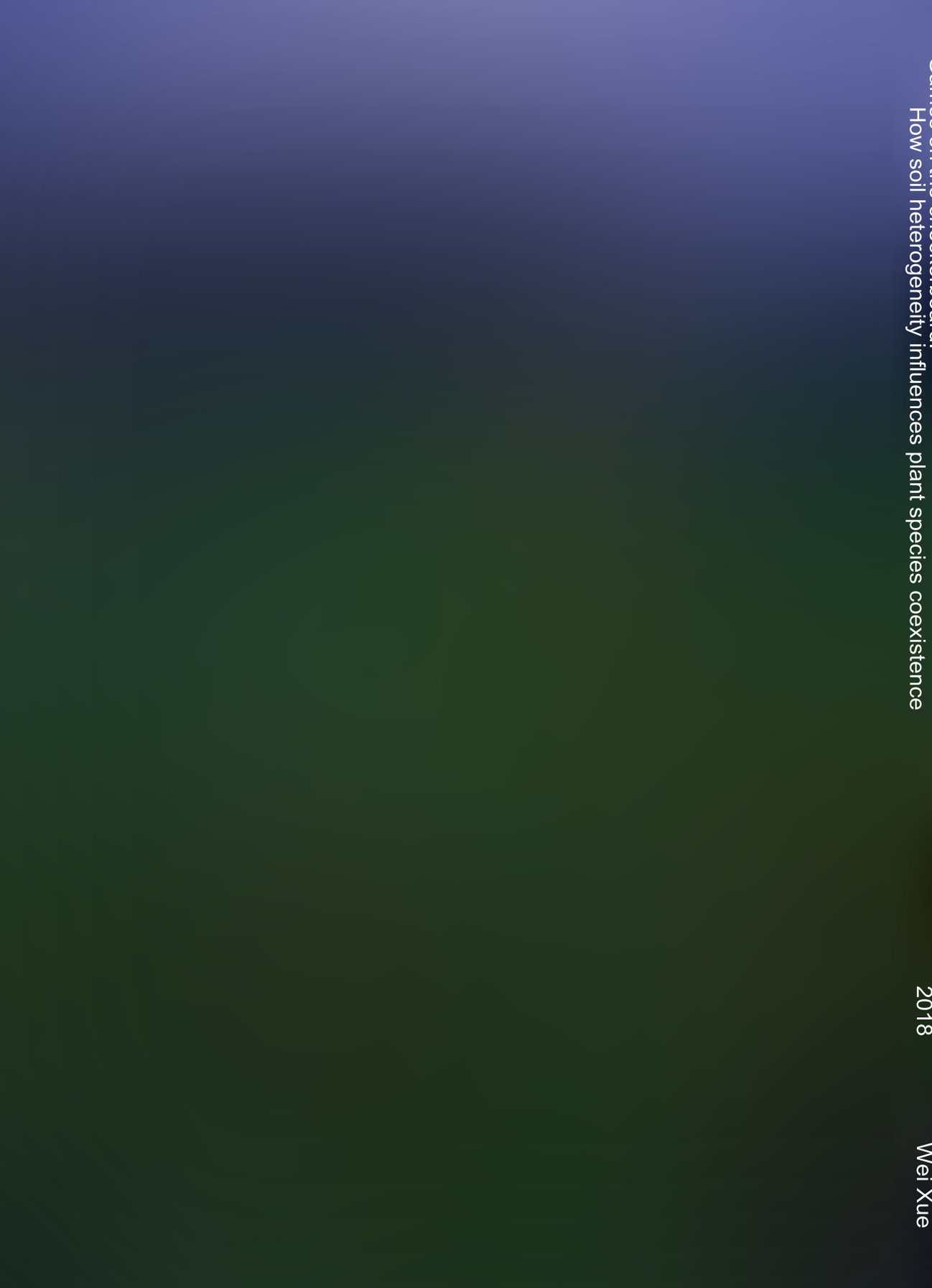
GAMES ON THE CHECKERBOARD

How soil heterogeneity influences plant species coexistence



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2018

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Games on the checkerboard

How soil heterogeneity influences plant species coexistence

Wei Xue

Thesis

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To my grandma

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Chapter 1

General introduction

The Convention on Biological Diversity of Rio de Janerio (1993) stressed the importance of biodiversity for ecosystem functions that are essential to humankind. Since then a steady flow of papers has been published about the impacts of biodiversity loss on plant production (e.g. Hector et al. 1999, Tilman et al. 2001, van Ruijven and Berendse 2005), decomposition (e.g. Gessner et al. 2010), soil respiration (e.g. Dias et al. 2010), invasion resistance (e.g. van Ruijven et al. 2003) and ecosystem stability (e.g. Gross et al. 2014). These experiments revealed worldwide a positive relationship between plant species diversity and ecosystem functioning, suggesting that biodiversity loss would be at the cost of crucial ecosystem functions, such as erosion resistance, production and provision of drinking water. Moreover, understanding plant species coexistence and the maintenance of plant species diversity might be of critical importance for the conservation of biodiversity and the maintenance of crucial ecosystem functions.

Heterogeneity-diversity hypothesis: the gap between ideal and reality

Many mechanisms have been proposed to explain species coexistence (Berendse 1979, Tilman and Pacala 1993, Hubbell 2005, Wilson 2011), in which environmental heterogeneity is thought to be one of the most important factors that contributes to plant species coexistence and maintenance of plant species diversity (Ricklefs 1977, Tilman 1982, Amarasekare 2003, Wilson 2011). Environmental heterogeneity, derived from the "habitat heterogeneity hypothesis" that was used to explain the variation in the diversity of animals (Simpson 1949, MacArthur and Wilson 1967), was first introduced in plant communities by Ricklefs in 1977 to explain the geographical patterns of tree species diversity (Ricklefs 1977). Since then, ecologists have long been trying to explore how important environmental heterogeneity is for plant species coexistence and the maintenance of species diversity.

Environmental heterogeneity is widely thought to promote plant species diversity through three main mechanisms. First, environment heterogeneity increases niche availability and the opportunities for resource partitioning so that plant species differing in resource requirements can coexist (Levine and HilleRisLambers 2009). Second, heterogeneous environments create more refuges and shelters for subordinate and rare species so that they can escape from the strong competition with dominant species (Chesson 2000, Hutchings et al. 2003). Finally, environment heterogeneity provides an opportunity for species diversification through

adaptation to different environmental conditions (Hughes and Eastwood 2006, Stein 2015), but this may only happen at evolutionary time scales.

Indirect evidence for the "heterogeneity-diversity hypothesis" in plants comes from studies comparing the performance of competing plants under various soil conditions (e.g. Reynolds et al. 1997, Fransen et al. 2001, Bliss et al. 2002, Brandt et al. 2013, Hendriks et al. 2015a, Burns et al. 2017). These studies have shown that spatial heterogeneity in soil characteristics or resources has implications for the coexistence of competing species by allowing preferential stolon or root placement of different plant species in different soil patches ("foraging behavior"; Hutchings and de Kroon 1994, Hutchings et al. 2003), reducing the dominance or competitive vigour of superior species (Fitter 1982, Fransen et al. 2001) and helping the establishment of species with competitive disadvantages (Burns et al. 2017).

More evidence comes from numerous observational studies that attempt to correlate soil environmental variation (e.g. the coefficient of variation or CV, of soil parameters) and/or the spatial structure of this variation (e.g. spatial grain or patch size calculated from semivariograms) with plant species diversity in natural ecosystems such as alpine meadows (e.g. Loneragan and Moral 1984, Tang et al. 2015), grasslands (e.g. (Bakker et al. 2003, Anderson et al. 2004), wetlands (e.g. Pollock et al. 1998, Shi et al. 2010) and forests (e.g. Pausas 1994, Honnay et al. 1999). Even though neutral, hump-shaped and negative relationships were found, most observational studies have reported positive relationships between a wide range of environmental heterogeneity characteristics and plant species richness and diversity (Lundholm 2009, Stein et al. 2014). For instance, in these observational studies, plant species richness and diversity are often positively correlated to heterogeneity in soil-based characteristics (e.g. soil types), land cover (e.g. habitat type), topography (e.g. elevation range) and climate (e.g. precipitation) (reviewed in Lundholm 2009, Stein et al. 2014 and more recently Schouten 2016, Xu et al. 2016, Zhou et al. 2017).

Given the wealth of indirect evidence, it is surprising that so far only a few experiments manipulating heterogeneity have tested this hypothesis directly (Table 1.1). In these experimental studies, soil type (Fitter 1982), microsite texture (Grime et al. 1987), soil nutrient supply (Collins and Wein 1998, Wijesinghe et al. 2005), light (Stevens and Carson 2002), nitrogen, soil depth (Baer et al. 2004, Baer et al. 2005, Baer et al. 2016), disturbance (Wilson and Tilman 1995, Questad and Foster 2008), soil profile (Williams and Houseman 2013) and plant-soil feedbacks (Wubs and Bezemer 2018) were manipulated at various spatial scales. However, these empirical studies found mixed results, varying from positive, neutral

to negative (Wijesinghe et al. 2005, Reynolds et al. 2007, Eilts et al. 2011, Gazol et al. 2013, Williams and Houseman 2013) effects of heterogeneity. However, non-positive (i.e. neutral or negative) heterogeneity effects on plant species diversity appear to be most common (Table 1.1; (Tamme et al. 2010). These results have raised an interesting question: why soil heterogeneity effects on plant species diversity differ among manipulated experiments.

Table 1.1. Summary of experimental studies testing the effects of spatial heterogeneity on plant species diversity. Experiment type: Field: outdoor experiment; Greenhouse: greenhouse experiment; Manipulated: spatial heterogeneity was manipulated; Observational: spatial heterogeneity was unmanipulated, usually different levels of soil heterogeneity in natural systems were compared. Factor: the manipulated variables. Effect: Negative, Neutral and Positive represent negative effect, no effect and positive effect of spatial heterogeneity on plant species diversity, respectively; hump-shaped represent greater species diversity in heterogeneous soil with small patches than in heterogeneous soil with large patches and in homogeneous soil. Reference: lists are references collected by Lundholm (2009) and several more recent references.

Study system	Experiment type	Factor	Effect	Reference
Grassland	Field, manipulated	Soil nutrient & clonal plant	Negative	Elits et al. 2011
Grassland	Greenhouse, manipulated	Soil nutrient	Negative	Gazol et al. 2013
Grassland	Field, manipulated	Soil nitrogen & depth	Neutral	Baer et al. 2004
Grassland	Field, manipulated	Soil nitrogen & depth	Neutral	Baer et al. 2005
Grassland	Field, manipulated	Soil nutrient	Neutral	Reynolds et al. 2007
Grassland	Field, manipulated	Soil nutrient	Neutral	Wijesinghe et al. 2005
Grassland	Greenhouse, manipulated	Microsite texture	Neutral	Grime et al. 1987
Grassland	Greenhouse, manipulated	Plant-soil feedback	Neutral	Wubs and Bezemer 2018
Grassland	Greenhouse, manipulated	Soil nutrient in three dimension	Hump-shaped	Liu et al. 2017
Grassland	Field, manipulated	Disturbance	Positive	Wilson 2000
Grassland	Field, manipulated	Disturbance & seeds addition	Positive	Questad and Foster 2008
Grassland	Field, manipulated	Soil nitrogen & depth & seed addition	Positive	Baer et al. 2016
Grassland	Field, manipulated	Soil profile	Positive	Williams and Houseman 2013
Grassland	Greenhouse, manipulated	Soil type	Positive	Fitter 1982
Old field	Field, manipulated	Light	Neutral	Stevens and Carson 2002
Old field	Field, manipulated	Soil nutrient	Neutral	Collins and Wein 1998
Wetland	Field, manipulated	Microtopography	Positive	Vivian-Smith 1997
Forest	Field, observational	Geomorphology	Positive	Burnett et al. 1998

Does spatial scale matter?

In the early nineteens, the famous ecologist Simon Levin proposed that: "The problem of pattern and scale is the central problem in ecology, unifying population biology and ecosystems science, and marrying basic and applied ecology. Applied challenges, such as the prediction of the ecological causes and consequences of global climate change, require the interfacing of phenomena that occur on very different scales of space, time, and ecological organization. Furthermore, there is no single natural scale at which ecological phenomena should be studied; systems generally show characteristic variability on a range of spatial, temporal, and organizational scales" (Levin 1992). Therefore, understanding ecological processes can only be done when studied at the appropriate spatial scale.

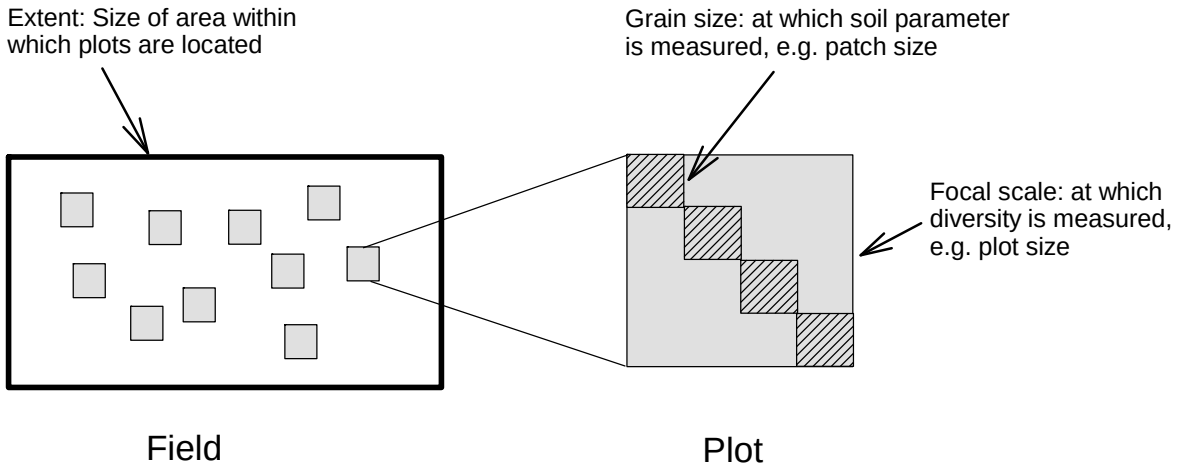

Fig. 1.1. Diagram showing the concepts of grain size, focal scale and extent, based on Wiens (1989) and Lundholm (2009). Within the field there are 10 randomly distributed plots (e.g., 1 m × 1 m each). Plant species diversity is measured within each plot. The environmental heterogeneity within each plot is calculated using the soil characteristics that are measured in each patch or grain (e.g., 25 cm × 25 cm).

Spatial scale has several components, including grain size, spatial extent and focal scale (Wiens 1989, Lundholm 2009). However, these components were confounded and defined differently in different studies (compare grain size in Lundholm 2009, Stein et al. 2014). Therefore, it is important to clarify exactly which definitions are used with respect to spatial scale. Here, I define spatial scale parameters following the approach used by Wiens (1989) and Lundholm (2009) (Fig. 1.1). For example, in an imaginary experiment, there are 10 plots (1 m × 1 m each) randomly distributed within a field. Within each plot, there are several patches that vary in nutrient availability (25 cm × 25 cm each). Plant species diversity is

measured in each plot. In this case, the area of the field is the spatial extent (i.e. size of area within which plots are located), the size of the soil nutrient patches is the grain size (i.e. scale at which soil parameters are measured) and the plot size is the focal scale (i.e. scale at which diversity is measured).

The effects of soil heterogeneity on plant species diversity can vary depending on the scale of soil heterogeneity (i.e. patch size or grain size) compared to the extent of plant rooting systems (Hutchings et al. 2003, Tamme et al. 2010, Eilts et al. 2011). When the scale of soil heterogeneity is smaller than the size of the plant rooting system, plant species can occupy their favored patches through selective replacements of ramets, roots and/or shoots ("foraging behavior"), integrating resources and thus outcompeting other plants (e.g. Hutchings and de Kroon 1994, Fransen et al. 2001, Day et al. 2003). By contrast, when the scale of soil heterogeneity is larger than the size of the plant rooting system, different soil patches may support different sub-communities, and the overall diversity will be higher than in equivalent homogeneous soils (Hutchings et al. 2003). The size of the rooting system of a plant is generally related to its foraging ability. For example, "guerilla" plants that have great foraging abilities may profit from exploiting high resource patches or patches without competitors in contrast to "phalanx" plants that have relatively poor foraging abilities (Navas and Garnier 1990, Campbell et al. 1991, Humphrey and Pyke 1998, Ye et al. 2006, Sammul 2011, Saiz et al. 2016). This would mean that plants with a guerrilla growth form may have a greater chance to exceed the scale of soil heterogeneity thus reducing plant species diversity. Indeed, in the presence of plants with long rhizomes or stolons, plant community species richness is generally depressed (e.g. Collins and Wein 1998, Stevens and Carson 2002, Baer et al. 2004, Eilts et al. 2011). Therefore, understanding the responses of plant species with different growth forms to heterogeneous environments would be a good starting point to unravel the mechanisms of plant coexistence in heterogeneous soil environments.

Soil heterogeneity effects on plant species diversity may also vary between different focal scales (spatial scale at which plant species diversity is quantified). As the focal scale (i.e. sample size) increases, a greater variety of patch types is included, which allows more species to coexist (MacArthur and Wilson 1967, Allouche et al. 2012). Alternatively, a larger focal scale means a greater chance to include a new plant species (i.e. sampling effects; Darlington 1957, May 1975, Connor and McCoy 1979). A meta-analysis of studies documenting relationships between plant species diversity and spatial heterogeneity showed that for observational studies positive heterogeneity-plant species diversity relationships were found

to be more common at larger focal scales, while for the few experimental studies there was no evidence of an effect of focal scale on heterogeneity-diversity relationships (Lundholm 2009). Two more recent studies have also illustrated that species richness and diversity (e.g. Wang et al. 2013), as well as plant community patterns (e.g. Li et al. 2016) differed when they were examined at different focal scales. Therefore, it is necessary to consider focal scale when effects of soil heterogeneity on plant species diversity are tested.

Stein et al. (2014) provided the first quantitative evidence that spatial extent also plays an important role in determining the relationship between environmental heterogeneity and species diversity of terrestrial plants and animals. However, so far spatial extent is thought to be important and is included in field observational studies (Field et al. 2009, Stein et al. 2014), but generally not in greenhouse or common garden experiments where environmental heterogeneity is controlled (Lundholm 2009, Tamme et al. 2010).

Environmental heterogeneity of resources and non-resources

Studies aiming to investegate soil heterogenity effects on plant species richness and diversity covered a variety of soil factors, e.g. soil nutrient supply (e.g. Gazol et al. 2013)), soil pH (e.g. Gough et al. 2000b), soil depth (Baer et al. 2004) and disturbance (Questad and Foster 2008). Schoolmaster (2013) proposed that effects of soil heterogeneity on plant species diversity may also depend on whether there is heterogeneity in resource (e.g. soil nutrient supply and water availability) or non-resource factors (e.g. soil pH and soil type). This is because plants consume a resource but experience a non-resource factor (Tilman and Pacala 1993). Previous studies reported that heterogeneity in soil resource factors generally failed to promote plant species diversity (e.g. Reynolds et al. 2007, Eilts et al. 2011, Gazol et al. 2013) while heterogeneity in non-resource factors often had a positive influence on plant species diversity (e.g. Fitter 1982, Vivian-Smith 1997, Williams and Houseman 2013). These results indicate that distinguishing resource and non-resource factors might be helpful to unravel negative and positive effects of soil heterogeneity on plant species diversity. However, comparing the results from studies that manipulated either resources or non-resource factors may be not sufficient because these studies differed in their study systems, e.g. they may differ in the ways to manipulate heterogeneity and the external environmental conditions may vary. As far as I am aware a soil heterogeneity study where resources and non-resources are manipulated within the same experiment is currently lacking.

Plant-soil feedbacks (PSFs) and spatial variation in PSFs

Soils vary spatially not only in abiotic factors (e.g. soil nutrients, pH) but also in biotic factors (e.g. soil biota). Soil biota have been suggested to be a key driver of plant species diversity (Bradley et al. 2008, de Kroon et al. 2012, Bever et al. 2015). Soil biota can enhance plant species diversity by reducing the dominance of particular plant species (e.g. soil pathogens; van der Putten et al. 1993), or by promoting the establishment of subordinate and rare plant species (e.g. via colonization by arbuscular mycorrhizal fungi; Gange et al. 1993, van der Heijden et al. 2008). However, other studies have shown that soil pathogens can also suppress rare plant species more than the dominant ones due to lower resistance of the former (Klironomos 2002, Mangan et al. 2010). Similarly, arbuscular mycorrhizal fungi can also promote the growth of dominant plant species (Hartnett and Wilson 1999, O'Connor et al. 2002), which can result in a reduction of plant species diversity. However, it is suggested that the effects of soil biota on plant species diversity rely on external abiotic factors, such as soil nutrients (Reynolds et al. 2003, De Deyn et al. 2004), as well as complex interactions among the soil biota (Reynolds et al. 2003, van der Heijden et al. 2008). Therefore, the soil community is generally viewed as a "black box" and in most studies only the net effects of soil community on plant responses i.e. plant-soil feedbacks are measured (Bever 2003, Brinkman et al. 2010).

Plant-soil feedback is the phenomenon that when a plant grows in the soil, it changes the soil properties, which in turn, can influence the performance of the same (conspecific plant-soil feedbacks) or other plant species (heterospecific plant-soil feedbacks) that grow later in the soil (Bever et al. 1997, Ehrenfeld et al. 2005, van der Putten et al. 2013). Most conspecific plant-soil feedbacks are negative (plants perform worse in soil conditioned by the same plant species than in soil conditioned by other plant species) even though positive plant-soil feedbacks are also regularly reported (reviewed in Kulmatiski et al. 2008). Negative plant-soil feedbacks are suggested to sustain species coexistence and thus promote the maintenance of plant species diversity (Bever et al. 1997, Bever 2003, Petermann et al. 2008), while positive plant-soil feedbacks are thought to promote plant dominance and homogenize plant communities (Hartnett and Wilson 1999, O'Connor et al. 2002). However, most evidence for this hypothesis is derived from theoretical models and from data obtained from experiments with plant monocultures. Plants virtually always grow in mixed communities in the field, and the presence of other species will not only affect how the focal plant will influence the soil, but will also affect how the focal plant will respond to changes in the soil (Kulmatiski et al.

2008, Bagchi et al. 2010, van de Voorde et al. 2011, Hol et al. 2013, Comita et al. 2014). For example, the influence of the focal plants on soil characteristics may be weakened in the presence of other plant species because negative plant-soil feedbacks might be plant density-dependent (Bell et al. 2006, Bagchi et al. 2010, van de Voorde et al. 2011, Kos et al. 2013, Comita et al. 2014). Besides, several other studies have shown that negative plant-soil feedbacks are enhanced when plants compete with other plants compared to when they grow alone (e.g. Callaway et al. 2004, Kulmatiski et al. 2008, Shannon et al. 2012, Hol et al. 2013), indicating that the presence of competitors may influence the responses of the focal plants to plant-soil feedbacks. Therefore, understanding how neighboring plants influence plant-soil feedbacks and how they respond to the plant-soil feedbacks differently in the presence of intra- and interspecific competitors would provide a complementary understanding of how plant-soil feedbacks influence plant species coexistence. However, so far, this has rarely been tested empirically.

It is important to note that plant-soil feedbacks vary spatially in the field as each plant individual influences its local soil in a specific manner. In theory, such spatial variation in plant-soil feedbacks (i.e. spatial plant-soil feedback heterogeneity) can influence plant performance and coexistence (Bonanomi et al. 2005, Mack and Bever 2014, Abbott et al. 2015, Zee and Fukami 2015). However, most of the empirical plant-soil feedback studies have ignored this inherent property of plant-soil feedbacks (but see Brandt et al. 2013, Burns et al. 2014, del Pino et al. 2015, Hendriks et al. 2015a, Hendriks et al. 2015b, Wubs and Bezemer 2016, Burns et al. 2017, Saar et al. 2018, Wubs and Bezemer 2018). Individual plants can benefit from spatial plant-soil feedback heterogeneity through foraging for nutrients in “foreign” soil patches thus avoiding contact with soil enemies in “own” soil patches (Hendriks et al. 2015b). However, in the presence of intraspecific competitors, the advantage (i.e. growing in “foreign” soil patches) may be less as competing individuals will employ the same strategy as the focal individual (Bliss et al. 2002, Bennett et al. 2017, Teste et al. 2017) and the performance of the plants may even be worse in monocultures in heterogeneous soils compared to homogeneous ones (Wubs and Bezemer 2016). In contrast, in soils that consist of patches of soil previously conditioned by different plant species, all competing plants can preferentially forage in “foreign” patches, and this may reduce competitive imbalances between species (Hendriks et al. 2015a), and promote species coexistence by slowing down competitive exclusion. So far, the effects of spatially heterogeneous plant-soil feedbacks on plant performance in plant mixtures have been rarely

addressed. The limited empirical evidence that is yet available impedes our understanding of how soil heterogeneity in plant-soil feedback may influence plant species coexistence. Studies that examine the influence of spatially heterogeneous plant soil feedbacks in mixed plant communities are urgently needed.

Aims of the thesis

In this thesis, I attempt to examine the effects of three types of soil heterogeneity, i.e. the spatial variation in soil nutrients, soil pH and plant-soil feedbacks, on plant competitive interactions, species coexistence and plant species diversity.

Specifically, I will try to answer the following questions:

(1) Does soil nutrient heterogeneity affect the competitive interactions between plant species with contrasting root architectures? If so, do these effects depend on intraspecific aggregation of the plant individuals?
(2) Does heterogeneity in soil nutrients and pH influence plant species richness and diversity? If they do, do the effects depend on the focal scale and the scale of soil heterogeneity, i.e. patch size?
(3) Does the abundance of a species in a plant community consisting of two species, via plant-soil feedback, influence the competitive interactions between two species when they grow later in the conditioned soils?
(4) Does spatial heterogeneity in plant-soil feedbacks influence the performance and competitive interactions of two plant species, and how do these effects depend on soil fertility?

Outline of the thesis

These questions are addressed by integrating two garden experiments (Chapter 2 and Chapter 3) and two greenhouse experiments (Chapter 4 and Chapter 5).

In **Chapter 2**, the growth of two clonal plant species with contrasting spatial architectures (one with aggregated ramets and one with diffuse ramets) is compared when grown in spatially homogenous soil, and in spatially heterogeneous soil consisting of low and high nutrient patches, after two growing seasons. The two plant species were planted either in

monocultures or in mixtures and in an even or a clustered distribution pattern. The competition between the two species was compared when they grow in spatially homogeneous and heterogeneous soils.

In **Chapter 3**, plant species diversity is investigated in field plots with homogeneously distributed soil nutrients and in heterogeneous plots consisting of low and high nutrient soil patches. There were two types of horizontally heterogeneous plots, one with small and one with large patches. The same design was used for homogeneous and heterogeneous plots that varied in soil pH. In addition, there were two vertically heterogeneous plots, with high and low nutrient soils located in the top and bottom layer, respectively, and one with high and low nutrient soils located in the bottom and top layer, respectively. In all plots I measured the plant species diversity over three seasons at both the plot and the patch scale.

In Chapter 4 and 5, I make use of a long-term field experiment where the abundance of two plant species, the grass *Anthoxanthum odoratum* and the forb *Centaurea jacea* was manipulated in plots with soil with high and low nutrient availability. I collected soil from the plots, and carried out two greenhouse plant-soil feedback experiments with these soils.

In **Chapter 4**, I examine the relationship between the abundance of the two plant species in the field plots (the conditioning phase) and the growth and competitive balance of the two species in pots in the greenhouse (the test or feedback phase). I also study the conspecific plant-soil feedback effects of the two species when they grow in monocultures and in mixtures in the greenhouse experiment.

Subsequently, in **Chapter 5**, I examine the performance of the two plant species grown in monocultures and in mixtures in pots with spatially heterogeneous soil, consisting of patches of soil collected from *Anthoxanthum odoratum* monocultures and from *Centaurea jacea* monocultures and in pots where the two soils are mixed homogeneously. The competitive interactions between the two species grown together in pots are also examined in the homogeneous and heterogeneous soils.

Finally, in **Chapter 6**, the main findings of chapter 2-5 are integrated and the implications for the effects of different types of soil heterogeneity on plant species coexistence and species diversity are discussed. I also discussed the results from a unique field observational study where I investigated the relationship between soil heterogeneity and plant species diversity. In addition, future research questions and the potential for using soil heterogeneity to restore plant species diversity are discussed.

Chapter 2

Intraspecific aggregation and soil heterogeneity: competitive interactions of two clonal plants with contrasting spatial architecture

Wei Xue*, Lin Huang*, Fei-Hai Yu and T. Martijn Bezemer

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*These authors contributed equally to this work

Abstract

Intraspecific aggregation of plant individuals can promote species coexistence by delaying competitive exclusions. However, such impacts may differ among species with contrasting spatial architecture and rely on the spatial distribution of resources.

We grew a phalanx clonal plant *Carex neutocarpa* (with aggregated ramets) and a guerilla one *Bolboschoenus planiculmis* (with diffused ramets) in monocultures or in 1:1 mixtures with an even or a clustered distribution pattern of the two species in homogeneous or heterogeneous soils.

After 16 months, shoot biomass and ramet number were greater in mixtures than in monocultures in *C. neutocarpa*, but smaller in *B. planiculmis*. However, the growth of neither *C. neutocarpa* nor *B. planiculmis* differed between even and clustered mixtures. Soil nutrient heterogeneity did not significantly affect the growth of either species, but increased relative yield of *B. planiculmis* and decreased that of *C. neutocarpa*.

The relative importance of intra- vs. interspecific competition depends on the spatial architecture of plants, and soil nutrient heterogeneity slows down competitive exclusion by decreasing differences in competitive ability between plants. However, our results do not support the idea that intraspecific aggregation of individuals alters competitive interactions between species.

Key words: aggregation, clonal growth form, clonal population, competition, environmental heterogeneity, guerilla and phalanx

Introduction

Intraspecific aggregation of plant individuals is a common phenomenon in plant communities (Greig-Smith 1979, Herben and Hara 2003, Lara-Romero et al. 2016). It can result from limited seed or clonal dispersal (Bolker et al. 2003, Seidler and Plotkin 2006), competitive interactions (Bolker and Pacala 1999, Xue et al. 2013), environmental heterogeneity (Seabloom et al. 2005, Lara-Romero et al. 2014) and positive plant-soil feedbacks (Hartnett and Wilson 1999, O'Connor et al. 2002). Spatial aggregation of conspecific plant individuals has profound impacts on ecological patterns and processes. For instance, it may change light interception and water use (Mokany et al. 2008), soil nutrient accumulation (Derner and Briske 2001) and litter decomposition (Yu et al. 2011). Hence, spatial aggregation of conspecific individuals can alter the relative importance of intra- vs. interspecific competition, affecting species coexistence (Tilman and Pacala 1993, Stoll and Prati 2001, Bolker et al. 2003, Lenssen et al. 2005, Wassmuth et al. 2009, Houseman 2013, Seahra et al. 2016). So far, studies testing the impact of spatial intraspecific aggregation of plant individuals have been mostly conducted in homogeneous environments (e.g. Stoll and Prati 2001, Lenssen et al. 2005, Monzeglio and Stoll 2008), without considering the inherent nature of environmental heterogeneity.

Natural environments are ubiquitously heterogeneous and essential resources for plant growth are commonly patchily distributed (Stuefer et al. 1996). A clonal plant may place more ramets in patches of higher resources by shortening inter-ramet distance (foraging strategy of a single clonal fragment, e.g. Slade and Hutchings 1987, Peng et al. 2013, Dong et al. 2015). A clonal plant may also increase the size of ramets in the higher resource patches by producing more leaves and roots, but without changing inter-ramet distance (consolidate strategy of a single clonal fragment, e.g. Lovett-Doust 1987, de Kroon and Schieving 1990, Birch and Hutchings 1994, Alpert and Mooney 1996). When several independent clonal fragments grow together, they may all sense resource heterogeneity and thus put more new ramets and/or increase ramet size in higher resource patches (foraging or consolidate strategies of several independent clonal fragments, e.g. Fransen et al. 2001, Day et al. 2003). Such responses, in turn, increase intraspecific aggregation (Maestre and Cortina 2002, Maestre et al. 2003, Seabloom et al. 2005, Lara‐Romero et al. 2016), and may further alter competitive interactions between intra- and interspecific individuals (Wijesinghe and Hutchings 1999, Maestre and Reynolds 2007, Monzeglio and Stoll 2008, Lara-Romero et al. 2016, Thomason

and Rice 2017). We therefore hypothesized that environmental heterogeneity will enhance the impact of intraspecific aggregation on competitive interactions between plant species. While many studies have tested the impacts of either environmental heterogeneity or spatial aggregation of intraspecific individuals on plant growth and competitive interactions, few have considered these two impacts simultaneously.

Plant species vary greatly in their spatial architectures and two contrasting spatial architectures have been identified for clonal plants, i.e., phalanx and guerilla (Lovett-Doust 1981). Clonal plants with a phalanx architecture produce no or short spacers connecting adjacent asexual individuals (ramets), so that ramets of the same genetic individual (genet) are spatially highly aggregated (Navas and Garnier 1990, Humphrey and Pyke 1998, Ye et al. 2006). By contrast, clonal plants with a guerilla architecture produce long spacers so that ramets of the same genet are widely spaced (Navas and Garnier 1990, Humphrey and Pyke 1998, Ye et al. 2006). Phalanx plants are expected to show advantages in acquiring local resources and thus may have competitive advantages in more crowded (with a higher spatial aggregation of individuals), homogeneous environments (Navas and Garnier 1990, Humphrey and Pyke 1998, Ye et al. 2006, Saiz et al. 2016, Lopp and Sammul 2017). By contrast, guerilla plants may have an advantage in exploiting open or high resource patches in heterogeneous environments through foraging (i.e. selective placement of ramets in high resource patches), but may benefit less in closed, homogeneous environments (Navas and Garnier 1990, Humphrey and Pyke 1998, Ye et al. 2006, Sammul 2011, Saiz et al. 2016). We are not aware of any studies that have tested simultaneously effects of environmental heterogeneity and spatial aggregation on the growth and competitive interactions of plants with contrasting spatial architectures. We hypothesized that impacts of environmental heterogeneity and intraspecific aggregation are different in phalanx and guerilla plants so that they alter competitive interactions between phalanx and guerilla plants.

To test our hypothesis, we grew a phalanx plant *Carex neurocarpa* and a guerilla plant *Bolboschoenus planiculmis* in monocultures and mixtures. The plants were grown either with an even or a clustered distribution pattern of the two species, and in either homogeneous soils or in heterogeneous soils consisting of high and low nutrient patches. Specifically, we addressed the following questions. (1) Does intraspecific aggregation of individuals affect the growth and competitive interactions of the two plants? (2) Does soil nutrient heterogeneity affect the growth and competitive interactions of the two plants with contrasting spatial

architecture? (3) Is there an interactive effect of soil nutrient heterogeneity and intraspecific aggregation on the growth and competitive interactions of the two plants?

Materials and methods

Plant species

Both the phalanx clonal plant *Carex neurocarpa* Maxim. and the guerilla clonal plant *Bolboschoenus planiculmis* (F. Schmidt) T. V. Egorova (synonym: *Scirpus planiculmis* F. Schmidt) are perennial sedges of the Cyperaceae family (Chen et al. 1999). *Carex neurocarpa* is a tussock-forming clonal plant and produces very short rhizomes (inter-ramet distance < 1 cm) and ramets of the same clone are closely spaced (Chen et al. 1999). In contrast, *B. planiculmis* forms long rhizomes (inter-ramet distance is ranging from 0.2 to 17 cm) so that ramets of the same clone are widely spaced (Chen et al. 1999). Rhizomes of *B. planiculmis* can branch intensively (Xue et al. 2013). Ramet height of *C. neurocarpa* is 0.2 to 1.0 m and that of *B. planiculmis* is 0.6 to 1.0 m. These two species are widely distributed and often coexist in wetlands in China (Chen et al. 1999).

Sampling and cultivation

On 15 June 2012, we collected more than 1800 ramets of *C. neurocarpa* and 1800 ramets of *B. planiculmis* from 20 natural populations along the north bank of Miyun reservoir in Beijing (40.533° N, 117.016° E). We then cut each ramet at 10 cm above shoot base and planted it in a small pot (10 cm in diameter) in an experimental garden (40.547° N, 117.010° E) a few kilometers away from the sampling places. After one month of cultivation, most of the ramets survived and produced new leaves. We then selected 864 similar-sized ramets of both *C. neurocarpa* and *B. planiculmis* and used them in the experiment described below. Initial biomass of the ramets was 0.132 ± 0.019 g (mean ± SE, n = 21) for *C. neurocarpa* and 0.119 ± 0.014 g (mean ± SE, n = 31) for *B. planiculmis*.

Experimental design

We pressed 48 wooden frames (50 cm wide × 50 cm long × 30 cm deep) into the soil to a depth of 25 cm in the experimental garden. The distance between adjacent frames was at least

0.5 m. The soil inside the wooden frames was removed and replaced with the experimental soil described below. Each frame was thereafter referred to as a plot.

The experiment consisted of two levels of soil heterogeneity (homogeneous vs. heterogeneous) crossed with four levels of planting types (monoculture of *C. neurocarpa,* monoculture of *B. planiculmis,* an even mixture of *C. neurocarpa* and *B. planiculmis,* and a clustered mixture of *C. neurocarpa* and *B. planiculmis*; Fig. 2.1). There were eight treatments in total and six replicates (plots) in each treatment.

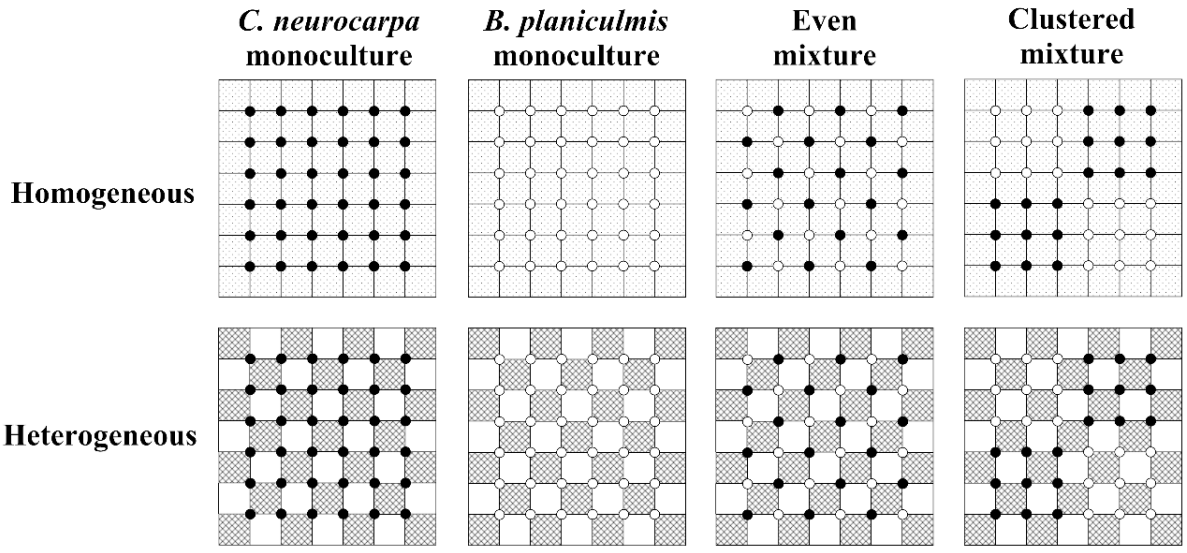

Fig. 2.1. Schematic representation of the experimental design. The experiment consisted of four homogeneous and four heterogeneous treatments with ramets grown in monocultures or mixtures, with the two species planted evenly or in clusters. In monocultures, 36 ramets of *Carex neurocarpa* (phalanx) or *Bolboschoenus planiculmis* (guerilla) were planted at the cross-points of the patches within each frame, and in mixtures, 18 ramets of both species were intraspecifically segregated or aggregated within each frame. In the heterogeneous treatments, open and shaded patches represent patches with low and high nutrients, respectively. Black and open dots mark the positions where the ramets of *C. neutocarpa* and *B. planiculmis* were initially planted.

In the heterogeneous treatments, each plot was divided into 49 equal patches (7.1 cm × 7.1 cm each) using a metal grid. Patches were filled with either an 1:1 (v:v) mixture of potting compost (total N: 13.39 g kg^{-1}; total P: 6.34 g kg^{-1}; total K: 24.45 g kg^{-1}) and sand (total N: 0.23 g kg^{-1}; total P: 1.01 g kg^{-1}; total K: 22.34 g kg^{-1}) (hereafter refer to as high nutrient soil) or an 1:9 (v:v) mixture of the compost and sand (hereafter refer to as low nutrient soil; Fig. 2.1). High and low nutrient soil patches were filled alternately. In total, 25 patches were filled with high nutrient soil (high nutrient patches) and 24 patches with the low nutrient soil (low

nutrient patches) in each plot. Thus, the high and low nutrient soils differed greatly in total N and total P. In the homogeneous treatments, the plot was also divided into 49 equal patches (7.1 cm × 7.1 cm), and in each patch, we filled a 25:24 (v:v) mixture of the high and low nutrient soils (Fig. 2.1). In this way, the total amount of nutrients per plot was the same in the homogeneous and the heterogeneous treatments. After filling the plots, we removed the metal grid so that roots could grow across patches. Before filling the plots with the soil mixtures, we placed, at the bottom of each plot, a piece of non-woven fiber (50 cm × 50 cm) which is widely used as rooting cloth to block roots from growing outside the plot but allow vertical movement of water.

We then planted ramets of *C. neurocarpa* and *B. planiculmis* at the cross-points of the patches within each plot (Fig. 2.1). In monocultures we planted 36 ramets of *C. neurocarpa* or *B. planiculmis* within a plot, and in mixtures we planted 18 ramets of both *C. neurocarpa* and *B. planiculmis* (Fig. 2.1). In even mixtures, ramets of the two species were planted in alternate positions (Fig. 2.1). In clustered mixtures, the 36 planting positions in a plot were divided into four clusters with nine planting positions each, and nine ramets of each species were planted within a cluster (Fig. 2.1). Thus, in even and cluster mixtures the 18 ramets of both *C. neurocarpa* and *B. planiculmis* were conspecifically segregated and aggregated, respectively (Fig. 2.1).

The experiment was maintained for 16 months (from 17 July 2012 to 4 November 2013). During the experiment, the mean precipitation from June to September was 329 mm in 2012 and 407 mm in 2013. Water was added to the plots when drought occurred in summer.

Harvest and measurements

Aboveground parts of each species were harvested at the end of experiment on 4 November 2013. We counted ramets of each species and harvested their aboveground shoots by cutting all plant material at ground level in each plot. For the guerilla plant (*B. planiculmis*) we also counted ramets and harvested aboveground shoots in each type of soil patches (high vs. low nutrient patches) separately in the heterogeneous treatments. In the homogeneous treatments we also counted ramets of *B. planiculmis* and harvested the aboveground shoots in the same way as in the heterogeneous treatment, i.e. separately in patches that were located at the same places as the high and low nutrient soil patches in the heterogeneous treatment. As the ramets of the phalanx plant (*C. neurocarpa*) did not grow off the locations where it was planted, we

harvested aboveground shoot biomass for this species in each plot but not separately for the two types of soil patches within each plot. Dry mass of all plant parts was determined after oven-drying at 70 °C for at least 48 h.

Data analysis

At the plot level, we first calculated shoot mass and ramet number per initial ramet of *C. neurocarpa* and *B. planiculmis* separately in each plot. Since the growth of the two species in the mixtures was not independent, we performed separate two-way ANOVAs to test the effects of soil nutrient heterogeneity (homogeneous vs. heterogeneous) and planting type (monoculture vs. even mixture vs. clustered mixture) on the growth measures of each of the two species at the plot level. Following ANOVA, planned contrasts were conducted to further separate the effect of planting type into the effect of competition type [intra- vs. interspecific competition, i.e. monoculture vs. (even mixture plus clustered mixture)] and the effect of intraspecific aggregation of plant individuals (even mixture vs. clustered mixture).

To directly examine the competitive interaction between the two species, we calculated relative yield of each species by dividing its shoot mass per initial ramet in each mixture (even or clustered mixture) by the mean shoot mass per initial ramet in monocultures across the six replicates. We used two-way ANOVA to test the effects of soil nutrient heterogeneity and intraspecific aggregation of plant individuals (even mixture vs. clustered mixture) on relative yield of each of the two species separately.

At the patch level, we first calculated shoot mass and ramet number per initial ramet per patch of the guerilla clonal plant *B. planiculmis* in both types of soil patches within the plots. We performed three-way ANOVA with repeated measures to test the effects of soil nutrient heterogeneity, planting type and patch type (high vs. low nutrient patches) within the plots on the growth of *B. planiculmis*. Following ANOVA, planned contrasts were conducted to further separate the effect of planting type into the effect of competition type and that of intraspecific aggregation. In this analysis, patch type was treated as a repeated variable as data in the high and low nutrient soil patches in the same plot were not independent.

Before analysis, data of shoot mass and number of ramets at the plot level and data of shoot mass of *B. planiculmis* at the patch level were square root transformed to improve normality

and homoscedasticity. All analyses were performed with R (version 3.3.2; http://www.r-project.org) in RStudio (version 1.0.44; http://rstudio.org).

Results

Shoot mass and ramet number were greater in mixtures than in monocultures in *C. neutocarpa* (Table 2.1A; Fig. 2.2A-B), but smaller in *B. planiculmis* (Table 2.1B; Fig. 2.2C-D). However, intraspecific aggregation significantly affected the growth of neither *C. neutocarpa* nor *B. planiculmis* (Table 2.1; Fig. 2.2). Soil nutrient heterogeneity or its interactions with planting type did not significantly affect shoot mass or ramet number (Table 2.1; Fig. 2.2).

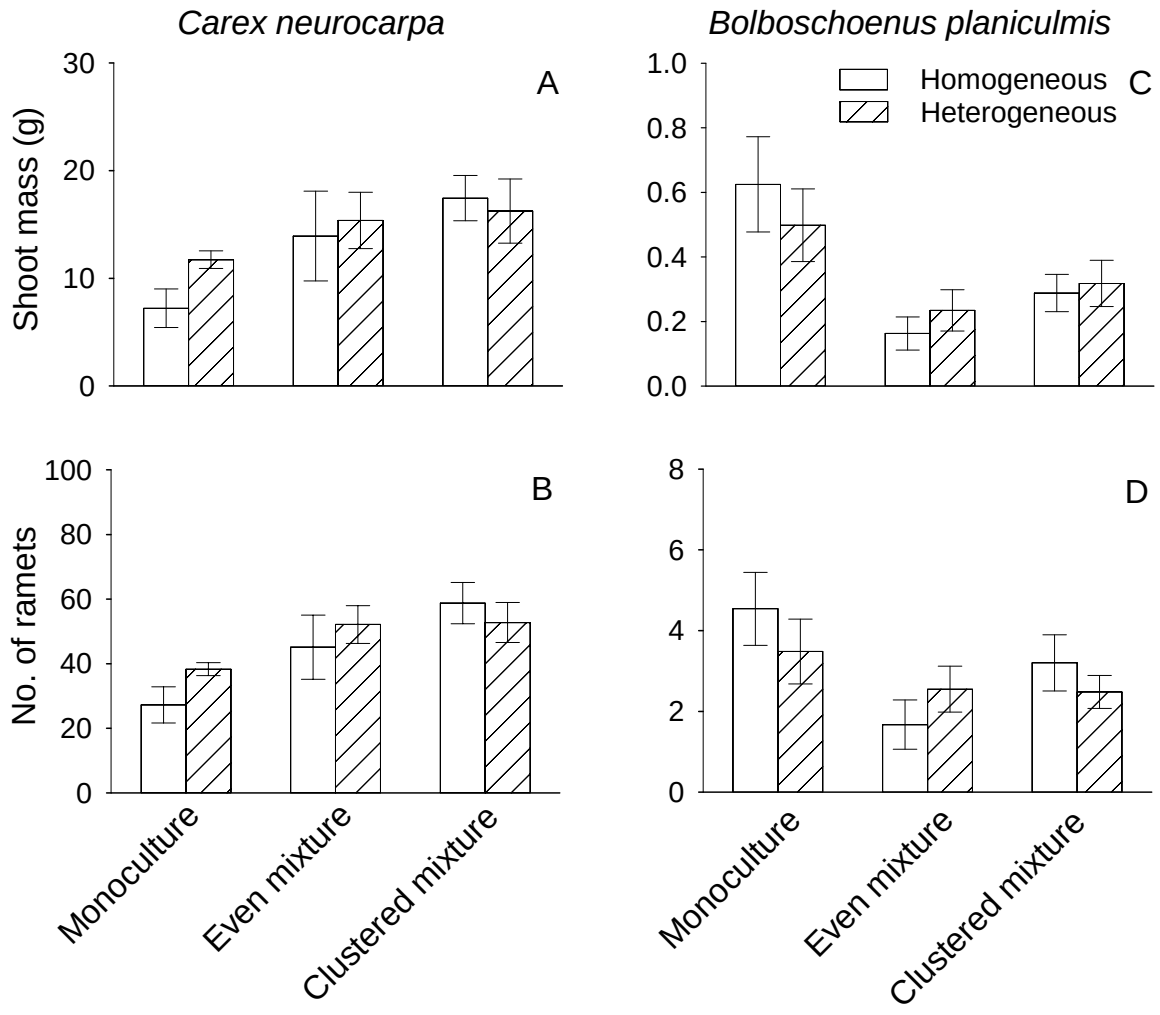

Fig. 2.2. Shoot biomass (A, C) and number of ramets (B, D) per initial ramet of *Carex neurocarpa* (phalanx, A, B) and *Bolboschoenus planiculmis* (guerilla, C, D) under the six treatments. Mean values (± 1 SE) are given.

Table 2.1. Effects of soil nutrient heterogeneity (homogeneous vs. heterogeneous) and planting type (monoculture vs. even mixture vs. clustered mixture) on the growth of (A) *Carex neurocarpa* (phalanx) and (B) *Bolboschoenus planiculmis* (guerilla) at the plot level. The effect of planting type was further separated into the effect of competition type [intra- vs. interspecific competition, i.e. monoculture vs. (even mixture plus clustered mixture)] and the effect of intraspecific aggregation (even mixture vs. clustered mixture) by planned contrasts

		Shoot mass[1]		No. of ramets[1]	
Effect	DF	*F*	*P*	*F*	*P*
(A) *Carex neurocarpa*					
Soil nutrient heterogeneity (H)	1	1.21	0.281	1.23	0.277
Planting type (P)	2	**4.22**	**0.024**	**6.65**	**0.004**
Competition type (C)	1	**7.51**	**0.010**	**12.04**	**0.002**
Intraspecific aggregation (A)	1	0.93	0.344	1.25	0.272
H × P	2	1.04	0.367	1.25	0.302
H × C	1	1.56	0.221	1.41	0.245
H × A	1	0.51	0.479	1.09	0.306
Residual	30				
(B) *Bolboschoenus planiculmis*					
Soil nutrient heterogeneity (H)	1	0.09	0.770	0.01	0.926
Planting type (P)	2	**5.65**	**0.008**	*2.96*	*0.067*
Competition type (C)	1	**9.57**	**0.004**	**4.57**	**0.041**
Intraspecific aggregation (A)	1	1.72	0.199	1.36	0.253
H × P	2	0.39	0.683	0.97	0.390
H × C	1	0.69	0.413	0.69	0.412
H × A	1	0.08	0.775	1.25	0.272
Residual	30				

[1] Data were square root transformed. Values are in bold when $P < 0.05$ and in italics when $0.05 < P < 0.1$.

Relative yield was significantly greater in homogeneous than in heterogeneous soils in *C. neutocarpa* (Table 2.2A; Fig. 2.3A), but tended to be significantly smaller in *B. planiculmis* (Table 2.2B; Fig. 2.3B). Intraspecific aggregation of individuals or its interactions with soil nutrient heterogeneity did not significantly affect relative yield (Table 2.2).

There were significant effects of soil nutrient heterogeneity × patch type on the growth measures of *B. planiculmis* (Table 2.3). Shoot mass and ramet number of *B. planiculmis* were greater in the high than in the low nutrient patches in the heterogeneous soil treatments, but did not differ between the mirrored high and low nutrient patches in the homogeneous soil treatment (Table 2.3; Fig. 2.4).

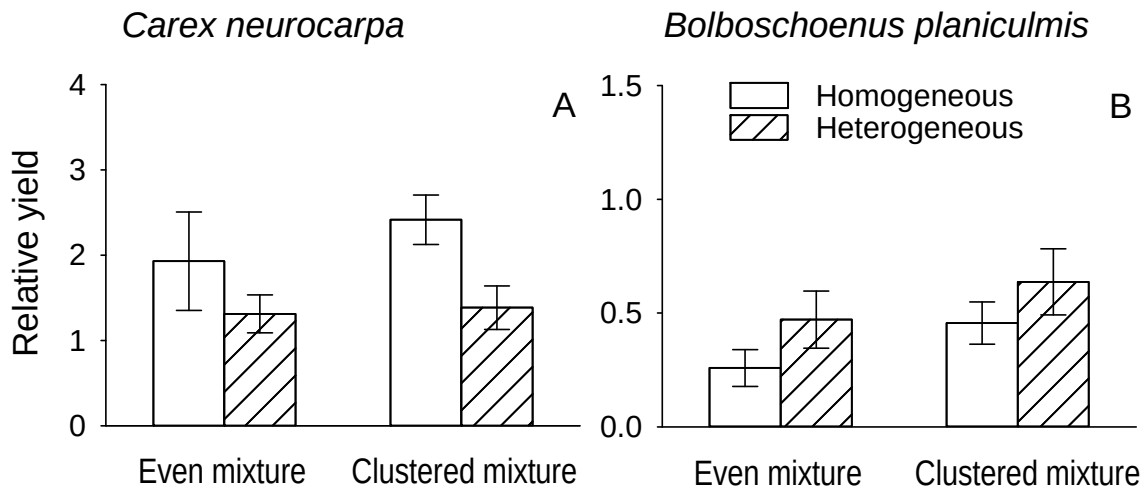

Fig. 2.3. Relative yield of *Carex neutocarpa* (phalanx, A) and *Bolboschoenus planiculmis* (guerilla, B). Mean values (± 1 SE) are given.

Table 2.2. Effects of soil nutrient heterogeneity (homogeneous vs. heterogeneous) and spatial intraspecific aggregation of plant individuals (even mixture vs. clustered mixture) on the relative yield of (A) *Carex neurocarpa* (phalanx) and (B) *Bolboschoenus planiculmis* (guerilla)

Effect	DF	*F*	*P*
(A) *Carex neurocarpa*			
Soil nutrient heterogeneity (H)	1	**5.11**	**0.035**
Intraspecific aggregation (A)	1	0.59	0.450
H × A	1	0.32	0.577
Residual	20		
(B) *Bolboschoenus planiculmis*			
Soil nutrient heterogeneity (H)	1	*2.99*	*0.099*
Intraspecific aggregation (A)	1	2.54	0.126
H × A	1	0.02	0.888
Residual	20		

Values are in bold when $P < 0.05$ and in italics when $0.05 < P < 0.1$.

Table 2.3. Effects of soil nutrient heterogeneity (homogeneous vs. heterogeneous), plant type (monoculture vs. even mixture vs. clustered mixture) and patch type (high vs. low nutrient patch) within plots on the growth of *Bolboschoenus planiculmis* (guerilla) at the patch level. The effect of planting type was further separated into the effect of competition type [intra- vs. interspecific competition, i.e. monoculture vs. (even mixture plus clustered mixture)] and the effect of intraspecific aggregation (even mixture vs. clustered mixture) by planned contrasts

		Shoot mass[1]		No. of ramets	
Effect	DF	*F*	*P*	*F*	*P*
Between subject					
Soil nutrient heterogeneity (H)	1	0.05	0.832	0.31	0.582
Planting type (P)	2	**5.79**	**0.007**	**3.94**	**0.030**
Competition type (C)	1	**9.86**	**0.004**	**6.74**	**0.015**
Intraspecific aggregation (A)	1	1.72	0.199	1.14	0.295
H × P	2	0.45	0.639	1.16	0.328
H × C	1	0.81	0.374	0.93	0.342
H × A	1	0.10	0.760	1.38	0.249
Residuals	30				
Within subject					
Patch type (PT)	1	**29.81**	**<0.001**	**31.73**	**<0.001**
H × PT	1	**13.60**	**<0.001**	**15.49**	**<0.001**
P × PT	2	0.36	0.698	0.26	0.776
C × PT	1	0.65	0.426	0.42	0.523
A × PT	1	0.08	0.785	0.10	0.761
H × P × PT	2	1.18	0.322	0.47	0.629
H × C × PT	1	1.86	0.183	0.94	0.340
H × A × PT	1	0.50	0.486	0.00	0.966
Residuals	30				

[1] Data were square root transformed. Patch type within plots was treated as a repeated factor. Values with $P < 0.05$ are in bold.

Discussion

The spatial architecture of plants can to some extent determine the uptake and the use of essential resources (Ye et al. 2006, Ikegami et al. 2009, Sammul 2011, Nacry et al. 2013, Xie et al. 2014, Lopp and Sammul 2017) and thus may affect competitive interactions between plant species (Schmid and Harper 1985, Humphrey and Pyke 1998, Sammul 2011, Liao et al. 2014, Lopp and Sammul 2017). Clonal plants can differ greatly in horizontal spatial architecture based on the distribution pattern of ramets of the same clone (Lovett-Doust 1981, Ye et al. 2006). Phalanx clonal plants show an aggregated distribution of ramets and are supposed to exhibit a competitive advantage when directly competing with other species (such as guerilla clonal plants with diffused distribution of ramets; Navas and Garnier 1990, Humphrey and Pyke 1998, Saiz et al. 2016, Lopp and Sammul 2017). By contrast, guerilla clonal plants exhibit an advantage to explore open areas by means of foraging to increase

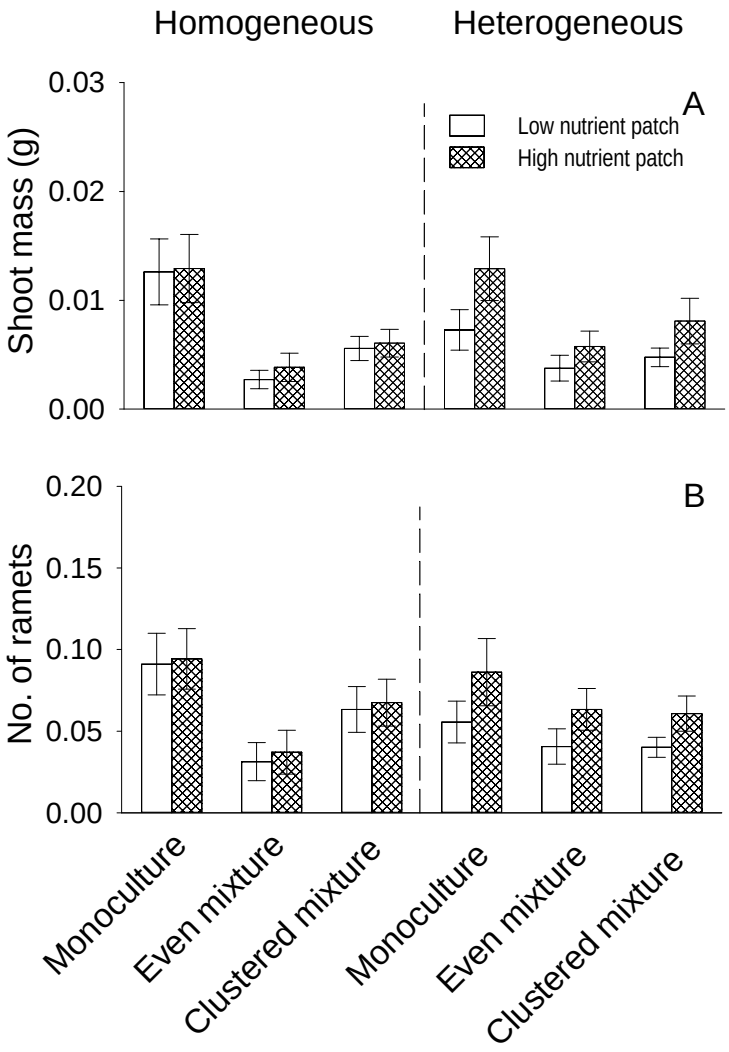

Fig. 2.4. Shoot biomass (A) and number of ramets (B) per initial ramet per patch of *Bolboschoenus planiculmis* (guerilla) in the high and low nutrient patches on homogeneous and heterogeneous soils. Mean values (± 1 SE) are given. The high and low nutrient soil patches in the homogeneous soil treatment represent the mirrored high and low nutrient patches at identical locations as those in the heterogeneous soil treatment.

resource uptake in heterogeneous environments (Rajaniemi and Reynolds 2004, Cahill and McNickle 2011, Sammul 2011, Xue et al. 2013, Dong et al. 2015, Lopp and Sammul 2017). Thus, the relative importance of intra- vs. interspecific competition is expected to differ between phalanx and guerilla clonal plants (Navas and Garnier 1990, Humphrey and Pyke 1998). We indeed found that the phalanx clonal plant *C. neutocarpa* and the guerilla clonal plant *B. planiculmis* showed contrasting responses to intra- vs. interspecific competition, i.e. the growth of the *C. neutocarpa* was greater in mixtures than in monocultures, but that of *B. planiculmis* was the opposite. Our results thus provide support for the view that the spatial architecture of plants can affect the relative importance of intra- vs. interspecific competition and thus the competitive interactions between plant species (Navas and Garnier 1990, Humphrey and Pyke 1998, Saiz et al. 2016).

Individuals of many plant species are distributed in aggregation, and such intraspecific aggregation of individuals is expected to alter the competitive ability of plants (Stoll and Prati 2001, Lenssen et al. 2005, Monzeglio and Stoll 2005, Hart and Marshall 2009, Thomason and Rice 2017). However, our results did not show any evidence that spatial aggregation of conspecific individuals affected the growth and competition ability of the two clonal plants, even though several previous studies showed that intraspecific aggregation benefited weaker competitors (Stoll and Prati 2001, Hart and Marshall 2009, Wassmuth et al. 2009, Lamošová et al. 2010). Intraspecific aggregation of plant individuals can influence plant growth because it can alter the relative importance of intra- vs. interspecific competition and slow down the competitive exclusion process. However, the phalanx clonal plant *C. neutocarpa* produced much more biomass than the guerilla clonal plant *B. planiculmis* at harvest. The overwhelming dominance of the phalanx clonal plant may have covered the potential positive effect of intraspecific aggregation on the competitive performance of the guerilla clonal plant. Consequently, we did not detect any impact of intraspecific aggregation. Therefore, the weaker competitor may not benefit from spatial aggregation of conspecific individuals due to the overwhelming suppression by the stronger competitors.

As expected, soil nutrient heterogeneity had little impact on the growth of the phalanx clonal plant *C. neutocarpa.* Unexpectedly, however, soil nutrient heterogeneity did not affect the growth of the guerilla plant *B. planiculmis* at the plot level. Guerilla clonal plants are thought to be able to benefit from soil nutrient heterogeneity because they can selectively place more roots/ramets in high nutrient patches (Birch and Hutchings 1994, Zhou et al. 2012, Dong et al. 2015), and exchange resources between interconnected ramets in patches of different resource levels through clonal integration (Alpert 1991, Song et al. 2013, Wang et al. 2017). The absence of soil heterogeneity effects on the growth of the guerrilla clonal plant at the plot level could be due to the mismatch between patch size and inter-ramet distance. However, we did find increased shoot mass and ramet number of *B. planiculmis* in the high nutrient patches at the patch level (i.e. showing foraging responses; Birch and Hutchings 1994, Wijesinghe et al. 2001, Rajaniemi and Reynolds 2004, Zhou et al. 2012), indicating that the guerilla clonal plant could respond to the heterogeneity treatment in our study. One possibility is that the benefits gained from foraging responses and resource integration may be offset by the presence of the conspecific and heterospecific competitors (Benot et al. 2013, Xue et al. 2013). At the end of the experiment, spaces were mostly occupied by the phalanx clonal plant, and hence only small patches of resources may remain. Thus, the effectiveness of exploiting

resources for the guerilla clonal species may have decreased (Wijesinghe and Hutchings 1999, Hutchings et al. 2003, Xue et al. 2013). Despite that, we found that soil nutrient heterogenity increased the relative competitive ability of the guerilla clonal plant *B. planiculmis* and decreased that of the phalanx clonal plant *C. neurocarpa*. This result indicates that soil nutrient heterogeneity may delay the competitive exclusion process though equalizing the competitive ability of the competing species.

Environmental heterogeneity in resource supply may have different effects on the growth of plants when their individuals are arranged in different spatial patterns (i.e. intraspecific aggregation or not). This is because intraspecific aggregation of plant individuals may alter their intra- and interspecific competition in communities and thus affect their responses to environmental heterogeneity (Monzeglio and Stoll 2008, Damgaard 2010, Lara-Romero et al. 2016). Unexpectedly, however, we did not find an interactive effect of soil nutrient heterogeneity and spatial aggregation of conspecific individuals on the performance of the phalanx or the guerilla clonal plant. Our results suggest that the responses of clonal plants to soil nutrient heterogeneity may not depend on the spatial patterns of the individuals.

We conclude that the relative importance of intra- vs. interspecific competition depends on the spatial architecture of plants, and soil nutrient heterogeneity can slow down the competitive exclusion through decreasing the relative difference in competitive ability between plants. However, our results do not support the idea that intraspecific aggregation of plant individuals can alter competitive interactions between species.

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Chapter 3

Soil heterogeneity and plant species diversity in experimental grassland communities: contrasting effects of soil nutrients and pH at different spatial scales

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Abstract

Soil heterogeneity is thought to promote plant species diversity. This hypothesis is well supported by numerous observational studies, but the evidence from manipulated experiments is limited. To test the heterogeneity-diversity hypothesis, we conducted a three-year field experiment in which a seed mixture of 16 common grassland species was sown in homogeneous soils with low, medium and high levels of soil nutrients or pH and in heterogeneous soils. We included horizontally heterogeneous soils consisting of 36 (small patch size) or 4 patches (large patch size) of low and high nutrients or low and high pH, and two vertically heterogeneous soils with low and high nutrient soils located in different soil layers. Soil nutrients and pH were manipulated separately.

We determined plant species richness and diversity at two spatial scales (40 cm× 40 cm plot scale and 10 cm × 10 cm patch scale). Plot-scale species richness or diversity was not influenced by soil heterogeneity. However, patch-scale species richness was lower in plots with soil with horizontally heterogeneous nutrients than in plots with soil where nutrients were distributed homogeneously. There was no difference between the two heterogeneous nutrient soils with different patch sizes. Patch-scale species diversity was higher in soils with heterogeneous pH with large patch size than in soils with heterogeneous pH with small patch size or the homogeneous pH soil at the final harvest. Patch-scale species diversity was lower in heterogeneous soil where high nutrient soil was located in the bottom half layer, than in heterogeneous soil where high nutrient soil was located in the top half layer or in homogeneous soil.

Within the horizontally heterogeneous nutrient soils, species richness was higher in high nutrient soil patches than in low nutrient soil patches. There was no difference between low and high pH soil patches within the two horizontally heterogeneous pH soils.

Our results show that soil heterogeneity can increase and decrease plant species diversity, depending on whether the soil varies in nutrients or pH, and on the spatial scale at which species diversity and soil heterogeneity are measured. We argue that understanding soil heterogeneity effects on plant species diversity requires studies that incorporate different soil factors that are manipulated and measured at multiple spatial scales.

Key words: soil heterogeneity, plant species richness, plant species diversity, soil nutrient, soil pH, spatial scales, patch size, focal scale

Introduction

Soil heterogeneity is widely thought to promote plant species coexistence and plant species diversity through increasing niche availability (Levine and HilleRisLambers 2009) and creating shelters and refuges from harsh environmental conditions (Chesson 2000, Hutchings et al. 2003). The hypothesis has been well supported in theory (Ricklefs 1977, Tilman and Pacala 1993, Chesson 2000, Hutchings et al. 2003) as well as in numerous observational studies (reviewed in Lundholm 2009, Stein et al. 2014). However, only a few experiments have directly tested the effects of soil heterogeneity on plant species diversity (reviewed in Lundholm 2009 and more recently Eilts et al. 2011, Gazol et al. 2013, Williams and Houseman 2013, Baer et al. 2016, Liu et al. 2017, Wubs and Bezemer 2018). These few experimental studies have reported mixed results, varying from positive to negative (e.g. Wijesinghe et al. 2005, Reynolds et al. 2007, Eilts et al. 2011, Gazol et al. 2013, Williams and Houseman 2013), in which, non-positive soil heterogeneity effects prevail (Lundholm 2009, Tamme et al. 2010).

The effects of soil heterogeneity on plant species diversity will depend on the scale of heterogeneity (i.e. patch size or grain size) and the extent of plant rooting systems (Hutchings et al. 2003, Tamme et al. 2010, Eilts et al. 2011). When the scale of soil heterogeneity is smaller than the size of the plant rooting system, plant species, especially clonal plants, can rapidly occupy their favored patches through selective replacements of ramets, roots or shoots, thus outcompeting other plant species (e.g. Hutchings and de Kroon 1994, Fransen et al. 2001, Day et al. 2003). Therefore, when certain species perform better in heterogeneous soils plant species diversity may decrease (i.e., the environment filter effect; Bazzaz 1991, Kraft et al. 2015). In contrast, Hutchings et al. (2003) predicted that when the scale of soil heterogeneity is larger than the size of the plant rooting system, different soil patches will support distinct sub-communities, and the overall diversity will be higher than in equivalent homogeneous soils. Soils can also be heterogeneous in a vertical dimension, but the effects of vertical heterogeneity on plant coexistence and plant species diversity are less well described (but see Berendse 1981, Fitter 1982, Maestre et al. 2006, Maestre and Reynolds 2006, Liu et al. 2017). Vertical heterogeneity in soils may promote plant species coexistence if different plant species can exploit soil resources at distinct soil layers (Fitter 1982). However, vertical heterogeneity in soils may also reduce plant species diversity when the soil resources are concentrated in

deeper soil layers because in this case only deep-rooting plant species can absorb the nutrients (Berendse 1979, 1981).

Soil heterogeneity effects on plant species diversity also depend on the focal scale: the spatial scale at which plant species diversity is quantified, i.e. the area of a sample. At greater focal scales, the number of microhabitats included in one sample may increase, and this can allow more species to coexist (MacArthur and Wilson 1967, Allouche et al. 2012). However, a meta-analysis on the few experimental studies examining soil heterogeneity effects on plant species diversity showed that the shape and magnitude of heterogeneity-diversity relationships were not related to the focal scale (Lundholm 2009). Therefore, it is still unresolved how soil heterogeneity influences plant species diversity at different spatial scales at which species diversity and soil heterogeneity are measured.

Recently, Schoolmaster (2013) proposed that effects of soil heterogeneity on plant species diversity may also depend on whether the soil varies in resource (e.g. soil nutrient or water availability) or non-resource factors (e.g. soil pH and soil type), since resources can be utilized by plant species while many non-resource factors have important impacts on the competitive vigour of plants (Tilman and Pacala 1993). Heterogeneity in soil resource factors generally fails to promote plant species diversity (e.g. Baer et al. 2005, Reynolds et al. 2007, Eilts et al. 2011, Gazol et al. 2013) while soil heterogeneity in non-resource factors often has a positive influence on plant species diversity (e.g. Fitter 1982, Reynolds et al. 1997, Vivian-Smith 1997, Williams and Houseman 2013). The contrasting effects observed in studies where soil resource factors and non-resource factors have been manipulated could be due to the type of factors but these experiments also differ greatly in how they were manipulated. So far, very few experimental studies have manipulated both soil resource and non-resource factors to test soil heterogeneity effects on plant species diversity (but see Baer et al. 2004, Baer et al. 2016).

Here, we conducted a three-year field experiment to test the effects of heterogeneity in different soil factors on plant species diversity at different spatial scales. We manipulated two soil factors, i.e. soil nutrients and soil pH that are both considered to be important factors affecting plant community structure (Tilman 1984, Tilman 1987, Gough et al. 2000b, Schaffers 2002, Isermann 2005, Laliberté et al. 2014). Soil nutrient availability and pH were manipulated separately. We sowed a seed mixture of 16 common grassland plant species in (i) homogenous soils with low, medium and high levels of nutrient availability or pH, in (ii)

horizontally heterogeneous soils consisting of low and high soil nutrient or pH patches that differed in patch size (small and large patch size), and in (iii) two vertically heterogeneous soils consisting of low and high soil nutrient patches in different soil layers. The experiment was carried out in poor sandy soils with low nutrient availability and each plot was divided into 6 × 6 patches of 10 cm ×10 cm each, irrespective of the heterogeneity treatments. Only the central 4 × 4 patches were used in the analysis.

Specifically, we made the following predictions: (1) Based on the heterogeneity-diversity hypothesis, plant species richness and diversity, determined both at 0.16 m^2 (40 cm × 40 cm plot scale) and 0.01 m^2 (10 cm × 10 cm patch scale) scale, will be higher in plots where high and low nutrient or pH soils are horizontally patchily distributed (heterogeneous soil) than in plots where the two soils are homogeneously mixed (homogeneous soil). Moreover, species richness and diversity determined at both spatial scales will be higher in plots with heterogeneous soil of large grain size (large patches) than in plots with heterogeneous soil of small grain size (small patches; Hutchings et al. 2003). (2) The variation in species composition among patches (10 cm × 10 cm) will be greater in heterogeneous plots than in homogeneous plots because different soil patches within the heterogeneous plots will support distinct sub-communities (Hutchings et al. 2003). (3) Plant species richness and diversity, at both 0.16 m^2 plot scale and 0.01 m^2 patch scale, will be smaller in plots where high and low nutrient soils are distributed in distinct top and bottom soil layers (vertically heterogeneous soils) than in plots where high and low nutrient soils are homogeneously mixed (homogeneous soils), because only deep-rooting or shallow-rooting species can utilize the nutrients. Therefore, (4) the variation in species composition among patches (10 cm × 10 cm) will be smaller in plots with vertically heterogeneous soils than in plots with homogeneous soil due to less intense species-species interactions in the vertically heterogeneous soils. (5) In plots where soil nutrients and pH are spatially homogeneous, plant species richness and diversity, at both 0.16 m^2 plot scale and 0.01 m^2 patch scale, will increase with increasing soil nutrient supply (due to the low nutrient availability of the background soil) and increasing soil pH (Schuster and Diekmann 2003). Hence, (6) the variation in species composition among patches (10 cm × 10 cm) will be larger in the homogeneous plots with higher soil nutrient supply and higher soil pH. This is due to stronger competition at higher soil nutrient availability or pH as we expect a greater productivity at the higher nutrient and pH levels.

Methods and materials

The experiment

In early spring 2015, original topsoil of an experimental field of Wageningen University, the Netherlands (51°59′N 5°39′E) was removed to a depth of 90 cm and refilled with a 1:4 (v:v) mixture of black soil and yellow sand. We then pushed 55 wooden frames (60 cm wide × 60 cm long × 40 cm deep) into the soil to a depth of 35 cm. The soil within each frame was removed and replaced by the experimental soils described below. Each frame is referred to as a plot. The 55 plots were arranged in five blocks with each block containing 11 plots. The distance between adjacent plots was 0.9 m. The paths between the plots were sown with a seed mixture of the grasses *Poa pratensis* and *Lolium perenne*.

We manipulated two soil factors, i.e. soil nutrients and soil pH, separately in this experiment. For each soil factor, there were five treatments i.e., three homogeneous soil treatments with different levels of soil nutrients or pH, and two horizontally heterogeneous soil treatments with different patch sizes (Fig. 3.1A). In addition, there were two vertically heterogeneous soil treatments consisting of two layers of low and high nutrient soils (Fig. 3.1B). In the three homogeneous soil nutrient treatments, each plot was filled with a 1:3 (v:v; low nutrient soil, "Low"), 1:1 (v:v; medium nutrient soil, "Medium") or 3:1 (v:v; high nutrient soil, "High") mixture of black soil and yellow sand (Fig. 3.1A). In the two horizontally heterogeneous soil nutrient treatments, each plot was equally divided into either 36 (each 10 cm × 10 cm; "Small patch") or four (each 30 cm × 30 cm; "Large patch") patches and each patch was filled with either low or high nutrient soil in a checkerboard manner (Fig. 3.1A). In the two treatments where heterogeneity was manipulated vertically, in each plot the upper or lower layer (20 cm in depth) was filled with either low or high nutrient soil (Fig. 3.1B).

In the three homogeneous soil pH treatments, each plot was filled with a 1:1 (v:v) mixture of black soil and yellow sand (low pH soil, "Low"), 2:1:1 (v:v:v) mixture of black soil, yellow sand and cyclone sand with 72 g $CaCO_3$ (200 g/m^2) (medium pH soil, "Medium") or 1:1 (v:v) mixture of black soil and cyclone with 144 g $CaCO_3$ (400 g/m^2) (high pH soil, "High"). The amount of $CaCO_3$ given to each pH treatment was based on Elberse et al. (1983). The two heterogeneous soil pH treatments were created using low and high pH soils in the same way as the horizontally heterogeneous soil nutrient treatments (Fig. 3.1). The total amount of nutrients in the medium nutrient treatment and the four heterogeneous nutrient soil treatments (two horizontally and two vertically heterogeneous nutrient treatments), as well as the total

amount of $CaCO_3$ in the medium pH treatment and the two heterogeneous pH treatments were equal. As the medium nutrient soil and the low pH soil shared the same treatment, there were 11 plots with different soil treatments randomly applied within each block.

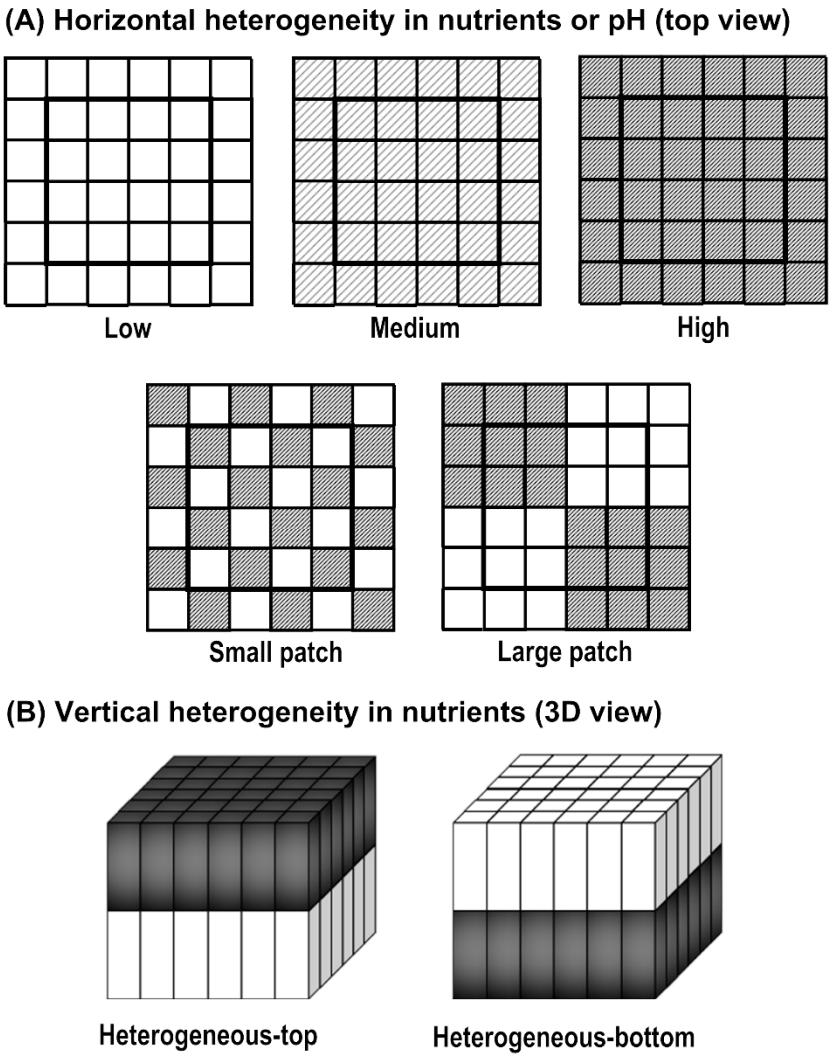


Fig. 3.1. Schematic representation of the experimental design. The experiment consisted of (A) three homogeneous soil treatments with low, medium and high levels of nutrient availability/pH and two horizontally heterogeneous soil treatments with low and high nutrient/pH soil patches and differing in patch size (small patch and large patch). (B) In addition, there were two vertically heterogeneous soil treatments with high nutrient soil in the top layer (0-20 cm; heterogeneous-top) or at the bottom layer (20-40 cm; heterogeneous-bottom). Soil nutrients and soil pH were manipulated separately. See the main text for the soils used in each treatment. Only plant growth in the central 16 patches (within the thick black line) was used for the analysis.

For each soil used in the experiment, we randomly took five soil samples for soil chemical analysis. Initial soil chemical characteristics are presented in Table S3.1. To ensure there was a distinct difference among the soil pH treatments, we further added 36 g and 72 g lime to the

plots with medium and high soil pH treatments, respectively, as well as 2 g lime in each of the 18 high pH patches within the two heterogeneous pH treatments. This was done twice a year, i.e. early during the growing season and after the harvest at the end of the growth season.

Table 3.1. Species used in the experiment with family name, growth form and germination rate.

Species name	Family name	Forb/grass	Germination rate (%)[1]
Anthoxanthum odoratum	Poaceae	Grass	42
Briza media	Poaceae	Grass	44
Festuca rubra	Poaceae	Grass	65
Luzula campestris	Juncaceae	Grass	21
Achillea millefolium	Asteraceae	Forb	90
Campanula rotundifolia	Campanulaceae	Forb	56
Centaurea jacea	Asteraceae	Forb	16
Hypochaeris radicata	Asteraceae	Forb	64
Knautia arvensis	Caprifoliaceae	Forb	31
Leontodon hispidus	Asteraceae	Forb	74
Leucanthemum vulgare	Asteraceae	Forb	65
Plantago media	Plantaginaceae	Forb	57
Prunella vulgaris	Lamiaceae	Forb	45
Rumex acetosa	Polygonaceae	Forb	92
Veronica chamaedrys	Plantaginaceae	Forb	49
Sanguisorba minor	Rosaceae	Forb	37

[1]Germination rate was tested by separately sowing 100 seeds of each plant species in a petri dish, and counting the number of seedlings after one week.

The plant community was created by evenly sowing a seed mixture of 16 common grassland species (96 seeds in total with six seeds of each species) in each patch (10 cm × 10 cm) within each plot. Seeds were purchased from Cruydthoeck, Nijeberkoop, The Netherlands. In total, we sowed 3456 seeds in each plot (a similar sowing rate applied as Wijesinghe et al. 2005). All species used in the experiment are native to the Netherlands and perennials with different growth forms and germination rates (Table 3.1). We did not include legume species because they can fix atmospheric N_2 (Trannin et al. 2000), which may potentially alter the nutrient availability and hence influence soil heterogeneity within plots. To introduce microbial communities, after sowing, the plots were evenly covered by 0.8 L of a 1:3 (v:v) mixture (sieved through 0.2 mm mesh) of live natural grassland soil (collected in a grassland two

kilometres away from the experiment garden) and low nutrient soil. To clarify that the treatment effects on plant species diversity were not caused by the difference in seed germination, an additional experiment was conducted in an unheated greenhouse. Seeds were germinated in pots filled with different soils used in the field experiment. After one month, we counted the species richness and calculated the species diversity. There were no significant differences among the soils (richness: $F_{4, 24}$=0.96, P=0.449; diversity: $F_{4, 24}$=0.76, P=0.563).

All weeds that emerged from the seed bank were removed by hand before sowing. After sowing, we weeded all plots at the beginning of each growing season. During the first three months of the experiment the plots were watered twice a day to promote the germination and establishment of the sown plant species. The experiment was maintained for three growing seasons. During the experiment the daily mean temperature and precipitation were 14.2 °C and 2.7 mm, respectively (http://www.knmi.nl).

Harvest and measurements

All aboveground parts in the central sixteen 10 cm × 10 cm patches were harvested separately at the end of each growing season (on 18th September 2015, 12th September 2016 and 10th August 2017, respectively) by cutting the vegetation at 1 cm above soil level. We determined whether an individual plant belonged to a patch or not, based on rooting, so that if a plant roots inside a patch, it belongs to this patch, regardless of whether the leaves are inside or outside this patch. We sorted the species in each of four randomly selected patches (or two randomly selected patches for each soil type in the heterogeneous plots) within each plot. To determine belowground biomass, at the final harvest, soil cores (4.5 cm in diameter, 40 cm deep) were taken from the same four randomly selected patches in each plot. Soil cores were divided into two different layers (0-20 cm and 20-40 cm) in the homogeneous and vertically heterogeneous plots. The belowground parts were carefully washed over a sieve (0.5 mm mesh). Separation of roots of the different plant species was not possible. Aboveground biomass of each plant species in each patch and belowground community biomass in each patch was determined after oven-drying at 70 °C for at least 48 h.

Data analysis

We determined plant species richness and diversity at two different spatial scales: 0.16 m^2 plot scale (40 cm × 40 cm) and 0.01 m^2 patch scale (10 cm × 10 cm). Plot-scale species richness was determined by summing the species in all sampled patches per plot. Diversity (*H*') was calculated as: $H' = -\sum_{i=1}^{S} P_i \ln P_i$, where *S* is species richness and P_i is aboveground biomass of species *i* divided by total aboveground biomass of all plant species in a plot. Patch-scale species richness was determined by averaging the species number over all sampled patches per plot (or in the case of horizontally heterogeneous soil treatments, averaged over each of the two patch types). Patch-scale diversity was determined by first calculating the diversity of each sampled patch using aboveground biomass of each species in each patch, then this value was averaged over all sampled patches in a plot (or in the case of horizontally heterogeneous soil treatments, averaged over each of the two patch types).

We also determined plant species composition at 0.16 m^2 plot scale (40 cm × 40 cm) and 0.01 m^2 patch scale (10 cm × 10 cm). For the plot-scale species composition, total aboveground biomass of each plant species in all sampled patches per plot was used while for the patch-scale species composition, mean aboveground biomass of each plant species over all sampled patches per plot was used. Plant species that occurred in less than 5% of the samples were excluded in the community composition analysis (McCune et al. 2002).

We determined the variation in species composition (beta diversity) within each plot (or in the case of horizontally heterogeneous soil treatments, within each type of soil patch). We first calculated a Bray-Curtis dissimilarity matrix based on square-root transformed aboveground biomass data. Then, we computed the mean pairwise Bray-Curtis dissimilarity between each pair of patches within each plot (or in the case of horizontally heterogeneous soil treatments, within each type of soil patch) for each sampling year. These mean pairwise dissimilarities were used as the variation in species composition (beta diversity) within each plot (or in the case of horizontally heterogeneous soil treatments, within each type of soil patch).

The calculated data were used in the following analyses.

We first tested the horizontal soil heterogeneity effects on plant community responses. In this analysis, we included the medium homogeneous soil nutrient or pH treatment and the two horizontally heterogeneous soil nutrient or pH treatments (i.e. the small patch and large patch treatments). Effects of horizontal soil heterogeneity in nutrients and pH were tested separately because we added $CaCO_3$ in the three pH heterogeneity treatments but not in the nutrient

heterogeneity treatments. We used a linear mixed-effects model to test the effects of the horizontal soil heterogeneity treatment (homogeneous vs. small patch vs. large patch), time (2015 vs. 2016 vs. 2017), and their interaction on both plot-scale and patch-scale species richness and diversity, as well as mean Bray-Curtis dissimilarity among patches. As we sampled the same experimental plot during three consecutive years, plot was included as a random factor to account for repeated measurements. Post-hoc comparisons among levels of horizontal soil heterogeneity treatment were tested using contrasts overall across all three years, as well as for each year separately if there was a significant interaction between the soil heterogeneity treatment and time.

We used unconstrained, principal component analysis (PCA) to explore plot-scale and patch-scale plant community composition under different levels of horizontal soil heterogeneity treatment. To assess whether the horizontal soil heterogeneity treatment influenced plant community composition we used constrained redundancy analysis (RDA). In the constrained redundancy analyses (RDA), Time was used as a covariate to define permutation blocks as we sampled the same experimental plot during three consecutive years. Significance was based on a permutation test (499 permutations).

We also compared the patch-scale plant species richness, diversity and mean Bray-Curtis dissimilarity between patch types (low vs. high nutrient or pH soil patches) within the two horizontally heterogeneous plots. We used a linear mixed-effects model to test the effects of time, grain size of soil heterogeneity (small patch vs. large patch), patch type (low vs. high nutrient or pH soil patches), and their interactions on the patch-scale species richness and diversity, as well as mean Bray-Curtis dissimilarity among each type of soil patches. As we sampled the same experimental plot during three consecutive years and because the two types of soil patches within the same plot are not independent, patch type nested within plot (plot/patch type) was included as a random factor. Then, we performed a PCA to explore plant community composition under different types of soil patches within the heterogeneous plots, and a RDA with time as a covariate to assess the effects grain size of soil heterogeneity and patch type on plant community composition.

To test the vertical soil nutrient heterogeneity effects on plant diversity and community composition, we included the homogeneous medium nutrient soil and the two heterogeneous plots with high and low nutrient soils distributed in distinct two layers (i.e. heterogeneous-top and heterogeneous-bottom soils). We repeated the analysis as we did in the horizontal soil heterogeneity analysis described above, but replaced the factor, horizontal soil heterogeneity

treatment, with vertical soil heterogeneity treatment (homogeneous vs. heterogeneous-top vs. heterogeneous-bottom) in the models.

We also examined the effects of differences in soil nutrients and pH in homogeneous soils on plant diversity and community composition by including the homogeneous low, medium and high nutrient or pH soil. We used the same methods as described above in the horizontal soil heterogeneity analysis, but replaced horizontal soil heterogeneity treatment in the model by soil nutrient or pH level (low vs. medium vs. high).

We performed linear mixed-effect models and computed Bray-Curtis dissimilarity metrics in R (version 3.3.2; http://www.r-project.org) in RStudio (version 1.0.44; http://rstudio.org). Linear mixed-effects models were fitted with the *nlme* package (version 3.1-128; Pinheiro et al. 2016) and all data were checked graphically for normality and homogeneity of variance. Bray-Curtis dissimilarity metrics were calculated using the *vegdist* function in the *vegan* package (version 2.4-4) and all abundance data were root square transformed prior to analysis. All multivariate analyses were conducted in Canoco 5.03 (Microcomputer Power, Ithaca NY, USA).

Results

Plant community responses to horizontal soil heterogeneity

Plot-scale (40 cm × 40 cm) species richness or diversity were not significantly different among the three horizontal soil nutrient heterogeneity treatments (homogeneous medium nutrient treatment, small patch and large patch heterogeneous nutrient treatments; Fig. S3.1A-B; Table S3.2A). Horizontal soil pH heterogeneity treatment also did not influence plot-scale species richness (Fig. S3.1C; Table S3.2B). However, the plot-scale diversity was significantly greater in heterogeneous pH plots with large patch sizes than in heterogeneous pH plots with small patch sizes and in homogeneous medium pH plots, but this was only significant for the last harvest (Fig. S3.1D; Table S3.2B).

Horizontal soil nutrient heterogeneity significantly influenced the patch-scale (10 cm × 10 cm) species richness, as indicated by the lower patch-scale richness in heterogeneous nutrient soils, both with small and large patches, than in homogeneous medium nutrient soil (Fig. 3.2A; Table S3.3A). However, the grain size of soil nutrient heterogeneity did not have a significant effect (Fig. 3.2A; Table S3.3A). The horizontal soil pH heterogeneity treatment did not

influence patch-scale species richness (Fig. 3.2D; Table S3.3B). However, at the final harvest, the patch-scale diversity was significantly greater in heterogeneous pH plots with large patches than that in heterogeneous pH plots with small patches and in homogeneous medium pH plots (Fig. 3.2E; Table S3.3B).

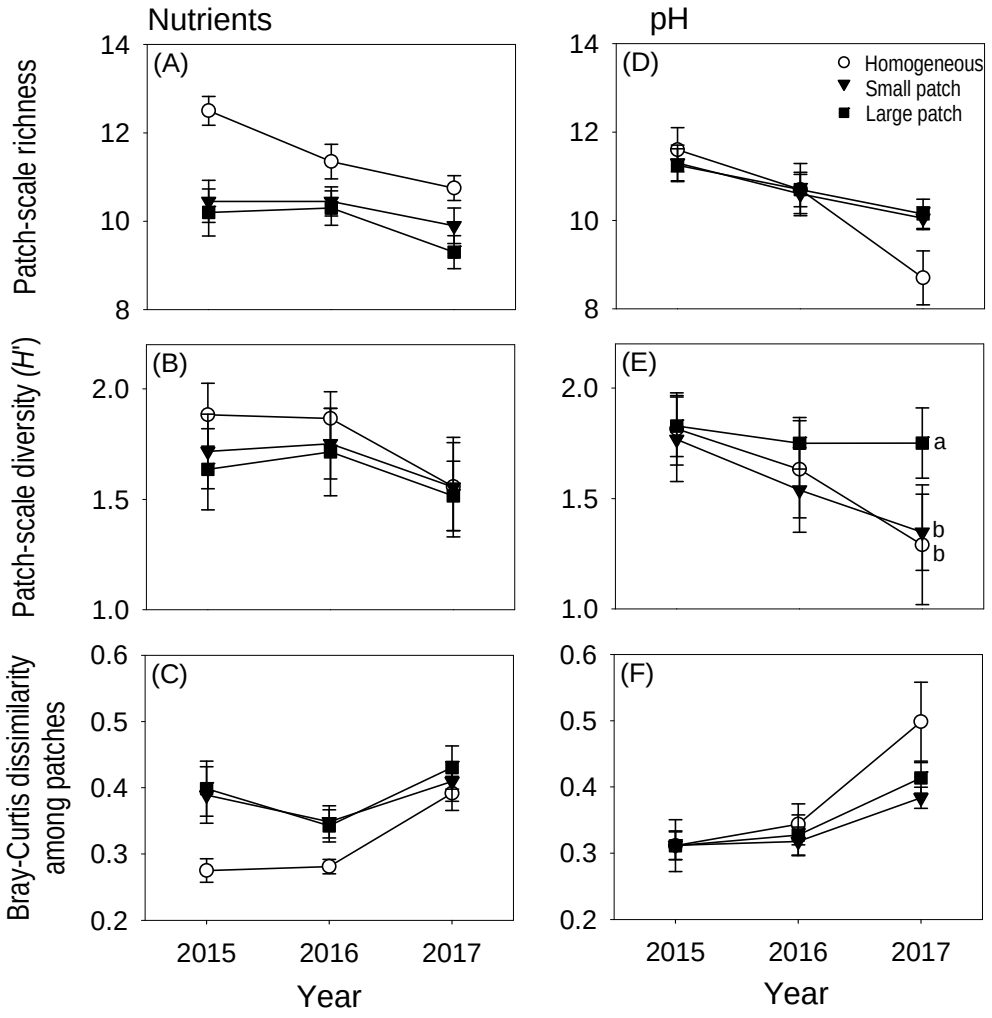


Fig. 3.2. Patch-scale (10 cm × 10 cm) species richness (A and D) and diversity (B and E), and mean Bray-Curtis dissimilarity among patches (C and F) in the horizontal soil heterogeneity treatments (i.e. the medium homogeneous soil, small patch and large patch heterogeneous soils) from 2015 to 2017. Mean values (±SE) are given. "Homogeneous", "Small patch" and "Large patch" represent homogeneous soil (medium nutrient/pH soil) and heterogeneous soil with small and large patch sizes, respectively. See Table S3.3 for statistical results. A significant time and soil heterogeneity treatment interaction occurred in (E), post-hoc comparisons among levels of horizontal soil heterogeneity treatment were made for each year separately: means that share the same letter (a–b) within a year are not significantly different at $P < 0.05$.

There was a significant horizontal heterogeneity treatment effect on plant species composition at both plot-scale and patch-scale (Fig. S3.2A-B, D-E). Moreover, horizontal soil nutrient

heterogeneity significantly influenced the variation in plant species composition (mean Bray-Curtis dissimilarity among patches). The mean Bray-Curtis dissimilarity was overall greater in both small- and large-patch heterogeneous nutrient soils than in homogeneous medium nutrient soil (Fig. 3.2C; Table S3.3A). However, there was no difference between the heterogeneous nutrient soils with small and with large patches, suggesting that the grain size of soil nutrient heterogeneity did not influence variation in species composition (Fig. 3.2C; Table S3.3A). The horizontal pH heterogeneity treatment did not influence the mean Bray-Curtis dissimilarity (Fig. 3.2F; Table S3.3B).

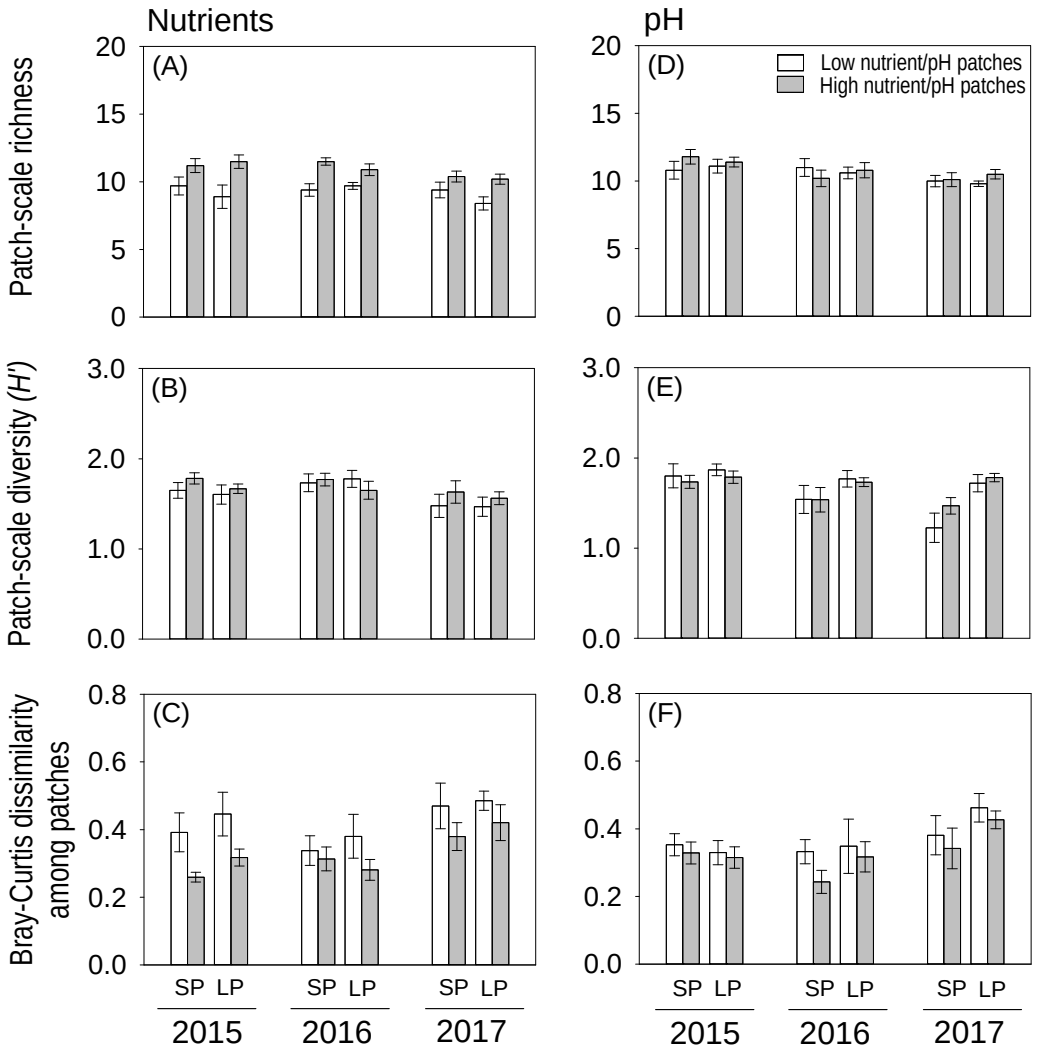


Fig. 3.3. Patch-scale (10 cm × 10 cm) species richness (A and D) and diversity (B and E), and mean Bray-Curtis dissimilarity among different types of soil patch (C and F) within the two horizontally heterogeneous soils of different grain sizes (i.e. small patch and large patch heterogeneous soils) from 2015 to 2017. Mean values (±SE) are given. "SP" and "LP" represent heterogeneous soil with small and large patch sizes, respectively. See Table S3.4 for statistical results.

Plant community responses to different types of soil patches within the horizontally heterogeneous soils

Patch-scale species richness was overall greater in high nutrient soil patches than in low nutrient soil patches within the two horizontally heterogeneous nutrient soils with different patch sizes (Fig. 3.3A; Table S3.4A). However, there was no difference between the two soil patches for patch-scale diversity (Fig. 3.3B; Table S3.4A). Patch type or its interaction with grain size of soil pH heterogeneity and/or time did not influence patch-scale species richness or diversity (Fig. 3.3D-E; Table S3.4B).

Neither in heterogeneous nutrient soils nor in heterogeneous pH soils did patch type significantly influence plant species composition (Fig. S3.2C, F). However, the variation in species composition, i.e., the mean Bray-Curtis dissimilarity was significantly greater among low nutrient soil patches than among high nutrient soil patches within the two heterogeneous nutrient soils (Fig. 3.3C; Table S3.4A), while the two different soil patches within the two heterogeneous pH soils did not differ significantly (Fig. 3.3F; Table S3.4B).

Plant community responses to vertical soil nutrient heterogeneity

The vertical soil nutrient heterogeneity treatment did not significantly influence plot-scale (40 cm × 40 cm) richness or diversity (Fig. S3.3; Table S3.5). However, there was a marginally significant vertical soil nutrient heterogeneity treatment effect on patch-scale richness and diversity, as indicated by the overall lower patch-scale (10 cm × 10 cm) species richness and diversity in the soils where high nutrient soils were located at the bottom half layer than in the homogenous medium nutrient soil (Fig. 3.4A-B; Table S3.6).

The vertical soil nutrient heterogeneity treatment significantly influenced plant species composition at both plot- and patch-scale (Fig. S3.4). The vertical soil nutrient heterogeneity treatment significantly reduced the variation in species composition (reduced mean Bray-Curtis dissimilarity) in both the heterogeneous-top treatment (where high nutrient soils were in the top half layer) and homogeneous medium nutrient treatment than in the heterogeneous-bottom treatment (where high nutrient soils were in the bottom half layer; Fig. 3.4C; Table S3.6).

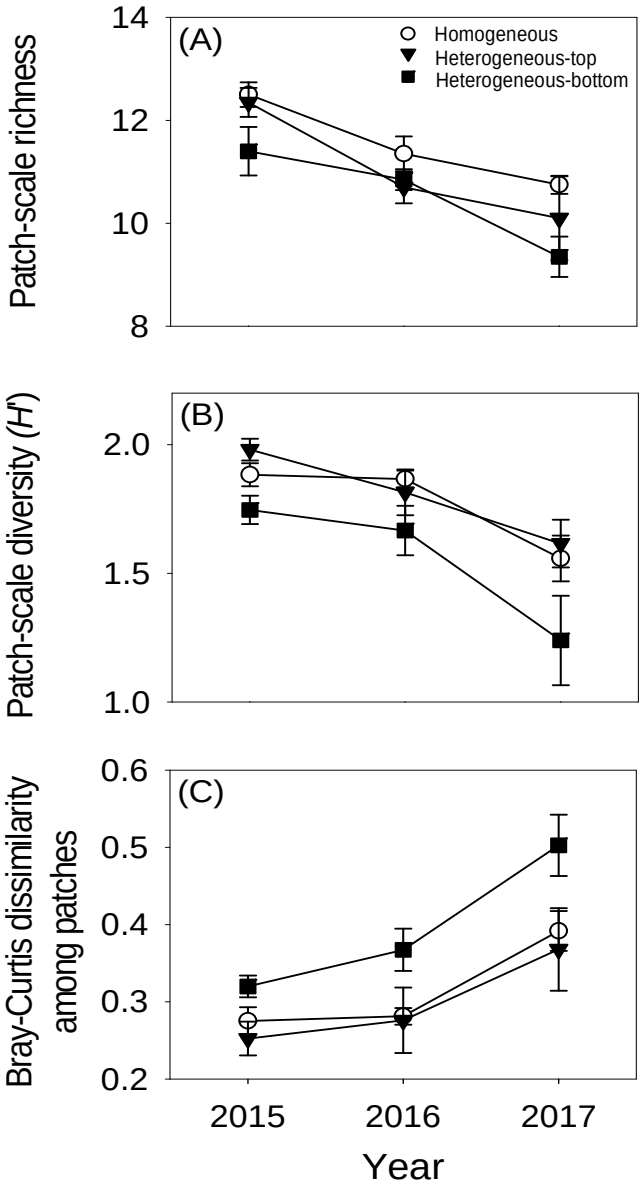


Fig. 3.4. Patch-scale (10 cm × 10 cm) species richness (A) and diversity (B), and mean Bray-Curtis dissimilarity among patches (C) in the vertical soil nutrient heterogeneity treatments (i.e. the medium homogeneous nutrient soil, heterogeneous-top and heterogeneous-bottom soils) from 2015 to 2017. Mean values (±SE) are given. "Homogeneous", "heterogeneous-top" and "heterogeneous-bottom" represent homogeneous soil (medium nutrient soil), heterogeneous soils where high nutrient soils were located at the top half and bottom half layer, respectively. See Table S3.6 for statistical results.

Plant community responses in homogeneous nutrient/pH soils

In homogeneous plots, neither soil nutrient level nor soil pH level significantly influenced plot-scale (40 cm × 40 cm) plant species richness or diversity (Fig. S3.5; Table S3.7).

At the patch-scale (10 cm × 10 cm), soil nutrient level significantly influenced richness and diversity. Plant species richness and diversity were higher in medium and high nutrient soils than in low nutrient soil (Fig. 3.5A-B; Table S3.8A). Soil pH level did not influence plant species richness or diversity at the patch-scale (Fig. 3.5D-E; Table S3.8B).

Soil nutrient and pH level had a significant effect on plant species composition at both plot (40 cm × 40 cm) and patch-scale (40 cm × 40 cm) (Fig. S3.6). Moreover, the variation in species composition, the mean Bray-Curtis dissimilarity among patches, was greater in low nutrient soil than in high nutrient soil (Fig. 3.5C; Table S3.8A). Soil pH level did not influence the mean Bray-Curtis dissimilarity among patches (Fig. 3.5F; Table S3.8B).

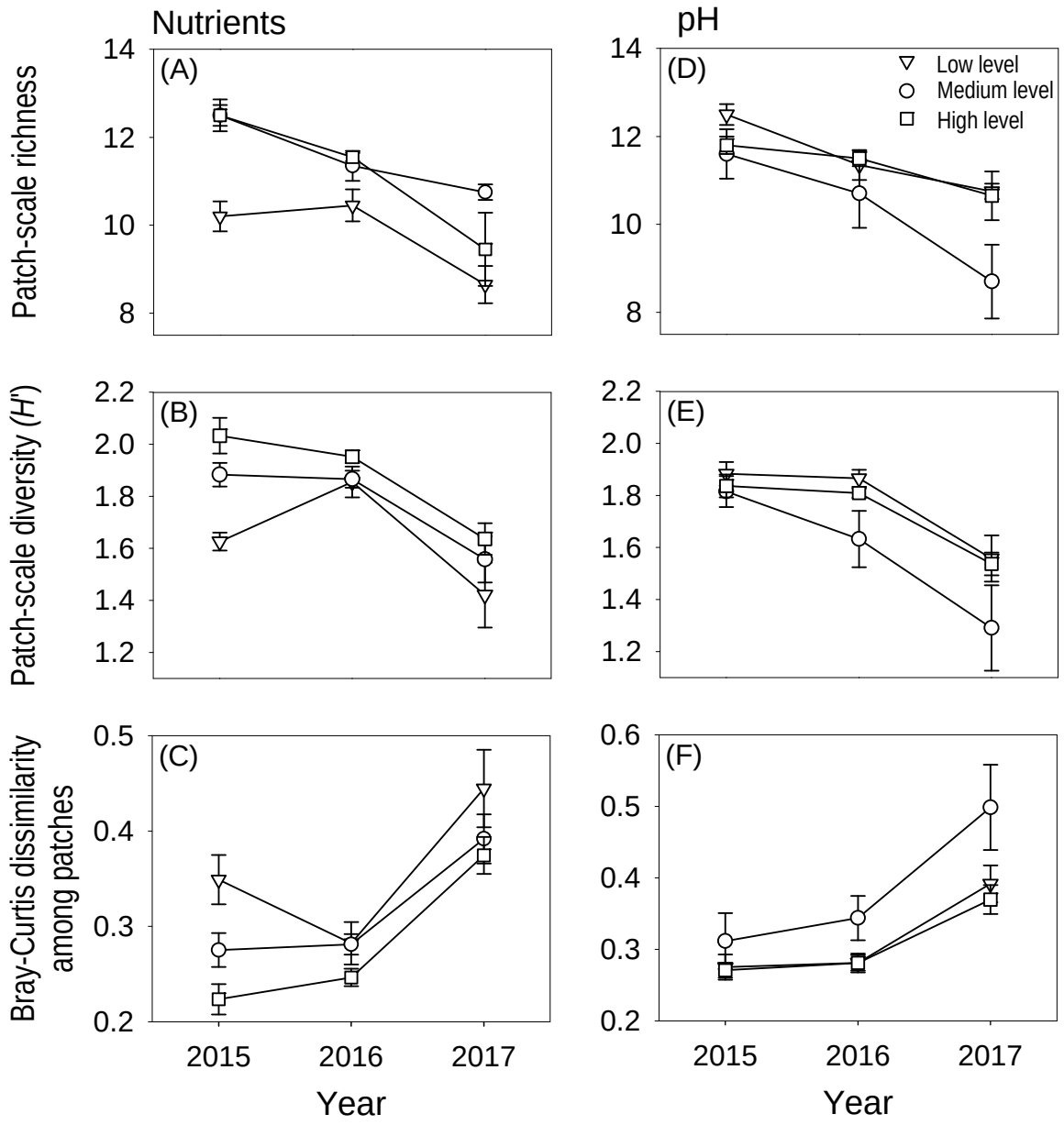


Fig. 3.5. Patch-scale (10 cm × 10 cm) species richness (A and D) and diversity (B and E), and mean Bray-Curtis dissimilarity among patches (C and F) in the homogenous soil treatments from 2015 to 2017. Mean values (±SE) are given. "Low level", "medium level" and "high level" represent the three homogeneous soil treatments with low, medium and high level of soil nutrient/pH, respectively. See Table S3.8 for statistical results.

Discussion

Our results show that both a horizontally and a vertically heterogeneous distribution of soil nutrients reduced plant species richness when compared to homogeneous soil that has the same amount of total nutrients. A spatially patchy arrangement of soil pH increased plant species diversity compared to the equivalent homogeneous pH soil when the grain size of the soil pH patches were large, even though this was only true at the final harvest. Within the two horizontally heterogeneous nutrient soils, plant species richness was overall greater in high nutrient soil patches than in low nutrient soil patches, but there was no difference between the low and high pH soil patches within the heterogeneous pH soils. In addition, plant species richness and diversity varied among homogeneous soils with different nutrient levels but did not do so among soils with different pH levels. These effects prevailed when species richness and diversity were determined at the patch scale but rather weak when measured at plot scale. Therefore, our results show that both changes in soil factors and changes in the heterogeneity of these factors influence plant species diversity (Klinkhamer and De Jong 1985, Gough et al. 2000a, Rajaniemi 2002, Bakker et al. 2003, Schuster and Diekmann 2003, Gross et al. 2005, Isermann 2005, Reynolds and Haubensak 2009). Further, these effects depend on the type of soil factors (resources vs. non-resources) that are manipulated as well as the spatial scales at which species diversity and soil heterogeneity are measured.

Horizontal heterogeneity in soil nutrient supply reduced plant species diversity, in agreement with other experimental studies (Baer et al. 2004, Gazol et al. 2013). Previous studies suggested that when the plant rooting system exceeds the soil patch size or grain size of the soil heterogeneity treatment, plants can integrate resources across patches and outcompete other plant species (Fransen et al. 2001, Hutchings et al. 2003, Eilts et al. 2011). Unfortunately we did not measure the actual root size of the plant species in our study. However, we did find a greater species richness (Fig. 3.3A) and community root biomass (Fig. S3.7C) in high nutrient than in low nutrient soil patches within the two horizontally heterogeneous soils with different patch sizes. This observation suggests that species, especially the dominant species *Hypochaeris radicata* benefited from the environment where soil resources are horizontally heterogeneously distributed by exploiting primarily its favourable microhabitats (Fig. S3.8; Fig. S3.11). This may have led to the reduction in plant species richness. In contrast to what we hypothesized, the grain size of soil heterogeneity did not influence plant species richness or diversity. We suggest that this is because the relative small difference in grain size used in our study. Hence, it is likely that the plant species in our

study can outgrow both patch sizes. Further studies should test whether soil heterogeneity imposed at larger grain sizes can promote plant species diversity.

As expected, different soil patches within the two horizontally heterogeneous nutrient soils supported distinct sub-communities, as indicated by the different species richness between the two types of soil patches. This may have led to a greater variation in species composition among the patches in the two horizontally heterogeneous soils than in homogeneous soil. Moreover, species composition varied more in the low nutrient soil patches than in the high nutrient soils patches within the two heterogeneous nutrient soils, and the same pattern was found between the homogeneous low and high nutrient soils. This is likely because on nutrient deficient soils, plant species are more sensitive to local-scale plant-plant and plant-soil interactions so that the species composition of the community can be more various. This result therefore indicates that soil nutrient level played an important role in structuring the plant community in our study.

Even though several studies have suggested that vertical heterogeneity in soil nutrients has the potential to promote plant species diversity (e.g. Fitter 1982), we found no evidence for this in our experiment. In contrast, compared to the homogeneous plots with an equivalent amount of nutrients, plant species diversity was reduced in the heterogeneous soils where high nutrient soils and low nutrient soils were distributed at the bottom half and top half layer, respectively, even though statistical evidence for this was weak. This is likely because only deep-rooting plant species, such as *Centaurea jacea* and *Sanguisorba minor* can use the resources that are available at the deeper layer only (Fig. S3.9: a greater relative abundance of these species in heterogeneous-bottom soils than in homogeneous soils), which may exclude other shallow-rooting plant species that have no access to soil nutrients in the deeper layer (Berendse 1981), resulting in a reduction in plant species diversity.

Even though this was only true for the final harvest, in heterogeneous pH plots with large grain size plant species diversity was higher than in plots with homogeneous soil, and this supports the heterogeneity diversity hypothesis (Ricklefs 1977, Tilman 1982, Tilman and Pacala 1993). One basic idea behind the positive effects of soil pH heterogeneity on plant species diversity is that different plant species prefer different soil microenvironments, and therefore a heterogeneous environment (with more microhabitats) will support more plant species. However, this does not seem to be true in our study, because we did not find significant differences in species richness or diversity (Fig. 3.3D, E), plant species composition (Fig. S3.2F) or the variation in species composition among patches (Fig. 3.3F),

between low and high pH soil patches within the two heterogeneous pH soils with small and large patch sizes. One possibility is that at the final harvest, the growth of plant species were more even in the large-patch heterogeneous pH soil than in homogeneous and small-patch heterogeneous pH soils. This view is supported by the observation that the relative abundance of the most dominant plant species *Centaurea jacea*, decreased from 27.4% in the homogeneous soil and 28.3% in the small-patch heterogeneous soil to 10.6% in the large-patch heterogeneous soil (Fig. S3.10C, D). This lower dominance of *C. jacea* may have allowed more space for subordinate species such as *Leontodon hispidus, Sanguisorba minor* and *Festuca rubra*, as well as the establishment of rare species such as *Briza media, Companula rotundifolia and Veronica chamaedrys.*

Plant species richness is generally thought to decline with increasing soil nutrient availability (e.g. Tilman 1987, Gough et al. 2000a, Rajaniemi 2002, Suding et al. 2005). This pattern can be explained by strong competition due to higher productivity in high nutrient soils (Grime 1973, Tilman 1982, Waide et al. 1999, Dooson and Gouon 2001). However, species richness can also increase with increasing soil nutrient availability when the productivity is low (Grime 1973, Klinkhamer and De Jong 1985). We expected a positive relationship between plant species diversity and soil pH levels in the present study. However, we did not find that soil pH influenced plant species richness or diversity, even though it influenced plant species composition and component plant species showed different responses to soil pH level (Fig. S3.10). Our results therefore suggest that soils with different pH levels may support different plant communities that are similar in species richness and diversity.

In conclusion, soil heterogeneity in nutrient supply decreased plant species richness and diversity, most likely due to competitive exclusion driven by the dominant species that benefited from the heterogeneous environment. In contrast, soil heterogeneity in pH increased plant species richness and diversity probably through providing refuges for subordinate and rare species. These effects prevailed when species richness and diversity were quantified at small spatial scale. Our study highlights that soil heterogeneity effects on plant species diversity depend on soil factor type, focal scale and the size of soil patches. Therefore, future studies testing soil heterogeneity-plant species diversity relationships should distinguish different soil factors at various spatial scales at which plant species diversity and soil heterogeneity are measured.

Acknowledgement

We thank Jan van Walsem and Frans Möller and many others for helping with the experiment. This work was funded by the China Scholarship Council (No. 201406510030).

Supporting information

Table S3.1. Soil chemical analysis of different soils. Means (±SE), sample size (n) and *F*-values of one-way ANOVA are given. Tukey post-hoc tests were made among the five soils, mean values sharing the same superscript (a-d) are not significantly different. Symbols give: *** $P<0.001$, ** $P<0.01$ and * $P< 0.05$. The amount of $N\text{-}NH_4$, $N\text{-}NO_3$ and $P\text{-}PO_4$ (mg/kg dry soil sample) were determined by adding 30.0 ml of 0.01 mol/L $CaCl_2$ solution to soil samples (3.0 g), shaking mechanically for at least 2 h at room temperature (20 ℃), filtering the solution and analyzing the nutrients in the soil extracts in a flow analyzer (SKALAR SAN plus system). Soil pH-H_2O was determined by adding 25.0 ml demi-water to soil samples (volume 5.0 ml), shaking for 5 min and measuring 2 h later. Soil organic matter was determined by measuring the difference between weights of the oven-dried (105 ℃) soil samples (5.0-10.0 g) before and after being heated in a furnace at 550 ℃.

Soil	$N\text{-}NH_4$ (mg/kg) (n=5)	$P\text{-}PO_4$ (mg/kg) (n=5)	$N\text{-}NO_3$ (mg/kg) (n=5)	K (mg/kg) (n=5)	pH (H_2O) (n=3)	Organic matter (%) (n=2)
Low nutrient soil	2.95 ± 0.29^{a}	0.35 ± 0.09	1.80 ± 0.16^{c}	22.64 ± 1.59^{b}	6.97 ± 0.01^{b}	0.98 ± 0.03^{c}
Medium nutrient/Low pH soil	2.09 ± 0.62^{ab}	0.11 ± 0.04	5.55 ± 0.79^{b}	30.70 ± 2.73^{ab}	6.86 ± 0.02^{c}	2.09 ± 0.06^{b}
High nutrient soil	2.22 ± 0.41^{ab}	0.29 ± 0.13	9.25 ± 1.52^{a}	35.98 ± 1.70^{a}	6.52 ± 0.01^{d}	3.46 ± 0.05^{a}
Medium pH soil	3.15 ± 0.44^{a}	0.16 ± 0.10	6.47 ± 0.68^{ab}	29.34 ± 3.57^{ab}	7.01 ± 0.04^{b}	2.19 ± 0.07^{b}
High pH soil	0.84 ± 0.07^{b}	0.19 ± 0.10	6.60 ± 0.52^{ab}	28.92 ± 2.62^{ab}	7.15 ± 0.00^{a}	2.37 ± 0.09^{b}
One-way ANOVA	4.95**	0.98	9.82***	3.50*	147.61***	190.86***

Table S3.2. Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), horizontal soil heterogeneity (homogeneous vs. small patch vs. large patch) and their interaction on plot-scale (40 cm × 40 cm) species richness and diversity. *F*-values, *P*-values and degrees of freedom of a linear mixed-effects model, and *t*-values and *P*-values of overall contrasts among levels of the horizontal soil heterogeneity treatment are presented.

			Plot-scale richness			Plot-scale diversity (*H'*)		
	DF	denDF	*F*	*t*	*P*	*F*	*t*	*P*
(A) *Soil heterogeneity in nutrients*								
Time (T)	2	24	4.04	-	**0.031**	5.67	-	**0.010**
Heterogeneity (H)	2	12	1.29	-	0.310	0.04	-	0.962
Homogeneous vs. Small patch	-	-	-	0.25	0.804	-	-0.05	0.964
Homogeneous vs. large patch	-	-	-	1.50	0.142	-	-0.26	0.796
Small patch vs. large patch	-	-	-	1.25	0.219	-	-0.22	0.831
T × H	4	24	1.04	-	0.406	1.46	-	0.245
(B) *Soil heterogeneity in pH*								
Time (T)	2	24	4.90	-	**0.016**	11.50	-	0.000
Heterogeneity (H)	2	12	0.31	-	0.740	3.71	-	0.056
Homogeneous vs. Small patch	-	-	-	-0.78	0.440	-	-0.76	0.455
Homogeneous vs. large patch	-	-	-	-0.31	0.757	-	1.89	0.067
Small patch vs. large patch	-	-	-	0.47	0.642	-	2.65	**0.012**
T × H	4	24	1.33	-	0.286	2.87	-	**0.045**

Table S3.3. Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), horizontal soil heterogeneity (homogeneous vs. small patch vs. large patch) and their interaction on patch-scale (10 cm × 10 cm) species richness and diversity, and mean Bray-Curtis dissimilarity among patches. *F*-values, *P*-values and degrees of freedom of a linear mixed-effects model, and *t*-values and *P*-values of overall contrasts among levels of the horizontal soil heterogeneity treatment are presented.

			Patch-scale richness			Patch-scale diversity (*H'*)			Mean dissimilarity[1]		
	DF	denDF	*F*	*t*	*P*	*F*	*t*	*P*	*F*	*t*	*P*
(A) *Soil heterogeneity in nutrients*											
Time (T)	2	24	7.25	-	**0.003**	12.88	-	**<0.001**	10.30	-	**0.001**
Heterogeneity (H)	2	12	12.25	-	**0.001**	1.99	-	0.179	4.60	-	**0.033**
Homogeneous vs. small patch	-	-	-	3.71	**<0.001**	-	1.26	0.215	-	-2.51	**0.017**
Homogeneous vs. large patch	-	-	-	4.69	**<0.001**	-	1.97	0.057	-	-2.73	**0.010**
Small patch vs. large patch	-	-	-	0.98	0.335	-	0.71	0.485	-	-0.23	0.820
T × H	4	24	1.26	-	0.313	0.80	-	0.535	1.54	-	0.221
(B) *Soil heterogeneity in pH*											
Time (T)	2	24	16.66	-	**<0.001**	24.07	-	**<0.001**	28.35	-	**<0.001**
Heterogeneity (H)	2	12	0.25	-	0.781	3.06	-	0.084	0.43	-	0.658
Homogeneous vs. small patch	-	-	-	-0.57	0.575	-	0.28	0.780	-	0.91	0.367
Homogeneous vs. large patch	-	-	-	-0.66	0.516	-	-1.99	0.055	-	0.62	0.542
Small patch vs. large patch	-	-	-	-0.09	0.929	-	-2.27	**0.030**	-	-0.30	0.767
T × H	4	24	2.07	-	0.117	4.17	-	**0.011**	1.44	-	0.252

[1] Data were ln-transformed

Table S3.4. Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), grain size of horizontal soil heterogeneity (small patch vs. large patch), patch type (low vs. high) within small- and large-patch heterogeneous soils and their interactions on patch-scale (10 cm × 10 cm) species richness and diversity, and mean Bray-Curtis dissimilarity among different types soil patch. *F*-values, *P*-values and degrees of freedom of a linear mixed-effects model are presented.

			Patch-scale richness		Patch-scale diversity (*H'*)		Mean dissimilarity[1]	
	DF	denDF	*F*	*P*	*F*	*P*	*F*	*P*
(A) *Soil heterogeneity in nutrients*								
Time (T)	2	32	3.45	**0.044**	7.09	**0.003**	9.14	**0.001**
Grain size (G)	1	8	0.75	0.412	0.38	0.557	0.60	0.462
Patch type (PT)	1	8	37.90	**<0.001**	1.28	0.290	8.56	**0.019**
T × G	2	32	0.26	0.776	0.10	0.902	0.44	0.646
T × PT	2	32	0.49	0.616	1.43	0.255	0.88	0.425
G × PT	1	8	0.36	0.563	0.87	0.379	0.06	0.812
T × G × PT	2	32	1.33	0.278	0.14	0.870	0.47	0.630
(B) *Soil heterogeneity in pH*								
Time (T)	2	32	6.44	**0.005**	9.62	**0.001**	6.36	**0.005**
Grain size (G)	1	8	0.02	0.883	7.68	**0.024**	1.20	0.305
Patch type (PT)	1	8	0.58	0.469	0.06	0.816	1.47	0.260
T × G	2	32	0.04	0.966	4.53	**0.019**	1.77	0.186
T × PT	2	32	1.13	0.336	2.15	0.133	0.28	0.754
G × PT	1	8	0.21	0.660	0.22	0.652	0.14	0.720
T × G × PT	2	32	0.92	0.409	0.32	0.726	0.15	0.862

[1] Data were ln-transformed

Table S3.5. Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), vertical soil heterogeneity (homogeneous vs. heterogeneous-top vs. heterogeneous-bottom) and their interaction on plot-scale (40 cm × 40 cm) species richness and diversity. *F*-values, *P*-values and degrees of freedom of a linear mixed-effects model, and *t*-values and *P*-values of overall contrasts among levels of the vertical soil heterogeneity treatment are presented.

			Plot-scale richness			Plot-scale diversity (*H*′)		
	DF	denDF	*F*	*t*	*P*	*F*	*t*	*P*
Time (T)	2	24	4.13	-	**0.029**	10.14	-	**0.001**
Heterogeneity (H)	2	12	0.43	-	0.660	1.64	-	0.235
Homogeneous vs. heterogeneous-top	-	-	-	0.91	0.369	-	0.52	0.610
Homogeneous vs. heterogeneous-bottom	-	-	-	0.61	0.548	-	-1.24	0.222
Heterogeneous-top vs. heterogeneous-bottom	-	-	-	-0.30	0.763	-	-1.76	0.088
T × H	4	24	0.82	-	0.528	0.49	-	0.741

Table S3.6. Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), vertical soil heterogeneity (homogeneous vs. heterogeneous-top vs. heterogeneous-bottom) and their interaction on patch-scale (10 cm × 10 cm) species richness and diversity, and mean Bray-Curtis dissimilarity among patches. *F*-values, *P*-values and degrees of freedom of a linear mixed-effects model, and *t*-values and *P*-values of overall contrasts among levels of the vertical soil heterogeneity treatment are presented.

			Patch-scale richness			Patch-scale diversity (*H*′)			Mean dissimilarity		
	DF	denDF	*F*	*t*	*P*	*F*	*t*	*P*	*F*	*t*	*P*
Time (T)	2	24	20.89	-	**<0.001**	29.79	-	**<0.001**	34.48	-	**<0.001**
Heterogeneity (H)	2	12	3.86	-	0.051	3.77	-	0.054	4.14	-	**0.043**
Homogeneous vs. Heterogeneous-top	-	-	-	1.34	0.188	-	0.35	0.731	-	0.48	0.636
Homogeneous vs. Heterogeneous-bottom	-	-	-	2.78	**0.009**	-	-2.18	**0.036**	-	-2.22	**0.033**
Heterogeneous-top vs. Heterogeneous-bottom	-	-	-	1.44	0.160	-	-2.53	**0.016**	-	-2.70	**0.011**
T × H	4	24	0.72	-	0.584	1.04	-	0.406	0.85	-	0.510

Table S3.7. Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), the homogenous soil treatment (low vs. medium vs. high) and their interaction on plot-scale (40 cm × 40 cm) species richness and diversity. *F*-values, *P*-values and degrees of freedom of a linear mixed-effects model, and *t*-values and *P*-values of overall contrasts among levels of the homogeneous soil treatment are presented.

			Plot-scale richness			Plot-scale diversity (*H'*)		
	DF	denDF	*F*	*t*	*P*	*F*	*t*	*P*
(A) *Soil nutrients*								
Time (T)	2	24	4.23	-	**0.027**	6.84	-	**0.004**
Nutrient level (L)	2	12	2.50	-	0.124	2.24	-	0.149
Low vs. medium	-	-	-	-2.22	**0.033**	-	0.15	0.879
Low vs. high	-	-	-	-1.33	0.192	-	1.90	0.065
Medium vs. high	-	-	-	0.89	0.381	-	1.75	0.089
T × L	4	24	0.09	-	0.986	1.09	-	0.383
(B) *Soil pH*								
Time (T)	2	24	4.99	-	**0.015**	15.60	-	**<0.001**
pH level (L)	2	12	0.38	-	0.695	0.52	-	0.606
Low vs. medium	-	-	-	0.65	0.517	-	-0.84	0.407
Low vs. high	-	-	-	-0.16	0.871	-	0.09	0.931
Medium vs. high	-	-	-	-0.82	0.419	-	0.93	0.361
T × L	4	24	1.34	-	0.283	0.95	-	0.454

Table S3.8. Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), the homogenous soil treatment (low vs. medium vs. high) and their interaction on patch-scale (10 cm × 10 cm) species richness and diversity, and mean Bray-Curtis dissimilarity among patches. *F*-values, *P*-values and degree of freedom of a linear mixed-effects model, and *t*-values and *P*-values of overall contrasts among levels of the homogeneous soil treatment are presented.

			Patch-scale richness			Patch-scale diversity (*H'*)			Mean dissimilarity		
	DF	denDF	*F*	*t*	*P*	*F*	*t*	*P*	*F*	*t*	*P*
(A) *Soil nutrients*											
Time (T)	2	24	23.79	-	**<0.001**	25.98	-	**<0.001**	67.95	-	**<0.001**
Nutrient level (L)	2	12	13.86	-	**<0.001**	8.87	-	**0.004**	4.23	-	**0.041**
Low vs. medium	-	-	-	-4.99	**<0.001**	-	2.37	**0.024**	-	1.60	0.118
Low vs. high	-	-	-	-3.95	**<0.001**	-	4.20	**<0.001**	-	2.90	**0.006**
Medium vs. high	-	-	-	1.04	0.308	-	1.84	0.075	-	1.30	0.202
T × L	4	24	2.08	-	0.115	1.60	-	0.206	2.59	-	0.062
(B) *Soil pH*											
Time (T)	2	24	18.78	-	**<0.001**	35.28	-	**<0.001**	46.36	-	**<0.001**
pH level (L)	2	12	2.82	-	0.099	2.45	-	0.128	2.82	-	0.099
Low vs. medium	-	-	-	2.23	**0.033**	-	-2.11	**0.043**	-	-1.92	0.063
Low vs. high	-	-	-	0.40	0.690	-	-0.46	0.648	-	0.25	0.804
Medium vs. high	-	-	-	-1.83	0.077	-	1.65	0.109	-	2.17	**0.037**
T × L	4	24	1.70	-	0.183	1.19	-	0.341	1.68	-	0.188

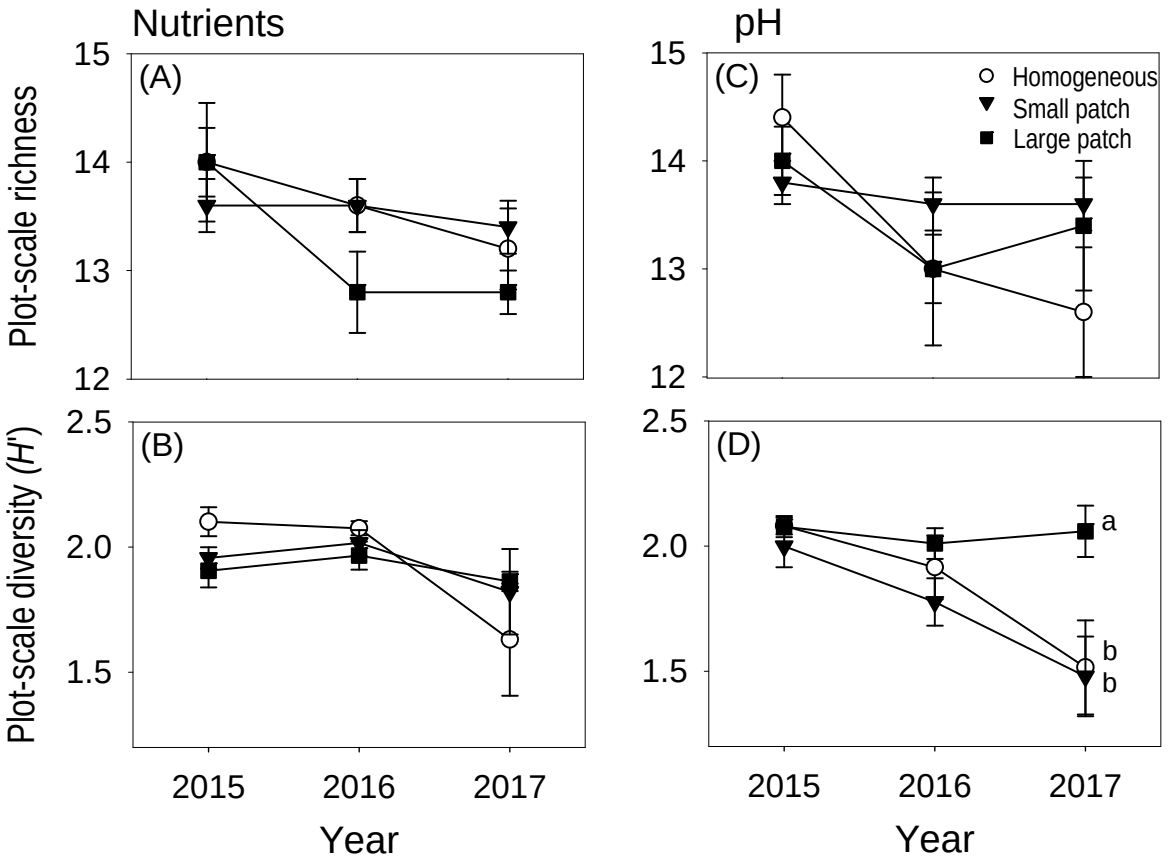


Fig. S3.1. Plot-scale (40 × 40 cm) species richness (A and C) and diversity (B and D) in the three horizontal soil heterogeneity treatments (i.e. the medium homogeneous soil, small patch and large patch heterogeneous soils) from 2015 to 2017. Mean values (±SE) are given. "Homogeneous", "Small patch" and "Large patch" represent homogeneous soil (medium nutrient/pH soil) and heterogeneous soil with small and large patch sizes, respectively. See Table S3.2 for statistical results. A significant time and soil heterogeneity treatment interaction occurred in (D), post-hoc comparisons among levels of the horizontal soil heterogeneity treatments were made for each year separately: means that share the same letter (a–b) within a year are not significantly different at $P < 0.05$.

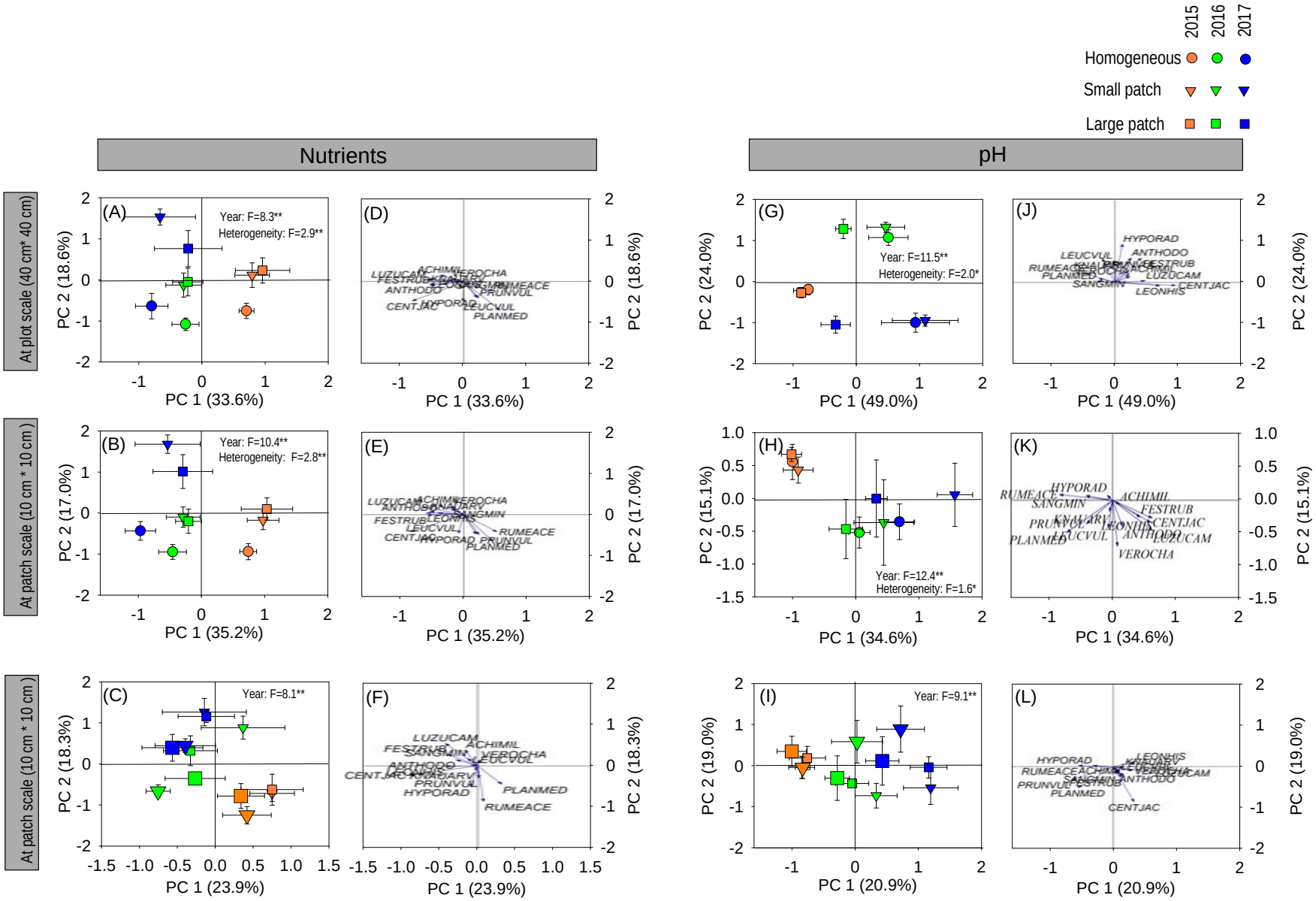

2015
2016
2017
Homogeneous
Small patch
Large patch
Nutrients
pH
At plot scale (40 cm* 40 cm)
At patch scale (10 cm * 10 cm)
At patch scale (10 cm * 10 cm)
(A)
Year: F=8.3**
Heterogeneity: F=2.9**
(B)
Year: F=10.4**
Heterogeneity: F=2.8**
(C)
Year: F=8.1**
(D)
(E)
(F)
(G)
Year: F=11.5**
Heterogeneity: F=2.0*
(H)
Year: F=12.4**
Heterogeneity: F=1.6*
(I)
Year: F=9.1**
(J)
(K)
(L)
PC 1 (33.6%)
PC 2 (18.6%)
PC 1 (35.2%)
PC 2 (17.0%)
PC 1 (23.9%)
PC 2 (18.3%)
PC 1 (49.0%)
PC 2 (24.0%)
PC 1 (34.6%)
PC 2 (15.1%)
PC 1 (20.9%)
PC 2 (19.0%)
ACHIMIL
ANTHODO
CENTJAC
FESTRUB
HYPORAD
LEUCVUL
LEONHIS
LUZUCAM
PLANMED
PRUNVUL
RUMEACE
SANGMIN
VEROCHA

Fig. S3.2. Principal Component analyses (unconstrained PCA) showing effects of time, horizontal soil heterogeneity treatments on plant community composition at plot-scale (A and G) and patch-scale (B and H), and effects of time, grain size of soil heterogeneity (small patch vs. large patch), and patch type (low vs. high nutrient/pH patch) within the small- and large-patch heterogeneous soils on plant community composition at patch-scale (C and I). Mean sample scores (±SEs for both axes) for the horizontal soil heterogeneity treatments (A-C and G-I) and for each plant species (D-F and J-L) are presented. Circles, triangles and squares represent homogeneous soils (medium nutrient/pH soil) and heterogeneous soils with small and large patch sizes, respectively. Yellow, green and blue represent samples in year 2015, 2016 and 2017, respectively. Small and large shapes (in C and I) separate low and high nutrient/pH soil patches within the two horizontally heterogeneous soils. *F*-values of significant effects of a constrained redundancy analysis (RDA) on year and horizontal soil heterogeneity treatment (Heterogeneity) or patch type (in C and F) are also presented for each panel. Asterisks indicate significance: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$. Abbreviations: ANTHODO- *Anthoxanthum odoratum*, FESTRUB-*Festuca rubra*, LUZUCAM-*Luzula campestris*, ACHIMIL-*Achillea millefolium*, CENTJAC-*Centaurea jacea*, HYPORAD-*Hypochaeris radicata*, KNAUARV-*Knautia arvensis*, LEONHIS-*Leontodon hispidus*, LEUCVUL-*Leucanthemum vulgare*, PLANMED-*Plantago media*, PRUNVUL-*Prunella vulgaris*, RUMEACE-*Rumex acetosa*, VEROCHA-*Veronica chamaedrys* and SANGMIN-*Sanguisorba minor*.

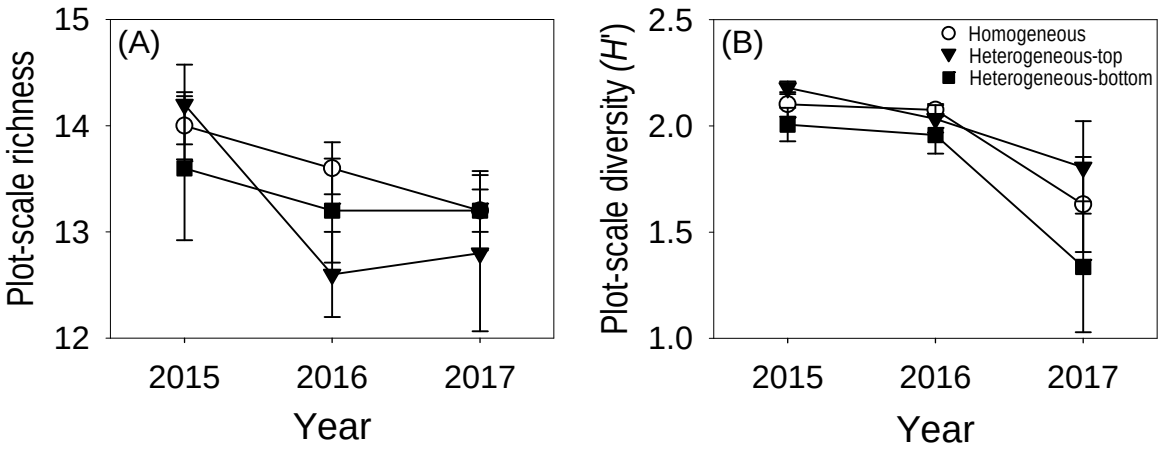


Fig. S3.3. Plot-scale (40 × 40 cm) species richness (A) and diversity (B) in the vertical soil nutrient heterogeneity treatments (i.e. the medium homogeneous nutrient soil, heterogeneous-top and heterogeneous-bottom soils) from 2015 to 2017. Mean values (±SE) are given. "Homogeneous", "heterogeneous-top" and "heterogeneous-bottom" represent homogeneous soil (medium nutrient soil), heterogeneous soils where high nutrient soils were located at the top half and bottom half layer, respectively. See Table S3.5 for statistical results.

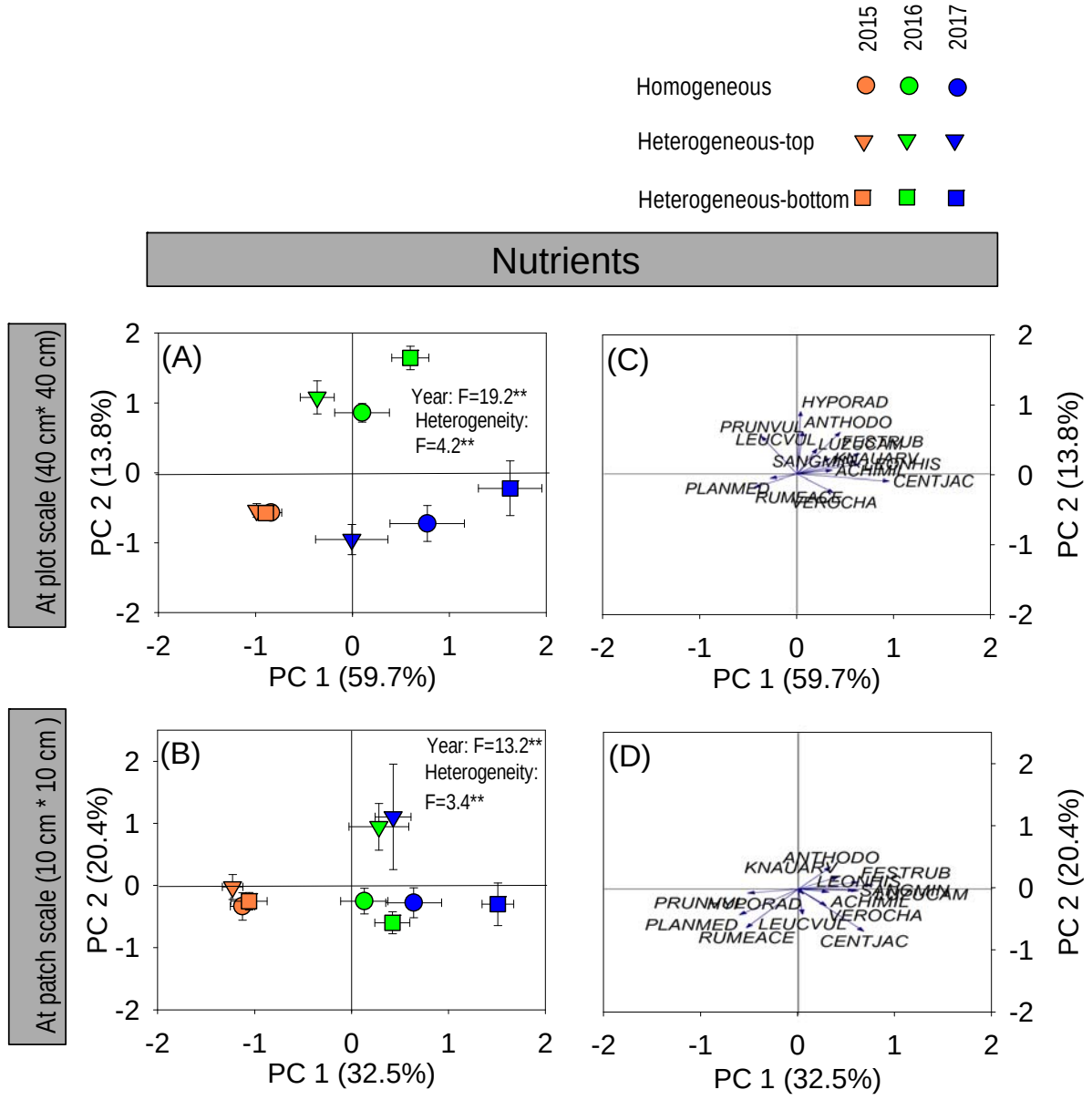


Fig. S3.4. Principal Component analyses (unconstrained PCA) showing effects of time, vertical soil heterogeneity in nutrients on plant community composition at plot-scale (A) and patch-scale (B). Mean sample scores (±SEs for both axes) for the three soil treatments (A-B) and for each plant species (C-D) are present. Circles, triangles and squares represent homogeneous soils (medium nutrient soil) and heterogeneous soils where high nutrient soils are located at the top and bottom layer, respectively. Yellow, green and blue represent samples in year 2015, 2016 and 2017, respectively. *F*-values of significant effects of a constrained redundancy analysis (RDA) on year and vertical soil heterogeneity in nutrient (vertical heterogeneity) are also presented for each panel. Asterisks indicate significance: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$. Abbreviations: ANTHODO-*Anthoxanthum odoratum*, FESTRUB-*Festuca rubra*, LUZUCAM-*Luzula campestris*, ACHIMIL-*Achillea millefolium*, CENTJAC-*Centaurea jacea*, HYPORAD-*Hypochaeris radicata*, KNAUARV-*Knautia arvensis*, LEONHIS-*Leontodon hispidus*, LEUCVUL-*Leucanthemum vulgare*, PLANMED-*Plantago media*, PRUNVUL-*Prunella vulgaris*, RUMEACE-*Rumex acetosa*, VEROCHA-*Veronica chamaedrys* and SANGMIN-*Sanguisorba minor*.

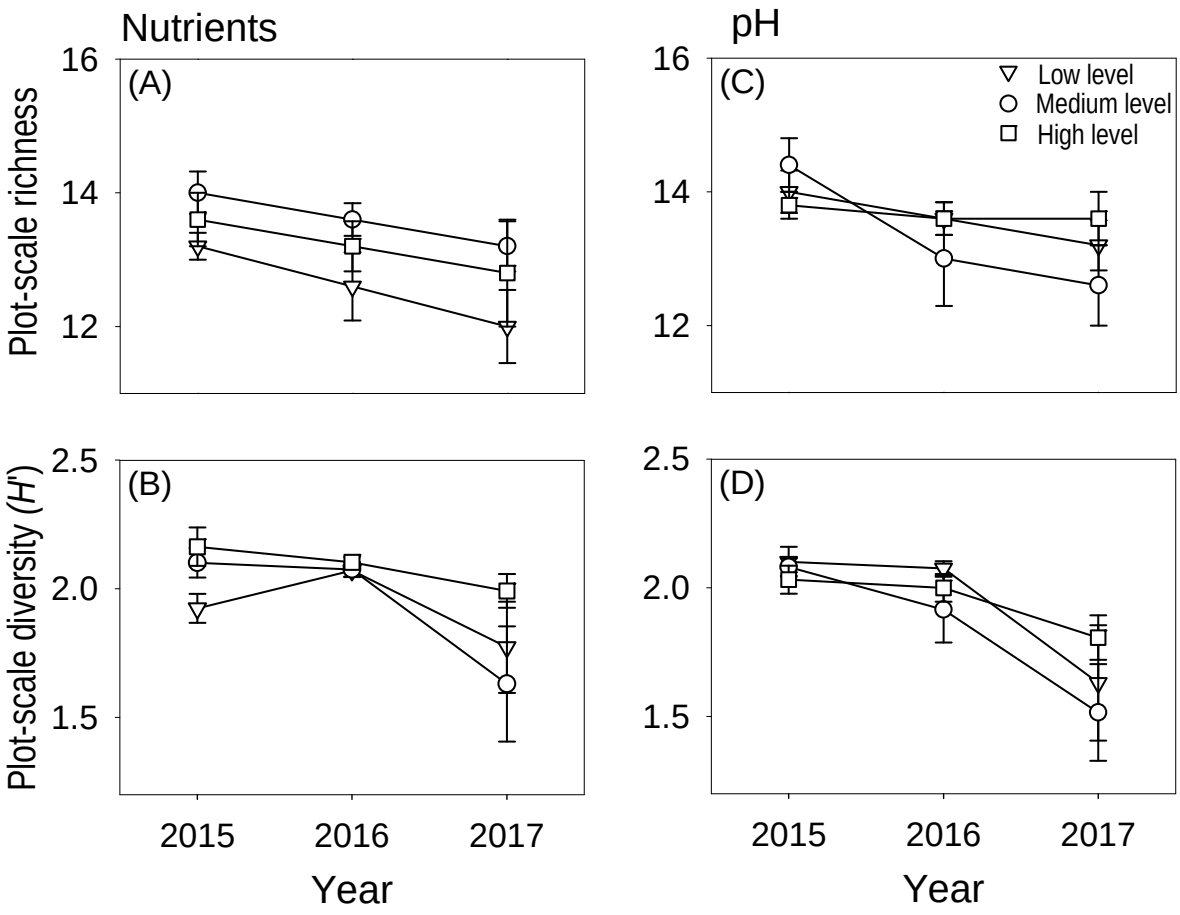


Fig. S3.5. Plot-scale (40 cm × 40 cm) plant richness (A and C) and diversity (B and D) in the three homogeneous soil treatments from 2015 to 2017. Mean values (±SE) are given. "Low level", "medium level" and "high level" represent the three homogeneous soil treatments with low, medium and high level of soil nutrient/pH, respectively. See Table S3.7 for statistical results.

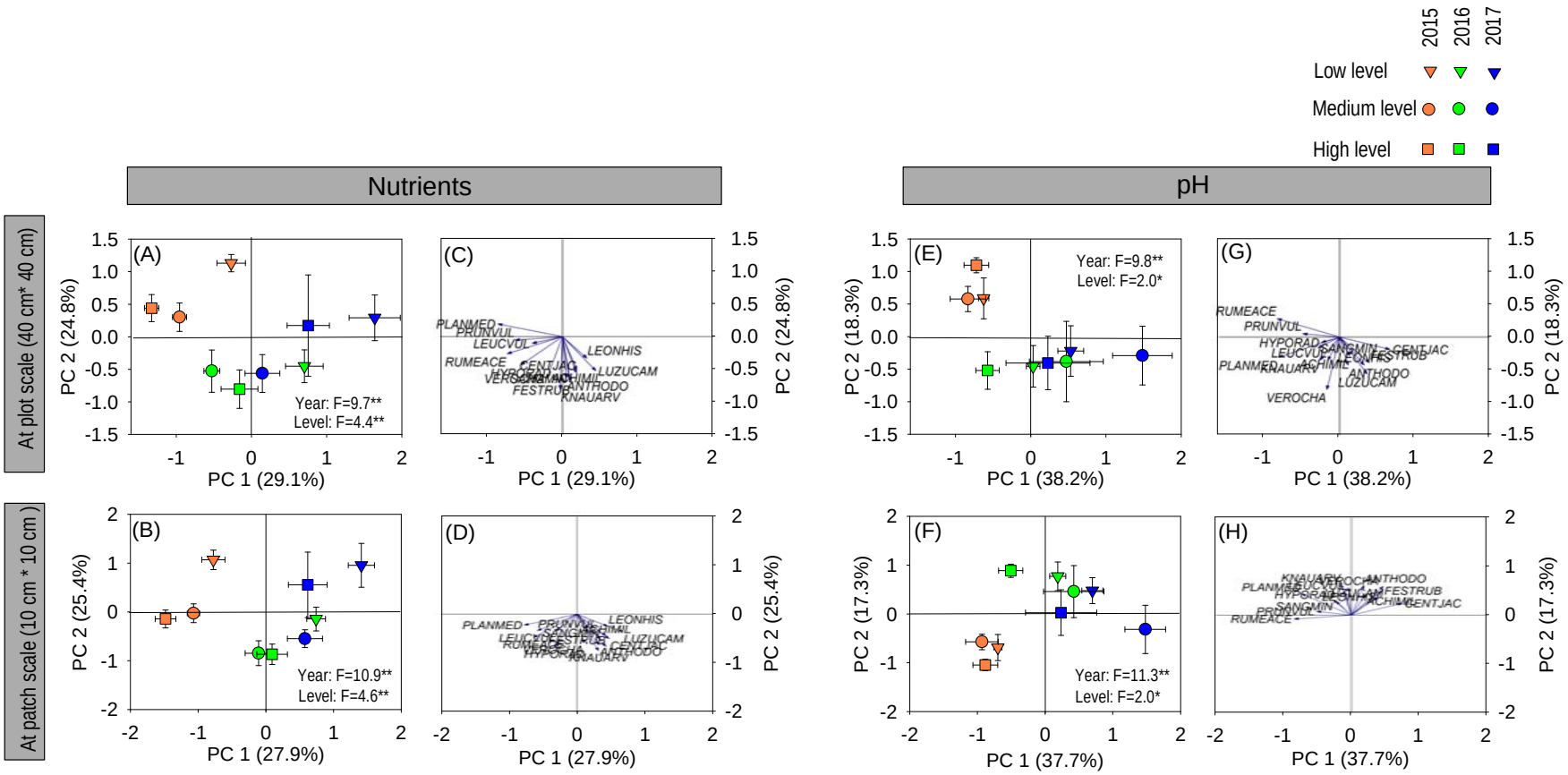


Fig. S3.6. Principal Component analyses (unconstrained PCA) showing effects of time and soil nutrient/pH level on plant community composition at plot-scale (A and E) and patch-scale (B and F). Mean sample scores (±SEs for both axes) for the three homogeneous soils (A-B and E-F) and for each plant species (C-D and G-H) are presented. Circles, triangles and squares represent low, medium and high nutrient/pH soil, respectively. Yellow, green and blue represent samples in year 2015, 2016 and 2017 respectively. *F*-values of significant effects of a constrained redundancy analysis (RDA) on year and soil nutrient/pH level are also presented for each panel. Asterisks indicate significance: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$. Abbreviations: ANTHODO- *Anthoxanthum odoratum*, FESTRUB-*Festuca rubra*, LUZUCAM-*Luzula campestris*, ACHIMIL-*Achillea millefolium*, CENTJAC-*Centaurea jacea*, HYPORAD-*Hypochaeris radicata*, KNAUARV-*Knautia arvensis*, LEONHIS-*Leontodon hispidus*, LEUCVUL-*Leucanthemum vulgare*, PLANMED-*Plantago media*, PRUNVUL-*Prunella vulgaris*, RUMEACE-*Rumex acetosa*, VEROCHA-*Veronica chamaedrys* and SANGMIN-*Sanguisorba minor*.

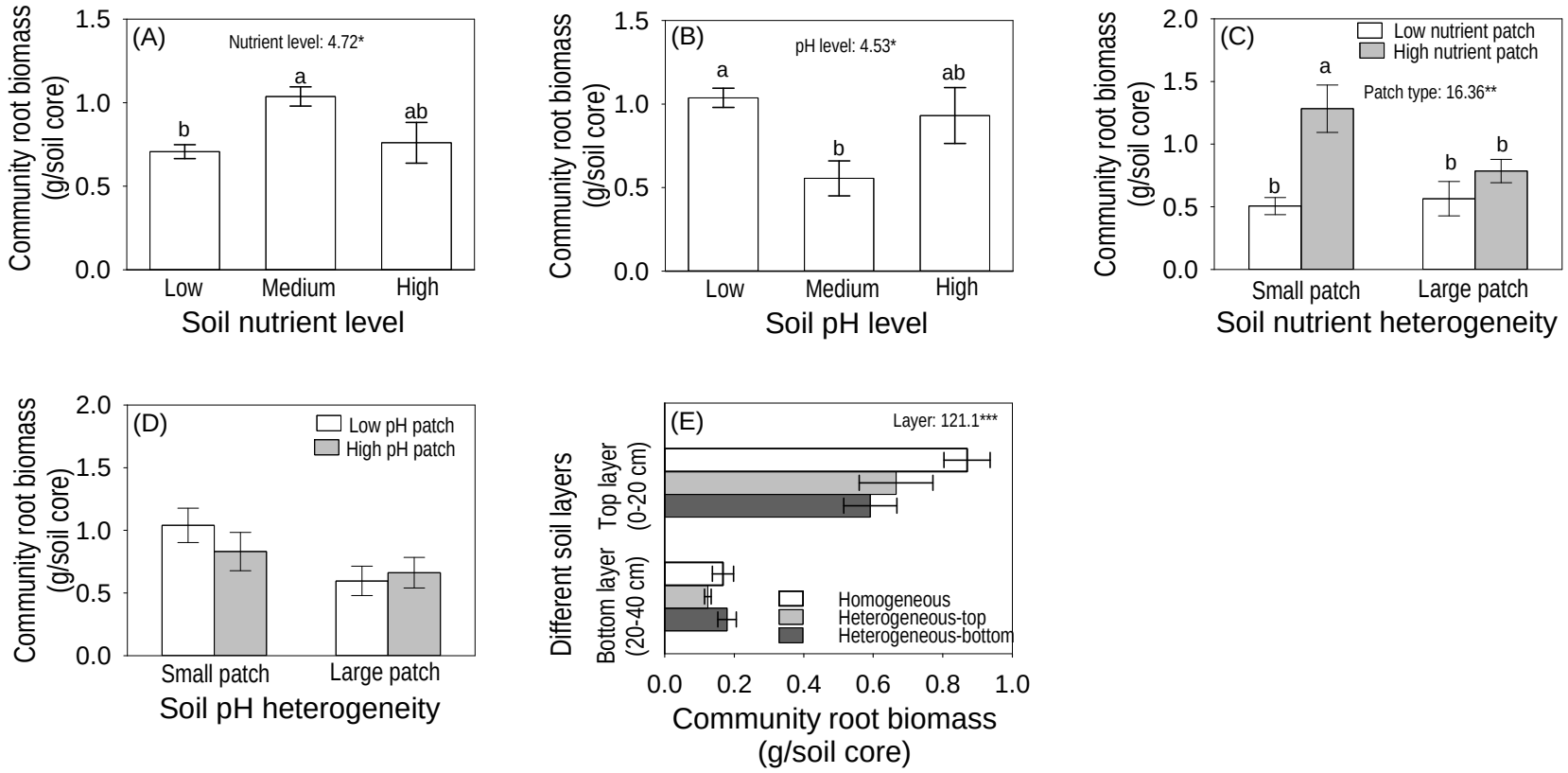


Fig. S3.7. Community root biomass (g/soil core) in the three homogeneous nutrient treatments (A) and pH treatments (B), in the low and high nutrient/pH soil patches within the two horizontally heterogeneous soils (C and D), as well as in the top (0-20 cm) and bottom layers (20-40 cm) within vertically heterogeneous soils (E) at the final harvest (2017). In A and B, "Low", "Medium" and "High" represent the three homogeneous soil treatments with low, medium and high level of soil nutrient/pH, respectively. In C and D, "Small" and "Large" represent heterogeneous soil with small and large patch sizes, respectively. In E, "Homogeneous", "heterogeneous-top" and "heterogeneous-bottom" represent homogeneous soil (medium nutrient soil), heterogeneous soils where high nutrient soils are located at the top and bottom layer, respectively. Mean values (±SE) and significant effects of one-way ANOVA with soil nutrient/pH level (A and B), two-way ANOVA with grain size of soil heterogeneity in nutrient/pH, patch type and their interaction (C and D) and two-way ANOVA with vertical soil heterogeneity in nutrient, soil layer and their interaction (E) are given: * P<0.05, ** P<0.01 and *** P<0.001. Tukey post-hoc tests were made among all soils in each panel. Mean values sharing the same letter (a-b) are not significantly different.

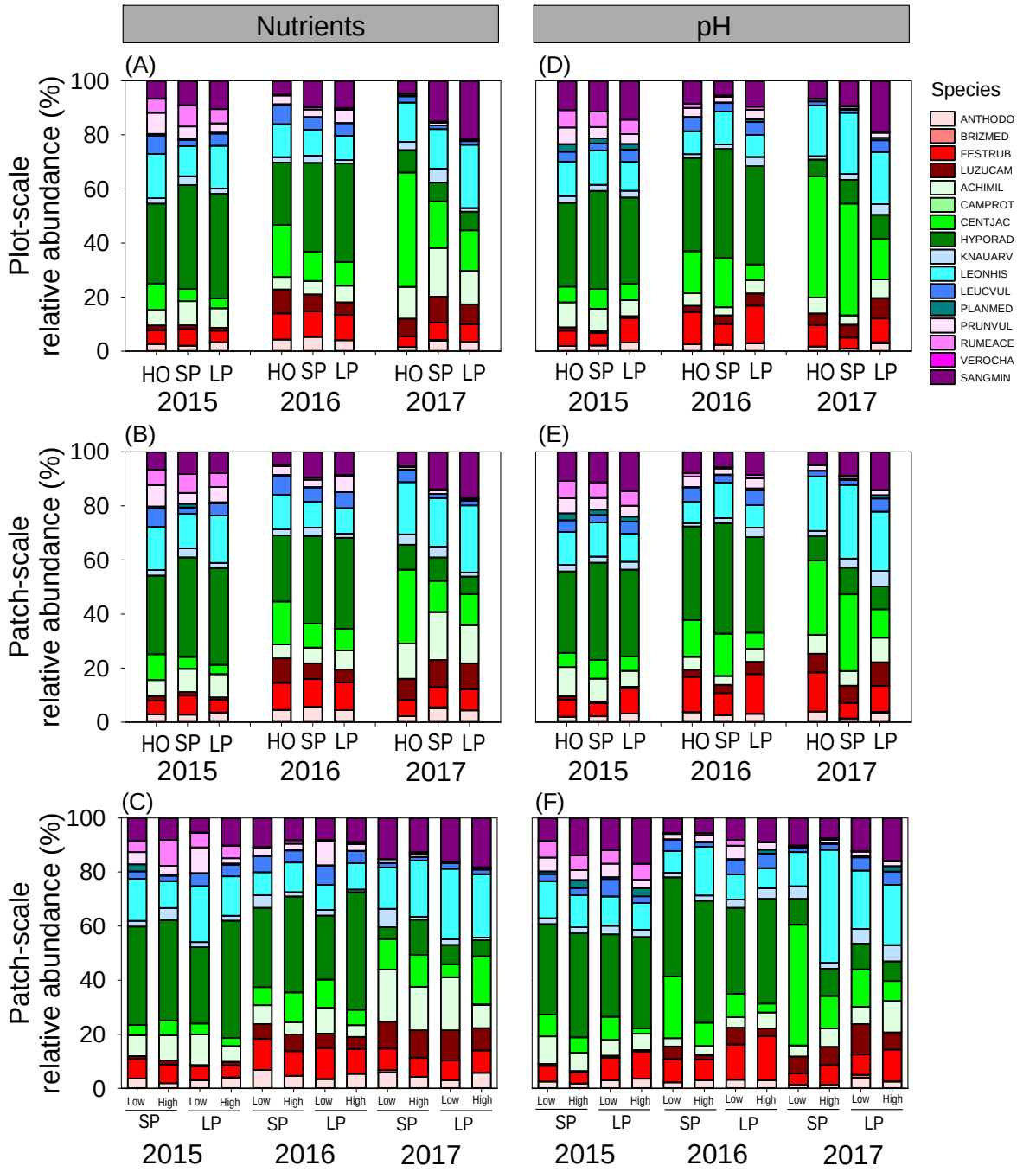


Fig. S3.8. Plot-scale (A and D) and patch-scale (B and E) relative abundance of the 16 plant species in the horizontal soil heterogeneity treatments (i.e. the medium homogeneous soil, small patch and large patch heterogeneous soils) from 2015 to 2017, as well as patch-scale (C and F) relative abundance of the 16 plant species in the low and high nutrient/pH soil patches within the two horizontally heterogeneous soil treatments from 2015 to 2017. Mean values are given. "HO", "SP" and "LP" represent homogeneous soil (medium nutrient/pH soil) and heterogeneous soil with small and large patch sizes, respectively. "Low" and "High" indicate low and high nutrient/pH soil patches within the two heterogeneous nutrient/pH heterogeneity treatments. Different colours represent different plant species. Abbreviations: ANTHODO- *Anthoxanthum odoratum*, BRIZMED- *Briza media*, FESTRUB-*Festuca rubra*, LUZUCAM-*Luzula campestris*, ACHIMIL-*Achillea millefolium*, CAMPROT-*Campanula rotundifolia*, CENTJAC-*Centaurea jacea*, HYPORAD-*Hypochaeris radicata*, KNAUARV-*Knautia arvensis*, LEONHIS-*Leontodon hispidus*, LEUCVUL-*Leucanthemum vulgare*, PLANMED-*Plantago media*, PRUNVUL-*Prunella vulgaris*, RUMEACE-*Rumex acetosa*, VEROCHA-*Veronica chamaedrys* and SANGMIN-*Sanguisorba minor*.

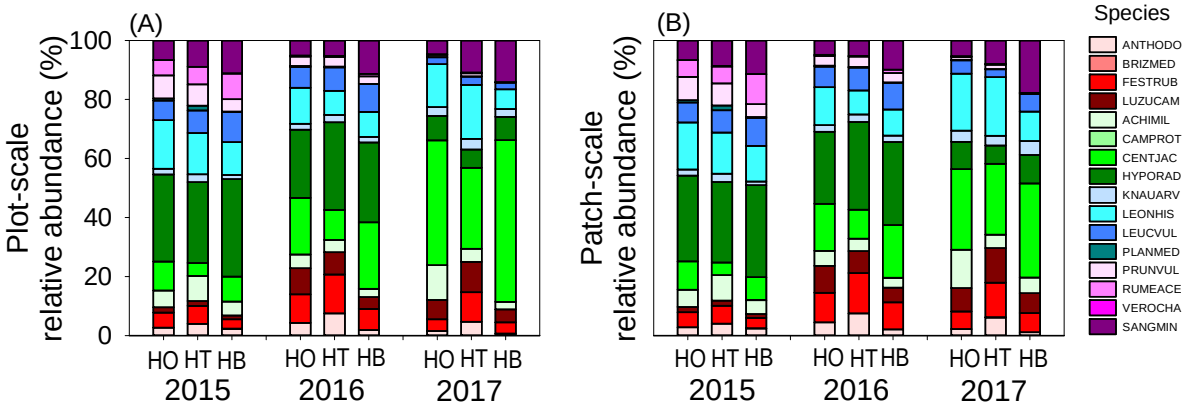


Fig. S3.9. Plot-scale (A) and patch-scale (B) relative abundance of the 16 plant in the vertical soil nutrient heterogeneity treatments (i.e. the medium homogeneous nutrient soil, heterogeneous-top and heterogeneous-bottom soils) from2015 to 2017. Mean values are given. "HO", "HT" and "HB" represent homogeneous soil (medium nutrient soil), heterogeneous soils where high nutrient soils are located at the top (heterogeneous-top) and bottom layer (heterogeneous-bottom), respectively. Different colours represent different plant species. Abbreviations: ANTHODO- *Anthoxanthum odoratum*, BRIZMED- *Briza media*, FESTRUB-*Festuca rubra*, LUZUCAM-*Luzula campestris*, ACHIMIL-*Achillea millefolium*, CAMPROT-*Campanula rotundifolia*, CENTJAC-*Centaurea jacea*, HYPORAD-*Hypochaeris radicata*, KNAUARV-*Knautia arvensis*, LEONHIS-*Leontodon hispidus*, LEUCVUL-*Leucanthemum vulgare*, PLANMED-*Plantago media*, PRUNVUL-*Prunella vulgaris*, RUMEACE-*Rumex acetosa*, VEROCHA-*Veronica chamaedrys* and SANGMIN-*Sanguisorba minor*.

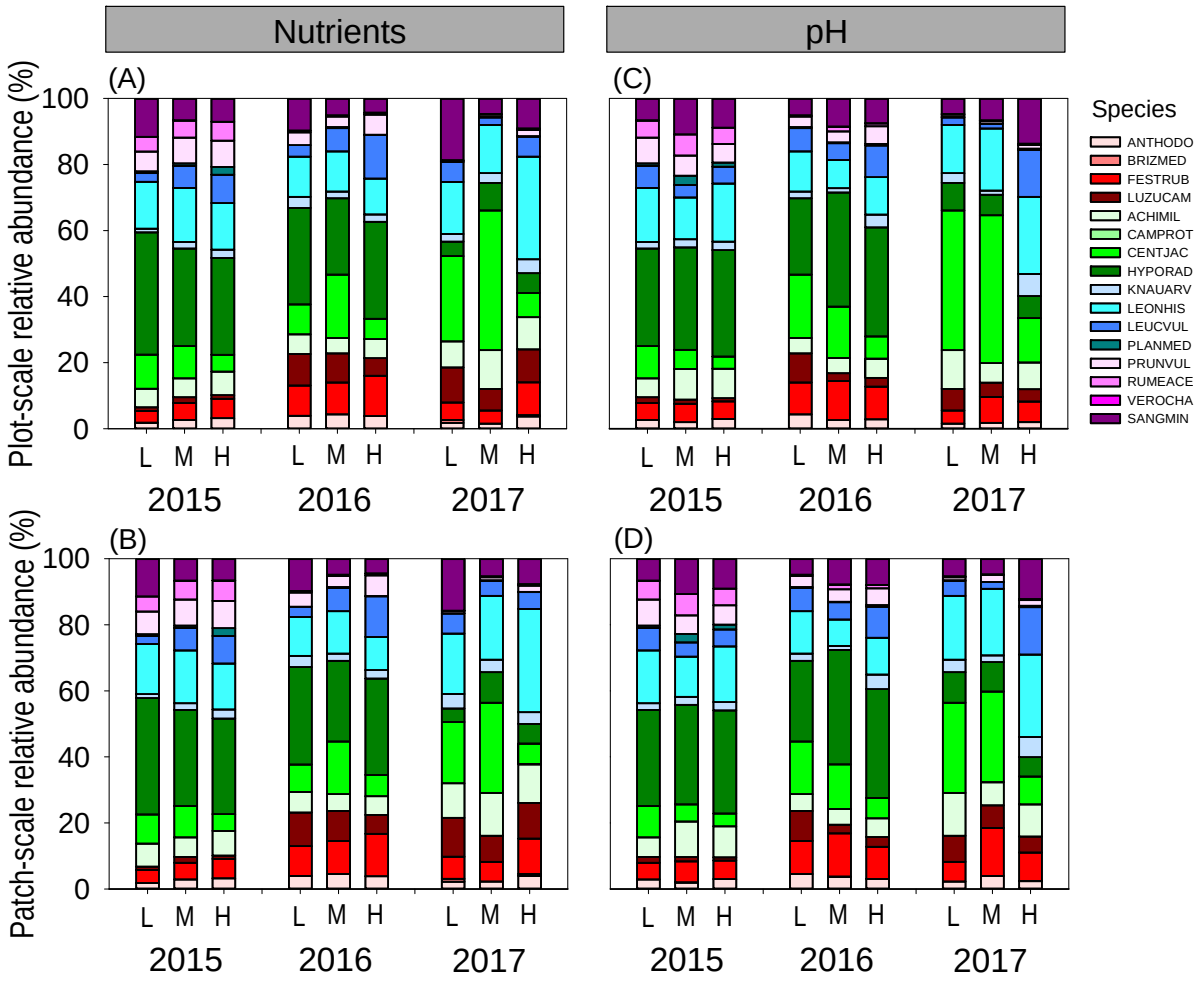


Fig. S3.10. Plot-scale (A and C) and patch-scale (B and D) relative abundance of the 16 plant species under the three homogeneous soil nutrient (A and B) and pH (C and D) treatments from 2015 to 2017. Mean values are given. "L", "M" and "H" represent the three homogeneous soil treatments with low, medium and high level of soil nutrient/pH, respectively. Different colours represent different plant species. Abbreviations: ANTHODO-*Anthoxanthum odoratum*, BRIZMED- *Briza media*, FESTRUB-*Festuca rubra*, LUZUCAM-*Luzula campestris*, ACHIMIL-*Achillea millefolium*, CAMPROT-*Campanula rotundifolia*, CENTJAC-*Centaurea jacea*, HYPORAD-*Hypochaeris radicata*, KNAUARV-*Knautia arvensis*, LEONHIS-*Leontodon hispidus*, LEUCVUL-*Leucanthemum vulgare*, PLANMED-*Plantago media*, PRUNVUL-*Prunella vulgaris*, RUMEACE-*Rumex acetosa*, VEROCHA-*Veronica chamaedrys* and SANGMIN-*Sanguisorba minor*.

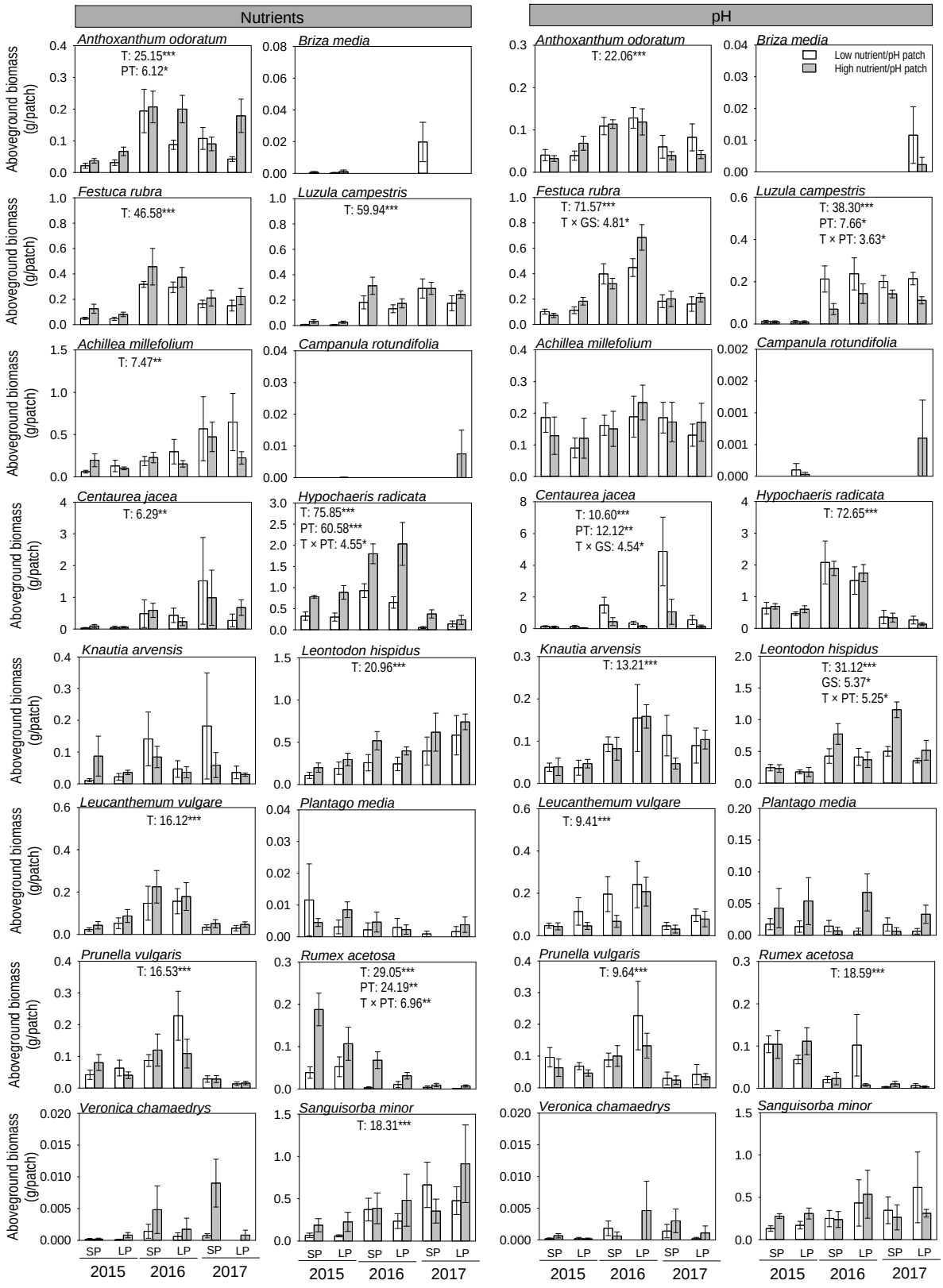
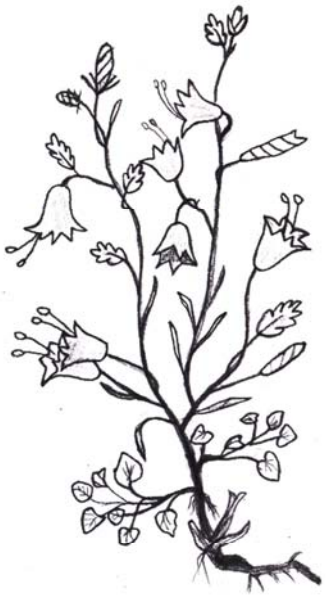


Fig. S3.11. Aboveground biomass (g/patch) in the low and high nutrient/pH soil patches within the two horizontally heterogeneous soil treatments from 2015 to 2017. "SP" and "LP" represent the heterogeneous soil with small and large patch sizes, respectively. Mean values (±SE), *F*-values and significant effects of three-way ANOVA with time (T), soil heterogeneity in nutrient/pH (H), patch type (PT) and their interaction are given: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$. All data of aboveground biomass were ln (x+1) transformed.



Chapter 4

Density-dependency and plant-soil feedback: former plant abundance influences competitive interactions between two grassland plant species through plant-soil feedbacks

Wei Xue, T. Martijn Bezemer and Frank Berendse

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Abstract

Negative plant-soil feedbacks (PSFs) are thought to promote species coexistence, but most evidence is derived from theoretical models and data from plant monoculture experiments.

We grew *Anthoxanthum odoratum* and *Centaurea jacea* in field plots in monocultures and in mixtures with three ratios (3:1, 2:2 and 1:3) for three years. We then tested in a greenhouse experiment the performance of *A. odoratum* and *C. jacea* in pots planted with monocultures and 1:1 mixtures and filled with live and sterile soils collected from the field plots.

In the greenhouse experiment, *C. jacea* produced less aboveground biomass in soil conditioned by *C. jacea* monocultures than in soil conditioned by *A. odoratum* monocultures, while the aboveground biomass of *A. odoratum* in general did not differ between the two monospecific soils. The negative PSF effect was greater in the 1:1 plant mixture than in plant monocultures for *A. odoratum* but did not differ for *C. jacea*. In the greenhouse experiment, the performance of *C. jacea* relative to *A. odoratum* in the 1:1 plant mixture was negatively correlated to the abundance of *C. jacea* in the field plot where the soil was collected from. This relationship was significant both in live and sterile soils. However, there was no relationship between the performance of *A. odoratum* relative to *C. jacea* in the 1:1 plant mixture in the greenhouse experiment and the abundance of *A. odoratum* in the field plots.

We show that the response of a plant to PSF depends on whether the focal species grows in monocultures or in mixtures and on the identity of the species. Interspecific competition can exacerbate the negative plant-soil feedbacks compared to intraspecific competition when a plant competes with a stronger interspecific competitor. Moreover, the abundance of a species in mixed plant communities, via plant-soil feedback, negatively influences the relative competitiveness of that species when it grows later in interspecific competition, but this effect varies between species. This phenomenon may contribute to the coexistence of competing plants under natural conditions through preventing the dominance of a particular plant species.

Key words: Interspecific competition, intraspecific competition, plant abundance, plant density, plant-soil feedbacks, plant-soil interactions, soil biota

Introduction

Plants can alter soil abiotic and biotic properties. These changes in the soil can subsequently influence the performance of the same or other plant species that grow on this substrate, which is known as plant-soil feedback (Bever et al. 1997, Ehrenfeld et al. 2005, van der Putten et al. 2013). Plant-soil feedbacks can influence plant growth positively through the accumulation of soil nutrients (Berendse 1990, Wardle et al. 1999, Chapman et al. 2006) or symbiotic mutualists (Klironomos 2002, van der Putten et al. 2016) and negatively by nutrient immobilization or depletion (Berendse 1994), or accumulation of soil pathogens (van der Putten et al. 2016). Positive feedbacks are thought to promote plant dominance and homogenize plant communities (Hartnett and Wilson 1999, O'Connor et al. 2002), while negative feedbacks allow species coexistence and increase plant species diversity (Bever et al. 1997, Bever 2003, Petermann et al. 2008).

Plant-soil feedbacks of a particular plant species are generally tested by comparing the performance of that species on soils that were planted with monocultures of the same species and on soils planted with monocultures of the other species (Bever et al. 1997, Kulmatiski et al. 2008, Brinkman et al. 2010). However, in the field, plants rarely grow in monocultures and often compete with other plant species. The presence of other species will not only affect the soil resources that are available for each of the component species (Casper and Jackson 2002, Hawkes et al. 2005), but also affect the composition of the soil community including both mutualistic and pathogenic organisms (Bartelt-Ryser et al. 2005, Hausmann and Hawkes 2009, Eisenhauer 2016). Conspecific plant-soil feedback effects may be weaker in soils conditioned by plant mixtures than in soils conditioned by monocultures of that species, due to the lower abundance of the focal species in plant mixtures (Hawkes et al. 2013). Several studies have suggested that negative plant-soil feedbacks in natural systems might be density-dependent (Bell et al. 2006, Bagchi et al. 2010, van de Voorde et al. 2011, Kos et al. 2013, Comita et al. 2014). If the local density of a species in a plant community increases, species-specific soil pathogens are expected to increase as well, thus decreasing the per capita fitness of that plant species (Bagchi et al. 2010). However, only one study has empirically examined this so far (Dudenhöffer et al. 2018).

The response of a plant to plant-soil feedback depends on whether the plant grows individually or in competition with other plants, and several studies have shown that negative plant-soil feedbacks are generally enhanced in the presence of competitors (e.g. Callaway et al. 2004, Kulmatiski et al. 2008, Shannon et al. 2012, Hol et al. 2013). However, whether plants

grown in monocultures (and hence experience intraspecific competition) and grown in plant mixtures (and experience interspecific competition) respond differently to plant-soil feedbacks is poorly understood. Casper and Castelli (2007) proposed that negative conspecific plant-soil feedbacks are expected to be more pronounced when plants compete with the same species (intraspecific competition) than with other plant species (interspecific competition). However, Kardol et al. (2007) and Petermann et al. (2008) reported that the negative response of plants to plant-soil feedback is stronger when the plants grew in interspecific competition than when they grew in intraspecific competition. Recently, Jing et al. (2015) demonstrated that when two plants compete, the soil feedback effects of one species can negatively influence the other competing species more than that it influences the conspecific species, even though the conspecific species suffers from negative plant-soil feedbacks when grown alone. Hence, in interspecific competition, it can be either advantageous or disadvantageous for a species to grow in the soil conditioned by the same species (Jing et al. 2015). It remains unresolved how the density of a plant species in a mixed plant community, via plant-soil feedback, influences the competition between two plant species when they grow later in the soil where these plants were previously growing.

Many plant-soil feedback studies compare the growth of a plant in sterilised soils with and without addition of a soil inoculum (e.g. van der Putten et al. 1993, Kardol et al. 2006, Hol et al. 2013). However, adding a small amount of live soil to a large amount of sterilised bulk soil may not result in representative soil communities. For example, the density of the soil community may be very different from that observed outdoors in the field (Brinkman et al. 2010). Alternatively, plant-soil feedbacks can be determined by comparing plant growth in sterilised and unsterilised pure soils. Sterilising typically results in increased availability of soil nutrients (Brinkman et al. 2010). Plant-soil feedbacks can be driven simultaneously by abiotic and biotic changes in the soil. Therefore, the difference between the performance of a plant in unsterilised and sterilised soil would be a net effect of the elimination of soil biota and the increase in soil nutrients.

The aim of the present study was to investigate how, via plant-soil feedbacks, the abundance of a species in a plant community consisting of two species influences the growth and competition between these two species when they grow later in the soil. We grew the grass *Anthoxanthum odoratum* and the forb *Centaurea jacea* in field plots in monocultures and in mixtures with three ratios (3:1, 2:2 and 1:3) for three years. We then tested the performance of *A. odoratum* and *C. jaceae* in a greenhouse experiment with the two species grown in

monocultures and in 1:1 mixtures in pots filled with either unsterilised or sterilized soils collected from the field plots. We specifically hypothesised that: (1) plants will produce less biomass in "own" soil (conditioned by conspecific monocultures) than in "foreign" soil (conditioned by heterospecific monocultures) as conspecific plant-soil feedbacks are generally negative. As a consequence, when two plant species grow in mixtures (interspecific competition), the plant species that encounters its "own" soil will be at competitive disadvantage. (2) Negative conspecific plant-soil feedback effects will increase with a greater abundance of that species during the previous growth phase (i.e. conditioning phase) in the plant community, e.g. due to the build-up of species-specific soil pathogens in the soil. (3) Negative conspecific plant-soil feedbacks will be stronger when plants grow in 1:1 plant mixtures than when they grow in monocultures, as the competing species will not suffer from negative plant-soil feedbacks and hence will be a stronger competitor. (4) Plant-soil feedbacks will be stronger in live soil than in sterile soil as sterilization will eliminate the soil biota that can drive the plant-soil feedbacks.

Methods and materials

Plant species

Our study species were *Anthoxanthum odoratum* L. (Poaceae) and *Centaurea jacea* L. (Asteraceae). *A. odoratum* is a perennial grass that produces closely connected ramets, while *C. jacea* is a long-lived perennial herb that has monocarpic shoots and can form extensive belowground branches (Jongejans and de Kroon 2005). Both species are native grassland species in western Europe. They have similar life history strategies, i.e., with clonal growth as well as sexual reproduction (Hartemink et al. 2004) and commonly coexist in meadows (van Ruijven and Berendse 2003).

Garden experiment

We performed a long-term competition experiment with *A. odoratum* and *C. jacea* in field plots from April 2013 to September 2015. In this experiment, we planted *A. odoratum* and *C. jacea* in monocultures as well as in mixtures at three planting ratios (3:1, 2:2 and 1:3) in plots (1×1 m^2). Black soil (total N: 2.13 g kg^{-1}; total C: 28.2 g kg^{-1}; total P: 2.39 g kg^{-1}) was used in each plot. Soils collected from these experimental plots were coded as Ao soil (conditioned

by monocultures of *A. odoratum*), Cj soil (conditioned by monocultures of *C. jacea*), 3Ao/1Cj soil (conditioned by a 3:1 mixture of *A. odoratum* and *C. jacea*), 2Ao/2Cj soil (conditioned by a 2:2 mixture of *A. odoratum* and *C. jacea*) and 1Ao/3Cj soil (conditioned by a 1:3 mixture of *A. odoratum* and *C. jacea*), respectively. The total number of seedlings planted in each plot was 144. Each treatment had five replicate blocks, yielding 25 plots. Plots were weeded regularly. In September 2015, we clipped each plant in the central 60 × 60 cm^2 at a height of 1 cm and determined the aboveground biomass of each of the two plant species in each plot after oven-drying it to constant weight. In February 2016, we collected the soil from the central area of 60 × 60 cm^2, to a depth of 20 cm of each experimental plot. The soil from each plot was sieved (1.5 cm mesh) and separated in two parts. Half of the soil from each plot was sterilized by γ-irradiation (minimum 25KGray, Isotron, Ede, the Netherlands) so that there were 50 different conditioned soils (5 planting treatments × 2 sterilization treatments × 5 replicate blocks).

Greenhouse experiment

Each of the 50 soil samples was used to fill three pots (21 cm in top-diameter and 18 cm in height) with 5.6 kg soil in each pot (Fig. 4.1) so that the entire experiment consisted of 150 pots. Pots filled with soils collected in the same field block were allocated to the same block in the greenhouse experiment so that there were five blocks corresponding to the blocks in the field experiment. Pots of different treatments were randomized within each block. Before filling the pots, we placed a piece of filter paper (15 cm in diameter) at the bottom of each pot to prevent soil from passing through holes in the bottom of the pot but allowing vertical movement of water. Each pot was placed on a tray to prevent possible contamination through leachate. For soil chemical analysis, we randomly selected three blocks, and took soil samples (5 planting treatments × 2 sterilization treatments) from each selected block. Unsterilised soils (not sterilised by γ-irradiation; live soil) and sterile soils (sterilized by γ-irradiation) were analysed separately. We measured soil organic matter content, nutrient content (NH_4, NO_3 and PO_4), water content and pH (Table 4.1; Methods S4.1).

We purchased seeds of *A. odoratum* and *C. jacea* from a specialized company (Cruydthoeck, Nijeberkoop, the Netherlands). All seeds of each plant species were evenly sown on plastic trays filled with steamed potting soil (0.03N-0.03P-0.03K, Seed Starting Potting Mix, Miracle-Gro Lawn Products, Inc., Marysville) that facilitates fast root development in a

heated greenhouse (20.0 ℃ average temperature, 70.2 % average relative humidity). The trays were watered daily. One week after germination, the trays with seedlings were moved to an unheated greenhouse (12.8 ℃ average temperature, 70.3 % average relative humidity) until they were transplanted into the pots.

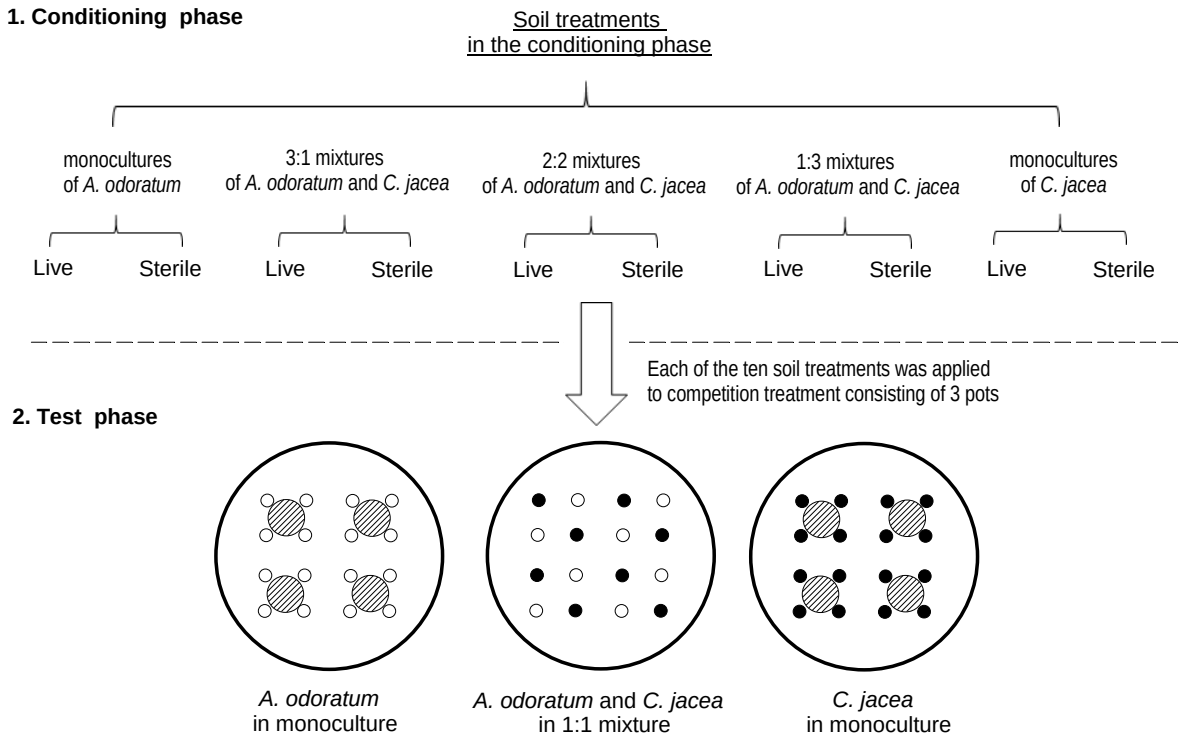

Fig. 4.1. Schematic representation of the experimental design. (1) Conditioning phase: conditioned soils were collected from a three-year field experiment, in which soils were conditioned separately by monocultures of *A. odoratum* and *C. jacea*, as well as mixtures of these two species at three planting ratios (3:1, 2:2 and 1:3). Conditioned soils were either sterilized or not (i.e., live and sterile), resulting in 10 soil treatments. (2) Test phase: we planted either 16 plants of species *A. odoratum* or species *C. jacea* in monocultures, or eight plants of each of the species in mixtures in each of the ten soil treatments in a greenhouse experiment. White and black dots represent the initial positions where *A. odoratum* and *C. jacea* were planted. The shaded circles within each pot represent the positions where we took soil samples.

We planted similar-sized seedlings of *A. odoratum* and *C. jacea* in each pot in either monocultures or 1:1 mixtures (Fig. 4.1). In monocultures, we planted 16 seedlings of *A. odoratum* or *C. jacea* in each pot. In the 1:1 mixtures, we planted eight seedlings of *A. odoratum* and eight seedlings of *C. jacea* in alternating positions (Fig. 4.1). After one week, we replaced dead seedlings. All other species emerging from the seed bank of the soil were removed manually during the experiment.

The experiment was maintained for 90 days (from 11 April to 11 July 2016) in the same unheated greenhouse. During the experiment, the mean temperature and the relative humidity in the greenhouse were 17.4 ℃ and 67.5 %. Water was added to all pots three times per week.

Harvest and measurement

Ninety days after transplanting, we clipped all plants at the soil level. The two different plant species in the 1:1 mixtures were harvested separately. We also took four soil cores (4.0 cm diameter, straight down to the bottom of pot) in each pot to measure the root mass (Fig. 4.1). We only took soil cores from pots planted with monocultures because it was not possible to separate roots of the two different plant species in the mixtures. We then washed all root samples over a 0.5 mm sieve. Aboveground parts and belowground parts of each plant species in each pot were oven-dried at 70 °C for 48 h and weighted.

Data analysis

In the garden experiment, we used replacement diagrams to show the biomass of the two species in all planting treatments (i.e., monocultures and mixtures at three different ratios: 3:1, 2:2 and 1:3), and used the relative crowding coefficient (k) of a species to assess its competitive ability in the mixtures relative to that in the monocultures (De Wit 1960). We calculated k as: $((z-1)/z)(O/M)(\frac{O}{M}-1)^{-1}$, in which z is the planting frequency of a species in the mixtures, O and M are the aboveground biomass of that species in mixtures and in monocultures, respectively. We then used linear regressions to assess whether k was dependent on the planting frequencies of each species.

In the greenhouse experiment, we first calculated aboveground biomass per plant of *A. odoratum* and *C. jacea* in each pot, since monocultures had twice as many individuals per species at the start of the experiment as mixtures. As belowground biomass was determined by taking soil cores, it was calculated as the mean of the root biomass in the four soil cores and not as the root biomass per pot. Data of aboveground and belowground biomass were log transformed to improve the normality and homogeneity of variance.

We first analysed the aboveground and belowground biomass of each plant species on the two soils collected from the monospecific plots in the field (i.e., Ao soil and Cj soil collected from

the garden experiment; monospecific soil) to test whether there was a soil type effect on the performance of the two species. We first performed a full-model analysis including species (*A. odoratum* vs. *C. jacea*), soil type (soil type was tested as "own" soil vs. "foreign" soil), sterilization (live vs. sterile), competition (monocultures vs. mixtures; only for aboveground biomass) and their interactions as fixed factors, with block as random factor. Subsequently, we separately analysed biomass of each species using a mixed-effect ANOVA with soil type, sterilization, competition (only for aboveground biomass) and their interactions as fixed factors, and block as a random factor.

To test if plant-soil feedback (PSF) differed in response to competition mode and the sterilization treatment, we calculated the PSF as the log-ratio of plant biomass on "own" and "foreign" soils ($\ln \frac{Biomass_{own}}{Biomass_{foreign}}$) for each combination of species, competition and sterilization. PSFs were calculated separately for each replicate and based on aboveground biomass (aboveground PSF) and belowground biomass (belowground PSF). We analysed the PSF-values using a full-model analysis including species, sterilization, competition (only for aboveground PSF) and their interactions as fixed factors, with block as random factor, followed by separate analyses using mixed-effect ANOVA with sterilization, competition (only for aboveground PSF) and the interaction (only for aboveground PSF) as fixed factors, and block as a random factor.

Since in the pots planted with plant mixtures the growth of the two species is not independent, we evaluated the competition between the two species by calculating for each pot the competitive balance index (CB). The CB was calculated as: $\ln \frac{MIX_{Ao}}{MIX_{Cj}}$ with MIX_{Ao} and MIX_{Cj} representing the biomass of *A. odoratum* and *C. jacea* in the 1:1 mixtures in the greenhouse experiment, respectively. Using this index, the performance of the two species in a pot was combined. CB will be equal to zero if the two species perform equally well in mixtures; CB will be positive if the biomass of *A. odoratum* is higher than *C. jacea* and negative if *C. jacea* biomass is higher. A one-sample *t*-test was used to test whether the CB differed from zero. We used two-way ANOVA to test the effects of soil type (Ao soil vs. Cj soil), sterilization and their interaction on the CB on the two monospecific soil types. Block was included as a random factor. Means of the CB values on different monospecific soils were compared using a post-hoc test for pairwise comparisons.

Further, the relationship between the growth of either *A. odoratum* or *C. jacea* in the greenhouse experiment and its former abundance in the field plots was analysed using linear

regression for each species, sterilization and competition (only for aboveground biomass) combination. The relationship between the CBs between the two species in the greenhouse experiment and the former abundance of either *A. odoratum* or *C. jacea* in the field plots was also analysed using linear regression separately for live and sterile soils.

We performed all data analysis using R (version 3.3.2) (http://www.r-project.org) in RStudio (version 1.0.44) (http://rstudio.org). Linear mixed-effect models were fitted with *nlme* (version 3.1-128) (Pinheiro et al. 2016). Post-hoc comparisons were tested as planned contrasts using the *multcomp* package (version 1.4-6) in R (Hothorn et al. 2008). All data were checked graphically for normality and homogeneity for variance.

Results

Biomass of the two plant species and soil properties in the field plots

In all mixtures, the total aboveground biomass of *C. jacea* per plot was significantly greater than that of *A. odoratum* (Fig. S4.1). *A. odoratum* showed an inverse sigmoid curve in the replacement diagram (Fig. S4.1) and its competitive ability (relative crowding coefficient, *k*) decreased with increasing frequency (Fig. S4.2A). There was no significant relationship between the competitive ability of *C. jacea* and its planting frequency (Fig. S4.2B).

The amount of P-PO_4, N-NH_4, and the pH (H_2O) were significantly higher in sterile soil than in live soil but there was no difference in the amount of N-NO_3, in soil moisture and in organic matter between live and sterile soils (Table 4.1). Overall, none of the measured properties except P-PO_4 was different among the five soils (Ao soil, 3Ao/1Cj soil, 2Ao/2Cj soil, 1Ao/3Cj soil and Cj soil; Table 4.1). The amount of P-PO_4 was overall higher in Cj soil than in other soils (Table 4.1).

Plant-soil feedback effects in monospecific soils in the greenhouse experiment

In the greenhouse experiment, *C. jacea* overall produced less aboveground biomass when grown in "own" soil (Cj soil) than in "foreign" soil (Ao soil), while aboveground biomass of *A. odoratum* did not differ between the two soils (Table S4.1A, S2A; Fig. 4.2A, B). *A. odoratum*

Table 4.1. Abiotic characteristics of live and sterile soils collected from the field plots. Means (±SE), and *F*- and *P*-values of two-way ANOVAs are given. Mean values sharing the same superscript (a-d) are not significantly different among the ten soils in each column (Tukey post hoc tests). Ao soil and Cj soil represent soils conditioned by monocultures of *A. odoratum* and *C. jacea*, respectively, while 3Ao/1Cj soil, 2Ao/2Cj soil and 1Ao/3Cj soil represent the soils conditioned by 3:1, 2:2 and 1:3 mixtures of *A. odoratum* and *C. jacea*, respectively. *** $P<0.001$, ** $P<0.01$, * $P<0.05$.

Sterilization	Soil	P-PO_4 (mg/kg)	N-NO_3 (mg/kg)[1]	N-NH_4 (mg/kg)	pH (H_2O)	Moisture (%)	Organic Matter (%)
Live soils	Ao soil	0.00 ± 0.00^{d}	0.49	6.14 ± 4.00^{b}	6.85 ± 0.11^{b}	14.45±1.64	2.94±0.48
	3Ao/1Cj soil	0.16 ± 0.12^{bcd}	0.50±0.14	6.74 ± 4.91^{b}	6.97 ± 0.16^{ab}	12.41±0.58	2.41±0.18
	2Ao/2Cj soil	0.06 ± 0.06^{cd}	0.57±0.14	5.23 ± 3.80^{b}	6.95 ± 0.15^{ab}	15.03±1.13	2.77±0.29
	1Ao/3Cj soil	0.06 ± 0.06^{cd}	0.56±0.27	7.02 ± 4.99^{ab}	7.02 ± 0.11^{ab}	11.98±2.04	2.50±0.58
	Cj soil	0.25 ± 0.12^{bcd}	0.66±0.25	5.76 ± 3.89^{b}	6.98 ± 0.20^{ab}	13.71±0.77	2.63±0.16
Sterile soils	Ao soil	0.67 ± 0.21^{abc}	0.90±0.33	23.78 ± 7.76^{a}	7.06 ± 0.05^{ab}	13.13±2.26	2.77±0.77
	3Ao/1Cj soil	0.22 ± 0.12^{bcd}	0.50±0.08	13.65 ± 1.30^{ab}	7.15 ± 0.10^{a}	11.53±0.72	2.15±0.17
	2Ao/2Cj soil	0.73 ± 0.15^{ab}	0.54±0.25	20.60 ± 1.13^{ab}	7.19 ± 0.08^{a}	11.34±0.88	2.04±0.14
	1Ao/3Cj soil	0.49 ± 0.16^{abcd}	0.58±0.30	17.54 ± 0.82^{ab}	7.16 ± 0.07^{a}	10.28±1.26	1.77±0.23
	Cj soil	1.09 ± 0.23^{a}	0.47±0.08	20.68 ± 5.17^{ab}	7.17 ± 0.11^{a}	14.75±2.06	2.60±0.55
ANOVA	Sterilization	37.10***	0.47	29.71***	21.17***	2.42	3.08
	Soil	3.36*	1.46	0.41	1.40	1.88	1.33
	Interaction	2.36	0.26	0.64	0.13	0.82	0.45

[1] Data was log-transformed. Data of N-NO_3 in the live soil conditioned by monoculture of *A. odoratum* (Ao soil) was based on only one sample.

produced more aboveground biomass in sterile soil than in live soil in both plant monocultures and the 1:1 plant mixture, but the difference was much bigger in the 1:1 plant mixture than in plant monocultures (Table S4.2A; Fig. 4.2A). In monocultures, *C. jacea* also produced more aboveground biomass in sterile soil than in live soil, but in the 1:1 plant mixture, there was no difference between these two sterilization treatments (Table S4.2A; Fig. 4.2B).

The belowground biomass of *A. odoratum* was significantly greater in live "foreign" soil than in live "own" soil, but did not differ in sterile "foreign" and "own" soil (Table S4.2B; Fig. 4.2C). In contrast, belowground biomass of *C. jacea* did not differ between "foreign" and "own" soil in either live or sterile soils (Table S4.1B, S4.2B; Fig. 4.2D).

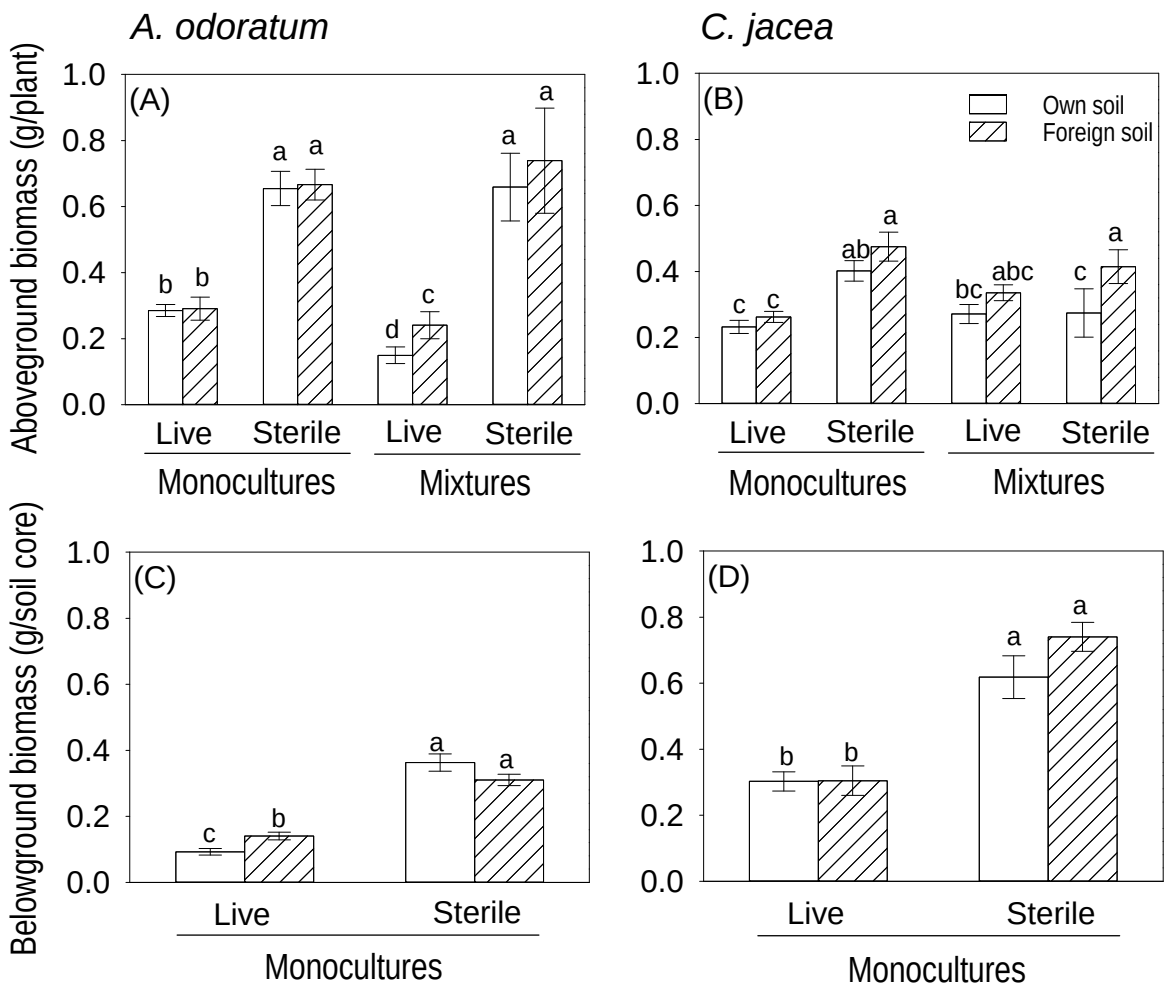

Fig. 4.2. Aboveground biomass per plant (A and B), and belowground biomass per soil core (C and D) of *A. odoratum* (A and C) and *C. jacea* (B and D) on "own" (soil conditioned by monocultures of the same species) and "foreign" soils (soil conditioned by monocultures of the other species) in the greenhouse experiment. "Sterile" and "Live" indicate sterilized soil and non-sterilized soil respectively. Plants were grown in monocultures and in 1:1 mixtures in the greenhouse experiment. Mean values (± 1 SE) are presented. Letters above the bars indicate significant differences in aboveground biomass among each panel.

The aboveground PSF of *A. odoratum* tended to be lower in the1:1 plant mixture than in the plant monoculture independent of sterilization treatment (Table S4.3A; Fig. S4.3A). Generally, the aboveground PSF of *C. jacea* was negative, but there was no difference between plant monocultures and 1:1 plant mixtures (Table S4.3A; Fig. S4.3B). The belowground PSF of *A. odoratum* was significantly greater in sterile soil than in live soil but there was no difference between these two soil types for *C. jacea* (Table S4.3B; Fig. S4.3C, D).

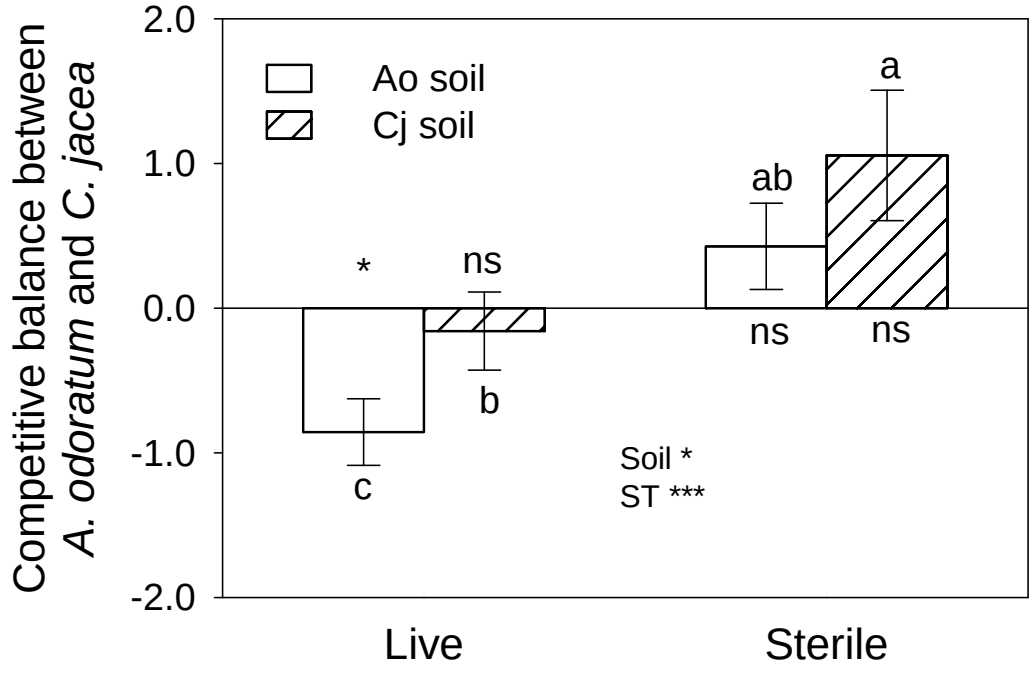

Fig. 4.3. Competitive balance (CB; $\ln\frac{MIX_{Ao}}{MIX_{Cj}}$) between *A. odoratum* and *C. jacea* in the 1:1 mixture on Ao soil (soils collected from field plots with *A. odoratum* monocultures) and Cj soil (soils collected from field plots with *C. jacea* monocultures) in the greenhouse experiment in sterile and live soil. Mean values (± 1 SE) and significant effects of a two-way ANOVA with soil type (Soil), sterilization (ST) and the interaction are presented, the superscript asterisks give *P*: * $P<0.05$ and *** $P<0.001$. Bars that share the same letters are not significant different based on a Tukey post-hoc comparison. Negative CB values indicate that the biomass of *C. jacea* is higher than that of *A. odoratum*, while positive CB values indicate that *A. odoratum* biomass is higher. The asterisk at the start of the first bar indicate that the value differs from zero ($P<0.05$) based on a one-sample *t*-test, ns indicates not significant.

Competitive balance between the two species grown in the 1:1 plant mixture in monospecific soils in the greenhouse experiment

The competitive balance index (CB: performance of *A. odoratum* in the 1:1 mixture relative to that of *C. jacea*) was greater in sterile soil than in live soil (Fig. 4.3). Overall, *C. jacea* was superior to *A. odoratum* in live soil while the reverse was true in sterile soil (Fig. 4.3). The competitive balance, CB was significantly smaller in live Ao soil than in live Cj soil, but there was no significant difference in CB between sterile Ao and sterile Cj soil. CB was significantly smaller than zero in live Ao soil, but did not differ from zero in live Cj soil, indicating that *C. jacea* was competitively superior in live Ao soil, but not in live Cj soil. In sterile soil, the pattern was similar but *A. odoratum* was superior over *C. jacea* (Fig. 4.3).

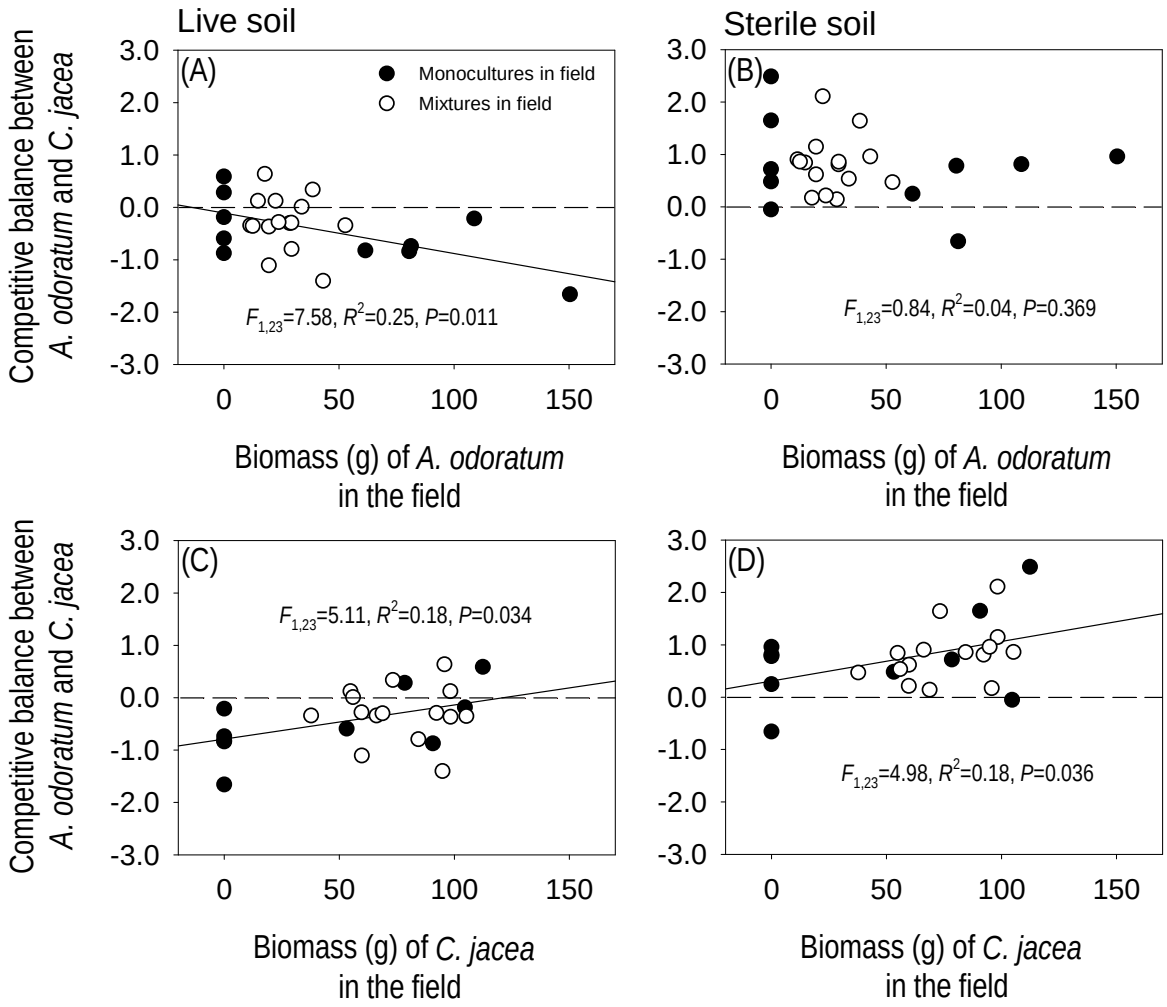

Fig. 4.4. Relationship between the biomass of *A. odoratum* (A and B) or *C. jacea* (C and D) in the field plots and the competitive balance (CB;$\ln\frac{MIX_{Ao}}{MIX_{Cj}}$) between *A. odoratum* and *C. jacea* in the 1:1 plant mixture in the greenhouse experiment. For the CB, negative values indicate that the biomass of *C. jacea* is higher than that of *A. odoratum*, while positive values indicate *A. odoratum* biomass is higher. Black and white dots represent soils collected from field plots planted with monocultures and mixtures, respectively. The *F*-, R^2- and *P*-values obtained from linear regressions are also presented.

Relationships between the growth and competitive balance in the greenhouse and abundance (biomass) in the field plots

For both species, there was no significant relationship between the growth (aboveground biomass and belowground biomass) of the species in the greenhouse experiment and the abundance of either species in the field experiment (all $P > 0.05$; Fig. S4.4-S4.6).

The CB between *A. odoratum* and *C. jacea* in the greenhouse experiment was negatively correlated to the abundance of *A. odoratum* in the field experiment in live soil (Fig. 4.4A), but not in sterile soil (Fig. 4.4B). However, this pattern was caused by one data point and was no

longer significant after removing this point (Fig. S4.7). There was a significant positive relationship between the CB between *A. odoratum* and *C. jacea* in the greenhouse experiment and the abundance of *C. jacea* in the field experiment in both live (Fig. 4.4C) and sterile soil (Fig. 4.4D).

Discussion

In the present study, we show that the competitive balance (the performance of *A. odoratum* relative to *C. jacea*) in the greenhouse was related to the former abundance of *C. jacea*, while it was independent of the former abundance of *A. odoratum* in the field. This result implies that the abundance of a plant species in mixed communities can influence the competitive interactions of later growing plants via plant-soil feedback effects, but that these effects vary between species.

Negative plant-soil feedback strength is often assumed to increase with previous plant density, but this has been rarely tested (e.g., Bell et al. 2006, Bagchi et al. 2010, Kos et al. 2013, Comita et al. 2014). In the present study, we therefore expected a negative relationship between the growth of a species in the greenhouse experiment and its former abundance in the field. However, we did not find such a relationship for either of the two species. A possible explanation is that *C. jacea* was competitively superior in all field plots and produced much more biomass than *A. odoratum*, even at low planting densities (Fig. S4.1). Hence, *C. jacea* may have played a dominant role in conditioning the soils in all mixtures, which may explain the lack of a relationship between the growth of a species in the greenhouse experiment and its former abundance in the field. However, it is also important to note that a low-productive plant species may have a much larger impact on the soil than a highly productive species. Another possible explanation may relate to the non-additivity of plant-soil feedbacks (Hawkes et al. 2013, Kuebbing et al. 2014). Growth of a test plant species in soil conditioned by different plant species simultaneously is not necessarily the same as the averaged effects those species have in monocultures.

We expected that the former plant abundance of one species may have a negative influence on its competitive performance later on. Indeed we observed that there was a negative relationship between the density in the field and the competitive performance for both *A. odoratum* and *C. jacea* although the significance of the relationship for *A. odoratum* was determined by one data point. In the present study, the two plants may have conditioned the

soil differently, it is possible that the conditioning effects of *A. odoratum* on the soil were overall weak even though its biomass varied strongly among the field plots (Fig. S4.4).

Remarkably, the negative effects of the former abundance of *C. jacea* on its competitive performance occurred both in live and sterile soils. In this study, *A. odoratum* benefited more from higher soil nutrient availability than *C. jacea* as indicated by the positive impact of sterilization on the performance of *A. odoratum* relative to *C. jacea*. Potentially, *C. jacea* which was more productive than *A. odoratum* in the field may have produced more labile soil organic matter leading to increased soil nutrient availability (Berendse 1990), which, in turn, could favour *A. odoratum* more than *C. jacea* (Fig. S4.8). However, in our study, we did not observe an increase in availability of nutrients in *C. jacea* soils (Table 4.1). Alternatively, we speculate that these negative plant-soil feedback effects may be driven by allelopathic effects (Callaway and Aschehoug 2000). It is possible that the root exudates of *C. jacea* in both live and sterile soils reduced the performance of *C. jacea* relative to *A. odoratum.* Our results would then suggest that allelopathy may inhibit or slow down the dominance of a particular species in interspecific competition, promoting the coexistence of species.

We hypothesized that negative plant-soil feedbacks would be stronger in the 1:1 plant mixture than in plant monocultures (van der Putten and Peters 1997, Kardol et al. 2007, Petermann et al. 2008). We found only limited evidence for this. The performance of *A. odoratum* was reduced more in live "own" soil than in live "foreign" soil when grown in competition i.e., in the 1:1 plant mixture than when grown in plant monoculture and a similar trend was observed for *C. jacea*. In the 1:1 plant mixture in the greenhouse experiment, *C. jacea* was competitively superior to *A. odoratum* in live soil, and the performance of *C. jacea* in its "own" soil was less reduced when grown together with *A. odoratum*, while the performance of *A. odoratum* in its "own" soil was much more reduced when grown with *C. jacea*. These results would suggest that interspecific competition can exacerbate negative plant-soil feedback effects compared to intraspecific competition, but only when a plant competes with a stronger competitor.

We expected that negative plant-soil feedbacks would be stronger in live soil than in sterile soil. In agreement with our hypothesis, the negative plant-soil feedback effects of *A. odoratum* were smaller or less negative in sterile soil than in live soil, but for *C. jacea* this was not true. This result indicates that the negative feedbacks encountered by *A. odoratum* appears to be biotic while that by *C. jacea* is abiotic. However, it should be noted that sterilization of soils can change soil features such as nutrient availability (Powlson and

Jenkinson 1976, Jakobsen and Andersen 1982, Brinkman et al. 2010), and fast-growing species of microorganisms can develop rapidly in sterilized soil (de Boer et al. 2003, Brinkman et al. 2010). Overall, our results regarding the effects of sterilization on plant-soil feedbacks effects are inconclusive, even though, sterilization *per se*, had a large effect on plant growth and plant competition.

We conclude that conspecific plant-soil feedbacks can negatively influence plant growth and that the negative effects tend to be stronger when the test plants grow in interspecific competition than when they grow in intraspecific competition. Moreover, the former abundance of a species in mixed plant communities, via plant-soil feedback, can negatively influence the relative competitiveness of that species when it grows later in interspecific competition. However, our study also shows that these plant-soil feedback effects depend on the identity of the plant species. In a broader context, the density dependent feedback effects may prevent the dominance of one species and promote the coexistence of competing plant species in natural systems.

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Supporting information

Table S4.1. Full model results of mixed-effect ANOVA testing the effects of species (*A. odoratum* vs. *C. jacea*), soil type ("own" soil vs. "foreign" soil), sterilization (live vs. sterile) and competition (monocultures vs. mixtures; only for aboveground biomass) on aboveground biomass (A) and belowground biomass (B) of *A. odoratum* and *C. jacea* in the greenhouse experiment. DF, DenDF, *F*-, and *P*- values are given.

	DF	DenDF	*F*	*P*
(A) *Aboveground biomass*[1]				
Species (SP)	1	60	9.23	**0.004**
Soil type (Soil)	1	60	9.74	**0.003**
Sterilization (ST)	1	60	108.02	**<0.001**
Competition (C)	1	60	5.22	**0.026**
SP × Soil	1	60	0.76	0.387
SP × ST	1	60	33.32	**<0.001**
Soil × ST	1	60	0.00	0.981
SP × C	1	60	1.54	0.220
Soil × C	1	60	3.69	0.059
ST × C	1	60	0.08	0.777
SP × Soil × ST	1	60	1.70	0.197
SP × Soil × C	1	60	0.02	0.877
SP × ST × C	1	60	13.86	**<0.001**
Soil × ST × C	1	60	0.06	0.808
SP × Soil × ST × C	1	60	1.54	0.220
(B) *Belowground biomass*[1]				
Species (SP)	1	28	150.36	**<0.001**
Soil type (Soil)	1	28	2.87	0.102
Sterilization (ST)	1	28	197.92	**<0.001**
SP × Soil	1	28	0.09	0.763
SP × ST	1	28	4.19	0.050
Soil × ST	1	28	1.79	0.192
SP × Soil × ST	1	28	8.50	**0.007**

[1] Data were based on the aboveground biomass and belowground biomass of *A. odoratum* and *C. jacea* on soils conditioned by monocultures of *A. odoratum* and *C. jacea* (monospecific soils) in the field.

Table S4.2. Results of mixed-effect ANOVA testing the effects of soil type ("own" vs. "foreign" soil), sterilization (live vs. sterile) and competition (monoculture vs. mixture; only for aboveground biomass) on aboveground biomass (A) and belowground biomass (B) of *A. odoratum* and *C. jacea* in the greenhouse experiment. DF, DenDF, *F*- and *P*-values are given.

			A. odoratum		*C. jacea*	
	DF	DenDF	*F*	*P*	*F*	*P*
(A) *Aboveground biomass*[1]						
Soil type (Soil)	1	28	3.22	0.084	8.34	**0.007**
Sterilization (ST)	1	28	166.22	**<0.001**	11.18	**0.002**
Competition (C)	1	28	7.91	**0.009**	0.57	0.456
Soil × ST	1	28	1.12	0.298	0.86	0.362
Soil × C	1	28	2.74	0.109	1.63	0.212
ST × C	1	28	7.52	**0.011**	8.41	**0.007**
Soil × ST × C	1	28	1.40	0.246	0.52	0.477
(B) *Belowground biomass*[1]						
Soil type (Soil)	1	12	2.90	0.114	0.84	0.378
Sterilization (ST)	1	12	189.03	**<0.001**	62.92	**<0.001**
Soil × ST	1	12	13.16	**0.004**	1.09	0.318

[1] Data were based on the aboveground and belowground biomass of *A. odoratum* and *C. jacea* on soils conditioned by monocultures of *A. odoratum* and *C. jacea* (monospecific soils) in the field. Values with $P < 0.05$ are in bold.

Table S4.3. Full model results of ANOVA testing the effects of species (*A. odoratum* vs. *C. jacea*), sterilization (live vs. sterile) and competition (monoculture vs. mixture; only for aboveground PSF) on the aboveground feedback (A) and belowground feedback (B) of *A. odoratum* and *C. jacea* in the greenhouse experiment. DF, DenDF, *F*-, and *P*- values are given.

	DF	DenDF	*F*	*P*
(A) *Aboveground PSF*				
Species (SP)	1	28	0.94	0.342
Sterilization (ST)	1	28	0.00	0.979
Competition (C)	1	28	4.55	**0.042**
SP × ST	1	28	2.10	0.159
SP × C	1	28	0.03	0.864
ST × C	1	28	0.07	0.788
SP × ST × C	1	28	1.90	0.180
(B) *Belowground PSF*				
Species (SP)	1	12	0.11	0.750
Sterilization (ST)	1	12	2.05	0.178
SP × ST	1	12	9.75	**0.009**

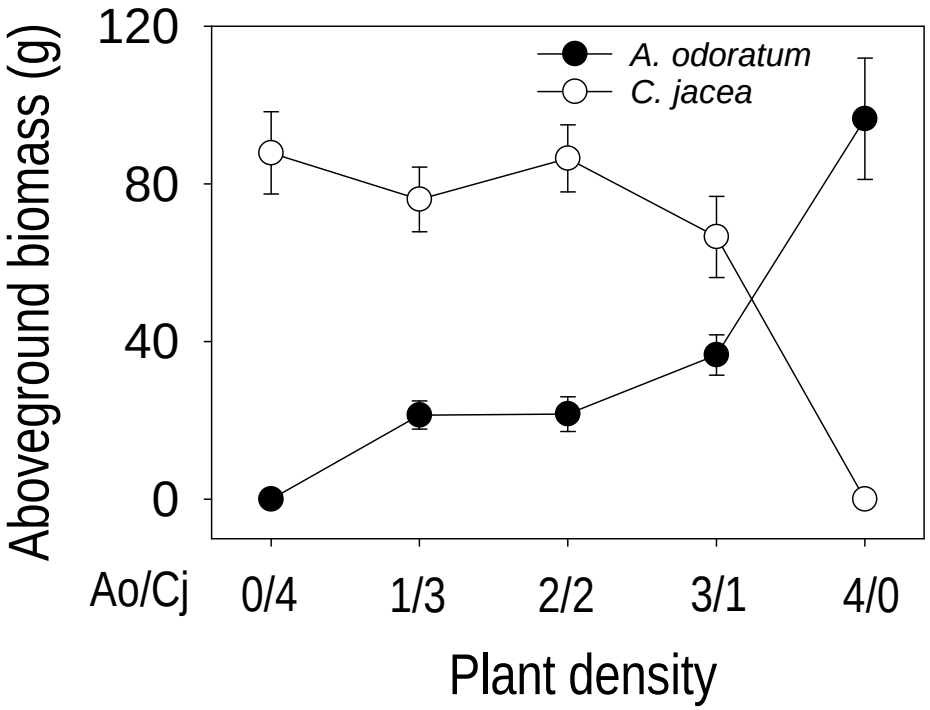

Fig. S4.1. Replacement diagram of the aboveground biomass of *A. odoratum* and *C. jacea* in the field experiment. The initial total seedling density was 144 seedlings/plot. Data of aboveground biomass was based on the central 60 × 60 cm^2 field collected after three growing seasons in 2015.

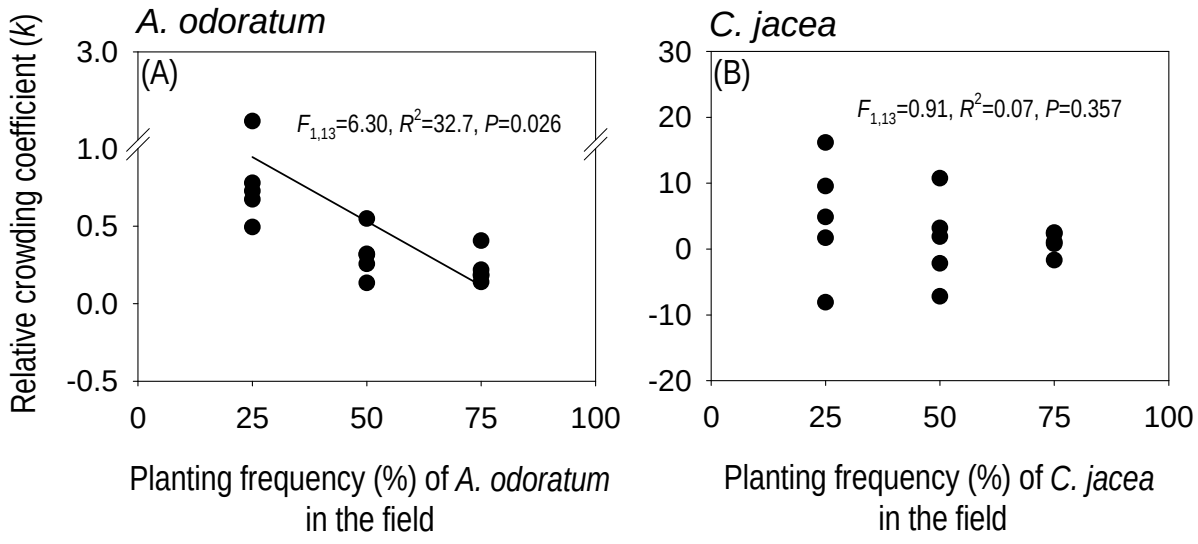

Fig. S4.2. Relationship between the relative crowding coefficient (*k*) of *A. odoratum* (A) and *C. jacea* (B) and their planting frequency in the mixtures in the field plot. *F*-, R^2- and *P*-values based on linear regressions are given.

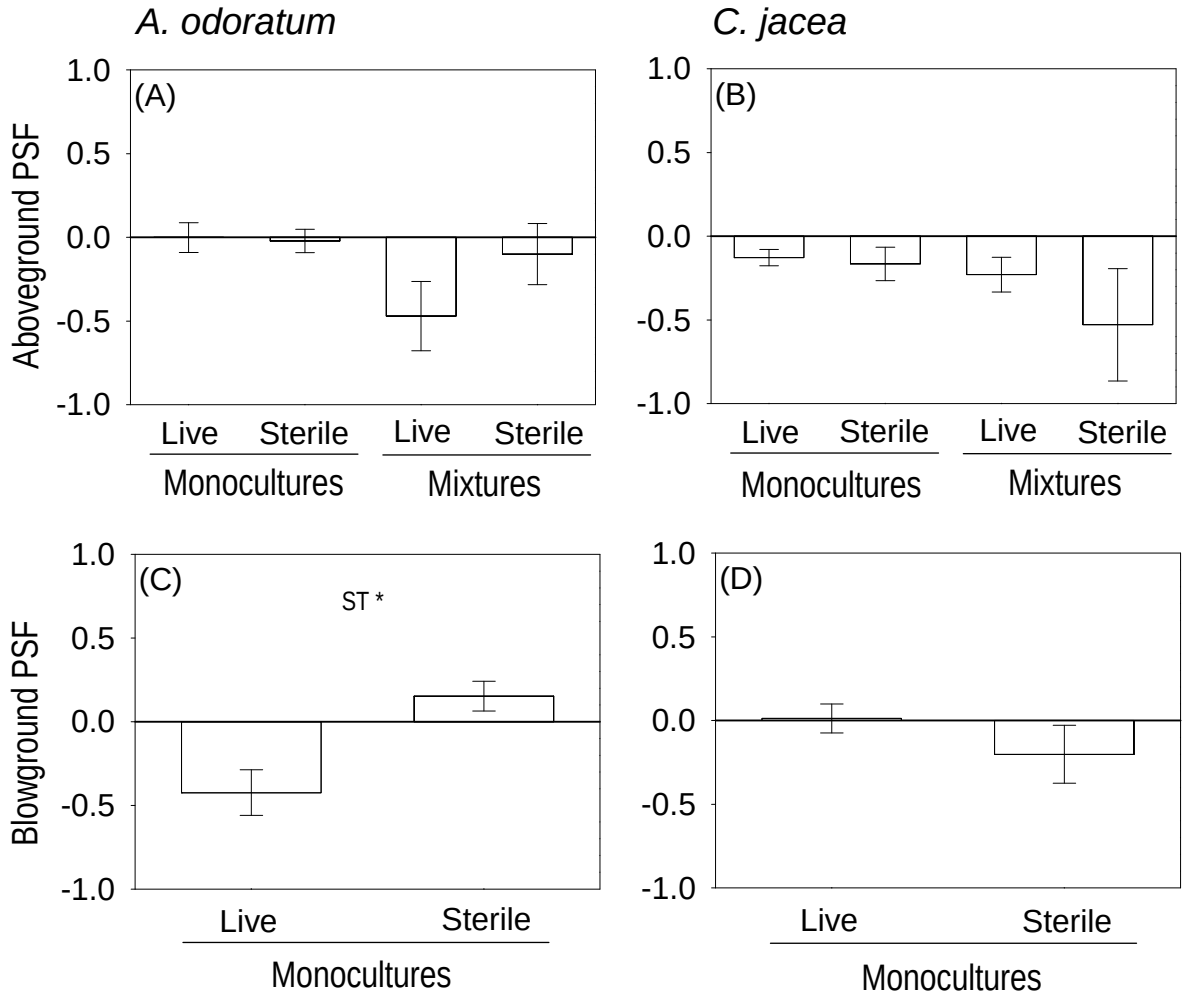

Fig. S4.3. Feedback strength (log-ratio of biomass in "own" and "foreign" soil) of *A. odoratum* and *C. jacea* in monocultures and mixtures. "Sterile" and "Live" indicate sterilized soil and non-sterilized soil respectively, respectively. Plants were grown in monocultures and in 1:1 mixtures in the greenhouse experiment. Mean values (± 1 SE) and significant effects of an ANOVA with sterilization (ST), competition (C; only for aboveground biomass) and the interaction are also presented: * $P<0.05$ (see Table S4.3 for full analysis).

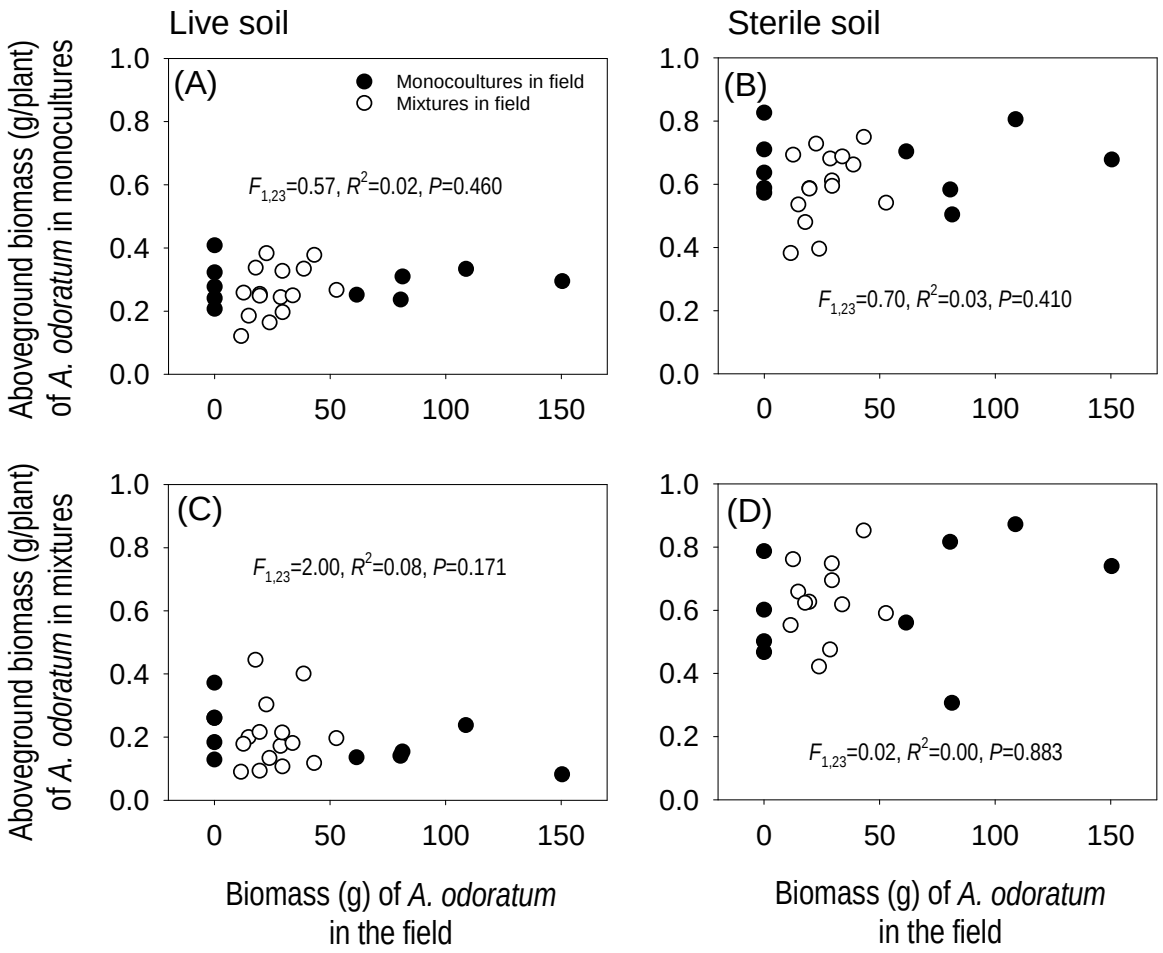

Fig. S4.4. Relationship between aboveground biomass per plant of *A. odoratum* in monocultures (A and B) or in 1:1 mixtures (C and D) in the greenhouse experiment and its aboveground biomass in the field plots. Black and white dots represent soils collected from field plots planted with monocultures and mixtures, respectively. *F*-, R^2- and *P*-values based on linear regressions are given.

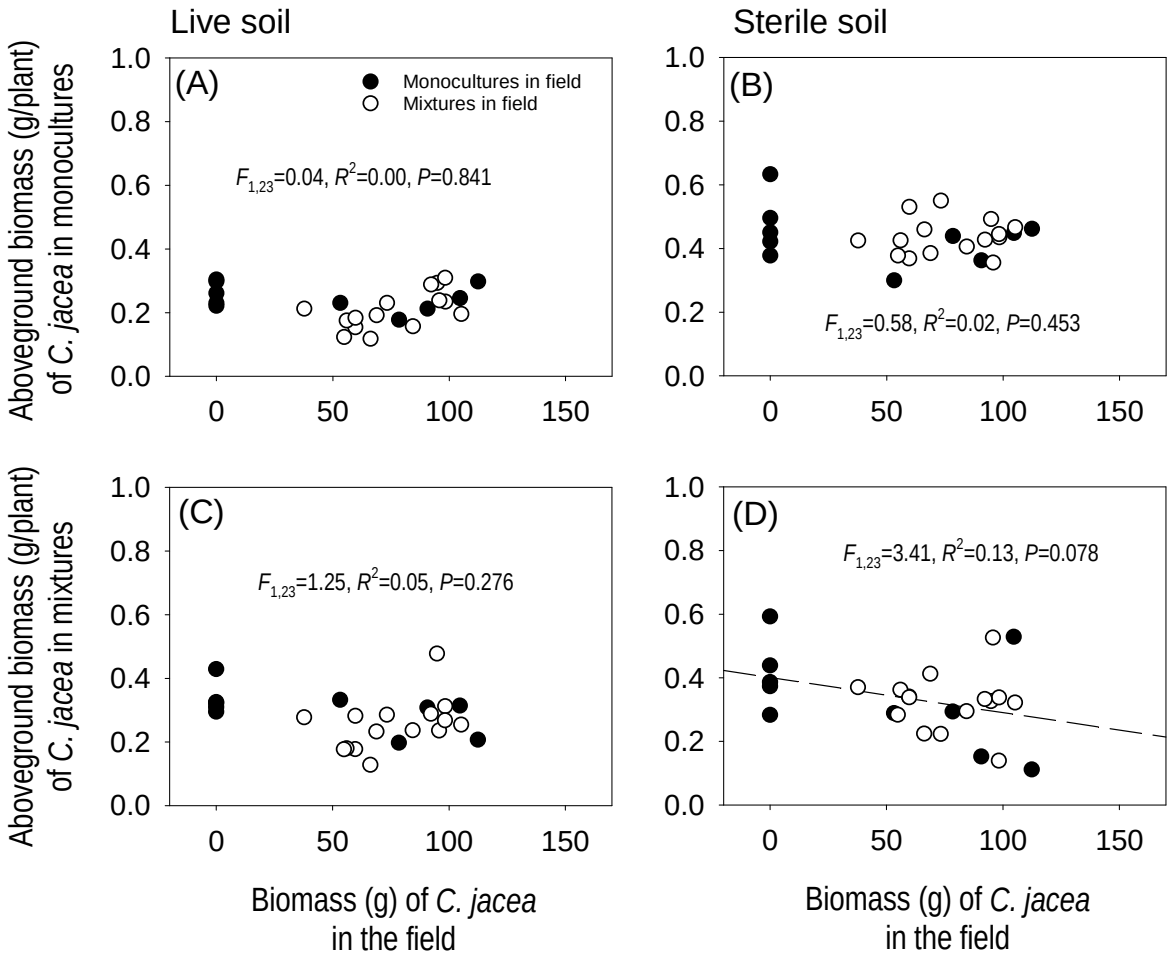

Fig. S4.5. Relationship between aboveground biomass per plant of *C. jacea* in monocultures (A and B) or in 1:1 mixtures (C and D) in the greenhouse experiment and its aboveground biomass in the field plots. Black and white dots represent soils collected from field plots planted with monocultures and mixtures, respectively. *F*-, R^2- and *P*-values based on linear regressions are given.

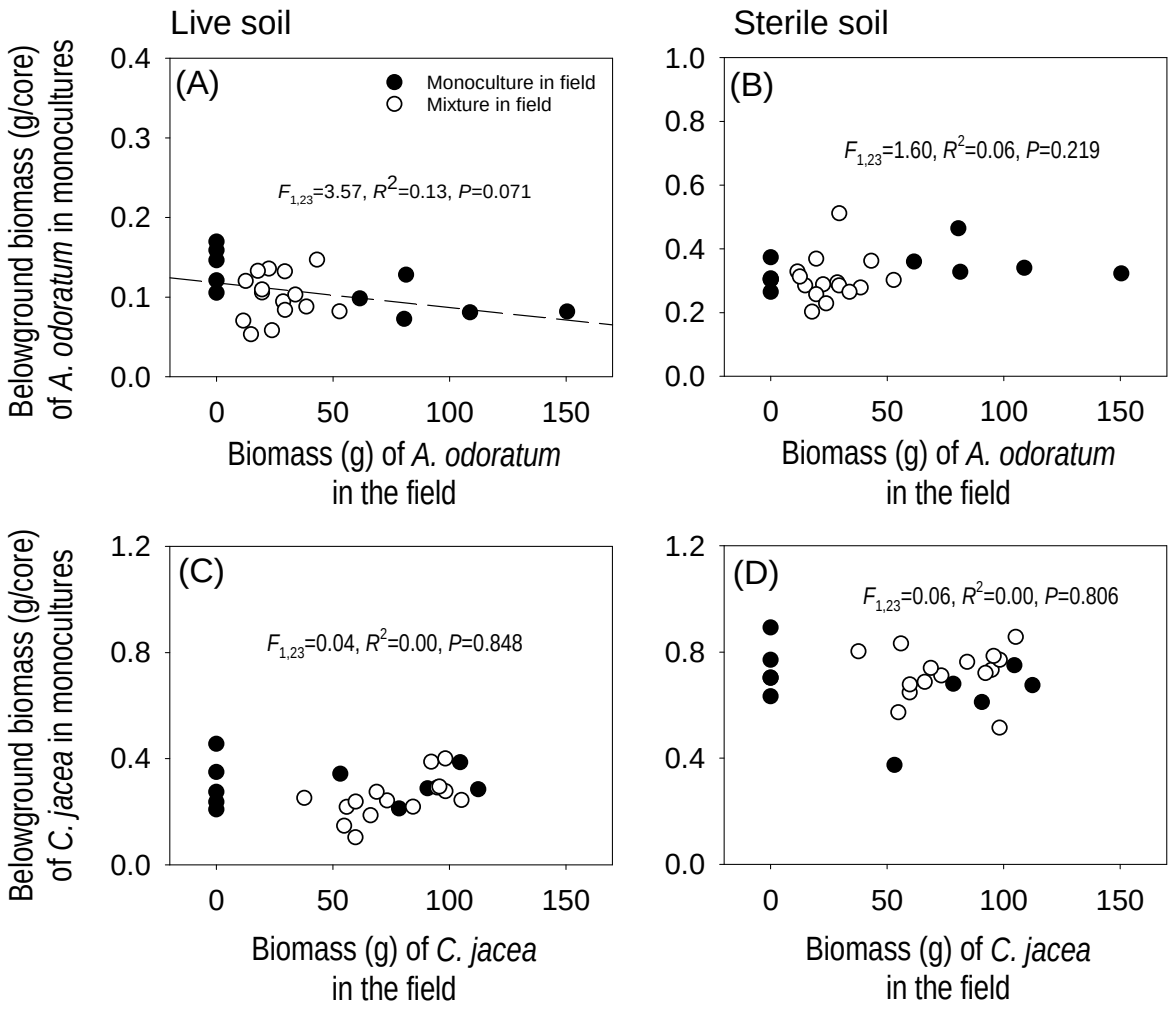

Fig. S4.6. Relationship between belowground biomass per soil core of *A. odoratum* (A and B) or *C. jacea* (C and D) in monocultures in the greenhouse experiment and its aboveground biomass in the field plots. Black and white dots represent soils collected from field plots planted with monocultures and mixtures, respectively. *F*-, R^2- and *P*-values based on linear regressions are given.

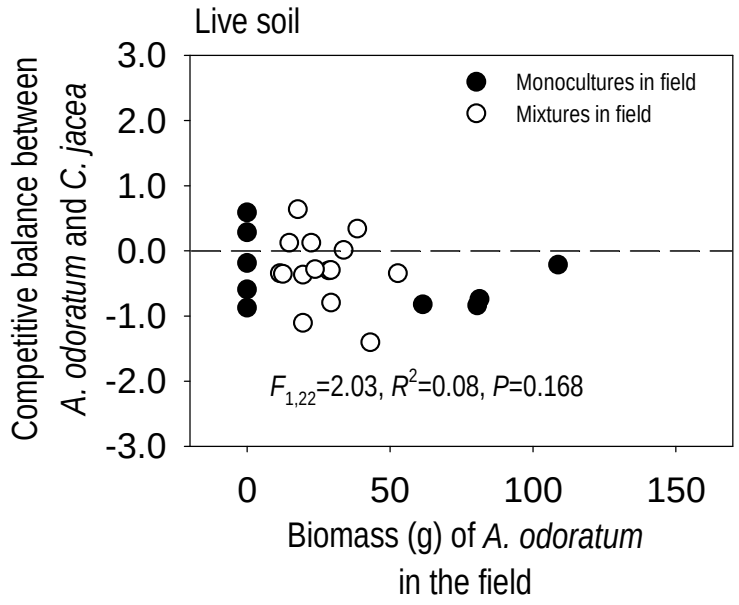

Fig. S4.7. Relationship between the biomass of *A. odoratum* in the field plot and competitive balance (CB;$\ln\frac{MIX_{Ao}}{MIX_{Cj}}$) between *A. odoratum* and *C. jacea* in the 1:1 mixtures in live soil in the greenhouse experiment after removing of an influential data point. Negative values indicate biomass of *C. jacea* is higher while positive values indicate *A. odoratum* biomass is higher. Black and white dots represent soils collected from field plots planted with monocultures and mixtures, respectively. *F*-, R^2- and *P*-values based on linear regressions are given.

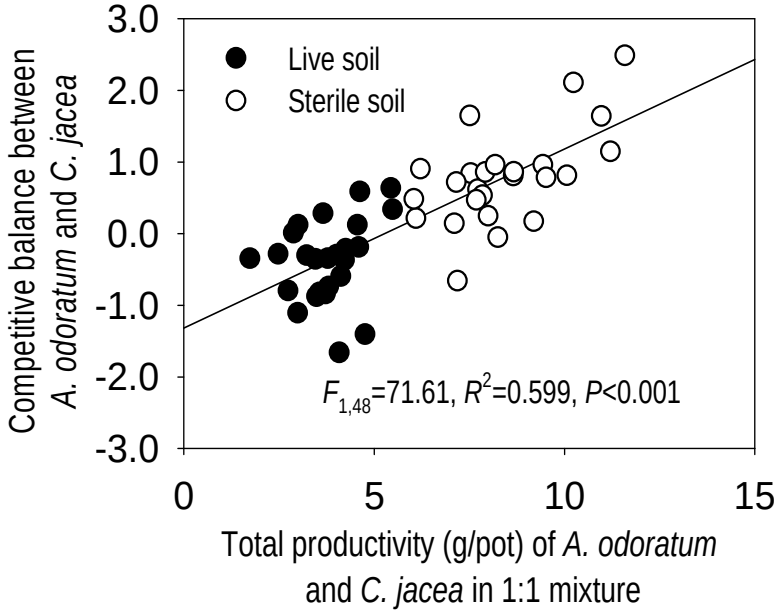

Fig. S4.8. Relationship between the total biomass per pot of *A. odoratum* and *C. jacea* and competitive balance ($\ln\frac{MIX_{Ao}}{MIX_{Cj}}$) between *A. odoratum* and *C. jacea* in the 1:1 mixtures in greenhouse experiment. *F*-, R^2- and *P*-values based on linear regressions are given.

Methods S4.1 Description of soil chemical analysis.

The amount of NH_4, NO_3 and PO_4 (mg/kg dry soil sample) were determined by adding 30.0 ml of 0.01 mol/L $CaCl_2$ solution to soil samples (3.0 g), shaking mechanically for at least 2 h at room temperature (20 ℃), filtering the solution and analyzing the nutrients in the soil extracts in a Skalar Segmented Flow Analyzer. Soil pH-H_2O was determined by adding 25.0 ml demi-water to soil samples (volume 5.0 ml), shaking for 5 min and wait 2 h before measuring. Soil organic matter was determined by measuring the difference between weights of the oven-dried (105 ℃) soil samples (5.0-10.0 g) before and after being heated in a furnace at 550 ℃. Weights of soil samples were determined and recorded after cooling down in the air to handwarm and further cooling for at least 45 min in a desiccator. Soil moisture content was determined by measuring the difference between the weights of soil samples before and after oven-drying (105 ℃) in the oven.

Chapter 5

Spatial heterogeneity in plant-soil feedbacks alters competitive interactions between two grassland plant species

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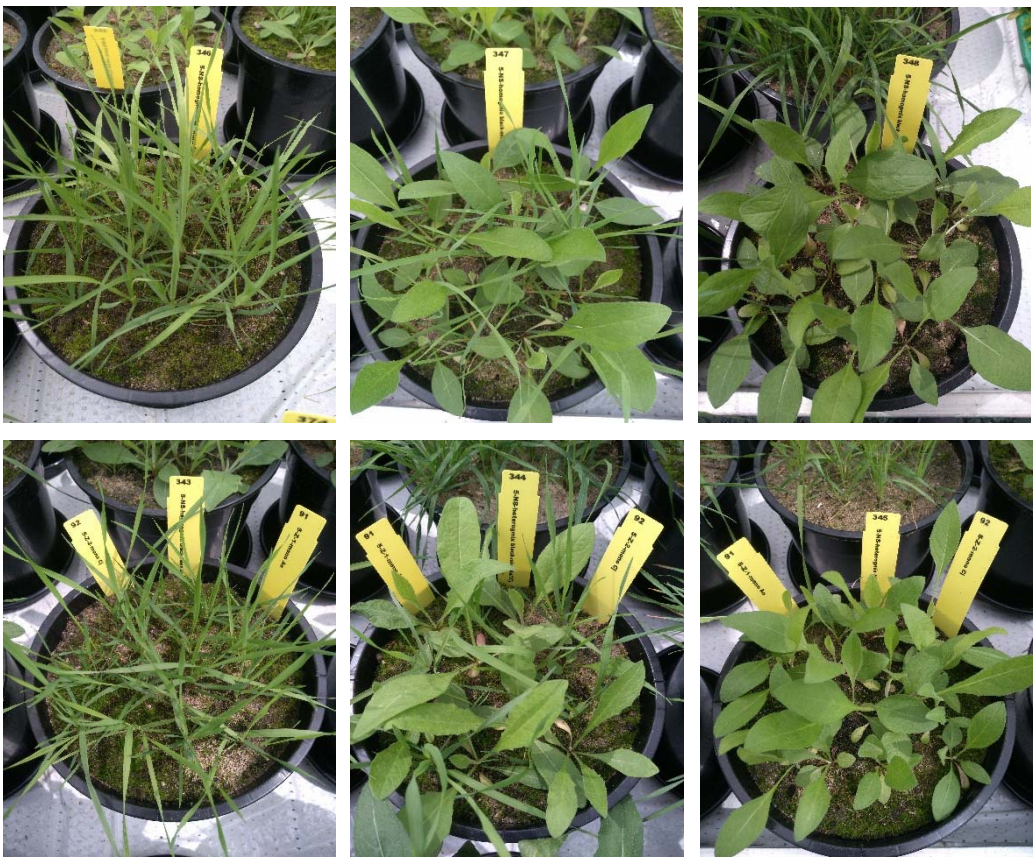

Abstract

The effects of plants on soil vary greatly between plant species and in mixed plant communities this can lead to spatial variation in plant-soil feedback (PSF) effects. Such spatial effects are thought to influence plant species coexistence, but the empirical evidence for this hypothesis is limited.

Here, we investigate how spatial heterogeneity in PSFs influences plant growth and competition. The experiment was carried out with high and low nutrient soils to examine how these effects depend on soil fertility. We collected soil from field plots planted for three years with monocultures of *Anthoxanthum odoratum* and *Centaurea jacea* and tested the performance of the two species in a greenhouse experiment in heterogeneous soils consisting of patches of "own" and "foreign" soils and in soils where the "own" and "foreign" soils were mixed homogeneously. In the test phase, plants were grown in monocultures and in 1:1 mixtures in live or sterilized soils.

Overall, *A. odoratum* in monocultures produced less aboveground biomass in heterogeneous soils than in homogeneous soils. *Centaurea jacea* produced less belowground biomass in live heterogeneous soils than in live homogeneous soils, but there was no difference between sterile heterogeneous and homogeneous soils. The belowground biomass per patch varied more in pots with live heterogeneous soils than in pots with live homogeneous soils for both plant species, but there was no difference between pots with sterile heterogeneous and homogeneous soils. In pots with plant mixtures, the difference in aboveground biomass between the two competing species tended to be smaller in heterogeneous than in homogeneous soils. In pots with heterogeneous soils, both plant species grown in mixtures produced more aboveground biomass in "foreign" soil patches than in "own" soil patches. The responses of plants to heterogeneous PSFs were not different between low and high nutrient soils.

Our results show that spatially heterogeneous PSFs can influence plant performance and competition via reducing the growth inequality between the two competing species by allowing selective growth in foreign soil patches, independent of initial soil nutrient availability. Such effect may slow down exclusion processes and thus promote the coexistence of competing species at the local scale in mixed plant communities.

Key words: soil heterogeneity, plant-soil feedback, intra- and interspecific competition, plant-plant interactions, plant-soil interactions, soil origin, soil nutrient, patchy distribution

Introduction

Plants change the properties of the soil they grow in and this can influence the performance of the same or other plant species that grow later in this soil, a phenomenon termed plant-soil feedback (Bever et al. 1997, van der Putten et al. 2013). Most plant species perform worse in soil where another individual of the same species grew previously ("own soil") than in soil where another plant species had been grown before ("foreign soil") and hence most conspecific plant-soil feedback effects are negative (Kulmatiski et al. 2008; but see Bennett et al. 2017, Teste et al. 2017). As each plant individual in a plant community influences its local soil in a specific manner, soil characteristics and plant-soil feedbacks may vary spatially in the field. Spatial variation in plant-soil feedbacks (i.e. spatial plant-soil feedback heterogeneity) has been theoretically suggested to influence plant performance and coexistence (Bonanomi et al. 2005, Fukami and Nakajima 2011, Mack and Bever 2014, Abbott et al. 2015, Zee and Fukami 2015). However, the vast majority of empirical plant-soil feedback studies so far have ignored such spatial aspects of plant-soil feedback (but see Brandt et al. 2013, Burns et al. 2014, del Pino et al. 2015, Hendriks et al. 2015a, Hendriks et al. 2015b, Wubs and Bezemer 2016, Burns et al. 2017, Wubs and Bezemer 2018).

In spatially heterogeneous soils, a plant can preferentially forage for nutrients in "foreign" soil patches thereby avoiding contact with its antagonists in "own" soil patches (Hendriks et al. 2015b). How plant-soil feedback heterogeneity will influence plant growth in the presence of neighbouring plants is less clear as competing plants may also change their foraging behaviour in heterogeneous soils (e.g. Cahill et al. 2010, Xue et al. 2013). In monospecific communities, spatial plant-soil feedback heterogeneity may not be beneficial because competing individuals will employ the same strategy (Bliss et al. 2002, Bennett et al. 2017, Teste et al. 2017). A recent study even reported that plants in monocultures performed worse in spatially heterogeneous soils than predicted from their performance in homogeneously conditioned soils (Wubs and Bezemer 2016).

When different plant species grow together in spatially homogeneous soils, interspecific competition generally enhances the plant-soil feedback effects (e.g. van der Putten and Peters 1997, Kardol et al. 2007, Petermann et al. 2008, Jing et al. 2015, Crawford and Knight 2016). Similar to what is observed when soil resources are distributed heterogeneously, in soils with spatially heterogeneous plant-soil feedbacks, plants growing in "own" soil patches will experience a competitive disadvantage and inferior competitors may benefit in these patches (Day et al. 2003, Hutchings et al. 2003, Hendriks et al. 2015a, Burns et al. 2017). Hence,

competing species may all preferentially forage in "foreign" patches and this may reduce competitive imbalances between species.

Several studies have shown that plants generally respond less strongly to plant-soil feedbacks in fertilized soils than in nutrient-poor soils (van der Putten and Peters 1997, De Deyn et al. 2004, Gustafson and Casper 2004, Manning et al. 2008, Kardol et al. 2013, Kos et al. 2013, Wubs and Bezemer 2017). However, how soil nutrient availability influences the impact of a plant on the soil (i.e. the soil conditioning effect in the conditioning phase) is less well understood. As plants generally interact more strongly with soil biota in nutrient-poor conditions (van der Heijden et al. 2008, Teste et al. 2017), we may also expect that the effects of plant-soil feedback heterogeneity on plant performance in the test phase will be stronger when the soil was originally nutrient-poor than when the soil was nutrient-rich during the conditioning phase.

In the present study, we examine how plant-soil feedback heterogeneity influences the performance and competitive interactions between two grassland plant species, and how these effects depend on soil fertility. We grew the grass *Anthoxanthum odoratum* and the forb *Centaurea jacea* in field plots in monocultures in either high nutrient or low nutrient soil. After three years, we collected soil from these monocultures and tested the performance of *A. odoratum* and *C. jacea* in monocultures and in 1:1 mixtures in a greenhouse experiment in homogeneous mixtures of "own" and "foreign" soil, and in spatially heterogeneous soils with distinct patches of "own" and "foreign" soil. The experiment was carried out with live and sterilized soil to test the impact of soil biota on the response of the two plant species to spatial plant-soil feedback heterogeneity. We tested four hypotheses: (1) in monocultures (intraspecific competition) plants will produce similar amounts of biomass in pots with two conditioned soils placed in discrete patches (heterogeneous soil) as in evenly mixed soil (homogenous soil) as, at the pot level, on average the biotic and abiotic composition of both soils are identical. However, there will be more variation in biomass among the soil patches within heterogeneous soils than within homogeneous soils. (2) In plant mixtures (interspecific competition), at the pot level, the difference in growth between the two competing species will be smaller in heterogeneous soils than in homogeneous soils, as each of the competing species will produce more biomass in "foreign" soil patches than in "own" soil patches within the heterogeneous soils. (3) Effects of plant-soil feedback heterogeneity in the test phase will be stronger when the soil was initially nutrient-poor than when the soil was initially nutrient-

rich during conditioning, as plant-soil feedback effects generally diminish with increasing soil fertility. (4) Plant-soil feedback heterogeneity effects will disappear in sterile soils.

Materials and methods

Plant species

We used a grass species, *Anthoxanthum odoratum* L. (Poaceae), and an herb, *Centaurea jacea* L. (Asteraceae). Both species can reproduce by seeds and vegetative growth (Hartemink et al. 2004). *Anthoxanthum odoratum* produces closely connected ramets while *C. jacea* forms extensive branches underground (Jongejans and de Kroon 2005). Both species are native in western Europe and commonly coexist in meadows (van Ruijven and Berendse 2003). Both plant species experience negative conspecific plant-soil feedbacks (supporting information: Fig. S5.1B, D: less root biomass in "own" than "foreign" live soils for *A. odoratum*, and less root and shoot biomass for *C. jacea*).

Soil conditioning in monoculture field plots

In an outdoor experimental garden (from April 2013 to September 2015), we planted monocultures (144 seedlings/plot) of *A. odoratum* and *C. jacea* in plots filled with either high nutrient soil ($N\text{-}NH_4$: 3.31 mg/kg; $P\text{-}PO_4$: 1.88 mg/kg; $N\text{-}NO_3$: 41.10 mg/kg) or low nutrient soil ($N\text{-}NH_4$: 2.44 mg/kg; $P\text{-}PO_4$: 0.36 mg/kg; $N\text{-}NO_3$: 0.09 mg/kg). There were 20 plots (2 levels of nutrient availability × 2 plant species × 5 replicate plots) of 1 m^2 each distributed over five replicated blocks in a randomized block design. Weeds were regularly removed during the experiment. In September 2015, all plants in the central 60 × 60 cm^2 of each plot were clipped at a height of 1 cm. Aboveground biomass in each plot was determined after being oven-dried to constant weight. Productivity of both plant species in high nutrient and low nutrient soils is shown in the supporting information (Fig. S5.2). In February 2016, we collected all topsoil (20 cm deep) from the central area of 60 × 60 cm^2 in each experimental plot and kept soil from different plots in different sealed bags. Then, soil collected from each plot was sieved (1.5 cm mesh) and further separated into two parts both kept in separate sealed bags. One of the two bags from each plot was sterilized by γ-irradiation (minimum 25KGray, Isotron, Ede, the Netherlands). Hence, there were 40 different conditioned soils (2 nutrient levels × 2 plant species × 5 replicate plots × 2 sterilization treatments). In the

greenhouse experiment, for each of the two nutrient levels and for sterile and non-sterile soil, we created two levels of PSF heterogeneity (spatially homogeneous PSF and spatially heterogeneous PSF) using soils conditioned by *A. odoratum* and *C. jacea* from the same field block (Fig. 5.1). A total of 120 pots (2 nutrient levels × 2 sterilization treatments × 2 PSF heterogeneity treatments (described below) × 3 planting treatments (described below) × 5 replicates) of 4.6 L each were used in the greenhouse experiment.

Greenhouse experiment

In the greenhouse experiment, two levels of PSF heterogeneity (spatially homogeneous PSF and spatially heterogeneous PSF) were created using soil conditioned by *A. odoratum* and *C. jacea* from the same field block (Fig. 5.1). In the heterogeneous soil treatments, each pot was equally divided into 4 patches using a metal grid and each patch was alternately filled with 1.4 kg soil conditioned by monocultures of *A. odoratum* or *C. jacea*. In the homogeneous soil treatments, each pot was filled with 5.6 kg of a 1:1 (w:w) homogenized mixture of soil conditioned by monocultures of *A. odoratum* and *C. jacea* (Fig. 5.1). In this way, there were pots that differed in spatial variation in plant-soil feedbacks while the abiotic and biotic soil conditions in the homogenous and heterogeneous soils were kept constant. We allocated pots filled with soils originated from the same field block in the same block in the greenhouse experiment so that there were five blocks. Pots of different treatments were randomized within each block. Holes were made in the bottom of each pot to allow vertical movement of water. To prevent soil from passing through holes, a piece of filter paper (15 cm in diameter) was placed at the bottom of each pot before filling the pot with soil. Each pot was placed on a tray to prevent possible contamination through leachate. The metal grid was removed after each pot was filled so that plants could grow freely across different patches. We randomly selected three field blocks, and collected subsamples from the soil of each plot in those blocks for soil chemical analysis. We measured soil organic matter content, nutrient content (NH_4, NO_3 and PO_4), water content and pH (Table S5.1). The amount of NH_4, NO_3 and PO_4 (mg/kg dry soil) were determined by adding 30.0 ml of 0.01 mol/L $CaCl_2$ solution to soil samples (3.0 g), shaking mechanically for at least 2 h at room temperature (20 ℃), filtering the solution and analyzing the nutrients in the soil extracts in a flow analyzer (SKALAR SAN plus system). Soil pH-H_2O was determined by adding 25.0 ml demi-water to soil samples (volume 5.0 ml), shaking for 5 min and measuring 2 h later. Soil organic matter was determined by measuring

the difference between weights of the oven-dried (105 ℃) soil samples (5.0-10.0 g) before and after being heated in a furnace at 550 ℃. The weight of each sample was determined after cooling it down in the air to handwarm temperature and further cooling it for at least 45 min in a desiccator. Soil moisture content was determined by measuring the difference between the weights of each soil sample before and after oven-drying (105 ℃).

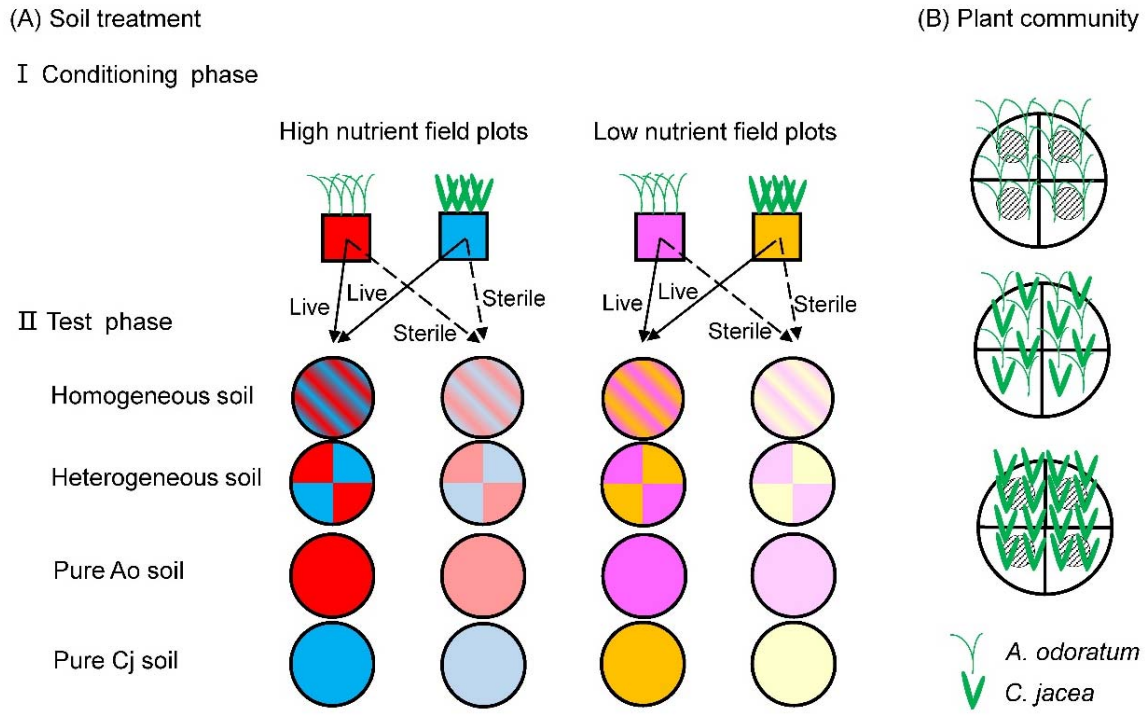

Fig. 5.1. Experimental design. (A) In the conditioning phase (Ⅰ), high nutrient and low nutrient soils were conditioned separately by monocultures of *A. odoratum* (Ao soil) and *C. jacea* (Cj soil) for three years in field plots. The initial planting density was 144 seedlings/plot. Soil was collected from the plots and conditioned soils were either sterilized or not (i.e., live and sterile), resulting in eight different soils (different colours). In the test phase (Ⅱ), pots with heterogeneous soils were created by filling with Ao soil and Cj soil in an alternated way, while pots with homogeneous soil (striped pot) were created by filling with 1:1 (w:w) mixtures of Ao soil and Cj soil. Additional pots were filled with pure Ao soil or pure Cj soil. The pure soil treatments (Pure Ao soil and Pure Cj soil) were not included in the main analysis; these results are presented in the supporting information. (B) Planting design. Each pot was planted with either 16 plants of *A. odoratum* or *C. jacea* in monocultures, or eight plants of each of the two species in mixtures. The shaded circles within the monoculture pots represent the positions where soil samples were taken.

In a heated greenhouse (20.0 ℃ average temperature, 70.2 % average relative humidity), seeds of *A. odoratum* and *C. jacea* (purchased from a wild seed supplier, Cruydthoeck, Nijeberkoop, the Netherlands) were sown on plastic trays filled with steamed potting soil that facilitates root development (0.03N-0.03P-0.03K, Seed Starting Potting Mix, Miracle-Gro Lawn Products, Inc., Marysville). The potting soil was watered daily so that the potting soil remained moist. One week after germination, the trays with seedlings were moved to an unheated greenhouse (12.8 ℃ average temperature, 70.3 % average relative humidity) until they were transplanted into the pots.

Similar sized seedlings of *A. odoratum* and *C. jacea* were used in the experiment. There were three planting treatments, i.e. the two species were planted in monocultures and in 1:1 mixtures (Fig. 5.1). In monocultures, we planted 16 seedlings (a similar planting density as applied in Wubs and Bezemer 2016) of *A. odoratum* or *C. jacea* in each pot. In mixtures, we planted eight seedlings of *A. odoratum* and *C. jacea* in alternating positions (Fig. 5.1). In this way, each seedling was surrounded by conspecific and heterospecific competitors. Dead seedlings were replaced during the first week of the experiment. We removed the dead seedlings, including the root system, and then planted a new seedling at the previous planting position. All other species emerging from the seed bank of the soil were removed manually during the experiment.

The experiment was maintained for 90 days (from 11 April to 11 July 2016) in the same unheated greenhouse. During the experiment, the mean temperature and the relative humidity in the greenhouse were 17.4 ℃ and 67.5 %, respectively. All pots were watered three times per week (300-800 ml per pot, each time depending on the weather conditions).

In this experiment, we analysed the effects of spatial plant-soil feedback heterogeneity by comparing spatially heterogeneous soils with homogeneously mixed soils that have the same origin. Hence, each pot consisted of the same initial nutritional and microbial composition. For completeness, in the experimental design we also included the two pure soil treatments (pure Ao soils and pure Cj soils; Fig. 5.1). In these two pure soil treatments, each pot was filled with 5.6 kg of soil conditioned by monocultures of *A. odoratum* (pure Ao soil treatment) or *C. jacea* (pure Cj soil treatment) growing in either high or low nutrient soil and originating from the same field block. The data of root and shoot biomass in these pure soils are presented in the supplementary information (Table S5.2; Fig. S5.1).

Harvest measurements

After 90 days, we clipped all plants at soil level. Plants growing in each patch within each pot were harvested separately. In the 1:1 mixtures, the two different species were also harvested separately. After clipping, we took one soil core (4.0 cm diameter, straight down to the bottom of pot) in each of the four soil patches in each pot to measure the root mass (Fig. 5.1). Soil cores were only taken from pots planted with monocultures since it was not possible to separate roots of the two different plant species in the mixtures. The soil samples were then washed by hand using a 0.5 mm sieve. Aboveground and belowground biomass of each plant species from each patch was oven-dried (70 °C) and weighed.

Data analysis

We analysed the aboveground biomass and belowground biomass in the greenhouse experiment at both pot level and patch level. Data of plant monocultures and mixed plant communities were analysed separately.

For plant monocultures, at the pot level, we first calculated aboveground biomass per plant (total aboveground biomass of a species in one pot divided by the number of seedlings in the pot), and belowground biomass per soil core of *A. odoratum* and *C. jacea* in each monoculture pot. Then we analysed aboveground biomass and belowground biomass separately for each of the two species planted in monocultures. We used a mixed-effect three-way ANOVA with nutrient availability (high vs. low), sterilization (live vs. sterile), soil heterogeneity (homogeneous vs. heterogeneous) and their interactions as fixed factors, and block as a random factor. A significant soil heterogeneity effect or a significant interaction with nutrient and/or sterilization would suggest that the growth of the species in monocultures is different between heterogeneous and homogeneous soils at the pot level.

The variation in aboveground and belowground biomass among the four patches within heterogeneous and homogeneous soils, was determined based on the coefficient of variation (CV) for each pot. CVs of aboveground biomass and of belowground biomass were analysed separately for each species, using a mixed-effect three-way ANOVA with nutrient availability, sterilization, soil heterogeneity and their interactions as fixed factors, and block as a random factor. A significant heterogeneity effect or a significant interaction with nutrient and/or

sterilization would suggest that the growth variation is different within heterogeneous and homogenous soils.

At the patch level, we first calculated aboveground biomass per plant (total aboveground biomass of a species in one patch divided by the number of seedlings in the patch), and belowground biomass per soil core of *A. odoratum* and *C. jacea* in each patch within each pot. Then, we analyzed the patch-level aboveground biomass and belowground biomass separately using a mixed-effect three-way ANOVA to test whether the two species grown in monocultures produced more biomass in "foreign" soil patches than in "own" soil patches within the heterogeneous soil. In this model, nutrient availability, sterilization, soil type ("own" vs. "foreign" soil) and their interactions were included as fixed factors, soil type nested in pot, and pot nested in block (block/pot/soil type) was included as a random effect to account for the non-independent of the growth in different patches within one pot.

For mixed plant communities, at the pot level, we first combined the growth of the two species in 1:1 mixtures in each pot by calculating the growth difference (*D*) to evaluate the effects of spatial plant-soil feedback heterogeneity on the competition between the two species. The *D*-value was calculated as the log-ratio of aboveground biomass of *A. odoratum* and *C. jacea* in mixtures. The *D*-value will be equal to zero if the two species perform equally well in mixtures; it will be positive if the biomass of *A. odoratum* is higher than *C. jacea*, and negative if *C. jacea* biomass is higher. We used three-way ANOVA to test the effects of nutrient availability, sterilization, soil heterogeneity and their interactions on *D*, block was included as a random factor. A one-sample *t*-test was used to test whether *D* for each combination of nutrient availability, sterilization and soil heterogeneity differed from zero. A significant soil heterogeneity effect or a significant interaction with nutrient and/or sterilization would suggest that the difference in the growth between the two competing species in the 1:1 mixture is different in heterogeneous and homogenous soils. We also analysed the plot-level aboveground biomass (total aboveground biomass of a species in one pot divided by the number of seedling in the pot) separately for each of the two species grown in the 1:1 mixture using a mixed-effect three-way ANOVA with nutrient availability, sterilization, soil heterogeneity and their interactions as fixed factors, and block as a random factor.

At the patch level, we tested whether the two species in the 1:1 mixtures produced more biomass in "foreign" soil patches than in "own" soil patches within the heterogeneous soils. We analysed the patch-level aboveground biomass (total aboveground biomass of a species in

one patch divided by the number of seedlings in the patch) separately for each of the two species grown in the 1:1 mixture, using a mixed-effect three-way ANOVA. Nutrient availability, sterilization, soil heterogeneity and their interactions were included as fixed factors, and soil type nested in pot, and pot nested in block (block/pot/soil type) as a random factor.

All data analysis were performed with R (version 3.3.2; http://www.r-project.org) in RStudio (version 1.0.44; http://rstudio.org). Linear mixed-effect models were fitted with *nlme* (version 3.1-128) (Pinheiro et al. 2016). All data were checked visually for normality and homogeneity of variance using Q-Q plots and residual plots, respectively.

Results

Effects of plant-soil feedback heterogeneity on the growth in monocultures

In monocultures, *A. odoratum* overall produced less aboveground biomass in heterogeneous soils than in homogeneous soils (Table S5.3A; Fig. 5.2A), but there was no significant difference in the aboveground biomass of *C. jacea* between the two soils (Table S5.3A; Fig. 5.2C). These results suggest that heterogeneity in PSFs did influence the aboveground biomass of *A. odoratum* but not of *C. jacea*. Both species produced much more aboveground biomass in sterile soil than in live soil (Table S5.3A; Fig. 5.2A, C), indicating that soil biota inhibited plant growth of both species.

PSF heterogeneity also influenced belowground biomass but the effect varied between the two species and soil sterilization. *A. odoratum* produced similar amounts of belowground biomass in heterogeneous and homogeneous soils (Table S5.3B; Fig. 5.2B). *C. jacea* produced less belowground biomass in live heterogeneous than in live homogeneous soils, but in sterilized soil there was no difference between these heterogeneity treatments (Table S5.3B: significant sterilization × heterogeneity effect; Fig. 5.2D). These results suggest that heterogeneity in PSFs influenced the belowground biomass of *C. jacea* but not of *A. odoratum*. Belowground biomass per soil core of both species was significantly greater in sterile soil than in live soil (Table S5.3B; Fig. 5.2B, D).

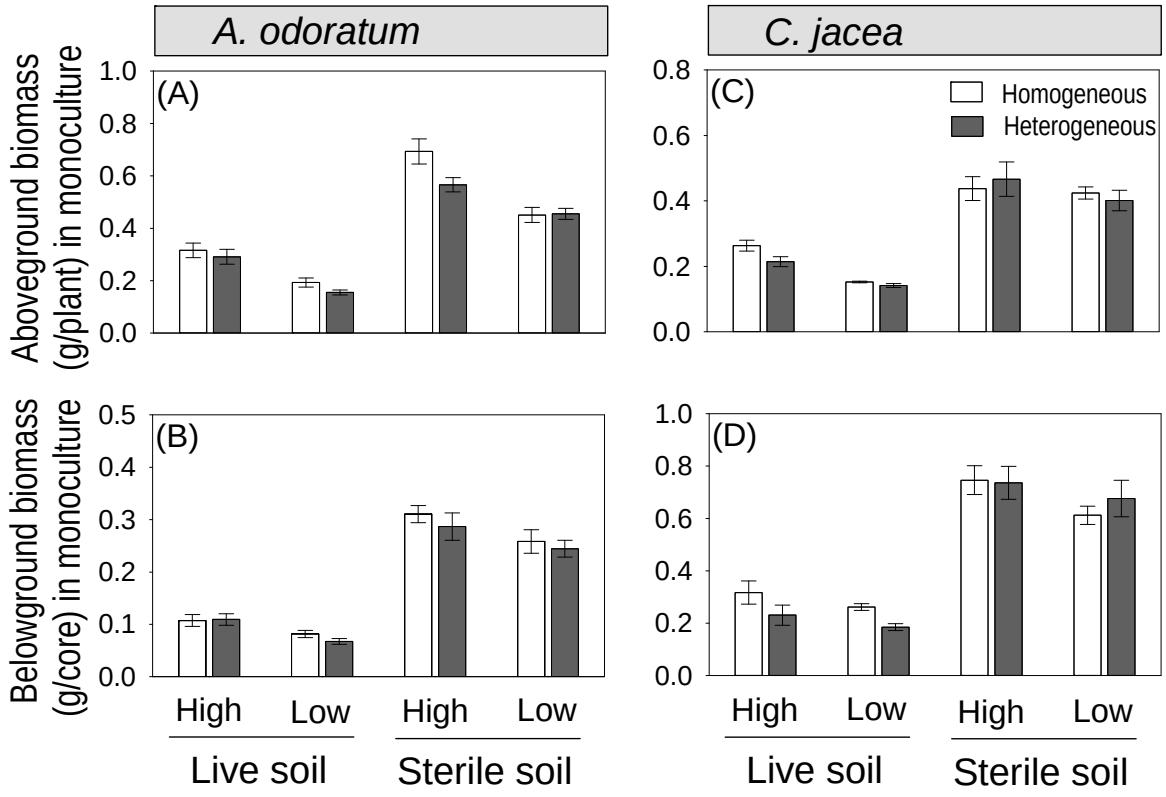

Fig. 5.2. Aboveground biomass per plant (A and C) and belowground biomass per soil core (B and D) of *A. odoratum* (A and B) and *C. jacea* (C and D) in plant monocultures in homogeneous and heterogeneous soils at the pot level. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1 SE) are presented. See Table S5.3A-B for statistical results.

Soil heterogeneity and the interaction with nutrients and/or sterilization did not affect the CV of aboveground biomass of either *A. odoratum* or *C. jacea* (Table S5.4A; Fig. 5.3A, C). CVs of belowground biomass of both plant species were significantly greater in live heterogeneous soil than in live homogeneous soil. In sterilized soil there was no difference between the two heterogeneity treatments (Table S5.4B: significant and marginally significant sterilization × heterogeneity effect for *A. odoratum* and *C. jacea*, respectively; Fig. 5.3B, D). Hence, PSF heterogeneity increased spatial variation in root growth in live soil but not when soil biota were excluded.

In monocultures, in pots with spatially heterogeneous soil, *A. odoratum* produced more aboveground biomass in live "foreign" soil patches than in live "own" soil patches when soil nutrient is low, but no difference was found between these two patches in high nutrient soil or in sterile soils (Table S5.5A: significant nutrient × sterilization × soil interaction effect; Fig. S5.3A). *C. jacea* produced more aboveground biomass in live "foreign" soil patches than in

live "own" soil patches but there was no difference between the two soil patches in sterile soils (Table S5.5A: significant sterilization × soil interaction effect; Fig. S5.3C). The same pattern was found for the belowground biomass of *A. odoratum*, while *C. jacea* overall produced less belowground biomass in "foreign" soil patches than in "own" soil patches (Table S5.5B; Fig. S5.3B, D). These results suggest that plant monocultures showed different responses to spatially heterogeneous PSFs.

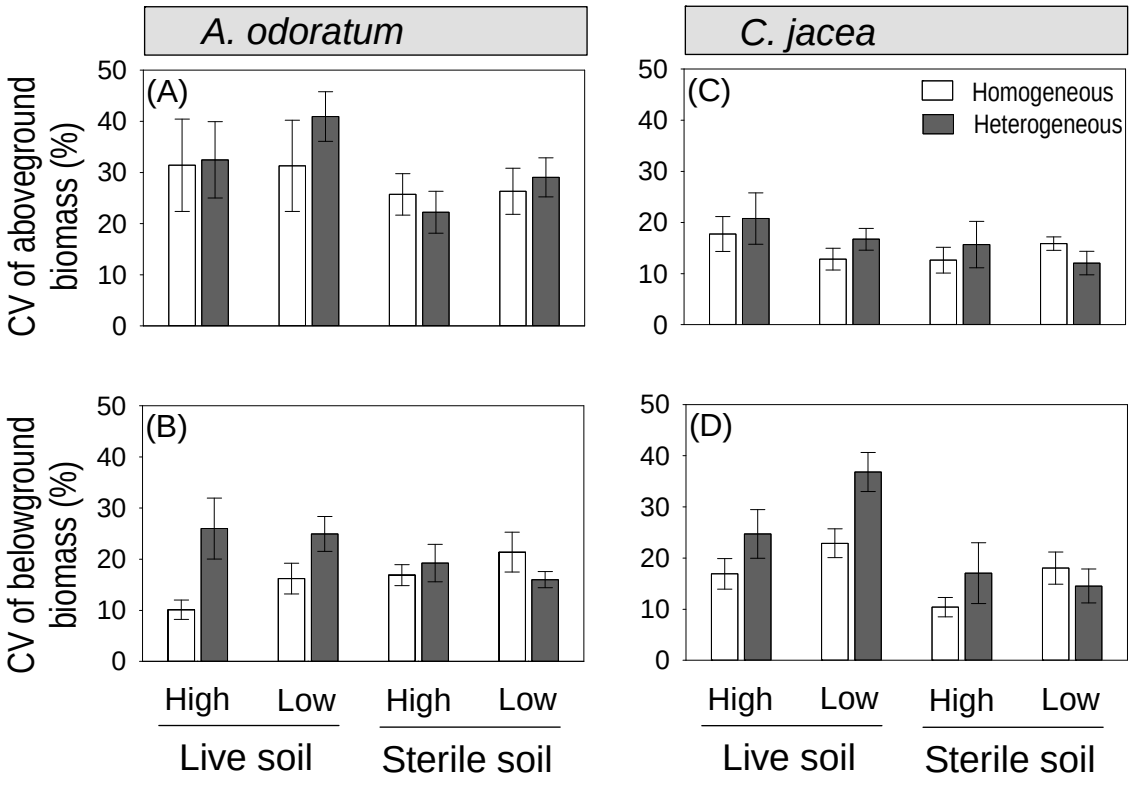

Fig. 5.3. Coefficient of variation (CV) of aboveground biomass (A and C) and CV of belowground biomass (B and D) of *A. odoratum* (A and B) and *C. jacea* (C and D) in plant monocultures among the four patches within homogeneous and heterogeneous soils. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1 SE) are presented. See Table S5.4 for statistical results.

Effects of plant-soil feedback heterogeneity on plant growth in mixtures

In mixtures, the growth difference between the two species tended to be smaller in heterogeneous soils than in homogeneous soils (Table S5.6: marginally significant heterogeneity effect; Fig. 5.4), indicating that the growth inequality between the two competing species was reduced in heterogeneous soils. The growth difference index (D) was generally negative in live soil but positive in sterile soil, i.e. *C. jacea* was superior to *A.*

odoratum in live soil while the reverse was true in sterile soil (Table S5.6; Fig. 5.4). The aboveground biomass of both species grown in mixtures is presented in the supporting information (Table S5.3C; Fig. S5.4).

In mixtures, in pots with spatially heterogeneous soil, *A. odoratum* produced more aboveground biomass in "foreign" soil patches than in "own" soil patches (Table S5.5C; Fig. 5.5A). A similar trend was observed for *C. jacea* but this was not significant (Table S5.5C; Fig. 5.5B). This result suggests that both plant species selectively grew in "foreign" soil patches in spatially heterogeneous soils.

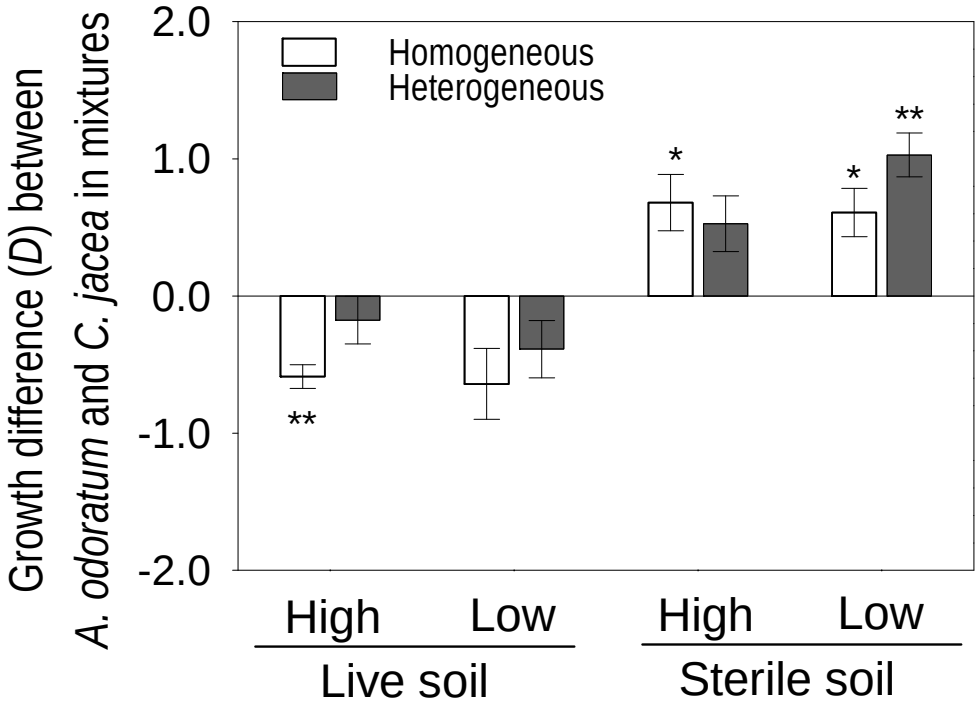

Fig. 5.4. Growth difference (*D*, log-ratio of aboveground biomass of *A. odoratum* and *C. jacea* in plant mixtures) in homogeneous and heterogeneous soils. Positive values indicate the biomass of *A. odoratum* is higher than *C. jacea* and negative values indicate the reverse is true. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1 SE) are presented. See Table S5.6 for statistical results. Asterisks at the end of bars indicate which means differed from zero (one-sample *t*-test). Symbols give *P*: ** $P<0.01$ and * $P<0.05$.

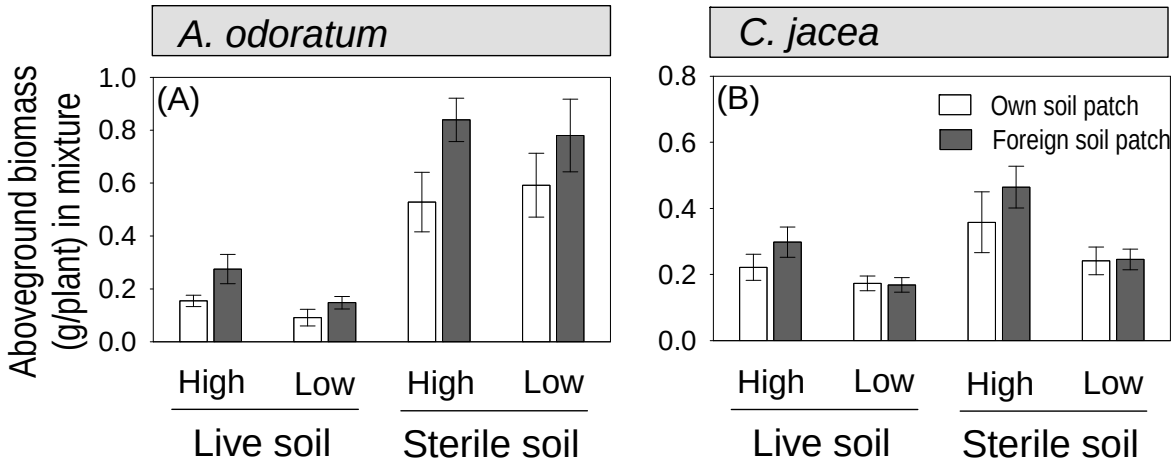

Fig. 5.5. Aboveground biomass per plant per patch of *A. odoratum* (A) and *C. jacea* (B) in plant mixtures in "own" and "foreign" soil patches for pots with heterogeneous soils. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1 SE) are presented. See Table S5.5C for statistical results.

Discussion

In this study we compared the growth of plants in pots with heterogeneous soils and homogeneous soils that consisted of the same component soils. Remarkably, even though the two soils had the same starting conditions regarding nutrients and microbial composition, we observed that in heterogeneous pots with conditioned soils that were spatially separated, the performance of plant monocultures was worse than in homogeneous pots with evenly mixed conditioned soils. When competing, the difference between the growth of the two species decreased in heterogeneous pots compared to homogeneous pots. Hence, our study implies that spatially heterogeneous PSFs, i.e. the spatial configuration of conditioned soils increase the negative effects for plant monocultures growing in "own" soil, and decrease the growth inequality between the two competing species.

Recently, Wubs and Bezemer (2016) reported a negative effect of spatial plant-soil feedback heterogeneity on plant growth in monocultures similar to what we found. In that study, the performance of six plant species grown in monocultures in soils with spatially heterogeneous PSFs and in monospecific conditioned soil was compared. The negative effect of heterogeneity in the study by Wubs and Bezemer (2016) was explained by the more diverse microbial communities present in heterogeneous soils (where four conditioned soils were present in a pot) than in monospecific soils where only one plant species had conditioned the

soil. Hence, spatial plant-soil feedback heterogeneity increased the chances of a plant to encounter specific soil pathogens, as well as the chances of co-infections by different soil pathogens (Wubs and Bezemer 2016). In contrast, in our study, the initial composition in each pot was similar irrespective of the heterogeneity treatment, since the same set of conditioned soils were used in pots with homogeneous and heterogeneous soil. Hence, the negative effect of spatially heterogeneous PSFs in our study is less likely due to the difference in the original composition of microbial communities. However, it is important to note that we did not measure the microbial composition in the soils, and hence we cannot exclude that mixing soil communities may have influenced the composition that established in these soils (Brinkman et al. 2010, Reinhart and Rinella 2016). Alternatively, evenly mixing the two soil communities implies that soil communities arranged in a patchy way in the heterogeneous pots may have been "diluted", which allows plant monocultures to grow more in homogenous soils than in heterogeneous soils (Hawkes et al. 2013, Hendriks et al. 2013).

In monocultures, plant growth varied more among the four patches within the heterogeneous soils than within the homogeneous soils, indicating that spatially heterogeneous PSFs promote growth divergence. This may be explained by the greater variety of microsites within the heterogeneous soils, i.e. there were two conditioned soils placed in discrete patches within the heterogeneous soils but the two conditioned soils were evenly mixed within the homogeneous soils. Hence, plants can avoid contact with their enemies by placing more shoots/roots in the "foreign" soil patches (Fig. S5.3; Hendriks et al. 2015b) in the heterogeneous soils, which increases the growth variations among these patches. Importantly, we only found such difference in live soil but not in sterile soil, indicating that soil biota were likely involved in the responses of plant monocultures to spatially heterogeneous PSFs. Further studies should aim to disentangle the role of the microbial community in creating spatial heterogeneity effects on plant growth.

We expected that in plant mixtures (interspecific competition), the growth difference between the competing species would be smaller in heterogeneous soils than in homogeneous soils. In our study, we only found weak evidence for this. In heterogeneous soils, both plant species encountered patches with "own" and "foreign" soils, potentially providing both plant species with enemy free space, i.e. the avoidance of contact with antagonists in "own" soil patches. Indeed in mixtures, we generally found a negative conspecific PSF (less growth in "own" than in "foreign" soil patches) even though this was only significant for one of the two species.

This result indicates that spatially heterogeneous PSFs can reduce the biomass inequality between competing species but also shows that the effects are plant species specific.

As expected, sterilizing the soil increased plant growth. Our results show that soil biota in our system have a negative effect on plant growth, i.e. there are more pathogenic or harmful microbes than beneficial ones present in conditioned soil. However, it is important to note sterilization of soils also increased the soil nutrient availability (Table S5.1), and this obviously promotes the growth of plant species. Unfortunately, we cannot distinguish to what extent the exclusion of soil biota and release of soil nutrients may have promoted the growth of the plant in sterilized soil, yet it must be a net effect of elimination of soil biota and an increase in soil nutrients (Brinkman et al. 2010). Remarkably, sterilization of soils changed the competition hierarchy of the two competing species, i.e., *C. jacea* is superior to *A. odoratum* in live soil while the reverse is true in sterile soil. One possible explanation is that *C. jacea* has a greater association with mycorrhizal fungi than *A. odoratum* under poor soil conditions as indicated by previous studies (the mycorrhizal fungi dependency of *C. jacea* and *A. odoratum* is about 64% and 35%, respectively; Grime et al. 1987, Tawaraya 2003, van der Heijden et al. 2008). Another possible explanation may be related to the competition for different resources. *Anthoxanthum odoratum* profits from the higher nutrient supply in the sterile soil treatments. In nutrient-rich environments, competition for light is important, thus species that can produce more leaves have a competitive advantage (Aerts 1999). *Anthoxanthum odoratum* is a species that can produce dense tillers rapidly (Lovett-Doust 1981, Humphrey and Pyke 1998) and they were taller than *C. jacea* plants in the greenhouse experiment (W. Xue, *pers. obs.*). This may explain why *A. odoratum* was the stronger competitor in sterile soil. In nutrient-poor environments (live soils in the present study), competition for nutrients prevails, and hence, species with larger rooting systems may have a competitive advantage (Aerts 1999, Grime 2006). *C. jacea* has a deeper root system than *A. odoratum*, thus most underground space was occupied by *C. jacea*, which may explain its competitive advantage in nutrient poor conditions.

We hypothesized that PSF heterogeneity effects in the test phase would be stronger when the soil was originally nutrient poor during conditioning, as PSF effects generally diminish with increasing soil fertility (van der Putten and Peters 1997, De Deyn et al. 2004). In contrast to our hypothesis, the effects of PSF heterogeneity did not differ between the two soil fertility levels as indicated by the absence of significant nutrient × heterogeneity effects. At the end of the conditioning period in the field, the amount of organic matter was higher in high nutrient

than in low nutrient soils, but there were no differences in other soil chemical properties between the two soil nutrient treatments (Table S5.1). This may explain why we did not observe stronger conditioning effects on PSF heterogeneity effects in low nutrient soils. More studies are needed to examine the role of spatial plant-soil feedback heterogeneity on plant performance and competition along a gradient of soil nutrient availability.

In conclusion, in soils with spatially heterogeneous plant-soil feedback plants produced less biomass than in homogeneously mixed soils. However, plant growth varied more among the patches within the heterogeneous soils than within the homogeneous soils. Moreover, spatially heterogeneous plant-soil feedbacks reduced the growth inequality between the two competing species by allowing them to grow more in "foreign" soil patches than in "own" soil patches. We did not find the evidence that initial soil fertility influences plant-soil feedback heterogeneity effects. Despite that, our results indicate that spatial plant-soil feedback heterogeneity could be a mechanism explaining species coexistence at the local scale.

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Author contributions

W.X., F.B. and T.M.B. designed the experiment; W.X. and F.B. collected the data; W.X. and T.M.B. analyzed the data; W.X. and T.M.B. wrote the first version of the manuscript. All authors discussed the results, contributed substantially to the draft and gave final approval for publication. There are no conflict of interests to declare.

Data Accessibility

Data are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.vm125vv (Xue, Berendse & Bezemer, 2018).

Supporting information

Table S5.1. Soil chemical analysis of different soils after three-years of conditioning in the field. Means (±SE), *F*- and *P*-values of three-way ANOVA are given. Tukey post-hoc tests were made among these soils, mean values sharing the same superscript (a-c) are not significantly different among the twelve soils. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase; "pure Ao soil" and "pure Cj soil" represent soils conditioned by monocultures of *A. odoratum* and *C. jacea*, respectively; "homogeneous soil" represents the 1:1 (v:v) mixture of pure Ao soils and pure Cj soil. Data of $N\text{-}NO_3$ was ln-transformed before analysis and the value of $N\text{-}NO_3$ in the live, high nutrient pure Ao soil is based on only one soil sample. Symbols give: *** $P<0.001$, ** $P<0.01$ and * $P<0.05$

Sterilization	Nutrient	Soil	$P\text{-}PO_4$ (mg/kg)	$N\text{-}NO_3$ (mg/kg)	$N\text{-}NH_4$ (mg/kg)	pH (H_2O)	Moisture (%)	Organic Matter (%)
(A) Live soils	High	pure Ao soil	0.00 ± 0.00^{b}	0.49	6.14 ± 4.00^{b}	6.85 ± 0.11^{b}	14.45 ± 1.64^{a}	2.94 ± 0.48^{a}
	High	pure Cj soil	0.25 ± 0.12^{ab}	0.66 ± 0.25	5.76 ± 3.89^{b}	6.98 ± 0.20^{ab}	13.71 ± 0.77^{a}	2.63 ± 0.16^{a}
	High	homogeneous soil	0.36 ± 0.21^{ab}	1.46 ± 0.73	5.86 ± 3.80^{b}	7.09 ± 0.11^{ab}	13.20 ± 2.08^{a}	2.69 ± 0.38^{a}
	Low	pure Ao soil	0.08 ± 0.08^{b}	1.01 ± 0.06	6.65 ± 4.85^{ab}	7.10 ± 0.23^{ab}	6.97 ± 0.74^{b}	1.05 ± 0.09^{b}
	Low	pure Cj soil	0.06 ± 0.03^{b}	0.44 ± 0.17	5.22 ± 3.29^{b}	7.02 ± 0.39^{ab}	7.06 ± 0.26^{b}	1.11 ± 0.13^{b}
	Low	homogeneous soil	0.07 ± 0.07^{b}	0.93 ± 0.10	5.97 ± 4.18^{b}	7.21 ± 0.13^{ab}	7.29 ± 0.49^{b}	1.10 ± 0.04^{b}
(B) Sterile soils	High	pure Ao soil	0.67 ± 0.21^{ab}	0.90 ± 0.33	23.78 ± 7.76^{a}	7.06 ± 0.05^{ab}	13.13 ± 2.26^{a}	2.77 ± 0.77^{a}
	High	pure Cj soil	1.09 ± 0.23^{a}	0.47 ± 0.08	20.68 ± 5.17^{ab}	7.17 ± 0.11^{ab}	14.75 ± 2.06^{a}	2.60 ± 0.55^{a}
	High	homogeneous soil	0.78 ± 0.16^{ab}	1.51 ± 0.17	16.78 ± 3.77^{ab}	7.20 ± 0.01^{ab}	12.69 ± 1.04^{a}	2.39 ± 0.27^{a}
	Low	pure Ao soil	0.81 ± 0.15^{ab}	0.55 ± 0.22	13.42 ± 4.86^{ab}	7.28 ± 0.05^{ab}	6.81 ± 0.64^{b}	1.18 ± 0.18^{b}
	Low	pure Cj soil	0.82 ± 0.35^{ab}	0.40 ± 0.14	12.38 ± 4.07^{ab}	7.30 ± 0.11^{ab}	6.63 ± 0.75^{b}	1.17 ± 0.11^{b}
	Low	homogeneous soil	0.86 ± 0.44^{ab}	0.99 ± 0.54	12.92 ± 3.11^{ab}	7.41 ± 0.02^{a}	6.62 ± 0.18^{b}	1.04 ± 0.09^{b}
(C) Three-way ANOVA		Nutrient (N)	0.40	2.74	2.98	5.56^{*}	121.38^{***}	110.91^{***}
		Sterilization (ST)	37.90^{***}	1.35	24.52^{***}	8.08^{**}	0.31	0.16
		Soil	0.73	2.56	0.34	1.88	0.32	0.48
		N × ST	0.27	0.60	3.02	0.14	0.02	0.51
		N × Soil	0.76	0.68	0.19	0.39	0.44	0.35
		ST × Soil	0.23	1.63	0.20	0.10	0.29	0.16
		N × ST × Soil	0.34	0.70	0.21	0.07	0.39	0.04

Table S5.2. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile) and soil type (pure Ao soil vs. pure Cj soil) on plot-level aboveground biomass (A and C) and belowground biomass (B) of *A. odoratum* and *C. jacea* in monocultures (A and B) and in 1:1 mixtures (C) in the pure soils. Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

			A. odoratum[1]		*C. jacea*[1]	
Effect	DF	denDF	*F*-value	*P*-value	*F*-value	*P*-value
(A) *Aboveground biomass in plant monocultures*						
Nutrient (N)	1	28	81.46	**<0.001**	64.09	**<0.001**
Sterilization (ST)	1	28	283.96	**<0.001**	303.39	**<0.001**
Soil type (Soil)	1	28	0.97	0.332	4.22	**0.049**
N × ST	1	28	1.04	0.317	27.13	**<0.001**
N × Soil	1	28	0.60	0.446	1.20	0.283
ST × Soil	1	28	0.01	0.932	1.03	0.319
N × ST × Soil	1	28	0.08	0.782	2.01	0.168
(B) *Belowground biomass in plant monocultures*						
Nutrient (N)	1	28	19.75	**<0.001**	17.50	**<0.001**
Sterilization (ST)	1	28	310.23	**<0.001**	281.23	**<0.001**
Soil type (Soil)	1	28	5.26	**0.030**	0.59	0.447
N × ST	1	28	0.49	0.490	9.16	**0.005**
N × Soil	1	28	0.04	0.846	0.67	0.421
ST × Soil	1	28	10.34	**0.003**	1.98	0.171
N × ST × Soil	1	28	1.56	0.222	10.31	**0.003**
(C) *Aboveground biomass in 1:1 plant mixtures*						
Nutrient (N)	1	28	20.60	**<0.001**	11.94	**0.002**
Sterilization (ST)	1	28	178.76	**<0.001**	2.99	0.095
Soil type (Soil)	1	28	9.32	**0.005**	13.24	**0.001**
N × ST	1	28	7.28	**0.012**	1.63	0.212
N × Soil	1	28	0.49	0.491	0.34	0.562
ST × Soil	1	28	6.68	**0.015**	0.01	0.937
N × ST × Soil	1	28	1.11	0.301	2.53	0.123

[1] Data were ln-transformed

Table S5.3. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile) and PSF heterogeneity (homogeneous soil vs. heterogeneous soil) on plot-level aboveground biomass (A and C) and belowground biomass (B) of *A. odoratum* and *C. jacea* in monocultures (A and B) and in 1:1 mixtures (C). Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

			A. odoratum[1]		*C. jacea*[1]	
Effect	DF	denDF	*F*-value	*P*-value	*F*-value	*P*-value
(A) *Aboveground biomass in plant monocultures*						
Nutrient (N)	1	28	96.59	**<0.001**	34.81	**<0.001**
Sterilization (ST)	1	28	366.29	**<0.001**	311.34	**<0.001**
Heterogeneity (H)	1	28	7.20	**0.012**	2.43	0.131
N × ST	1	28	6.70	**0.015**	17.46	**<0.001**
N × H	1	28	0.22	0.639	0.00	0.955
ST × H	1	28	0.37	0.547	2.13	0.156
N × ST × H	1	28	3.59	0.068	1.76	0.195
(B) *Belowground biomass in plant monocultures*						
Nutrient (N)	1	28	21.42	**<0.001**	5.13	**0.031**
Sterilization (ST)	1	28	362.26	**<0.001**	244.60	**<0.001**
Heterogeneity (H)	1	28	1.69	0.205	5.28	**0.029**
N × ST	1	28	2.87	0.101	0.03	0.856
N × H	1	28	0.50	0.487	0.11	0.746
ST × H	1	28	0.01	0.905	7.94	**0.009**
N × ST × H	1	28	1.13	0.297	0.20	0.660
(C) *Aboveground biomass in 1:1 plant mixtures*						
Nutrient (N)	1	28	27.59	**<0.001**	26.39	**<0.001**
Sterilization (ST)	1	28	416.70	**<0.001**	16.18	**<0.001**
Heterogeneity (H)	1	28	2.18	0.151	2.28	0.142
N × ST	1	28	6.64	**0.016**	0.03	0.855
N × H	1	28	1.20	0.283	0.08	0.773
ST × H	1	28	0.08	0.780	0.92	0.345
N × ST × H	1	28	2.45	0.129	0.68	0.417

[1] Data were ln-transformed

Table S5.4. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile), PSF heterogeneity (homogeneous soil vs. heterogeneous soil) on CV (coefficients of variation) of aboveground biomass (A) and belowground biomass (B) of *A. odoratum* and *C. jacea* in monocultures. Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

			A. odoratum		*C. jacea*	
Effect	DF	denDF	*F*-value	*P*-value	*F*-value	*P*-value
(A) *CV of aboveground biomass in plant monocultures*						
Nutrient (N)	1	28	0.90	0.350	1.10	0.304
Sterilization (ST)	1	28	3.90	0.058	1.75	0.196
Heterogeneity (H)	1	28	0.36	0.555	0.47	0.497
N × ST	1	28	0.00	0.955	0.92	0.346
N × H	1	28	0.80	0.379	0.45	0.508
ST × H	1	28	0.48	0.495	0.74	0.398
N × ST × H	1	28	0.02	0.888	0.74	0.396
(B) *CV of belowground biomass in plant monocultures*						
Nutrient (N)	1	28	0.42	0.524	5.09	**0.032**
Sterilization (ST)	1	28	0.15	0.706	16.11	**<0.001**
Heterogeneity (H)	1	28	4.88	**0.036**	5.85	**0.022**
N × ST	1	28	0.15	0.699	1.58	0.219
N × H	1	28	2.33	0.138	0.15	0.697
ST × H	1	28	7.99	**0.009**	3.26	0.082
N × ST × H	1	28	0.00	0.952	2.50	0.125

Table S5.5. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile), soil type ("own" vs. "foreign" soil within the heterogeneous soils) on patch-level aboveground biomass (A and C) and belowground biomass (B) of *A. odoratum* and *C. jacea* in monocultures (A and B) and in 1:1 mixtures (C) within the heterogeneous soil. Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

			A. odoratum[1]		*C. jacea*[1]	
Effect	DF	denDF	*F*-value	*P*-value	*F*-value	*P*-value
(A) *Aboveground biomass in plant monocultures*						
Nutrient (N)	1	12	31.76	**<0.001**	10.49	**0.007**
Sterilization (ST)	1	12	134.54	**<0.001**	123.20	**<0.001**
Soil type (Soil)	1	16	4.51	0.050	1.12	0.307
N × ST	1	12	7.43	**0.018**	2.64	0.130
N × Soil	1	16	6.27	**0.024**	1.09	0.312
ST × Soil	1	16	8.28	**0.011**	9.59	**0.007**
N × ST × Soil	1	16	6.00	**0.026**	0.01	0.930
(B) *Belowground biomass in plant monocultures*						
Nutrient (N)	1	12	14.06	**0.003**	1.72	0.215
Sterilization (ST)	1	12	193.53	**<0.001**	139.14	**<0.001**
Soil type (Soil)	1	16	1.63	0.221	6.92	**0.018**
N × ST	1	12	3.95	0.070	0.34	0.570
N × Soil	1	16	2.11	0.166	0.28	0.604
ST × Soil	1	16	5.28	**0.035**	0.84	0.373
N × ST × Soil	1	16	0.19	0.671	0.04	0.850
(C) *Aboveground biomass in plant mixtures*						
Nutrient (N)	1	12	7.87	**0.016**	14.10	**0.003**
Sterilization (ST)	1	12	135.83	**<0.001**	10.73	**0.007**
Soil type (Soil)	1	16	16.96	**0.001**	3.09	0.098
N × ST	1	12	6.96	**0.022**	0.17	0.688
N × Soil	1	16	0.00	0.950	2.85	0.111
ST × Soil	1	16	0.88	0.363	0.14	0.718
N × ST × Soil	1	16	1.48	0.242	0.03	0.865

[1] Data were ln-transformed

Table S5.6. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile), PSF heterogeneity (homogeneous soil vs. heterogeneous soil) on growth difference (*D*) between *A. odoratum* and *C. jacea* in 1:1 mixtures. Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

			Growth difference *(D)*	
Effect	DF	denDF	*F*-value	*P*-value
Nutrient (N)	1	28	0.10	0.756
Sterilization (ST)	1	28	79.41	**<0.001**
Heterogeneity (H)	1	28	3.20	0.085
N × ST	1	28	1.77	0.194
N × H	1	28	0.64	0.431
ST × H	1	28	0.59	0.448
N × ST × H	1	28	1.97	0.172

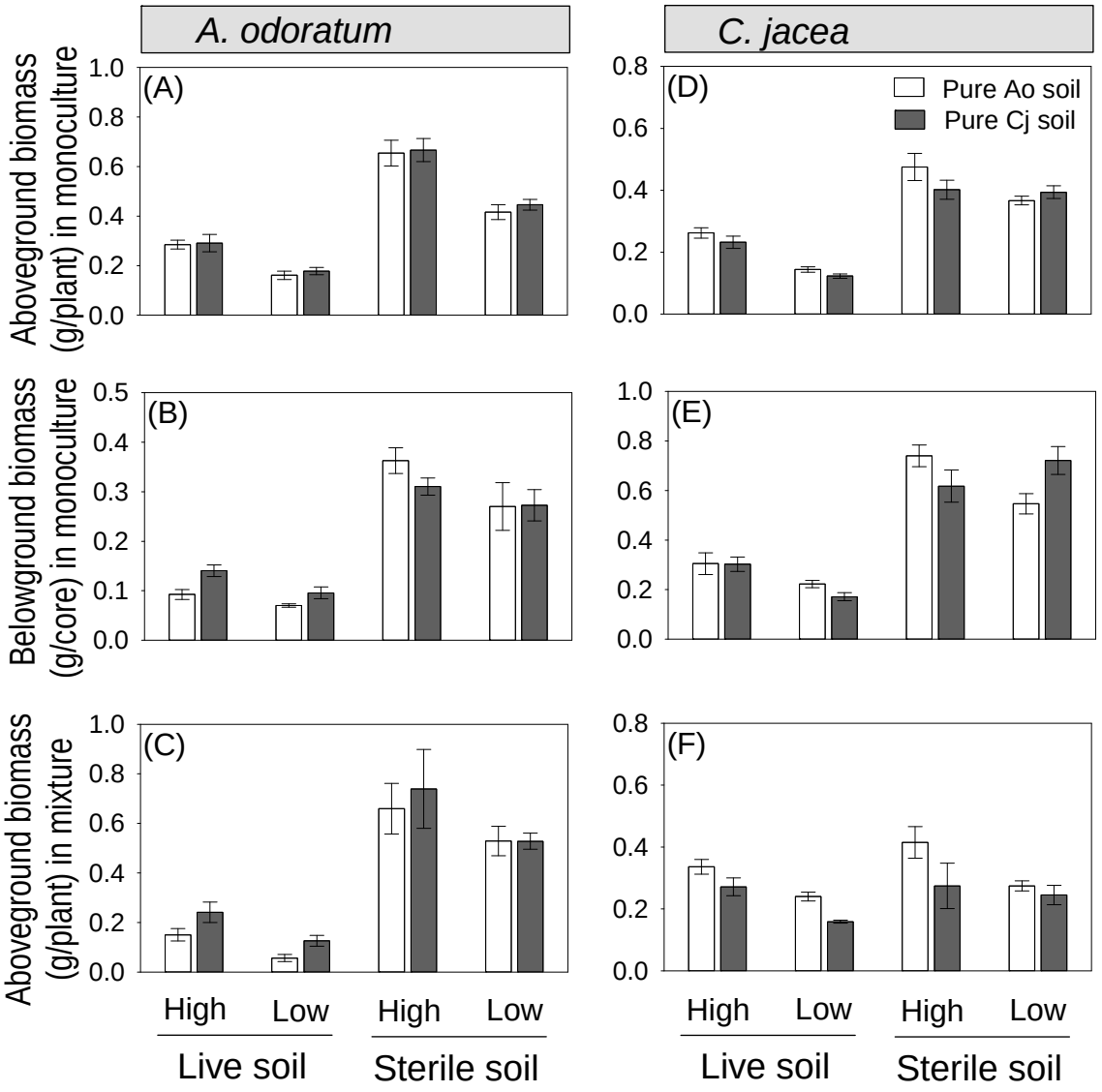


Fig. S5.1. Aboveground biomass per plant (upper and bottom panels) and belowground biomass per soil core (middle panels) of *A. odoratum* and *C. jacea* in monocultures and mixtures in pure Ao soil and pure Cj soils. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field- collected soil and sterilized field-collected soil, respectively. Plants were grown in monocultures and in 1:1 mixtures. Mean values (± 1 SE) are presented. See Table S5.2 for statistical results.

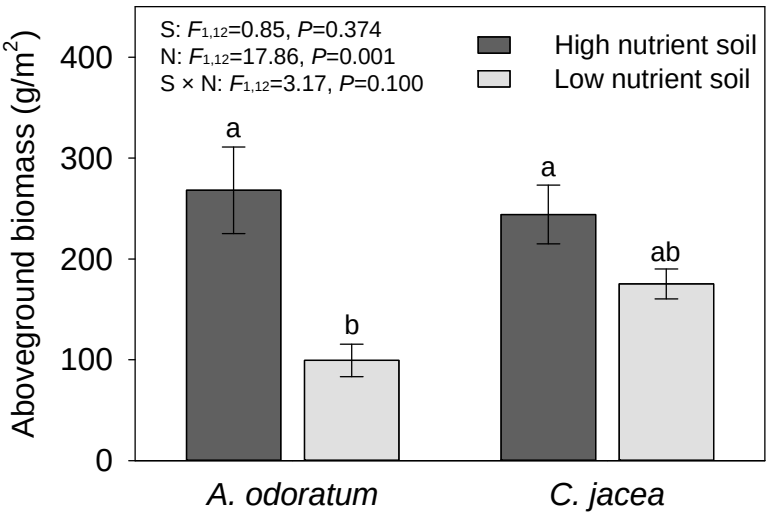


Fig. S5.2. Aboveground biomass in the field plots at the end of the conditioning period for *A. odoratum* and *C. jacea* monocultures in high nutrient and low nutrient soils. Mean dry weight (± 1 SE), *F*- and *P*-values of a two-way ANOVA with species (S), nutrient (N) and the interaction (S × N) are also presented: ** P<0.01. Bars sharing the same superscript (a-b) are not significantly different based on a Tukey post-hoc test.

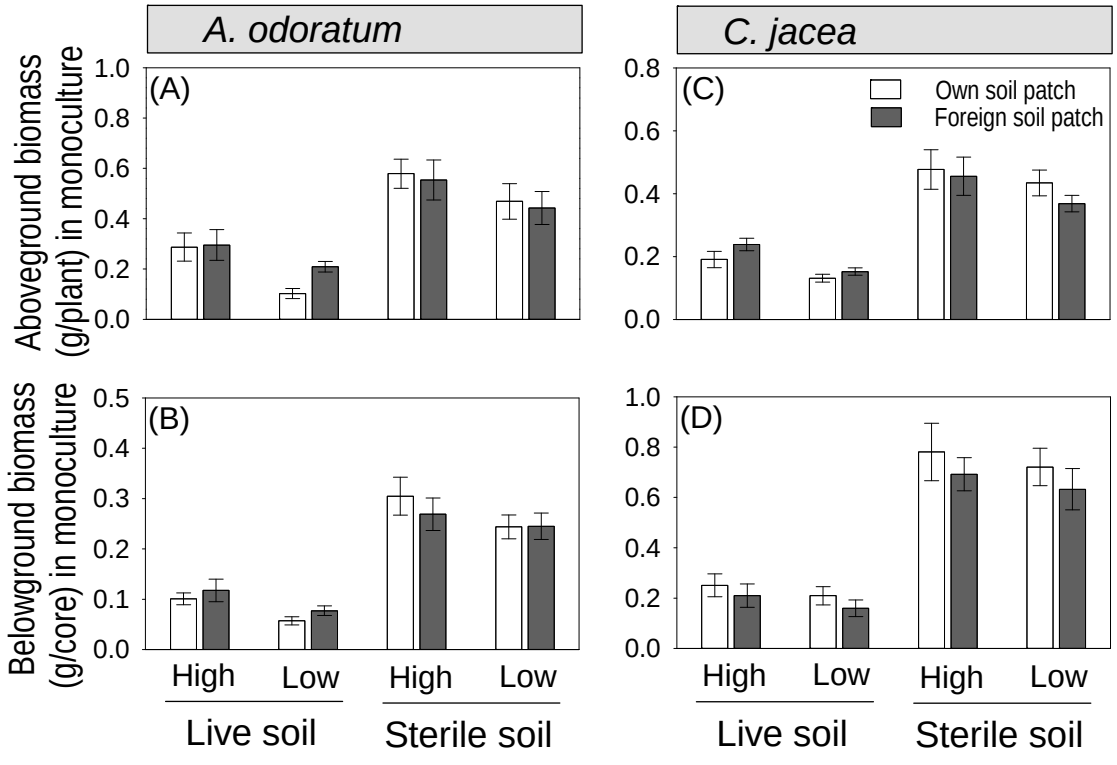


Fig. S5.3. Aboveground biomass per plant per patch (A and C) and belowground biomass per soil core per patch (B and D) of *A. odoratum* and *C. jacea* in the greenhouse experiment. Data are for monocultures with heterogeneous soils. "Own" and "foreign" soil patches refer to conspecific and heterospecific soil patches respectively. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1 SE) are presented. See Table S5.5A-B for statistical results.

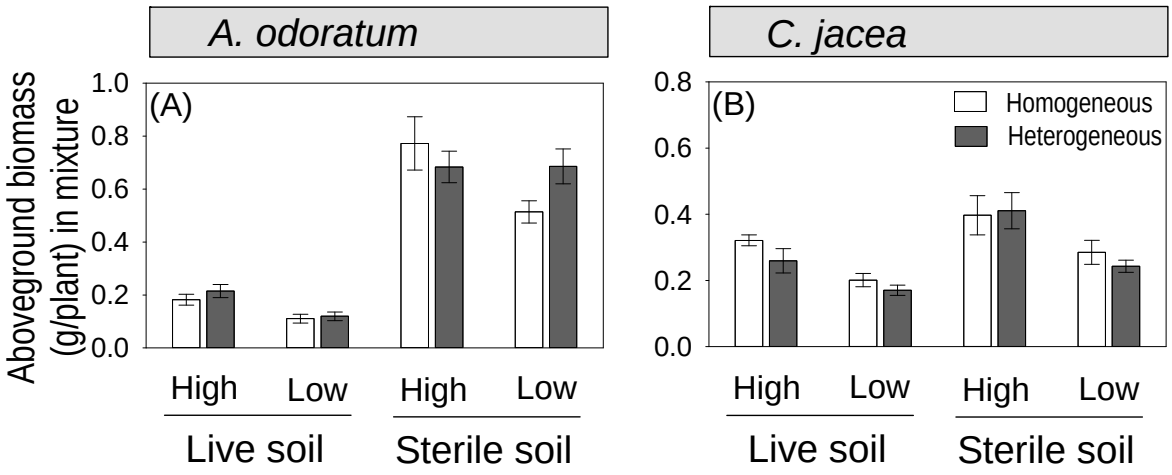


Fig. S5.4. Aboveground biomass per plant of *A. odoratum* (A) and *C. jacea* (B) in 1:1 mixtures in homogeneous and heterogeneous soils at the pot level. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1 SE) are presented. See Table S5.3C for statistical results.

Chapter 6

General discussion

One of the central aims in ecology is to understand the mechanisms that are responsible for plant species coexistence under various environmental conditions (Tilman and Pacala 1993, Rosenzweig 1995). Such knowledge is urgently needed to predict whether environmental changes will lead to plant diversity loss, and to develop strategies to restore species diversity. A classic mechanism is that spatial heterogeneity in resources and other environmental factors allows species coexistence by increasing niche availability (Wilson 2011), creating refuges and shelters for subordinate and rare species (Chesson 2000, Hutchings et al. 2003) and providing species diversification opportunities (Hughes and Eastwood 2006, Stein 2015). This has been well supported in theoretical studies (Ricklefs 1977, Chesson 2000, Hutchings et al. 2003) as well as in many observational studies (Pausas 1994, Bakker et al. 2003, Shi et al. 2010, Wang et al. 2013). However, the experimental evidence for this hypothesis is scarce (see Table 1.1 in Chapter 1). In this thesis, I aimed at testing how different types of spatial heterogeneity, i.e. soil heterogeneity in nutrient supply, pH and plant-soil feedbacks may influence plant species coexistence and diversity by integrating the results of two greenhouse experiments and two garden experiments.

As a starting point, in a two-year common garden experiment, I investigated the growth of two clonal plants with contrasting spatial architectures (i.e. phalanx with aggregated ramets vs. guerilla with diffuse ramets), as well as the competition between the two species planted in even or clustered mixtures on homogeneous and heterogeneous soils (Chapter 2). I found that spatial heterogeneity in soil nutrients did not significantly influence the growth of either plant species at the plot level, possibly due to the mismatch between patch size and inter-ramet distance, i.e. because the plants could exploit resources across patches. However, within the heterogeneous soils, the guerilla plant *Bolboschoenus planiculmis* produced more biomass in high nutrient soil patches than in low nutrient soil patches, indicating that the guerilla plant did respond to soil nutrient heterogeneity in this study. Importantly, soil nutrient heterogeneity increased the relative competitive ability of the guerilla plant *B. planiculmis* but decreased that of the phalanx plant *Carex neurocarpa*, regardless of whether they were growing in even mixtures or clustered mixtures. These results suggest that soil nutrient heterogeneity may delay the competitive exclusion process through equalizing the competitive ability of the competing species.

In order to directly test whether soil heterogeneity can promote plant species diversity, I investigated plant species richness and diversity of an experimental grassland community in field plots with homogeneously distributed soil nutrients and in horizontally heterogeneous

plots consisting of 36 or 4 patches of both low and high nutrient soils arranged in a checkerboard manner. The same design was used for homogeneous and heterogeneous plots that varied in soil pH (Chapter 3). In addition, I also investigated the plant species richness and diversity on two vertically heterogeneous plots consisting of high and low nutrient soils arranged in distinct layers (top and bottom layer). I determined plant species richness and diversity at two spatial scales: 0.16 m^2 plot scale and 0.01 m^2 patch scale. I did not find a significant soil heterogeneity effect on plot-scale plant species diversity. However, horizontal soil heterogeneity in nutrients reduced patch-scale plant species richness as indicated by the lower species richness in soils with heterogeneously distributed nutrients than in equivalent homogeneous soil. Similarly, vertical soil heterogeneity in nutrients also reduced patch-scale plant species richness as indicated by the lower species richness in vertically heterogeneous soils where high nutrient soil was located in the bottom layer as compared to the equivalent homogenous soil. In contrast, spatial heterogeneity in pH increased patch-scale plant species diversity. I measured a higher plant species diversity in heterogeneous pH soil with large patches than in homogeneous pH soil.

Within the horizontally heterogeneous soils, patch-scale plant species richness was higher in high nutrient soil patches that in low nutrient soil patches, but there was no difference in the high and low pH soil patches. These results indicate that spatial heterogeneity in soil characteristics influences plant species diversity, but also that these effects depend on the soil factor that is investigated, and on the spatial scales at which the plant species diversity and soil heterogeneity are measured.

One of the most important sources of spatial variation in soils of natural plant communities is the small-scale spatial distribution of plant species within the community. This can lead to strong spatial variation in the abundance of plant-species-specific soil pathogens. I investigated the potential implications of plant-soil feedbacks and their spatial variation for plant species coexistence. I collected soils from a three-year field experiment where the grass *Anthoxanthum odoratum* and the forb *Centaurea jacea* were planted in monocultures and in different mixtures on soils with high and low nutrient availability. I examined the relationship between the abundance of the two plant species in the field plots (the conditioning phase) and the competitive balance between the two species in pots in the greenhouse (the test or feedback phase). I also examined the conspecific plant-soil feedback effects of the two species when they were grown in monocultures and in 1:1 mixtures in the greenhouse experiment (Chapter 4). I showed that there was a negative relationship between the

competitive performance of *C. jacea* relative to *A. odoratum* in the greenhouse experiment and the abundance of *C. jacea* in the field plots where the soil was collected. However, there was no relationship between the abundance of *A. odoratum* in the field plot and its competitive performance in the greenhouse experiment. Moreover, the negative conspecific feedback tended to be stronger in the plant mixtures than in plant monocultures for *A. odoratum* but did not differ for *C. jacea*. These results indicate that the former abundance of a species in mixed plant communities, via plant-soil feedback, influences the relative competitiveness of that species when it grows later in competition with other species. I conclude that such effects may contribute to plant species coexistence by preventing the dominance of one species.

In a subsequent pot experiment I used soils collected from *Anthoxanthum odoratum* monocultures and from *Centaurea jacea* monocultures in the field plots. I created homogeneous soils by evenly mixing these two collected soils, and heterogeneous soils by arranging the two collected soils in discrete patches in a checkerboard manner. Then, I examined the performance and competitive interactions between the two species grown in monocultures and in mixtures in pots with homogeneous soils and in pots with heterogeneous soils (Chapter 5). I found that spatial heterogeneity in plant-soil feedbacks reduced the growth of both species in monocultures, and reduced the growth difference between the two species in mixtures by allowing them to preferentially grow in "foreign" over "own" soil patches. These results indicate that spatial heterogeneity in plant-soil feedbacks influence plant performance and may promote plant species coexistence through reducing the growth inequalities between the competing species.

On the basis of these results, I discuss below how spatial heterogeneity in the soil may influence plant species coexistence and plant species diversity (Table 6.1).

Soil resource heterogeneity and species coexistence

Soil resource heterogeneity can alter competitive interactions between plants (Fransen et al. 2001, Novoplansky and Goldberg 2001, Bliss et al. 2002), but see Cahill and Casper 1999). Plants may respond to soil resource heterogeneity by selectively growing more roots in nutrient rich patches (i.e. foraging behavior; e.g. de Kroon and Hutchings 1995, Wijesinghe et al. 2001, Hodge 2004, Rajaniemi and Reynolds 2004, Semchenko et al. 2008, Cahill and McNickle 2011), and some plant species are more able to do this than other ones. Therefore,

Table 6.1. Impacts of different kinds of soil heterogeneity on plant species coexistence and diversity and the possible underlying mechanisms discussed in this thesis. Effects: "?" indicate no direct empirical evidence yet.

Soil heterogeneity	Effect	Possible mechanism
Abiotic factor		
Resource: soil nutrients	Positive	When soil nutrient patches are small (e.g. 10 cm × 10 cm and 30 cm × 30 cm), inferior species benefit from soil heterogeneity by integrating resources across patches, enhancing competitive ability of competitive inferior species (Chapter 2)
	Negative	When soil nutrient patches are small (e.g. 10 cm × 10 cm and 30 cm × 30 cm), superior species benefit from soil heterogeneity by integrating resources across patches, promoting dominance of species at competitive advantages (Chapter 3)
	? Positive	When soil nutrient patches are large, species cannot integrate resources across patches, rich and poor-resource patches will support different sub-communities, but overall species diversity will be higher than in homogeneous soils with equivalent resource (Hutchings et al. 2003)
	Neutral	When high-nutrient soil patches are located at top layer, both shallow- and deep-rooting plant species can utilize soil nutrients (Chapter 3)
	Negative	When high-nutrient soil patches are located at bottom layer, only deep-rooting plant species can utilize soil nutrient, outcompeting other plant species (Chapter 3)
Non-resource: soil pH	Neutral	When soil pH patches are small (e.g. 10 cm × 10 cm), no refuges or shelters for subordinate and rare species are available due to intense competition (Chapter 3)
	Positive	When soil pH patches are large (e.g. 30 cm × 30 cm), refuges and shelters are available for subordinated and rare species (Chapter 3)
Biotic factor		
Plant-soil feedback	Positive	Density-dependent negative plant-soil feedback prevents dominance of particular plant species (Chapter 4)
	Positive	Plant-soil feedback heterogeneity reduces growth inequalities of competing plant species by allowing them grow more in "foreign" soil patches (Chapter 5)

the outcome of competition may be largely influenced by the plasticity and the foraging abilities of the competing plant species (Bliss et al. 2002). It is generally thought that plants with guerilla growth form have an advantage in heterogeneous soils compared to plants with phalanx growth form (Navas and Garnier 1990, Humphrey and Pyke 1998, Ye et al. 2006, Sammul 2011, Saiz et al. 2016). The "guerilla" plants can produce long rhizomes or stolons that connect the adjacent ramets so that they can exploit a much larger area than the "phalanx" plants that produce no or very short rhizomes or stolons (Lovett-Doust 1981). The findings in Chapter 2 support this idea. Soil nutrient heterogeneity increased the relative competitive ability of the "guerilla" plant *Bolboschoenus planiculmis* and decreased that of the "phalanx" plant *Carex neurocarpa*. However, such benefits are not strong enough to shift the competitive hierarchy between the two species, i.e. the "phalanx" plant always wins from the "guerilla" plant, regardless of the distribution patterns of soil nutrients (Chapter 2). Nevertheless, soil nutrient heterogeneity may delay the competitive exclusion process through equalizing the competitive abilities of the competing species, which has important implications for plant species coexistence.

An important question is whether soil nutrient heterogeneity promotes plant species diversity in wild plant communities. Numerous observational studies have reported a general positive relationship between soil resource variation and plant species diversity in natural systems (Lundholm 2009, Stein et al. 2014). However, only a few experimental studies have directly tested the effects of soil resource heterogeneity on plant species diversity and they often reported negative or neutral effects (e.g. Baer et al. 2004, Reynolds et al. 2007, Eilts et al. 2011, Gazol et al. 2013). In agreement with previous experimental studies, increasing spatial heterogeneity in soil nutrients in the horizontal dimension in my study also reduced plant species diversity (Chapter 3). A widely recognized mechanism underlying this negative effect is that plant size may have exceed the scale of soil nutrient heterogeneity, i.e. the patch size or grain size (Tilman and Pacala 1993, Hutchings et al. 2003, Eilts et al. 2011). Some plant species can benefit from integrating resources across patches and outcompete other plant species (Hutchings and de Kroon 1994, Fransen et al. 2001, Song et al. 2013). A reduction in plant species diversity due to dominance of particular plant species has been reported in several previous studies (Baer et al. 2004, Reynolds et al. 2007, Eilts et al. 2011, Gazol et al. 2013). This view is also supported by the results in Chapter 3 in this thesis, as the dominant plant species *Hypochaeris radicata* benefited from the heterogeneous environment where soil resources were horizontally heterogeneous distributed, by showing a greater growth in high

nutrient soil patches than in low nutrient soil patches, independent of the soil patch sizes (10 cm × 10 cm and 30 cm × 30 cm) used in my thesis.

The negative effects of horizontally spatial heterogeneity in soil nutrients on plant species diversity found in Chapter 3 does not necessary mean that spatial heterogeneity in soil resources cannot promote plant species diversity in a community. Hutchings et al. (2003) proposed that when soil nutrient patches are large, high-nutrient soil patches will support lower diversity than low-nutrient soil patches. Nevertheless, different soil patches will support distinct sub-communities, so that the overall diversity will be higher in heterogeneous than in homogeneous habitats with equivalent amounts of soil nutrients. However, we still lack direct empirical evidence for this hypothesis. Therefore, more studies manupulating soil resource heterogeneity at a wide range of nutrient patch sizes are needed to test the heterogeneity-diversity hypothesis.

Compared to the few experimental studies examining the effects of horizontal heterogeneity in soil nutrients on plant species diversity, the effects of vertical heterogeneity in soils are even less well described (but see Berendse 1981, Fitter 1982, Maestre et al. 2006, Maestre and Reynolds 2006, Liu et al. 2017). In the experiment reported in Chapter 3 I manipulated the presence of high nutrient soil in the top or bottom layer. I found a reduction in plant species diversity in the treatment where high-nutrient soil was located in the bottom layer as compared to the treatment where high-nutrient soil was located in the top layer and the homogeneous treatment where the low and high nutrient soils were evenly mixed. In the treatment with nutrient-rich bottom soil, only deep-rooting plant species can utilize the soil nutrients in the deeper layer, so that they may outcompete the shallow-rooting plant species that can only exploit the nutrient-poor shallow layer. This view is supported by the greater relative abundance of deep-rooting plant species such as *Centaurea jacea* and *Sanguisorba minor*, but the smaller relative abundance of shallow-rooting plant species such as *Anthoxanthum odoratum* and *Festuca rubra* in heterogeneous-bottom soil than in heterogeneous-top soil.

The results in Chapter 2 and 3 provided direct evidence that the spatial configuration of soil nutrient patches, in both horizontal and vertical dimensions, can play an important role in driving plant species coexistence and plant community diversity.

Heterogeneity of non-resources in the soil and species coexistence

As Schoolmaster (2013) proposed, soil heterogeneity in resource and non-resource factors may have different effects on plant species diversity. As one of the most important non-resource factors, soil pH has long been thought to influence plant species diversity since it can influence germination, establishment and growth of plant species directly as well as indirectly through changing soil microbial communities (e.g. Palazzo and Duell 1974, Buchanan et al. 1975, Brouwer 1978, Gough et al. 2000b, Isermann 2005, Lauber et al. 2009). This idea has been supported by many observational studies that investigated the correlation between mean soil pH level or variation in soil pH (heterogeneity) and plant species richness in natural systems (Giesler et al. 1998, Gough et al. 2000b, Isermann 2005, Schuster and Diekmann 2005, Zhang et al. 2015). In Chapter 3, to my knowledge for the first time, I provided direct experimental evidence for a causal relationship, as soil heterogeneity in pH increased plant species diversity of an experimental community. As species co-existence theory suggests, spatial variation in soil factors to which plant species respond differently, will favor different species in various microenvironments (Hutchinson 1959, Tilman and Pacala 1993). However, this seems not to be true in our garden experiment, as I did not find significant differences in species richness or composition between the low and high soil pH patches within the heterogeneous pH soils. Alternatively, the increased plant species diversity in the heterogeneous pH plots with large patches in Chapter 3 could be due to more even growth of each of the component plant species. This view is supported by the reduction in relative abundance of the most dominant species *Centaurea jacea* from 27.4% in homogeneous soil and 28.3% in small-patch heterogeneous soil to 10.6% in the plots with large heterogeneous patches. The lower relative abundance of *C. jacea* may have provided opportunities for the growth of subordinate species such as *Leontodon hispidus, Sanguisorba minor* and *Festuca rubra* and the establishment of rare species such as *Briza media, Companula rotundifolia and Veronica chamaedrys*.

Negative plant-soil feedback, plant-soil feedback heterogeneity and species coexistence

So far, I have focused on the effects of soil heterogeneity in abiotic factors, both resource factors, i.e. soil nutrient supply and non-resource factors, i.e. pH, on plant species coexistence and diversity. However, soil biota also influence plant species diversity (Bradley et al. 2008,

de Kroon et al. 2012, Bever et al. 2015). Moreover, it is suggested that the effects of soil biota on plant species diversity depend on abiotic factors (e.g. soil nutrients) and complex interactions among the soil biota (Reynolds et al. 2003, De Deyn et al. 2004, van der Heijden et al. 2008). Therefore, in many studies, a plant-soil feedback approach is followed in which the net effects of the soil community on plant growth is measured (Bever 2003, Brinkman et al. 2010). Negative plant-soil feedbacks are thought to promote plant species coexistence and increase plant species diversity (Bever et al. 1997, Bever 2003, Petermann et al. 2008) similar to the well-known Janzen-Connell effects (Janzen 1970, Connell 1971). If the local density of a species in a plant community increases, species-specific soil pathogens are expected to increase as well, thus decreasing the fitness of that plant species. Such density-dependent negative plant-soil feedbacks have been recorded in several field studies (Packer and Clay 2000, Mangan et al. 2010, Kos et al. 2013). In this thesis, I report in Chapter 4 experimental evidence of a negative relationship between the density of *Centaurea jacea* in field plots (conditioning phase) and the competitive performance of this species in a greenhouse experiment (test phase). As this relationship was significant in both unsterilized and sterilized soils, I speculate that these negative plant-soil feedback effects may be driven by allelopathic effects (Callaway and Aschehoug 2000). Therefore, combining the evidence from both field and experimental studies, I expect that density-dependent negative feedback effects may promote the coexistence of competing plant species through preventing the dominance of one of the competing species.

For a long time, spatial variation in plant-soil feedback, an inherent characteristic of plant-soil feedback, has been ignored in plant-soil feedback studies (Kulmatiski et al. 2008), even though its importance for plant performance and coexistence has been documented in theoretical studies (Bonanomi et al. 2005, Mack and Bever 2014, Abbott et al. 2015, Zee and Fukami 2015). Recently, the effects of spatial heterogeneity in plant-soil feedbacks have been tested empirically. These studies show that spatial heterogeneity in plant-soil feedback has important influences on plant performance such as germination and seedling survival (Brandt et al. 2013), invasibility (Burns et al. 2014), specific leaf area (del Pino et al. 2015), root distribution patterns (Hendriks et al. 2015b), root traits (Saar et al. 2018), population growth (Wubs and Bezemer 2016) and establishment (Burns et al. 2017). Moreover, plant-soil feedback heterogeneity can shift the competitive hierarchy between competing species (Hendriks et al. 2015a). In Chapter 5, I report an experimental study to assess the potential of spatially heterogeneous plant-soil feedbacks to promote plant species coexistence. I showed

that spatial heterogeneity in plant-soil feedbacks has implications for species coexistence through reducing the inequalities between the growth of the competing species. In heterogeneous soils both plant species encountered patches with “own” and “foreign” soils, potentially providing both plant species with enemy free space, i.e. the avoidance of contact with antagonists in “own” soil patches (Chapter 5).

Recently, Wubs and Bezemer (2018) studied experimental plant communities consisting of four common grassland species, and found no direct proof that heterogeneity in plant-soil feedback enhanced plant species diversity (evenness) in these communities. In their study, Wubs and Bezemer (2018) grew these plant communities in soils that were conditioned by mixtures of these four species and in soils that consisted of patches of monoculture soil of the same four species. The diversity of the test plant communities appeared to be driven by the number of the plant species that conditioned the soil rather than by the spatial arrangement of these soils. The lack of direct evidence has impeded our understanding of how plant-soil feedback heterogeneity may influence plant species diversity. Therefore, I propose that more empirical studies, both in the greenhouse and in the field, are needed to further test the effects of spatial heterogeneity in plant-soil feedbacks on plant species coexistence and diversity.

Soil heterogeneity effects in the real world: a unique long-term field study

In this thesis, so far I have reported the results from garden experiments and greenhouse experiments. However, does spatial soil heterogeneity influence plant species diversity in the field? I have mentioned above several times that a huge number of observational studies have examined the relationship between the variation in soil characteristics (soil heterogeneity) and plant species diversity in natural systems, and they generally report positive relationships. Nevertheless, we lack long-term data on plant species establishment and development in spatially heterogeneous soil environments.

In 1966, the Dutch botanist Dr. Ger Londo created an extremely variable habitat at Scherpenzeel in The Netherlands and recorded the establishment and development of grassland plant species in this habitat. In this habitat, he used several soil types (e.g. humous sand, calcareous clay, loam, loamy sand and dune sand) and arranged them in a mosaic pattern. He divided the habitat into 28 plots (each 1 m × 1 m) and each of them has its own micro-environment (i.e. composition of soil types, soil depth and soil gradients; Fig. 6.1). This grassland is naturally maintained without disturbance except for mowing twice (in July

and October) every year. He recorded the number of plant species in each plot since the establishment of the habitat. These unique observations provided us with an opportunity to test whether spatial soil heterogeneity can maintain diverse plant communities in the long term and to investigate how plant species diversity may relate to the spatial variation of different soil variables.

I first investigated the temporal pattern (from 1966 to 2016) of plant species richness at two different spatial scales, i.e. at the regional scale (total number of plant species across the 28 plots) and at the plot scale (mean number of plant species across the 28 plots). I found that, at both regional and plot scales, there was a steady increase in the number of plant species from 1966 (establishment of the experiment) to 1973 (Fig. 6.2A). After that, from 1973 to 2016, the plot-scale species richness (i.e. the mean plant species richness) remained steady while there was a slight fluctuation in the total number of plant species during this time period (Fig. 6.2A). These long-term monitoring results suggest that it is possible to establish and maintain species-rich plant communities by creating mosaic habitats.

Fig. 6.1. Photograph of the grassland experiment of Ger Londo. (A) Plant species richness was recorded in each 50 cm × 50 cm subplot: the plot-level richness refers to the number of species in each plot; subplot-level richness refers to the number of species in each subplot. Soil cores (1.5 cm in diameter) were taken from the top layer (0-10 cm) of the soil (B). Each subplot was divided into 4 quadrants (each 25 cm × 25 cm) and in each quadrant two soil cores were taken and pooled. Plot-level soil heterogeneity was calculated as the CV of each of the soil properties across the 16 subplots within each plot; Subplot-level soil heterogeneity was calculated as the CV of each of the soil properties across the four quadrants within each subplot. Photo credits: Wei Xue.

Then I examined the relationships between soil heterogeneity (CV) in several soil variables ($N\text{-}NH_4$, $P\text{-}PO_4$, $N\text{-}NO_3$, organic matter and pH) and plant species richness. I determined the heterogeneity-richness relationships at two different spatial scales (1 m × 1 m plot scale and

50 cm × 50 cm subplot scale). At the plot level, plant species richness was positively related to the CV of the pH of the soil, but did not relate to CVs of other soil properties (Fig. 6.2B). At subplot level, plant species richness was positively related to CVs of pH (Fig. 6.2C) and N-NO_3 (Fig. 6.2D), but did not relate to CVs of other soil properties. The positive relationships between plant species richness and CV of pH are in agreement with the common garden experiment that I described in Chapter 3, indicating that spatial heterogeneity in soil pH plays an important role in influencing plant species diversity. It is far more complicated to detect the underlying mechanisms for the observed relationships in a field study than in an experimental study. However, in a field study, both deterministic processes such as soil nutrient mineralization, facilitation and competition (e.g. Tilman and Pacala 1993, Silvertown 2004, Levine and HilleRisLambers 2009) and stochastic processes such as colonization, dispersal and extinction (e.g. Blomqvist et al. 2003, Chave 2004, Hubbell 2005, Gravel et al. 2006) interact to shape the structure of plant communities. Therefore, more experimental studies aiming to unravel the underlying mechanisms of soil heterogeneity-species diversity relationship are needed (Ortega et al. 2018).

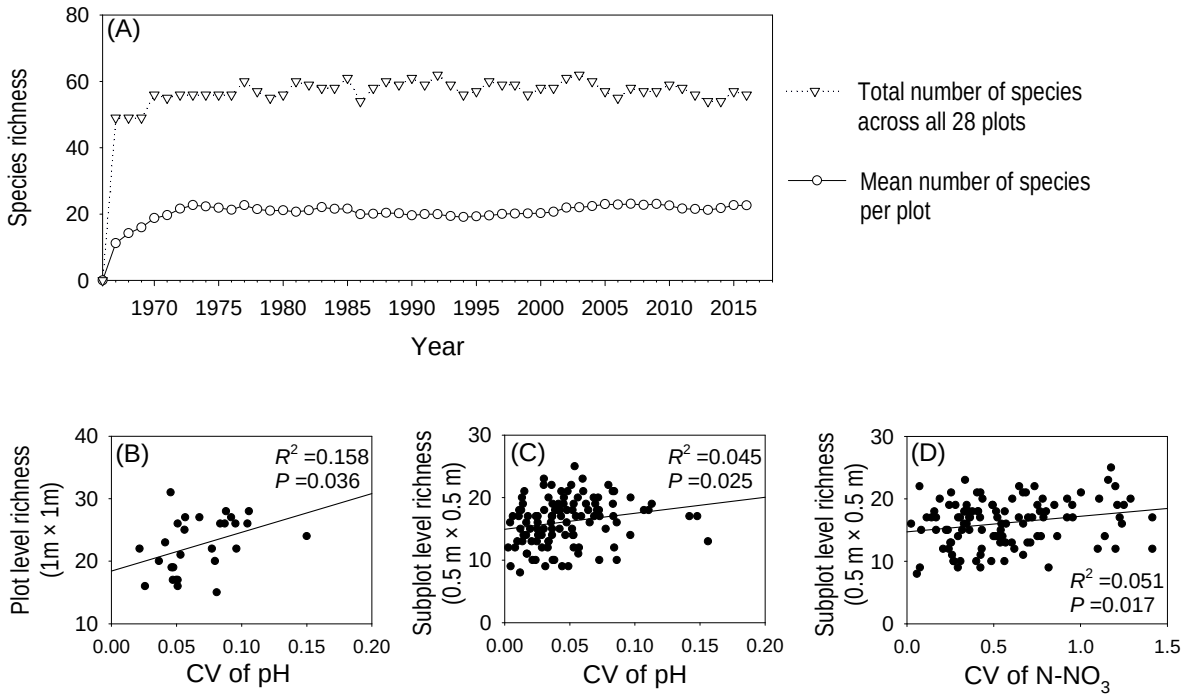


Fig. 6.2. Temporal changes in total and mean plant species richness across the 28 plots (A), and plot-level (B) and subplot-level (C-D) soil heterogeneity-plant species richness relationships in the grassland. Only significant relationships are presented. R^2 and *P*-values based on linear regressions are also presented. See Fig. 6.1 for the calculation of plant species richness and CV of soil properties at plot and subplot scales.

Implications for plant diversity restoration

On the basis of the results of this thesis (Chapter 2-5), I conclude that soil heterogeneity does promote plant species coexistence and diversity. An important question, however, is whether this insight can help to restore plant species diversity? For example, in an abandoned agricultural field in North America, heterogeneity in soil depth, in soil nutrients, and in the combination of both depth and nutrients were manipulated to test whether increasing soil heterogeneity helps to restore plant species diversity after reintroducing a native prairie vegetation by seeding (Baer et al. 2005). After three years, the authors did not find that soil heterogeneity increased plant species diversity because it appeared to promote the dominance of one of the sown species *Panicum virgatum*. The experiments in this thesis describe a similar result (Chapter 3). However, Baer et al. (2016) found that plant species richness was higher in heterogeneous soils after a second seeding 8 years after the initial sowing (Baer et al. 2016). These results suggest that increasing soil heterogeneity alone might not be sufficient to increase plant species diversity, but that it can help to restore plant species diversity in combination with other practices such as mowing and grazing to reduce the dominance of particular plant species (Collins et al. 1998).

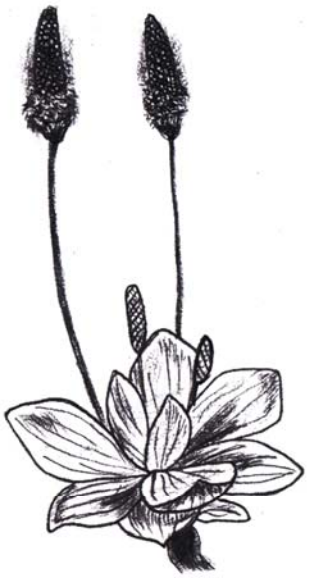
As the results of Chapter 4-5 suggest, plant-soil feedbacks may also play an important role in driving plant community assembly. Recently, Wubs et al. (2016) applied this ecological theory in an abandoned farmland area in the Netherlands, aiming to restore this degraded ecosystem (Wubs et al. 2016). They removed the top soil of this former arable field and inoculated new soils from either a heathland or a grassland nearby. After six years, they found that the plant communities could be steered towards either a heathland or a grassland target community depending on the donor soil. The underlying mechanisms are not clear yet, but should be the net effect of complex interactions as the soil still is a proverbial “black box”.

In summary, management that integrates both abiotic (e.g. resources and other physical and chemical factors) and biotic (e.g. soil biota and its interaction with other organisms) factors is essential for the restoration of plant species diversity (Benton et al. 2003, Heneghan et al. 2008).

Concluding remarks

In this thesis, I show that soil heterogeneity can influence plant species coexistence and diversity. However, such influence depends on the type of soil factor and the plant species that are considered. The underlying mechanisms might differ among the different factors of which the spatial variation is manipulated (Table 6.1). Spatial heterogeneity in soil nutrients can promote plant species coexistence through increasing the competitive ability of competitive inferior plant species and decreasing that of the superiors. However, spatial heterogeneity in soil nutrient supply can also reduce plant species diversity when the dominant plant species benefit from the heterogeneous supply of nutrients. Spatial heterogeneity in soil pH can increase plant species diversity only when the patch size is sufficiently large, probably through providing refuges and shelters for the growth of subordinate and rare species. Density-dependent negative plant-soil feedbacks can promote plant species coexistence through preventing the dominance of a particular plant species. Further, the spatial heterogeneity in plant-soil feedbacks can also promote plant species coexistence via reducing the growth inequalities between the competing species by allowing them to selectively grow in "foreign" soil patches.

In this thesis, I manipulated soil nutrient, soil pH, and plant-soil feedbacks independently. In the real world, different soil factors interact to influence plant coexistence and plant species diversity (Reynolds and Haubensak 2009). Moreover, the effects of soil heterogeneity on plant species coexistence and plant species diversity may also depend on other non-edaphic factors such as CO_2 (Maestre et al. 2007, García-Palacios et al. 2012), temperature and light intensity (del Pino et al. 2015) and herbivory (Tsunoda et al. 2014, van der Waal et al. 2016). Therefore, studies integrating different types of soil heterogeneity, as well as other key environmental factors are urgently needed to guide the restoration of plant species diversity.



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Summary

The loss of biodiversity can greatly influence ecosystem functions. Hence understanding the mechanisms behind plant species coexistence and maintenance of plant species diversity has important implications for biodiversity conservation and ecosystem function improvement. Environmental heterogeneity has long been thought to promote plant species coexistence and plant species diversity through increasing niche availability. Theoretical and observational studies have supported this hypothesis. However, convincing evidence for the heterogeneity-diversity hypothesis delivered by appropriate experiments is very scarce. The aim of my thesis was to examine the effects of different types of soil heterogeneity, i.e. the spatial variation in soil nutrients, soil pH and plant-soil feedbacks, on plant competitive interactions, species coexistence and plant species diversity.

As a first step, I tested the potential of soil nutrient heterogeneity to promote plant species coexistence. In an experimental garden, two plant species with contrasting growth forms (i.e. phalanx vs. guerilla growth form) were planted in monocultures and in mixtures on homogenous and heterogeneous substrates consisting of low and high nutrient soil patches. In the plant mixtures, the two plants were either evenly distributed or planted in a clustered pattern. After two growing seasons, I found that soil nutrient heterogeneity increased the competitive ability of the competitive inferior species (guerilla growth form) and decreased that of the competitive superior species (phalanx growth form). The species with the guerilla strategy benefited more from the heterogeneous soil environment by selectively growing in high nutrient soil patches within heterogeneous soils. Apparently, soil nutrient heterogeneity does have the potential to promote plant species coexistence by slowing down the competitive exclusion process.

Further, I tested whether soil nutrient heterogeneity can promote plant species diversity in an experimental community and whether these effects depend on the spatial scale at which species diversity (focal scale) and soil heterogeneity (grain size or patch size) are measured. I found that horizontal soil heterogeneity in soil nutrients reduced plant species richness, regardless of the patch size of the heterogeneity treatments. This decline in species richness is likely because the dominant species benefited from the heterogeneous environment, and outcompeted other plant species. Moreover, vertical heterogeneity in soil nutrients also reduced plant species diversity when high nutrient soil was located in the bottom layer. In this

case only deep-rooting plant species had access to high nutrient soil, and could outcompete other plant species that cannot utilize the resources in the deeper layers. Spatial heterogeneity in soil nutrients only influenced species diversity when determined at the 10 cm × 10 cm patch scale but not when determined at the 40 cm × 40 cm plot scale. Therefore, soil nutrient heterogeneity indeed influenced plant species richness but the effect was negative in these experimental communities. Such negative effects were more common when species diversity was quantified at the small scale, but diversity were not influenced by the spatial scale at which soil heterogeneity was measured (i.e. patch size), at least at the spatial scales used in this thesis.

In a parallel experiment, I repeated the horizontal heterogeneity experiment by manipulating soil pH. I found that soil heterogeneity in pH promoted plant species diversity when the soil pH patches were large, and that heterogeneous pH soil with large patches sustain a higher species diversity than heterogeneous pH soil with small patches, even though these effects were only significant at the final harvest. This is likely because heterogeneous pH soil with large patch sizes provided refuges for the subordinate and rare species. Such positive effects were only significant when species diversity was quantified at a small (10 cm × 10 cm patch) scale. These results thus support the classic heterogeneity-diversity hypothesis, and highlight the importance of spatial scales at which species diversity and soil heterogeneity are measured in heterogeneity-diversity relationships.

Subsequently, I focused on the effects of biotic factors, i.e. plant-soil feedbacks and spatial variation in plant-soil feedback on plant species coexistence. I examined how the abundance of a species in a plant community consisting of two species in long-term field plots (conditioning phase), via plant-soil feedbacks influences competition between these two species when they grow later in a greenhouse experiment (test phase) on soils collected from the field experiment. There was a negative relationship between the abundance of a species in the field plot and its relative competitiveness in the greenhouse experiment, probably due to allelopathic effects because this relationship was also true after elimination of soil biota. This negative density-dependent feedback effect varied between plant species, yet it has the potential to promote the coexistence of competing plant species through preventing the dominance of particular plant species.

Using soils collected from monoculture plots in the field experiment, in a greenhouse experiment, I created heterogeneous soil consisting of discrete patches of “own” soil (conditioned by the same plant species as the focal species) and “foreign” soil (conditioned by

another plant species), and homogeneous soil where the “own” and “foreign” soils were evenly mixed. The difference in growth between the two competing plant species was smaller in heterogeneous soil than in homogenous soil, indicating that spatial heterogeneity in plant-soil feedback can reduce plant growth differences so that it may promote plant species coexistence. I also found that both competing plant species grew better in “foreign” soil patches than in “own” soil patches within the heterogeneous soil treatment. This can contribute to reduced growth differences in spatially heterogeneous plant-soil feedback conditions.

In conclusion, soil heterogeneity had an important influence on plant species coexistence and plant species diversity. However, the effects varied depending on the type of soil factors that were manipulated, as well on the spatial scales at which species diversity and soil heterogeneity were measured. Soil nutrient heterogeneity can promote plant species coexistence through equalizing the competitive ability between competing plant species, and reduce plant species diversity by promoting the dominance of particular plant species. Heterogeneity in soil pH may promote plant species diversity when the patch size of soil heterogeneity is sufficiently large by proving refuges for subordinate plant species. Plant-soil feedback is a key process in influencing plant species coexistence. These negative density-dependent feedbacks can promote plant species coexistence by preventing the predominance of one of the plant species. The spatial variation in plant-soil feedbacks also has the potential to promote coexistence by reducing the growth inequalities between the competing plant species. Future studies integrating spatial heterogeneity in different soil properties at various spatial scales are urgently needed to guide the restoration of plant species diversity.

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Wageningen, the Netherlands

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About the Author

Wei Xue was born in Jianyang, Sichuan Province, China, on September 22, 1988. He grew up in a small village. In 2007, he left his hometown and started to study at Beijing Forestry University in Beijing, majoring in Management of Wildlife and Nature Reserve. After four years, in the same university, he continued his master program studying the effects of soil heterogeneity on competitive interactions of wetland clonal plants, supervised by prof. Ming-Xiang Zhang and prof. Fei-Hai Yu.

In 2014, he obtained the financial support from China Scholarship Council (CSC) and moved to Wageningen University, The Netherlands, to do his PhD research. During his PhD, he worked on the effects of different types of soil heterogeneity on plant species coexistence and plant species diversity, supervised by prof. Frank Berendse and prof. T. Martijn Bezemer. During the first two years of his PhD, he worked at Wageningen University and conducted his experiments there. After finishing his experiments at the university, in December 2016, he moved to the Netherlands Institute of Ecology (NIOO-KNAW) and there he finished his writing work.

Publications

Peer-reviewed publications

Luo, F.-L., L. Huang, T. Lei, **W. Xue**, H.-L. Li, F.-H. Yu, and J. H. C. Cornelissen. 2016. Responsiveness of performance and morphological traits to experimental submergence predicts field distribution pattern of wetland plants. Journal of Vegetation Science **27**:340-351.

Wang, P., Y.-S. Xu, B.-C. Dong, **W. Xue**, and F.-H. Yu. 2014. Effects of clonal fragmentation on intraspecific competition of a stoloniferous floating plant. Plant Biology **16**:1121-1126.

Xue, W., L. Huang, and F.-H. Yu. 2016. Spatial heterogeneity in soil particle size: does it affect the yield of plant communities with different species richness? Journal of Plant Ecology **9**:608-615.

Xue, W., L. Huang, F.-H. Yu, and T. M. Bezemer. 2018. Intraspecific aggregation and soil heterogeneity: competitive interactions of two clonal plants with contrasting spatial architecture. Plant and Soil **425**: 231-240.

Xue, W., F. Berendse, and T. M. Bezemer. 2018. Spatial heterogeneity in plant-soil feedbacks alters competitive interactions between two grassland plant species. Functional Ecology. https://doi.org/10.5061/dryad.vm125vv.

Xue, W., T. M. Bezemer, and F. Berendse. 2018. Density-dependency and plant-soil feedback: former plant abundance influences competitive interactions between two grassland plant species through plant-soil feedbacks. Plant and Soil **428:** 441-452.

Zhang, Q., Y.-S. Xu, L. Huang, **W. Xue**, G.-Q. Sun, M.-X. Zhang, and F.-H. Yu. 2014. Does mechanical disturbance affect the performance and species composition of submerged macrophyte communities? Scientific Reports **4**:4888.

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PE & RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (5.0 ECTS)

- Soil heterogeneity and plant species richness: a review of literature

Writing of Project proposal (4.5 ECTS)

-How soil heterogeneity affects plant species diversity

Post-graduate courses (4.6 ECTS)

- Structural Equation Modelling (SEM); PE&RC (2018)
- Life History Theory; GELIFES (2018)
- Meta-analysis; PE&RC (2018)

Deficiency, Refresh, Brush-up courses (1.5 ECTS)

- Basic Statistics; PE&RC (2016)

Competence strengthening/skills courses (1.8 ECTS)

- Scientific Writing (SWR); Wageningen in'to Languages (2017)

PE&RC annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

-Vegetation-soil interactions Symposium (2014)
-PE&RC Day-optimization of science: Pressure & Pleasure (2014)
-PE&RC First year weekend (2015)
-PE&RC Day-preventing the end of the world (2017)
-PhD Symposium: frontiers in Ecology: the soil-plant interphase (2017)
-WGS PhD Workshop carousel (2017)

Discussion groups/local seminars /other scientific meetings (9.6 ECTS)

-Current rhemes- biodiversity research at the crossroads (2014)

-Wageningen plant microbiome network (2016-2017)

-Plant soil interactions discussion group (2016-2018)

-NIOO Research day; poster presentation (2017)

-NIOO Monday seminar (2017-2018)

International symposia, workshops and conferences (5.4 ECTS)

-NAEM meeting; poster presentation (2017)

-BES Joint annual meeting: ecology across borders; poster presentation (2017)

-NAEM meeting; oral presentation (2018)

Colophon

The research described in this thesis was conducted at Nature Conservation and Plant Ecology group at Wageningen University, and the Department of Terrestrial Ecology of the Netherlands Institute of Ecology (NIOO-KNAW).

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Propositions

1. Spatial soil heterogeneity has heterogeneous effects on plant species diversity.
   (this thesis)

2. Soil heterogeneity effects on plant species richness and diversity depend on the spatial scales at which these variables are measured.
   (this thesis)

3. The sustainable development of society requires heterogeneous and diverse cultures.

4. In science, publishing a high-impact paper is more important than publishing a paper in a high-impact factor journal.

5. Scientific journals should encourage researchers to publish a plain language summary when they publish a scientific paper, to help the public to understand the complicated scientific results.

6. Climate change leads to change of diet culture.

Propositions belonging to the thesis, entitled:

'Games on the checkerboard: How soil heterogeneity influences plant species coexistence'

Wei Xue

Wageningen, 10 September 2018