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Effects of spatial plant–soil feedback heterogeneity on plant performance in monocultures

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Summary

1. Plant–soil feedback (PSF) effects have almost exclusively been quantified on homogeneous soils, but as different plant species will influence their local soil differently, in reality PSF effects will be spatially heterogeneous. Whether plant performance in soils with spatially heterogeneous PSF can be predicted from pot experiments with homogeneous soils is unclear.

2. In a glasshouse experiment, we tested the response of monocultures of six grassland species (two grasses, two legumes and two forbs) to three spatially explicit treatments (fine-grained heterogeneity, coarse-grained heterogeneity and homogeneous). Sixteen patches of conditioned soil (~6 × 6 cm) were placed within each container. For homogeneous treatments, all patches contained the same conditioned soil within a container. The fine-grained heterogeneous treatment contained four differently conditioned soils that were applied following a Latin square design, while for the coarse-grained heterogeneous treatment, four contiguous square blocks of four cells each were created in each container.

3. In general, species grew worse on soil conditioned by conspecifics. However, when the biomass production on all homogeneous soil treatments (own and foreign soils) was averaged and compared to the heterogeneous treatments, we found that biomass production was lower than expected in the heterogeneous soils. This effect of heterogeneity depended on both the conditioning and test species, but most heterogeneity effects were negative. The grain of the heterogeneity (coarse vs. fine: at the chosen spatial scale) did not affect plant performance.

4. We hypothesize that a more diverse soil community is present in spatially heterogeneous soils. This increases (i) the chance of plants to encounter its antagonists, which may then rapidly increase in numbers; and (ii) the scope for synergistic co-infections. Together, this may lead to non-additive responses of plants to spatial heterogeneity in PSF.

5. *Synthesis.* Plant performance was lower in spatially heterogeneous soils than predicted by spatially homogeneous soils. In natural grasslands that have mixed plant communities conditioning the soil, plant–soil feedback (PSF) effects on plant performance may therefore be more negative than what is predicted from pot experiments. Our results emphasize the need to incorporate the spatial dynamics of PSF both in empirical and modelling studies if we are to understand the role of PSF in plant–plant interactions and plant community dynamics.

Key-words: grasslands, heterogeneous soil, plant–plant interactions, plant–soil (below-ground) interactions, soilborne antagonists, spatial grain, spatial interactions, upscaling

Introduction

A rapidly increasing number of studies have argued that plant–soil feedbacks (PSFs) may play a profound role in determining plant performance and the composition of natural vegetation (Van der Putten *et al.* 2013). Mathematical models have shown that spatial heterogeneity in PSF effects at the plot level can greatly influence plant community composition (Bonanomi, Giannino & Mazzoleni 2005; Eppinga *et al.* 2006; Mack & Bever 2014). However, so far empirical PSF studies have almost exclusively been conducted in spatially homogeneous soils and whether those empirical PSF data can reliably be used to extrapolate to spatially heterogeneous conditions is an open question.

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Spatial heterogeneity has long been investigated as a driver of plant community composition (Lundholm 2009; Reynolds & Haubensak 2009; Tamme *et al.* 2010; Allouche *et al.* 2012), but this work has almost completely been restricted to heterogeneity in abiotic conditions (e.g. acidity, water-table, nutrient availability and form). However, it is becoming increasingly clear that at small scales (Bezemer *et al.* 2010), spatial heterogeneity may be largely be driven by biotic interactions and this may strongly affect plant–plant interactions (Tamme *et al.* 2010; De Kroon *et al.* 2012). The biotic drivers could be, for example, localized plant–microbe interactions that affect nutrient mineralization or accumulation of pathogens.

Plant–soil feedback studies assess the consequences of plant-induced changes in the soil's biotic as well as abiotic conditions for plant performance (Bever 1994; Bardgett *et al.* 1999; Ehrenfeld, Ravit & Elgersma 2005; Casper & Castelli 2007; Van der Putten *et al.* 2013). Soil microbial communities change in response to differences in root-associated rhizodeposits among plants species (Raaijmakers *et al.* 2009; Bever, Platt & Morton 2012; Philippot *et al.* 2013). These rhizodeposits include carbon sources as well as secondary compounds involved in plant defence that differentially affects the population growth rates of different microbes. The effect of soil conditioning on plant performance, that is the feedback, can be positive and negative depending on both conditioning and responding plant species (Bever, Westover & Antonovics 1997; Bever 2003; Van der Putten *et al.* 2013). Furthermore, PSF can arise from conditioning by conspecific individuals (direct or conspecific PSF) or heterospecific individuals (indirect or heterospecific feedback; Bever, Westover & Antonovics 1997; Van de Voorde, Van der Putten & Bezemer 2011). Most direct (conspecific) plant–soil feedbacks are negative (Kulmatiski *et al.* 2008; Petermann *et al.* 2008).

The composition of the soil community is strongly heterogeneous in space across a range of scales (Ettema & Wardle 2002; Bever *et al.* 2010), from changes in rhizosphere communities along individual roots (Folman, Postma & Van Veen 2001) up to macroecological patterns in species assemblages (Green & Bohannan 2006). At relatively small scales, individual plants can alter the composition of the soil community (Bever 1994; Grayston *et al.* 1998; Bever *et al.* 2010; Van der Putten *et al.* 2013). As different plant species induce different rhizosphere conditions, they promote different soil communities and this leads to a patchy below-ground 'mosaic' of soil communities (Bever *et al.* 2010). In line with this, distinct soil communities under different plant species have been observed at the level of individual plants (5 cm diameter soil cores) in diverse plant communities in the field (Bezemer *et al.* 2010) and the density of conspecifics in the field affects the feedback strength (Kos, Veendrick & Bezemer 2013). However, besides the identity of the conditioning plant, the composition of the local soil community is also to some extent influenced by the composition of the surrounding plant community (Bezemer *et al.* 2010). Consequently, while the mosaic structure of plant species-specific conditioning effects can clearly be observed below-ground in field, in reality also neighbouring plants contribute to local soil conditioning as a plant's zone of influence typically extends beyond its neighbouring plants (Casper, Schenk & Jackson 2003). We use the term spatial PSF heterogeneity here to indicate that adjacent patches differ in their feedback effects and we contrast this with homogeneous soil that consists of a larger patch conditioned uniformly by one species.

To date, the vast majority of studies of the consequences of PSFs for plant communities have relied on homogeneous soils. However, a few recent experiments have directly addressed PSF in spatially explicit settings. These studies have shown that small-scale PSF heterogeneity can alter plant vital rates and affect rates of invasion by competitors (Brandt *et al.* 2013; Burns & Brandt 2014). Furthermore, analogous to root foraging for nutrients, plants also respond to spatial differences in soil community composition by selectively placing their roots in patches conditioned by heterospecifics ('foreign' soil) over patches conditioned by conspecifics ('own' soil; Hendriks *et al.* 2015).

While these studies established that small-scale spatial PSF heterogeneity can affect plant performance, it is still unclear whether spatially heterogeneous PSF effects are predictable from pot experiments with spatially homogeneous soils. This is a critical next step in upscaling the role of individual plant–soil interactions to whole plant communities in field settings (Kardol *et al.* 2013; Hawkes *et al.* 2013). In addition, recent modelling work has shown that the spatial scale over which PSF affects plants (i.e. feedback neighbourhood size) can significantly influence the consequences of PSF for plant communities (Mack & Bever 2014), but this has not been tested experimentally.

Here we test explicitly whether plant performance on soils with spatial heterogeneous PSF effects can be predicted from performance on homogeneous soils. In addition, we test for the first time how the grain of spatial PSF heterogeneity influences plant growth. We quantified plant performance of six focal species grown in monoculture on soils conditioned by these species. The conditioned soils were placed in three spatial PSF treatments (fine-grained heterogeneity, coarse-grained heterogeneity and homogeneous; Fig. 1). In each treatment, containers were filled with 16 patches of conditioned soil. Spatial PSF heterogeneity was introduced by placing multiple differently conditioned soil patches within a single container. In the heterogeneous treatments, the local soil patch quality (texture, nutrient availability, soil biotic community) was the same as in the homogeneous treatments as the same soil was used to create the soil patches (i.e. no conditioned soils were mixed) – only their spatial arrangement differed. Our null model was that the performance of a plant on a local patch of soil in a heterogeneous set of patches would be identical to its performance on the same soil in a homogeneous set of patches. Hence, we expected that overall performance of a plant monoculture in spatially heterogeneous PSF soils would be isometrically (1:1) predictable as the mean performance of that plant on each of the constituent soils in homogeneous conditions (when the calculation is weighted by the proportion of each conditioned soil type in the heterogeneous soil).

This null model assumes that all plant–soil interactions are local (i.e. within a patch) and that the soil communities in the differently conditioned soils have no significant interactions that affect plant performance. These are assumptions commonly made in models of PSF (e.g. Bonanomi, Giannino & Mazzoleni 2005; Eppinga *et al.* 2006). Furthermore, we expected that if spatial heterogeneity alters the effects of PSFs, the difference would be most pronounced in containers with fine-scale heterogeneity, while containers with coarse-scale heterogeneity would be intermediate relative to spatially homogeneous soil. Finally, we expected that the strength of direct PSF ('own' vs. 'foreign' comparison) would become less strong as the grain of heterogeneity becomes smaller (PSF: homogeneous > coarse- > fine-grained heterogeneity).

Materials and methods

We conducted a plant–soil feedback glasshouse experiment in which we grew six focal plant species on six conditioned soils and where we explicitly manipulated the spatial heterogeneity of conditioned soils in the containers (Fig. 1). Six plant species were selected that are typical for old fields on sandy soils in north-west Europe, with two representatives each for grass, forb and legume functional groups. They were as follows: *Agrostis capillaris* L. and *Festuca rubra* L., *Hypochaeris radicata* L. and *Jacobea vulgaris* Gaertn. (syn. *Senecio jacobea* L.), and *Lotus corniculatus* L. and *Trifolium pratense* L. Plant–soil feedback experiments typically consist of two phases, first one where plants condition the soil (conditioning phase) and subsequently a phase where the effects of the soil conditioning on plant growth are tested (test or feedback phase).

PHASE 1: CONDITIONING PHASE

Soil was collected from a grazed old-field restoration grassland (Mossel, Planken Wambuis, Ede, the Netherlands, GPS: 52°04′N 05°45′E) where agricultural practices were ceased in 1995, in September 2012. In total, approximately 2500 kg soil was collected from the topsoil (to 30 cm depth) and sieved over a 5-mm mesh. The soil was used to fill large square containers (length × width × height: 17 × 17 × 17 cm) with 5 kg soil per container (Fig. 1). Seeds for each of the species were obtained from a specialized company that provides seeds from wild plants (Cruydt-Hoeck, Assen, the Netherlands) or collected from the same field as the soil (*J. vulgaris*). All seeds were surface-sterilized (1 min. in <2.5% NaClO solution), rinsed with water and allowed to germinate on sterilized glass beads in a climate chamber (16:8-h day–night cycle, continuous 20 °C) for two weeks. Seedlings of each of the six species were planted in monocultures (16 individuals per container) creating 58 containers per species, except for *A. capillaris* and *J. vulgaris* with 77 containers each. More soil of the latter two species was needed to create the spatially heterogeneous treatments in the test phase (see below). All containers were randomly located within a glasshouse compartment, but each container was *a priori* allocated to one of three replicates and these replicates were maintained throughout the experiment. The plants were allowed to grow in the glasshouse (16:8 h day: night, natural light supplemented with 600 W metal-halide lamps, 1 per 4 m^{-2}, approx. 225 µmol light quanta m^{-2} s^{-1} at plant level, 21:16 °C day: night, 50–70% relative humidity) for 8 weeks. Subsequently, all above-ground biomass was clipped flush with the soil, dried (72 °C, 48 h) and weighed. We created three independent soil replicates for each of the six conditioning species by pooling and homogenizing the soil only from those containers that were *a priori* allocated to the same replicate. During homogenization, all root systems were removed. To obtain a sufficient amount of soil for the test phase, each of the 18 soil replicates (6 conditioned soils × 3 replicates) was mixed with sterilized (>25KGray gamma radiation, Isotron, Ede, the Netherlands) field soil collected from the same site in a 8.4:1.6 (conditioned: sterile w:w) ratio. From each of the homogenized soil replicates, a sample (200 g) was taken for chemical analysis upon addition of the sterilized soil. We measured NH_4, NO_3 (both KCl extraction), PO_4 (P, Olsen extraction) and soil organic matter (ashed at 430 °C for 24 h) content as well as soil acidity (in 1:2.5 w:

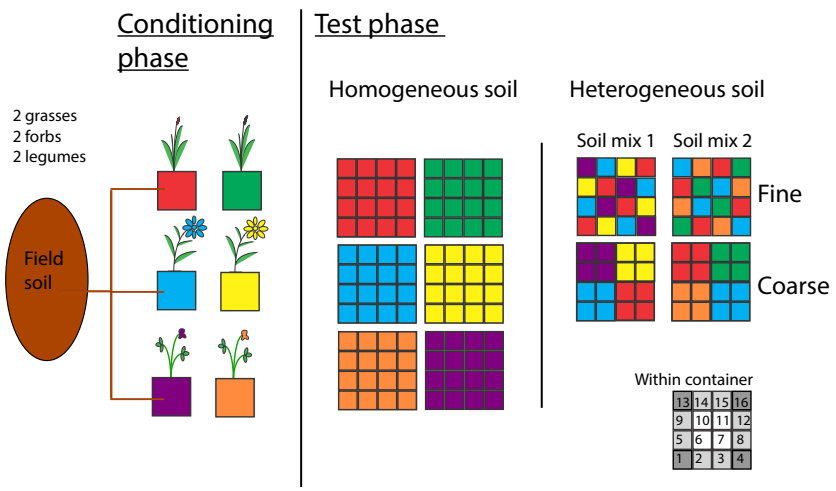

Fig. 1. Conceptual representation of the experimental design. Common field soil was conditioned by monocultures of six plant species. The six soil conditioning treatments are identified by having the colour of the soils (squares) corresponding to the colours of the flowers. Three independent soil replicates were made for each of the conditioned soils. The conditioned soils were used to create spatially homogeneous as well as heterogeneous (coarse and fine) soil treatments in the test phase. The heterogeneous treatments were created using two soil combinations, each containing soil conditioned by four species. Each of the ten soil treatments was planted with each of the six focal species in monoculture full factorially in the test phase. In the inset, the numbering of the gridcells is shown, and the different shadings show how the within-container design has rotational symmetry. For further details, see main text.

w dry soil: water suspensions; see Table S1 in Supporting Information).

PHASE 2: TEST PHASE

In the test phase, three different levels of spatial PSF heterogeneity were created (spatially homogeneous, spatially heterogeneous coarse-grained and spatially heterogeneous fine-grained) each applied to different large containers (Fig. 1). Each container (length × width x height: 26 × 22 × 22 cm) was divided with a custom-made metal grid into 4 × 4 cells, each with a surface area of ~35 cm^2 (the length and width of the cells differed slightly to account for the rounded corners of the containers), extending to the bottom of the container. The size of the gridcells was chosen because at this grain size, systematic differences in soil community composition were detected in open communities in the field (Bezemer *et al.* 2010). In each container, independent of the treatment, all 16 gridcells were filled individually and any given gridcell was always filled with soil conditioned by a single species. Immediately after filling the containers, the grids were removed so that during the experiment the soil patches in each container were in full contact. One of the corners of each of the containers was marked as a point of reference. For the spatially homogeneous treatment, all cells in a container were filled with soil conditioned by the same species, while for spatially heterogeneous treatments (coarse- and fine-grained) gridcells were filled with soil conditioned by four different species (Fig. 1). The four soils in the fine-grained treatment were applied following a Latin square design, while for the coarse-grained treatment, four contiguous square blocks of four cells each were created in each container. The two spatially heterogeneous treatments (coarse- and fine-grained) were created with two different mixes of soil conditioned by four species (soil mix). Soil mix one consisted of soils conditioned by *A. capillaris*, *J. vulgaris*, *H. radicata* and *L. corniculatus*; soil mix two consisted of *A. capillaris*, *J. vulgaris*, *F. rubra* and *T. pratense*. Consequently, both soil mixes had at least one representative each of the grass, forb and legume plant functional types. Soils from all six focal species were used separately to create spatially homogeneous containers. Subsequently, each of the ten soil-by-spatial heterogeneity treatments (six homogeneous, two coarse and two fine) was planted with monocultures of each of the six test species. The whole set-up was replicated three times, using the three independent soil replicates. In total, there were 180 containers in the test phase (10 soil treatments × 6 test plant species × 3 replicates).

Each container was filled with 2.5 kg sterilized gravel (quartz, 4–8 mm) and then with 8 kg of conditioned soil (500 g per gridcell). For each treatment, the containers were filled with conditioned soil in the same way: weighing 500 g of the appropriate conditioned soil type and then carefully pouring the soil into the respective gridcell and continuing until all cells of the container were filled. Each container was planted with 32 seedlings, planting two individuals into each gridcell (each seedling 1 cm from the gridcell mid-point). Seeds were germinated in the same way as in the conditioning phase. Seedlings that died upon transplantation were replaced once during the first week. The containers were placed in the glasshouse in a complete randomized design under the same conditions as during the conditioning phase and allowed to grow for eight weeks. The soil was kept moist by regular watering (two or three times per week depending on evapotranspiration rates). After 8 weeks of growth, above-ground plant biomass was clipped flush with the soil, dried (72 °C, 48 h) and weighed separately per gridcell for each of the containers (i.e. 16 observations per container, with known locations of each observation within the container). Below-ground biomass was sampled by inserting a soil corer (Ø 3.3 cm) into the middle of a gridcell and gently pushing it to the bottom of the container. While extracting the corer, it was made sure that all soil in the column, down to the gravel underneath, was collected. To make sure the soil cores were taken exactly in the middle of each gridcell, a metal grid (same dimensions as before, but only 1 cm high) was placed on top of the soil when taking soil cores. Roots were extracted from the soil cores by careful washing over a sieve (2-mm mesh) and subsequently dried and weighed. For the spatially heterogeneous treatments (coarse- and fine-grained), all sixteen gridcells were sampled, while from for the spatially homogeneous treatment, eight cores were taken (cell numbers 2, 4, 5, 7, 10, 12, 13 and 15, Fig. 1).

DATA ANALYSIS

Differences in abiotic conditions and shoot biomass at the end of the conditioning phase were tested with one-way ANOVA. Spearman correlations were used to assess the relationship of the abiotic soil variables at the end of the conditioning phase and the above- and below-ground biomass of each of the test species at the end of the test phase. Because each test species was grown on the same set of soils (six conditioned soils * three soil replicates), these correlations were calculated for each test species separately. Plant biomass data from the test phase were analysed at container level and at gridcell level separately to study overall effects of changing spatial heterogeneity and specific responses to differently conditioned soils in detail.

Container level

Above-ground biomass at container level was calculated as the sum of the biomass of all sixteen gridcells. As below-ground biomass was determined in soil cores and not in the entire soil volume, it was analysed as mean biomass per soil core. To test for the effect of spatial PSF heterogeneity on plant performance, we compared plant biomass across the three levels of spatial heterogeneity. Containers in each heterogeneous treatment contained four soils, while in the homogeneous treatment, they contained only one. To facilitate comparison, we calculated an expected plant biomass from the same four soils in the homogeneous treatment assuming, as a null expectation, that there was no additional effect of spatial PSF heterogeneity. Therefore, we calculated the null expectation as the mean performance of the test species in spatially homogeneous containers on the same four soils that constituted the heterogeneous treatment. The null expectation was calculated separately for both soil mixes and each independent soil replicate. The calculated expected biomass values were used in an analysis to act as the control treatment (i.e. reflecting plant performance on the constituent soils if no spatial interaction were to occur). The biomass in the heterogeneous treatments was included in the analyses as it was observed. Consequently, the analyses were based on 108 data points: 6 test species × 2 soil mixes × 3 spatial treatments (fine-, coarse-grained heterogeneous and expected-from-homogeneous) × 3 replicates. The effect of spatial heterogeneity on container level was analysed in separate general linear models (GLMs) for above- and below-ground biomass. Test plant species, soil mix and spatial heterogeneity treatment (fine, coarse and expected-from-homogeneous) and their interactions were included as fixed effects. Post hoc comparisons among levels of spatial heterogeneity were tested as planned contrasts both overall across all six test species and for each test species separately (Crawley 2002; Brinkman *et al.* 2010; Galecki & Burzykowski 2013).

To determine how soil conditioning affected plant growth (plant–soil feedback), we analysed data from the spatially homogeneous containers separately. Whole container above- and below-ground biomass was analysed using two-way ANOVA (6 soils × 6 species × 3 replicates = 108 containers), with both conditioned soil type and test plant species as fixed effects. Within the overall model, the effect of 'own' relative to 'foreign' soil was analysed using planned contrasts for each test species separately.

Gridcell level

We analysed the above- and below-ground biomass on gridcell level using linear mixed models (LMMs). Test plant species, conditioned soil type and spatial PSF heterogeneity treatment (fine-, coarse-grained heterogeneity and homogeneous) and their interactions were included as fixed effects. The models included nested random effects for soil replicates, container and gridcell. The gridcell factor was introduced to account for positional effect within containers, but given the rotational symmetry in the within-container design, the gridcell factor had three levels [corner (four per container), edge (eight) or centre (four) of the container; Fig. 1]. Analyses with a 16-level random effect for gridcell lead to the same qualitative conclusions (c.f. Tables 2 and S2). The above-ground analysis was based on 2880 data points: 6 test species × 10 soil treatments (six homogeneous, two coarse-scale heterogeneous treatments and two fine-scale heterogeneous treatments) × 3 replicates × 16 observations per container. For root biomass, there were 2016 data points as in the six homogeneous soils, only eight instead of 16 gridcells were sampled. Differences among levels of spatial heterogeneity were tested as planned contrasts both overall and for each test species on each conditioned soil separately.

Plant–soil feedback has often been characterized as the difference in plant performance on self-conditioned or 'own' soils vs. the performance on soil conditioned by heterospecifics or 'foreign' soil (direct PSF; Van der Putten *et al.* 2013). To test whether the direct PSF changed in response to different levels of spatial heterogeneity, we calculated the feedback as the log ratio of plant biomass on own and foreign soils (Brinkman *et al.* 2010) per test species in each of the three different heterogeneity treatments. This PSF index was calculated separately for each soil replicate (i.e. $n = 6$ test species × 3 spatial heterogeneity treatments × 3 soil replicates = 54). The feedback values were analysed using a two-way linear model with fixed effects for test plant species and spatial heterogeneity. Differences in PSF among spatial heterogeneity treatments were tested using planned contrasts. This was done for each test species separately.

In a number of gridcells, some of the plants had died during the experiment. Mortality was treated as a binary variable which takes the value one when either one or both of the plants of a given gridcell had died. Seedling mortality was analysed at the gridcell level using a generalized linear mixed model (GLMM) with the same fixed and random effects as the LMM on shoot biomass above but with a binomial error distribution. The GLMM was fitted by maximum likelihood and the significance of model terms was evaluated using likelihood ratio tests. In addition, the LMMs on plant biomass were refitted and evaluated using only those gridcells where both seedlings survived until harvest. Finally, to check that plant rooting patterns were consistent across the spatial heterogeneity treatments, we regressed below-ground biomass on shoot biomass across all treatment combinations at container level. We compared the fit to a model with three separate slopes for each of the three treatments by means of an F-test for coincidental regressions (Zar 1999, p. 375).

All analyses were conducted in R version 2.15.3 (R Core Team 2013). Linear (mixed) models were fitted with the NLME [version 3.1-108 (Pinheiro *et al.* 2013)], GLMMs in LME4 [v1.0-4 (Bates *et al.* 2013)] R-packages. All models were graphically checked for homogeneity of variances and their error distributions. When the multi-species (test species) models were heteroscedastic, separate variances per test species were included in the models using generalized least squares following Pinheiro & Bates (2000) and Zuur *et al.* (2009). Post hoc comparisons and planned contrasts were made using the MULTCOMP (v1.2-18) package in R (Hothorn, Bretz & Westfall 2008). Post hoc tests were corrected using the method of Benjamini & Hochberg (1995).

Results

EFFECT OF SPATIAL PSF HETEROGENEITY ON PLANT PERFORMANCE – CONTAINER LEVEL

Spatial PSF heterogeneity significantly affected plant performance (Table 1), which was reduced both in fine- and coarse-grained heterogeneity treatments relative to the performance on spatially homogeneous soils (Fig. 2a). On average, plants produced less above-ground (7.4%) and below-ground

Table 1. Analysis of variance (SS type I) table of GLMs on plant shoot and root biomass at the container level in the spatial plant–soil feedback (PSF) heterogeneity experiment. Presented are degrees of freedom (d.f.), *F*-values, *Z*-values for overall planned contrasts among spatial PSF heterogeneity treatments and *P*-values

		Shoot biomass			Root biomass		
Term	d.f.	*F*	*Z*	*P*-value	*F*	*Z*	*P*-value
Plant species (PS)	5	130.42	–	<0.0001	83.66	–	<0.0001
Soil mix (SM)	1	1.87	–	0.18	1.47	–	0.23
Spatial heterogeneity (SH)	2	5.56	–	0.006	9.32	–	0.0003
Coarse vs. Homogenous		–	−2.86	0.004	–	−4.15	<0.0001
Fine vs. Homogenous		–	−3.18	0.0001	–	−3.46	0.0005
Coarse vs. Fine		–	−0.95	0.34	–	0.69	0.49
PS × SM	5	0.92	–	0.48	2.49	–	0.04
PS × SH	10	2.79	–	0.006	1.76	–	0.08
SM × SH	2	0.23	–	0.79	0.88	–	0.42
PS × SM × SH	10	0.77	–	0.66	0.93	–	0.51
Residual	72						

(17.0%) biomass in spatially heterogeneous conditions than predicted from their performance on the same soils in homogeneous conditions (Table 1; Fig. 2a). There was, however, variation in the strength of the response of test species to PSF heterogeneity (Table 1). In terms of above-ground biomass, *L. corniculatus* and *A. capillaris* experienced significant reductions in the spatially heterogeneous treatments, while for the other species, there was no strong effect (Fig. 2b). Below-ground, the effect of spatial heterogeneity was more general, only the root biomass of *F. rubra* was not significantly affected by spatial heterogeneity (Fig. 2c). For both above- and below-ground biomass, however, the grain (patch size) of spatial PSF heterogeneity (coarse vs. fine) did not have a significant effect (Fig. 2a; Table 1). Regressions of root biomass as a function of shoot biomass for each of the test species showed that the model fit was not improved significantly by including separate slopes for each of the three levels of spatial heterogeneity (Table S3, Fig. S1). In addition, the confidence intervals of the regression slopes for all three spatial heterogeneity treatments overlap for each of the test species. This indicates that the relationship between root and shoot biomass was consistent across the treatments. Shoot biomass was overall a significant predictor of root biomass for most species (Table S3). For the two species where this was not the case, the variation in shoot biomass was very small, making it hard to detect a relationship. The regression slopes were, however, positive also in these cases (Fig. S1).

EFFECT OF SPATIAL PSF HETEROGENEITY ON PLANT PERFORMANCE – GRIDCELL LEVEL

Also at the level of individual gridcells above- and below-ground plant biomass were generally lower in the heterogeneous than the homogeneous treatments (respectively: −8.0% and −16.4%; Table 2). However, the effect of spatial heterogeneity differed between the test plant species and depended on the soil it was growing in (plant x soil interaction; Table 2; Figs 3 and 4). While the majority of heterogeneity effects were negative, we also observed some cases of positive influence (Figs 3 and 4). These were, however, only significant in three cases (shoot biomass in combinations: plant × soil: *F. rubra* × *J. vulgaris*, *H. radicata* × *F. rubra* and *J. vulgaris* × *F. rubra*; Fig. 3), indicating that negative heterogeneity effects dominated. Contrary to our expectation, the spatial grain of the heterogeneity did not significantly affect the magnitude of the heterogeneity effect (Table 2, Figs 3 and 4).

Plant mortality differed among test plant species and was affected by soil conditioning (Fig. S2; Table S4). There was a weakly significant interaction between test plant species and spatial heterogeneity ($P = 0.04$; Table S4), but no consistent pattern across the test species. Mortality rates were generally low (0–10% of gridcells affected), but were quite high in *J. vulgaris*, particularly on its own soil (up to 75%). We re-analysed the gridcell level LMMs on plant biomass using only those gridcells where no plants had died. This led to the same qualitative conclusions as the full analyses with the exception that for shoot biomass, the main effect of space was now only marginally significant (Table S5). There were, however, clear interactions with spatial heterogeneity, indicating that it still affected the plant responses in the reduced data set.

EFFECT OF SPATIAL HETEROGENEITY ON THE STRENGTH OF DIRECT PLANT–SOIL FEEDBACK

Both above- and below-ground plant biomass per container were strongly affected by the species conditioning the soil

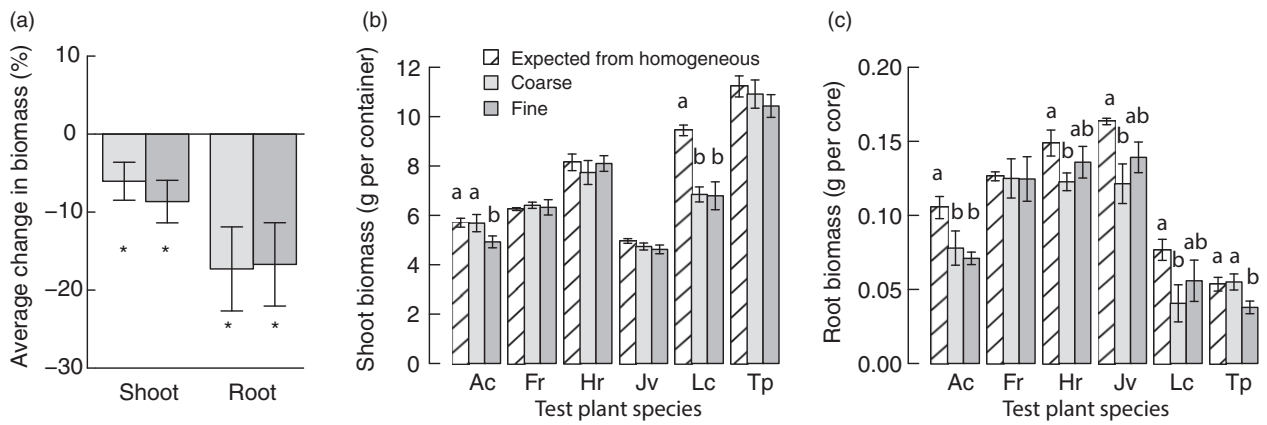

Fig. 2. Effect of different levels of spatial plant–soil feedback (PSF) heterogeneity (container level) on plant performance. (a) The effect of spatial PSF heterogeneity on plant performance averaged over the test species. Presented is the change in biomass relative to the expected biomass calculated from spatially homogeneous soils. The effects of both coarse (light grey)- and fine (dark grey)-scale heterogeneity are shown. (b and c) Mean shoot (b) and root (c) biomass for each of six different test species in the three levels of spatial PSF heterogeneity. Observed biomass is given for coarse and fine spatial treatments, while the calculated expected biomass based on plant performance in spatially homogeneous soils is given for reference (indicated by the hatched bars). In both spatially heterogeneous treatments (coarse and fine), the test plants grew on soil patches conditioned by four species (c.f. Fig. 1). Plant performance on the same four soils in the spatially homogeneous treatment was used to calculate the expected plant performance in the heterogeneous treatments (see Methods for details). Stars indicate significant differences from zero (a; see Table 1 for contrast results) and different letters above the bars (b and c) indicate significant differences in biomass among spatial PSF heterogeneity treatments within each test species (i.e. no among-species comparisons were made; for the overall analyses, see Table 1). Ac: *Agrostis capillaris*, Fr: *Festuca rubra*, Hr: *Hypochaeris radicata*, Jv: *Jacobea vulgaris*, Lc: *Lotus corniculatus*, Tp: *Trifolium pratense*.

Table 2. Analysis of variance (SS type I) table of mixed models of plant biomass (shoots and root separately) in the spatial plant–soil feedback (PSF) experiment at gridcell level. The models include nested random effects for soil replicate, container and gridcell. Presented are degrees of freedom (d.f.), *F*-values, *Z*-values for overall planned contrasts among spatial PSF heterogeneity treatments and *P*-values

		Shoot biomass			Root biomass		
Term	d.f.	*F*	*Z*	*P*-value	*F*	*Z*	*P*-value
Plant species (PS)	5, 276	134.62	–	<0.0001	128.08	–	<0.0001
Conditioned soil (CS)	5, 12	11.98	–	0.0003	3.04	–	0.05
Spatial heterogeneity (SH)	2, 276	5.211	–	0.006	15.16	–	<0.0001
Coarse vs. Homogenous		–	−3.02	0.003	–	−5.09	<0.0001
Fine vs. Homogenous		–	−4.11	<0.0001	–	−4.06	<0.0001
Coarse vs. Fine		–	−0.90	0.37	–	1.00	0.32
PS × CS	25, 276	5.10	–	<0.0001	4.25	–	<0.0001
PS × SH	10, 276	3.19	–	0.0007	2.62	–	0.005
CS × SH	10, 276	3.33	–	0.0004	2.01	–	0.03
PS × CS × SH	50, 276	1.10	–	0.32	0.86	–	0.74

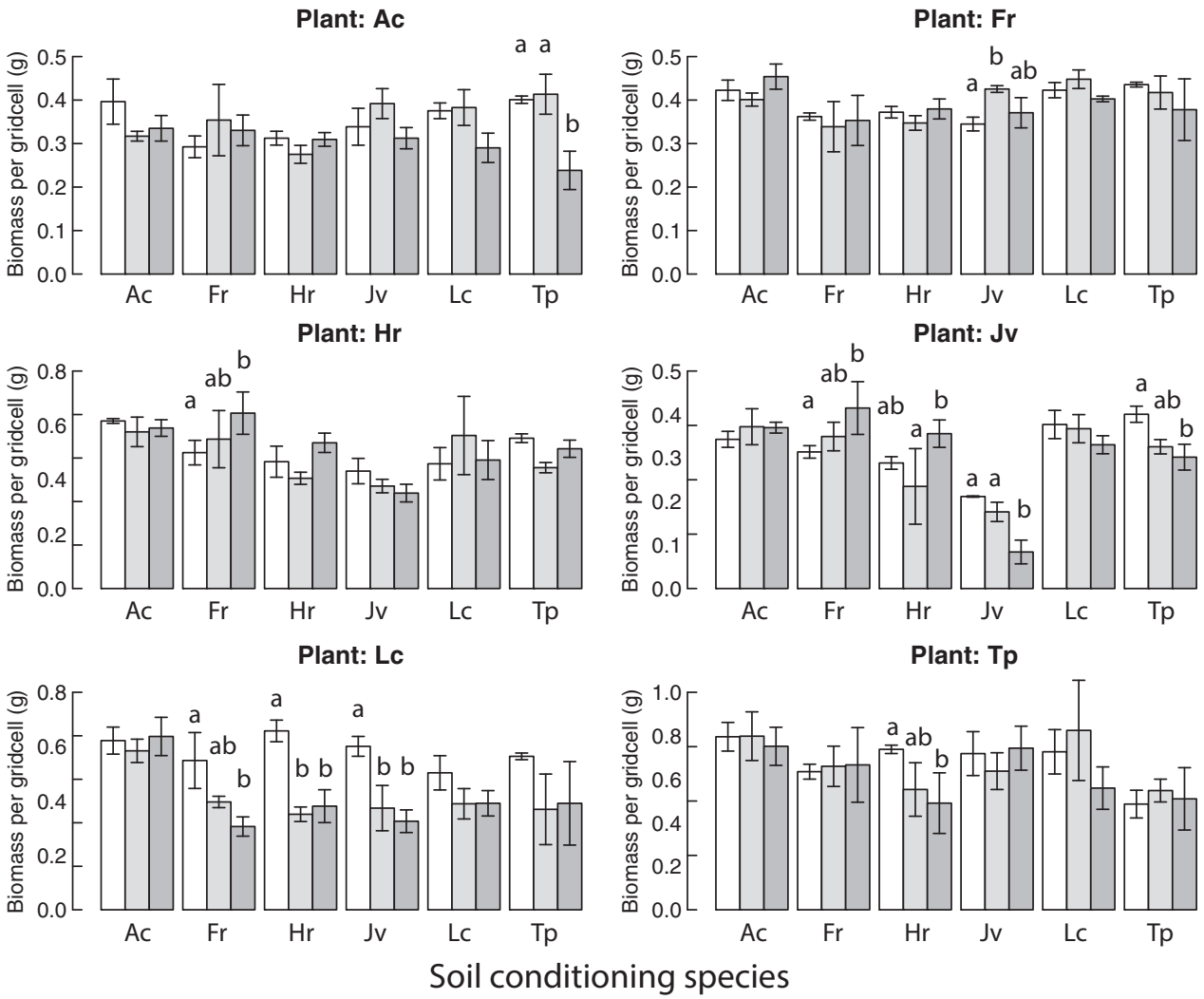

Fig. 3. Above-ground biomass per gridcell (mean±SE) for each test plant species on each of the six conditioned soils in three levels of spatial plant–soil feedback heterogeneity (homogeneous: white bars, coarse grained: light grey bars and fine grained: dark grey bars). Different letters above bars indicate significant differences among treatments within each test species and conditioned soil – based on gridcell level mixed model analyses (Table 2). Abbreviations as in Fig. 2.

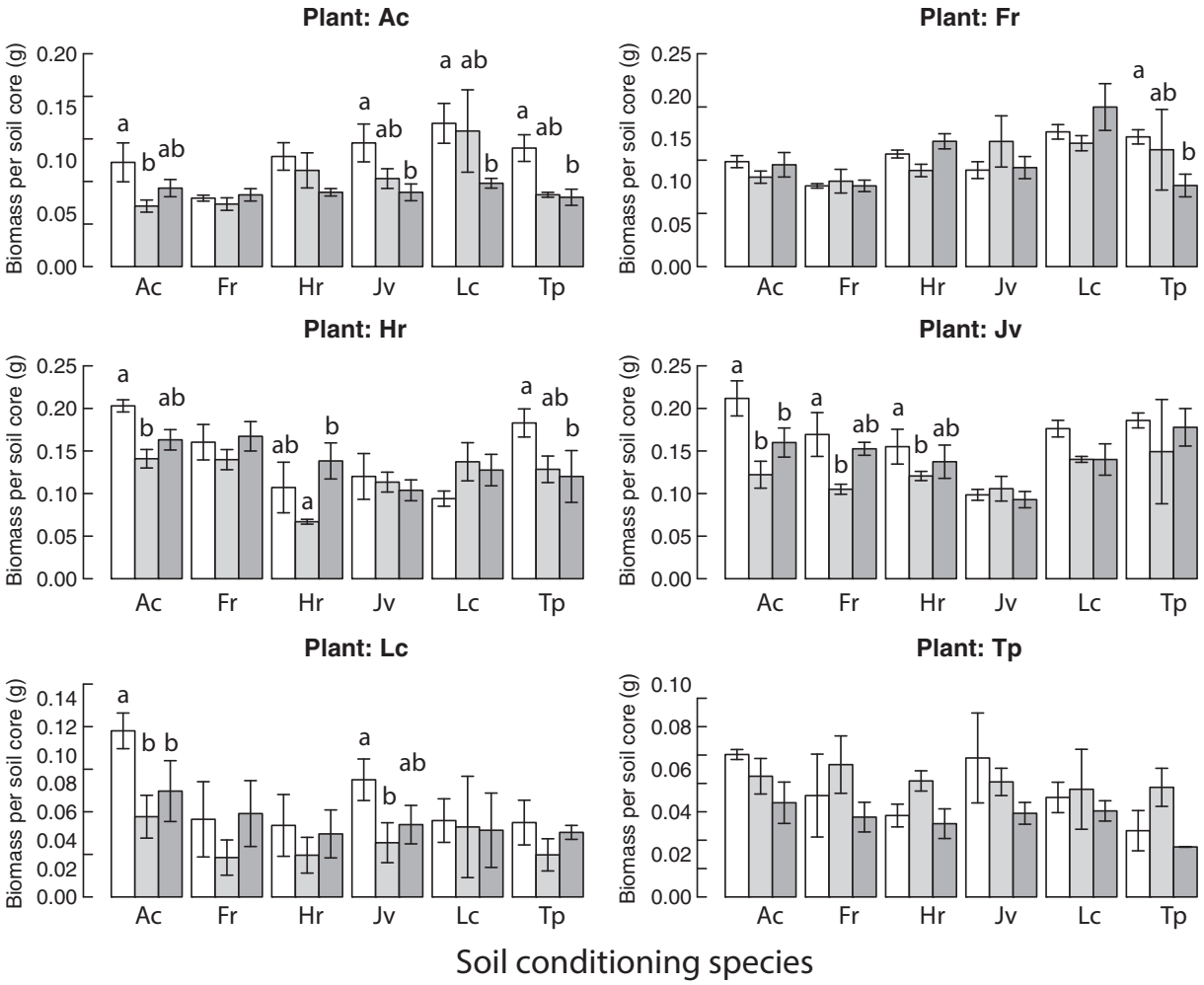

Fig. 4. Below-ground biomass (mean±SE) per gridcell for each test plant species on each of the six conditioned soils in three levels of spatial plant–soil feedback heterogeneity (homogeneous: white bars, coarse grained: light grey bars and fine grained: dark grey bars). Different letters above bars indicate significant differences among treatments within each test species and conditioned soil – based on gridcell level mixed model analyses (Table 2). Abbreviations as in Fig. 2.

($F_{5,72}$ = 17.16, $P < 0.0001$ and $F_{5,72}$ = 5.77, P = 0.0002 resp.; Table S6), but the response to each soil differed among the test species (above-ground biomass: $F_{25,72}$ = 2.05, P = 0.0096; below-ground biomass: $F_{25,72}$ = 3.21, P = 0.0001; Fig. 5). The majority of plant species grew worse on their own soil than on other soils (Fig. 5; Table S6). Only above-ground biomass of *A. capillaris* was slightly higher (but this was not significant) on its own soil and this was not the case for below-ground biomass. In addition, there were also clear indirect feedbacks. For instance, biomass of *F. rubra* was equally low in *J. vulgaris*-conditioned soil as in its own soil (Fig. 5). Similarly, *A. capillaris* biomass was relatively high on its own soil, but this species produced 26% and 34% less biomass above- and below-ground, respectively, in soil conditioned by *F. rubra*.

In contrast to the amount of biomass produced, the strength of the direct plant–soil feedback (own–foreign comparisons) did not change significantly among the spatial heterogeneity treatments (Table S7). As was the case in spatially homogeneous pots, most direct PSF values were negative (Fig. S3), and only in the case of *J. vulgaris* shoot biomass, did fine-grained heterogeneity lead to more strongly negative PSF values than observed under homogeneous conditions. Consequently, while plants produced on average less biomass in soils with spatially heterogeneous PSF, the generally negative effect of growing on self-conditioned soil was not aggravated by spatial PSF heterogeneity.

EFFECT OF SOIL CONDITIONING ON SOIL ABIOTIC COMPOSITION

Soil conditioning caused differences in soil nitrate content and acidity (Table S1), but the relationship of the biomass of the test plant species at the end of phase two with the abiotic conditions was not consistent across species (Table S8). There was a generally positive influence of nitrogen and soil acidity on the biomass of non-legumes, while for the two legume species, these relationships were reversed.

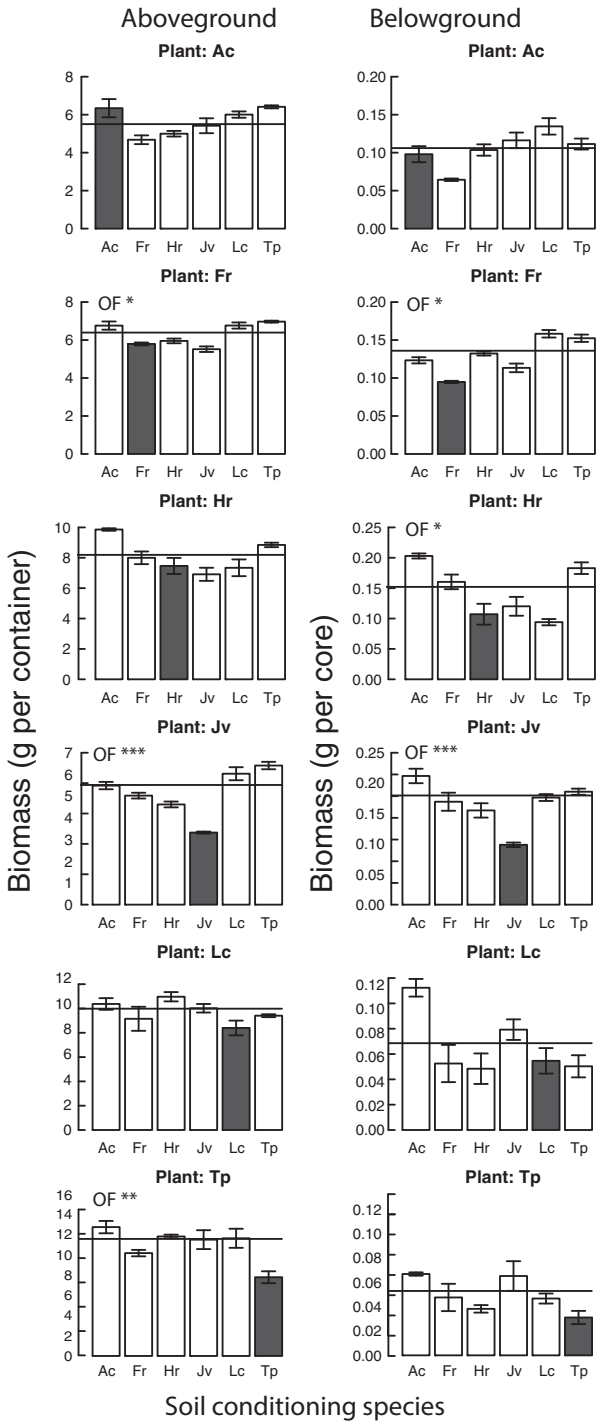

Fig. 5. Effect of induced plant–soil feedback on plant performance (±SE) in monocultures under spatially homogeneous conditions (i.e. one soil per container). Above- (left) and below-ground (right) biomass of each of the six test species (panels) on each of the six conditioned soils (bars) are shown. Dark grey bars indicate a test species growing on the soil conditioned by conspecifics ('own' soil). The horizontal lines give the mean biomass of each species on its respective 'foreign' soils as a visual aid. Significance of the planned contrasts 'own' vs. 'foreign' (OF) soil performance is indicated in the panels (for full analysis see Table S6). Abbreviations as in Fig. 2.

Discussion

Our results show that spatially heterogeneous plant–soil feedback effects result in lower plant biomass than predicted from spatially homogeneous treatments. In line with other studies, most plant species grew worse on soil conditioned by conspecifics ('own' soil) than on heterospecific conditioned soil ('foreign' soil; Petermann *et al.* 2008; Kulmatiski *et al.* 2008). However, when the biomass production on all four relevant homogeneous soil treatments (own and foreign soils) was averaged and compared to the biomass produced in heterogeneous PSF soils, we found that the observed biomass was in general lower than expected. The effect of PSF heterogeneity on plant performance did, however, vary depending on the test plant species as well as on the species that conditioned the soil. The reduction in plant biomass due to spatial PSF heterogeneity did occur both on soil conditioned by conspecifics ('own') and heterospecifics ('foreign') and was insensitive to the grain of the heterogeneity (fine vs. coarse). If our study, with six different monocultures and six species-specific conditioned soils, can be extrapolated, the results suggest that the spatial PSF heterogeneity expected in the field (Ettema & Wardle 2002; Bezemer *et al.* 2010) may influence plant performance more strongly than was expected previously. However, as PSF can alter competitive ability (Casper & Castelli 2007), multispecies experiments that allow interspecific competition are needed to test this prediction.

In our experiment, the spatially heterogeneous treatments consisted of four differently conditioned soils in the same container, while each of these soils in the homogeneous treatment was placed in different containers. Plants cultivate different soil communities (Bever 1994; Grayston *et al.* 1998; Bezemer *et al.* 2010) by allowing different soil taxa to increase in abundance, which can affect plant performance when their populations exceed a certain threshold (Van der Putten, Van Dijk & Troelstra 1988; Hendriks *et al.* 2015). Consequently, the spatially heterogeneous treatments likely had a higher diversity of taxa exceeding this threshold than the containers in the homogeneous treatment. In our experiment, plant roots grew intermingled throughout whole containers (E.R.J. Wubs, pers. obs.). Hence, all plant individuals in the monocultures may have faced a more diverse pool of antagonists in spatially heterogeneous than in homogeneous soils. As a result, this may increase the chances of a plant to encounter the specific pests and pathogens it is susceptible to. When triggered by the presence of their host, the densities of antagonists may rapidly increase and hence this may explain why we observed that plant performance was reduced more in the heterogeneous treatments than what was predicted from the performance in spatially homogeneous soils. Importantly, the density of specific antagonists that are present in a conditioned soil will be lower in heterogeneous soils (e.g. lower volume of 'own' soil). Hence, this argument is only valid if the severity of the antagonists' effects is not strongly related to their initial density in the soil. Nevertheless, the higher

level of diversity in the soil in the heterogeneous treatments also increases the scope for interactions among soil organisms, which may have increased the chances of co-infections. Co-infections of multiple pathogens can greatly aggravate diseases (De Rooij-Van der Goes 1995; Castillo *et al.* 2003; Tatineni *et al.* 2010) and have recently been suggested to play an important role in mediating Janzen–Connell effects (Benítez *et al.* 2013). As we grew plants in monocultures, transmission rates of pathogens from plant to plant are likely to have been high (Mitchell, Tilman & Groth 2002; Schnitzer *et al.* 2011). The higher diversity of antagonists in heterogeneous soils combined with transmission of potentially co-infecting antagonists from plant to plant may have resulted in the observed additional negative effect of PSF on plant performance in the heterogeneous treatments. Our results are in contrast to those of Hendriks *et al.* (2015) who found that single plants had higher total biomass in pots with spatial PSF heterogeneity. We speculate that this may be due to the fact that the homogeneous control treatment in the study of Hendriks *et al.* (2015) consisted of an artificially homogenized mixture of the four soils used in the heterogeneous treatment. Due to this mixing, all soil biota were more or less homogeneously distributed around the plant, thus providing no enemy-free spaces in which the plant roots could avoid contact with their antagonists. In our study, none of the conditioned soils were mixed. Instead, we tested plant performance on each of the conditioned soils separately, thus also allowing for enemy-free spaces in the spatially homogeneous treatment.

To test whether the observed negative heterogeneity effects could be explained by the soil biota from the most growth-repressing soil (typically 'own' soil) colonizing the whole container, we also calculated the expected performance of plants on heterogeneous soils based not on the mean but on the performance in the homogeneous soil treatment with the strongest negative PSF. We expected that if the antagonists of the most growth-repressing soil take over the (heterogeneous) soil in the entire container, they would drive the PSF effect at container level and consequently that this would predict plant performance in the heterogeneous containers. However, we found that on average plants performed better than predicted by the most growth-repressing soil (Fig. S4). Thus, plant biomass of monocultures on spatially heterogeneous soils was less than the mean performance on those soils in homogeneous condition, but not as low as their biomass on the soil least conducive to its growth. Apparently, PSFs in heterogeneous soils are not a simple function of the PSF measured in their constituent soils and include important non-additive interactions (Brandt *et al.* 2013; Hendriks *et al.* 2013). Our study suggests that monoculture growth will be poorer on soils uniformly conditioned by conspecifics ('own') than in heterogeneous soils where the direct PSF effect is somewhat diluted or delayed (Hendriks *et al.* 2013, 2015). Importantly, however, plant establishment success will vary across different uniformly conditioned soils as the observed indirect feedbacks in our study underline. Moreover, in the field, plant performance will also be importantly affected by competition with other species, which in turn are influenced by PSF (Casper & Castelli 2007).

The observed negative effects of spatial PSF heterogeneity could, alternatively, also have been caused by altered rooting patterns. Spatial heterogeneity in the soil can cause plants to adjust their rooting patterns, both in response to abiotic (Hutchings, John & Wijesinghe 2003; De Kroon & Mommer 2006) and biotic changes (Hendriks *et al.* 2015). Altered rooting patterns could have different costs of resource investment (Grime & Mackey 2002), which could have altered the biomass production over the longer term. It is important to point out that in our study, root biomass was sampled using root cores and that not entire grids were measured. It is therefore possible that the changes in biomass observed in fact reflect only repositioning of the roots within the containers in response to patch quality (Hutchings, John & Wijesinghe 2003; De Kroon & Mommer 2006; Hendriks *et al.* 2015). However, given that many root cores were sampled per container and that we did not observe that a particular conditioned soil consistently attracted more roots than others, we believe this may be unlikely. In addition, regression models predicting root biomass from shoot biomass show that including a separate slope for each of the levels of spatial heterogeneity does not significantly improve the model for any of the six test species (Table S3, Fig. S1). This suggests that the relationship between root and shoot biomass was consistent across the spatial PSF heterogeneity treatments. Even though this is indirect evidence, these data suggest that the observed heterogeneity effects below-ground reflect differences in biomass production rather than differences in rooting patterns. More studies are needed to untangle the relative impact of different mechanisms and understand the consequences of spatially heterogeneous PSFs for plant community composition (Bever *et al.* 2010; Van der Putten *et al.* 2013). For instance, in contrast to the early- and mid-succession species used here, it would be interesting to test whether later-successional plant species may benefit from spatial heterogeneity, because it can be expected that the diversity of mutualists (e.g. fungal endophytes) is also higher in heterogeneous soils and later-successional species tend to have positive direct PSF (Kardol, Bezemer & Van der Putten 2006).

Contrary to our expectation, we did not find consistent differences in performance due to fine-grained or coarse-grained heterogeneity (Mack & Bever 2014). This supports the idea that the negative impact of heterogeneity in our study was caused by the higher soil biodiversity in heterogeneous settings: in both coarse- and fine-grained heterogeneity treatments, the introduced soil biodiversity per container was the same. This does suggest that at the scale of our experiment (0.0572 m^2 containers), the exact grain of the heterogeneity is unimportant, provided that the heterogeneity itself is present within a plant's zone of influence (Casper, Schenk & Jackson 2003). As with heterogeneity in abiotic conditions (Hutchings, John & Wijesinghe 2003), plant root systems can easily grow across these smaller patches and integrate over the differences in the biotic soil conditions, even though this does strongly

affect the overall performance of the plant as antagonistic populations are likely to increase in time (Hendriks *et al.* 2015). Future experiments should study whether plant roots respond differentially to abiotic and biotic soil heterogeneity, for instance in response to (volatile) signalling molecules released by the microbial community. This could provide insight into whether potential gains of soil exploration are actively weighed against the risks of encountering soil-borne antagonists. In addition, the spatial dynamics of PSFs – that is, how far and how fast does PSF spread – is an open question. This will particularly depend on the mobility of the soil biota involved in the feedback (Bever, Platt & Morton 2012). A better understanding of the spatial PSF dynamics would improve our understanding of spatial patterns in natural plant communities (Bever, Westover & Antonovics 1997; Molofsky *et al.* 2002; Van der Putten 2003; Mack & Bever 2014), with potential application in intercropping designs.

In line with other studies on plant–soil feedback, we observed that in homogeneous soils for most of the tested plant species, direct feedbacks ('own' vs. 'foreign' soil) were strongly negative (Bever 2003; Kulmatiski *et al.* 2008; Petermann *et al.* 2008). However, we also show that indirect feedbacks – the effect of a soil conditioned by one species on another species – can also have strong and variable effects. Both direct and indirect feedback can importantly change the performance of interacting plants and consequently affect community composition (Bever, Westover & Antonovics; Bever 2003), for instance during secondary succession (Van de Voorde, Van der Putten & Bezemer 2011). Interestingly, the strength of direct PSF itself was not affected by spatial heterogeneity in most cases. Only for shoot biomass of *J. vulgaris,* did the strength of direct PSF increase as the grain of heterogeneity became finer. Consequently, while spatial PSF heterogeneity did reduce plant biomass in general, it did not affect plant biomass more strongly in soil patches conditioned by conspecifics ('own') than in the spatially homogeneous treatment. The stronger direct PSF of *J. vulgaris* in the fine-grained heterogeneous treatment is likely a consequence of increased plant mortality which was observed in that treatment. In general, however, plant mortality was not affected by spatial heterogeneity. We believe that plant mortality is an integral, albeit extreme, consequence of PSF. Since we replaced all seedlings that had died due to plant handling during transplantation, we assume that the changes in mortality rates reported here are indeed a consequence of PSF. In any case, when plant biomass was analysed using only gridcells where no seedling had died, the outcomes remained qualitatively unchanged.

Plant–soil feedbacks can be mediated both by changes in soil community composition and changes in abiotic conditions (Ehrenfeld, Ravit & Elgersma 2005). Soil conditioning in our experiment did lead to differences in abiotic conditions, particularly soil nitrogen content and acidity, and abiotic conditions were correlated to plant performance in the test phase to some extent. However, the relationships were not consistent across the test species. For instance, nitrogen content did promote plant growth for most species at least to some extent, especially so for *F. rubra*, but it reduced the performance of the legumes. In combination with the observation that most of the test species (five out of six) had a strong negative direct PSF, we interpret the lack of consistent relationships of plant performance with the measured abiotic conditions across species as evidence for the differential impact of altered soil communities during soil conditioning on plant performance (Kardol, Bezemer & Van der Putten 2006; Hendriks *et al.* 2013). It is important to note that although differences in abiotic conditions among the soils may have partly been responsible for the plant–soil feedbacks themselves, the effect of spatial PSF heterogeneity on plant performance described here is independent of these abiotic differences. This is because the comparison between plant performance in homogeneous and heterogeneous soils was always made while the plants were rooted in patches with the same soil, and these same soils were placed in containers with varying levels of spatial heterogeneity.

We conclude that spatial PSF heterogeneity affects plant performance in monocultures and that plants on average grow less well relative to homogeneous soils. However, spatial heterogeneity did not aggravate the generally negative direct PSF found in most test species. Instead, plant biomass was on average reduced in both 'own' and 'foreign' soil patches. In addition, the spatial grain of the PSF heterogeneity did not further affect plant performance. We hypothesize that the negative effect of spatial heterogeneity on plant performance results from the greater soil diversity encountered in spatially heterogeneous soils, making it more likely that plants will encounter their antagonists and increase the risk of co-infections with several pathogens. Our data suggest that spatial PSF heterogeneity in the field may negatively impact plant performance over and above the effects suggested by classical PSF studies. There are, however, important differences in direct and indirect PSFs in homogeneous treatments and thus soil patch quality as well. Finally, the spatial dynamics and dimensions of PSF effects need to be addressed in empirical and modelling studies to understand its consequences for plant communities in the field.

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Data accessibility

Data deposited in the Dryad Digital Repository: https://datadryad.org/resource/doi:10.5061/dryad.8g940 (Wubs & Bezemer 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Soil chemistry and shoot biomass at the end of the conditioning phase

Table S2. Results of gridcell level LMMs on plant biomass using a 16-level random effect for positional effects within containers

Table S3. Results of F-test for coincidental regressions between root and shoot biomass across levels of spatial heterogeneity

Table S4. Results of GLMMs on plant mortality

Table S5. Results of gridcell level LMMs on plant biomass using only gridcells where both seedlings survived until harvest

Table S6. ANOVA table for effects of soil conditioning on plant biomass in the spatially homogeneous treatment, including own–foreign contrasts

Table S7. ANOVA table for PSF strength in different spatial heterogeneity treatments

Table S8. Spearman correlations between plant biomass at harvest in the test phase and soil chemistry and shoot biomass at the end of the conditioning phase

Figure S1. Relationship between root and shoot biomass for each of the six test species.

Figure S2. Plant mortality in the different experimental treatments.

Figure S3. PSF strength (own–foreign) in response to spatial heterogeneity treatments.

Figure S4. Effect of spatial heterogeneity as predicted by poorest plant performance on homogeneous soils.

