

Chapter 2

Can root trait diversity explain complementarity effects in a grassland biodiversity experiment?

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2.1 Abstract

The positive relationship between plant biodiversity and community productivity is well established. However, our knowledge about the mechanisms underlying these positive biodiversity effects is still limited. One of the main hypotheses is that complementarity in resource uptake is responsible for the positive biodiversity effects: plant species differ in resource uptake strategy, which results in a more complete exploitation of the available resources in space and time when plant species are growing together. Recent studies suggest that functional diversity of the community, i.e. the diversity in functional characteristics ('traits') among species, rather than species richness *per se*, is important for positive biodiversity effects. However, experimental evidence for specific trait combinations underlying resource complementarity is scarce. As the root system is responsible for the uptake of nutrients and water, we hypothesize that diversity in root traits may underlie complementary resource use and contribute to the biodiversity effects. In a common garden experiment, 16 grassland species were grown in monoculture, 4-species mixtures differing in root trait diversity and 16-species mixtures. The 4-species mixtures were designed to cover a gradient in average rooting depth. Above-ground biomass was cut after one growing season and used as a proxy for plant productivity to calculate biodiversity effects. Overall, plant mixtures showed a significant increase in biomass and complementarity effects, but this varied greatly between communities. However, diversity in root traits (measured in a separate greenhouse experiment and based on literature) could not explain this variation in complementarity effects. Instead, complementarity effects were strongly affected by the presence and competitive interactions of two particular species. The large variation in complementarity effects and significant effect of two species emphasizes the importance of community composition for positive biodiversity effects. Future research should focus on identifying the traits associated with the key role of particular species for complementarity effects. This may increase our understanding of the links between functional trait composition and biodiversity effects as well as the relative importance of resource complementarity and other underlying mechanisms for the positive biodiversity effects.

2.2 Introduction

Many biodiversity experiments have shown a positive relationship between plant species richness and productivity (Hooper *et al.*, 2005, Balvanera *et al.*, 2006, Cardinale *et al.*, 2012). However, the mechanisms underlying this positive biodiversity effect are still debated (Cardinale *et al.*, 2011, Schnitzer *et al.*, 2011, de Kroon *et al.*, 2012, Atwater *et al.*, 2015, Kuebbing *et al.*, 2015). One of the main hypotheses is that positive biodiversity effects on productivity are the result of resource complementarity. Different plant species differ in resource uptake strategy, which results in complementarity in resource uptake when plant species are growing together. In species-rich plant communities, resources will thus be more completely exploited in space and time than in species-poor plant communities (e.g. Berendse, 1982, Tilman *et al.*, 1997b, Loreau *et al.*, 2001, Cardinale *et al.*, 2007, Cardinale *et al.*, 2011, Roscher *et al.*, 2012).

It has been suggested that resource complementarity occurs mainly belowground (van Ruijven *et al.*, 2005, de Kroon *et al.*, 2012, Bardgett *et al.*, 2014, Fischer *et al.*, 2014, Yang *et al.*, 2015). However, empirical studies that have investigated resource complementarity belowground are scarce and the results are mixed. The most classical example of resource complementarity is in differential root distribution patterns (i.e. vertical niche differentiation) among individual plant species (Parrish *et al.*, 1976, Berendse, 1982, Fitter, 1986, Silvertown *et al.*, 2015). Differentiation in rooting depth could imply that species are able to acquire water and nutrients from separate parts of the soil (e.g. shallow and deep soil layers), thereby decreasing resource competition and increasing resource exploitation. However, experimental tests of vertical niche differentiation in terms of nutrient uptake (von Felten *et al.*, 2009, Schultz *et al.*, 2012, Bachmann *et al.*, 2015, Hoekstra *et al.*, 2015) or root biomass distribution (Mommer *et al.*, 2010, Ravenek *et al.*, 2014) have yielded little evidence supporting resource complementarity in grassland biodiversity experiments.

An alternative approach to reveal resource complementarity is to focus on the functional traits of the species involved. Several studies have shown that species composition (Hector *et al.*, 2011, Avolio *et al.*, 2014) and functional group richness (Tilman *et al.*, 1997a, Hooper *et al.*, 2004, Marquard *et al.*, 2009) greatly influence the biodiversity effects. The consensus is that differences between species in functional traits, rather than species richness per se, can enhance total resource capture (Cardinale *et al.*, 2012), but experimental evidence that links trait differences to biodiversity effects is limited (but see Flynn *et al.* 2011). One of the outstanding questions is whether the average trait value of the community (community weighted mean; CWM) or the diversity in traits is more important for the complementarity effects. Given the fact that resource complementarity is based on differences among species, one would expect that trait diversity is more important than the mean trait value. However, the few studies that linked the traits of the species to community performance found that CWMs explained more variation in biomass (Finegan *et al.*, 2015) and biodiversity effects (Roscher *et al.*, 2012) than functional diversity. However, these studies mainly focused on aboveground traits, whereas resource complementarity is predominantly expected to occur belowground. Indeed, recent studies showed that the inclusion of root traits can be important for predicting monoculture and mixture biomass (Roscher *et al.*, 2012, Hernandez *et al.*, 2016, Schroeder-Georgi *et al.*, 2016). Revealing the importance of root traits for mixture community biomass is a next step, which we aim to tackle in the current study.

Here we investigate the importance of root traits for positive biodiversity effects by linking key root traits to complementarity effects in plant mixtures, focusing on rooting depth. We used a pool of 16 plant species to establish communities of four species that differed in average rooting depth as well as in diversity of rooting depths – ranging from only shallow rooting species to only deep rooting species. In addition we grew all monocultures and 16-species mixtures. We assessed biodiversity effects of the mixtures, based on aboveground biomass production, after one growing season. Other root traits, such as specific root length (Ryser, 2006, Fort *et al.*, 2013, Leuschner *et al.*, 2013, Fort *et al.*, 2014, Schroeder-Georgi *et al.*, 2016), root mass density (amount of root biomass per soil volume), or root tissue density (Craine *et al.*, 2001, Leuschner *et al.*, 2013), which could represent alternative nutrient acquisition strategies, increasing resource exploitation (Roumet *et al.*, 2006, Bardgett *et al.*, 2014, Mommer *et al.*, 2015, Ravenek *et al.*, 2016, Roumet *et al.*, 2016), were measured in a separate greenhouse experiment (15 weeks) and included in the community trait diversity analyses, together with rooting depth. Although a specific trait may be a key trait in resource complementarity, it is likely that multiple traits influence resource complementarity, so that a certain trait combination of root traits could best predict the increased biomass in mixtures. We tested 1) if root trait composition (community trait averages) and trait diversity are linked to complementarity effects. In addition, we tested 2) if specific plant species contribute significantly to complementarity effects, and compared their trait values to those of the other species.

2.3 Materials & Methods

2.3.1 Common garden experiment

A common garden experiment consisting of 198 plots was established in April 2014 at the experimental fields of Wageningen University, the Netherlands (51°99'N 5°66'E). Average annual temperature is 10.2 ± 0.7 °C, with an average annual precipitation of 847.3 mm (www.klimaatatlas.nl). The original field soil was removed until 80 cm below the field margin and replaced with a mixture of pure river sand and soil from an old field (3:1) in the upper 50 cm layer, and pure river sand in the lower layer (50-80 cm depth). The topsoil layer (0-50 cm) was rather nutrient-poor (Table 2.1). Wooden frames were pushed into the soil (22 cm deep) to create plots of 70 cm x 70 cm. The plots were arranged in 3 blocks with each 3 rows of 22 plots. Within the blocks, the distance between the plots was 80 cm in the length direction, and 100 cm in the width direction. The soil between the plots was sown with the grasses *Poa pratensis* and *Lolium perenne*.

Sixteen grassland species were grown in monocultures (96 plots), 4-species mixtures (90 plots) and 16-species mixtures (12 plots). The design focused on variation in traits and responses within the 4-species mixtures, rather than the effect of species richness. Each block contained 16 monocultures (all species), 15

Table 2.1: Soil characteristics (means ± standard error) at the start of the common garden and greenhouse experiment.

	Common garden		**Greenhouse**
	sand	**mixed soil**	**mixed soil**
C (g/kg)	8.26 ± 0.26	15.11 ± 0.48	7.01 ± 0.10
N (g/kg)	0.83 ± 0.03	1.23 ± 0.08	0.87 ± 0.05
P (g/kg)	0.12 ± 0.01	0.18 ± 0.01	0.05 ± 0.02
OM %	0.60 ± 0.03	1.45 ± 0.04	1.39 ± 0.05
pH-H_2O	7.24 ± 0.01	7.08 ± 0.07	5.94 ± 0.01

different 4-species mixtures (three of each rooting depth treatment) and two different 16-species mixtures (differing in planting positions and planting abundance, i.e. number of individuals, of the species); replicated twice. The positions of the plots within each block were randomized. The species used are perennial grassland species, of which eight are grass species and eight herb species (Table 2.2). All species occur in the vegetation class Molinio-Arrhenatheratea, a class of moderately nutrient-rich hay meadows (Schaminée *et al.*, 1996). Species were selected to have a gradient in maximum rooting depth (RD; Van Duuren *et al.*, 2003). Due to seed contamination in *Leontodon hispidus*, two species of the same genus, *Leontodon hispidus* and *Leontodon autumnalis*, were present in the experiment. Therefore, the experiment contained 17 species instead of 16, but we will continue to refer to "4-species mixtures" and "16-species mixtures", as the two *Leontodon* species shared one position in the design. For both species, however, we use separate trait and biomass values in the analyses outlined below, based on the number of individual plants per plot.

We explicitly used the root trait RD as a factor in the design of our study. Therefore, 4-speciecommunities were designed to cover a gradient in RD. RD was taken mainly from "Biobase 2003" (Van Duuren *et al.*, 2003) that used five maximum rooting depth classes: 0-0.10 m, 0.10-0.20 m, 0.20-0.50 m, 0.50-1.00 m and >1.00 m, based on Kutschera (1960), Kutschera *et al.* (1982), Beringen *et al.* (1986) and Ellenberg (1952). We classified species with maximum rooting depth values between 0.10 -0.50 m as shallow species, and species with a maximum rooting depth deeper than 0.50 m as deep species for our experimental design. We created 4-species mixtures with five levels of rooting depth by randomly selecting either four, three, two, one or zero shallow-rooting species (or vice versa, deep-rooting). The plant communities were established by planting five-week old seedlings, similar in size (c. 2-5 cm shoot, depending on the species, and c. 5-10 cm deep roots) and grown on the same soil as used in the field, in a grid of 8 x 8 plants (64 per plot) to ensure similar overall plant densities in each plot. This plant density is similar to other biodiversity experiments with planted seedlings (e.g. van Ruijven *et al.*, 2003, Berendse *et al.*, 2015). The inner grid of 6x6 plants was used for harvests. In the 4-species mixtures, the positions of the species within a plot were assigned randomly, with equal density of each species. In addition, we maximised interspecific competition, by planting species such that individual plants were standing directly next to plants of all other species present.

The species positions in the 16-species mixtures were also chosen randomly. The 16-species plots were planted in six different random positions (with two replicas), to account for plant neighbour effects. In the first months after planting, sprinklers were used in dry periods to prevent seedling desiccation. All plots were frequently weeded to maintain species composition.

In September 2014, aboveground biomass was harvested per plot per species. The plants were clipped two cm above the soil surface. The biomass samples were washed to remove sand and oven dried for 48 hours at 70 °C.

2.3.2 Greenhouse experiment

Individual plants of the same plant species as used in the common garden experiment were grown in the greenhouse in Wageningen, the Netherlands. Most species grew from January 2015 onwards, but three species (*Sanguisorba officinalis, Leontodon hispidus* and *Ranunculus sardous*) followed 3-5 weeks later due to slow germination. All plants were grown in 3L (19 cm diameter; 15.5 cm high) pots, one plant per pot, with a similar mixture of pure river sand and field soil (3:1) as the common garden experiment (see Table 2.1 for soil characteristics). The temperature in the greenhouse was set to 17/19°C (18.3 ±1.5 °C) respectively following an 8-16 hour dark-light cycle. Growth lamps (600 W; c.80 μmol m^{-2} s^{-1}) were automatically turned on when light levels were lower than 85 W·m^{-2} during the day. The pots were watered 2-3 times per week from below to maintain a moisture content of about 17.9 (±2.3)% (gravimetric; slightly above field capacity). The replicates, 10 pots with 1 plant per species, were divided over 10 blocks and placed in a random position within the block. After 15 weeks, the plants were harvested and the roots were washed. All green, fully developed leaves were scanned with a leaf scanner (Li-3100 Area Meter) to determine specific leaf area (SLA). A representative root subsample of 50 mg fresh material was taken from each plant for the root trait measurements (see below). Only fine roots (< 2 mm) were included in the root trait measurements. Root storage organs of *S. officinalis* were thereby excluded. The root samples were stored in 70% ethanol before they were rinsed and coloured with neutral red (0.07 g L^{-1}) for 24 h. They were scanned at a resolution of 600 dpi (EPSON Perfection V700/V750 3.92) and analysed automatically with WinRHIZO (Pro V 2013e). All fresh plant material was dried for 48 hours at 70°C and weighted.

2.3.3 Selection of root traits

We measured root traits that represent different aspects of the root system and are potentially linked to belowground resource complementarity (Bardgett *et al.*, 2014, Hernandez *et al.*, 2016, Roumet *et al.*, 2016, Schroeder-Georgi *et al.*, 2016). As potential morphological key traits, we measured specific root length (SRL; $m \cdot g^{-1}$) and root tissue density (RTD; $g \cdot cm^{-3}$). A high SRL (and low RTD) is associated with a more competitive strategy with higher mobile nutrient uptake and low longevity (Ryser, 2006, Mommer *et al.*, 2011, Ravenek *et al.*, 2016), while a high RTD reflects a more conservative and stress tolerant resource acquisition strategy (Eissenstat, 1992, Craine *et al.*, 2001, Fort *et al.*, 2013), although these relations are not always consistent (Weemstra *et al.*, 2016). SRL appeared to be an important trait predicting monoculture biomass (Schroeder-Georgi *et al.*, 2016) and potentially important for complementary water uptake (Barkaoui *et al.*, 2016). Rooting depth (RD; m) was taken as an important root distribution trait (Lynch, 1995) to reflect vertical differentiation in resource uptake (Berendse, 1982, Fitter, 1986, von Felten *et al.*, 2008, Mommer *et al.*, 2010, von Felten *et al.*, 2012), a potentially important trait in the positive biodiversity effect mechanism (Mueller *et al.*, 2013, Hernandez *et al.*, 2016). Root mass density (RMD; g root L^{-1} soil) was used as a root performance measure, reflecting the root investment per soil volume of the pot. We included the specific leaf area (SLA; $m^2 \cdot kg^{-1}$) as an aboveground reference trait. CWM of SLA was the best predictor of biomass in Finegan *et al.* (2015). SRL, RTD, RMD and SLA were measured in the greenhouse experiment, and RD was taken from maximum rooting depth classes from literature (Van Duuren *et al.*, 2003) as mentioned above. In order to calculate a numeric species specific RD, we used the average of the maximum rooting depth classes that were given by "Biobase 2003" (Van Duuren *et al.*, 2003), which were based on the lowest and highest maximum rooting depth values found in Kutschera (1960), Kutschera *et al.* (1982), Beringen *et al.* (1986) and Ellenberg (1952). We used 1.25m for the highest class of >1 m to facilitate the calculation of community trait means and diversity (see below).

2.3.4 Calculations

We used the planted species abundance (relative density) to calculate community weighted means (CWMs) and trait diversity, because we expected that early after establishment (one growing season in this case) the number of planted individuals provides a better estimate of abundance (of traits) than aboveground biomass. Moreover, aboveground abundance may not be similar to belowground abundance. Hence, relative abundance was 25% and 6.25% in 4- and 16-species mixtures respectively, for all species except the *Leontodon* species, for which the abundance was calculated based on the actual number of individual plants, as they shared one position in the design due to seed pollination (see 'common garden experiment' above). Trait diversity in plant mixtures, both for single traits and the five root traits combined, was calculated as functional dispersion (FDis; Laliberté & Legendre 2010). We used FDis as the index for functional trait diversity, as it incorporates the dispersion or spread of trait values within the plant communities in relation to the average trait values (weighted mean distance to the weighted mean), a good proxy for trait diversity of the community, independent of its average. Moreover, FDis is independent of species richness (Laliberté *et al.*, 2010). We did calculate other trait diversity indices – functional richness, Rao's Q, functional evenness, and functional divergence (Villéger *et al.*, 2008, Mason *et al.*, 2013), but these were closely correlated to FDis and did not change the results. All traits were first standardized (mean = 0, stdev = 1) and checked for trait correlations in order to include only uncorrelated traits (Villéger *et al.*, 2008, Lefcheck *et al.*, 2015). The functional diversity indices were calculated with the 'FD' package using R (3.1.3).

The additive partitioning approach (Loreau *et al.*, 2001) was used to calculate the biodiversity effects. The Net Effect (NE) measures the overall difference between the observed yield in mixture and the expected yield based on the mean of the monocultures. This effect is partitioned into Complementarity Effects (CE) and Selection Effects (SE) to distinguish between the average increase in species performance in mixture (CE) and effects of highly productive species dominating species mixtures (SE). Here, we focus on CE as this effect is most closely associated with resource complementarity (Loreau & Hector 2001). We used the relative CE (rCE; CE/mean monoculture biomass) to have a measure that is independent of differences in mean monoculture biomass (Craven *et al.*, 2016). NE, CE and SE were calculated within blocks, as the monoculture biomass was significantly lower in block one (ANOVA on monoculture biomass with block and species as fixed effects. Block: $F_{2, 48} = 9.305$, $P < 0.001$, independent of the species ($F_{30,48}=0.8$, $P = 0.743$ for block*species interaction).

Species performance in mixtures was calculated per plot using Deviation (D_i) following Loreau (1998). D_i is the proportional deviation of a species yield in mixture from its expected yield: $D_i = \frac{O_i - E_i}{E_i}$. The expected yield of a species (E_i) was calculated as its proportion of individuals planted in the mixture multiplied by its monoculture biomass. The advantage of using D_i is that it is independent of species richness and composition (Loreau, 1998), allowing comparison of 4-species mixtures and 16-species mixtures.

2.3.5 Statistical analyses

The effect of species richness on community biomass was tested with a Linear Mixed Model (LME) with species richness as fixed factor and species composition as random factor. Differences between the species richness levels were tested with pairwise comparisons (LSD). The same LME model was used for NE, SE and CE in 4- and 16-species mixtures.

Trait correlations were assessed using Spearman correlation coefficients, as the trait values were not always distributed normally. The relationships between complementarity effects (rCE) and CWMs or trait diversity (FDis) were calculated with an LME with rCE as dependent variable and the CWM or FDis as fixed factor (covariate), species richness as fixed factor to test for potential differences between the 4- and 16-species mixtures, and species composition as random factor.

To investigate whether complementarity effects depended on the presence of particular species, we used an LME with presence/absence of each species as separate fixed factors and species composition as random factor. We only included the 4-species mixtures in these analyses, as all species were always present in the 16 species mixtures. Species that were significant were included in a second LME in which interactions between species were also included, and the species effects were tested by pairwise comparisons. Species performance in mixtures (D_i) was analysed per species using an LME similar to the one described above for NE, CE and SE. The performance data (D_i) of *Leucanthemum vulgare* was log(x+1) transformed to meet model assumptions. Statistical analyses were done in IBM SPSS Statistics 21, and in R 3.3.0 using the package "nlme".

2.4 Results

2.4.1 Biomass patterns

Species richness had a positive effect on aboveground community biomass ($F_{2,\ 63} = 4.5$, $P < 0.05$; Fig. 2.1a). Shoot biomass was significantly higher in the 4-species mixtures and 16-species mixtures than in the monocultures ($t = 2.7$, $P < 0.01$ and $t = 2.2$, $P < 0.05$ respectively), but did not increase from 4-species to 16 species mixtures ($t = 0.7$, $P = 0.511$).

Similarly, NE, CE and SE were significantly greater than zero in 4-species and 16-species mixtures ($F_{1,\ 51} = 11.3$, $P < 0.001$, $F_{1,\ 51} = 15.0$, $P < 0.001$ and $F_{1,\ 51} = 39.7$, $P < 0.001$ for NE, CE and SE respectively; Fig. 2.1b), but did not differ between 4-species mixtures and 16-species mixtures ($F_{1,\ 49} = 0.6$, $P = 0.455$ $F_{1,\ 49} = 1.9$, $P = 0.174$ and $F_{1,\ 49} = 0.3$, $P = 0.565$ for NE, CE and SE respectively). On average, a large part of the NE was attributed to the CE (49% for the 4-species mixtures and 75% for the 16-species mixtures). Importantly, the 4-species mixtures showed a wide range in CE (from -46 to +109 g m^{-2} in 4-species mixtures and -25 to + 88 g m^{-2} in 16-species mixtures), indicating that complementarity depends on species composition.

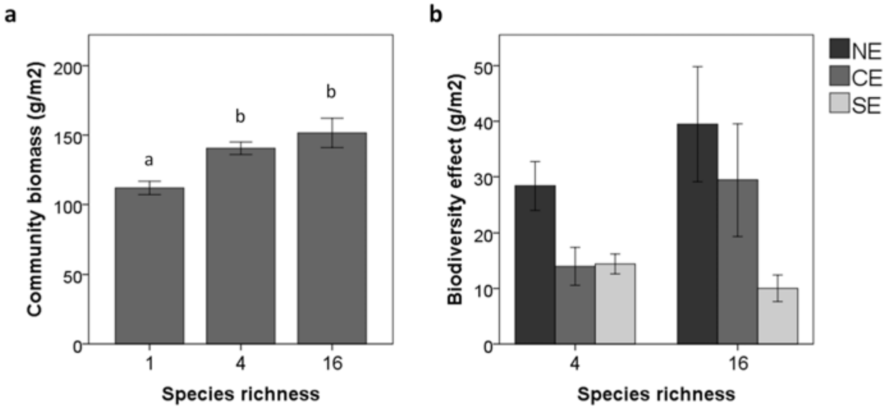


Figure 2.1 a) Community biomass (g·m^{-2}) was significantly higher in mixtures than in the monocultures. b) Consequently, NE, CE and SE are greater than zero in both mixture types. Bars show means ± standard error.

2.4.2 Linking complementarity and traits

Species differed significantly in SRL, RMD, RTD, RD and SLA (Table 2.2; Fig. S2.1). The species covered a relatively large range in trait values. For example, SRL ranged from 116 (*C. jacea*) to 610 $m \cdot g^{-1}$ (*T. flavescens*) and RD ranged from 0.15 to 1.25 m, with *A. stolonifera, A. odoratum, B. media, L. autumnalis, L. hispidus,* and *P. vulgaris* as shallow, and *A. elatius* and *G. mollugo* as the deepest rooting species (Table 2.2; Fig. S2.1). No significant correlations between the traits were found ($P > 0.143$).

Despite these differences in traits, no relationships between the relative complementarity effect (rCE) of the mixtures and their CWMs were found (Fig. 2.2). Also functional trait diversity, based on single traits (Fig. 2.2) or the combination of traits (Fig. 2.3), could not explain the variation in overyielding among communities.

2.4.3 Species effects on overyielding

In contrast to traits, we did find significant effects of species on rCE. Two species, the herbs *A. millefolium* and *L. vulgare*, had a significant effect on rCE, but in opposite directions (Fig. 2.4): in 4-species mixtures, the presence of *A. millefolium* enhanced overyielding ($F_{1, 43} = 7.1$, $P < 0.05$), while the presence of *L. vulgare* reduced it ($F_{1, 43} = 4.1$, $P < 0.05$). Interestingly, these two effects depended on each other ($F_{1, 41} = 15.536$, $P < 0.001$). The positive effect of *A. millefolium* on overyielding disappeared completely when *L. vulgare* was present ($F_{1, 22} = 21.8$, $P < 0.001$), whereas the presence of *L. vulgare* had no effect on rCE when *A. millefolium* was absent ($F_{1, 31} = 0.1$, $P = 0.707$). However, 16-species mixtures, in which both species are present, did show significantly positive rCE ($t = 2.8$, $P < 0.01$; Fig. 2.4).

Although *L. vulgare* had a negative effect on rCE of the whole community, *L. vulgare* itself performed significantly better in mixtures than in monocultures ($D_i > 0$; $F_{1, 15} = 66.4$, $P < 0.001$; Fig. 2.5). The same was true for *A. millefolium*. Also several other species – *A. stolonifera, A. odoratum, F. pratensis, F. rubra and L. hispidus* – performed overall significantly better in mixtures ($F_{1, 15} > 4.5$, $P < 0.05$). About the same amount of species performed worse in mixtures: *A. elatius, B. media, G. mollugo, P. vulgaris, S. officinalis* and *T. flavescens* ($F_{1, 15} > 9.5$, $P < 0.01$).

Table 2.2 The species used in the experiment and their trait values, as measured in the greenhouse experiment (SRL, RMD, RTD, and SLA) or based on literature (RD). RD: rooting depth, SRL: specific root length, RTD: root tissue density, SLA: specific leaf area., FG= functional group. See Material and Methods for more information. Values show means ± standard error.

Species	Species code	FG	max RD class experimental design	average maximum RD (m)*	RMD ($g \cdot L^{-1}$)	SRL ($m \cdot g^{-1}$)	RTD ($g \cdot cm^{-3}$)	SLA ($m^2 \cdot kg^{-1}$)
Agrostis stolonifera	Agr	Grass	shallow	0.15	0.071 ± 0.01	494 ± 28	0.093 ± 0.005	27.8 ± 1.0
Anthoxanthum odoratum	Ant	Grass	shallow	0.15	0.129 ± 0.012	485 ± 33	0.093 ± 0.004	21.9 ± 1.4
Arrhenatherum elatius	Arr	Grass	deep	1.25	0.092 ± 0.009	240 ± 19	0.134 ± 0.005	31.1 ± 1.6
Briza media	Bri	Grass	shallow	0.15	0.045 ± 0.004	378 ± 22	0.106 ± 0.006	22.6 ± 1.1
Festuca pratensis	Fpra	Grass	deep	0.55	0.132 ± 0.011	287 ± 20	0.139 ± 0.006	17.2 ± 0.9
Festuca rubra	Frub	Grass	shallow	0.25	0.073 ± 0.004	361 ± 26	0.112 ± 0.008	6.6 ± 1.4
Phleum pratense	Phle	Grass	deep	0.55	0.145 ± 0.014	435 ± 25	0.112 ± 0.006	23.2 ± 1.2
Trisetum flavescens	Tri	Grass	shallow	0.35	0.061 ± 0.006	610 ± 32	0.093 ± 0.006	28.9 ± 1.3
Achillea millefolium	Ach	Herb	deep	0.35	0.096 ± 0.013	207 ± 18	0.125 ± 0.009	20.2 ± 0.7
Centaurea jacea	Cen	Herb	shallow	0.35	0.079 ± 0.007	116 ± 8	0.14 ± 0.007	26.7 ± 1.3
Galium mollugo	Gal	Herb	deep	1.25	0.03 ± 0.004	402 ± 23	0.103 ± 0.006	23.9 ± 2.0
Leontodon autumnalis	Leo aut	Herb	shallow	0.15	0.059 ± 0.006	208 ± 23	0.119 ± 0.01	33.7 ± 1.7
Leontodon hispidus	Leo his	Herb	shallow	0.15	0.096 ± 0.013	246 ± 23	0.087 ± 0.004	25.8 ± 1.8
Leucanthemum vulgare	Leu	Herb	deep	0.75	0.078 ± 0.006	266 ± 20	0.096 ± 0.007	18.0 ± 0.9
Prunella vulgaris	Pru	Herb	shallow	0.15	0.042 ± 0.003	258 ± 14	0.097 ± 0.003	22.3 ± 1.6
Ranunculus sardous	Ran	Herb	shallow	0.25	0.045 ± 0.005	252 ± 9	0.056 ± 0.001	22.8 ± 1.1
Sanguisorba officinalis	San	Herb	deep	0.75	0.047 ± 0.007	263 ± 29	0.092 ± 0.006	27.3 ± 1.2

Notes: * Based on literature.

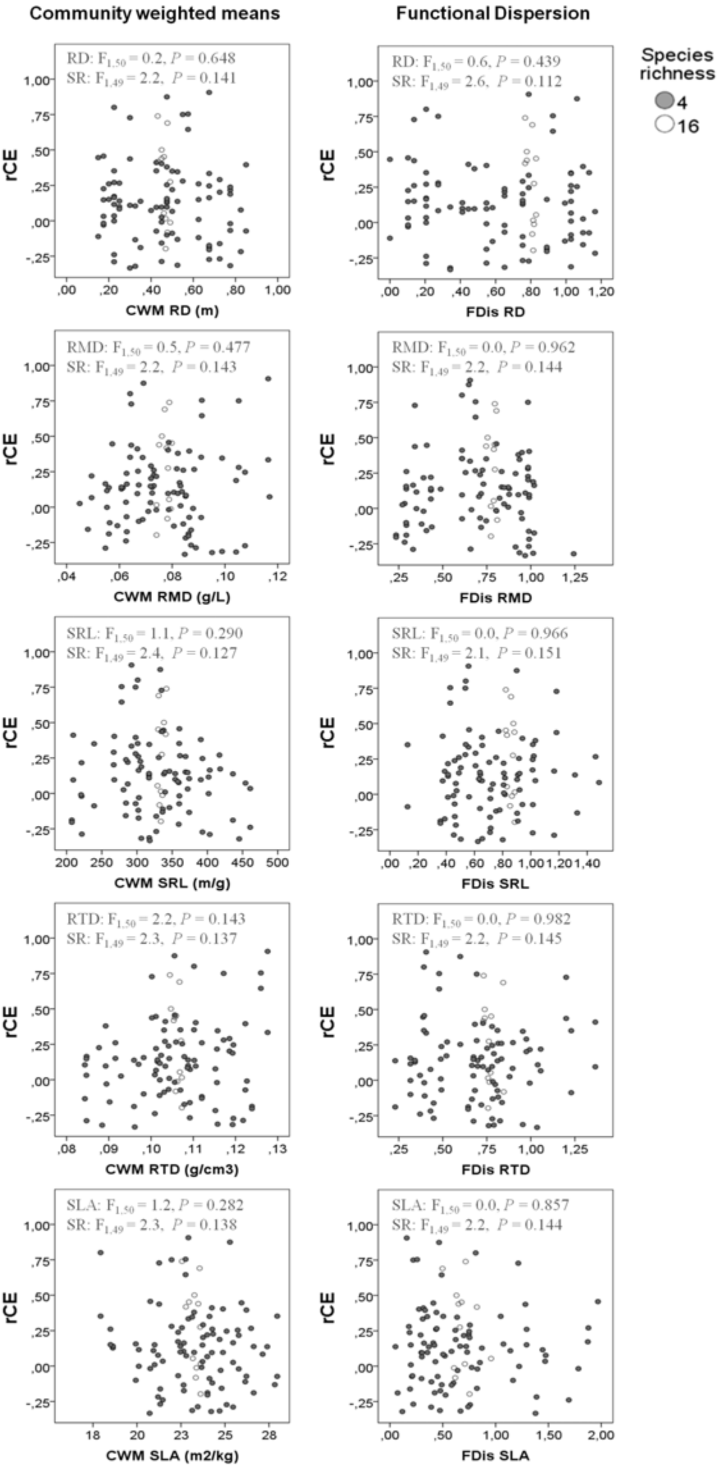


Figure 2.2 Relative complementarity effect (rCE) was not related to community weighted trait means (CWM – left panels) or trait diversity (FDis– right panels). Species richness (SR) did not affect the results. For trait abbreviations, see Table 2.2.

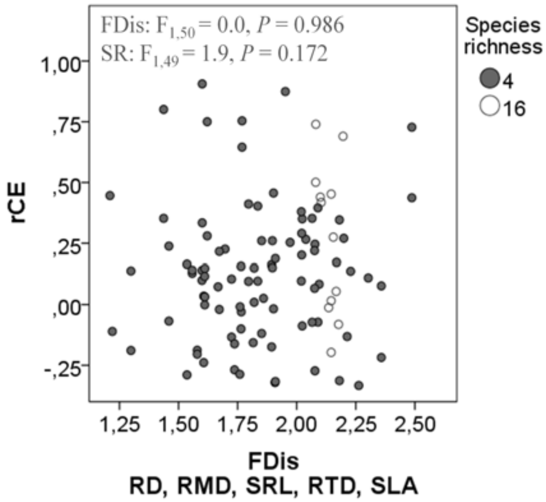


Figure 2.3 Relative complementarity effect (rCE) is not related to the functional diversity (FDis) of all root traits (RD, RMD, SRL, RTD, SLA) combined. Species richness (SR) did not affect the results.

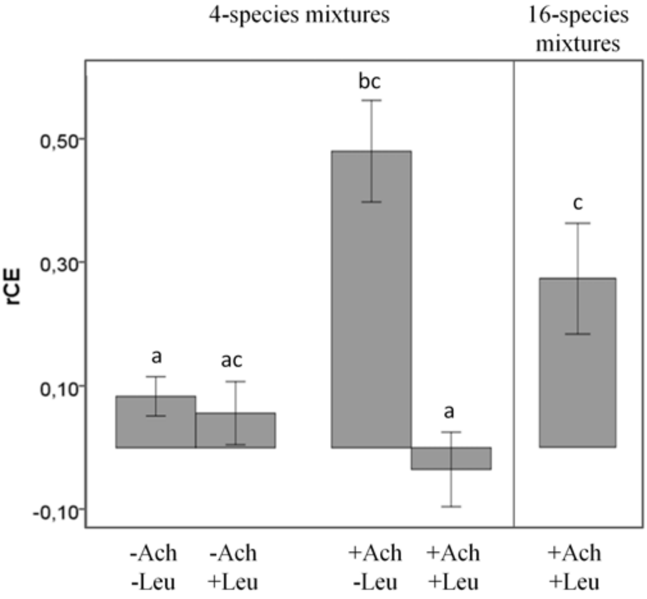


Figure 2.4 Strong interactive effects of two species on relative complementarity effect (rCE). In 4-species mixtures, rCE was increased by the presence of *A. millefolium* (Ach). The presence of *L. vulgare* (Leu) strongly decreased the positive effect of *A. millefolium*. However, in the 16-species mixtures, which contained both *A. millefolium* and *L. vulgare*, complementarity effects were greater than in 4-species mixtures containing these two species (see also Fig 2.1b). Bars show means ± standard errors.

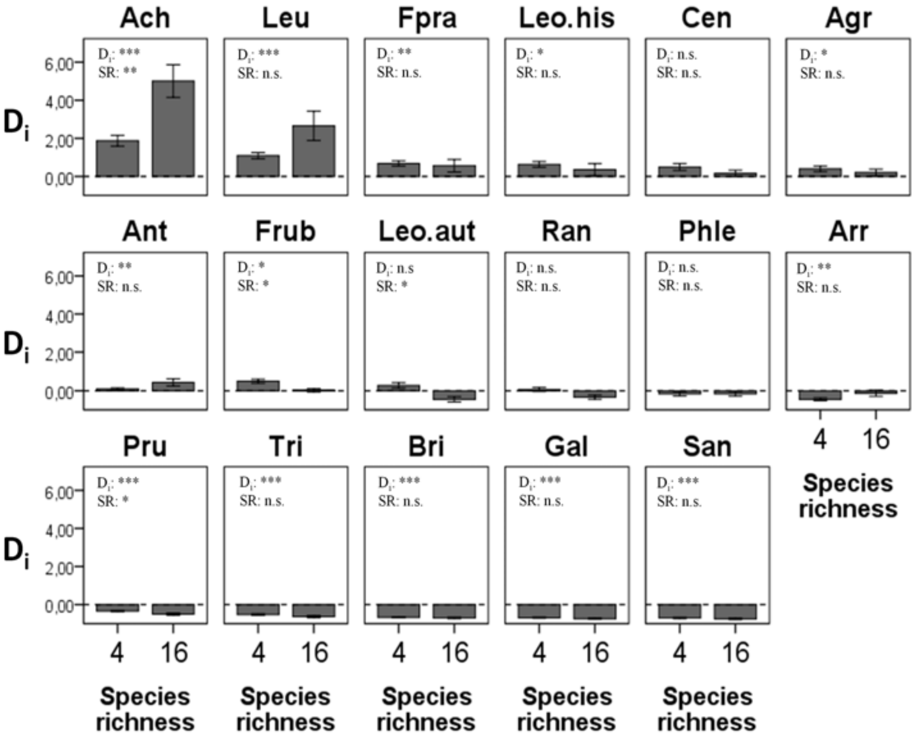


Figure 2.5 The species performance in mixtures (D_i) differs greatly between species, ranging from a four-fold increase (*A. millefolium* (Ach) in 16-species mixtures) to more than 50% reductions *T. flavescens* (Tri), *B. media* (Bri), *G. mollugo* (Gal) and *S. officinalis* (San). Asterisks next to D_i and species richness (SR) indicate deviations from zero and effects of species richness, respectively. *** = P < 0.001, ** = P < 0.01, * = P <0.05, n.s. = not significant. Bars show means ± standard errors.

2.5 Discussion

Overall, species mixtures performed significantly better than expected, and positive complementarity effects (CE) occurred. However, this could not be explained by gradients in root trait CWMs or root trait diversity. Instead, the CE depended on species composition. In particular, the presence and interactions of two particular species did explain differences in CE among mixtures.

A meta-analysis of Cardinale *et al.* (2007) showed that community productivity increased with increasing species richness in 79% of the 44 biodiversity experiments included in the analysis. Indeed, our study confirmed these results: the community biomass increased with species richness and we found significant biodiversity effects in the 4-species and 16-species mixtures. Notable is the low productivity compared to other experiments in Cardinale *et al.* (2007): the monocultures yielded on average 115 ± 5 g m^{-2} compared to 226 g m^{-2} in literature. This is probably the result of the low amounts of nutrients in the soil (Table 2.1) and relative young age of the plants. Despite low productivity, NE was on average 0.21 in mixtures (30 ± 4 g m^{-2} NE of 142 ± 4 g m^{-2} community biomass), which matches the average NE of 0.26 (0.21 - 0.31 CI) that is found in other studies when comparing mixtures with their monocultures (Cardinale *et al.*, 2007). Similar to the majority of studies, both CE and SE contributed to NE. The ratio between CE and SE in this study is smaller (1.0; 14 ± 3 g m^{-2} CE and 14 ± 2 g m^{-2} SE) in the 4-species mixtures than is usually found (1.9; Cardinale *et al.* 2007), but bigger (2.9; 29 ± 10 g m^{-2} CE and 10 ± 2 g m^{-2} SE) in the 16-species mixtures. However, interestingly, these positive CE occurred already after the first growing season, whereas several experiments have reported that positive biodiversity effects become only apparent after the second year (e.g.Tilman *et al.*, 2001, van Ruijven *et al.*, 2005, Fargione *et al.*, 2007, Marquard *et al.*, 2009), and increase in time (Cardinale *et al.*, 2007). Therefore, the relative contribution of CE to NE is fairly high compared to the first year of other experiments. More importantly, in our study, the four species mixtures showed a large variation in rCE, indicating that species composition influences complementarity effects substantially.

In contrast to our hypothesis, the variation in rCE could not be explained by the root trait composition of the communities. Neither CWMs of several important root traits – RD, RMD, RTD and SRL – nor functional diversity in these traits (alone or in combination) showed clear relationships with rCE. There might be several explanations as to why we were not able to establish relationships between functional traits and complementarity effects, which can broadly be grouped into two categories: 1) the traits we used do not capture resource complementarity, and 2) other mechanisms than resource complementarity are more important for positive biodiversity effects. Below, we will discuss these in detail.

2.5.1 Traits that capture resource complementarity

In our experiment, we focus on morphological traits associated with spatial resource uptake differences, which have been shown to be related to community biomass: RD (Mueller *et al.*, 2013, Hernandez *et al.*, 2016), SRL (Schroeder-Georgi *et al.*, 2016), RMD and RTD. In addition, traits related to temporal differences in nutrient uptake (phenology) could be important to include (e.g. Ebeling *et al.*, 2014). Additional insights in traits may come from studies focusing on competitive ability rather than complementarity. For example, Ravenek *et al.* (2016) found that high growth rates (RGR) and high root length densities (RLD), rather than SRL, determine competitive ability. RLD is the product of two traits we used in our study (RMD and SRL), and as such less likely to contribute to explain complementarity effects, but including RGR (and relative root growth rates in particular) in future studies may improve the predictive power of trait indices.

2.5.2 Trait values

Another reason why we did not find a link between root traits and complementarity effects may be that the trait values we used do not accurately reflect the traits in the actual mixtures. Although we used rooting depth values from a data base that used multiple maximum rooting depth sources, rooting depth values in our experiment might have been different. Different species values would result in different community trait values, affecting the relationship between rooting depth and rCE. Similarly, although we measured most of the traits on the same species (from the same seed populations) in a separate greenhouse experiment rather than obtaining them from databases, we cannot rule out that the trait values in the common garden experiment were different due to intraspecific trait variability or trait plasticity (Jung *et al.*, 2010, Carmona *et al.*, 2015). More specifically, differences in abiotic conditions between the greenhouse experiment and common garden experiment may have affected the root trait values. In general, nutrient poor habitats stimulate root growth compared to shoot growth, resulting in an increased root mass fraction (Freschet *et al.*, 2015) and root to shoot ratio (Boot *et al.*, 1990, Forde *et al.*, 2001). Several studies showed that morphological root traits such as root diameter or SRL (Zobel *et al.*, 2007, Leuschner *et al.*, 2013), root length (Ryser *et al.*, 1995), and RTD (Leuschner *et al.*, 2013) could respond to nutrient changes. However, other studies have shown that root trait values are relatively stable within species. For example, variability in SRL in response to changes in nutrient availability has been shown to be relative moderate or even insignificant for fine roots (Boot *et al.*, 1990, Hutchings *et al.*, 1994, Freschet *et al.*, 2015, Poorter *et al.*, 2015). In addition to trait variability within a species due to responses to the environment, trait values might also vary within a root system of an individual plant, depending on root diameter (Zobel, 2003, Drouet *et al.*, 2005), branching order (Picon-Cochard *et al.*, 2012) or age (Drouet *et al.*, 2005). We cannot rule out that trait values differed between the common garden and the greenhouse experiment due to changes in root branching and the relative proportions of different orders in response to differences in environmental conditions (Hutchings *et al.*, 1994, Forde *et al.*, 2001) and plant age (Drouet *et al.*, 2005) between the greenhouse and common garden experiment.

Furthermore, compared to the greenhouse experiment, the species in the common garden experiment may have different trait values due to plastic trait responses to heterospecific neighbours. Indeed, several studies showed that trait values can change in plant mixtures compared to single plants or plant monocultures (Gubsch *et al.*, 2011, Nord *et al.*, 2011, Mueller *et al.*, 2013, Lipowsky *et al.*, 2014, Zuppinger-Dingley *et al.*, 2014, Belter *et al.*, 2015). For example, plant species richness has been found to increase SLA (Lipowsky *et al.*, 2014). Similarly, community rooting depth distribution has been shown to change with species richness (Skinner *et al.*, 2006, Mommer *et al.*, 2010, Mueller *et al.*, 2013, Hernandez *et al.*, 2016). Yet, species-specific changes in rooting depth distribution in plant mixtures remain unknown.

In general, information on how root trait values change in species mixtures is very scarce, due to the fact that it is very difficult to identify roots in communities with multiple species. The lack of knowledge on if or how root traits change in species mixtures, and how these changes depend on species identity and environmental conditions, makes it difficult to determine how root trait plasticity would change our results. Nonetheless, we think it is unlikely that trait value differences between the common garden and the greenhouse experiment are the main determinants of our results. We expect that other reasons (see paragraph above and below) are more likely to explain why we did not find a relation between root traits and complementarity effects. However, new experimental research, aimed at measuring trait values for a range of species under different abiotic conditions and in different competitive settings, will be needed to really determine the suitability of single trait values to predict species and community performance.

2.5.3 Alternative mechanisms

It is also possible that we did not find a relationship between traits and complementarity because resource complementarity is not the main mechanism underlying the observed positive biodiversity effects. There is empirical evidence for vertical differentiation in nutrient and water uptake among plant species (Parrish *et al.*, 1976, Berendse, 1982, Fitter, 1986, Silvertown *et al.*, 2015), but perhaps it does not lead to increased NE and CE (von Felten *et al.*, 2009, Mommer *et al.*, 2010, Schultz *et al.*, 2012, Turnbull *et al.*, 2013, Bachmann *et al.*, 2015, Hoekstra *et al.*, 2015, Hernandez *et al.*, 2016). Several studies suggest that other mechanisms than resource complementarity are responsible for the positive biodiversity effect (de Kroon *et al.*, 2012, Fischer *et al.*, 2014, Yang *et al.*, 2015). In many cases, these mechanisms involve interactions with other organisms (Eisenhauer, 2011): the accumulation of species-specific pathogens at low diversity (Maron *et al.*, 2011, Schnitzer *et al.*, 2011, Hendriks *et al.*, 2013), or increased beneficial soil biota (mycorrhiza) diversity in mixtures (Balvanera *et al.*, 2006, König *et al.*, 2010, Hiiesalu *et al.*, 2014). We assume that the effects of soil biota will become more important in the long-term, and that plant-plant interactions determined most of the patterns in productivity and biodiversity effects we observed in the first growing season, but we cannot rule out that soil biota played a role. Either way, traits that can capture the interactions with soil biota would constitute a valuable addition to a trait-based approach to explain positive biodiversity effects.

2.5.4 The importance of particular species for complementarity

The large variation in rCE that we observed in the 4-species mixtures shows that, similar to most other biodiversity studies (e.g.Tilman *et al.*, 1997a, Hooper *et al.*, 2004, Hooper *et al.*, 2005, Hector *et al.*, 2011, Avolio *et al.*, 2014, Finegan *et al.*, 2015, Hernandez *et al.*, 2016), not only species richness, but also species composition is very important for productivity and positive biodiversity effects. That differences among species are important for biodiversity effects, is also clearly illustrated by the significant effects of two particular species on community rCE. Interestingly, both *A. millefolium* and *L. vulgare* strongly increased in performance in mixtures, but their effects on community overyielding were contrasting: rCE was strongly increased by the presence of *A. millefolium*, but decreased by the presence of *L. vulgare.* The question is how to explain these effects. Both species had their root trait values in the central part of the range of trait values across all species (Fig. S2.1). Therefore, differences in root traits are unlikely to explain the important effects of *A. millefolium* and *L. vulgare* in mixtures. Interestingly, both species are from the same tribe (Anthemideae) within the Asteraceae family (Thompson, 2007). This suggests that they may share other important traits, related to the rhizobiome (Wehner *et al.*, 2014) or root exudates (Mommer *et al.*, 2016), which enhanced their competitive ability in this experiment, and affected the complementarity effects at the community level. Elucidating the traits associated with the success of these two species, and their contrasting effects on complementarity in mixtures, may enhance our understanding of the relationships between trait composition and positive biodiversity effects.

In conclusion, we show positive biodiversity effects in plant mixtures, but found no relationship between root trait diversity and complementarity effects despite a clear gradient in biodiversity effects across species compositions. Although the consensus is that functional differences are more important for biodiversity effects than species richness per se, experimental evidence identifying the key functional differences remains limited. On the other hand, it is clear that species composition is important. This is illustrated here by the strong effects of two particular species from the Asteraceae family on rCE. Future research, investigating the traits of key species, in relation to the traits of the other species, may increase our understanding of the links between functional trait composition and biodiversity effects. This is likely to also shed light on the relative importance of resource complementarity and other underlying mechanisms for the positive biodiversity effects.

2.6 Acknowledgements

We would like to thank colleagues and students from our group, and the department of Experimental Plant Ecology, Radboud University, Nijmegen, for their help with planting and collecting data. Special thanks to Jan van Walsem, Frans Moller and Jan-Willem van der Paauw for all their help in the common garden experiment. This study was supported by the Research School for Socio-Economic and Natural Sciences of the Environment (SENSE), the Netherlands. Liesje Mommer is supported by a NWO-Vidi grant.

2.7 Supplementary figures

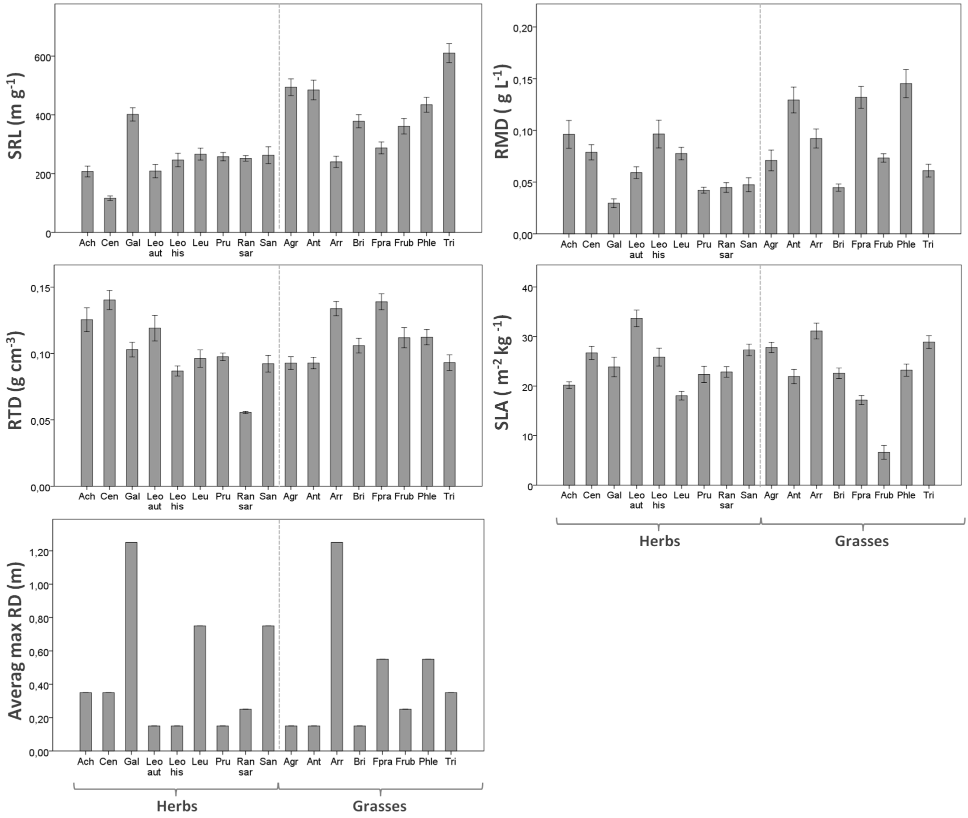


Figure S2.1 Species trait values measured in the greenhouse experiment (SRL, RMD, RTD, and SLA) and based on literature (RD). RD: rooting depth, SRL: specific root length, RTD: root tissue density, SLA: specific leaf area. Bars show means ± standard error. Ach: *Achillea millefolium*, Cen: *Centaurea jacea*, Gal: *Galium mollugo*, Leo aut: *Leontodon autumnalis*, Leo his: *Leontodon hispidus*, Leu: *Leucanthemum vulgare*, Pru: *Prunella vulgaris*, Ran sar: *Ranunculus sardous*, San: *Sanguisorba officinalis*, Agr: *Agrostis stolonifera*, Ant: *Anthoxanthum odoratum*, Arr: *Arrhenatherum elatius*, Bri: *Briza media*, Fpra: *Festuca pratensis*, Frub: *Festuca rubra*, Phle: *Phleum pratense*, Tri: *Trisetum flavescens*.

Chapter 3

Using root traits to understand temporal changes in biodiversity effects in grassland mixtures

Lisette M. Bakker, Liesje Mommer, Jasper van Ruijven

3.1 Abstract

Biodiversity-ecosystem functioning (BEF) studies typically show that species richness enhances community biomass, but the underlying mechanisms remain debated. Here, we combine metrics from BEF research that distinguish the contribution of dominant species (selection effects, SE) from those due to positive interactions such as resource partitioning and facilitation (complementarity effects, CE) with a functional trait approach in an attempt to reveal the functional characteristics of species that drive community biomass in species mixtures. In a biodiversity experiment with 16 plant species in monocultures, 4-species and 16-species mixtures, we used aboveground biomass to determine the relative contributions of CE and SE to biomass production in mixtures in the second, dry year of the experiment. We also measured root traits (specific root length (SRL), root length density (RLD), root tissue density (RTD) and the deep root fraction (DRF)) of each species in monocultures and linked the calculated community weighted mean (CWM) trait values and trait diversity of mixtures to complementarity and selection effects. In the second year of the experiment, community biomass and CE and SE increased compared to the first year. The increase in biomass was stronger in mixtures than in monoculture and the contribution of SE to this positive effect was greater than that of CE. The increased contribution of relative SE was associated with root traits: SE increased most in communities with high abundance of species with deep, thick and dense roots. In contrast, changes in CE were not related to trait diversity or CWM trait values. *Synthesis:* Positive effects of species richness on community biomass were mainly driven by increased dominance of deep-rooting species in a dry year, supporting the insurance hypothesis of biodiversity. Other positive interactions did occur, but the lack of relationships with root traits suggests that belowground resource partitioning or facilitation via root traits were not important for community productivity in our biodiversity experiment.

3.2 Introduction

Biodiversity research has focused on the positive effects of species richness on ecosystem functioning (Hooper *et al.*, 2005, Balvanera *et al.*, 2006, Cardinale *et al.*, 2012, Tilman *et al.*, 2014). Experiments in grasslands have shown that increased plant species richness typically results in higher community productivity (Marquard *et al.*, 2009, Van Ruijven and Berendse, 2009, Reich *et al.*, 2012). Although the focus has traditionally been on species richness, biodiversity experiments can also be considered as a test of how interactions between species affect community productivity (Finn *et al.*, 2013, Brophy *et al.*, 2017). An important question that remains is which interactions between the different species drive the positive effects of plant species richness on productivity. For example, enhanced productivity may mainly be driven by increased dominance of a few productive species (Grime, 1998, Mokany *et al.*, 2008) or due to complementary interactions, such as resource partitioning among species (Hector *et al.*, 1999, Fargione *et al.*, 2007, von Felten and Schmid, 2008).

A first step to identify the importance of interactions between different species for ecosystem functioning is the use of metrics developed in the field of biodiversity-ecosystem functioning research. The additive partitioning method of Loreau and Hector (2001) allows discriminating between the effect of increased dominance of productive species (selection effects, SE) and effects due to positive interactions such as resource partitioning or facilitation (Tilman *et al.*, 1997b, Cardinale *et al.*, 2011), commonly referred to as complementarity effects (CE). Meta-analysis has shown that in general, both complementarity and selection effects contribute to positive biodiversity effects on ecosystem functions, although the contribution of complementarity effects tends to increase with time (Cardinale *et al.*, 2007).

To determine which biological mechanism(s) determine the positive effects of species richness on productivity, calculating the relative contribution of both types of effects is not sufficient. Instead, applying a functional trait approach to patterns of selection and complementarity effects may help to further understand these interactions in diverse plant communities, since there is consensus in biodiversity-ecosystem functioning research that it is not plant species richness *per se*, but the value and range of functional traits of the species and their interactions that determine ecosystem functioning (Dıaz and Cabido, 2001). Functional traits of a community consist of two main components: 1) the community weighted mean trait value (CWM) and 2) the variation in trait values within the community (Díaz *et al.*, 2007). Particularly CWM functional traits have successfully been used to predict ecosystem functions, such as community biomass (Mokany *et al.*, 2008, Roscher *et al.*, 2012, Mueller *et al.*, 2013, Finegan *et al.*, 2015, Barkaoui *et al.*, 2016, Hernandez and Picon-Cochard, 2016). However, traits have rarely been linked directly to metrics like complementarity and selection effects, which specifically quantify the contribution of different species interactions. Here, we determine how functional traits are related to selection and complementarity effects to enhance our understanding of the interactions underlying the positive effects of species richness on ecosystem functioning.

The selection effect (SE) was originally developed to determine whether positive effects of species richness on biomass production could be explained by increased dominance of certain productive species in mixtures (Aarssen, 1997, Huston, 1997, Tilman, 1999b, Loreau and Hector, 2001). This is closely linked to the mass-ratio hypothesis (Grime, 1998), which states that ecosystem functioning is mainly determined by the trait values of the dominant species in the community and can be predicted by the CWM of traits. Linking selection effects to functional traits can identify the traits that lead to positive effects on community biomass via increased dominance of certain species in mixtures. When a certain trait value is favourable and allows species to become dominant in mixtures, selection effects should show a relationship with the CWM trait values (Fig. 3.1A), while the role of variation in that trait is expected to play a minor role (Fig. 3.1B).

The complementarity effect (CE), on the other hand, is considered to be due to positive biotic interactions among plant species, such as resource partitioning (Tilman *et al.*, 2001, Hooper *et al.*, 2005, Cardinale *et al.*, 2007, Cardinale *et al.*, 2011). Resource partitioning can occur in mixtures in which species differ in their resource acquisition strategies (in space or time), leading to an increased exploitation of the available resources (Cardinale *et al.*, 2007, Levine and HilleRisLambers, 2009). For example, a plant community with both shallow and deep-rooting species may be able to explore a larger soil volume for resources than each species in monoculture (Berendse, 1982, Dimitrakopoulos and Schmid, 2004). If complementarity effects are indeed due to resource partitioning among species, then complementarity effects will depend on variation between species in traits associated with resource uptake.

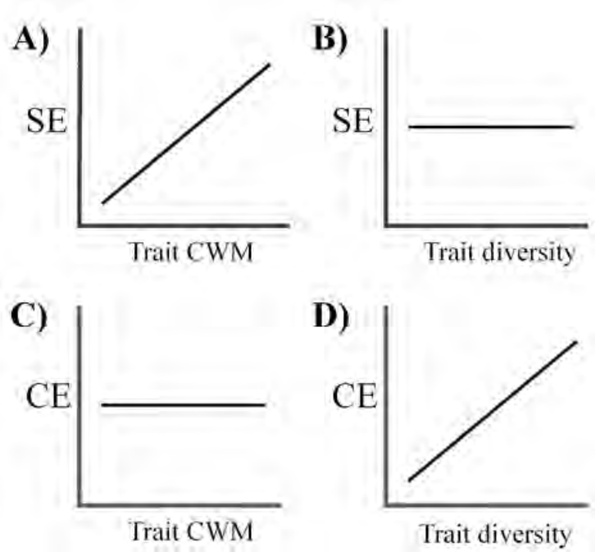


Figure 3.1 Conceptual figure showing the expected relationships between functional traits and selection effects (SE) and complementarity effects (CE) in plant communities. If productive species with particular traits benefit from interactions in mixtures, SE is expected to increase with the CWM of those traits (A), but show no relationship with trait diversity (B). In contrast, if CE is linked to resource partitioning, it should increase with diversity in traits associated with resource uptake diversity (D), but show no clear relationship with CWM traits (C). Note that the relationship between CWM and SE could also be negative if low trait values are related to competitive dominance. See text for further explanation.

Hence, we would expect complementarity effects to increase with increasing diversity in functional trait values (Fig. 3.1D), but show no clear relationship with the average trait value (CWM) of the community (Fig. 3.1C).

Recent research suggests that belowground interactions among plant species are an important, yet hidden driver of biodiversity effects (de Kroon *et al.*, 2012, Bardgett *et al.*, 2014, Fischer *et al.*, 2015, Yang *et al.*, 2015, Mommer *et al.*, 2016). Root characteristics such as rooting depth and SRL have been shown to be important predictors of community productivity (Barkaoui *et al.*, 2016, Schroeder-Georgi *et al.*, 2016). Therefore, we focus on *root traits* that are associated with resource partitioning belowground to identify the interactions that drive community biomass. Root traits are particularly relevant in our experiment, where a drought occurred during the growing season. During a drought, access to belowground resources such as water and nutrients is particularly important. For example, deep-rooting may provide certain species access to additional water, which could benefit neighbouring shallow-rooting species via complementary water use or facilitation, potentially leading to increased complementarity effects in communities consisting of species differing in rooting depths (Fig. 3.1D). However, access to water in deeper soil layers may also give particular species a competitive benefit, which may lead to large selection effects in communities with high CWM rooting depth (Fig. 3.1A).

Here, we use experimental plant communities differing in species richness and composition to determine if combining the additive partitioning method (to determine complementarity and selection effects) with a functional trait approach can help to explain which interactions between species lead to enhanced productivity in plant mixtures. We focus on the changes in biomass and biodiversity effects from the first to the second, dry year of the experiment. Specifically, we test if:

1) community biomass increases with species richness and if this increase becomes stronger in the second year of the experimen
2) increased biomass is associated with significant complementarity and selection effects, and if their contributions to increased biomass in mixtures change from the first to the second year
3) shifts in complementarity and selection effects from the first to the second year can be explained by variation in root traits

3.3 Materials & Methods

3.3.1 Common garden experiment

A biodiversity experiment was established in April 2014 at an experimental field of Wageningen University, the Netherlands (51°99'N 5°66'E). For full details about the experimental design, we refer to chapter 2. Briefly, a total of 198 plots, arranged in three blocks were created by pushing wooden frames of 70 cm x 70 cm into the soil (22 cm deep). Sixteen grassland species were used to establish 96 monocultures (six per species), 90 4-species mixtures (45 different species compositions), and 12 16-species mixtures (six different planting positions and planted species abundances, so six different species compositions), equally divided over the three blocks, so that each block contained two monoculture plots per species, 15 4-species mixtures (replicated twice) and two 16-species mixtures (replicated twice).

In each plot, 64 individuals were planted in a regular 8 x 8 grid, using five-week-old seedlings, to have similar plant densities among species and species richness levels (Balvanera *et al.*, 2006). We used eight perennial grass and eight forb species that are common in moderately nutrient-rich hay meadows in the Netherlands (Table 3.1; Schaminée *et al.*, 1996). Seeds were obtained from a native seed supplier

(Cruydt-hoeck, Nijeberkoop, the Netherlands). Unfortunately, well after planting, the perennial *Ranunculus repens* plants turned out to be individuals of the annual *Ranunculus sardous*. In March of the second year of the experiment, the senesced *R. sardous* individuals were replaced by seedlings of *R. repens*. Further, approximately 30% of the planted *Leontodon hispidus* individuals turned out to be another species from the same genus: *L. autumnalis*. Therefore, the experiment contained 17 species instead of the planned 16 species. We will continue to refer to monocultures, 4-species mixtures and 16-species mixtures, as the two *Leontodon* species shared one position in the design. However, the two species were harvested separately to allow differentiation in the analyses. We harvested aboveground biomass of all plots in September 2014 and in July 2015 by clipping the vegetation at two cm above the soil surface. Biomass was sorted to species per plot and oven dried at 70°C for at least 48h.The inner 6 x 6 plants were used for analyses to account for potential plot edge effects. Plots were weeded regularly to maintain species composition.

3.3.2 Root traits

We measured four root traits of the 16 species in the monoculture plots: specific root length (SRL; $m \cdot g^{-1}$), root length density (RLD; $m \cdot cm^{-3}$ soil), root tissue density (RTD; g cm^{-3}) and deep rooting fraction (DRF). SRL is associated with resource uptake, and is known to respond to nutrient and water availability (Padilla *et al.*, 2013, Fort *et al.*, 2014, Poorter and Ryser, 2015, de Vries *et al.*, 2016). High RTD values are associated with nutrient poor and infrequently disturbed environments, reflecting a conservative resource strategy (Craine *et al.*, 2001). A large root surface per soil volume (RLD) could increase nutrient uptake rate (Mommer *et al.*, 2011) and make plants successful competitors for nutrients and water (Ravenek *et al.*, 2016). DRF, the fraction of roots in the deeper layer, is selected to reflect the vertical distribution of the root system (rooting depth). Especially during drought, when water is only available in the deeper soil layers, DRF could determine nutrient competition and survival (Zwicke *et al.*, 2015, Barkaoui *et al.*, 2016).

To measure root traits, three soil cores (50 cm deep * 2.5 cm diameter) were taken per plot in all monocultures immediately after the harvest in 2015. The soil cores were divided in four soil layers: 0-5 cm, 5-15 cm, 15-30 cm and 30-50 cm. The three cores were pooled per plot per layer and carefully washed with a 0.5 mm sieve to collect the roots. Of each sample, a representative subsample was stored in 70% ethanol and coloured with neutral red (0.07 g·L^{-1}) for 24h before being scanned (EPSON Perfection v700/750) at 600 dpi. The scans were analysed using WinRHIZO (Pro V 2013e) to determine root length and root volume. The remaining samples and the scan subsamples were oven dried for 72h at 70°C and weighted. Root length and dry mass of scan subsamples were used to calculate SRL. RTD was determined using root volume and biomass of the same subsamples. The average SRL and RTD per plot were calculated as the average of the 4 soil layers, weighted by the root biomass of the layers. DRF was calculated as the fraction of root biomass in layer 30-50 cm compared to total root biomass in 0-50 cm. RLD was calculated by multiplying the SRL and root biomass per soil volume.

CWM and trait diversity values were calculated for each mixture plot, using species relative abundances aboveground (% biomass) and monoculture trait values. Trait diversity was calculated as functional dispersion (FDis), an index that incorporates the spread of trait values within a community relative to the average trait value (Laliberté and Legendre, 2010). This proxy for trait diversity is independent of the CWM and species richness (Laliberté and Legendre, 2010). CWMs and FDis were calculated using the "FD" package (Laliberté and Shipley, 2011) in R statistics (version 3.1.3).

3.3.3 Environmental conditions

In the growing season of the second year (June 10th to July 14th 2015) a drought manipulation was set up using temporary rainout shelters and supplementary watering of control plots (see supplementary methods for details). However, due to the warm weather during the treatment period, the control plots also suffered from drought. Measurements revealed that the moisture content at the start of the simulated drought (7.5% ± 0.1) was already well below field capacity (16.1% ± 1). During the drought, soil moisture content of both drought and control plots declined. At the end of the drought, soil moisture content was very low in both treatments, albeit a bit higher in control plots (5.7% ± 0.1) than in drought plots (3.1% ±0.1). Probably due to the overall dry conditions in both the control and the drought treatment, there were no significant differences in biomass between control and drought plots in 2015 (see Table S3.1 for an overview). Therefore, we decided to analyse all plant communities – control and drought – together. Effects of the experimental drought will not be discussed in the remainder of the manuscript.

3.3.4 Biodiversity effects

Complementarity (CE) and selection effects (SE) of all mixture plots were calculated using the additive partitioning method of Loreau and Hector (2001), to assess the performance of the mixtures compared to the monocultures. According to this approach, the net effect of biodiversity (NE) can be partitioned into (CE) and (SE). NE, or delta yield (ΔY), measures the overall difference between the observed yield in the mixture (Y_O) and the expected yield (Y_E), which is the average monoculture yield of the component species. CE and SE can then be calculated using equation 3.1 (Loreau and Hector, 2001).

$$\Delta Y = Y_O - Y_E = N\overline{\Delta RY}\,\overline{M} + \mathrm{N\ cov}(\Delta \mathrm{RY}, M) \qquad (eq\ 3.1)$$

in which N is the number of species in the mixture, and $\overline{\Delta RY}$ is the difference between the observed and expected relative yield in mixture (RY_O - RY_E), averaged across species. The observed RY of a species is its biomass in mixture divided by its biomass in monoculture, whereas the expected RY is the species' planted relative abundance, which in our case was equal to 1/N. $\overline{M}$ is the average monoculture yield of the species that are present in the mixture. In equation 3.1, the left component ($N\,\overline{\Delta RY}\,\overline{M}$) measures CE, while the right term ($\mathrm{N\ cov}(\Delta \mathrm{RY}, M)$) measures SE as the covariance in deviation from expected yield (ΔRY) and monoculture yield (M) across species multiplied with the number of species in mixture (N) (Loreau and Hector, 2001). The RY and M of *Leontodon* species were corrected using the actual number of planted individuals, as they shared one position in the design due to seed pollution (see above). We used the average monoculture biomass per block to calculate NE, CE, and SE, because a lower monoculture biomass was found in block one in the first year ($F_{2,48}$ = 9.3, P < 0.001 with an ANOVA on monoculture biomass with block and species as fixed effects).

It is important to note that CE (and SE) in mixtures not only depend on shifts in biomass production of species (ΔRY), but also on their average monoculture yield. This means that, on average, these biodiversity effects may differ between mixtures simply because the component species differ in monoculture yield. As we are specifically interested in overyielding of different species mixtures, we corrected for differences in monoculture biomass by dividing biodiversity effects by the average monoculture yield ($\bar{M}$) (Craven *et al.*, 2016). These standardized effects give the contribution of CE and SE to changes in biomass in mixtures relative to monocultures and will be referred to as the relative CE (rCE) and SE (rSE). These relative biodiversity effects are independent of community biomass. For example, an rCE of 0.25 means that complementarity effects increased mixture biomass by 25% compared to the average monoculture biomass.

Finally, as we are specifically interested in the biodiversity effect - trait relationships that occurred during the second, dry year, we calculated the change in relative CE and SE from the first year to the dry year as the difference in rCE or rSE between the dry year (2015) and the previous year (2014). These differences will be referred to as ΔrCE and ΔrSE. We will present both results of absolute biodiversity effects (NE, CE, SE) and relative biodiversity effects (rNE, rCE, rSE), and focus on ΔrCE and ΔrSE to link to the community traits.

3.3.5 Statistical analyses

Differences in trait values between the species and between grass and forb species were tested with a Linear Mixed Effect (LME) model with species or plant functional group (PFG; grass or forb) as fixed factor, and block as random factor. We corrected for unequal variance between the PFGs by adjusting the variance structure using 'weights' for the PFGs (Zuur *et al.*, 2009). Correlations between root traits and with root biomass across species were tested using Spearman's correlation coefficient.

The effect of species richness and year on community biomass, biodiversity effects (NE, CE and SE) and relative biodiversity effects (rNE, rCE and rSE) were tested with a LME model with species richness, year and their interaction as fixed factors, and species composition, block and plot number as random factors, using 'REML'. Pairwise comparisons were made per year using Tukey Contrasts with the "multcomp" package (Hothorn *et al.*, 2008). Biodiversity effects were tested with a similar LME model: NE, CE and SE were used as response variable, with species richness, year and their interactions as fixed factors, and species composition and plot number as random factors (REML). Community biomass and biodiversity effects

(NE, CE, and SE) were "ln" transformed to meet model assumptions, after adding the absolute minimal value plus one to have only positive values (for biodiversity effects).

Relationships between community root traits and ΔrCE or ΔrSE were tested using a LME with the trait CWM or trait diversity (FDis) as fixed factor and species composition as random factor (ML). Finally, as two species significantly affected rCE in the first year of the experiment (chapter 2) and significant SE indicates some species are more important than others, we also determined the effects of each species on ΔrCE and ΔrSE in separate models, using a LME with presence/absence of a species as fixed factor and species composition as random factor (ML).

Statistics were done in R statistics (version 3.1.3), using the "nlme" package (Pinheiro *et al.*, 2016) to make linear mixed effect models (LME), and type III ANOVAs to test the effects.

3.4 Results

3.4.1 Community biomass and biodiversity effects

Biomass was positively affected by species richness in both years: species mixtures had on average more biomass than the monocultures in both years (Fig. 3.2A and Table 3.2). This effect of species richness increased in the second year (Fig. 3.2A and Table 3.2). In both years, biomass was not significantly different between 4- and 16-species mixtures. Plant biomass was significantly higher in the second year compared to the first year (Fig. 3.2A and Table 3.2).

Positive biodiversity effects were found in the 4- and 16-species mixtures in both years, with an increase in NE, CE and SE in the second year (Fig. 3.2B and Table 3.2). In the first year, the contribution of CE and SE to the observed NE was approximately equal (53% CE and 47% SE), but in the second year, SE became more important (62% SE vs 38% CE; see Fig. 3.2B). The relative biodiversity effects, standardized for differences in average monoculture biomass between mixtures (rNE, rCE and rSE), showed very similar patterns, with an increase from the first to the second year, and an increased contribution of rSE in the second year (Table 3.2; Fig. S3.1). The increase from the first to the second year (Δ) was greater for rSE than for rCE. On average, ΔrSE was twice as large as ΔrCE: 0.28 versus 0.14. This shows that the positive effect of plant species richness on plant biomass in the second year increased by 42%, of which 1/3 (14%) was due to increased rCE and 2/3 (28%) due to greater rSE (see also Fig. S3.1).

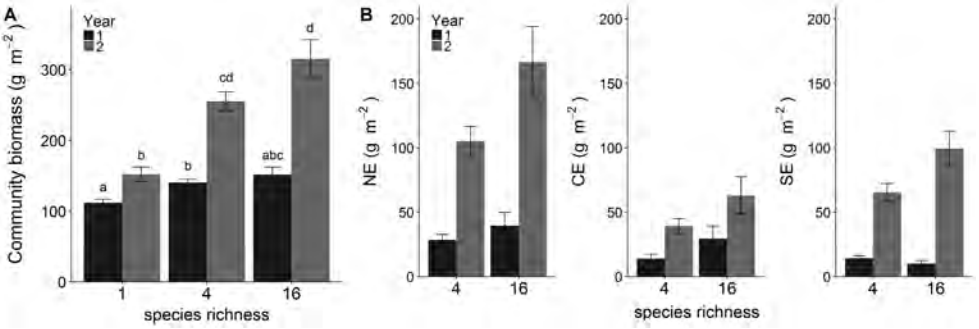


Figure 3.2 Community biomass (A) and biodiversity effects NE, CE, SE (B) at different levels of species richness in the first (black bars) and second year (grey bars) of the experiment. In all cases, the increase from the first to the second year was significant. Community biomass was greater in mixtures than in monocultures in both years (A), but differences between 4- and 16-species mixtures were not significant for biomass, NE, CE and SE (B). Different letters (A) indicate significant differences between years and species richness levels using Tukey Contrasts. Bars show mean ± standard error.

Table 3.2 Effects of species richness (sr) and year on community biomass, absolute net (NE), complementarity (CE) and selection effects (SE), and the relative biodiversity effects (rNE, rCE and rSE). *** = P < 0.001, ** = P < 0.01, * = P <0.05, ns = not significant.

	Intercept	**year**	**sr**	**year x sr**
Biomass (ln)	$F_{1,195}$ = 4584.9 (***)	$F_{1,195}$ = 10.8 (**)	$F_{2,64}$ = 4.2 (*)	$F_{2,195}$ = 12.2 (***)
NE (ln)	$F_{1,100}$ = 17319.9 (***)	$F_{1,100}$ = 78.4 (***)	$F_{1,49}$ = 0.4 (ns)	$F_{1,100}$ = 3.4 (ns)
CE (ln)	$F_{1,100}$ = 32075 (***)	$F_{1,100}$ = 25.3 (***)	$F_{1,49}$ = 1.3 (ns)	$F_{1,100}$ = 0.3 (ns)
SE (ln)	$F_{1,100}$ = 5450 (***)	$F_{1,100}$ = 74.2 (***)	$F_{1,49}$ = 0.1 (ns)	$F_{1,100}$ = 6.6 (*)
rNE	$F_{1,100}$ = 16.2 (***)	$F_{1,100}$ = 48.1 (***)	$F_{1,49}$ = 0.3 (ns)	$F_{1,100}$ = 3.5 (ns)
rCE	$F_{1,100}$ = 12.3 (***)	$F_{1,100}$ = 18.6 (***)	$F_{1,49}$ = 1.9 (ns)	$F_{1,100}$ = 0 (ns)
rSE	$F_{1,100}$ = 13.1 (***)	$F_{1,100}$ = 48.8 (***)	$F_{1,49}$ = 0.2 (ns)	$F_{1,100}$ = 6.7 (*)

3.4.2 Root traits

Species differed significantly in the root traits SRL, RLD, RTD and DRF ($F_{15, 61} > 5.0$, $P < 0.001$; Table 3.1, Fig. S3.2). Differences between species were large, with up to 12, 15, 2 and 6 fold differences between the minimum and maximum trait values of SRL, RLD, RTD and DRF, respectively. The forb *C. jacea* showed the lowest SRL (50 m·g^{-1}) and highest RTD (0.22 g·cm^{-3}), while the grass *A. odoratum* had the highest SRL (601 m·g^{-1}) and lowest RTD (0.11 g·cm^{-3}). Highest RLD was 0.25 m·cm^{-3} for the grass *F. rubra*, whereas the forb *R. repens* reached only 0.02 m·cm^{-3}. Finally, the forb *A. millefolium* had highest DRF, with more than 40% of its root biomass in the 30-50 layer, whereas the grasses *B. media* and *T. flavescens* had less than 10%. On average, forb species had significantly lower SRL and RLD, and higher DRF compared to the

Table 3.1 Species' trait values (mean ± stand error) measured in the monoculture plots (n = 6). FG = functional group, SRL = specific root length, RLD = root length density, RTD = root tissue density, DRF = deep root fraction.

Species	**Species code**	**FG**	**SRL (m·g^{-1})**	**RLD (m·cm^{-3} soil)**	**RTD (g·cm^{-3})**	**DRF**
Agrostis stolonifera	Agr	Grass	359 ± 38	0.15 ± 0.02	0.16 ± 0.01	0.17 ± 0.03
Anthoxanthum odoratum	Ant	Grass	601 ± 13	0.14 ± 0.01	0.11 ± 0.00	0.12 ± 0.02
Arrhenatherum elatius	Arr	Grass	171 ± 9	0.16 ± 0.01	0.16 ± 0.01	0.20 ± 0.04
Briza media	Bri	Grass	317 ± 13	0.21 ± 0.02	0.14 ± 0.01	0.07 ± 0.02
Festuca pratensis	Fpra	Grass	249 ± 16	0.15 ± 0.02	0.15 ± 0.01	0.21 ± 0.03
Festuca rubra	Frub	Grass	292 ± 19	0.25 ± 0.02	0.16 ± 0.02	0.22 ± 0.04
Phleum pratense	Phle	Grass	395 ± 25	0.24 ± 0.02	0.14 ± 0.01	0.15 ± 0.03
Trisetum flavescens	Tri	Grass	434 ± 17	0.22 ± 0.01	0.15 ± 0.01	0.08 ± 0.02
Achillea millefolium	Ach	Forb	99 ± 8	0.08 ± 0.01	0.21 ± 0.00	0.42 ± 0.07
Centaurea jacea	Cen	Forb	50 ± 4	0.05 ± 0.00	0.22 ± 0.01	0.38 ± 0.03
Galium mollugo	Gal	Forb	256 ± 21	0.18 ± 0.02	0.15 ± 0.01	0.30 ± 0.05
Leontodon autumnalis *Leontodon hispidus*	Leo	Forb	136 ± 11	0.08 ± 0.01	0.12 ± 0.00	0.32 ± 0.03
Leucanthemum vulgare	Leu	Forb	196 ± 7	0.11 ± 0.01	0.12 ± 0.01	0.21 ± 0.03
Prunella vulgaris	Pru	Forb	211 ± 9	0.11 ± 0.02	0.12 ± 0.01	0.17 ± 0.04
Ranunculus repens	Ran	Forb	144 ± 28	0.02 ± 0.00	0.14 ± 0.02	0.16 ± 0.02
Sanguisorba officinalis	San	Forb	109 ± 9	0.04 ± 0.01	0.19 ± 0.02	0.35 ± 0.05

grass species ($F_{1, 61}$ = 5.4; $P < 0.001$). However, forb and grass species overlapped in their trait values and covered a wide range of values within their functional group. For example, species' SRL differed five-fold (from 50 ± 4 to 256 ± 21 m·g^{-1}) and 3.5 fold (from 171 ± 9 to 601 ± 13 m·g^{-1}) within forbs and grasses, respectively (See Table 3.1)

SRL was positively related to RLD (ρ = 0.75, $P < 0.01$, n = 16) and negatively to DRF (ρ = -0.79, $P < 0.01$, n=16), which means that species with high SRL had in general high RLD and less roots in the deeper soil layers. The species root trait values were not correlated with species average root biomass (ρ < 0.49, $P > 0.064$, n = 16). See Fig. S3.3 for trait correlations.

3.4.3 Biodiversity effect-root trait relationships

Biodiversity effects differed considerably between plant mixtures. Although the average change in complementarity effects from the first to the second year (ΔrCE) was significantly positive, values of individual plots ranged from -0.60 to 1.24 among the 4-species mixtures. This suggests that biodiversity effects strongly depend on species composition. However, this variation in ΔrCE could not be attributed to variation in root traits. Against our expectations, we could not find any relationships between root trait diversity (FDis) and ΔrCE. Relationships between CWM traits and ΔrCE were also not significant (Fig. 3.3 and Table 3.3).

In contrast, ΔrSE, which also showed considerable variation between mixtures, was clearly related to CWM root traits: communities with low SRL, high RTD, low RLD, and high DRF averages showed the greatest increase in rSE (Fig. 3.4). Of these CWMs, DRF was the best predictor of ΔrSE (lowest AIC; Table 3.3). In addition, changes in rSE were also related to trait diversity: ΔrSE decreased with increasing diversity in SRL and RLD in mixtures. No significant relationships with diversity in RTD and DRF were found (Fig. 3.4). In all cases, the trait diversity models were poorer predictors of ΔrSE than the CWM models (Table 3.3).

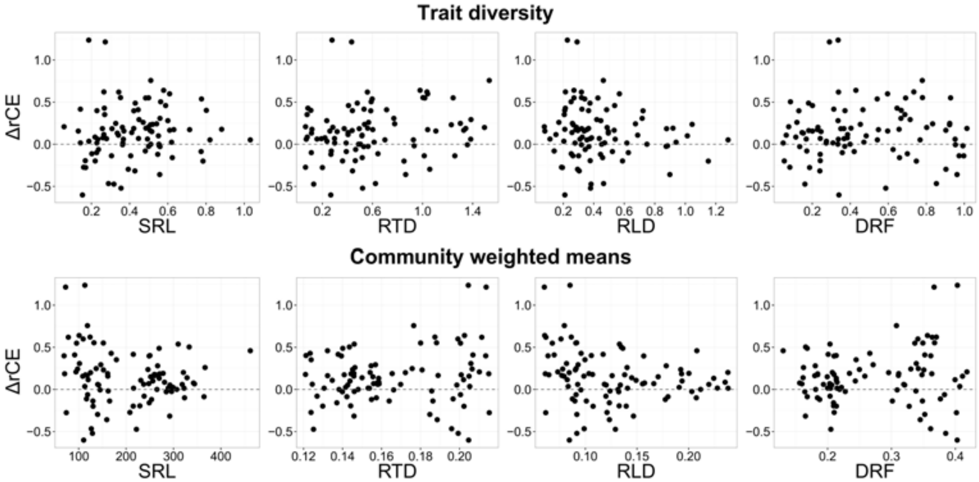


Figure 3.3 The change in relative complementarity effects from the first to the second year (ΔrCE) as a function of root trait diversity (FDis, top panels) and average community root traits (CWM, bottom panels). No significant relationships were found. See Table 3.3 for statistics. SRL = specific root length, RLD = root length density, RTD = root tissue density, DRF = deep root fraction.

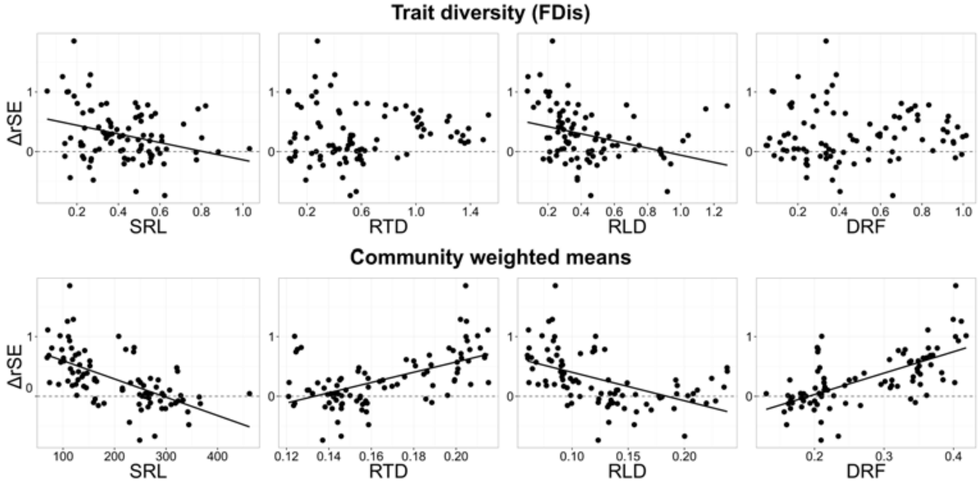


Figure 3.4 The change in relative selection effects from the first to the second year (ΔrSE) as a function of root trait diversity (FDis, top panels) and average community root traits (CWM, bottom panels). Significant relationships are shown by solid lines. See Table 3.3 for statistics. SRL = specific root length, RLD = root length density, RTD = root tissue density, DRF = deep root fraction.

3.4.4 Species contributions

In the 4-species mixtures, the presence of three species significantly affected ΔrCE. The presence of *B. media* had a negative effect, while that of *C. jacea* and *G. mollugo* enhanced ΔrCE (Table 3.3). These species were better predictors of ΔrCE than root trait diversity or CWM (lower AIC; Table 3.3). These three species did not show clear overlap in trait values: the forb *C. jacea* has relative high DRF and RTD, and low SRL and RLD, whereas the forb *G. mollugo* is characterized by intermediate values for each root trait. The grass *B. media* was one of the most shallow-rooting species (low DRF), but had intermediate values for the other root traits (Table 3.1, Fig. S3.1).

Similarly, we found significant effects of the presence of four species on ΔrSE. The presence of the forbs *A. millefolium* and *C. jacea* enhanced ΔrSE, while *P. vulgaris* and *B. media* reduced it (Table 3.3). However, in contrast to ΔrCE, the presence of these four species was a poorer predictor of ΔrSE than the best performing CWM root trait (DRF). The species with a positive effect on ΔrSE*, A. millefolium* and *C. jacea,* were the two species with highest DRF (Table 3.1, Fig. S3.1), whereas the species with a negative effect, *P. vulgaris* and *B. media,* have intermediate and lowest DRF, respectively.

3.5 Discussion

In this study, we found positive effects of species richness on community biomass, which increased from the first to the second, dry year. Application of the additive partitioning method revealed that both positive interactions (complementarity effect, CE) and increased dominance of productive species (selection effect, SE) contributed to the positive effect of species richness on community biomass. However, particularly the selection effect increased strongly from the first to the second year, and contributed most to the positive plant mixture effects in the second year. Importantly, the increase in selection effect (relative; corrected for biomass) was linked to the CWM of the deep root fraction (DRF): communities with a high abundance of deep rooting species showed greater selection effects. In addition, selection effects also increased with increasing root tissue density (RTD) and decreased with specific root length (SRL) and root length density (RLD). In contrast, we found no evidence for

Table 3.3 Effects of root traits and presence of species on the change in relative complementarity and selection effects from the first to the second year (ΔrCE and ΔrSE, respectively). Effect indicates whether the slope of the significant relationship is positive (+) or negative (-). AIC gives the Akaike Information Criterion, which indicates the most parsimonious model (lowest AIC value). For root trait abbreviations, see Table 3.1.

		ΔrCE				**ΔrSE**			
Factor		**Effect**	**F-value**	**p-value**	**AIC**	**Effect**	**F-value**	**p-value**	**AIC**
Trait CWM	SRL		1.75	0.193	56.06	-	33.94	<0.001	53.07
	RTD		1.89	0.176	55.91	+	26.40	<0.001	57.95
	RLD		4.01	0.051	53.86	-	20.28	<0.001	61.98
	DRF		1.10	0.300	56.70	+	43.37	<0.001	46.82
Trait diversity (FDis)	SRL		0.44	0.510	57.39	-	8.64	<0.01	71.69
	RTD		2.33	0.134	55.56		1.28	0.264	78.95
	RLD		1.48	0.231	56.32	-	8.60	<0.01	71.71
	DRF		0.00	0.970	57.82		5.20	0.028	76.02
Species	*A. millefolium*		2.62	0.113	55.21	+	8.11	<0.01	72.47
	A. stolonifera		0.84	0.363	56.96		1.99	0.165	78.09
	A. odoratum		1.15	0.290	56.66		0.83	0.366	79.24
	A. elatius		0.79	0.380	57.02		0.06	0.809	80.02
	B. media	-	5.57	<0.05	52.45	-	7.16	<0.05	73.30
	C. jacea	+	7.92	<0.01	50.37	+	5.53	<0.05	74.76
	F. pratensis		0.01	0.942	57.81		0.07	0.789	80.01
	F. rubra		2.80	0.102	55.04		2.28	0.138	77.80
	G. mollugo	+	14.81	<0.001	44.28		3.41	0.072	76.72
	Leontodon sp.		3.15	0.083	54.71		0.62	0.435	79.45
	L. vulgare		0.02	0.893	57.80		1.63	0.209	78.45
	P. pratense		0.04	0.834	57.77		1.66	0.204	78.41
	P. vulgaris		0.06	0.804	57.75	-	7.65	<0.01	72.87
	R. repens		0.41	0.523	57.40		0.34	0.562	79.73
	S. officinalis		0.59	0.447	57.23		2.12	0.153	77.97
	T. flavescens		0.64	0.427	57.16		0.28	0.599	79.79

relationships between complementarity effects (relative) and root traits. Together, our findings suggest that positive effects of plant interactions in mixtures in a dry year are predominantly due to increased dominance of deep-rooting species.

3.5.1 Temporal increase in biodiversity effects

The net effect (NE) and complementarity effect increased over the first two years of our experiment, consistent with previous studies (Cardinale *et al.*, 2007). However, we also found an increase in the selection effect over time, while most other studies could not identify temporal patterns in selection effects (van Ruijven and Berendse, 2005, Cardinale *et al.*, 2007, Roscher *et al.*, 2016). In the second year of our study, the selection effect was greater than the complementarity effect, which is inconsistent with the general pattern that the complementarity effect is greater than the selection effect (Cardinale *et al.*, 2007). Yet, the sign and size of the selection effect appear to be highly variable among biodiversity studies (Loreau and Hector, 2001, Dimitrakopoulos and Schmid, 2004, Hooper and Dukes, 2004, Roscher *et al.*, 2005, van Ruijven and Berendse, 2005, Cardinale *et al.*, 2007, Vermeulen *et al.*, 2016). We suggest that environmental conditions may play a role in explaining this variability of the selection effect. The drought in the second year may have allowed a few species, which are productive in monoculture, to dominate in mixtures, leading to a large contribution of selection effects to positive effects on community biomass in mixtures. This increase in selection effects is related to root traits, and deep rooting (DRF) in particular. Thus, a summer drought led to increased dominance and productivity of deep-rooting species, which may have maintained community productivity in mixtures.

3.5.2 The role of root traits in biodiversity effects

To enhance our understanding of the belowground aspects behind the positive effects of species interactions, we focused on belowground characteristics of the plant species in our experiment and measured four root traits that are related to nutrient and water uptake (de Vries *et al.*, 2016, Fort *et al.*, 2017) and can play a role in species interactions (Fort *et al.*, 2014, Ravenek *et al.*, 2016, Semchenko *et al.*, 2017). However, these root traits were correlated across species. As such, it is difficult to identify which root trait is most important. Using model comparisons, we found that DRF was the best predictor of mixture effects. Therefore, we focus on that trait in the discussion. However, it is important to note deep- rooting (high DRF) was also associated with thick (low SRL) and dense (high RTD) roots and a low density of roots in the soil (low RLD). As such, we cannot rule out that these other root traits also contributed to the relationships between DRF and plant mixture effects discussed below.

In our experiment, selection effects (relative) increased from the first to the second year, and this increase was strongest in mixtures with a high deep root fraction (CWM-DRF). In order to understand the functionality of this correlation, it is important to realise that selection effects are calculated as the covariance between monoculture productivity and the relative performance of species in mixture (Loreau and Hector, 2001). Thus, a positive selection effect means that the most productive species in monoculture also have the highest relative performance in mixture. In other words, the most productive species in monoculture dominate in mixtures. Here, we show that, in line with our hypothesis, selection effects can be predicted by CWM root traits: the change in selection effects were positively related to the mean DRF of the community. This suggests that particularly deep-rooting species were productive in monoculture and increased in biomass in mixture. This pattern is further illustrated by the contributions of individual species: the two species that enhanced selection effects most were the species with highest DRF (*C. jacea* and *A. millefolium*). In the dry conditions of the second year, it may be advantageous to have deep roots, which may provide access to water in deeper soil layers (e.g. Comas *et al.*, 2013, Barkaoui *et al.*, 2016). This may allow these species to not only maintain biomass production in monoculture, but also to increase their dominance in mixtures during drought.

Increased dominance of deep-rooting species may go at the expense of shallow-rooting species, but may also lead to complementary water use (Caldeira *et al.*, 2001) if deep-rooting species predominantly take up water in the deeper layers and leave the water in the shallow layer for the other species or facilitation by bringing water from the deeper soil layers to the surface, thereby enhancing the total amount of available water for the shallow-rooting species (Prieto *et al.*, 2012). In line with such positive interactions, we did find significantly positive complementarity effects that also increased with time. Although selection effects showed a stronger increase in the second year and contributed more, complementarity effects also contributed significantly to the positive mixture effects. This shows that although the expansion of highly productive, deep rooting species in mixtures (i.e. positive selection effects) was most important, other positive interactions also played a role. However, in contrast to selection effects, we could not detect significant relationships between complementarity effects and root traits. We found no evidence that complementarity effects were greater in mixtures consisting of shallow and deep-rooting species (high diversity in DRF), rejecting our hypothesis. This result highlights two important implications. One is that the traits that are important for biomass production in monocultures and competitive dominance in mixtures (in this case, DRF), do not necessarily play a role in other positive interactions, as captured by complementarity effects. This is also illustrated by the contribution of individual species. The two species that significantly enhanced complementarity effects (*C. jacea* and *G. mollugo*) differed considerably in root traits: *C. jacea* has the second highest DRF, highest RTD and lowest SRL, whereas *G. mollugo* is characterized by intermediate values for these traits. Similarly, one of the two deep-rooting species that enhanced selection effects (*C. jacea*) also enhanced complementarity effects, but the other (*A. millefolium*) did not. Second, our findings suggest that belowground resource partitioning is not the main driver of complementarity effects in our study. This conclusion is consistent with that of several tracer studies (von Felten *et al.*, 2009, Bachmann *et al.*, 2015, Hoekstra *et al.*, 2015), and trait studies that found no significant overyielding in grass mixtures with complementary traits (Hernandez and Picon-Cochard, 2016) or effect of rooting depth diversity on biodiversity effects (chapter 2, Roscher *et al.*, 2012). Perhaps other interactions, such as light partitioning aboveground (Anten and Hirose, 1999, Lorentzen *et al.*, 2008, Vojtech *et al.*, 2008) or reduced negative effects of plant-soil feedback in mixtures (Maron *et al.*, 2011, Schnitzer *et al.*, 2011, de Kroon *et al.*, 2012, Hendriks *et al.*, 2013) were more important. Identifying the relationships between complementarity effects and other traits of the species involved may help to identify the positive interactions between plant populations underlying complementarity effects.

3.5.3 Species interactions and drought resistance

Several studies have investigated the effects of species interactions on resistance to (experimental) drought. Most of these focused on species richness, and a recent meta-analysis concluded that resistance against drought increases with increasing species richness (Isbell *et al.*, 2015). Our experiment is not a true drought experiment: we report data from a dry year and compare the response to the year before (see alsoVan Ruijven and Berendse, 2010, Wright *et al.*, 2015). Nevertheless, our results show a similar pattern as drought experiments: the increase in biomass production from the first to the second, dry year was greater in mixtures than in monocultures, suggesting higher drought resistance in mixtures (see Isbell *et al.*, 2015). The mechanisms underlying this pattern have remained obscure, but our results suggest that enhanced biomass production of deep-rooting species in mixtures is important. This is consistent with Barkaoui *et al.* (2016), who showed that drought resilience of grass mixtures increased with CWM rooting depth, and Skinner *et al.* (2004) and Hernandez and Picon-Cochard (2016), who showed that the presence of a deep-rooting species was more important for community water use than species richness or root diversity.

The contribution of complementarity effects to increased biomass production in mixtures in the dry year was significant, but smaller than selection effects and not related to root traits. The latter suggests that mechanisms associated with root traits, such as complementary resource use belowground, did not play an important role in the interactions between species in a dry year. This is in line with (tracer) studies, which could not find clear evidence for complementary water use in mixtures (Kahmen *et al.*, 2006, Bachmann *et al.*, 2015, Hoekstra *et al.*, 2015). Together, these findings confirm the insurance hypothesis of biodiversity, which states that biodiversity can maintain ecosystem functioning under fluctuating environmental conditions via the increased probability that particular species, which maintain functioning when others decline, are present (Yachi and Loreau, 1999).

3.5.4 Conclusion

In our approach, we combined the additive partitioning method from biodiversity-ecosystem functioning research and a functional root trait analysis to identify the main interactions that determine community biomass in experimental mixtures. This approach revealed that positive effects of species interactions on community biomass were mainly driven by selection effects (SE): increased dominance of deep-rooting species in mixtures. Other positive interactions also occurred, as illustrated by positive complementarity effects (CE), but their contribution was smaller and not related to root traits. We propose that the large contribution of selection effects relative to other positive interactions is due to the dry conditions in the second year of our experiment, but this remains to be tested directly. Long term studies manipulating both species richness and environmental conditions (such as summer drought), which can disentangle annual dynamics from the effects of environmental fluctuations, will increase our understanding of the interactions underlying the positive effects of biodiversity on ecosystem functioning.

3.6 Acknowledgements

We would like to thank everyone that helped with planting, harvesting and collecting the data. Special thanks to Jan van Walsem, Frans Moller and Jan-Willem van der Paauw and Natalie Oram for their help in the common garden experiment. This study was supported by the Research School for Socio-Economic and Natural Sciences of the Environment (SENSE), the Netherlands.

3.7 Supplementary methods

The experiment was initially set up as a drought manipulation experiment. All blocks (and plots) were covered with transparent rainout shelters (6.00 m x 36.00 m x 2.60 m (w x l x h)) to 40 cm above the soil surface to allow air circulation. The shelters were made of an aluminium frame, covered with a transparent plastic sheet (Solar EVA, 180 μm thick) with a light transmission of 90% and 2% reduction of the red-far red ratio on a sunny day. Aluminium gutters were installed at the bottom of the sheets to lead rainwater away from the plots. Temperature was recorded automatically every 10 minutes within the rain shelters (three thermocouples per block) and between the rain shelters (two thermocouples) with a logger (Datataker DT85). On average, the shelters increased the temperature with 0.95 ± 0.06°C on a 24h cycle, ranging from 3.85°C in the middle of a sunny day to 0.31°C on a cloudy day. All plots were thus covered to reduce potential shelter effects (temperature, relative humidity) between treatments (Vogel *et al.*, 2013, Kreyling *et al.*, 2016). One of the two replicates of each composition in each block received 5-7.5 mm rain every 2-3 days, with a total of 68.4 mm, being the average June precipitation over the last 30 years (www.klimaatatlas.nl). During the period that the plant communities experienced drought (June 6th to July 10th 2015), outside temperature was on average 16.9 ± 2.2 °C, with an average relative humidity of 70 ± 1 % (weather station Deelen, 19 km from field site). The rain shelters blocked 38.2 mm of precipitation from the start of drought to the harvest (June 6th to July 6th) and 35.6 mm during harvest (6th to 10th of July). Before the drought manipulation started, conditions were already dry, with 24.5 mm precipitation in April and 57.5 mm in May, compared to 46.5 mm and 66.2 mm in April and May respectively on average (1981-2010) (www.klimaatatlas.nl).

Soil moisture content of the top 15 cm soil was measured in each plot, every week (5 times) during the simulated drought, using a TDR probe (TRIME-PICO64; HD2 meter), and a retention (pF) curve of the soil was made to get an indication of available soil moisture for plant uptake. For the retention curve, sampling rings of 100 cc with undisturbed soil were taken at different locations in between plots at a depth of 5 cm (n=5; mixed soil), 25 cm (n=9; mixed soil) and 65 cm (n=9; sand) below the soil surface.

3.8 Supplementary figures

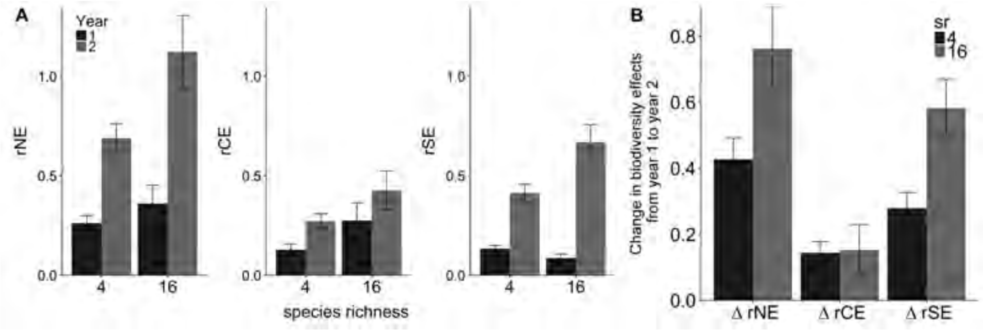


Figure S3.1 A) Relative biodiversity effects (rNE, rCE, rSE) in the first and second year of the experiment. The relative biodiversity effects, which are the biodiversity effects corrected for differences in monocultures productivity (see material and methods), show similar patterns to NE, CE, and SE. See Table 3.2 for statistics. B) Change in relative biodiversity effects (rNE, rCE and rSE) from year 1 to year 2 for 4-species mixtures (black bars) and 16-species mixtures (grey bars). sr = species richness. Bars show mean ± standard error.

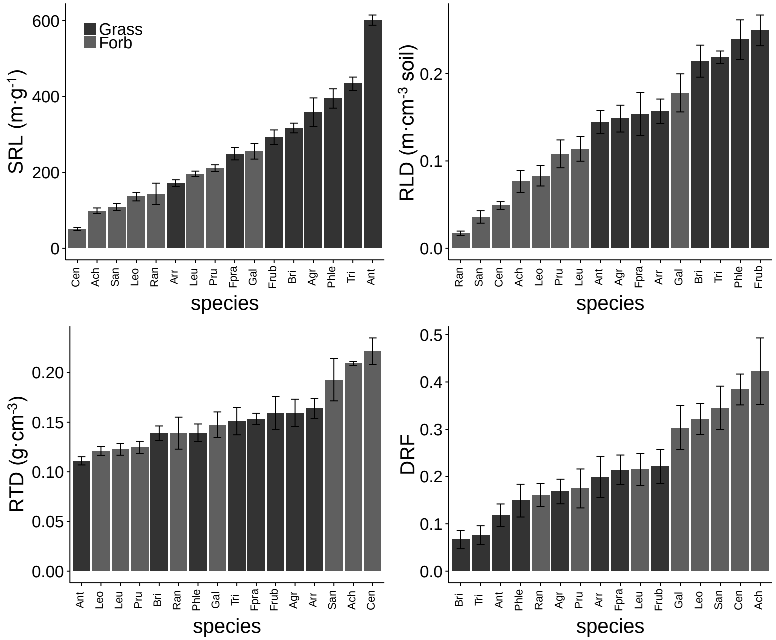


Figure S3.2: Species trait values measured in monocultures (n = 6). The average species values are used to calculate root community averages and root trait functional diversity of the mixtures. SRL = specific root length, RLD = root length density, RTD = root tissue density, DRF = deep root fraction. Bars show means ± standard errors.

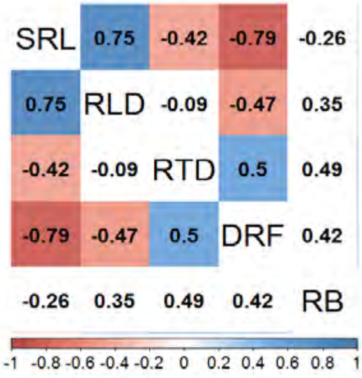


Figure S3.3 Species root trait correlations (n = 16). A significant ($P < 0.05$) positive correlation is shown in blue, a significant negative correlation in red, and no correlation ($P > 0.05$) in white. Numbers are Spearman's correlation coefficients. SRL = specific root length, RLD = root length density, RTD = root tissue density, DRF = deep root fraction, RB = root biomass.

3.9 Supplementary tables

Table S3.1 Linear mixed effect model results with the effect of drought treatment (drought) on community biomass, biodiversity effects and monoculture traits in the second year. Species or species richness (sr) was included in the model as well, together with block as a random factor (REML). Drought treatment had no significant effect on community biomass, complementarity effect (CE), selection effect (SE) and monoculture traits in the second year. The effect of drought treatment on RLD differed among species, with positive and negative effects of drought treatment on RLD. The response variables were (ln or square root) transformed to meet model assumptions. *** = P < 0.001, ** = P < 0.01, * = P <0.05, ns = not significant.

Response variable (transformation)	**Drought effect**	**Additional fixed factors in the models and their significance**
Biomass monocultures year 2 (ln)	$F_{1,62}$ = 0.0 (ns)	species (***), species x drought (ns)
Biomass year 2 (ln)	$F_{1,190}$ = 0.2 (ns)	sr (***), sr x drought (ns)
CE year 2 (ln)	$F_{1,96}$ = 0.6 (ns)	sr (ns), sr x drought (ns)
SE year 2 (ln)	$F_{1,96}$ = 0.0 (ns)	sr (ns), sr x drought (ns)
SRL (sqrt)	$F_{1,61}$ = 0.4 (ns)	species (***), species x drought (ns)
RTD (sqrt)	$F_{1,61}$ = 0.4 (ns)	species (***), species x drought (ns)
RLD (sqrt)	$F_{1,61}$ = 1.7 (ns)	species (***), species x drought (*)
DRF (sqrt)	$F_{1,60}$ = 3.6 (ns)	species (***), species x drought (ns)

Chapter 4

Deep or shallow: does the root distribution of neighbours affect the performance of individual plants?

Lisette M. Bakker, Liesje Mommer, Katie Barry, Jasper van Ruijven

4.1 Abstract

Biodiversity studies have tried to find evidence that resource partitioning is one of the driving mechanisms of the positive relationship between plant diversity and productivity. Resource partitioning suggest that species differences in resource acquisition can lead to increased resource uptake and performance of diverse mixtures (overyielding). For example, a community consisting of deep and shallow-rooting species is expected to acquire a larger part of the available soil resources than species monocultures or mixed communities containing only shallow or only deep-rooting species. However, experimental evidence that such species differences in root distribution lead to positive biodiversity effects is scarce. Most of these studies so far focused on community responses at the plot scale, averaging species differences and responses. We hypothesized that this approach may conceal the effects of species differences on species performance and overyielding. In this study, we determined the performance of almost 1700 individual plants of 16 species in monocultures, 4-species and 16-species mixtures of a biodiversity experiment, and related their relative performance in mixtures (compared to monocultures) to the rooting depths of the neighbouring plants. We expect that deep-rooting individuals would increase in yield with shallow-rooting neighbours, and vice versa, due to reduced root competition in the different soil layers. Overall, individual performance of deep-rooting species increased in mixtures, whereas that of shallow-rooting species did not. However, the increased performance of deep-rooting species depended on neighbour rooting depth: deep-rooting species performed better with shallow-rooting neighbours. Shallow-rooting species showed no response to neighbour rooting depth. Our study shows that the presence of deep and shallow-rooting species is important for positive biodiversity effects, which is consistent with evidence for resource partitioning. We propose that future research focused on individual plant performance in local plant neighbourhoods in relation to the functional traits of the component species has the potential to elucidate the mechanisms underlying positive biodiversity effects.

4.2 Introduction

Numerous biodiversity experiments have shown an increase in plant community productivity with plant diversity (Hooper *et al.*, 2005, Marquard *et al.*, 2009, Van Ruijven and Berendse, 2009, Cardinale *et al.*, 2012). Although this positive relationship between species richness and productivity is well established, the underlying mechanisms remain debated (Hooper *et al.*, 2005, Cardinale *et al.*, 2011, Schnitzer *et al.*, 2011, Tilman *et al.*, 2014, Wright *et al.*, 2017, Barry *et al.*, in prep). One of the main mechanisms proposed to underlie the positive effect of biodiversity on productivity is resource partitioning (Tilman *et al.*, 1997b, Fridley, 2001, Hooper *et al.*, 2005, Barry *et al.*, in prep). This hypothesis assumes that differences in resource uptake strategies between plant species allow them to exploit a larger amount of the resources when growing together compared to growing in monocultures.

A classic example of resource partitioning is differences in rooting depth (Parrish and Bazzaz, 1976, Yeaton *et al.*, 1977, Berendse, 1979, Berendse, 1982): if species with different rooting depths grow together in mixtures, resources can be acquired from shallow and deep soil layers. This may result in a more complete soil exploration, increased nutrient uptake and ultimately, enhanced biomass production (Tilman *et al.*, 1997b, Hooper *et al.*, 2005, Cardinale *et al.*, 2011). In grasslands, grasses are often assumed to root shallower than forb species (Berendse, 1979, Berendse, 1982, Wardle and Peltzer, 2003), but this difference in rooting depth between functional groups is not always apparent (Mommer *et al.*, 2010, Ravenek *et al.*, 2014, Oram *et al.*). At the species level, however, several studies have shown clear differences in rooting depth (Van Duuren *et al.*, 2003, Wardle and Peltzer, 2003, Dimitrakopoulos and Schmid, 2004, von Felten and Schmid, 2008, chapter 3).

Although plant species differ in rooting depth, experimental evidence that these differences in rooting depth contribute to the positive effect of biodiversity on plant productivity is limited. Mueller *et al.* (2013) found that the increase of community biomass with species richness coincided with an increase in community root biomass in deep soil layers (0.3-1m). Oram *et al.* (2018) showed that increased root biomass in mixtures (measured as the complementarity effect; Loreau *et al.*, 2001) was positively related to the average rooting depth of the community. These two studies suggest that deep rooting is important for community biomass production.

However, they do not necessarily show that interspecific differences in rooting depth between species contribute to the positive biodiversity-productivity relationship. For example, the positive link between rooting depth and increased productivity in mixtures as found by Mueller *et al.* (2013) and Oram *et al.* (2018) could also be due to deep-rooting species performing better in diverse plant communities than shallow-rooting species. In addition, both studies focus at the community level. Patterns observed at the community level are the net result of a range of species interactions, depending on the abundance and identity of the species involved, which may obscure the effect of specific interactions. To determine if differences in rooting depth between species can contribute to increased biomass production, we need to focus on the performance of individual species in diverse plant communities. Assessing the performance of individual species in mixtures and their relationships with species' own traits and those of the neighbouring species may elucidate the interactions that contribute to enhanced biomass production in diverse communities.

Therefore, in this study, we focus on species differences in rooting depth to explain species-specific plant performance in mixtures. First, we assess whether deep-rooting species perform better in mixtures compared to shallow-rooting species. This may be due to the fact that deep-rooting species can reach more nutrients and water in the deeper layers, which may be particularly beneficial if nutrients and water are limited (Comas *et al.*, 2013, Barkaoui *et al.*, 2016, Zeiter *et al.*, 2016). This would lead to a positive relationship between rooting depth and species' performance in diverse plant communities (Fig. 4.1A). However, the performance of a species in a mixture is not only determined by its own functional traits, but probably also by those of its neighbours. A deep-rooting species is less likely to increase in performance if the majority of neighbours is deep-rooting as well, since they have to share the available resources of the deeper layers. Following the resource partitioning hypothesis, deep-rooting will be particularly beneficial in a community that consists of shallow-rooting species. A deep-rooting species may be able to tap into additional resources in the lower soil layers and increase its own performance. At the same time, the performance of shallow-rooting species with deep-rooting neighbours may also increase because competition for resources in the shallow soil layer is relaxed if deep-rooting species preferentially use deeper soil layers to take up resources. Thus, we would expect that shallow-rooting species increase in yield with deeper rooting neighbour species,

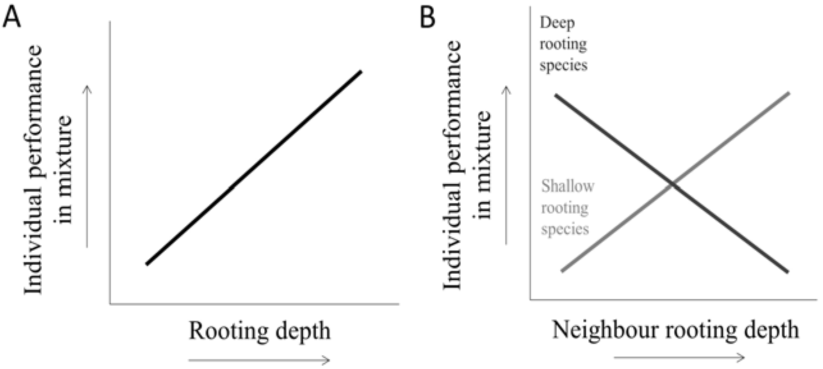


Figure 4.1 Conceptual figure showing the expected relationships between performance of individual plants in mixtures and their rooting depth (A) and neighbour rooting depth (B). We expect that species performance increases with its rooting depth as a result of increased water (and nutrient) availability for the deeper rooting species (see graph A). In mixtures, shallow-rooting individuals are expected to have larger yields when standing next to deep-rooting neighbour species as a result of released competition for nutrients and water in the shallow soil layers (increasing line in B). In contrast, deep-rooting species are expected to experience less competition and thus increased yield when standing next to shallow-rooting neighbours (declining line in B).

while deep-rooting species increase in yield when having shallow-rooting neighbour species (Fig. 4.1B).

Here, we measured plant species performance (aboveground biomass production in mixture compared to that in monoculture) in plant communities differing in species richness and composition. Rather than using plot-level data, we determined the biomass of almost 1700 individual plants of 16 different species in monocultures and mixtures. We used these data to test whether the performance of individual plants in plant mixtures can be explained by 1) their own rooting depth and 2) the rooting depth of their neighbours.

4.3 Material and methods

4.3.1 Individual plants from a common garden biodiversity experiment

A biodiversity experiment with monocultures, 4-species mixtures and 16-species mixtures was established in April 2014 at an experimental field of Wageningen University, the Netherlands (51°99’N 5°66’E). The design of this biodiversity experiment is described in more detail in chapter 2. In short, we established 198 plots of 70 x 70 cm on sandy soil (organic matter content = 1.45 ± 0.04 %, pH = 7.08 ± 0.07, N = 1.23 ± 0.08 ($g{\cdot}kg^{-1}$), P = 0.18 ± 0.01 $g{\cdot}kg^{-1}$, and C = 15.11 ± 0.48 g kg^{-1} in the upper 50 cm). 96 plots were assigned to species monocultures (six per species), 90 to 4-species mixtures (45 different species compositions) and 12 to 16-species mixtures. The monocultures and mixtures were equally divided over three blocks.

We used eight grasses and eight forbs that are common in Dutch hay meadows (Schaminée *et al.*, 1996). The grass species were *Agrostis stolonifera* (L.), *Anthoxanthum odoratum* (L.), *Arrhenatherum elatius* (L.), *Briza media* (L.), *Festuca rubra* (L.), *Festuca pratensis* (Huds.), *Phleum pratense* (L.), *Trisetum flavescens* (L.), and the forb species *Achillea millefolium* (L.), *Centaurea jacea* (L.), *Galium mollugo* (L.), *Leucanthemum vulgare*, *Leontodon hispidus* (L.), *Prunella vulgaris* (L.), *Sanguisorba officinalis* (L.), *Ranunculus repens* (L.). Seeds were bought from a native seed supplier (Cruydt-hoeck, Nijeberkoop, the Netherlands). In each plot, seedlings (five weeks old) were planted in a grid of 8 x 8, with similar planting positions within each richness level (Fig. S4.1). In mixtures, the planting positions were randomly assigned to the species of that particular species composition. In the 4-species mixtures, species were planted in such a way that each individual plant was standing next to all three heterospecific neighbours (Fig. S4.1). In 4-species mixtures, individual plants were directly surrounded by five to eight heterospecific neighbours, and in the 16-species mixtures by six to eight heterospecific neighbours (Fig. S4.1).

Unfortunately, seeds of one forb species, *L. hispidus*, turned out to be contaminated (70%) with seeds from another species of the same genus, *L. autumnalis*. As it was not possible to replace the *L. autumnalis* plants in time, both species share one position in the design. Therefore, the experiment actually contained 17 species instead of 16 species. For reasons of clarity, we still refer to 4-species mixtures and 16-species mixtures. In addition, well after planting, the perennial species *R. repens* turned out to be an annual species of the same genus: *R. sardous*. As these plants died after the first growing season, their positions in each plot were replanted with *R. repens* seedlings in March 2015. In this study, biomass data of *R. sardous* and *R. repens* were excluded.

In total, 1690 individual plants were selected for this experiment. Only individuals planted in the inner 6 x 6 grid were included to avoid potential edge effects. Of these 36 individuals, we randomly selected six individuals in each monoculture and two individuals of each species in 4-species mixtures. In the 16-species mixtures, all individuals (two or three per species; 0-3 for the two *Leontodon* species) were included.

Aboveground biomass of these individuals was collected in September 2014, July 2015, and October 2016 by clipping them at two cm above the soil surface. We included all aboveground biomass of a species that was found at the original planting position (a square measuring 8.75 x 8.75 cm). Three species (*A. millefolium*, *A. stolonifera*, *F. rubra*) are capable of clonal spread of 1 to 25 cm per year (Klimešová *et al.*, 2017) and may have had biomass outside the original planting position, but this was impossible to determine without disturbing our experiment. After harvesting, biomass was oven dried at 70 °C for at least 48 h and weighted.

To determine species-specific rooting depth, three soil cores were taken (50 cm deep x 2.5 cm diameter) in each monoculture in 2015. The three soil cores were divided in four soil layers, 0-5 cm, 5-15 cm, 15-30 cm and 30-50 cm, and pooled per plot. The samples were carefully washed with a 0.5 mm sieve to collect the roots, oven dried at 70 °C for 72 h and weighted.

4.3.2 Calculations

To assess individual plant performance in the different mixed plant communities relative to the performance of a species growing in monoculture, we calculated the relative yield of each individual in mixture (dY) for each species, mixture and year. This was calculated as the difference in natural logarithm (ln) between the biomass (g) of an individual of species *i* in mixture *j* ($Y_{i,\ \mathrm{mix}\ j}$) and the average biomass of the individuals of the same species *i* in monoculture ($Y_{\mathrm{i,\ mono}}$): $dY = \ln(Y_{i,\ \mathrm{mix}\ j} + 1) - (\overline{\ln(Y_{i,\ \mathrm{mono}} + 1)})$.

Species-specific rooting depth values were calculated as the deep root fraction (DRF), which is the fraction of root biomass in the deep (30-50 cm) layer compared to total root biomass in the entire 0-50 cm soil layer. These monoculture DRF values are used as a species' own rooting depth, hereafter referred to as $\mathrm{DRF}_{\mathrm{own}}$.

For each individual, we also calculated the average neighbour deep root fraction ($\mathrm{DRF}_{\mathrm{neighb}}$), using the deep-rooting fraction (based on monoculture values) of the neighbouring plants that surrounded the measured individuals (see Fig. S4.1). Including the DRF of conspecific neighbours resulted in a significant correlation between the rooting depth of the measured individual ($\mathrm{DRF}_{\mathrm{own}}$) and the average rooting depth of the neighbours ($\rho = 0.23$, $P < 0.001$), which makes it hard to disentangle the effects of a species' own rooting depth and that of its neighbours. To avoid that this correlation would affect our results, the rooting depth of the neighbours (DRF_{neighb}) was calculated as the average DRF of the surrounding heterospecific individuals only.

4.3.3 Statistics

Differences in DRF among species and plant functional group (PFG; grass or forb) were tested with a linear mixed effect model with block as random factor after a square root transformation. We corrected for unequal variance between the PFGs by adjusting the variance structure using 'weights' for the PFGs (Zuur *et al.*, 2009). Differences in biomass between species and the effect of year were first tested in monocultures, using linear mixed effect model (restricted maximum likelihood (REML)) with biomass as response variable and species, year and their interaction as fixed factors, and plant ID nested in plot number and subsequently nested in block as random factors. Next, we included the mixtures and added species richness and its interactions as fixed factor and species composition as random factor to the model to test the additional effects of species richness on biomass. In case of significant interactions, separate tests were done for each species, species richness level or year.

Further, we tested the effect of own rooting depth on individual biomass in monoculture and relative performance in mixture (dY), using a similar linear mixed model with own rooting depth as fixed factor instead of species (REML). The effect of own rooting depth on dY was also tested per year per species richness level (four or 16-species mixture), with a linear mixed model with DRF_{own} as fixed factor and block as random factor. As a next step in explaining performance in mixture (dY), we included the neighbour rooting depth in a full model with own rooting depth, neighbour rooting depth, species richness and year and all their interactions as fixed factors, and plant ID nested in plot number and subsequently nested in block as random factors (Burnham, 2002, Fijen *et al.*, 2015; Maximum likelihood (ML)), and used model selection, using the function "dredge" of R package "MuMIn (version 1.15.6)" (Bartoń, 2016) to find the best model explaining dY. The best model was chosen based on lowest AIC, with a minimum difference of 2 ΔAIC compared to other competing models (Burnham, 2002). The relative importance of the variables were calculated according to Burnham (2002), and the variables that were included in the best explaining model were subsequently tested for significance (REML). See Table S4.1 for model selection procedures. To illustrate the interactive effect of own rooting depth and neighbour rooting depth on overyielding, we plotted the relationship (model predictions) between overyielding and neighbour rooting depth for four different own rooting depths (DRF 0.1, 0.2, 0.3, and 0.4), using year 2014 and 4-species mixtures as example. We used the package "visreg" (Breheny and Burchett, 2017) to visualize this. In all analyses, we excluded zeros from the data set: individuals were no longer taken into account when they died during the three years of the experiment (see Fig. S4.2 for survival). Biomass was ln (x + 1) transformed to meet model assumptions.

All statistics were done in R (version 3.1.3) with R studio (version 1.0.143), using the "lme4" (Bates *et al.*, 2015) or "nlme" (Pinheiro *et al.*, 2016) package. We used anova type III with the "anova" function of the "stats" package (R CoreTeam, 2016) to test the models.

4.4 Results

4.4.1 Biomass and rooting depth in monocultures

On average, deep root fraction (DRF) was 0.22 ± 0.03, meaning that 22% of the root biomass (0-50 cm) was found in the deeper (i.e. 30-50 cm) soil layer. Consequently, 78% of the roots was located in the top (0-30 cm) soil layer. However, the fraction of roots in the deeper layer differed significantly among species ($F_{14, 72} = 8.1$, $P < 0.001$), ranging from 7 (*B. media*) to 42% (*A. millefolium;* see Table 4.1). On average, forbs had twice as many roots in the deeper soil layer than the grasses (31 % ± 2 compared to 15 % ± 1; $F_{1, 85} = 43.9$, $P < 0.001$), but also within these two functional groups, species differed significantly in DRF ($F_{7, 38} = 3.4$, $P < 0.01$ for grasses and $F_{7, 37} = 6.1$, $P < 0.001$ for forbs).

In monocultures, average individual biomass differed between species ($F_{15, 77} = 7.0$, $P < 0.001$), ranging from 0.06 ± 0.1 (*L. autumnalis* in 2016) to 4.5 ± 1.1 gram (*A. millefolium*, in 2016; see Fig. 4.2). Overall, individual biomass increased with year (year: $F_{2, 1050} = 17.3$, $P < 0.001$), but this effect differed between species (species x year: $F_{30, 1035} = 18.1$, $P < 0.001$ (Fig. 4.2). Nine out of the sixteen species significantly

Table 4.1 Species' functional group and deep root fraction (DRF): the proportion of biomass that was found in the 30-50 cm soil layer compared to total root biomass in the 0-50 cm soil layers. These values were obtained in species monocultures (n=6 per species).

Species	**Functional group**	**Deep root fraction (DRF)**
Agrostis stolonifera	Grass	0.17 ± 0.03
Anthoxanthum odoratum	Grass	0.12 ± 0.02
Arrhenatherum elatius	Grass	0.20 ± 0.04
Briza media	Grass	0.07 ± 0.02
Festuca pratensis	Grass	0.21 ± 0.03
Festuca rubra	Grass	0.22 ± 0.04
Phleum pratense	Grass	0.15 ± 0.03
Trisetum flavescens	Grass	0.08 ± 0.02
Achillea millefolium	Forb	0.42 ± 0.07
Centaurea jacea	Forb	0.38 ± 0.03
Galium mollugo	Forb	0.30 ± 0.05
Leontodon autumnalis/hispidus	Forb	0.32 ± 0.03
Leucanthemum vulgare	Forb	0.21 ± 0.03
Prunella vulgaris	Forb	0.17 ± 0.04
Sanguisorba officinalis	Forb	0.35 ± 0.05

increased in biomass in 2015 compared to 2014, while three species (*A. stolonifera*, *L. autumnalis*, and *T. flavescens)* decreased. In 2016, most species showed an increase in biomass compared to the previous year, but three species (*C. jacea, L. vulgare* and *P. vulgare*) declined (Fig. 4.2).

The relationship between rooting depth (DRF_{own}) and individual biomass of species in monoculture depended on year (DRF_{own} x year: $F_{2,\ 1058} = 10.7$, $P < 0.001$). Analyses per year revealed a positive relationship between DRF_{own} and individual biomass that strengthened over time. This relationship was not significant in the first year ($F_{1,\ 84} = 1.5$, $P = 0.\ 23$), marginally significant in 2015 ($F_{1,\ 87} = 3.5$, $P = 0.06$), and significant in 2016 ($F_{1,\ 80} = 12.2$, $P < 0.001$).

4.4.2 Species performance in mixtures

The effect of species richness on individual biomass depended on the species (species x sr: $F_{30,\ 98} = 3.5$, $P < 0.001$; Fig. 4.2). In addition, these effects were dependent on time (species x sr x year: $F_{60,\ 3057} = 8.5$, $P < 0.001$). In 2014, individuals of two species

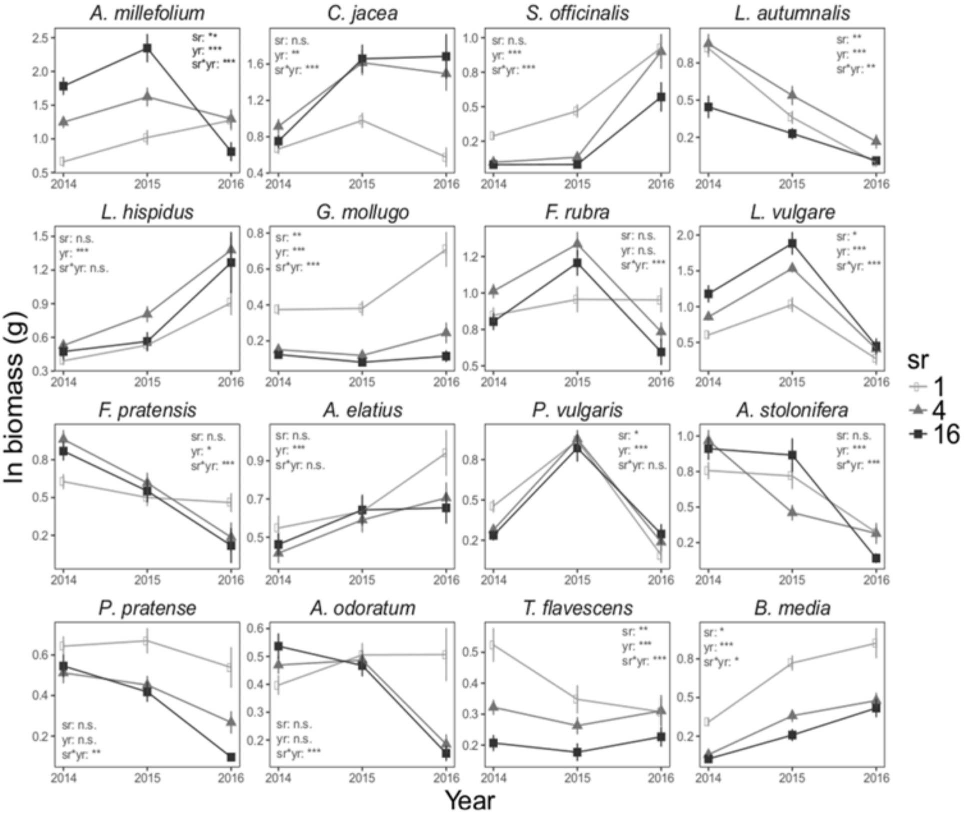


Figure 4.2 Biomass of individual plants of each species in monocultures, 4-species mixtures and 16-species mixtures, measured over three growing seasons (2014-2016). Species are ordered from deep (*A. millefolium*) to shallow (*B. media*), based on rooting depth of species in monocultures (see Table 4.1). sr = species richness, yr = year, *** = P < 0.001, ** = P < 0.01, * = P < 0.05, ns = not significant. Means ± standard errors are shown.

increased in biomass in 4- and 16-species mixtures compared to monocultures (*A. millefolium* and *L. vulgare*), two species showed higher biomass in 4-species mixtures only (*F. rubra* and *L. autumnalis*), while five species showed significant biomass reductions in mixtures (*B. media*, *G. mollugo*, *P. vulgaris, S. officinalis* and *T. flavescens*; Fig. 4.2). In 2015, the effects of species richness on individual biomass were relative similar to 2014, with seven species that were significantly affected. Three species (*A. millefolium* and *L. vulgare, F. rubra*) showed increased biomass in both mixtures types, and one species (*A. stolonifera)* increased in 16-species mixtures, but decreased in 4-species mixtures. Three species that 'underyielded' in mixtures in 2014 were still performing significantly better in monocultures (*B. media*, *G. mollugo*, and

S. officinalis) in 2015 (Fig 4.2). In 2016, the positive effects of species richness were not statistically significant (anymore) for several species (*C. jacea*, *L. autumnalis*, *L. hispidus*, *L. vulgare*, *P. vulgaris*), despite their higher average yields in mixtures. Four species were significantly and negatively affected by species richness (*A. odoratum*, *B. media*, *G. mollugo*, and *P. pratense*) (Fig. 4.2).

Relative performance of individual plants in mixtures (dY) increased with their own deep root fraction (DRF_{own}: $F_{1,\ 1097} = 155.3$, $P < 0.001$), from very shallow species performing worse in mixtures than in monoculture (dY < 0) to deep-rooting species performing better in mixtures (dY > 0; see Fig. 4.3).

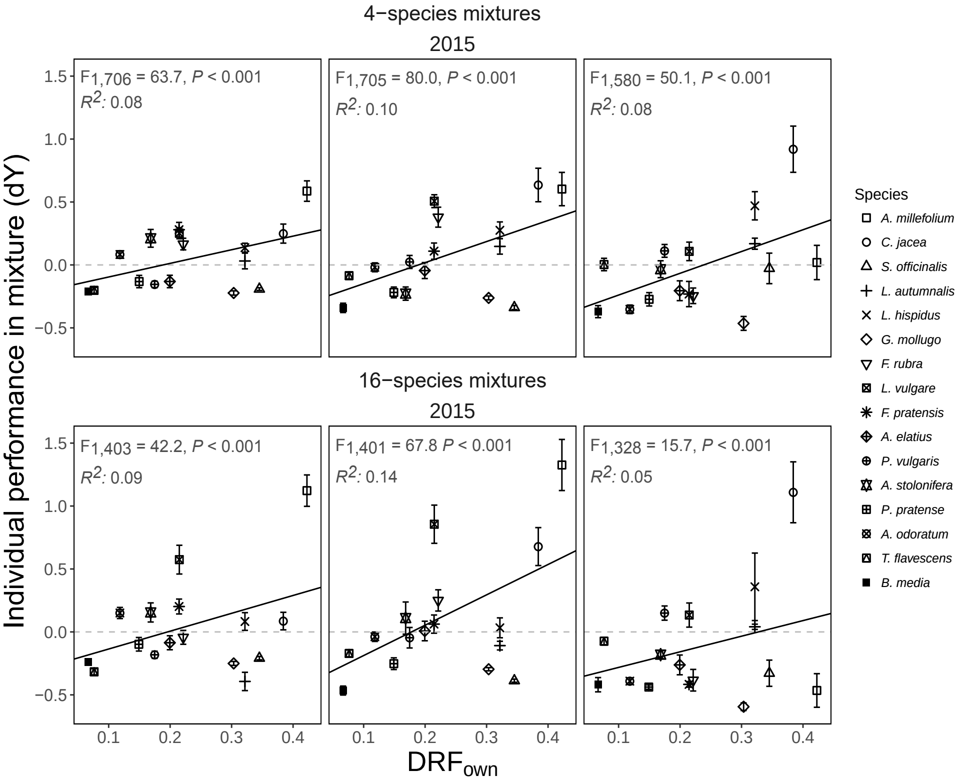


Figure 4.3 Species relative performance in mixture compared to monoculture (dY) in relation to species' own rooting depth (DRF_{own}) in 4-species mixtures (above) and 16-species mixtures (below) in three subsequent years. In each year, a positive relationship between dY and DRF_{own} was found: individuals with deeper roots (higher DRF_{own}) performed better in mixtures than species with shallow roots. Deep-rooting species had higher performance in mixtures than monocultures (dY > 0; above grey dotted line), while shallow-rooting species performed worse (dY < 0; below grey dotted line). Species means ± standard errors are shown. *** = P < 0.001, ** = P < 0.01, * = P < 0.05, ns = not significant. The grey dotted line indicates that species performance in mixture is equal to that in monoculture.

Although the slope of the relationship depended on year (DRF_{own} x year: $F_{2, 2056} = 11.3$, $P < 0.001$) and on the interaction between year and species richness (sr x year x DRF_{own}: $F_{2, 2056} = 6.6$, $P < 0.01$), it was significant in all three years and at both levels of species richness (Fig. 4.3). Thus, deep rooting species (higher DRF_{own}) performed better in mixtures than species with shallow roots.

There was no overall relationship between performance of individual plants in mixtures and the average rooting depth of the neighbours (DRF_{neighb}; Table 4.2; see Table S4.1 for model selection procedures). However, in line with our hypothesis we found that the effect of the rooting depth of the neighbours depended on the rooting depth of the individual (see DRF_{neighb} x DRF_{own} in Table 4.2). Deep-rooting species performed better with shallow-rooting neighbours than with deep-rooting neighbours. However, shallow-rooting neighbours were not affected by the rooting strategy of the neighbours (Fig. 4.4).

Table 4.2 Selected linear mixed effect model (see Table S4.1 for model comparison) testing the effects of species' own rooting depth (DRFown), neighbour rooting depth (DRFneighb), species richness (sr) and year on plant performance in mixture compared to monoculture (dY). df = degrees of freedom. Significant effects are marked in bold.

	Individual plant performance (dY)		
	df	**F-value**	**P-value**
Intercept	1, 2012	4.9	**0.027**
DRF_{own}	1, 2012	21.4	**< 0.001**
DRF_{neighb}	1, 1011	0.5	0.468
species richness (sr)	1, 98	0.5	0.487
Year	2, 2012	7.2	**< 0.001**
DRF_{own} x DRF_{neighb}	1, 2012	7.9	**0.005**
DRF_{own} x sr	1, 1011	0.5	0.497
DRF_{own} x year	2, 2012	5.2	**0.006**
SR x year	2, 2012	1.0	0.358
DRF_{own} x sr x year	2, 2012	6.6	**0.001**

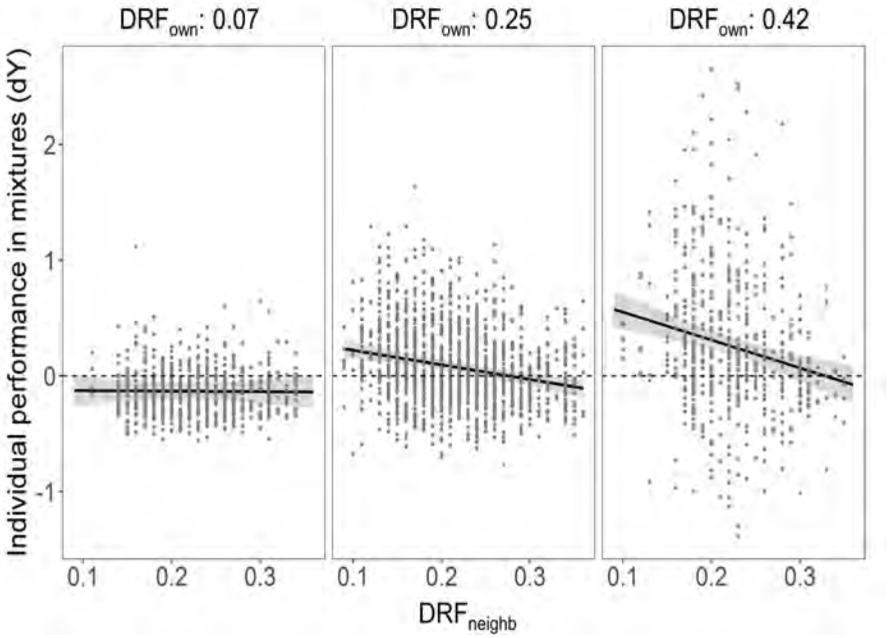


Figure 4.4 The relationship between individual performance in mixture (dY) and the average rooting depth of the neighbours (DRF_{neighb}), shown for a shallow (DRF_{own}: 0.07), intermediate (DRF_{own}: 0.25), and deep-rooting (DRF_{own}: 0.42) species. The effect of the rooting depth of the neighbours depended on the rooting depth of the species considered: individuals of deep-rooting species (DRF_{own}: 0.42) performed better when surrounded by shallow neighbours (low DRF_{neigh}). In contrast, the performance of individuals of shallow-rooting species (DRF_{own}: 0.07) was not affected by the rooting depth of their neighbours. This graph illustrates the significant interaction between DRF_{own} and DRF_{neighb} based on model predictions (model in Table 4.2; see methods and Table S4.1 for model selection) using 2014 and 4-species mixtures as an example, showing the expected value (black line), confidence interval (grey band), and partial residuals (dots).

4.5 Discussion

Deep-rooting plant species performed significantly better in mixtures than shallow-rooting species. Our approach, in which we focus on the performance of individual plants in response to their direct neighbours, showed that these benefits for deep-rooting species depended on the rooting strategy of their neighbours: overyielding of deep-rooting species was larger when growing with shallow-rooting neighbours than with deep-rooting neighbours. The reverse did not occur, since the performance of shallow-rooting species in mixtures was not affected by the rooting depth of their neighbours. This suggests that in particular combinations of both shallow and deep-rooting species are important for community productivity in diverse plant communities.

4.5.1 Rooting strategy and performance in mixtures

As hypothesized, species' rooting depth was an important predictor for performance in mixture: the deep-rooting individuals performed on average better in mixtures compared to monocultures than shallow-rooting species. This suggests that rooting depth is an important trait related to plant performance in species-rich plant communities. Several empirical studies also indicate that rooting depth is important for plant performance in grasslands. For example, rooting depth was positively related to water uptake (Fort *et al.*, 2017) and species biomass production under drought (Hoekstra *et al.*, 2015, Zeiter *et al.*, 2016) in grasslands. The advantage for deep-rooting species may be the result of access to additional resources in the deeper layers compared to shallow species (Maeght *et al.*, 2013), but also to the ability to switch to nutrient and water uptake from deeper layers during dry conditions (Kulmatiski and Beard, 2013, Hoekstra *et al.*, 2014). Environmental conditions could therefore determine the benefits of being deep rooted in mixtures. In our study, plants were grown on a sandy soil, in which water shortages in the upper layers are likely to occur during dry periods. Especially the dry summer of 2015, before the harvest of that year may have increased the importance of rooting depth for maintaining biomass production in diverse pant communities. To investigate the generality of the advantage of deep-rooting, we recommend future studies to investigate the link between rooting depth and plant performance under different environmental conditions, varying in soil type, hydrology and climate.

4.5.2 The effect of neighbour rooting depth on species' performance in mixtures

Deep-rooting individuals showed highest overyielding when growing next to shallow neighbours. This shows that besides a species' own traits, the traits of the neighbours determine plant performance in mixtures. This is consistent with Herz *et al.* (2017), who showed that predictions of root and shoot biomass of individual plants in grassland communities were improved by including functional traits (e.g. root magnesium and calcium concentration, root mass per volume, leaf dry matter content) of the local neighbourhood. More importantly, our results suggest that particularly the difference in traits between a focal species and its neighbours is important for its performance.

In contrast to the deep-rooting species, shallow-rooting species showed no response to the rooting depth of the neighbours. This raises the question why the shallow-rooting species did not profit from standing next to species with a different – deeper – rooting strategy, while the deep-rooting species did? This may be related to the general distribution of roots over depth in grasslands. In grasslands, the majority of the roots are found in the shallow soil layers (0-30 cm) (Jackson *et al.*, 1996, Bessler *et al.*, 2009, Mueller *et al.*, 2013). Also so-called deep-rooting species have a large proportion of their roots in the upper soil layers. For example, in this study, the deep-rooting species (DRF > 0.30) still allocated 65% of their roots in the upper 30 cm soil. In Oram *et al.* (2018), the deepest-rooting species had still 40% of its roots in the upper five cm of the soil. Thus, shallow species, even when surrounded by deep-rooting species, have to compete with many other roots in the upper soil layers (Wardle and Peltzer, 2003, Nippert and Knapp, 2007a, Frank *et al.*, 2010). In contrast, deep-rooting species surrounded by shallow species will also take up nutrients in deeper layers from which the shallow species are virtually absent (Nippert and Knapp, 2007a). This potential release from competition in deeper layers may allow deep-rooting species to increase in performance in mixtures with shallow species.

It is important to note that we used the rooting depth measured in monocultures to predict species performance in mixtures. As such, we assume that rooting depths are similar in mixture and monoculture. Recent work in other biodiversity experiments, in which species-specific root distributions in plant mixtures was determined using molecular methods, suggests that this is not necessarily the case (Mommer *et al.*, 2010, Oram *et al.*, 2018). Roots are plastic and known to respond to e.g. nutrient availability and neighbours (Hodge, 2004, Mueller *et al.*, 2013, Belter and Cahill, 2015). On the other hand, there is little evidence that shifts in rooting depth are large enough to qualitatively affect the outcome of our analyses. In the study by Oram *et al.* (2018), the actual rooting depth measured in mixtures was no better predictor of biodiversity effects than the average rooting depth based on monocultures, and both rooting depth values were correlated. Moreover, if shifts in rooting depth in mixtures were large enough to change how species are ranked in terms of rooting depth, it is unlikely that we would have found effects of monoculture rooting depth on species performance in mixtures. On the other hand, using the actual rooting depth of the individuals in mixtures may also strengthen the observed relationships between rooting depth and species performance.

In addition, incorporating other root traits that are linked to resource uptake (Fitter, 1986, Fort *et al.*, 2017, Guderle *et al.*, 2018) may improve our predictions of species performance. Especially for the shallow-rooting species, which are likely competing with all surrounding neighbours, partitioning via other root traits related to resource uptake may be more important than rooting depth. For example, Ravenek *et al.* (2016) showed that species' initial competitive success was positively linked to root length density (RLD). Other traits that may play a role in resource uptake are root diameter (Comas *et al.*, 2013), specific root length (Gross *et al.*, 2008, Hernández *et al.*, 2010), root to shoot ratio (Wang *et al.*, 2010), the amount of root hairs (Zhu *et al.*, 2010), or traits related to plasticity in resource uptake over soil depth (Volkmann *et al.*, 2016, Kulmatiski *et al.*, 2017). Future studies integrating root traits and their plasticity at the species level to resource uptake and species performance, may enhance our understanding of the interactions between species that contribute to positive effects of plant diversity on ecosystem functioning.

4.5.3 Implications for biodiversity-ecosystem functioning relationships

Our study suggests that the presence of deep-rooting species is important for increased biomass production in diverse communities. This is consistent with previous studies showing positive effects of deep-rooting legumes (Mueller *et al.*, 2013, Hernandez and Picon-Cochard, 2016), and deep-rooting forbs (Reich *et al.*, 2004, Skinner *et al.*, 2004) on biodiversity effects. In our study, on average forbs rooted deeper than grasses and showed greater performance in mixtures (see chapter 3). However, we also found that within these two groups, there is considerable variation in rooting depth and performance in mixtures. This shows that investigating the effects of trait differences at the species level can provide more insights than focusing on functional groups. This is in line with Mueller *et al.* (2013), who concluded that the actual rooting depth of the community better explained increased community biomass production in mixtures than the presence of deep-rooting legumes. Similarly, Oram *et al.* (2018) found a positive relationship between rooting depth and biodiversity effects, while the two main functional groups (grasses and forbs) did not differ in rooting depth.

More importantly, our study shows that overyielding of deep-rooting species predominantly occurs in the presence of shallow neighbours. Since this benefit for deep rooting did not lead to a decline of the shallow species, this will probably lead to positive effects on biomass production at the community level. A few studies at the community level have tried to link differences in rooting depth among species (measured as functional diversity) to community biomass or complementarity effects, but found no relationships (Roscher *et al.*, 2012, Barkaoui *et al.*, 2016, Wagg *et al.*, 2017a, Oram *et al.*, 2018, chapter 2 and 3). An important reason for the discrepancy between these findings and our results may be the difference in resolution between the studies. At the level of communities, effects on individual plants and species in local neighbourhoods are averaged, potentially concealing differential responses within and among species. By investigating the effects of rooting depth on individual plants in local neighbourhoods, i.e. surrounding neighbouring plants, the spatial resolution was increased. Assuming individual plants mainly interact with directly surrounding plants, then species or functional trait composition of the direct neighbours will explain shifts in performance much better than species richness or composition at the plot level (Fichtner *et al.*, 2017). Further, the fact that we found a significant relationship may also be due to the large number of data points. We were able to include almost 1700 individuals in a range of local neighbourhoods, while biodiversity studies focusing at the community level are typically restricted to a limited number of plots (e.g. 82 plots in Jena main biodiversity experiment (Roscher *et al.*, 2012), 138 plots in the Jena Trait Based Experiment (Oram *et al.,* 2018), and 152 plots in Mueller *et al.*, 2013), and a single species composition (measurement) per plot. Thus, research at the species level, using local neighbourhoods, i.e. traits of directly surrounding plants, instead of plot averages, can enhance our understanding of the importance of trait differences between species for ecosystem functioning at the community level.

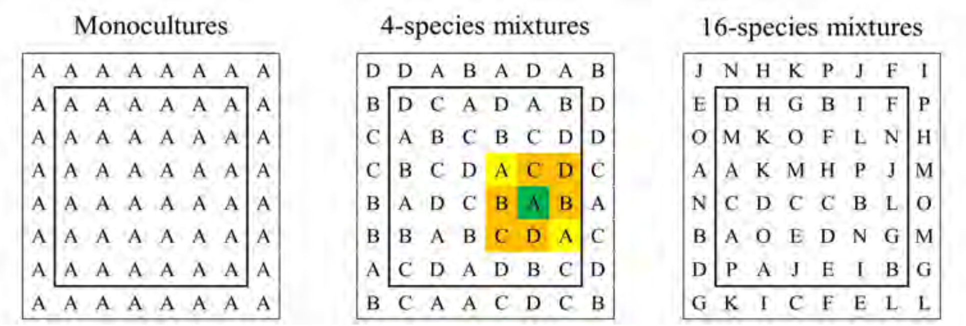
4.5.4 Conclusions

Our results show that both the rooting depth of a species and rooting depth of the neighbours can explain aboveground performance of plant species in grassland communities. Particularly deep-rooting individuals surrounded by shallow-rooting neighbours showed increased performance in mixtures. Shallow species showed no response to neighbour rooting depth and did not show increased performance in mixtures. These results suggest that spatial resource partitioning via rooting depth contributes – at least partially – to the positive effects of plant species richness on plant productivity. We see two directions for future research: 1) strengthening the link between rooting depth and resource uptake, for example by incorporating other root traits, and 2) establishing relationships between trait differences between species and performance in local neighbourhoods across resource gradients. Combining these may further enhance our understanding of the mechanisms underlying the positive effects of biodiversity on ecosystem functioning.

4.6 Acknowledgements

We would like to thank everyone that helped with planting, harvesting and collecting the data. Special thanks to Jan van Walsem, Frans Moller and Jan-Willem van der Paauw, Natalie Oram and Anne Jansma for their help in the common garden experiment. L.M.B. was supported by the Research School for Socio-Economic and Natural Sciences of the Environment (SENSE), the Netherlands. L.M. was supported by a NWO VIDI grant 864.14.006.

4.7 Supplementary figures



Monocultures

A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	A

4-species mixtures

D	D	A	B	A	D	A	B
B	D	C	A	D	A	B	D
C	A	B	C	B	C	D	D
C	B	C	D	A	C	D	C
B	A	D	C	B	A	B	A
B	B	A	B	C	D	A	C
A	C	D	A	D	B	C	D
B	C	A	A	C	D	C	B

16-species mixtures

J	N	H	K	P	J	F	I
E	D	H	G	B	I	F	P
O	M	K	O	F	L	N	H
A	A	K	M	H	P	J	M
N	C	D	C	C	B	L	O
B	A	O	E	D	N	G	M
D	P	A	J	E	I	B	G
G	K	I	C	F	E	L	L

Figure S4.1 Species planting position schemes of the monocultures, 4-species mixtures and 16-species mixtures. Species were randomly assigned to a planting position (a letter) for each different species composition. As an example, the green square represents a measured individual that we harvested, of which its own rooting depth (DRF_{own}) was estimated using the rooting depth (deep root fraction) found in monocultures. Neighbour rooting depth (DRF_{neighb}) was calculated based on the heterospecific neighbours that directly surrounded the measured individual (shown in orange), excluding the conspecific neighbours (shown in yellow). We only measured individuals that were planted in the inner 6 x 6 rows.

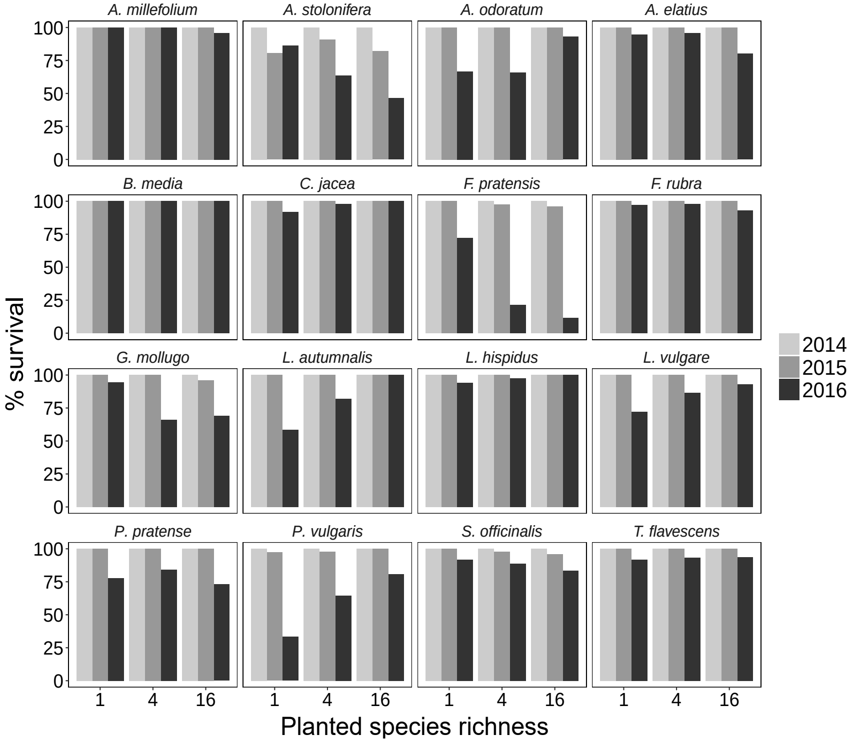


Figure S4.2 Average survival of the selected individuals per species in monocultures, 4-species mixtures and 16-species mixtures in three subsequent years (2014 - 2016).

4.8 Supplementary tables

Table S4.1 Competing models explaining individual performance in mixtures (dY), using the estimated rooting depth of the individuals (DRF_{own}), average rooting depth of the neighbouring plants (DRF_{neigh}), species richness (sr), year (yr) and all their interactions as explaining factors. The models with weight sum up to 0.96 are shown. The model with only DRF_{own} and the intercept-only model are shown as well for comparison. The last row shows the relative importance of the variables (w + (j)) (Burnham, 2002). The best model (model 1) is further used for statistical analyses.

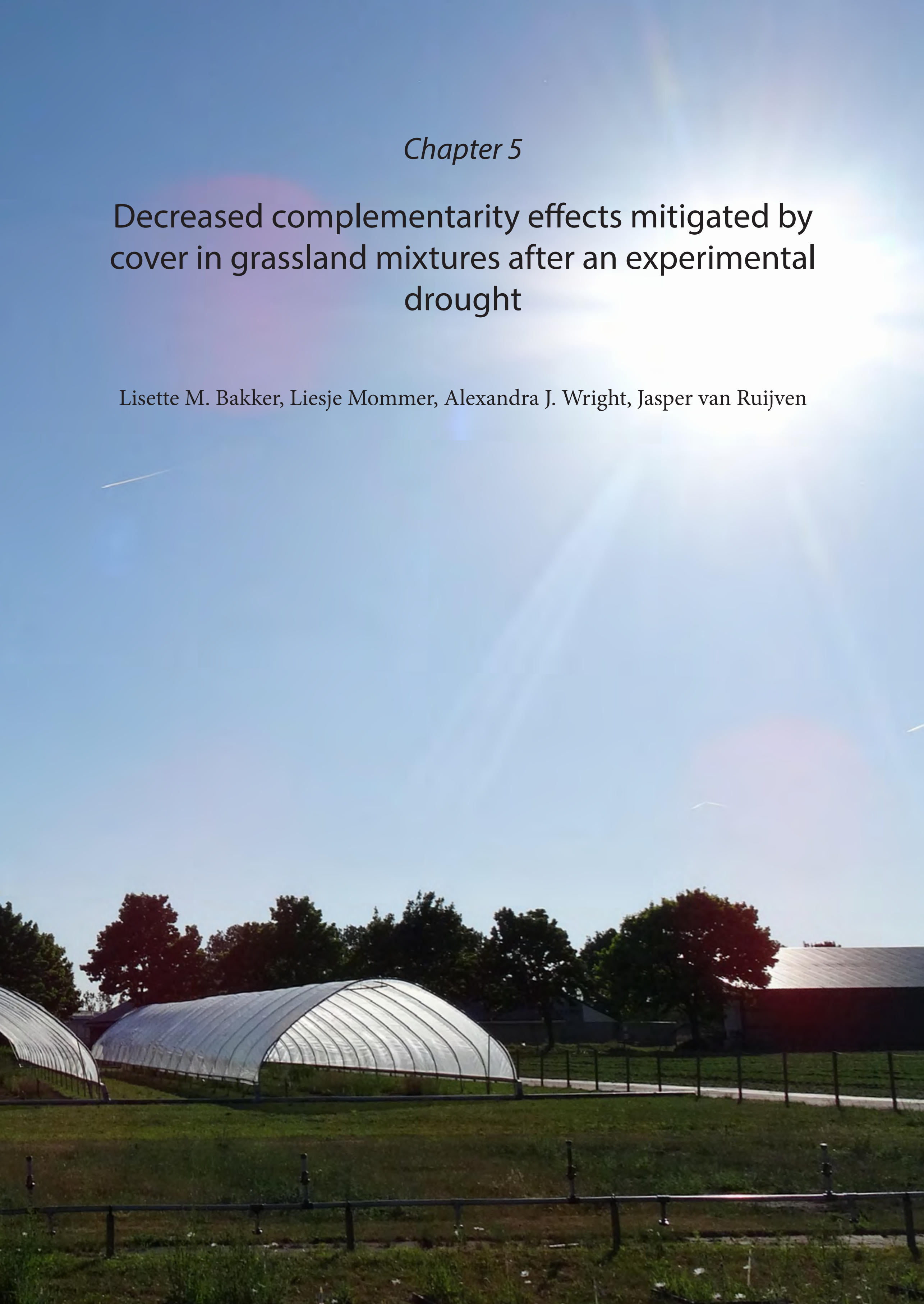
Model	**Intercept**	**DRF_{own}**	**DRF_{neigh}**	**sr**	**year**	**DRF_{own} *DRF_{neigh}**	**DRF_{own} *sr**	**DRF_{own} *year**	**DRF_{neigh} *sr**
1	-0.31	2.62	0.43	+	+	-6.78	+	+	
2	-0.30	2.61	0.41	+	+	-6.76	+	+	+
3	-0.27	2.62	0.29	+	+	-6.78	+	+	
4	-0.27	2.49	0.29	+	+	-6.20	+	+	+
5	-0.27	2.62	0.27	+	+	-6.76	+	+	+
6	0.03	1.13	-1.09	+	+		+	+	
7	-0.24	2.50	0.14	+	+	-6.21	+	+	+
8	-0.32	2.70	0.43	+	+	-6.79		+	
9	-0.24	2.46	0.12	+	+	-6.02	+	+	
10	0.03	1.13	-1.12	+	+		+	+	+
11	-0.22	2.42	0.04	+	+	-5.85	+	+	+
12	-0.32	1.54							
13	0.03								
w+(j)		1.00	1.00	1.00	1.00	0.95	0.98	1.00	0.34

$\text{DRF}_{\text{neigh}}$ *year	sr *year	DRF_{own} *$\text{DRF}_{\text{neigh}}$ *sr	DRF_{own} *$\text{DRF}_{\text{neigh}}$ *year	DRF_{own} *sr *year	$\text{DRF}_{\text{neigh}}$ *sr *year	DRF_{own} *$\text{DRF}_{\text{neigh}}$ *sr *year	df	ΔAIC	weight
	+			+			18	0.000	0.493
	+			+			19	2.008	0.181
+	+			+			20	3.327	0.093
	+	+		+			20	3.743	0.076
+	+			+			21	5.337	0.034
	+			+			17	5.838	0.027
+	+	+		+			22	7.076	0.014
	+						15	7.220	0.013
+	+		+	+			22	7.226	0.013
	+			+			18	7.774	0.010
+	+	+	+	+	+	+	28	17.955	0.000
							6	121.329	0.000
							5	271.623	0.000
0.19	1.00	0.10	0.02	0.97	0.01	0.00			

Chapter 5

Decreased complementarity effects mitigated by cover in grassland mixtures after an experimental drought

Lisette M. Bakker, Liesje Mommer, Alexandra J. Wright, Jasper van Ruijven



5.1 Abstract

The positive relationship between plant diversity and productivity, and the negative effect of drought on plant productivity are well established. However, the role of plant diversity in buffering plant productivity against drought events remains less clear. On the one hand, higher plant diversity may reduce drought stress via an increased chance of including drought-tolerant species or complementary water use. In addition, higher productivity and leaf area may reduce drought stress by reducing soil evaporation. On the other hand, increased productivity in species rich communities may also increase drought stress via increased transpiration due to higher leaf area. In this study, we experimentally exposed monocultures and 4-species mixtures of 16 different plant species to drought in a field experiment. Half of the communities experienced a six-week drought, while the other half, with similar species composition, were watered 2-3 times a week. Aboveground biomass was used to determine drought resistance, and additive partitioning was used to calculate complementarity effects. Further, we examined the role of plant community cover in these drought effects. Drought reduced aboveground biomass by 35%, irrespective of plant diversity. However, drought led to a significant loss of complementarity effects in mixtures, which suggests that species' responses to drought differed between monocultures and mixtures. Our analyses revealed that these responses were mitigated by community cover at the start of the drought: communities with low cover showed the largest decrease in complementarity, whereas communities with high cover were able to maintain complementarity effects at a level that was similar to those in control communities. These results suggest that plant cover plays an important role in drought resistance and potential positive effects of plant diversity during drought events. Future research specifically aimed at disentangling the contributions of cover, evapotranspiration and species composition along a drought gradient, may further increase our insights into the effect of biodiversity and drought resistance.

5.2 Introduction

Global climate change is likely to increase the frequency of extreme weather events in the coming century (Stocker *et al.*, 2013). Future climate scenarios predict decreased precipitation in summer and increased occurrence of prolonged summer droughts in Europe (Dankers and Hiederer, 2008, Klein Tank *et al.*, 2014). Research has shown that prolonged droughts can strongly reduce ecosystem productivity (Morecroft *et al.*, 2004, Ciais *et al.*, 2005, Reichstein *et al.*, 2007) and may also lead to biodiversity losses (Tilman and El Haddi, 1992).

The loss of biodiversity can subsequently decrease important ecosystem functions such as community productivity (Loreau *et al.*, 2001). Two decades of biodiversity research in experimental grasslands has shown that plant productivity increases with increasing plant species richness (Isbell *et al.*, 2011, Cardinale *et al.*, 2012, Tilman *et al.*, 2014). To further understand the underlying mechanisms of this positive effect of biodiversity on plant productivity, the additive partitioning method (Loreau and Hector, 2001) is often used. This method allows a distinction to be made between the contribution of positive interactions such as facilitation and resource partitioning (referred to as "complementarity effect") and that of increased dominance of productive species (referred to as "selection effect"). Meta-analyses have shown that both effects can be important, but that complementarity effects are often greater than selection effects (Cardinale *et al.*, 2007).

Importantly, diversity and drought may also interact in their effects on productivity. A recent meta-analysis (Isbell *et al.*, 2015) showed that in general, increasing biodiversity enhances resistance to natural drought (i.e. the ability to withstand a decrease in productivity during drought). However, they also reported "substantial variability in the effect of biodiversity on resistance". In addition, experimental drought studies reported neutral or negative effects of diversity on drought resistance (Pfisterer and Schmid, 2002, De Boeck *et al.*, 2008, Vogel *et al.*, 2012). How plant diversity affects drought resistance is still unclear. Multiple co-occurring mechanisms (Yachi and Loreau, 1999, Caldeira *et al.*, 2001, Van Peer *et al.*, 2004, Leimer *et al.*, 2014, Wright *et al.*, 2017) that shift in importance over time and space (Wardle *et al.*, 2004, Tylianakis *et al.*, 2008, Hiddink *et al.*, 2009) may explain the mixed results found for the effects of biodiversity on drought resistance.

Perhaps the most straightforward explanation for a positive effect of diversity on drought resistance is based on the 'insurance hypothesis' of biodiversity (Yachi and Loreau, 1999). Following this hypothesis, more diverse communities have a greater probability of containing drought-resistant species that can maintain community productivity during drought. Note that this is analogous to the effect of increased dominance of particular productive species on productivity described above (i.e. the selection effect). As such, this 'insurance' effect on resistance may be detected by comparing selection effects in communities with and without drought.

In addition, plant diversity may enhance drought resistance via two types of positive interactions between species, which can be assessed by calculating complementarity effects. The first is partitioning of water uptake in diverse mixtures (Silvertown *et al.*, 2015, Guderle *et al.*, 2018), for example via differences in rooting depth among species. This may decrease competition for water within the communities, and as such, enhance drought resistance. Several studies suggest this water partitioning can contribute to community performance during a drought (Caldeira *et al.*, 2001, Van Peer *et al.*, 2004, Postma and Lynch, 2012, Silvertown *et al.*, 2015). A second mechanism that can lead to positive interactions in diverse communities, is facilitation (Wright *et al.*, 2017). For example, plant species richness has been shown to increase relative air humidity and decrease temperature and vapour pressure deficit in the canopy, favouring the growth of small plants during drought (Wright *et al.*, 2014). In addition, increased leaf area in more diverse communities (Hector *et al.*, 1999, Weisser *et al.*, 2017, Guderle *et al.*, 2018) can reduce soil evaporation by covering the soil surface, increasing soil moisture in the upper layers, and thus reducing drought stress in more diverse communities (Rosenkranz *et al.*, 2012, Wright *et al.*, 2014).

Conversely, increased leaf area may also decrease drought resistance. Greater leaf area in higher diversity plots may lead to increased transpiration (Verheyen *et al.*, 2008, Milcu *et al.*, 2016, Guderle *et al.*, 2018), reducing water availability in the soil (De Boeck *et al.*, 2006, Mokany *et al.*, 2008, Leimer *et al.*, 2014) and potentially decreasing drought resistance in diverse communities.

In this study, we use an experimental drought in a grassland biodiversity experiment to test if plant species richness can affect drought resistance. To determine which of the mechanisms mentioned above contribute to this effect, we apply the additive partitioning method (Loreau and Hector, 2001) to aboveground biomass in drought and control plots. If drought-resistant species maintain biomass production of mixtures (and are most productive in monoculture), we would expect increased selection effects under drought. If complementary water use and/or facilitation are important, we would expect to see an increase in complementarity effects in the drought plots. In addition, we measure species' rooting depths and plant community cover and soil moisture to test if resistance and biodiversity effects are affected by variation in rooting depth among species and/or by plant cover via soil evapotranspiration.

5.3 Material and Methods

5.3.1 Common garden experiment

A biodiversity experiment with monocultures, 4-species mixtures and 16-species mixtures was established in Wageningen, the Netherlands, in April 2014 (51°99'N 5°66'E). See chapter 2 for more detailed information on the design. In short, original soil of the field was removed and replaced by a mix of agricultural soil and river sand (1:3) in the upper 50 cm layer (0-50 cm) and pure river sand in the 50-80 cm layer. In total, 198 plots of 70 x 70 cm, divided over three blocks, were created by pushing wooden frames in the soil (22 cm deep). We used sixteen grassland species to create 90 monocultures (six monocultures per species), 90 4-species mixtures (45 different species combinations, replicated twice), and 12 16-species mixtures (six mixtures differing in number of individuals per species and planting positions, replicated twice). The monocultures and mixtures were equally divided over the blocks, and in such a way that each block contained two monocultures per species, 15 different 4-species mixtures (replicated twice) and two different 16-species mixtures (replicated twice). The second replica of the species mixtures was placed within the same block for the use of two different water treatments (see below). The communities were planted in a random position within the block. In this chapter, we focus on the 4-species mixtures and the monocultures as we are primarily interested in the effects of species composition: presence/absence of drought tolerant species. Therefore, we excluded the 16-species mixtures, which share the same species composition, from the analyses in this study.

The sixteen grassland species contained eight grasses and eight herbs that are common in poor to moderately rich natural grasslands in the Netherlands (Table 5.1). Seeds were obtained from the seed supplier Cruydt-Hoeck, Nijeberkoop, the Netherlands. In each plot, 64 five-week old seedlings were planted in an 8 x 8 grid to start with equal plant densities. Unfortunately, well after planting we discovered that one of the species, *Leontodon hispidus*, was contaminated with another species of the same genus, *Leontodon autumnalis,* due to seed pollution via the seed supplier. As a consequence, there were actually 17 species in the experiment, with the *Leontodon* species sharing one position in the design. In addition, one of the species, *Ranunculus repens*, turned out to be the annual species *Ranunculus sardous*. After one growing season, *R. sardous* was replaced by *R. repens*.

In April 2017, each plot was fertilized twice (three weeks in between) with 4.7 L nutrient solution (20 mg L^{-1} NH_4, 483 mg L^{-1} NO_3, 46 mg L^{-1} P, 96 mg L^{-1} SO_4, 200 mg L^{-1} K, 120 mg L^{-1} Ca, and 21 mg L^{-1} Mg), which corresponds to 15 kg N/ha in total). This was done to compensate for nutrient losses via the aboveground biomass harvests of 2014 to 2016.

Table 5.1 Species that are used is the experiment and their plant functional group (PFG) and their deep root fraction (DRF; means and standard error), measured in species monocultures in 2015 (n = 6). DRF is the fraction root biomass found in the 30-50 cm soil layer compared to total biomass in the 0-50 cm layers (see methods). Nomenclature follows Van der Meijden (2005).

Species	**PFG**	**DRF**
Achillea millefolium	Forb	0.42 ± 0.07
Agrostis stolonifera	Grass	0.17 ± 0.03
Anthoxanthum odoratum	Grass	0.12 ± 0.02
Arrhenatherum elatius	Grass	0.20 ± 0.04
Briza media	Grass	0.07 ± 0.02
Centaurea jacea	Forb	0.38 ± 0.03
Festuca pratensis	Grass	0.21 ± 0.03
Festuca rubra	Grass	0.22 ± 0.04
Galium mollugo	Forb	0.30 ± 0.05
Leontodon autumnalis/hispidus	Forb	0.32 ± 0.03
Leucanthemum vulgare	Forb	0.21 ± 0.03
Phleum pratense	Grass	0.15 ± 0.03
Prunella vulgaris	Forb	0.17 ± 0.04
Ranunculus sardous	Forb	0.16 ± 0.02
Sanguisorba officinalis	Forb	0.35 ± 0.05
Trisetum flavescens	Grass	0.08 ± 0.02

5.3.2 Drought treatment

From May 5th to June 16th 2017, half of the plots were subjected to an experimental drought. All blocks (all plots) were covered by rainout shelters (6.00 m x 36.00 m x 2.60 m (w x l x h)) made of an aluminium frame and a transparent plastic sheet (Solar EVA, 180 µm thick). We covered all plots to control for potential side effects of the rainout shelters (Vogel *et al.*, 2013, Kreyling *et al.*, 2016). The plastic sheet was attached to aluminium gutters at 40 cm above the soil surface, to lead rain water away and to allow air circulation under the shelters. Light transmission of the plastic sheet was 90%. Temperature measurements within and between the shelters, using shielded thermocouples and a Datataker DT85 logger (every 10 minutes from 18th of May till 13th of June), revealed an average temperature increase of 2.0 ± 0.1 °C on a 24h basis, ranging from 0.75 ± 0.1 °C during the night and under clouded conditions, up to 5.0 ± 0.6 °C in the middle of a sunny day.

During the drought period, half of the plots, i.e. half of each species composition (three monoculture plots per species and one mixture plot per species composition), were randomly assigned to a drought treatment – the drought plots (D). The other replicate per composition per block served as control plots (C) and received additional water. The drought plots did not receive any water for 43 days, which resembles a drought that occurred once in the last 60 years (since daily precipitation measurements were done; KNMI, 2015), but is expected to occur more often in the future (Dankers and Hiederer, 2008, Klein Tank *et al.*, 2014). The control plots were watered 2-3 times a week, with on average 3.9 mm water (groundwater) per day (168 mm in total) to maintain soil moisture content. We assume that the plants could not reach the groundwater level, which was 146 ± 1 cm below the surface in the middle of May, since Schenk and Jackson (2002) showed that grass dominated plant communities had 95% of their biomass in the first 89 cm. To start with similar moistures content in all plots at the start of the drought, all plots received 22 mm water at the start of the drought period.

5.3.3 Data collection

Aboveground plant material was harvested by cutting the plants two cm above the soil surface at the end of the drought (12th to16th of June), sorted to species and dried in the oven for 48h at 70 °C before weighing. In addition, pictures were taken at the start of the drought with an RGB camera (Sony DSC HX50) at 1.3 m above the soil surface to estimate plant community cover using the vegetation index "Excess Green minus Excess Red (ExG−ExR)" of Meyer and Neto (2008). The vegetation index was calculated using the packages "rgdal" (Bivand *et al.*, 2017) and "raster" in R (Hijmans, 2015), using a pixel intensity threshold value of 80 for the vegetation index after visual checking and comparison with cover percentages using green band pictures and pixel thresholding in ImageJ (1.51f) (Rasband, 1997-2017).

The rooting depth of each species was determined using root biomass sampled in July 2015 in each monoculture by taking three soil cores per plot (50 cm deep x 2.5 cm diameter), divided into four layers: 0-5, 5-15, 15-30 and 30-50 cm. Samples were pooled per plot per layer and carefully rinsed with tap water using a 0.5 mm sieve to collect the fine roots (<2 mm). The root samples were oven dried for 72h at 70°C and weighted. The deep rooting fraction (DRF), the fraction of roots that was found in the deepest layer (30-50 cm) compared to total root biomass (0-50 cm), was calculated for each (monoculture) plot. Species' deep rooting fractions are shown in Table 5.1.

In addition, soil moisture was determined gravimetrically (Reynolds, 1970) in four soil layers, 0-15, 15-30, 30-50 and 50-60 cm, by taking soil samples with a soil core (1.5 cm diameter) at the start of the drought and at the end of the drought period. Samples were collected in plastic bags, fresh weighted, dried for at least 48h at 105 °C, and weighted again for dry weight.

Temperature of the plant community was measured on a warm, sunny day in the fourth week of the drought, using an infra-red camera. The thermal camera was placed on a two meter high tripod to capture the temperature of the inner 0.32 m^2 of the communities. We assume that community temperature is related to the drought stress of the community, partly because leaf temperature increases when leaf transpiration is reduced due to water stress (Jackson and Hillel, 1982). In addition, community temperature shows the severity of the micro conditions that the communities experience (heath stress).

5.3.4 Calculations

Biodiversity effects (net effects (NE), complementarity effects (CE) and selection effects (SE) were calculated for all mixtures using the additive partitioning method of (Loreau and Hector, 2001). In this method, the following equation (eq. 5.1) is used,

$$\Delta Y = Y_O - Y_E = N\overline{\Delta RY}\,\overline{M} + \mathrm{N}\ \mathrm{cov}(\Delta \mathrm{RY}, M) \qquad (eq.5.1)$$

in which delta yield (ΔY) measures the overall difference between the observed yield in the mixture (Y_O) and the expected yield (Y_E). N is the number of species present in the mixture at the end of the drought, and ΔRY the difference between the observed and expected relative yield in mixture (RY_O - RY_E), averaged across species. RY_O is the observed yield of a species in mixture, divided by its monoculture yield, while the expected relative yield (RY_E) is 1/N. is the average monoculture yield of the species that are present in the mixture. In this equation, ΔY is similar to NE, while the left component $(N\,\overline{\Delta RY}\,\overline{M})$ measures CE and the right component $(\mathrm{N}\ \mathrm{cov}(\Delta \mathrm{RY}, M))$ SE. Positive biotic interactions in mixtures, such as complementary water use, will be measured by CE. As is shown in equation 1, SE is calculated as the covariance between deviation from expected yield and monoculture biomass. Thus, it measures if increased (or decreased) yield in mixtures is related to productivity in monoculture across the species present in a mixture. If under drought, drought tolerant species are the most productive species in monoculture and become dominant in mixtures (and thereby maintain community biomass during drought), this will lead to increased positive SE values. We calculated the average monoculture yield ($\overline{M}$) per drought treatment, so that biodiversity effects of control mixture plots were compared with their control monocultures and drought mixture plots with drought monocultures. It is important to note that biodiversity effects depend on monoculture biomass (M): all else equal, a larger M will mean larger biodiversity effects (see eq. 5.1). This means that if drought leads to a reduction in (monoculture) biomass, biodiversity effects will decrease as well, even if the species interactions underlying these effects do not change. To be able to compare biodiversity effects between control and drought plots, we standardized biodiversity effects by dividing them by their average monoculture yield (Craven *et al.*, 2016). These will be referred to as relative NE (rNE) relative CE (rCE) and relative SE (rSE). Note that the value of these relative biodiversity effects can be interpreted as the relative contribution to mixture biomass, compared to the average monocultures biomass. For example, an rNE of 0.5 means that the net effect increased mixture biomass by 50% compared to the average monoculture biomass.

All species that died before or during the drought and had zero biomass during biomass collection (see Fig. S5.3 for survival) were excluded from the analyses. As *Leontodon* species shared one position in the design due seed contamination (see above), their RY_E and M were corrected using the actual number of planted individuals: RY_E of *Leontodon* species was calculated as the number of individuals planted divided by the total number of individuals planted in the inner 6 x 6 rows (36 individuals). To calculate M, monoculture biomass was weighted per plot using the number of individuals planted in monoculture, by multiplying the observed plot yield by the number of total planted individuals divided by the number of planted individuals in that plot. Unfortunately, all *L. autumnalis* individuals died in the monocultures, but not in mixtures. To determine biodiversity effects for mixtures containing this species, we used its average biomass in mixtures, separately for drought and control plots and corrected for the different planting densities, as its monoculture yield in the calculations. Thus, on average, *L. autumnalis* did not affect the biodiversity effects in mixtures.

Next, relative species performance in mixtures was calculated per plot using proportional deviation (D_i; Loreau, 1998) . D_i is the proportional deviation of species biomass in mixture (O_i) from its expected biomass (E_i): $D_i = (O_i - E_i) / E_i$. The expected biomass of a species (E_i) was calculated as the proportion of individuals planted in the mixture multiplied by its monoculture biomass. This measure is independent of the number of individuals planted, which is necessary to compare the performance of *L. hispidus*. Further, we calculated monoculture drought resistance for each species as the difference in logs between the average monoculture biomass in control plots and the average biomass in drought plots.

Finally, for each mixture we calculated the average rooting depth as the community weighted mean (CWM) deep root fraction (DRF; the fraction of root biomass in 30-50 cm compared to total root biomass in the 0-50 cm soil profile). In addition, we calculated variation in rooting depth among the species in each community as functional diversity in DRF. This diversity in DRF was calculated as functional dispersion (FDis), a functional diversity index that is independent of the

CWM, but includes the spread of trait values within the community relative to the community weighted mean (Laliberté and Legendre, 2010). Communities' CWMs and FDis in DRF were calculated using the "FD package" in R (Laliberté and Shipley, 2011), using species specific DRF values (Table 5.1) and species relative abundances. Species relative abundances were based on the harvested aboveground biomass (species aboveground biomass compared to whole community biomass).

5.3.5 Statistics

Soil moisture at the start and end of the drought was analysed using an linear mixed model (LME) with drought treatment, species richness and soil layer and their interactions as fixed factors and plot nested in block as random factor. Differences between layers were tested using post hoc tests (Tukey) with the "lsmeans" package (Lenth, 2016). The effects of species richness and drought on community biomass were tested using an LME with planted species richness and drought treatment and their interaction as fixed factors and block and species composition as random factors (REML). Similarly, the effect of drought treatment on rNE, rCE and rSE was tested with an LME with drought treatment as a fixed factor and species composition as a random factor.

To identify drought-tolerant species and potential shifts in species' drought resistance in mixtures, we also analysed species-specific biomass in monoculture and mixture. Differences in the drought response between species in monoculture were tested with an LME using log biomass as response variable and species and drought treatment as fixed factors. Block was included as random factor. For the mixtures, the effect of the drought treatment on species' D_i was tested using an LME with drought treatment as fixed variable and species composition as random factor. Thereafter, a similar test was done for all species separately. *L. autumnalis* was excluded from these analyses as this species did not have a monoculture yield to compare mixture yield with.

To examine the role of rooting depth in species and community drought resistance, we tested the effect of DRF on monocultures drought resistance (biomass difference between C and D plots) with an linear model. We expected that species with high DRF, indicating a vertical root distribution with a high proportion of roots in deeper layers, have a higher drought resistance, since species with a high DRF have potentially higher water uptake from deeper layers, where water availability is higher during drought. Further, we investigated the importance of the average DRF values (CWMs) and diversity (FDis) in DRF within a community on performance of the mixture communities (rCE) under drought and control conditions: we coupled rCE to DRF CWM and DRF FDis and drought treatment in an LME with species composition as a random factor (Maximum Likelihood).

To determine if plant cover played a role in the community drought response, we first determined the relationships between cover, soil moisture at the start and at the end of the drought, and community temperature with Pearsons correlation coefficient (package " Hmisc" (Harrell Jr and others, 2016), using all plots. Next, we tested whether cover affected changes in rCE due to drought by including cover (continuous) and its interaction with drought as fixed factors in the LME used for rCE as described above. As a second step, we analysed the relationships between rCE and cover separately for the control and drought plots using linear models.

In all models, species richness was included as a factor with two levels: monocultures and mixtures. To meet model assumptions, biomass was ln transformed. Similarly, rNE, rCE, rSE, and D_i were log10 transformed after adding the lowest value plus one to have only positive values. In the analyses of biodiversity effects, extreme outliers – defined as values that were five or more times the interquartile range above the third quartile – were removed. This led to the removal of three rNE and rSE (same plots) and one rCE value. All statistics were done in R (version 3.1.3) with R studio (version 1.0.153), using the "lme4" (Bates *et al.*, 2015) or "nlme" (Pinheiro *et al.*, 2016) package. We used anova type III with the "anova" function of the "stats" package (R CoreTeam, 2016) to test the models.

5.4 Results

5.4.1 Drought treatment: soil moisture and temperature

At the start of the drought treatment, gravimetric soil moisture was similar for control and drought plots (on average 6.2 ± 0.1 %; drought: $F_{1, 182} = 0.0$, $P = 0.889$) and also for monocultures and mixtures (species richness (SR): $F_{1, 182} = 0.1$; $P = 0.717$) over the whole 60 cm depth profile. However, the experimental drought treatment significantly reduced soil moisture ($F_{1, 182} = 199.2$; $P < 0.001$). At the end of the drought treatment, soil moisture content was 4.6 ± 0.1 % in the control plots and 2.3 ± 0.1 % in the drought plots. This decrease in moisture in drought plots depended on soil layer (drought x soil layer: $F_{3, 542} = 61.4$, $P < 0.001$): experimental drought significantly decreased soil moisture for all soil layers, except for the deepest (50-60 cm) layer, which showed no effect of drought (Fig. S5.1). At the end of the drought treatment, the effect of species richness on soil moisture depended on drought treatment (SR x drought: $F_{1, 182} = 6.0$; $P < 0.05$). In the drought plots, there was no difference in soil moisture between monocultures and mixtures (SR: $F_{1, 91} = 0.3$, $P = 0.57$), but under control conditions, plant mixtures had a slightly lower soil moisture than monocultures (SR: $F_{1, 91} = 6.6$, $P < 0.05$; Fig. S5.1). Furthermore, community temperature was significantly increased in drought plots (drought: $F_{1, 176} = 51.6$, $P < 0.001$). In the middle of a sunny day during the drought, control plots had an average temperature of 33.3 ± 0.4 ^{o}C, while the temperature in drought plots increased to 42.6 ± 0.5 ^{o}C.

5.4.2 Effects of species richness and drought on community biomass

Biodiversity increased plant productivity: on average, four-species mixtures had 65% more biomass than monocultures (SR: $F_{1, 57} = 13.5$, $P < 0.001$). The drought treatment decreased community biomass by 35% on average compared to the control (drought: $F_{1, 127} = 70.1$, $P < 0.001$; Fig. 5.1A), independent of species richness (drought x SR: $F_{1, 127} = 0.3$, $P = 0.57$; Fig. 5.1A).

The positive effect of species richness on biomass was reflected in overall positive biodiversity effects (Fig. 5.1B). However, the biodiversity effects were strongly affected by drought. The relative net effect (rNE) was significantly reduced in drought plots compared to control plots (drought: $F_{1, 42} = 16.7$, $P < 0.001$). This reduction in rNE with drought was mainly caused by a strong decrease in relative complementarity effects (rCE) in drought plots ($F_{1, 43} = 9.6$, $P < 0.01$): from 0.47 in the control to 0.21 under drought (Fig. 5.1B). Thus, positive interactions (measured as CE) increased mixture biomass by 47% compared to the average monoculture in the control, but under drought this decreased to only 21%. Drought did not affect the relative selection effect rSE ($F_{1, 42} = 0.1$, $P = 0.73$; Fig. 5.1B).

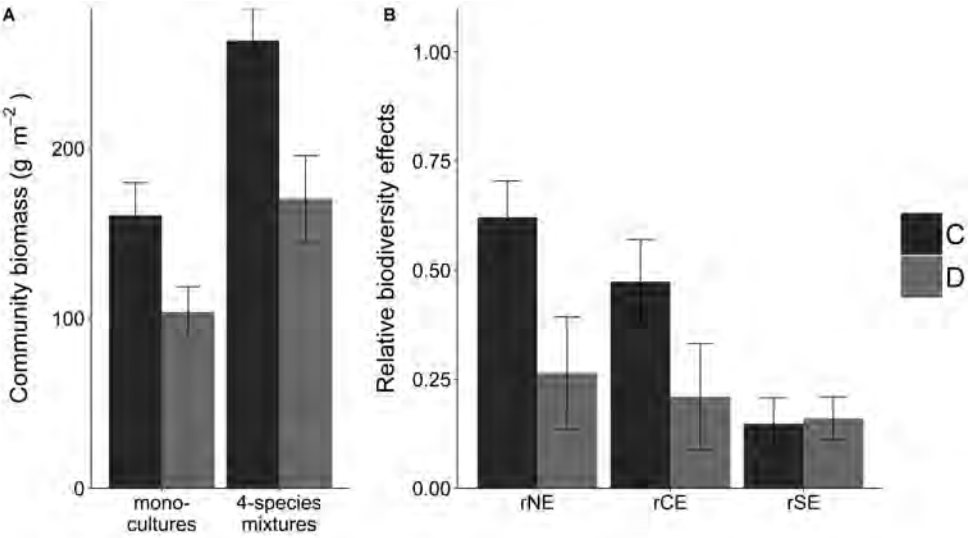


Figure 5.1 Community biomass of monocultures and 4-species mixtures (A) and relative biodiversity effects in the 4-species mixtures (B) under watered control conditions (C; dark grey bars) and drought conditions (D; light grey bars). A) Community biomass was significantly reduced by the drought treatment. This reduction was similar for monoculture and mixtures (35%). B) The relative net effect (rNE), complementarity effect (rCE) and selection effect (rSE) in the control (C) and drought (D) mixtures plots. rCE is significantly reduced by drought, while rSE is similar in C and D plots. The decrease in rCE in D plots means that drought reduces the positive biodiversity effects in mixtures that result from positive biotic interspecific interactions such as facilitation, so that the effect of species richness on community biomass decreases. Bars show means ± standard errors.

5.4.3 Species drought responses

Overall, species biomass in monoculture was significantly reduced by the drought treatment (drought: $F_{1,\,62} = 25.0$, $P < 0.001$). Although a large gradient in drought resistance across species was found, ranging from -74% for *P. vulgaris* to +33% for *A. stolonifera* (see drought resistance in Fig. 5.2), the effect of drought did not differ between species (drought x species: $F_{1,\,60} = 0.8$, $P = 0.69$ for effect). Moreover, no relationship was found between species' drought resistance in monoculture (C-D difference) and their DRF ($F_{1,\,14} = 1.4$, $P = 0.25$).

Overall, drought reduced relative performance of species in mixtures compared to monocultures (D_i) (Fig. 5.3; $F_{1,\,247} = 8.0$, $P < 0.01$). Although drought seemed to affect relative performance of some species more than others (e.g. compare *A. millefolium* and *C. jacea* in Fig. 5.3), the effect of drought on relative performance did not differ significantly among species ($F_{15,\,243} = 0.7$, $P = 0.81$). When analysing the species separately, however, five species (*F. rubra*, *A. millefolium*, *A. elatius*, *A, odoratum* and *G. mollugo*), showed a significant decrease in relative performance in mixtures in drought plots (Fig. 5.3).

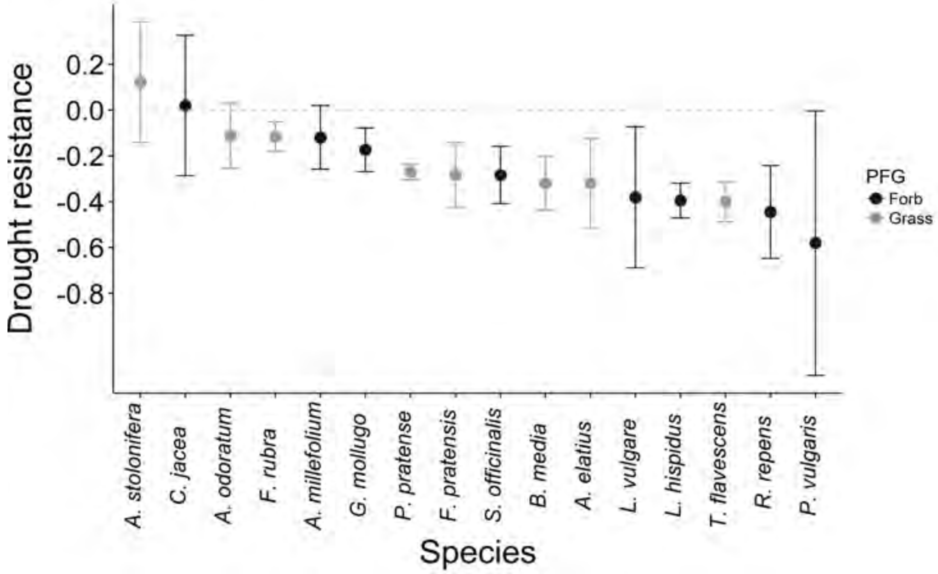


Figure 5.2 Drought resistance (difference in log biomass between drought and watered control plots; n = 3) of each species in monoculture. Although there seems to be a gradient in resistance, differences between species were not significant. Mean ± standard errors are shown. PFG = plant function group.

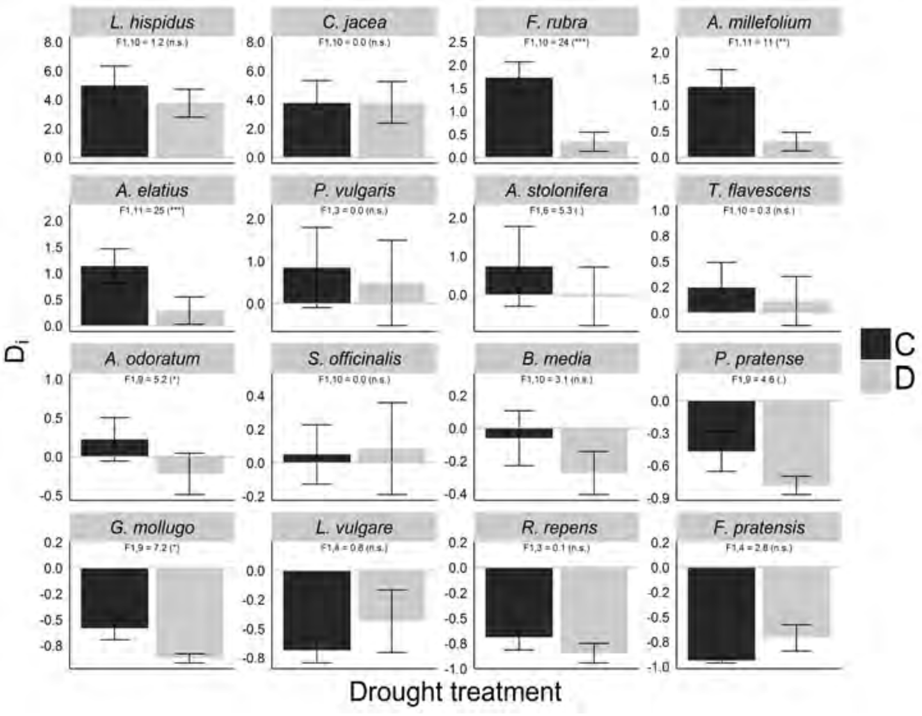


Figure 5.3 The relative performance of species in mixtures compared to monocultures (D_i) was often reduced by drought. This reduction was significant for five species: *F. rubra, A. millefolium, A. elatius, A. odoratum* and *G. mollugo*. Positive D_i values indicate better performance in mixtures than in monocultures. Negative D_i values imply that species performed worse in mixtures. C = watered control plots (dark grey bars), D = drought plots (light grey bars). Species are sorted based on their D_i in the control plots. Bars show mean ± standard errors. Asterisks indicate significance: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, . = $P < 0.1$, n.s. = non-significant.

5.4.4 The role of rooting depth and plant cover for community drought responses

We did not find a relationship between relative complementarity effects (rCE) and the rooting depths of the different communities. Neither the community weighted mean (CWM) nor the functional diversity (FDis) in deep root fraction (DRF) were significantly associated with rCE, irrespective of drought (CWM: $F_{1, 41} = 0.0$, $P = 0.84$ and CWM x drought: $F_{1, 41} = 1.5$, $P = 0.22$; FDis: $F_{1, 41} = 1.1$, $P = 0.29$ and FDis x drought: $F_{1, 41} = 0.0$, $P = 0.88$; Fig. S5.4).

At the start of the drought treatment, plant cover was greater in species mixtures compared to monocultures (SR: $F_{1, 180} = 30.4$, $P < 0.001$). On average, cover was 14% greater in mixtures, although cover values in mixtures ranged from 12 to 84% (Fig. 5.4). Higher plant cover (of monocultures and mixtures) was weakly associated with lower soil moisture over the whole layer depth (0-60cm) at the start of the drought ($\rho = -0.16$, $P < 0.05$), so that the highest covered mixtures plots had on average 1.2% lower moisture than the plots with very low cover. At the end of the drought, soil moisture was significantly reduced in drought plots compared to control plots, but in both soil moisture decreased with plant cover ($\rho = -0.21$, $P < 0.01$; Fig. S5.2C and S5.2D; -1.6% over the whole cover range). Community temperature was also found to be higher in communities with lower soil moisture ($\rho = -0.42$, $P < 0.001$; Fig. S5.2B). At the same time, however, plant cover reduced community temperature during drought ($\rho = -0.38$, $P < 0.001$). This effect was found in control and drought plots (Fig. S5.2A).

Including cover in the analysis of rCE revealed that the negative effect of drought on rCE was marginally significantly affected by cover (cover x drought: $F_{1, 41} = 3.5$, $P = 0.07$), suggesting that the negative effect of drought on rCE depended on community cover. Indeed, when analysing control and drought plots separately, rCE increased with cover in drought plots ($F_{1, 43}= 8.1$, $P < 0.01$), whereas no effect of cover on rCE was found in the control plots ($F_{1, 42} = 0.2$, $P < 0.62$). As a result, rCE was strongly reduced compared to control plots (and negative) at low plant cover and increasingly positive with increasing cover. In communities with a high cover, rCEs were as large as or even larger in drought plots compared to control plots (Fig. 5.4).

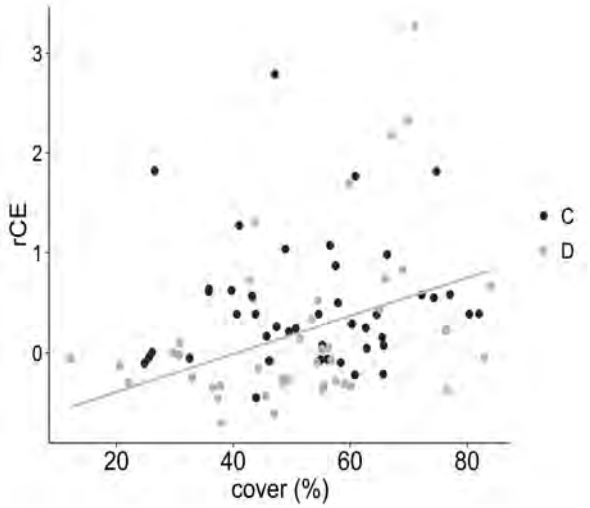


Figure 5.4 Relationships between the relative complementarity effect (rCE) and community biomass cover. In the drought plots (D; light grey dots), rCE was reduced in communities with low cover (light grey line). In control plots (C; dark grey dots), no significant relationship between rCE and cover was found. Dotted grey horizontal line shows the expected yield in mixtures based on monoculture yield (rCE=0).

5.5 Discussion

In our study, plant species richness increased aboveground productivity, but did not affect drought resistance at the plot level. However, biodiversity effects, and complementarity effects in particular, strongly decreased with drought. These negative effects of drought on complementarity effects were mitigated by plant cover: there were strong reductions in complementarity effects at low plant community cover, but similar or even greater complementarity effects at high cover. We found no evidence that increased dominance of productive drought-resistant species (following the insurance hypothesis) played an important role in the drought response of plant mixtures. Together, these results suggest that effects of diversity on drought resistance depend on the extent to which plant mixtures can decrease soil evaporation and/or ameliorate microclimatic conditions in the canopy.

5.5.1 Biodiversity and drought resistance

In our study, the relative biomass reduction due to the drought treatment was independent of plant species richness. This is in line with several other experimental studies that showed no effect of plant species richness on drought resistance (proportional; Pfisterer and Schmid, 2002, De Boeck *et al.*, 2008, Vogel *et al.*, 2012, Craven *et al.*, 2016), but it contradicts the results of a recent meta-analysis, in which resistance to natural droughts increased with plant diversity across a range of biodiversity experiments in grasslands (Isbell *et al.*, 2015). However, in the latter there was considerable variation among experiments, ranging from negative to positive relationships between species richness and drought resistance. We hypothesized that this variation may be related to changes in the relative contribution of different mechanisms to diversity-dependent drought resistance. Below, we will review the available evidence for each of these mechanisms.

5.5.2 Potential mechanisms related to drought resistance

First, increased drought resistance in diverse communities may be due to an increased chance of including drought-resistant species that maintain community productivity, following the insurance hypothesis of biodiversity (Yachi and Loreau, 1999). We hypothesized that this mechanism should be reflected in an increase in positive selection effects under drought. However, the drought treatment did not increase (relative) selection effects. Also at the species level, we did not find strong evidence for the insurance hypothesis in our experiment. Although two species appeared drought-resistant in monoculture (*A. stolonifera* and *C. jacea*; see Fig. 5.2), these species did not significantly increase their relative performance in mixtures (D*i*) under drought (Fig. 5.3). These species did not compensate reductions of other drought-sensitive species in our experiment. It is important to note that increased dominance of drought-resistant species can also lead to negative selection effects, if the drought tolerant species are low productive species in monoculture and increase in dominance under drought (Caldeira *et al.*, 2005, Mariotte *et al.*, 2013). However, we also found no evidence for reductions of selection effects (i.e. more negative rSE) under drought. Although our results provide no support for the insurance hypothesis (Yachi and Loreau, 1999), this mechanism may operate in other experiments that do contain productive drought-resistant species that increase in performance in mixtures (e.g.Spence *et al.*, 2016).

Second, increased drought resistance in mixtures compared to monocultures may also be due to positive interactions between species, such as complementary water use and facilitation. If these mechanisms are important under drought, we would expect to find an increase in complementarity effects, which measure the outcome of positive interactions (Loreau and Hector, 2001). In contrast, we found a decrease in complementarity effects in our study. This suggests that positive interactions are not sufficiently strong to enhance drought resistance of mixtures compared to monocultures. If anything, the reduction of complementarity effects suggests that drought decreased positive interactions in mixtures. This may be related to increased transpiration due to increased leaf area (Hector *et al.*, 1999, Weisser *et al.*, 2017). Several studies have shown increased water use (Caldeira *et al.*, 2001, Van Peer *et al.*, 2004), water uptake (Guderle *et al.*, 2018) or transpiration (Verheyen *et al.*, 2008) in plant mixtures, leading to decreased soil moisture (Mokany *et al.*, 2008, Leimer *et al.*, 2014). In the control plots, we did find decreased soil moisture in mixtures compared to monocultures, perhaps due to increased transpiration via greater plant cover in mixtures compared to monocultures. However, this negative effect of species richness on soil moisture was absent in the drought treatment, perhaps because both monocultures and mixtures approached minimum soil moisture content (Fig. S5.1). These results are in line with Verheyen *et al.* (2008), who showed that the pattern of increased evapotranspiration in mixtures compared to monocultures in control conditions disappeared or even reversed after drought. Moreover, our findings suggest that under drought, cover positively affected drought resistance: despite the decrease in soil moisture with increased cover, community temperature decreased with cover under drought, suggesting that drought stress was more severe at low rather than high cover within mixtures. In addition, complementarity effects increased with cover under drought. Complementarity effects were not affected by drought at the highest levels of cover within our study, but only decreased under drought at low cover. This suggests that cover plays a role in diversity-dependent variation in drought resistance, with reduced resistance in mixtures compared to monocultures at low cover (due to a reduction in complementarity effects), but similar resistance at high cover (where complementarity effects are not affected by drought).

If drought stress is indeed most severe at lower cover, as our temperature data suggest, we would expect differential responses of species in mixtures, depending on cover in their monocultures. All else equal, species with relatively high cover in monoculture will experience increased drought stress in low cover mixtures, reducing their relative performance in these mixtures under drought. In contrast, species with low cover in monoculture will experience reduced drought stress in high cover mixtures, which may increase their relative performance. We tried to test this by determining if the average change in species performance in mixture (D_i) between drought and control plots was related to their cover in monoculture, but no relationship was found (linear model: $F_{1,14} = 0.5$, $P = 0.51$)). However, this may also be due to the fact that in our design, each mixture has a different species composition, which may also strongly affect the relative performance of individual species in mixtures. It is important to note that this facilitative effect of cover, which may be due to increased air humidity and decreased vapour pressure deficit in the canopy or decreased soil evapotranspiration (Wright *et al.*, 2014), may also lead to positive effects of species richness on drought resistance. In our experiment, plant mixtures spanned a large gradient in plant cover (12-84%), with reduced performance in low cover mixtures (due to reduced performance of species with high cover in monoculture) and increased performance in mixtures with high cover. The net result can be negative (as in our study), but may shift to positive if plant mixtures are predominantly characterized by high cover (compared to monocultures). Future research should determine the importance of (changes in) cover for diversity-dependent drought resistance, for example by independently manipulating leaf area and plant cover along a drought gradient.

5.5.3 The role of rooting depth in drought resistance

We found no evidence that species' drought resistance was related to their deep root fraction (DRF), and in contrast to community cover, community weighted mean DRF and diversity in DRF did not affect complementarity effects. These results are not in line with studies that found higher drought resistance for grassland species or communities with deeper roots (e.g. Hoekstra *et al.*, 2015, Barkaoui *et al.*, 2016, Zeiter *et al.*, 2016), or with chapter 3, in which deep-rooting species increased in monocultures and mixtures after a dry period. One explanation for this discrepancy may be that we measured roots until a depth of 50 cm. Our soil moisture results show that the deeper layer we used to determine DRF (30-50 cm) was affected by the drought treatment to the same extent as the shallower layers, whereas the layer below (50-60 cm) showed no effect of drought. Also, the groundwater level at our field site is well below 50 cm. Perhaps the roots below 50 cm were more important during drought. This may explain why Barkaoui *et al.* (2016), who measured roots until 120 cm depth, did find a relationship between rooting depth and drought resilience. Further, we cannot exclude the possibility that plasticity in rooting depth over time and in response to species richness (Mommer *et al.*, 2010, Mueller *et al.*, 2013) and drought (Padilla *et al.*, 2013) have resulted in differences between our estimated community rooting depth based on measurements in monoculture and the actual rooting depths in mixtures. However, in chapter 3, the same rooting depth estimates from monocultures did predict species overyielding in mixtures, which would be very unlikely if they do not accurately capture rooting depth in mixtures.

5.5.4 Implications and conclusions

Together, our findings suggest that plant diversity did not increase drought resistance. On the one hand, the decreased complementarity effects indicates decreased drought resistance in mixtures, perhaps as a result of decreased soil moisture due to increased transpiration. On the other hand, the mitigation of complementarity effect reductions in high-covered plots, suggest that facilitation via increased cover in mixtures may have increased drought resistance. We did not find an important role of rooting depth in drought resistance, nor did we find evidence for drought-resistance species that increased their performance in mixtures, maintaining community productivity. We suggest that variation in the role of community cover may also explain the differences in the effects of biodiversity on drought resistance between studies (Pfisterer and Schmid, 2002, De Boeck *et al.*, 2008, Isbell *et al.*, 2015, Craven *et al.*, 2016). In studies in which plant diversity increases cover substantially, plant diversity could positively affect drought resistance via microclimate amelioration. In contrast, studies with plant community that have low cover could find decreased drought resistance with species richness. These differential effects of community cover deserve further research.

Differences in the effects of biodiversity on drought resistance among studies may also be due to differences in the extent of the drought conditions. The drought in our study was quite severe: the drought plots did not receive any water for 43 days, a drought that only occurred once in the last 60 years (since daily precipitation measurements were done), while outside conditions were relatively dry and sunny. In contrast, Isbell *et al.* (2015) used a return time of 10 years in their definition of drought. In addition, soil type may play a role. Our study was done on a sandy soil, which is known to have a low water holding capacity (Rawls *et al.*, 1982, Hudson, 1994) and thus less possibility for facilitation through hydraulic redistribution (Neumann and Cardon, 2012). At medium wetness levels or in soil types with a better holding capacity complementarity effects could be optimized (Neumann and Cardon, 2012, Isbell *et al.*, 2015). Our extreme dry conditions could be the reason that we found no positive effect of biodiversity on resistance, in contrast to the meta-analysis by Isbell *et al.* (2015). It may also explain why we found a decrease in complementarity effects under drought, while De Boeck *et al.* (2008), using drought as result of warming (on silt loam), and Craven *et al.* (2016), including several studies with variable results, found no effect. Finally, the extreme conditions in our experiment may also be the reason that the insurance hypothesis did not play a large role in drought resistance in our study. If all species are affected by drought and stop growing, even the drought-resistant species will not be able to compensate for the reduced performance of drought-sensitive species. An important direction for future research is investigating the effect of plant diversity on ecosystem functioning along a drought gradient on different soil types. By manipulating species richness, drought intensity and productivity and cover, we may disentangle the relative contributions of cover, evapotranspiration and species composition along gradients of environmental stress, and further increase our insights into the effect of biodiversity on drought resistance.

5.6 Acknowledgements

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5.7 Supplementary figures

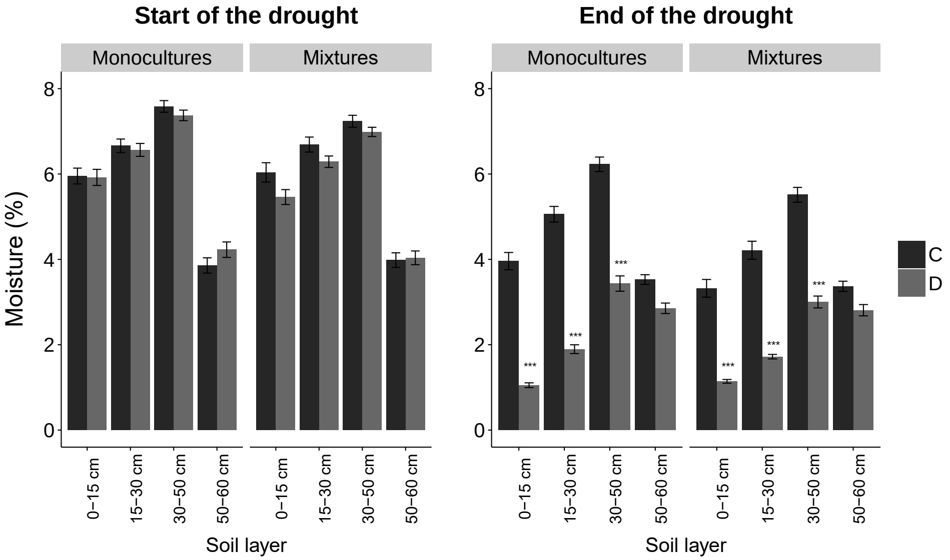


Figure S5.1 Soil moisture (gravimetric) at the start of the drought (left picture) and at the end of the drought (right picture). At the start of the drought, soil moisture content did not differ between control and drought plots or species richness levels. However, drought strongly reduced soil moisture in all soil layers except for the 50-60 cm layer. Only in control plots, soil moisture was lower in mixtures than in monocultures at the end of the drought ($F_{1, 91} = 6.6$, $P < 0.05$). C = watered control plots (dark grey bars), D = drought plots (light grey bars). Asterisks indicate significance *** = P <0.001 for differences between C and D plots. Bars show means ± standard error.

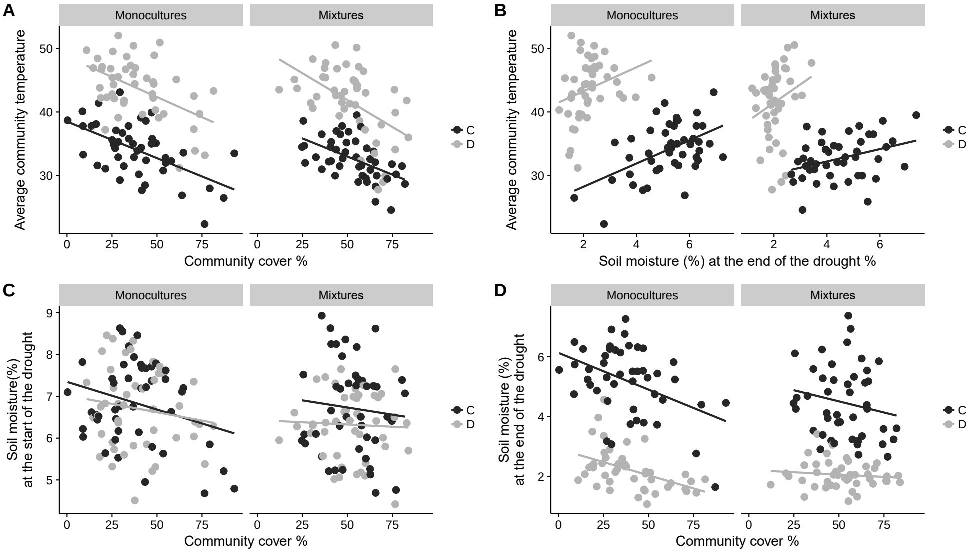


Figure S5.2 Relationships between community cover, temperature and soil moisture in monocultures and mixtures in drought (D; grey dots) and control (C; black dots) plots.

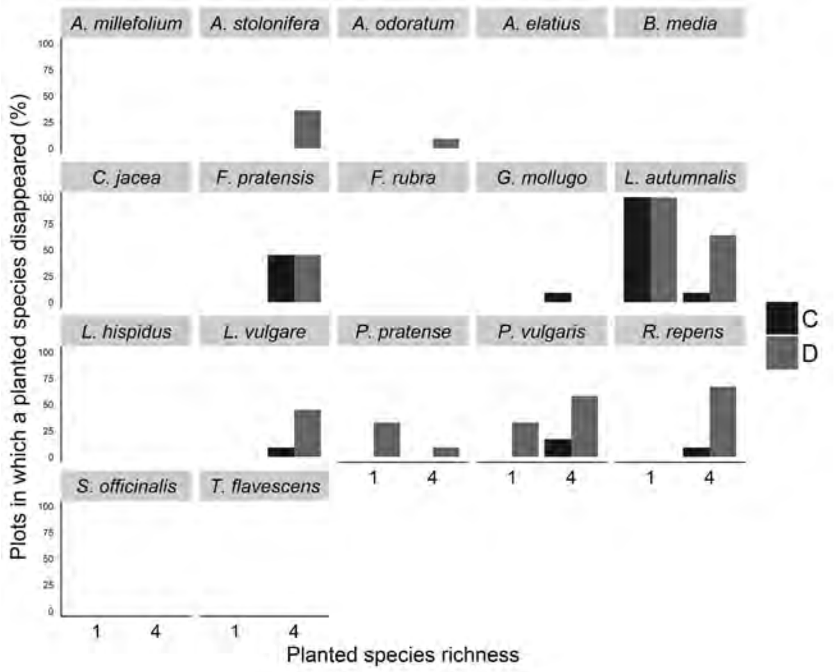


Figure S5.3 Species survival in monocultures and 4-species mixtures in drought plots (D; light grey bars) and watered control plots (C; dark grey bars). Nine species, four grasses and five forbs, disappeared completely from at least one monoculture or mixture. Species disappearance was more common in drought (75% of the disappearances) than in control plots (25%) and in species mixtures (86% of the disappearances) than in monoculture (14%).

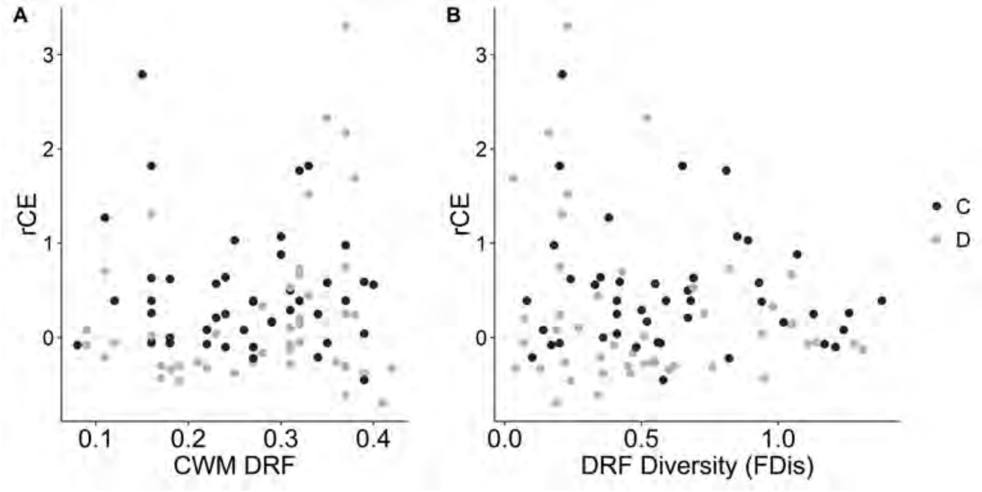


Figure S5.4 Relative complementarity effects (rCE) and deep root fraction community weighted means (DRF CWM) and deep root fraction diversity (DRF FDis) of the plant mixtures were not linked. C = control plots (dark grey dots), D = drought plots (light grey plots), dotted grey line = expected yield based on monoculture yields (rCE=0).

Chapter 6

General discussion

6.1 Introduction

In this thesis, I investigated the mechanisms underlying positive biodiversity effects on productivity in experimental grassland communities. I aimed to get more insight into the role of resource partitioning in positive effects of plant diversity using a functional trait approach. Resource partitioning is hypothesized to occur when species that differ in their resource uptake strategy grow together, which results in a more complete exploitation of the available resources (Tilman *et al.*, 1997b, Hooper *et al.*, 2005, Cardinale *et al.*, 2011). As the root system is responsible for the acquisition of nutrients and water (Lynch, 1995, Fort *et al.*, 2017), I hypothesized that diversity in root traits such as rooting depth may underlie resource partitioning and contribute to the biodiversity effects. For example, a community consisting of deep and shallow-rooting species is expected to acquire a larger part of the available soil resources (and increase productivity) than species monocultures or mixed communities containing only shallow or only deep-rooting species. Therefore, in this thesis, I applied a functional trait approach to answer the main research question: can positive biodiversity effects in experiments grassland communities be explained by root traits? I examined this question in the first three chapters of this thesis using traits measured in a pot and field experiment. I focused in particular on variation in rooting depth, representing the potential range of water and nutrient uptake from different vertical soil layers. The root traits were specifically coupled to selection effects (biomass increases in mixtures due to the dominance of productive species) and complementarity effects (biomass increases in mixtures due to positive interactions), following the "additive partitioning method" of Loreau and Hector (2001) (see Box 1.1).

In a biodiversity experiment, I demonstrated that species mixtures had on average significantly higher yields than monocultures ("overyielding") (chapter 2, 3, and 5). This finding serves as the baseline for the rest of my thesis. In chapter 2, complementarity effects (i.e. relative complementarity effects, so standardized with communities' average monoculture yield; see Box 1.1; hereafter just called complementarity effects) could not be explained by diversity in root traits from a pot experiment and rooting depth from literature. Also in the next chapter (chapter 3), I found that *diversity* in root traits, now estimated with root traits measured in the monocultures of the biodiversity experiment, could not explain changes in complementarity effects from the first to the second year. Although root traits could not explain complementarity effects, the *average* root trait values (community weighted means; CWMs) did explain changes in relative selection effects (hereafter called selection effects). This means that root traits were coupled to the productivity in monoculture ánd dominance, and thus increased yield, in mixtures. In chapter 4, in which I measured biomass of individual plants in species monocultures and mixtures over three years, species with deep roots showed the highest overyielding in mixtures. More importantly, deep-rooting species showed highest overyielding when neighbouring plants were shallow-rooting species. In contrast, the shallow-rooting species did not respond to the rooting depth of their neighbours. Thus, when measuring at a smaller scale than the plot level, diversity in rooting depth was important for species-specific overyielding in plant mixtures via increased performance of deep rooting species.

Further, I hypothesized that species' differences in rooting depth may especially play a large role during a drought (Comas *et al.*, 2013), so that diverse communities have increased access to water when total available water (and nutrients) is low (Nippert and Knapp, 2007b). This way, drought resistance, i.e. the ability to maintain biomass production during a drought, may be increased in plant mixtures. I tested this effect of plant diversity on drought resistance in chapter 5, by studying the effect of drought on biodiversity effects using the partitioning method of Loreau and Hector (2001). I examined whether rooting depth or other mechanisms, such as the role of community plant cover, play a role in drought resistance. In chapter 5, I could not find a positive effect of plant diversity on drought resistance. Plant mixtures and monocultures had a similar proportional decrease in productivity after an experimental drought. We found no evidence that diversity in root traits played an important role in the drought response of plant mixtures. Instead, the drought treatment reduced complementarity effects in mixtures, and this reduction was mitigated in mixtures that had high community plant cover at the start of the drought.

6.2 Biodiversity effects in the biodiversity experiment

6.2.1 Increased productivity in species mixtures

The biodiversity experiment that I established for this thesis showed an increase in aboveground biomass in species mixtures compared to species monocultures in all years (chapters 2, 3, and 5; Fig. 6.1). Mixtures had on average over all years 60 ± 6% higher yield than monocultures (excluding drought plots in 2017), which is consistent with the average 54% (C.I. = 45% to 64%) increase reported in a meta-analysis (Cardinale *et al.*, 2007). The increased productivity in mixtures was already shown in the first year of establishment, whereas most biodiversity experiments detected only significant increases from the second year after establishment (e.g. Tilman *et al.* (2001), and van Ruijven and Berendse (2005), Fargione *et al.* (2007)). The early positive biodiversity effects in this thesis may be the result of the relative poor nutrient conditions, i.e. relatively low organic matter content (1.5%; Table 2.1), since biodiversity effects have reported to be stronger with less nutrients (no fertilization) (von Felten and Schmid, 2008, Craven *et al.*, 2016). The species richness effect became stronger in the second year, and remained similar in the third and fourth year (Fig. 6.1A). Strengthening of biodiversity effects over time can occur via increased performance of species-rich communities over time (Cedar Creek biodiversity experiment in Tilman *et al.*, 2014), a decrease in monoculture biomass over time (Marquard *et al.*, 2009, Van Ruijven and Berendse, 2009) or both progressive decreases in monocultures and increases in species-rich communities (van Ruijven and Berendse, 2005, Meyer *et al.*, 2016). In my experiment, the biodiversity-productivity relationship mainly strengthened through relative increases in biomass in mixtures.

6.2.2 Increase in complementarity effects over time

Positive complementarity effects were found in all years, and increased over time (Fig. 6.1B). Selection effects showed a less consistent pattern: both positive and negative effects were found and effects differed from year to year (Fig. 6.1B). These patterns support the previous observations that complementarity effects generally increase over time, while selection effects are more variable and less predictable over

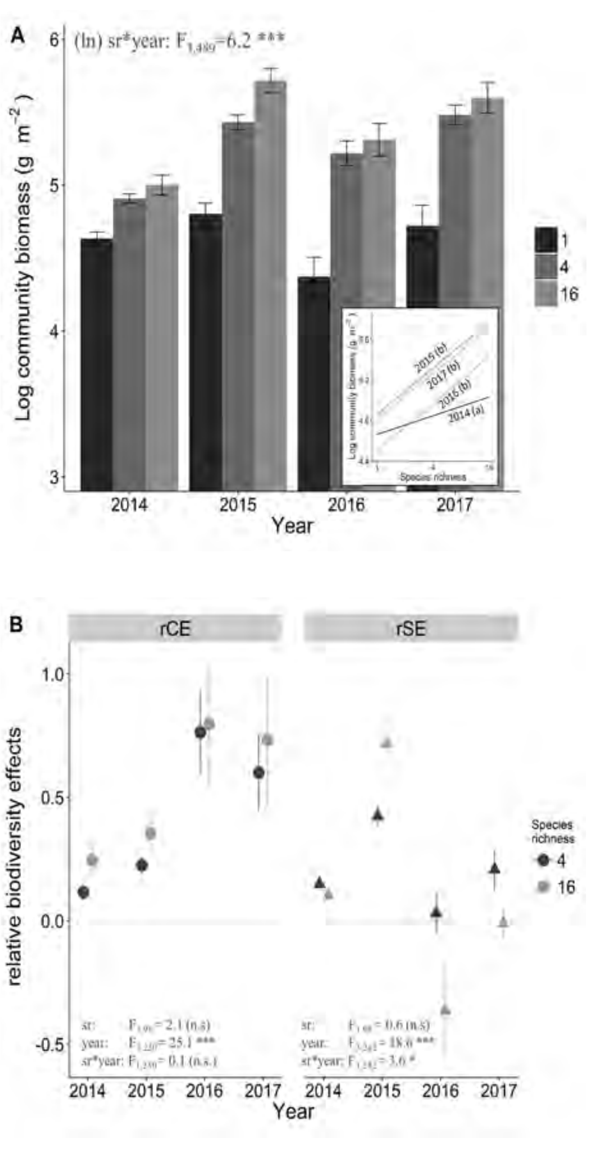


Figure 6.1. A) Community productivity (ln transformed) of monocultures, 4-species mixtures, and 16-species mixtures in four subsequent years (2014 – 2017) and B) Relative complementarity effects (rCE; circles) and relative selections effects (rSE; triangles) over time. The effect of species richness differed per year, with stronger effects in the second, third and fourth year compared to the first (see inset). Complementarity effects (relative) were positive in all years, and increased over time, independent of species richness. Selection effects (relative) were more variable over time and differed significantly between years.

Biomass data were analysed using a linear mixed effect model (LME) used with community biomass (ln transformed) as response variable, log species richness (numeric) and year (factor) as fixed factors and plot number nested in block as random factor. Species richness effects were subsequently tested per year and confidence intervals of the species richness estimates were compared. The temporal patterns of rCE and rSE were tested using a similar LME with species richness, year and their interaction as fixed factors. In 2017, only control plots are included (drought plots excluded). Extreme outliers with values of 20 or more time the interquartile range above the 3th quartile or below the first quartile were removed from analyses (three rCE and seven rSE values). Letters indicate significant different species richness effects, sr = species richness.

time (Loreau, 2000, Cardinale *et al.*, 2007, Fargione *et al.*, 2007, Van Ruijven and Berendse, 2009). The increase in complementarity effects over time may be the result of increasing positive plant-plant interactions. For example, deep rooting species may reach deeper soil layers in absolute terms, increasing the access to nutrients in the deeper soil layers. This increase in biotope (resource) space over time may have strengthened the complementarity effects (Dimitrakopoulos and Schmid, 2004).

An alternative hypothesis is that changes in plant-soil interactions over time, such as an increase in positive plant-associated microbes like arbuscular mycorrhizal fungi (AMF), have increased complementarity effects (Reynolds *et al.*, 2003, Van Der Heijden *et al.*, 2008, Wagg *et al.*, 2011). Further, variation in selection effects over time may be the result of changes in plant-plant interactions, such as species' competitive advantage, due to changes in environmental conditions.

6.3 Using root traits to explain biodiversity effects

6.3.1 Species specific root traits

Many experiments have provided evidence that grassland species have inherent differences in their rooting system: they differ in their vertical root distribution (called "rooting depth" hereafter) and many other root traits, such as root diameter, specific root length (SRL), root length density (RLD) or root tissue density (RTD) (Fort *et al.*, 2013, Iversen *et al.*, 2017). Similarly, in this thesis, species differed in SRL, root mass density (RMD), RTD, deep root fraction (DRF) and specific leaf area (SLA), in both a controlled pot experiment with one plant per pot (chapter 2) and in species monocultures in a common garden biodiversity experiment (chapter 3). Overall, grasses had thinner roots (higher SRL), more root length per soil volume (higher RLD) and shallower roots than forbs (lower DRF), but there were still significant differences within these two functional groups. These species root trait differences resulted in plant mixtures that clearly differed in their root trait values (community weighted means), and root trait diversity. Therefore, if these root trait differences reflect differences in resource uptake (Prieto *et al.*, 2015, Fort *et al.*, 2017), these grassland species are likely to show resource partitioning, potentially resulting in positive complementarity effects. Simultaneously, if the root traits determine species performance in monocultures and dominance in mixtures, these root traits are likely linked to selection effects.

6.3.2 Root traits and biodiversity effects

This thesis provided mixed results for the relevance of root traits to explain biodiversity effects. In the first year (chapter 2), we could not find a link between root trait diversity and complementarity effects when using traits of young plants from a separate pot experiment. Also in the second year (chapter 3), using traits from the two-year old monocultures, complementarity effects were not coupled to root trait diversity. However, chapter 3 did show that plant communities with deep, thick and dense roots (high DRF, low SRL and high RTD community weighted means (CWMs)) had highest selection effects. This suggests that species with deep, thick and dense roots performed well in monocultures ánd mixtures, and that root traits can be important predictors of the increased yield in mixtures. The importance of the deep rooting species was confirmed in chapter 4, in which I showed that individual plants of the deep rooting species increased in performance in mixture. In contrast, shallow-rooting species had a lower yield in mixtures than in monocultures. Increases in community biomass in mixtures were thus likely driven by the deep rooting species. Interestingly, in this investigation, I revealed that diversity in rooting depth was important: the deep-rooting species performed better in mixtures with shallow-rooting neighbours, while their performance was not increased when standing next to deep rooting neighbours. This suggests that both the actual rooting depth values and diversity in rooting depth within a community, via differences between neighbouring plants, are important for positive biodiversity effects, but that the importance of root diversity is not necessary visible at the plot level.

The fact that rooting depth is important for positive biodiversity effects is consistent with Mueller *et al.* (2013), who reported increased community productivity in communities with an increased proportion of root biomass in the deeper layers. In addition, Oram *et al.* (2018) found the largest relative complementarity effects in deep-rooting plant communities. They also found that selection effects were more negative in deep-rooting communities, in contrast to the more positive selection effects in deep-rooting communities in this thesis (chapter 3). The contrasting effect of deep-rooting on selection effects in Oram *et al*. (2018) and this thesis may indicate that, despite the advantage of deep rooting in mixture, deep rooting does not always lead to highest productivity in monoculture. In this thesis, the high productivity in monoculture of deep-rooting species may have been the result of the extreme dry period prior to harvest.

Further, the finding that root trait diversity could not explain complementarity effects at the plot level (chapter 2 and 3) is in agreement with Oram *et al.* (2018) and

Wagg *et al.* (2017a). This result supports the idea that when measuring at the plot level, root trait CWMs are better predictors of community biomass (Mokany *et al.*, 2008, Barkaoui *et al.*, 2016) and biodiversity effects (Roscher *et al.*, 2012) than root trait diversity.

6.3.3 Considerations regarding the use of functional diversity indices

The study of individuals (chapter 4) showed that differences in rooting depth between neighbouring plants can be important for overyielding in mixtures. However, this link between root trait diversity and overyielding was not found when using functional trait diversity (functional dispersion; FDis) at the community (i.e. plot) level (chapter 2 and 3). As explained in chapter 4, this discrepancy may be due to differences in resolution between the two approaches: both the spatial resolution and the number of data points. In addition, the fact that community (plot) level studies found no relationship between complementarity effects and functional diversity in rooting depth (chapter 2 and 3, Wagg *et al.*, 2017a, Oram *et al.*, 2018) may also be due to how functional diversity is calculated and related to positive biodiversity effects. Consider, for example, four-species communities consisting of a different numbers of deep and shallow-rooting species. According to several functional diversity indices, including "functional dispersion"(FDis) and "Rao's quadratic diversity" (RaoQ) (Mason *et al.*, 2013), which are used in the studies that linked root diversity to biodiversity effects (RaoQ in Roscher *et al.* (2012) and Wagg *et al.* (2017b); FDis in Oram *et al.* (2018) and this thesis), a community consisting of two shallow and two deep species is most diverse. Moreover, a community that contains one shallow species and three deep rooting species is as diverse as a community that contains one deep rooting species and three shallow rooting species (Table 6.1). The assumption is that overyielding increases with functional diversity. Hence, the community with two deep and two shallow species is expected to show greatest overyielding, and a community with three shallow species and one deep species would yield as much as a community with three deep and one shallow species. However, chapter 4 showed that species do not always respond similarly to root trait differences: deep rooting species showed greater overyielding with more shallow neighbours, while shallow rooting species did not respond to rooting depth of their neighbours at all. When species respond differently to trait differences, the highest yields may be found with different species ratios (e.g. one deep and three shallow species). This is probably the case when some species have larger positive effects on the growth of other species than the other way around. A modelling study of Vermeulen *et al.* (2016) indeed showed that complementarity effects can occur if the species interaction is beneficial for only one of the two competitors. Alternatively, more uneven communities with lower diversity values may also be most productive when the resources are not equally divided over the biotope space (soil layers). For example, when more resources are found in the upper soil layers, communities with three shallow and one deep species may be most efficient in nutrient uptake, resulting in highest productivity. As such, overyielding may not be linearly related the functional diversity indices used. As such, functional diversity in rooting depth is unlikely to capture overyielding. My results and these examples suggest that we need to look critically at the use of single indices like functional dispersion and the assumption that most diverse communities are the most productive (positive linear trait diversity-CE relationship). Experiments using different species (trait) ratios in combination with different resource distributions may give more insight on the use of diversity indices at the community level.

Table 6.1. Functional diversity calculated as Rao's quadratic diversity (RaoQ) and Functional Dispersion (FDis) of three hypothetical communities consisting of four species that are shallow-rooting (DRF: 0.10) or deep-rooting (DRF: 0.35). These two functional diversity indices are used to link root trait diversity to biodiversity effects (RaoQ in Roscher *et al.* (2012) and Wagg *et al.* (2017b), FDis in Oram *et al.* (2018) and this thesis). Diversity values are calculated using the "FD" package (Laliberté and Shipley, 2011) in R (version 3.3.1).

Community	**Species richness**	**Number of shallow species**	**Number of deep species**	**Functional diversity RaoQ**	**Functional diversity FDis**
1	4	1	3	0.63	0.68
2	4	3	1	0.63	0.68
3	4	2	2	0.83	0.91

6.3.4 Trait plasticity

In all chapters in this thesis, I used species *average* root trait values, obtained from plants that I grew in a separate pot experiment (chapter 2) and monocultures in the biodiversity experiment (chapter 3), to explain community responses. While I could not explain community responses in the field with traits measured in a pot experiment (chapter 2), root trait values from the monocultures were linked to selection effects (chapter 3) and performance of individual plants (chapter 4). This suggest that average species values measured in monocultures can be used to predict community responses, but that species values obtained in a pot experiment, or at least the ranking of the species values, may be different than traits (ranking) in the field (Mokany and Ash, 2008). Indeed, trait values for pot grown plants differed from those measured on plants in the field (SRL and RTD in Table 2.2 compared to Table 3.1), possibly due to root plasticity in response to environmental conditions (Zobel *et al.*, 2007, Leuschner *et al.*, 2013, de Vries *et al.*, 2016). However, when I used trait values of the pot grown plants (chapter 2) in a new analysis to predict selection effects in the second year, results were similar to those found in chapter 3 using monoculture traits: SRL, RTD, and rooting depth CWMs based on root trait values from the pot experiment and literature were significantly linked to changes in selection effects from the first to the second year (ΔrSE).

Further, by using average species traits, the important assumption is made that these obtained species values, or ranking, would be similar in species mixtures. Multiple studies have shown that this is not always the case; root traits can be plastic in response to neighbours (heterospecific) (Belter and Cahill, 2015, Abakumova *et al.*, 2016). For example, plant species growing in mixtures have shown to root shallower (Mommer *et al.*, 2010) or deeper than expected based on the monocultures (Mueller *et al.*, 2013, Hernandez and Picon-Cochard, 2016, Oram *et al.*, 2018). Still, the relationships between root trait values from the monocultures and selection effects (chapter 3) and between root trait values and overyielding of individual plants (chapter 4) suggest that average species values can be used to predict responses in mixtures. Therefore, I expect that root plasticity in response to species richness or environmental conditions was relative low in this study, with higher variation between species than variation within individuals of the same species (larger interspecific variation than intraspecific variation). Alternatively, changes to environmental conditions or neighbours were relative similar among species. Nevertheless, by including root plasticity, i.e. intraspecific variability, root traits may explain more variation in community responses. To understand the importance of the inclusion of intraspecific variability in trait approaches, we need to determine the size of intraspecific trait variability compared to interspecific variability, and include intraspecific variability in calculating community trait values when this is a large contributor to the total variation.

6.4 Resource partitioning as a mechanism underlying the positive complementarity effects

The increased yield of deep-rooting species with shallow-rooting neighbours (chapter 4) suggest that rooting depth differences contribute – at least partially – to the positive effects of plant species richness on plant productivity. Although this finding is consistent with the hypothesis of spatial resource partitioning, assuming that rooting depth differences reflect different spatial resource uptake strategies, it does not provide direct evidence that root trait differences act via resource partitioning. So far, several model studies (Northfield *et al.*, 2010, Postma and Lynch, 2012), evapotranspiration studies (De Boeck *et al.*, 2006, Verheyen *et al.*, 2008, Milcu *et al.*, 2016, Guderle *et al.*, 2018) and trait diversity studies (Barkaoui *et al.*, 2016, and Bu *et al.*, 2017 in forest stands) support resource partitioning, but it has been difficult to demonstrate the role of resource partitioning experimentally. A straightforward, but complex and labour intensive method to investigate resource partitioning is measuring resource uptake of species via isotope labelling: inserting labelled resources in different soil layers and measuring how much of these resources is used in the plant material (von Felten *et al.*, 2009, Hoekstra *et al.*, 2014, Bachmann *et al.*, 2015, Guderle *et al.*, 2018). So far, these tracer studies have also not found clear evidence that species uptake differences contributed to the increased resource uptake or yield in mixtures (Kahmen *et al.*, 2006, Schultz *et al.*, 2012, Bachmann *et al.*, 2015). However, note that these studies focussed on spatial or chemical partitioning to explain the positive biodiversity effects, while several studies suggest that temporal uptake differences may perhaps be more important for resource partitioning (Hooper, 1998, Mamolos and Veresoglou, 2000, Weigelt *et al.*, 2008). In line with differences in temporal resource acquisition, studies have reported that species can be plastic in their resource uptake, so that species can switch their "uptake behaviour", for example from shallow to deeper soil layers, without changing their physical root systems (Hamblin and Tennant, 1987, Garrigues *et al.*, 2006, Hoekstra *et al.*, 2014, Guderle *et al.*, 2018). In this case, rooting depth is just reflecting the potential for resource uptake (depth). Therefore, the link between rooting depth, other root traits and resource uptake, or root trait differences and resource partitioning, may depend on environmental conditions, such as soil moisture (Hoekstra *et al.*, 2014, Guderle *et al.*, 2018). Investigating which root traits are most important for resource uptake under different environmental conditions, and how trait plasticity and plasticity in uptake (independent of root morphology and architecture) can affect resource uptake, will be important next steps to find out whether the positive effect of rooting depth diversity acts via resource partitioning.

Alternatively, the positive effect of differences in root traits on community productivity could act via different mechanisms, such as plant-microbe interactions (Van Der Heijden *et al.*, 2008, de Kroon *et al.*, 2012, Eisenhauer, 2012, Hendriks *et al.*, 2013, Bardgett, 2017, Luo *et al.*, 2017). Root traits may determine the identity and quantity of root associated or soil organisms (Legay *et al.*, 2014), and hence diversity in root traits could increase the diversity of soil organisms, affecting many soil processes (Bardgett *et al.*, 2014). Increased soil diversity could positively affect productivity, since research has suggested that dilution of host-specific pathogens in plant mixtures (Maron *et al.*, 2011, Schnitzer *et al.*, 2011) or the increase of beneficial soil organisms (e.g. AMF) in plant mixtures (Scherber *et al.*, 2010, Walder *et al.*, 2012) is driving the positive biodiversity effects. Thus, diversity in root traits could play an important role in belowground 'abiotic' or 'biotic facilitation' (Wright, 2017), positively affecting productivity.

6.5 Plant diversity and drought resistance

6.5.1 Plant diversity and the negative impact of drought on productivity

In this thesis (chapter 5), plant diversity did not increase drought resistance: monocultures and mixtures had similar (proportional) aboveground biomass reductions (35%) after an experimental drought. These results support previous findings of several other biodiversity-drought experiments that also did not find a significant effect of plant diversity on drought resistance (using proportional changes in aboveground productivity; Pfisterer and Schmid, 2002, Caldeira *et al.*, 2005, Kahmen *et al.*, 2005, Van Ruijven and Berendse, 2010, Vogel *et al.*, 2012). On the contrary, these results are not consistent with the conclusion of a recent meta-analysis of Isbell *et al.* (2015), who evaluated the effect of species richness on resistance to natural weather extremes, including drought. Although they reported a "substantial variability in the effect of biodiversity on resistance among studies and among years within studies", overall community drought resistance increased with plant diversity. It is important to note that the meta-analysis of Isbell *et al.* (2015) used natural droughts (and wet events), which occurred three to 24 months before biomass sampling. In contrast, experimental studies excluding precipitation using rainout shelters and sampling biomass immediately after the experimental drought found no effect of plant species richness on drought resistance (chapter 5, Pfisterer and Schmid, 2002, Kahmen *et al.*, 2005, Vogel *et al.*, 2012). Although Isbell *et al.* (2015) could not find an effect of duration (event within 3, 6, 9, 15, 18, 21, or 24 months before harvest) on resistance, the discrepancy in results between Isbell *et al.* (2015) and experimental studies may have been caused by the time period between the drought and harvest: studies of Isbell *et al.* (2015) may have include a short period of recovery after drought, while the experimental studies did not. If recovery takes only a few months (Schwalm *et al.*, 2017) and species-rich communities recover more quickly after drought than monocultures (Van Ruijven and Berendse, 2010), the time period between the drought and harvest may explain the different findings. Alternatively, differences in drought intensity between experimental and natural studies could have led to different effects of plant diversity on drought resistance. Indeed, Isbell *et al.* (2015) showed a marginally significant interaction between the intensity of the event and biodiversity on resistance: resistance was higher during moderate climate events compared to extreme ones. Moreover, Isbell *et al.* (2015) defined a drought as occurring once in more than 10 years, whereas most experimental droughts, which typically exclude rain completely for 6-8 weeks prior to harvest, are more extreme. For example, the experimental drought I imposed in chapter 5 is expected to occur once in 60 years. Future research should investigate how the timing and intensity of droughts affects the biodiversity-drought interaction.

6.5.2 The impact of drought on biodiversity effects

Despite the decrease in biomass due to drought, the positive biodiversity-plant productivity relationship persisted, a result that has also been found in other biodiversity-drought studies (Pfisterer and Schmid, 2002, De Boeck *et al.*, 2008, Van Ruijven and Berendse, 2010, Isbell *et al.*, 2015, Craven *et al.*, 2016). In chapter 5, I found a greater absolute reduction in biomass in mixtures compared to monocultures and as a consequence, *absolute* net effects (in $g{\cdot}m^{-2}$) also decreased. However, mixtures also produced more biomass under control conditions, and the *relative decrease* in biomass due to drought was similar in monocultures and mixtures: 35%. Consequently, one would expect that the *relative* net effect also would not change under drought. However, in chapter 5 I showed a decrease in relative net effects under drought. This suggests that not all species responded similar to drought in monocultures and mixtures, and that shifts in relative performance in mixtures occurred.

The decrease in biodiversity net effects was the result of a decrease in relative complementarity effects (chapter 5) , while no significant changes in relative selection effects were found under drought. To my knowledge, only two biodiversity studies tested how climate events affected relative biodiversity effects. Caldeira *et al.* (2005) found a greater decline of absolute complementarity effects in higher diversity communities after an extreme dry winter with increased number of days with frost (consistent with a greater absolute decrease in biomass in diverse communities), but no effects on relative biodiversity effects. Similarly, Craven *et al.* (2016) found no effect of drought on relative net, complementarity and selection effects. The fact that these studies did not find an effect of drought on relative changes while I did, suggests that different mechanisms or processes were driving the positive biodiversity effects, perhaps due to different environmental conditions in the studies (Tylianakis *et al.*, 2008, Scheepens *et al.*, 2017). As mentioned before, drought intensity may have played a role in the different outcomes, if the role of different mechanisms – such as water partitioning, transpiration, protection via cover, or compensatory dynamics by drought resistant species – depend on environmental conditions. I will elaborate on this in the following paragraphs.

6.5.3 Mechanisms affecting complementarity effects

The decline in relative complementarity effects under drought (chapter 5) suggests that the positive interactions that can lead to positive complementarity effects, such as resource partitioning or facilitation (Loreau and Hector, 2001) are sensitive to drought, and did not contribute to drought resistance of plant mixtures. A similar effect was found in the second year of my experiment, in which a low average contribution of complementarity effect to the positive net effect was found after a drought of four weeks (chapter 3). Further, no role of rooting depth (deep root fraction; DRF) was found in explaining complementarity effects during drought (chapter 5). Together, this indicates that water partitioning via diversity in rooting depth did not significantly contribute to drought resistance. These results are in line with Hoekstra *et al.* (2014), who could not find a clear link between interspecific differences in water uptake depth and drought resistance, and Barkaoui *et al.* (2016), who found only a very marginal role of diversity in root traits on productivity under drought.

Instead, the decrease in relative complementarity effects and lower soil moisture in mixtures suggests that plant diversity may have decreased the positive interactions in mixtures via increased soil water depletion (Van Peer *et al.*, 2004). Increased water use and transpiration in mixtures (Verheyen *et al.*, 2008, Milcu *et al.*, 2016, Guderle *et al.*, 2018), possibly the result of water partitioning and/or increased biomass in mixtures (Van Peer *et al.*, 2004), reduces available soil moisture and can thus result in earlier or more severe drought (Verheyen *et al.*, 2008). When I measured soil moisture at the end of the drought treatment, the lower soil moisture in mixtures was primarily seen in the control plots, since both monocultures and mixtures were very dry in the drought plots, probably almost reaching minimum soil water content (chapter 5). However, soil moisture data that I used for monitoring soil moisture in the upper 15 cm (using a TDR probe) showed that mixtures did indeed have lower soil moisture during the drought than monocultures, and thus experienced earlier and more extreme drought (Fig. 6.2). This earlier decrease in soil moisture in mixtures could have resulted in increased competition for water in mixtures, decreasing the complementarity effect.

Besides the negative effect of increased biomass (leaf area) in mixtures on drought stress (and complementarity effects) via increased soil water loss through

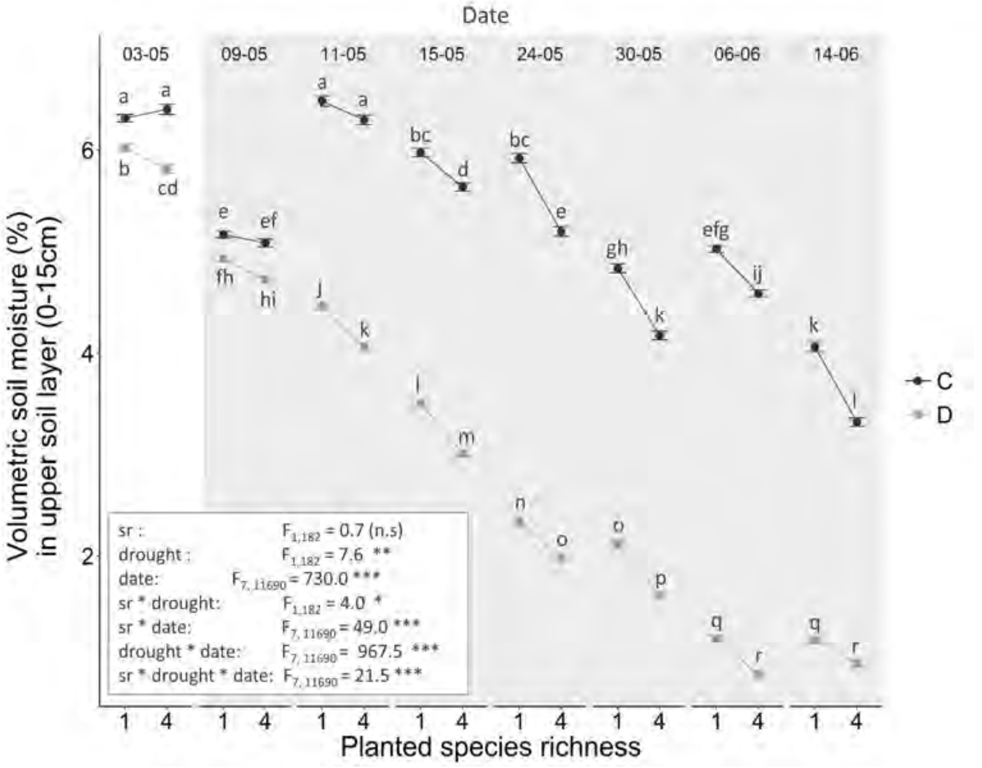


Figure 6.2. Soil moisture (volumetric) over time in the monocultures and 4-species mixtures in control (C; dark grey dots) and drought plots (D; light grey dots) during the experimental drought in the fourth year of the biodiversity experiment (2017; grey background). Soil moisture was measured once in each plot in the upper soil layer (0-15 cm) using a TDR probe (TRIME-PICO64; HD2 meter). The effects of species richness, drought treatment and date (factor) and their interactions on soil moisture were tested with a linear mixed effect model with plot number nested in block as random variable to account for repeated measures in time (using restricted maximum likelihood) and an anova type III ("nlme" package (Pinheiro et al., 2016) in R version 3.3.1). Multicomparisons (Tukey) were made among treatments ("lsmeans" package (Lenth, 2016)). Means ± standard errors are shown. sr = species richness, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$. Letters show significant differences among the treatments ($P < 0.05$).

transpiration, the increases in biomass in mixtures may also positively affect the drought stress by affecting the microclimate conditions. Similar to previous studies (Tilman *et al.*, 1996, Hector *et al.*, 1999, Spehn *et al.*, 2000), community plant cover was increased in mixtures compared to monocultures (chapter 5). Although increases in cover were related to decreases in soil moisture, community temperature was lower in plots with high cover, indicating decreased drought stress via temperature. In the low covered plots, high temperatures (up to 50 °C was reached on average) can decrease productivity further by increasing vapour pressure deficits and evapotranspiration, leading to earlier stomatal closure (Arnone *et al.*, 2008) and reduced growth. Indeed, chapter 5 showed that the reduction in complementarity effects due to drought were largest in communities with low cover. Only mixtures with a cover higher than approximately 65 % could maintain complementarity effects at a level similar to that of control mixtures (see Fig. 5.4). Increased cover may have ameliorated the micro-climate conditions by decreasing water loss via soil evaporation, and by increasing relative humidity in the canopy, decreasing vapour pressure deficits (Wright *et al.*, 2014). It has recently been hypothesized that this is an important facilitative mechanism underlying the positive biodiversity-productivity relationship (Wright *et al.*, 2014, Wright *et al.*, 2017). Although I did not measure evapotranspiration over time, vapour pressure deficits or relative humidity in the communities, the results in this thesis (increased temperature and decreased complementarity effects in low cover plots) support the hypothesis that increased plant cover in mixtures plays an important role in abiotic facilitation via the amelioration of micro-climate conditions.

6.5.4 Contrasting effects of biomass and cover during drought

Since complementarity effects decreased on average with drought, negative species interactions in mixtures, such as increased competition due to increased soil drying, may have decreased the positive interactions in mixtures. I hypothesize that the balance between these positive and negative effects of plant diversity depends on the duration or intensity of the drought via two important processes that influence drought stress: 1) increased water uptake and transpiration in mixtures via water partitioning and increased biomass, and 2) decreased soil evaporation and temperature in mixtures via increased cover. Note that while soil water loss via transpiration will mostly depend on the amount of leaf area (biomass) and plants stomatal behaviour, soil evaporation cannot be directly controlled by plants, and depends largely on the heating of soil. However, plants control evaporation indirectly by covering the soil. During a drought, plant transpiration is likely to decrease due to soil water shortage, whereby soil evaporation becomes increasingly important in increasing drought stress. Especially at this point, community soil cover may play a significant role in reducing negative drought effects.

As an example, I illustrated in Figure 6.3 how these two processes and the duration or intensity of a drought could affect the drought resistance of monocultures vs mixtures. In the beginning of a drought (moderate drought) increased water uptake in mixtures (Verheyen *et al.*, 2008, Milcu *et al.*, 2016, Guderle *et al.*, 2018), possibly via water partitioning (Van Peer *et al.*, 2004), may lead to increased performance compared to monocultures. However, when the drought continues, increased water uptake and transpiration in mixtures can decrease soil water availability (De Boeck *et al.*, 2006, Mokany *et al.*, 2008, Leimer *et al.*, 2014; Fig. 6.2). As a consequence, mixtures will reach critical soil moisture values earlier, favouring slower drying monocultures. This may be the reason why Verheyen *et al.* (2008) detected that the pattern of increased evapotranspiration in mixtures compared to monocultures in control conditions was reversed during drought. Also Leimer *et al.* (2014) reported that species richness effects on the water balance were less pronounced during periods with low moisture. Then, if the drought continues, soil water availability will continue to decrease, also in monocultures (see Fig. 6.2) and facilitation via cover may become increasingly important. Mixtures, which have highest cover, will have an advantage again by reducing soil evaporation and temperature. Thus, the net effect of drought on complementarity effects can depend on the length of the drought and strength of the opposing interactions. The 'switches in time' (advantage for monocultures or mixtures, see Fig 6.3), which may also depend on environmental conditions such as soil type (water holding capacity; Rawls *et al.*, 1982, Hudson, 1994), make it difficult to detect an overall effect of drought on biodiversity effects in studies that differ in duration, intensity and timing of the drought, and often also in other environmental characteristics. It is therefore important to further investigate which processes are the main drivers of complementarity effects under different environmental (moisture) conditions. This can for example be done by investigating the role of cover and water partitioning for complementarity effects along a moisture gradient on different soil types. To be able to compare the stress intensities among studies, it is necessary to include microclimatic conditions such as temperature, air humidity and soil water status, and not only focus on precipitation shortages to define drought intensity.

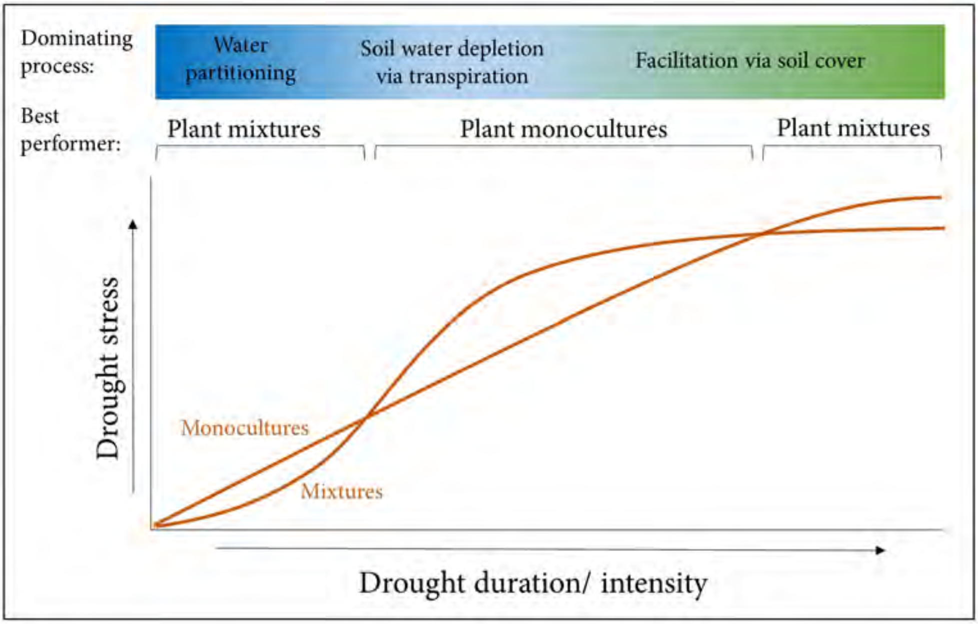


Figure 6.3. Conceptual diagram showing complementarity effects in mixtures can be influenced by multiple processes, the importance of which could be affected by the length or intensity of the drought. Whether plant mixtures or monocultures have the best performance at the end of the drought may thus depend on the length of the drought and relative importance of the processes during the drought.

6.5.5 Drought resistance via the insurance hypothesis

I expected that species mixtures would have increased drought resistance via the insurance hypothesis (Yachi and Loreau, 1999). Following this theory, diverse communities have a higher change of containing species that can maintain biomass production during drought and can compensate for the reduced growth of species that are less drought tolerant, increasing drought resistance of the community. If this drought tolerant species also maintains high biomass in monoculture, its compensatory performance in mixtures should be reflected in increased (positive) selection effects under drought. This may explain the relatively large increase in selection effects from the first to the second, dry year (chapter 3; see also Fig. 6.1B). However, no significant changes in selection effects were found in after the experimental drought in the fourth year (chapter 5), in line with findings of Craven *et al.* (2016) and De Boeck *et al.* (2008). This means that it were not necessarily productive species that gained dominance in mixtures under drought. Moreover, analyses at the species level showed that no species significantly increased their dominance under drought, despite the fact that some species seemed more affected by drought than others (chapter 5).

Together, these findings suggest that productivity was not maintained under drought as a result of increased productivity of more drought tolerant species. An important explanation for the fact that we could not find support for the insurance hypothesis, may again be related to the intensity of the drought. If the drought was extreme very rapidly, all species may quickly have stopped growing, preventing the more drought resistant species from increasing in dominance in mixtures. Perhaps the insurance hypothesis plays a bigger role after the drought, when compensatory dynamics can occur during community recovery (Van Ruijven and Berendse, 2010). Aboveground effects may have also been delayed, for example when drought first increases belowground productivity under drought (Kahmen *et al.*, 2005), so that compensatory effects on the aboveground productivity will especially been seen after drought by affecting the recovery and resilience. Investigating the survival of individuals and species along a species richness gradient, and how species richness affects recovery could give additional insights on the longer-term impact of drought on grassland productivity via the insurance hypothesis.

6.6 Concluding remarks and future directions

6.6.1 Explaining positive biodiversity effects using root traits

This thesis was one of the first to link diversity in root traits to biodiversity effects in an attempt to elucidate the mechanisms underlying positive biodiversity effects. Chapter 3 and 4 showed that community rooting depth and (correlated) morphological traits such as RTD, SRL and RLD are indeed useful traits to predict biodiversity effects. At the community level, root traits were related to selection effects via community weighted means, suggesting that belowground plant-plant interactions play an important role in biodiversity-ecosystem functioning relationships. However, in contrast to my hypotheses, the predictive power of interspecific variation in root traits for complementarity effects was limited: no relationships between root trait diversity and complementarity effects were found.

Yet, my thesis provides two ways in which the approach of linking root trait variation and complementarity effects can be improved. First, in chapter 4 I showed that at the species level, differences in rooting depth between neighbouring plants were important for increased performance in mixtures, and thus likely also for increased biomass of mixtures. These contrasting results indicate that it matters at which spatial resolution the measurements are done to explain the importance of trait differences in species interactions. The use of individuals has increased our resolution of plant-plant interactions, first by using the local neighbourhood, i.e. traits of directly surrounding plants, instead of a larger community, and second by including the variation in growth of individuals of the same species within a community. This indicates that measurement on a smaller scale – on the species or individual level – may give more insight in the effects of trait interactions on plant performance than measurements at the community level. Hence, biodiversity research could benefit from including the local neighbourhood interactions to elucidate the mechanisms.

Second, in this thesis I also showed that changes in the environmental conditions can affect the relative importance of particular biological mechanisms for biodiversity effects: drought decreased complementarity effects, resulting in an increased relative contribution of selection effects to net effects. This suggests that the relationships between traits and biodiversity effects also depend on abiotic conditions. For example, if nitrogen is the main limiting resource, variation in traits related to light capture may be less relevant for predicting community performance. Instead, high values of a single trait (e.g. the ability to fix nitrogen) may predict community performance much better. In contrast, in conditions were multiple resources are limiting, several different resource uptake strategies may contribute to community performance. This would probably make diversity in traits related to these resource uptake strategies a better predictor of community performance. As a first step, therefore, it is important to determine which traits are most important for resource uptake and growth under the given environmental conditions. As a second step we can test whether diversity in those traits contributes to positive complementarity effects under those environmental conditions, in experiments in which both biodiversity and abiotic conditions are manipulated. By choosing the "right" traits in specific conditions, relationships between trait differences and the effects of species interactions in communities (such as increased yield) may be improved.

6.6.2 Biodiversity and drought

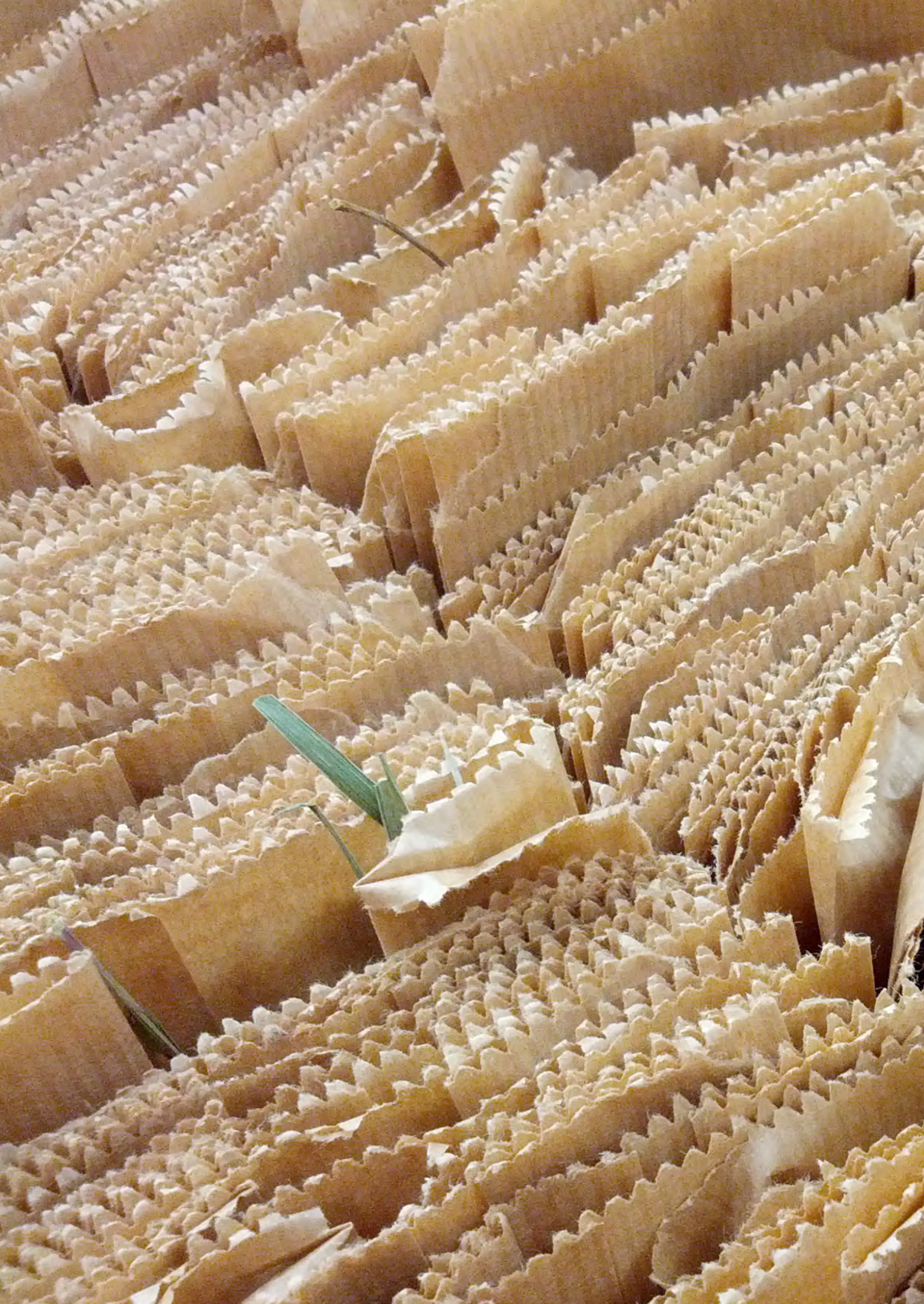
The last part of the thesis demonstrates that species richness does not necessarily increase drought resistance. A prolonged drought reduced biomass independent of species richness, but did reduce complementarity effects. This reduction in complementarity effects was mitigated in communities with high plant cover. Increased transpiration and plant cover in mixtures may thus play a large role in the drought resistance of plant mixtures versus monocultures, and need further investigation. Moreover, the relative importance of the mechanisms that are involved in the positive biodiversity effects and the biodiversity-drought interaction, such as cover and increased transpiration, may depend on the drought intensity and duration. Combined, experiments in which leaf area (transpiration) and plant cover (soil evaporation) are independently manipulated and exposed to different drought intensities can give useful insights on this issue.

6.6.3 Conclusions

Altogether, the results of this thesis indicate that rooting depth and some related morphological and architectural root traits are relevant to explain the positive biodiversity effects in grassland mixtures. Trait averages (CWMs) predicted biomass responses of mixtures (via selection effects), but trait diversity (FDis) could not explain complementarity effects at the plot level. However, when measuring at the scale of the individual rather than at the plot level, root trait diversity appeared to be important, since deep-rooting species increased biomass when growing with shallow-rooting neighbours.

Drought resistance was not increased with species richness, and I neither found evidence for an important role of deep-rooting species, nor of diversity in root traits in increasing drought resistance. Complementarity effects decreased under drought. This reduction was mitigated in diverse communities with high plant cover, suggesting that plant cover plays an important role in drought resistance and potential positive effects of plant species richness during drought events.

The next step is to examine the role of functional traits for complementarity and selection effects over different resource gradients to further elucidate the mechanisms underlying positive biodiversity effects, and the interaction between diversity and drought. This would allow a better understanding of the importance of diversity for ecosystem productivity and predicting the impacts of more frequently occurring droughts on the functioning of our natural grasslands.



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Summary

Biodiversity-ecosystem functioning studies typically show that plant species richness enhances community biomass. In addition, species richness has been shown to mitigate the negative impact of prolonged drought on community productivity. The biological mechanisms that drive these biodiversity effects are, however, still debated. One of the main hypotheses is that resource partitioning is responsible for the positive biodiversity effect on community productivity: plant species differ in resource uptake strategy, which results in a more complete exploitation of the available resources in space and time when plant species are growing together. For example, a community consisting of deep and shallow-rooting species is expected to acquire a larger part of the available soil resources than species monocultures or mixed communities containing only shallow or only deep-rooting species. This mechanism may especially play a role when resources such as water are scarce, for example during a drought. However, empirical studies that have investigated belowground resource partitioning are scarce and the results are mixed.

In the last two decades, two important approaches have been developed to better understand which mechanisms underlie the increased yield in species mixtures, or 'overyielding'. First, Loreau and Hector (2001) developed the additive partitioning method. This method allows discriminating between the effect of increased dominance of productive species (selection effects, SE) and effects due to positive interactions such as resource partitioning or facilitation, commonly referred to as complementarity effects (CE). Meta-analysis has shown that in general, both CE and SE contribute to positive biodiversity effects on ecosystem functions. Second, biodiversity research started to use species' inherent characteristics or "traits" to understand the role of community composition and "functional diversity" instead of species richness per se for community productivity. Functional traits of a community consist of two main components: 1) the community weighted mean trait value (CWM) and 2) the variation in trait values within the community. Most biodiversity studies found that both trait CWMs and trait diversity could explain variation in biomass or drought resilience, but found a larger importance of CWMs. Although traits have been successfully coupled to productivity in biodiversity experiments, traits have rarely been linked directly to metrics like complementarity and selection

effects, which specifically quantify the contribution of different species interactions. For example, since the selection effect reflects the dominance of certain species, and dominant species greatly influence the average trait values of the community (CWMs), I expect the CWM of traits related to competitive performance to be linked to selection effects. In contrast, complementarity effects are due to positive interactions between species, for example due to resource partitioning between species that differ in resource acquisition. Therefore, I expect complementarity effects will be linked to variation in traits (i.e. trait diversity) rather than trait means. As the root system is responsible for the uptake of nutrients and water, both of which are limiting in my experiment, I hypothesized that (diversity in) root traits are particularly important for biodiversity effects.

In this thesis, I further investigated the mechanisms underlying positive biodiversity effects in two research lines. In the first research line, I aimed to get more insight into the role of resource partitioning in overyielding by combining the additive partitioning method with a functional trait approach: can we explain positive biodiversity effects in grassland mixtures using (diversity in) root traits? I examined this question using pot and field traits, and on the community and species level to get a comprehensive understanding. In the second research line, I focussed on the interaction between plant species richness and drought to investigate how drought affects the biodiversity effects, and which potential mechanisms are involved. To address these questions, a new biodiversity experiment was established in which 16 grassland species were grown in monocultures, 4-species and 16-species mixtures. I used aboveground biomass to calculate the contributions of complementarity and selection effects to biomass production in mixtures.

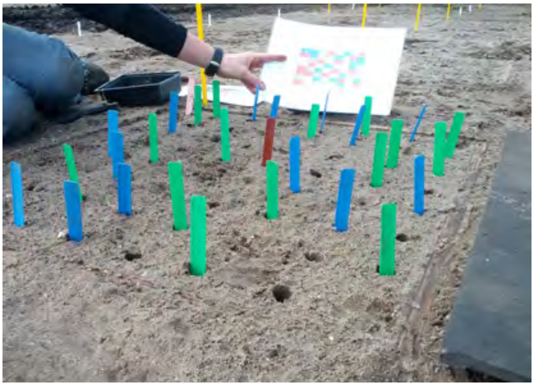
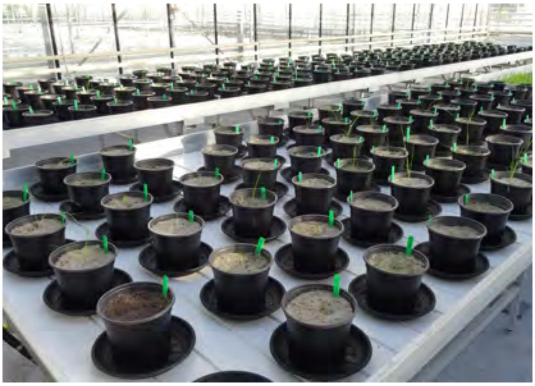
In the first year of the biodiversity experiment, plant mixtures showed an increase in biomass compared to monocultures and positive biodiversity effects. However, diversity in traits that were obtained from a separate pot experiment and literature (specific leaf area (SLA), specific root length (SRL), root mass density (RMD), root tissue density (RTD), and maximum rooting depth (RD)) could not explain variation in complementarity effects. Instead, complementarity effects were strongly affected by the presence and competitive interactions of two particular species. Interestingly, both species strongly increased in performance in mixtures, but their effects on community overyielding were contrasting: complementarity was strongly increased by the presence of *A. millefolium* but decreased by the presence of *L. vulgare*. The large variation in complementarity effects and significant effect of two species in the first year shows that, similar to most other biodiversity studies, not only species richness but also species composition is very important for positive biodiversity effects.

In the second year of the experiment, community biomass and complementarity and selection effects increased compared to the first year. The increase in biomass was stronger in mixtures than in monoculture and the contribution of selection effects to this positive effect was greater than that of complementarity effects. The increased contribution of (relative) selection effects was associated with root traits measured in monocultures (specific root length (SRL), root length density (RLD), root tissue density (RTD) and the deep root fraction (DRF)): selection effects increased most in communities with high abundance of species with deep, thick and dense roots. These traits were better predictors of selection effects than the presence of species. In contrast, changes in complementarity were not related to trait diversity (functional dispersion) or CWM trait values.

Most biodiversity studies that investigated whether species trait differences lead to overyielding focused on the plot scale (e.g. one to several m^2). By doing so, species responses are averaged across a range of plant individuals and local species neighbourhoods, possibly concealing effects of species differences on species performance and overyielding. Therefore, I measured the aboveground biomass of almost 1700 plants of 16 species in the first three growing seasons in the biodiversity experiment to examine the importance of species' differences for overyielding at a more local scale than a whole community. I focused on the performance of individual plants in response to the rooting depth of their direct neighbours. The results showed that species rooting depth (measured in monocultures in the second year) was important for performance in mixtures: individual performance of deep-rooting species increased in mixtures, whereas that of shallow-rooting species did not. More importantly, the increased performance of deep-rooting species depended on neighbour rooting depth: deep-rooting species performed best with many shallow-rooting neighbours. Shallow-rooting species showed no response to neighbour rooting depth. By looking at the level of individual plants, I was able to show that variation in root traits (i.e. rooting depth) is important for positive biodiversity effects.

The communities in the biodiversity experiment were exposed to an experimental drought to investigate the effect of plant species richness on drought resistance, i.e. the ability to maintain biomass productivity during a drought. All plots were covered with rainout shelters for several weeks to simulate a prolonged drought. During this period, half of the plots were watered, while the other half with similar species compositions did not receive any water. Drought reduced aboveground biomass by 35%, irrespective of plant species richness. However, drought led to a significant loss of complementarity effects in mixtures, which suggests increased drought stress in mixtures compared to monocultures. We found no evidence that increased dominance of productive drought-resistant species (following the insurance hypothesis), or diversity in root traits played an important role in the drought response of plant mixtures. However, our analyses revealed that this response was mitigated by community cover at the start of the drought: the decrease in complementarity effects was strongest in mixtures with low cover, and disappeared in mixtures with high cover. These results suggest that community cover plays an important role in drought resistance and potential positive effects of plant species richness during drought events. Further, mixed results among biodiversity-drought studies suggest that the duration and intensity of a drought may strongly affect how diversity affects drought resistance. Future research specifically aimed at disentangling the contributions of cover, evapotranspiration and species composition along a drought gradient may further increase our insights into the effect of biodiversity and drought resistance.

Overall, I found increased yield in mixtures compared to monocultures in all years. Large variation in complementarity effects and selection effects among mixtures indicates that species composition is an important determinant of biodiversity effects. Further, species showed significant differences in root traits, possibly reflecting different resource uptake strategies. These root traits could explain community (selection effects) and individual performance, showing that rooting depth and related root traits such as RTD, SRL and RLD are important traits that can predict species responses in mixtures and capture the effect of species composition on biodiversity effects. While complementarity effects could not be explained by functional trait diversity on the community level, the importance of diversity in root traits was shown through measurements on a more local scale, using individual plants. The increased performance of deep-rooting species with shallow-rooting neighbours suggest that spatial resource partitioning via rooting depth contributes - at least partially - to the positive effects of plant species richness on plant productivity. During a drought, I found no evidence for an important role of deep-rooting species and diversity in root traits in increasing drought resistance (i.e. maintaining productivity under drought). I propose that we should strengthen the link between variation in root traits such as rooting depth and key processes that determine plant productivity, such as resource uptake. This may involve incorporating other (root) traits, but it also requires critically rethinking the hypothesis that ecosystem functioning would show a linear increase with trait diversity. The next step is to focus on establishing the relationships between trait differences between species and performance in local neighbourhoods across environmental gradients, to elucidate the mechanisms underlying positive biodiversity effects, and the interactive effects of plant species richness and drought on grassland productivity. This allows us to better understand the importance of diversity for ecosystem productivity and to predict the impacts of more frequently occurring droughts on the functioning of our grasslands.





Dutch summary

Samenvatting

Verschillende biodiversiteitstudies van over de hele wereld hebben aangetoond dat de biomassaproductie van een plantengemeenschap toeneemt met het aantal verschillende plantensoorten. Ook heeft onderzoek aangetoond dat het aantal plantensoorten het negatieve effect van een langdurige droogte op plantengroei kan beperken. We weten echter nog niet door wat voor biologische mechanismen deze positieve effecten van hogere plantensoorten rijkdom worden veroorzaakt.

Een belangrijke theorie is dat het positieve effect van biodiversiteit op biomassaproductie (ook wel overyielding genoemd) wordt veroorzaakt door het onderling verdelen van voedingsstoffen ('resource partitioning'): plantensoorten verschillen in hun strategieën om voedingsstoffen op te nemen, zodat er in totaal meer voedingstoffen opgenomen kunnen worden als verschillende plantensoorten naast elkaar groeien. Zo zou een plantengemeenschap met zowel diep- als ondiepwortelende soorten een groter deel van de beschikbare voedingsstoffen op kunnen nemen dan een plantengemeenschap met slechts één van beide soorten (alleen diep- of ondiepwortelend). Dit mechanisme zou vooral belangrijk kunnen zijn als de voedingsstoffen, zoals water en nutriënten, schaars zijn, bijvoorbeeld tijdens een droogte. Er is tot nu toe echter weinig bewijs gevonden voor het belang van de ondergrondse verdeling van voedingsstoffen voor de positieve biodiversiteiteffecten. Een andere theorie, de insurence hypothesis genoemd, zegt dat soortenrijke plantengemeenschappen de biomassaproductie over de tijd en bijvoorbeeld tijdens een droogte beter op peil kunnen houden dan soortenarme gemeenschappen vanwege de verhoogde kans dat de gemeenschap een soort bevat die goed groeit in die (droge) milieuomstandigheden.

In de afgelopen twee decennia zijn er twee belangrijke benaderingen ontwikkeld om de mechanismen achter de positieve biodiversiteitseffecten op biomassa beter te kunnen begrijpen. Ten eerste ontwikkelden Loreau en Hector (2001) de additive partitioning method. Met deze methode wordt er onderscheid gemaakt in een biomassatoename in plantenmengsels door positieve interacties tussen soorten, zoals bijvoorbeeld voedingstoffenverdeling (dit wordt het complementariteitseffect genoemd; CE), en een biomassatoename door een toename van dominante productieve soorten (dit wordt het selectie-effect genoemd; SE). Een meta-analyse van Cardinale et al. (2007) heeft aangetoond dat beide effecten, SE en CE, bijdragen aan de positieve effecten van biodiversiteit. Met deze methode kon dus worden aangetoond dat de hogere productie in mengsels niet alleen wordt veroorzaakt door de verhoogde kans op productieve soorten, maar dat er positieve biotische interacties spelen. Ten tweede is biodiversiteitonderzoek gebruik gaan maken van inherente kenmerken of eigenschappen (traits) van plantensoorten om het belang te begrijpen van soortensamenstelling en functionele diversiteit in plaats van het aantal soorten voor de productiviteit van de plantengemeenschap. Een planteigenschap is bijvoorbeeld de dikte van de bladeren of de maximale bewortelingsdiepte. De functionele planteigenschappen van een plantengemeenschap bestaan uit twee componenten: 1) het gewogen gemiddelde van een eigenschap (trait community weighted mean) en 2) de variatie in een eigenschap binnen de gemeenschap (eigenschap diversiteit; trait diversity). Biodiversiteitonderzoek heeft aangetoond dat én gemiddelden én diversiteit van functionele planteigenschappen biomassaproductie en droogtebestendigheid van plantengemeenschappen kunnen verklaren. De gemiddelden verklaarden de meeste variatie in productie. Alhoewel planteigenschappen succesvol zijn gekoppeld aan biomassaproductie, weten we nog niet welke combinaties van eigenschappen leiden tot de positieve effecten van soortenrijkdom op biomassa. Om hier achter te komen kunnen de eigenschappen worden gekoppeld aan complementariteit- en selectie-effecten. Er is echter nog weinig onderzoek dat (diversiteit in) eigenschappen aan complementariteit- en selectie-effecten heeft gekoppeld, terwijl deze effecten de bijdrage van verschillende mechanismen van de positieve interactie tussen soortenrijkdom en biomassaproductie kwantificeren. Complementariteiteffecten worden veroorzaakt door positieve interacties tussen soorten, bijvoorbeeld door voedselverdeling tussen soorten met verschillende opname strategieën. Er kan daarom verwacht worden dat complementariteiteffecten gerelateerd zijn aan de diversiteit in eigenschappen. Met name diversiteit in worteleigenschappen zou belangrijk kunnen zijn voor biodiversiteiteffecten, gezien de belangrijke rol van het wortelstelsel voor de opname van water en nutriënten. Daarentegen reflecteert het selectie-effect de dominantie van bepaalde soorten. Aangezien dominante soorten voor een groot deel de gemiddelde eigenschapwaarde van een plantengemeenschap bepalen, wordt verwacht dat gewogen gemiddelden van planteigenschappen (gerelateerd aan concurrentievermogen) zijn gekoppeld aan selectie-effecten.

In dit proefschrift heb ik in twee onderzoekslijnen nader onderzoek gedaan naar de mechanismen die de positieve biodiversiteiteffecten veroorzaken. In de eerste onderzoekslijn probeer ik meer inzicht te krijgen in de rol van voedselverdeling bij de biomassatoename in soortenmengsels door de additive partitioning method te combineren met de benadering via functionele plant eigenschappen. De vraag was of ik positieve biodiversiteitseffecten kan verklaren met (diversiteit in) worteleigenschappen. Om deze vraag te beantwoorden heb ik een nieuw biodiversiteitexperiment opgezet, waarin zestien graslandsoorten zijn geplant in monoculturen (één soort), 4-soortenmengsels en 16-soortenmengels (plots van 70 x 70 cm). De bovengrondse biomassaproductie is gebruikt om de bijdrage van selectie-effecten en complementariteiteffecten aan de biomassatoename in mengsels uit te rekenen. De worteleigenschappen heb ik gemeten in een potexperiment in kassen, maar ook buiten in de monoculturen van het biodiversiteitsexperiment. Ik heb bovendien op verschillende schalen gekeken naar relaties tussen overyielding en worteleigenschappen: op plotniveau en op soortsniveau met individuele planten. In de tweede onderzoekslijn heb ik de interactie tussen plantdiversiteit en droogte bestudeerd door te onderzoeken hoe droogte de biodiversiteiteffecten (SE en CE) beïnvloedt en welke mogelijke biologische mechanismen hierin een rol spelen.

In het eerste jaar van het biodiversiteitexperiment vertoonden de 4- en 16-soortenmengsels een toename in biomassa vergeleken met de monoculturen, en positieve selectie- en complementariteiteffecten (hoofdstuk 2). De complementariteiteffecten konden echter niet worden verklaard met diversiteit in planteigenschappen (specifieke bladoppervlakte (SLA), specifieke wortellengte (SRL), bewortelingsdichtheid (RMD) en de weefseldichtheid van de wortel (RTD); gemeten in het een potexperiment). In plaats daarvan waren complementariteiteffecten gerelateerd aan de aanwezigheid van twee bepaalde plantensoorten, namelijk *L. vulgare* (margriet) en *A. millefolium* (duizendblad). Interessant is dat beide soorten beter in mengsels groeiden dan in monoculturen, maar dat het effect van de soorten tegenovergesteld was: complementariteiteffecten van de plantengemeengschappen waren verhoogd bij de aanwezigheid van duizendblad en verlaagd bij de aanwezigheid van margriet. De grote variatie in complementariteiteffecten en de significante effecten van de twee soorten in het eerste jaar laten zien dat, zoals in de meeste biodiversiteitstudies, niet alleen het aantal soorten van belang is voor positieve biodiversiteitseffecten, maar ook de soortensamenstelling zelf.

In het tweede jaar van het biodiversiteitsexperiment was er een toename van biomassa en biodiversiteitseffecten vergeleken met het eerste jaar (hoofdstuk 3). In de soortenmengsels was er een gemiddeld grotere toename in selectie-effecten dan complementariteiteffecten. De toename in selectie-effecten was gekoppeld aan worteleigenschappen (SRL, wortellengte dichtheid (RLD), RTD, en diep wortelfractie (DRF)) gemeten in de monoculturen: selectie-effecten waren het grootst in mengsels waar soorten met diepe, dikke en dichte (compacte) wortels veel voorkomen. Deze eigenschappen konden selectie-effecten beter voorspellen dan de aanwezigheid van specifieke soorten. Daarentegen konden complementariteiteffecten niet verklaard worden met de diversiteit of gemiddelde waarden in deze worteleigenschappen. Zoals in het eerste jaar konden complementariteiteffecten beter voorspeld worden met de aanwezigheid van drie specifieke soorten (andere soorten dan in jaar 1) dan met de eigenschappen van de soorten.

De meeste biodiversiteitstudies hebben op plotschaal (bijvoorbeeld één tot een aantal m^2) gekeken of verschillen in planteigenschappen tot overyielding leiden. Door op deze schaal te kijken worden de verschillende responsen en effecten van soorten en meerdere individuele planten gemiddeld. Het middelen van de groei van individuen en soorten kan echter de effecten van verschillen in planteigenschappen op de groei verbergen. Daarom heb ik in de eerste drie groeiseizoenen van het biodiversiteitexperiment de bovengrondse biomassa gemeten van bijna 1700 individuele planten, van zestien verschillende soorten in monoculturen en mengsels, om te onderzoeken of verschillen in eigenschappen belangrijk zijn voor overyielding op een kleinere schaal dan de hele plot (gemeenschap). De soortspecifieke bewortelingsdiepte (gemeten in monoculturen in het tweede jaar) bleek een goede voorspeller voor de groei van individuele planten in mengsels: individuen van diep-wortelende soorten deden het beter in mengsels dan monoculturen. Daarentegen deden individuen van ondiep-wortelende soorten het beter in monoculturen. Het meest interessant was dat de groei van de diepwortelende soorten afhing van de bewortelingsdiepte van hun buurplanten: diepwortelende soorten waren het productiefst wanneer ze omringd werden door ondiepe buren. De ondiep-wortelende soorten reageerden echter niet op de bewortelingsdiepte van de buren. Door op dit niveau van individuele planten en hun directe buren te kijken i.p.v. alle planten en soorten van de gemeenschap samen heb ik aan kunnen tonen dat variatie in worteleigenschappen (bewortelingsdiepte) wel degelijk belangrijk kan zijn voor verhoogde groei in mengsels en dus voor positieve biodiversiteitseffecten.

Om te onderzoeken of plantdiversiteit de droogtebestendigheid van een gemeenschap beïnvloedt zijn de plantengemeenschappen in het biodiversiteit experiment blootgesteld aan een experimentele droogte (hoofdstuk 5). Met droogtebestendigheid bedoel ik het vermogen om de biomassaproductie tijdens een droogte op peil te houden. Om een droogte te simuleren zijn alle plots voor een aantal weken bedekt met droogtekappen. Tijdens deze periode heeft de helft van de plots met de hand water gekregen, terwijl de andere helft (met dezelfde soortensamenstellingen) géén water kreeg. De droogte verlaagde de bovengrondse biomassa met gemiddeld 35%, onafhankelijk van het aantal plantensoorten. In tegenstelling tot de verwachting, leidde de droogte echter tot een significante reductie in complementariteiteffecten in mengsels, wat suggereert dat de droogtestress in mengsels groter was dan in monoculturen. Ik heb geen bewijs kunnen vinden voor de hypothese dat productieve droogte-tolerante soorten in dominantie toenemen in mengsels en zo de droogtebestendigheid verbeterden (volgens de insurance hypothesis). Ik vond ook geen bewijs voor de hypothese dat diversiteit in worteleigenschappen een belangrijke rol speelt bij de droogtebestendigheid van mengsels. De analyses toonden echter wel aan dat de reductie in complementariteiteffecten afhankelijk was van de vegetatiebedekking (plantbedekking van de bodem) aan het begin van de droogte: de reductie in complementariteiteffecten was het grootst in mengsels met een lage bedekking, en verdween in mengsels met een hoge bedekking. Deze resultaten suggereren dat plantbedekking een belangrijke rol kan spelen in de droogtebestendigheid van een gemeenschap, met potentiële positieve effecten van planten soortenrijkdom tijdens droogte. Verder suggereert een vergelijking van de uiteenlopende resultaten van verschillende biodiversiteit-droogte studies dat de duur en intensiteit van de droogte sterk bepaalt hoe plantendiversiteit de droogtebestendigheid van plantengemeenschappen beïnvloedt. Onze inzichten wat betreft het effect van biodiversiteit op droogtebestendigheid zouden verbeterd kunnen worden door ons in toekomstig onderzoek te richten op het ontrafelen van de effecten van bedekking en soortensamenstelling over een droogtegradiënt.

Kortom, soortenmengsels vertoonden in alle jaren een toename in biomassa vergeleken met monoculturen. De grote variatie in complementariteiteffecten en selectie-effecten binnen de mengsels wijzen erop dat soortensamenstelling – en niet alleen soortenrijkdom – biodiversiteitseffecten voor een groot deel bepaalt. Verder verschilden de onderzochte graslandsoorten significant in hun worteleigenschappen, hetgeen zou kunnen duiden op verschillende voedselopname-strategieën van plantensoorten. Deze worteleigenschappen konden de biomassa-productie van individuele planten in mengsels en de selectie-effecten gedeeltelijk verklaren. Dit toont aan dat bewortelingsdiepte en gerelateerde worteleigenschappen belangrijke planteigenschappen zijn om het gedrag van soorten in soortenmengsels en het effect van soortensamenstelling op biodiversiteiteffecten te voorspellen. Complementariteiteffecten, die nu juist het effect meten van positieve interacties tussen soorten, zoals voedselverdeling, konden echter niet verklaard worden met functionele diversiteit in worteleigenschappen. Daarentegen laat mijn onderzoek zien dat diversiteit in worteleigenschappen wel degelijk van belang is als er op een kleinere schaal wordt gekeken. Niet op plotniveau, maar op het niveau van individuele planten bleek dat diepwortelende soorten beter groeien als ze omringd worden door ondiep- in plaats van diepwortelende soorten. Dit suggereert dat ruimtelijke voedselverdeling via bewortelingsdiepte wellicht bijdraagt aan het positieve effect van soortenrijkdom op biomassaproductie.

Tegen mijn verwachting in vond ik tijdens een droogte geen bewijs voor de hypothese dat de diepwortelende soorten en diversiteit in worteleigenschappen een rol spelen bij droogtebestendigheid van plantengemeenschappen. In toekomstig onderzoek is het aan te raden biodiversiteitsexperimenten te doen over verschillende milieugradienten. Zo kunnen we onderzoeken hoe de biodiversiteiteffecten van milieuomstandigheden (bv vocht) afhangen, welke planteigenschappen belangrijk zijn in bepaalde milieuomstandigheden, en of variatie in die eigenschappen in soortenmengsel de biomassaproductie verhoogt. Verder moet er een kritische blik geworpen worden op de hypothese dat plantenmengsels het beste functioneren met een maximale diversiteit in (wortel)eigenschappen. Misschien is een combinatie van hoge en lage waarden van een eigenschap al voldoende, zoals ondiep- en diepwortelend. Dat vereist echter een andere analyse/manier van kijken naar de relaties tussen eigenschappen en CE. Daarnaast blijkt uit mijn onderzoek dat het gecombineerde effect van plantdiversiteit en droogte op grasland productie beter kunnen onderzoeken op een kleine schaal (directe buurplanten) dan op plot niveau. Zulke observaties en inzichten vergroten ons begrip betreffende het belang van diversiteit voor de productiviteit van ecosystemen. Het stelt ons bovendien in staat te voorspellen hoe het functioneren van onze graslanden wordt beïnvloed door steeds vaker voorkomende droogte.

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SENSE-matties, I also got a good start in Wageningen thanks to you! It's great that so many of us could start a PhD or ended up on other interesting projects. I'm proud to be the first of us having the defense; luckily Arie did not beat us, girls ;), although it was a close call. Good luck with finishing your theses (or other projects), and let's keep having SENSE-drinks and dinners.

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Tibi gratiam ago!


Lisette

About the author

Lisette Marleen Bakker was born on the 16th of October 1988 in Alkmaar, the Netherlands. She spent most of her childhood in Oudorp, running around, painting with her grandmother (see painting made in 1996 on the left), playing sports and spending lots of time with her family and friends. She followed in the footsteps of her grandfather and parents, by attending Murmellius Gymnasium Alkmaar (secondary school). Her interest in natural processes and life around her made her passionate to attain a bachelor degree in biology. After visiting many study information days on several universities, her choice was certain: Biology at the Vrije University Amsterdam. And no single moment of regret about this choice, her love for biology grew and grew. The enormous diversity of plants and their adjustments to so many (harsh) conditions made her realize that plants are very impressive organisms. During this time Lisette assisted the plant breeding department at the seed company Bakker Brothers during her summer holidays. Her growing love for plant biology and old love for scouting activities (camp fires) were perfectly combined during her bachelor thesis about the flammability of heathland species (supervised by prof.dr. Hans Cornelissen, System Ecology, VU). After receiving her BSc Biology (cum laude), Lisette decided it was time for a small study break to do what she loved: traveling. She went for almost three months to Chili, to travel from north to south and encounter a whole set of different biomes: from the desert to the snow. The rest of the year was spent on a language course in Oxford, UK, and a job as student assistant at the Vrije Universiteit Amsterdam.

Lisette continued her education by studying for the master degree Ecology at the Vrije Universiteit, Amsterdam. After submitting her first master thesis regarding plant transpiration reduction, water availability and plant traits (supervised by dr.ir. Bob Douma and prof.dr.ir. Peter van Bodegom from Systems Ecology, VU University, and dr.ir. Ruud Bartholomeus and prof.dr.ir. Flip Witte from KWR Watercycle Research Institute), Lisette had the chance to participate in the SENSE honours programme during the second year of her master. This programme gave her the opportunity to write a PhD-proposal together with one of the departments and supervisors that joined the programme. She moved from Amsterdam to Wageningen to join the

Nature Conservation and Plant Ecology group at Wageningen University. Together with prof.dr.ir. Liesje Mommer and dr. Jasper van Ruijven, she wrote the proposal that led to this PhD-thesis. At the same time, she was introduced to the biodiversity research field, writing her second master thesis (supervised by Liesje Mommer and Jasper van Ruijven): "exploring the role of pathogens in the positive biodiversity-productivity relationship". The PhD-proposal was chosen, i.e. the grant honored, so this very interesting research project could begin! Lisette quickly finished her MSc Ecology (cum laude). To start the PhD project fresh and enthusiastic, Lisette decided to first travel for several weeks through Asia before starting the PhD-project. After this travel, the PhD-adventure began, starting with designing and establishing a new multiyear biodiversity-drought experiment. Throughout the years, she supervised several bachelor and master students, and assisted various practicals of bachelor courses (Ecology I, Forest and Nature Conservation, and Ecohydrology). She also visited many (international) conferences to present her work through poster and oral presentations. Lisette will continue her career working at the seed company Bakker Brothers, where she can use her interest in feeding the world in a sustainable way, and enthusiasm for knowledge on plant and environment and for designing new projects, in a more applied setting.

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SENSE PhD Courses

- Environmental research in context (2014)
- Research in context activity: ‘Government review of the IPCC AR5 SYR report, for the Ministry of Infrastructure and Environment’ (2014)
- Soil Ecology and Planetary boundaries (2016)

Other PhD Courses

- Introduction in R (2014), Wageningen University (2014)
- Mixed linear Models, Wageningen University (2014)
- Structural Equation Modelling, Wageningen University (2015)

Management Skills Training (o.a.)

- Project and Time Management, Valley Consult, Wageningen (2014)
- Workshop Personal Leadership ‘The Choice’, Wageningen University (2014)
- Competence Assessment, Maas Organisation & Career development (2014)
- Organization of the student colloquia of Plant Ecology and Nature conservation group (2015-2017)
- Organizing the ‘Plant-Soil Interaction’ discussion group (2016-2017)

External training at a foreign research institute

- Plant Ecology Department, University of Bayreuth, Germany (2015)

Didactic Skills Training (o.a.)

- Assisting practicals of the BSc courses ‘Ecology I’, ‘Forest and Nature Conservation’ and Ecohydrology (2014-2016)
- Supervising four BSc students (2015) and three MSc students (2015-2017)

Selection of Oral Presentations

- *Using root traits to explain changes in biodiversity effects over time*. NEAM (NERN) Conference, 14-15 February 2017, Lunteren, The Netherlands
- *Decreased complementarity effects in grassland mixtures after an extreme drought*. Ecology across borders, 11-14 December 2017, Ghent, Belgium

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Propositions

1. Deep rooting plant species play an important role in determining the positive biodiversity effects in grassland.
   (this thesis)

2. Interpreting the "Complementarity Effect" (sensu Loreau & Hector, 2001) as evidence for resource complementarity hinders progress in understanding the mechanisms underlying positive biodiversity effects.
   (this thesis)

3. The way how scientific breakthroughs are communicated is at least as important for scientific credibility and impact on society as the scientific content that is being conveyed.

4. A better understanding of the relations between microbial diversity and soil functions requires not only taxonomic and functional identification of soil microbes, but also the distinction between inactive and active microbial cells.

5. Studying a plant without its environment is like reading a word without the full sentence.

6. Critical thinking should already be included in education at primary schools.

7. Trust in society starts with trustworthy leadership.

Propositions belonging to the thesis, entitled *"The positive effect of biodiversity: using root traits to understand effects of plant diversity and drought on grassland productivity".*

Lisette Marleen Bakker

Wageningen, 30th of May 2018