Spatial heterogeneity of plant-soil feedback affects root interactions and interspecific competition

Marloes Hendriks[1§], Janneke M. Ravenek[1*§], Annemiek E. Smit-Tiekstra[1], Jan Willem van der Paauw[1,4], Hannie de Caluwe[1], Wim H. van der Putten[2,3], Hans de Kroon[1], Liesje Mommer[4]

[1] Radboud University Nijmegen, Institute for Water and Wetland Research, Department of Experimental Plant Ecology, PO Box 9100, 6500 GL Nijmegen, The Netherlands

[2] Netherlands Institute of Ecology, Department of Terrestrial Ecology, PO Box 50, 6700 AB Wageningen, The Netherlands

[3] Laboratory of Nematology, Wageningen University, PO Box 8123, 6700 ES, The Netherlands

[4] Nature Conservation and Plant Ecology, Wageningen University, PO Box 47, 6700 AA Wageningen, The Netherlands

*Corresponding author: j.ravenek@science.ru.nl

Phone: +31-24-3652902

§ M.H. and J.M.R. contributed equally to this study

Word count

Summary	196	
Body total	6133	
Introduction	950	
Materials & methods	2557	
Results	943	
Discussion	1683	
Acknowledgements	67	
# Figures	4 (all colour)	
# Tables	0	
# Supporting Information	S. Methods	1
	S. Tables	3
	S. Figures	4

Summary

- Plant-soil feedback is receiving increasing interest as a factor influencing plant competition and species coexistence in grasslands. However, we do not know how spatial distribution of plant-soil feedback affects plant belowground interactions. We examined how spatial heterogeneity of soil biota affects competitive interactions in grassland plant species.
- We performed a pair-wise competition experiment combined with heterogeneous distribution of soil biota using four grassland plant species and their soil biota. Patches were applied as quadrants of 'own' and 'foreign' soils from all plant species in all pairwise combinations. To evaluate interspecific root responses, species-specific root biomass was quantified using real-time polymerase chain reaction (RT-PCR).
- All plant species suffered negative soil feedback, but strength was species-specific, reflected by decrease in root growth in own compared to foreign soil. Reduction in root growth in own patches by the superior plant competitor provided opportunities for inferior competitors to increase root biomass in these patches. These patterns did not yet cascade into aboveground effects during our experiment.
- We show that root distributions can be determined by spatial heterogeneity of soil biota, affecting plant belowground competitive interactions. Thus, spatial heterogeneity of soil biota may contribute to plant species coexistence in species-rich grasslands.

Key words: soil heterogeneity, plant-soil feedback, root competition, grasslands, soil biota, coexistence

Introduction

Evidence is accumulating that plant-soil feedback interactions (Bever *et al.*, 1997) contribute to plant species coexistence (Bever, 2003) and plant community dynamics in natural vegetation (van der Putten, 2003). Many studies report plant-soil feedback effects that are plant species-specific and negative (Kulmatiski *et al.*, 2008; van der Putten *et al.*, 2013). This means that a particular plant species changes abiotic and/or biotic soil conditions such that establishment and growth of individuals of that species is reduced, while other plant species that are less harmed by the specific soil conditions are favoured (Bever *et al.*, 2012). Therefore, negative plant-soil feedback may promote local plant species richness (Chesson, 2000; Petermann *et al.*, 2008; Mangan *et al.*, 2010b; Mack & Bever, 2014), and on a longer term contribute to succession (van der Putten, 2003; Kardol *et al.*, 2006).

Studies investigating plant-soil feedback have largely focused on homogeneous soil conditions for individual plants in pots (Augspurger & Wilkinson, 2007; Petermann *et al.*, 2008; Mangan *et al.*, 2010b; Hendriks *et al.*, 2013). However, soil nutrients are known to be heterogeneously distributed in soil (Cain *et al.*, 1999; Farley & Fitter, 1999). Similarly, soil biota are distributed heterogeneously as well (Ettema & Wardle, 2002; Bever *et al.*, 2010; Bezemer *et al.*, 2010), for example because each plant species has its own soil microbial community in and around the roots (Mangan *et al.*, 2010b; Philippot *et al.*, 2013). This spatial patterning will lead to spatial variation in plant-soil feedback, so that a diverse plant community, such as species-rich grasslands will consist of a mosaic of different soil microbial and faunal communities (Bezemer *et al.*, 2010). These mosaics of soil biota may cause spatial patterns of (negative) plant-soil feedback within plant communities, and even within different parts of the root system of an individual plant. Little is known about how heterogeneity affects plant-soil feedback and interspecific competition between plant species (but see Brandt *et al.*, 2013; Burns & Brandt, 2014)).

This lack of knowledge contrasts with what we know about plant's root responses towards soil nutrient patches, affecting competitive outcomes (Robinson, 1996; Fransen *et al.*, 2001; Rajaniemi, 2007; Mommer *et al.*, 2012) and, consequently, the potential to affect species coexistence (Maestre *et al.*, 2005; Wijesinghe *et al.*, 2005; Lundholm, 2009; García-Palacios *et al.*, 2012). It has been shown that plant roots respond to heterogeneous distributions of plant soil feedback (Hendriks *et al.*, 2015). Changes in root distribution induced by soil biota appeared plant species-specific (Hendriks *et al.*, 2013), suggesting that competitive relations may change if plant-soil feedback is distributed heterogeneously in plant communities. Shifts in competitive dominance between plants due to plant-soil feedback have

been demonstrated both under controlled conditions (van der Putten & Peters, 1997) and in the field (Casper & Castelli, 2007). If these shifts lead to increased opportunity for competitively inferior plant species to exploit soil patches where competitive pressure is reduced, spatial heterogeneity of plant-soil feedback may enhance plant species coexistence.

Currently, there is no proof of principle that heterogeneity of plant-soil feedback may enhance plant co-existence in grasslands. Therefore, the aim of the present study was to test if heterogeneous distributions of patches with different plant-soil feedback effects, representing heterogeneity of soil biota and possibly also of nutrients, cause shifts in species-specific root distribution and competitive relationships among plant species. We examined the following hierarchical set of hypotheses.

(1) A prerequisite for our present study is that plants grown in monoculture produce less (root) biomass in soil conditioned by conspecifics ('own' soil) than with soil conditioned by heterospecifics ('foreign' soil).

(2) Plants grown in interspecific competition will be at a competitive disadvantage when confronted only with patches of own soil.

(3) As a consequence of (1), plants grown in monoculture confronted with a combination of patches of own and foreign soil produce more (root) biomass than when confronted with own soil only.

(4) In interspecific competition in soils with patches of *different* soil origins, plants will produce less root biomass in their own soil patch and increase root biomass in the others; this alleviates negative plant-soil feedback and reduces the strength of interspecific competition.

To test these hypotheses, we combined a classic pairwise plant competition experiment (Wilson & Keddy, 1986) with a plant-soil feedback approach (Brinkman *et al.*, 2010). Four plant species were grown in all pairwise combinations in heterogeneous soils containing two patches of conditioned soil and two patches of background soil. The patches were created from conditioned soils from monocultures of each of these four plant species and all pairwise combinations of conditioned soil were used (Fig. 1). We used molecular techniques to quantify plant species specific root biomass in each conditioned soil section (Mommer *et al.*, 2008).

Materials and Methods

Plant species

We used two grass species, *Anthoxanthum odoratum* L. and *Festuca rubra* L., and two forb species, *Leucanthemum vulgare* L. and *Plantago lanceolata* L. Different degrees of negative plant-soil feedback effects have been demonstrated for these plant species in previous studies (Hendriks *et al.*, 2013; Hendriks *et al.*, 2015). All four are common perennial grassland species in western Europe and mostly occur in traditional hay meadows (van Ruijven & Berendse, 2003).

Seed and pot preparations

Seeds of *A. odoratum*, *F. rubra* and *L. vulgare* were obtained from a seed company (Cruydthoeck, Nijeberkoop, The Netherlands) that collects seeds from wild populations. *Plantago lanceolata* seeds were collected from previous experiments (Mommer *et al.*, 2010). Prior to germination, seeds were surface-sterilized for five hours in a desiccator of 3 l by adding 1.5 ml HCl (37-38 %; v:v) to each of the two beakers with 50 ml sodium hypochlorite (10-15 % chlorine). Subsequently, seeds were germinated on γ-sterilized sand (25 kGy at Synergy Health, Ede, the Netherlands) that was kept moist with sterilized deionized water in small containers (previously sterilized with 70 % EtOH) at 22°C (light conditions 175 µmol PAR m^{-2} s^{-1}, day/night regime: 12 h light/ 12 h dark). Seedlings were transplanted to pots 15 days after germination. This procedure was followed for both phases of the experiment. Pots were sterilized prior to the experiment with a sodium hypochlorite solution (Cl^- concentration 0.05 %).

Soil preparations

Like in previous plant-soil feedback studies (Bever *et al.*, 1997; Kulmatiski & Kardol, 2008; Brinkman *et al.*, 2010; Hendriks *et al.*, 2013), we used a conditioning phase, followed by a feedback phase. The main purpose of the conditioning phase was to obtain soils with plant species-specific soil communities of each of the four plant species, which could be used in the feedback phase. In the conditioning phase, on average 25 % (v:v) inoculum of specific soil from 7-year-old monocultures of each of the four plant species from a previous experiment (Mommer *et al.*, 2010) was added to sterilized soils (loamy sand with sandy sand (2:1 v:v)) (Bever *et al.*, 1997; Kulmatiski & Kardol, 2008; Brinkman *et al.*, 2010; Hendriks *et al.*, 2013). The soil of the original plant monocultures was a mixture of loamy sand, sandy sand

158 and potting soil (Mommer *et al.*, 2010). On these soils, plant monocultures of each of the four
159 species also used in the present experiment were established and grown for seven years, with
160 regular weeding. No nutrients were added to the soil during this period. We used the soil from
161 monocultures from a previous experiment as inoculum, instead of neutral soil, to extend the
162 conditioning phase of the experiment and produce soils with strong plant-soil feedback effects
163 (Hendriks *et al.*, 2013; Hendriks *et al.*, 2015).

164 In the conditioning phase, 6-8 seedlings were planted in 2 L pots. Pots were watered
165 with deionized water and placed in a climate chamber at 16 h 22°C (day) and 8 h 18°C
166 (night). Light was supplied at 230 µmol PAR m^{-2} s^{-1}. Once a week, a random selection of pots
167 was weighed and re-set to initial moisture content by initial weight. After two months,
168 aboveground plant biomass was harvested and soils (including roots that served as inoculum
169 source of soil biota) were cut into ± 4 cm^3 pieces and stored in the dark at 4 °C for three
170 months.

171 For the feedback phase, we created two different types of soil: background soil and
172 patch soil. The background soil was created by mixing sandy soil with γ-irradiated loamy
173 sandy soil (3:1 v:v). For the patch soils, we mixed sandy soil and γ-irradiated loamy sandy
174 soil (2:1 v:v), and subsequently added conditioned soil of one of the four plant species (1:1
175 v:v), creating four different soil types, one for each plant species. Roots of the plants in the
176 conditioning phase were present in the soil of the feedback phase, which is common practice
177 in plant-soil feedback experiments, since microorganism in the roots and rhizosphere serve as
178 inoculum for the soil biota community. Concentrations of extractable N and P, as a proxy for
179 differences in all nutrient concentrations, were measured in all soils (see below).

180

181 *Experimental setup*

182 Pots of 15 cm diameter (top) x 15 cm (2.4 L) were split into four compartments (quadrants)
183 using an iron frame and filled with designated soils (Fig. 1). After filling of each quadrant
184 with soil, the iron frame was removed; hence, no physical boundaries between the quadrants
185 were present during the experiment.

186 Two opposite quadrants (Q1 and Q3, the 'plant' quadrants) were filled with
187 background soil, so that plants could establish in the pot before being confronted with
188 conditioned soils. Moreover, plants are expected to have a lower chance to establish in own
189 patches than in soil of other plant species due to negative plant-soil feedback. If own soil
190 would have been present in the plant quadrants, the plant growth would have been hampered
191 immediately, as demonstrated in Van der Putten & Peters (1997) and Hendriks *et al.* (2013).

Having background soil, rather than own or mixed soil in the home quadrant also decreased variation in size among plant species, which would also have affected competition. The compartments in between the 'plant' quadrants (Q2 and Q4) were filled with conditioned soil of one of the four plant species (Fig. 1) in different combinations. All ten possible soil combinations of soil types (four 'mono soils' and six 'mixed soils') were used and patch soil types were randomly assigned to quadrants (Q2 and Q4).

In each 'plant' quadrant (Q1 and Q3), a 15-day-old seedling of either *A. odoratum*, *F. rubra, L. vulgare* or *P. lanceolata* was placed (Fig. 1), allowing for all ten possible plantings (again, four intraspecific 'plant monocultures' and six interspecific 'mixed plant communities'). Plants were randomly assigned to plant quadrants (Q1 and Q3). The plant species under investigation will be referred to as the target species, the other one will be referred to as competitor species. Since roots grow rapidly and can reach all parts of a pot in a matter of weeks, it is likely that roots competed also in the 'plant' quadrants (Q1/Q3). However, our hypotheses addressed the effect of root competition in conditioned soils of the vacant patches (Q2/Q4).

So, the experiment consisted of 10 plantings x 10 soil combinations, resulting in 100 different treatments. Each treatment was replicated six times. The entire experiment was equally divided into two blocks over time with a two-week delay between blocks. Each block contained three replicates of each treatment. Plants were grown for seven weeks in a climatized greenhouse in September/October 2013 in the greenhouse facility of Radboud University (Nijmegen, the Netherlands). During the day (8.00 am – 8.30 pm), temperature was on average 22.4 °C, during the night (8.30 pm – 8.00 am), temperature was on average 18 °C. Light levels varied between 20 – 470 µmol PAR m^{-2} s^{-1}. The watering procedure was similar to the conditioning phase.

Nutrient concentrations of the soils

As we used four different plant species in the conditioning phase, nutrient content of the patch soil types may have differed (Kardol *et al.*, 2006; Brinkman *et al.*, 2010). Therefore, we analyzed the concentrations of available nutrients (NO_3^-, NH_4^+, and PO_4^{3-}) in these four patch soil types and in the background soil separately. Differences between the four patch soil types were absent or small (significant only for NO_3^-). We tested for correlations between the fraction of total root biomass per pot and the fraction of available NO_3^- per patch by calculating Pearson's r. For NO_3^-, the correlation was weak and negative (r = -0.16). Therefore, it is unlikely that the difference in NO_3^- affected root distributions rather than

differences in plant-soil feedback. Nutrient analyses and results are given in the Supplementary Information (Table S1 and Supplementary Methods).

Harvest

At plant harvest, shoots were clipped at soil level. Soil cores (4 cm diameter) were taken in the middle of each of the four quadrants (30 % of quadrant volume) in order to avoid edge effects. Roots from the patch quadrants (Q2 and Q4) were carefully washed by the authors and experienced technicians and helpers who could recognize and remove old root fragments that originated from the conditioning phase, using 0.5 mm sieves. The remaining roots were dried between tissues and weighed fresh. Two subsample of ± 50 mg fresh weight were taken for molecular analysis to determine species-specific root abundance (Mommer *et al.*, 2008) and frozen in liquid nitrogen before storing at -80 °C. The remainder of the roots was re-weighed for fresh weight. The unwashed samples from the plant quadrants (Q1 and Q3) were stored at 4 °C no longer than two weeks and washed after all Q2 and Q4 samples for molecular analysis were washed. Shoot and root material was dried at 65 °C to constant weight and weighed. We calculated a fresh:dry weight ratio for roots.

Molecular analyses

To estimate the proportion of each of the plant species in the mixed root samples in Q2 and Q4, we applied the RT-PCR method of Mommer *et al*. (2008). DNA was extracted using a DNeasy 96 Plant Mini Kit following the manufacturer's protocol (Qiagen, Venlo, the Netherlands); and DNA concentrations were measured using a Qubit Fluorimeter (Invitrogen© through Life Technologies, Carlsbad, CA, United States of America). Each plant species was separately amplified in triplicate from each extract using RT-PCR using primer pairs described in (Mommer *et al.*, 2008), with the exception of *P. lanceolata* where a different primer pair ("Pl3") was used (5'-GAGAAAGCAGTAGGAAACCACAGTG-3', 5'-GATCGAGATCTCTCACTCAAAACCC-3'). RT-PCR reactions were performed with HOT FIREPol Eva Green (Solis BioDyne, Tartu, Estonia) qPCR Mix Plus with an addition of 0.94 µM $MgCl_2$, a primer concentration of 120 nM for *F. rubra*, *L. vulgare* and *P. lanceolata* and 60 nM for *A. odoratum*, and 4 ng genomic DNA for *P. lanceolata* or 1 ng genomic DNA for the other species, in a reaction volume of 20 µl. The RT-PCR program was as follows: 15 min at 95 °C; then 45 cycles of 20 s at 95 °C, 30 s at 62 °C and 15 s at 72 °C; and finally a melting curve analysis of 5 s per cycle with an increase of 0.5 °C per cycle, starting at 70 °C and

ending at 91 °C. RT-PCR analyses were performed on a CFX96 Touch Real-Time PCR Detection System (Bio-rad Laboratories, Hercules, California, USA).

In order to test the robustness of the RT-PCR estimates of abundances of roots, reference curves were produced (Fig. S1). Twenty six standards were made using pooled monocultural roots originating from all soil types. Ten of these standards contained equal proportions of all plant species (25% each) and in the remainder, individual plant abundances were between 5-80%. The four best fitting standards with equal proportion of all plant species (based on smallest summed discrepancy between measured and actual presence) were used as reference standards on all 96 well qPCR plates in which samples were run. The correlations between actual species proportion (from hand-made mixed samples from monoculture roots) and estimated species proportions in the reference curves appeared linear, with R^2 values of 0.89, 0.90, 0.95 and 0.85 for *A. odoratum*, *F. rubra*, *L. vulgare* and *P. lanceolata* respectively (see Fig. S1).

Root biomasses per species per conditioned quadrant were determined based on fresh weight of the root sample multiplied by the fraction of the plant species in that sample as determined by RT-PCR, and transformed to dry weight using the fresh:dry weight ratio of each plant species on each soil type (as in Mommer *et al.*, 2010).

Statistics

All statistical analyses were performed in R (R-Core-Team, 2014), using the nlme (Pinheiro, 2011), car and agricolae packages. All plant biomass data were analyzed with linear mixed effects ANOVA (type III sums of squares). The random part of the models consisted of block, with pot nested within block and quadrant within pot when applicable, to account for dependence of measurements of both plant individuals in one pot. In addition to full-model analyses, we split all analyses for individual plant species or plant mixed communities, when relevant. All plant biomass data were square-root transformed to meet assumptions of ANOVA, unless mentioned otherwise.

To assess plant-soil feedback for all plant species (hypothesis 1), we first analyzed monoculture root biomass (two individuals per pot) of all four plant species on the four mono soils (A=*A. odoratum*, F=*F. rubra*, L=*L. vulgare*, P=*P. lanceolata*). This analysis was also run with soil types defined as own or foreign (Petermann *et al.*, 2008; Hendriks *et al.*, 2013).

In order to evaluate hypothesis 3, we analyzed plant monoculture root biomass (two individuals per pot) on *all mono and mixed* soils to assess release from plant-soil feedback as

292 expected from hypothesis 1. Soil combinations were defined as ‘own-own’, ‘foreign-own’ or
293 ‘foreign-foreign’.
294 Subsequently, we analyzed individual plant responses, for root as well as for shoot
295 biomass. To check whether differences in root mass and distribution patterns occurred based
296 on interspecific competition (hypotheses 2 and 4), root biomass of individual plants was
297 analyzed using the following fixed factors: target species, competitor species, target soil (soil
298 type in target quadrant, the quadrant from which the root biomass was analyzed), and opposite
299 soil (soil type in opposite quadrant). This analysis was also performed with the soil factors
300 defined own or foreign.
301 We tested whether, in a mixed plant community on mixed soils, both plant species of a
302 competing pair respond differently to the soils in the conditioned quadrants within a pot. We
303 did this with a separate ANOVA analysis for each mixed plant community on every mixed
304 soil separately (target species x target soil, two levels each per analysis). An uneven root
305 distribution over conditioned soils (indicated by a target species x target soil interaction)
306 might indicate root mass re-distribution belowground as hypothesized under 4. A different
307 distribution could indicate differential effects of different plant-soil feedback on root growth
308 (Hendriks *et al.*, 2015).
309 Finally, we analysed individual shoot biomass using target species, competitor species
310 and soil combination as fixed factors, the latter being merged from target and opposite soil
311 (ten levels).
312

Results

Interspecific and intraspecific competition on mono soils

Root biomass in plant monocultures differed among plant species (Species effect: $F_{3,79}$=30.99, P<0.001, Table S2A,B, Fig. 2), and depended on type of mono soil (i.e. two quadrants with same conditioned soil) (Plant species x Mono soil type $F_{9,79}$=7.74, P<0.001, Table S2A, Fig. 2). Overall, plant species produced less biomass in own than foreign mono soils, but negative plant-soil feedback was not apparent in all four species, as indicated by the significant interaction between plant species and soil type (Plant species x Mono soil type (own vs foreign): $F_{9,87}$=12.58, P<0.001, Table S2B, Fig.2). The plant species with the highest biomass production (*P. lanceolata*) also had the strongest negative plant-soil feedback: a 54% reduced root biomass in own compared to foreign mono soil. *Festuca rubra* had 38% reduced biomass in own compared to foreign mono soil, while the plant-soil feedback effects were not significant for *A. odoratum* and *L. vulgare* (8% and 13% reduction, respectively in own compared to foreign mono soil).

In interspecific competition in mixed plant communities on mono soil (i.e. two quadrants with the same conditioned soil) *P. lanceolata*, *F. rubra* and *L. vulgare*, but not *A. odoratum* produced less root biomass in own compared to foreign mono soil (Table S3C, target x soil (own vs foreign): $F_{3,1245}$=26.5, P<0.001; Fig. S2). Concomitantly, the interspecific competitor species produced more root biomass in these two patches of own soil of *P. lanceolata, F. rubra* and *L. vulgare* than in two patches of soil foreign to both plant species (Fig. S2, Table S3C: target x competitor x soil (own vs foreign), $F_{9,1245}$=2.90, P = 0.002). Hence, on own soil, all species except *A. odoratum* produced less root biomass against an interspecific competitor.

Interspecific and intraspecific competition in mixed soils

In plant monocultures in pots where at least one of the two quadrants was filled with foreign soil, three plant species were released from negative plant-soil feedback as total root biomass increased from own-own, to foreign-own, to foreign-foreign soil (Species x Soil combination: $F_{6,227}$=12.92, P<0.001 with soil type defines as own-own, foreign-own, or foreign-foreign; Fig. S3). *Festuca rubra* and *P. lanceolata* both showed a gradual decrease of root biomass from foreign-foreign, to foreign-own, and again to own-own soil, but the total reduction was stronger for *P. lanceolata* than for *F. rubra* (54% and 38%, respectively). Root biomass of *L. vulgare* was 18% higher in soils with two foreign patches compared to situations where own soil was present in one (foreign-own) or two quadrants (own-own). *A.*

odoratum was unresponsive to 'own' soils as it produced similar amounts of root biomass in foreign-foreign, foreign-own and own-own soil (Fig. S3).

The outcome of belowground interspecific competition on mixed soils (i.e. two quadrants with different patches of conditioned soil) depended significantly on the combination of soils in the quadrants (Fig. 3). For example, *P. lanceolata* was the strongest competitor (Table S3B, target: $F_{3,1245}$=322, P<0.001), producing high root biomass in foreign patches, irrespective of competitor species. Interestingly, in pots with quadrants with own and foreign soil, *P. lanceolata* root biomass in the own quadrant was significantly lower than in the foreign quadrant. This negative effect of own soil biota on root biomass of *P. lanceolata* allowed an increase of root biomass of the neighbouring plant species in the patch with *P. lanceolata* soil (which was thus a foreign patch for this neighbouring plant species). This shift in root distribution was particularly significant in cases where the foreign patch for *P. lanceolata* was the own soil of the neighbouring plant species (Fig. 3c,e,f; highlighted treatments, all P < 0.01). These shifts occurred only in mixed plant communities with *P. lanceolata* and not among the three other plant species (Fig. 3a, b, d).

Plant mixtures: aboveground biomass in competition

Monoculture shoot biomass of *F. rubra* and *P. lanceolata* growing in mono soils was (marginally) significantly reduced in own compared to foreign soil (Species x Soil type (own vs foreign): $F_{3,87}$=2.447, P=0.069), but the shoot biomass of other two plant species was not affected by mono soil type (Fig. S4). Therefore, aboveground responses were similar to belowground responses with regard to effects of negative plant-soil feedback in plant monocultures on mono soils (Fig 2). Also aboveground, *F. rubra, P. lanceolata*, and to a lesser degree *L. vulgare* showed release from negative plant-soil feedback in monoculture when one or two quadrants contained foreign instead of own soil (Fig. S4; Planting x Soil combination, defined as own-own, foreign-own, or foreign-foreign: $F_{6,227}$=3.88, P=0.001). Shoot biomass in interspecific competition was not affected by soil combination (Table S3A, Soil combination: $F_{9,586}$=0.69, P=0.717, Fig. 4 and S4), neither by mono soils nor the mixed soils. The effect of soil combination (10 pairwise combinations) on shoot biomass differed, depending on plant species: soil combination affected shoot biomass of *P. lanceolata* ($F_{9,199}$=2.20, P=0.024) while it did not affect shoot biomass of the grasses (*A. odoratum*: $F_{3,196}$=0.64, P=0.762 and *F. rubra* $F_{9,198}$=1.58, P=0.122, respectively) and of *L. vulgare* ($F_{9,197}$=1.76, P=0.078).

We found a clear competitive hierarchy since the effect of competitor on shoot biomass (Table S3A, Competitor: $F_{3,447}$=3.11, P=0.026; Figure 4 and S4) did not differ between target plant species (Target x Competitor: $F_{9,447}$=0.35, P=0.957), or on different soil combinations (Target x Competitor x soil combination: $F_{81,447}$=0.48, P=0.999). Independent of soil combinations, shoot biomass was always larger when plant species were competing with *F. rubra* than with *L. vulgare* and *A. odoratum*. Additionally, shoot biomass was always lowest when competing with *P. lanceolata.* This indicated a clear hierarchy of competitive effect strengths of the competitors, with the largest plant species also being the strongest competitor: *P. lanceolata* → *A. odoratum* & *L. vulgare* → *F. rubra*.

Discussion

Until now, effects of plant-soil feedback on competition have been studied in soils that were uniformly conditioned by only one of the competing plant species (van der Putten & Peters, 1997; Casper & Castelli, 2007; Kulmatiski *et al.*, 2008; Petermann *et al.*, 2008; van der Putten *et al.*, 2013). In such cases, plants exposed to negative soil feedback are replaced by other species leading to directional or cyclic succession (van der Putten & Peters, 1997; Casper & Castelli, 2007; Bever *et al.*, 2012). Our study provides evidence that spatial heterogeneity of plant-soil feedback may lead to different belowground plant-plant interactions, potentially affecting community dynamics. When the strongest competitor is confronted with own soil (i.e. conspecifically conditioned soil), root growth of this plant species is inhibited, giving the roots of the inferior competitor an advantage in that specific patch. Under heterogeneous conditions, negative plant-soil feedback thus creates competitive opportunities for inferior species, with roots able to escape from other soil patches where they lose competition. Soils heterogeneous in the abundance of species-specific soil biota seem the rule in species-rich plant communities (Ettema & Wardle, 2002; Bever *et al.*, 2010; Bezemer *et al.*, 2010). If the responses that we observed hold in the field, this heterogeneity may contribute to plant community dynamics and species coexistence.

Differential plant-soil feedback patches within the rooting zone affect plant interactions belowground

Negative plant-soil feedback was strongest for *P. lanceolata*. This plant species was the only one grown from seeds collected from a previous experiment with the same soil. Possibly, the seed provenance might have affected the degree of plant-soil feedback, for example by adaptation of *P. lanceolata* to its soil biota by selection, or by selection effects of the plants on the soil biota. There are only very few studies on plant adaptation to soil biota (Lankau, 2011; Schweitzer *et al.*, 2014; terHorst *et al.*, 2014), so that it is not well predictable which of these two options might provide a better explanation. Moreover, strong negative plant-soil feedback of *P. lanceolata* in our study was consistent with other plant-soil feedback studies, which did not necessarily started from such locally collected plant seeds (Petermann *et al.*, 2008; Harrison & Bardgett, 2010; Hendriks *et al.*, 2015). *Plantago lanceolata* was also the plant species with the largest amount of total biomass, giving it a significant advantage in belowground competition over the other three plant species (Cannell *et al.*, 1984; Bartelheimer *et al.*, 2008). Interestingly, the distribution of soil biota within the rooting zone of *P. lanceolata* lead to changes in root distribution over patches in mixed soils: reduced root

423 growth of the strongest competitor (*P. lanceolata*) in own soil compared to foreign patches,
424 allowing opportunities for its competitors in the avoided patches. This increased opportunity
425 for root growth even resulted in the inferior competitor (*L. vulgare*) surpassing the dominant
426 competitor in terms of root mass in that patch. Belowground competition is an important part
427 of plant competition (Wilson, 1988; Mariotte *et al.*, 2012; Kiær *et al.*, 2013). In due course,
428 the strong negative plant-soil feedback and reduced competitiveness of the potentially
429 strongest competitor also may enhance the aboveground competitive abilities of subordinate
430 competitors (Mariotte *et al.*, 2012).

431 In the present study, soil conditioning by different plant species was used as a proxy
432 for changing relative abundance of soil biota. Identifying the soil biota was outside the scope
433 of this study, since the aim was to test whether heterogeneity of plant-soil feedback within a
434 plant's rooting zone changes belowground interactions and competitive performance.
435 Negative plant-soil feedback effects can be induced by fungal pathogens (Raaijmakers *et al.*,
436 2009) and nematodes (van Ruijven *et al.*, 2003; de Deyn *et al.*, 2004), whereas little is known
437 about bacterial diseases that may cause plant-soil feedback (Mendes *et al.*, 2013). Negative
438 plant-soil feedback effects may be alleviated by beneficial soil biota such as arbuscular
439 mycorrhizal fungi (van der Heijden *et al.*, 1998; Bever, 2002; Helgason *et al.*, 2002; Mangan
440 *et al.*, 2010a). The soil biota involved in plant-soil feedback can also affect patch dynamics by
441 their persistence in the soil in the absence of host plants. For example, when fungi with highly
442 persistent spores are causing negative feedback it may take several years for fungal densities
443 to decrease and new seedlings can establish (Van der Putten *et al.*, 2001). When short-living
444 nematodes are the main cause for negative plant-soil feedback, establishment of seedlings
445 may potentially occur much faster (Van der Putten *et al.*, 2001; van der Putten, 2003). Thus,
446 to understand temporal patch-dynamics in natural plant communities, identifying soil biota
447 that cause negative plant-soil feedback will be relevant in future studies.

448 There are two major alternative mechanistic explanations for plant-soil feedback
449 effects in our study. Detrimental effects of soil on roots might also have been caused by
450 autotoxic compounds. Such effects might be due to chemicals exuded by the dead root tissues,
451 or by microbial breakdown products (Mazzoleni *et al.*, 2015). Generally, non-biotic
452 autotoxicity is very difficult to be demonstrate. Alternatively what is considered as
453 autotoxicity might still be a result of microbial degradation of dead plant tissues (Bais *et al.*,
454 2006). Another possibility is that the plant-soil feedback effects are due to nutrient limitation
455 (other than N and P) caused during the conditioning phase. For example, Bezemer et al.
456 (2006) proposed that in specific cases potassium might have been a limiting factor, However,

457 in our approach, the inoculation of 25% conditioned soil with sterilized background soil will
458 have largely avoided such strong effects of nutrient limitation.
459
460 *Belowground vs aboveground competitive responses*
461 In monoculture soils (i.e. two patches of the same conditioned soil), negative effects of soil
462 biota on belowground plant biomass were reflected in growth reductions aboveground. In
463 mixed soil (i.e. patches containing different types of conditioned soil), however, soil biota
464 also affected root distribution, but this did not yet cascade into aboveground effects, leaving
465 the aboveground competitive hierarchy outcome unaffected at least for the duration of the
466 experiment. One likely reason is that two unsuitable 'own' soil patches in monoculture soils,
467 where on average 46% of the roots were located, induced stronger aboveground responses
468 than one 'own' soil patch in mixed soils. Another explanation is that plastic root responses to
469 soil biota have buffered aboveground biomass responses. The negative effects of soil
470 microbes might be compensated for by increased nutrient uptake rates in other quadrants in
471 mixed soil combinations, as shown for these plant species in (Hendriks *et al.*, 2015), or by a
472 change in allocation leading to different root-shoot ratios (Mommer *et al.*, 2010; Padilla *et al.*,
473 2013). Additionally, the set-up of the present study contained 'plant' quadrants of neutral
474 (unconditioned; Q1 and Q3) soil which might have been additional 'escapes' for plant roots,
475 in both monoculture soil combinations as well as mixed soil combinations.
476 Compared to aboveground, belowground plant competition is less likely to cause
477 competitive exclusion due to size symmetry (Weiner & Thomas, 1986; Hautier *et al.*, 2009;
478 Lamb *et al.*, 2009) and thus might have less immediate effects on plant community structure.
479 Moreover, Price *et al.* (2012) suggested that belowground rooting patterns can temporarily be
480 neutralized by competitive interactions aboveground (Price *et al.*, 2012). However, also the
481 opposite has been demonstrated as detrimental effects of plant-soil feedback on belowground
482 plant structures appeared to have a lag phase of 3 months before affecting aboveground
483 competitive ability (van der Putten & Peters, 1997). Future studies will have to reveal on what
484 spatial and temporal scales plant-soil feedbacks operate and drive plant competitive
485 interactions below and aboveground in natural grasslands.
486
487 *Consequences for species coexistence in natural ecosystems*
488 Until now, plant-soil feedback studies have not considered the interactive effects of different
489 soil legacies within the root system of a single plant. Plants occupy different patches in
490 vegetation at different timescales. Roots, however, expand further horizontally than shoots

(Pecháčková *et al.*, 2004; Hiiesalu *et al.*, 2012), and will explore soil patches with different strengths of plant-soil feedback (Hendriks *et al.*, 2015). Price *et al.* (2012) found that both biotic and abiotic heterogeneity positively affected plant species coexistence belowground. Our results suggest that plants do not need to perish due to their own soil enemies, but can escape on small spatial scales to better resorts in terms of plant-soil feedback. Bezemer *et al.* (2010) showed that entire soil food webs can differ in small-scale soil patches under individual plants, investigating semi-natural grasslands of the same type as where our plant species originate from. Small-scale patches of soil biota are thus likely to occur naturally in these grassland systems.

The plant species used in the present study may occur in the field as rather isolated individuals, as well as larger patches of individuals of the same species. In the absence of information on soil biotic patchiness in the field, we have chosen grids of single sizes that may be either too small or too large to represent all natural conditions. However, our approach provides a proof of concept and subsequent studies may be needed in order to determine how grid size may influence plant-plant interactions through spatial variation in plant-soil feedback. It also remains to be investigated how these small-scale root-soil and root-root interactions will affect the shifting mosaics on small spatial scales induced by soil-borne pathogens (Olff *et al.*, 2000; Bonanomi *et al.*, 2005; Bever *et al.*, 2012; Mack & Bever, 2014).

In the field, negative plant-soil feedback might also be diluted when plant roots grow intermingled. Recent studies that showed the role of plant-soil feedback suppression as a possible cause of overyielding in species-diverse plant communities (Maron *et al.*, 2011; Schnitzer *et al.*, 2011) proposed that the main cause was a dilution of soil pathogens. Here, we show that this effect might as well be due to patchy distribution of negative plant-soil feedback effects. There are relatively few field studies where plant-soil feedback effects have been studied in relation to plant competition (Casper & Castelli, 2007). More of these types of studies are needed, to investigate how root responses allow plants to escape from their own plant-soil feedback, and how heterogeneity in soil biota changes competitive balances and contribute to plant species coexistence in the field.

Acknowledgements

The authors thank Laura Reiniers, Peter Cruijsen, Raoul Luijten, Jo – Ruud – José Hendriks and Dorine Schoonhoven-van Ewijk for their help during the harvest, and Jasper van Ruijven, three anonymous referees and Editor Dr Maarja Öpik for feedback on previous versions of this manuscript. L.M. was supported by a NWO Veni grant. M.H. was supported by NWO

525 open competition grant 819.01.001. J.R. was supported by the DFG as part of the Jena
526 Experiment Phase IV (FOR456/1451).

References:

Augspurger CK, Wilkinson HT. 2007. Host specificity of pathogenic Pythium species: implications for tree species diversity. *Biotropica* **39**(6): 702-708.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in rhizosphere interations with plants and other organisms. *Annual Review of Plant Biology* **57**: 233-266.

Bartelheimer M, Steinlein T, Beyschlag W. 2008. 15N-nitrate-labelling demonstrates a size symmetric competitive effect on belowground resource uptake. *Plant Ecology* **199**(2): 243-253.

Bever JD. 2002. Negative feedback within a mutualism: host–specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **269**(1509): 2595-2601.

Bever JD. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist* **157**(3): 465-473.

Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M. 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution* **25**(8): 468-478.

Bever JD, Platt TG, Morton ER. 2012. Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual Review of Microbiology* **66**(1): 265-283.

Bever JD, Westover KM, Antonovics J. 1997. Incorporating the soil community into plant population dynamics: The utility of the feedback approach. *Journal of Ecology* **85**(5): 561-573.

Bezemer TM, Fountain MT, Barea JM, Christensen S, Dekker SC, Duyts H, van Hal R, Harvey JA, Hedlund K, Maraun M, et al. 2010. Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. *Ecology* **91**(10): 3027-3036.

Bonanomi G, Giannino F, Mazzoleni S. 2005. Negative plant–soil feedback and species coexistence. *Oikos* **111**(2): 311-321.

Brandt AJ, de Kroon H, Reynolds HL, Burns JH. 2013. Soil heterogeneity generated by plant–soil feedbacks has implications for species recruitment and coexistence. *Journal of Ecology* **101**(2): 277-286.

Brinkman PE, van der Putten WH, Bakker EJ, Verhoeven KJ. 2010. Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology* **98**(5): 1063-1073.

Burns JH, Brandt AJ. 2014. Heterogeneity in plant–soil feedbacks and resident population dynamics affect mutual invasibility. *Journal of Ecology* **102**(4): 1048-1057.

Cain ML, Subler S, Evans JP, Fortin M-J. 1999. Sampling spatial and temporal variation in soil nitrogen availability. *Oecologia* **118**(4): 397-404.

Cannell M, Rothery P, Ford E. 1984. Competition within stands of *Picea sitchensis* and *Pinus contorta*. *Annals of Botany* **53**(3): 49-62.

Casper BB, Castelli JP. 2007. Evaluating plant–soil feedback together with competition in a serpentine grassland. *Ecology Letters* **10**(5): 394-400.

Chesson P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* **31**(ArticleType: research-article / Full publication date: 2000 / Copyright © 2000 Annual Reviews): 343-366.

de Deyn GB, Raaijmakers CE, Van Ruijven J, Berendse F, van der Putten WH. 2004. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. *Oikos* **106**(3): 576-586.

Ettema CH, Wardle DA. 2002. Spatial soil ecology. *Trends in Ecology & Evolution* **17**(4): 177-183.

Farley RA, Fitter AH. 1999. Temporal and spatial variation in soil resources in a deciduous woodland. *Journal of Ecology* **87**(4): 688-696.

Fransen B, de Kroon H, Berendse F. 2001. Soil nutrient heterogeneity alters competition between two perennial grass species. *Ecology* **82**(9): 2534-2546.

García-Palacios P, Maestre FT, Bardgett RD, de Kroon H. 2012. Plant responses to soil heterogeneity and global environmental change. *Journal of Ecology* **100**(6): 1303-1314.

Harrison KA, Bardgett RD. 2010. Influence of plant species and soil conditions on plant–soil feedback in mixed grassland communities. *Journal of Ecology* **98**(2): 384-395.

Hautier Y, Niklaus PA, Hector A. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* **324**(5927): 636-638.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *Journal of Ecology* **90**(2): 371-384.

Hendriks M, Mommer L, de Caluwe H, Smit-Tiekstra AE, van der Putten WH, de Kroon H. 2013. Independent variations of plant and soil mixtures reveal soil feedback effects on plant community overyielding. *Journal of Ecology* **101**(2): 287-297.

Hendriks M, Visser EJW, Visschers IGS, Aarts BHJ, de Caluwe H, Smit-Tiekstra AE, van der Putten WH, de Kroon H, Mommer L. 2015. Root responses of grassland species to spatial heterogeneity of plant–soil feedback. *Functional Ecology* **29**(2): 177-186.

Hiiesalu I, ÖPik M, Metsis M, Lilje L, Davison J, Vasar M, Moora M, Zobel M, Wilson SD, PÄRtel M. 2012. Plant species richness belowground: higher richness and new patterns revealed by next-generation sequencing. *Molecular Ecology* **21**(8): 2004-2016.

Kardol P, Bezemer TM, van der Putten WH. 2006. Temporal variation in plant-soil feedback controls succession. *Ecology Letters* **9**(9): 1080-1088.

Kiær LP, Weisbach AN, Weiner J. 2013. Root and shoot competition: a meta-analysis. *Journal of Ecology* **101**(5): 1298-1312.

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM. 2008. Plant-soil feedbacks: a meta-analytical review. *Ecology Letters* **11**(9): 980-992.

Kulmatiski A, Kardol P. 2008. Getting plant-soil feedbacks out of the greenhouse: experimental and conceptual approaches. *Progress in Botany*: 24.

Lamb EG, Kembel SW, Cahill JF, Jr. 2009. Shoot, but not root, competition reduces community diversity in experimental mesocosms. *Journal of Ecology* **97**(1): 155-163.

Lankau RA. 2011. Resistance and recovery of soil microbial communities in the face of Alliaria petiolata invasions. *New Phytologist* **189**(2): 536-548.

Lundholm JT. 2009. Plant species diversity and environmental heterogeneity: spatial scale and competing hypotheses. *Journal of Vegetation Science* **20**(3): 377-391.

Mack KML, Bever JD. 2014. Coexistence and relative abundance in plant communities are determined by feedbacks when the scale of feedback and dispersal is local. *Journal of Ecology* **102**(5): 1195-1201.

Maestre FT, Bradford MA, Reynolds JF. 2005. Soil nutrient heterogeneity interacts with elevated CO2 and nutrient availability to determine species and assemblage responses in a model grassland community. *New Phytologist* **168**(3): 637-649.

Mangan SA, Herre EA, Bever JD. 2010a. Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant–soil feedback. *Ecology* **91**(9): 2594-2603.

Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI, Bever JD. 2010b. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* **466**(7307): 752-755.

Mariotte P, Buttler A, Johnson D, Thébault A, Vandenberghe C. 2012. Exclusion of root competition increases competitive abilities of subordinate plant species through root–shoot interactions. *Journal of Vegetation Science* **23**(6): 1148-1158.

Maron JL, Marler M, Klironomos JN, Cleveland CC. 2011. Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters* **14**(1): 30-41.

Mazzoleni S, Bonanomi G, Incerti G, Chiusano ML, Termolino P, Mingo A, Senatore M, Giannino F, Cartenì F, Rietkerk M, et al. 2015. Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negative plant–soil feedbacks? *New Phytologist* **205**(3): 1195-1210.

628 **Mendes R, Garbeva P, Raaijmakers JM. 2013.** *The rhizosphere microbiome: significance of plant*
629 *beneficial, plant pathogenic, and human pathogenic microorganisms*.
630 **Mommer L, van Ruijven J, de Caluwe H, Smit-Tiekstra AE, Wagemaker CAM, Ouborg NJ, Bögemann**
631 **GM, van der Weerden GM, Berendse F, de Kroon H. 2010.** Unveiling below-ground species
632 abundance in a biodiversity experiment: a test of vertical niche differentiation among
633 grassland species. *Journal of Ecology* **98**(5): 1117-1127.
634 **Mommer L, van Ruijven J, Jansen C, van de Steeg HM, de Kroon H. 2012.** Interactive effects of
635 nutrient heterogeneity and competition: implications for root foraging theory? *Functional*
636 *Ecology* **26**(1): 66-73.
637 **Mommer L, Wagemaker CAM, de Kroon H, Ouborg NJ. 2008.** Unravelling below-ground plant
638 distributions: a real-time polymerase chain reaction method for quantifying species
639 proportions in mixed root samples. *Molecular Ecology Resources* **8**(5): 947-953.
640 **Olff H, Hoorens B, de Goede RGM, van der Putten WH, Gleichman JM. 2000.** Small-scale shifting
641 mosaics of two dominant grassland species: the possible role of soil-borne pathogens.
642 *Oecologia* **125**(1): 45-54.
643 **Padilla FM, Mommer L, de Caluwe H, Smit-Tiekstra AE, Wagemaker CAM, Ouborg NJ, de Kroon H.**
644 **2013.** Early root overproduction not triggered by nutrients decisive for competitive success
645 belowground. *PLoS ONE* **8**(1): e55805.
646 **Pecháčková S, Albrechtová M, Hadincová V, Krahulec F, Herben T. 2004.** Horizontal and vertical
647 distribution of root absorption zones of four common grass species in a mountain grassland.
648 *New Phytologist* **161**(1): 303-312.
649 **Petermann JS, Fergus AJF, Turnbull LA, Schmid B. 2008.** Janzen-Connell effects are widespread and
650 strong enough to maintain diversity in grasslands. *Ecology* **89**(9): 2399-2406.
651 **Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. 2013.** Going back to the roots: the
652 microbial ecology of the rhizosphere. *Nature Reviews Microbiology* **11**(11): 789-799.
653 **Pinheiro J, Bates, Douglas, DebRoy, Saikat, Sarkar, Deepayan, the R Development Core Team 2011.**
654 Nlme: linear and nonlinear mixed effects models. R package version 3.1-98.
655 **Price JN, Hiiesalu I, Gerhold P, Paertel M. 2012.** Small-scale grassland assembly patterns differ above
656 and below the soil surface. *Ecology* **93**(6): 1290-1296.
657 **R-Core-Team 2014**. R: A Language and Environment for Statistical Computing. Version 3.1.2. Vienna,
658 Austria: R Foundation for Statistical Computing.
659 **Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moenne-Loccoz Y. 2009.** The rhizosphere: a
660 playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and*
661 *Soil* **321**(1-2): 341-361.
662 **Rajaniemi T. 2007.** Root foraging traits and competitive ability in heterogeneous soils. *Oecologia*
663 **153**(1): 145-152.
664 **Robinson D. 1996.** Resource capture by localized root proliferation: why do plants bother? *Annals of*
665 *Botany* **77**(2): 179-186.
666 **Schnitzer SA, Klironomos JN, HilleRisLambers J, Kinkle LL, Reich PB, Xiao K, Rillig MC, Sikes BA,**
667 **Callaway RM, Mangan SA, et al. 2011.** Soil microbes drive the classic plant diversity-
668 productivity pattern. *Ecology* **92**(2): 296-303.
669 **Schweitzer JA, Juric I, van de Voorde TFJ, Clay K, van der Putten WH, Bailey JK. 2014.** Are there
670 evolutionary consequences of plant-soil feedbacks along soil gradients? *Functional Ecology*
671 **28**(1): 55-64.
672 **terHorst CP, Lennon JT, Lau JA. 2014.** The relative importance of rapid evolution for plant-microbe
673 interactions depends on ecological context. *Proceedings of the Royal Society B-Biological*
674 *Sciences* **281**(1785).
675 **van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken**
676 **A, Sanders IR. 1998.** Mycorrhizal fungal diversity determines plant biodiversity, ecosystem
677 variability and productivity. *Nature* **396**(6706): 69-72.
678 **van der Putten WH. 2003.** Plant defense belowground and spatiotemporal processes in natural
679 vegetation. *Ecology* **84**(9): 2269-2280.

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, et al. 2013. Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology* **101**(2): 265-276.

van der Putten WH, Peters BAM. 1997. How soil-borne pathogens may affect plant competition. *Ecology* **78**(6): 1785-1795.

Van der Putten WH, Vet LEM, Harvey JA, Wäckers FL. 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology & Evolution* **16**(10): 547-554.

van Ruijven J, Berendse F. 2003. Positive effects of plant species diversity on productivity in the absence of legumes. *Ecology Letters* **6**(3): 170-175.

van Ruijven J, de Deyn GB, Berendse F. 2003. Diversity reduces invasibility in experimental plant communities: the role of plant species. *Ecology Letters* **6**(10): 910-918.

Weiner J, Thomas SC. 1986. Size variability and competition in plant monocultures. *Oikos* **47**(2): 211-222.

Wijesinghe DK, John EA, Hutchings MJ. 2005. Does pattern of soil resource heterogeneity determine plant community structure? An experimental investigation. *Journal of Ecology* **93**(1): 99-112.

Wilson JB. 1988. Shoot competition and root competition. *Journal of Applied Ecology*: 279-296.

Wilson SD, Keddy PA. 1986. Species competitive ability and position along a natural stress/disturbance gradient. *Ecology*: 1236-1242.

Supplementary Information

Supplementary Methods: Description of soil nutrient (NO_3^-, NH_4^+ and PO_4^{3-}) extraction and analysis and statistical analysis of the nutrient concentrations.

Table S1: Soil nutrient concentrations (NO_3^-, NH_4^+ and PO_4^{3-}) in µmol per kg dry soil in all soils.

Table S2: Results of linear mixed-effects ANOVA (type III sums of squares) of plant monoculture community root biomass (in 30 % of soil volume) in g dry weight per pot.

Table S3: Results of linear mixed-effects ANOVA (type III sums of squares) of individual shoot biomass (A) and root biomass (B and C) on all soils.

Figure S1. Reference curves of estimated plant species proportion (y-axis) against actual species proportion in sample (x-axis) for all four plant species in the mixed root samples, used for estimating species proportions after RT-PCR analysis.

Figure S2. Species-specific root mass per soil quadrant (Q2 and Q4) in mixed plant communities on mono soils.

Figure S3. Plant monoculture community root biomass (in 30 % of soil volume) in g dry weight in all soil combinations.

Figure S4. Species-specific shoot biomass in g per individual in monoculture communities in all soil combinations.

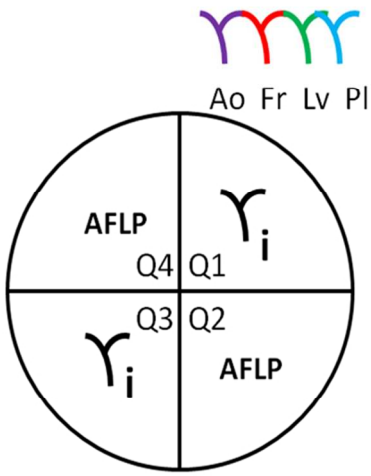
720 **Figure 1.** Experimental design. Four plant species (*A. odoratum, F. rubra, L. vulgare* and *P.*
721 *lanceolata*) were planted in interspecific and intraspecific competition. Plants were planted in
722 quadrants with 'neutral' background soil (Q1 and Q3), the patches in between (Q2 and Q4)
723 contained conditioned soil (soil origin: A, F, L, P). This resulted in a full-factorial design in
724 which we combined ten plant community compositions with ten soil combinations.
725 Abbreviations and symbols: Ao=*A. odoratum*, Fr=*F. rubra*, Lv=*L. vulgare*, Pl=*P. lanceolata*.
726 A= soil of *A. odoratum*, F=soil of *F. rubra*, L=soil of *L. vulgare* or P=soil of *P. lanceolata*.

727 **Figure 2**. Plant monoculture community root biomass (g dry weight in 30% of total soil
728 volume) in four different mono soils (A, F, L, P). Each panel (a-d) represents a different plant
729 monoculture. Shaded bars show biomass in own-own soil. Abbreviations used: A=soil of *A.*
730 *odoratum*, F=soil of *F. rubra*, L=soil of *L. vulgare*, P=soil of *P. lanceolata*. Values are means,
731 error bars depict + 1 SE. Different letters above bars indicate significant differences as
732 determined by Tukey's HSD test.

733 **Figure 3.** Species-specific root mass per soil quadrant (Q2 and Q4) in mixed plant
734 communities (a) *Ao-Fr* (b) *Ao-Lv* (c) *Ao-Pl* (d) *Fr-Lv* (e) *Fr-P*. (f) *Lv-Pl* on all mixed soils.
735 Mixed soils are separated by the soil used in each conditioned soil quadrant (Q2 or Q4).
736 Purple bars = *A. odoratum*; red bars = *F. rubra*; green bars = *L. vulgare*; blue bars = *P.*
737 *lanceolata*. Abbreviations used: *Ao*= *A. odoratum*, *Fr*= *F. rubra*, *Lv*= *L. vulgare*, *Pl*= *P.*
738 *lanceolata* and A=soil of *A. odoratum*, F=soil of *F. rubra*, L=soil of *L. vulgare*, P=soil of *P.*
739 *lanceolata*. Values are means, error bars depict + 1 SE. Dense shading indicates roots
740 growing in own soil. Highlighted (boxed) sets of bars indicate competition of a plant species
741 pair on both their own soils. Asterisks indicate a statistically significant plant species x soil
742 interaction within a mixed soil (tested for each pairwise combination of plants and soils
743 separately, two species and two soils per analysis), hence, a different distribution of root mass
744 over the quadrants; § 0.10 < P 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0.001 < P <
745 0.

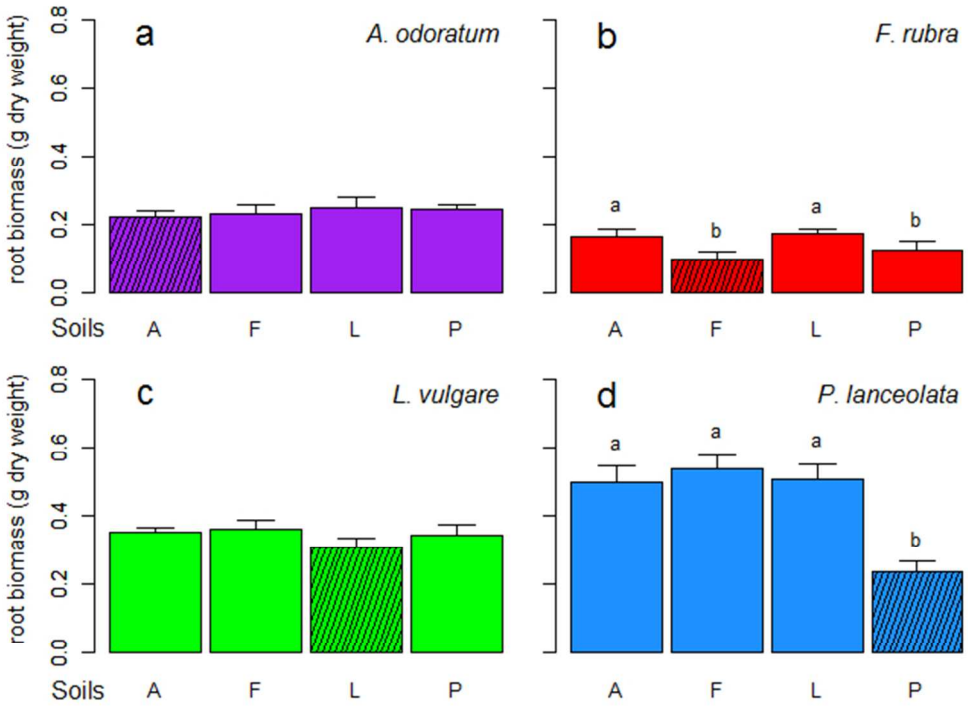
746 **Figure 4**. Species-specific shoot biomass in g per individual in mixed plant communities on
747 all soil combinations. (a) *Ao-Fr* (b) *Ao-Lv* (c) *Ao-Pl* (d) *Fr-Lv* (e) *Fr-P*. (f) *Lv-Pl*. Purple bars
748 = *A. odoratum*; red bars = *F. rubra*; green bars = *L. vulgare*; blue bars = *P. lanceolata*.
749 Abbreviations used: *Ao= A. odoratum*, *Fr= F. rubra*, *Lv= L. vulgare*, *Pl= P. lanceolata* and
750 A=soil of *A. odoratum*, F=soil of *F. rubra*, L=soil of *L. vulgare*, P=soil of *P. lanceolata*.
751 Values are means, error bars depict + 1 SE. Dense shading indicates a plant growing on own-
752 own soil; wide shading indicates a plant on foreign-own soil.

753

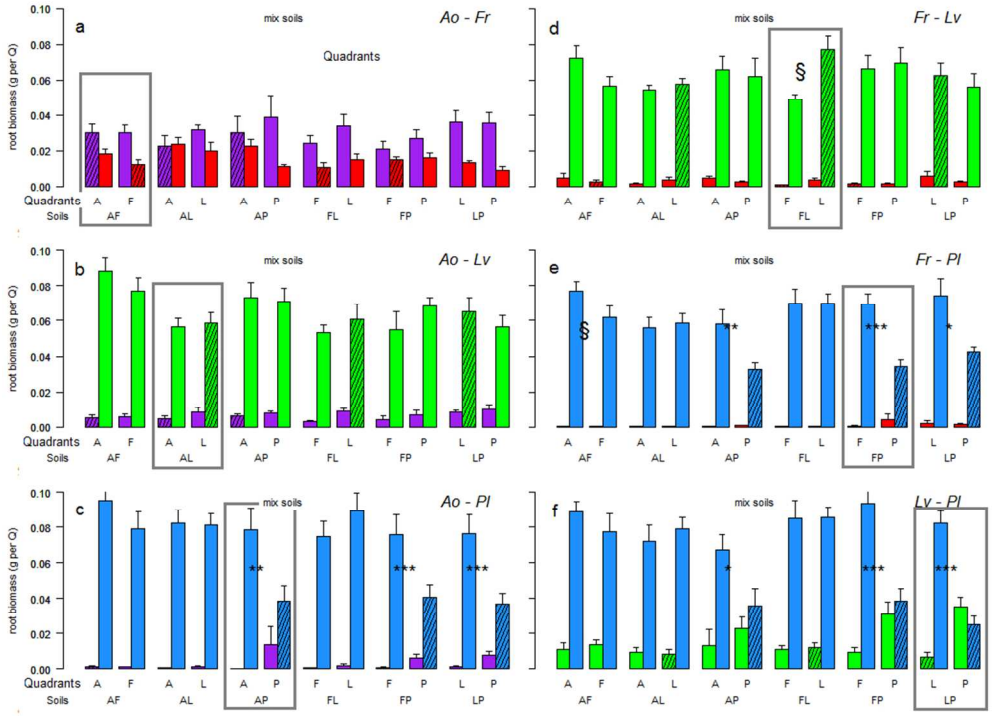


Planting (10) X	Soil combination (10)
Ao – Ao	A – A
Ao – Fr	A – F
Ao – Lv	A – L
Ao – Pl	A – P
Fr – Fr	F – F
Fr – Lv	F – L
Fr – Pl	F – P
Lv – Lv	L – L
Lv – Pl	L – P
Pl – Pl	P – P

Experimental design. Four plant species (A. odoratum, F. rubra, L. vulgare and P. lanceolata) were planted in interspecific and intraspecific competition. Plants were planted in quadrants with 'neutral' background soil (Q1 and Q3), the patches in between (Q2 and Q4) contained conditioned soil (soil origin: A, F, L, P). This resulted in a full-factorial design in which we combined ten plant community compositions with ten soil combinations. Abbreviations and symbols: Ao=A. odoratum, Fr=F. rubra, Lv=L. vulgare, Pl=P. lanceolata. A= soil of A. odoratum, F=soil of F. rubra, L=soil of L. vulgare or P=soil of P. lanceolata.
266x137mm (96 x 96 DPI)

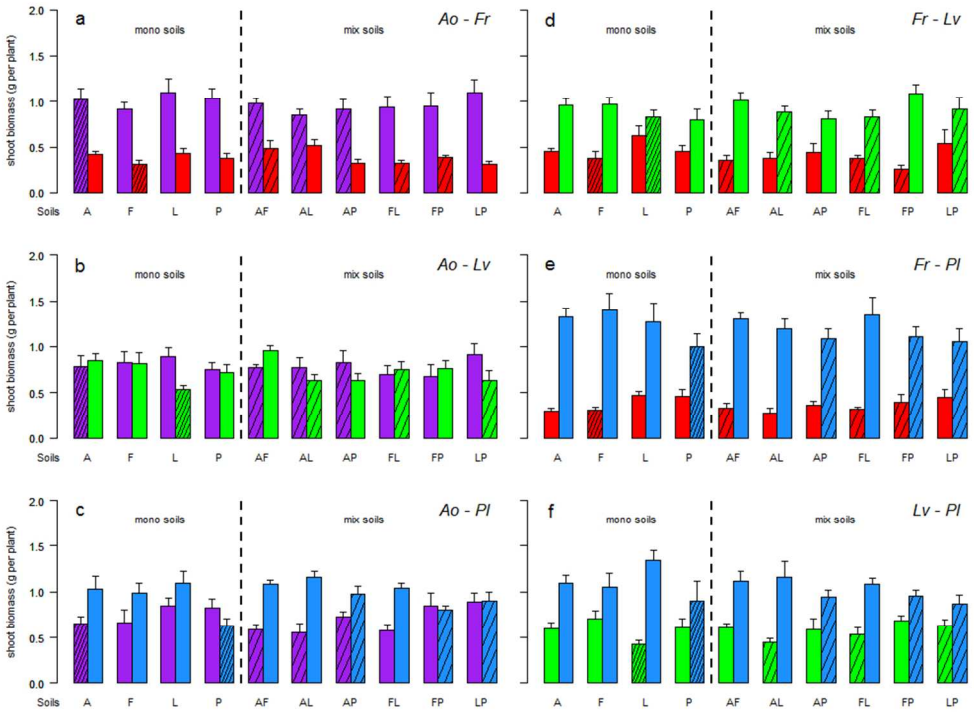


Plant monoculture community root biomass (g dry weight in 30% of total soil volume) in four different mono soils (A, F, L, P). Each panel (a-d) represents a different plant monoculture. Shaded bars show biomass in own-own soil. Abbreviations used: A=soil of A. odoratum, F=soil of F. rubra, L=soil of L. vulgare, P=soil of P. lanceolata. Values are means, error bars depict + 1 SE. Different letters above bars indicate significant differences as determined by Tukey's HSD test.
174x132mm (96 x 96 DPI)



Species-specific root mass per soil quadrant (Q2 and Q4) in mixed plant communities (a) Ao-Fr (b) Ao-Lv (c) Ao-Pl (d) Fr-Lv (e) Fr-P. (f) Lv-Pl on all mixed soils. Mixed soils are separated by the soil used in each conditioned soil quadrant (Q2 or Q4). Purple bars = A. odoratum; red bars = F. rubra; green bars = L. vulgare; blue bars = P. lanceolata. Abbreviations used: Ao= A. odoratum, Fr= F. rubra, Lv= L. vulgare, Pl= P. lanceolata and A=soil of A. odoratum, F=soil of F. rubra, L=soil of L. vulgare, P=soil of P. lanceolata. Values are means, error bars depict + 1 SE. Dense shading indicates roots growing in own soil. Highlighted (boxed) sets of bars indicate competition of a plant species pair on both their own soils. Asterisks indicate a statistically significant plant species x soil interaction within a mixed soil (tested for each pairwise combination of plants and soils separately, two species and two soils per analysis), hence, a different distribution of root mass over the quadrants; § 0.10 < P 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0.001 < P < 0.

277x198mm (96 x 96 DPI)



Species-specific shoot biomass in g per individual in mixed plant communities on all soil combinations. (a) Ao-Fr (b) Ao-Lv (c) Ao-Pl (d) Fr-Lv (e) Fr-P. (f) Lv-Pl. Purple bars = A. odoratum; red bars = F. rubra; green bars = L. vulgare; blue bars = P. lanceolata. Abbreviations used: Ao= A. odoratum, Fr= F. rubra, Lv= L. vulgare, Pl= P. lanceolata and A=soil of A. odoratum, F=soil of F. rubra, L=soil of L. vulgare, P=soil of P. lanceolata. Values are means, error bars depict + 1 SE. Dense shading indicates a plant growing on own-own soil; wide shading indicates a plant on foreign-own soil.
268x198mm (96 x 96 DPI)