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

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Abstract

1. The effects of plants on soil vary greatly between plant species and in mixed plant communities this can lead to spatial variation in plant-soil feedback (PSF) effects. Such spatial effects are thought to influence plant species coexistence, but the empirical evidence for this hypothesis is limited.
2. Here, we investigate how spatial heterogeneity in PSFs influences plant growth and competition. The experiment was carried out with high and low nutrient soils to examine how these effects depend on soil fertility. We collected soil from field plots planted for three years with monocultures of *Anthoxanthum odoratum* and *Centaurea jacea* and tested the performance of the two species in a greenhouse experiment in heterogeneous soils consisting of patches of "own" and "foreign" soils and in soils where the "own" and "foreign" soils were mixed homogeneously. In the test phase, plants were grown in monocultures and in 1:1 mixtures in live or sterilized soils.
3. Overall, *A. odoratum* in monocultures produced less aboveground biomass in heterogeneous soils than in homogeneous soils. *Centaurea jacea* produced less belowground biomass in live heterogeneous soils than in live homogeneous soils, but there was no difference between sterile heterogeneous and homogeneous soils. The belowground biomass per patch varied more in pots with live heterogeneous soils than in pots with live homogeneous soils for both plant species, but there was no difference between pots with sterile heterogeneous and homogeneous soils. In pots with plant mixtures, the difference in aboveground biomass between the two competing species tended to be smaller in heterogeneous than in homogeneous soils. In pots with heterogeneous soils, both plant species grown in mixtures produced more aboveground biomass in "foreign" soil patches than in "own" soil patches. The responses of plants to heterogeneous PSFs were not different between low and high nutrient soils.

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

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

Introduction

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

In spatially heterogeneous soils, a plant can preferentially forage for nutrients in "foreign" soil patches thereby avoiding contact with its antagonists in "own" soil patches (Hendriks et al., 2015b). How plant-soil feedback heterogeneity will influence plant growth in the presence of neighbouring plants is less clear as competing plants may also change their foraging behaviour in heterogeneous soils (e.g. Cahill et al., 2010; Xue, Huang, Dong, Zhang, & Yu, 2013). In monospecific communities, spatial plant-soil feedback heterogeneity may not be beneficial because competing individuals will employ the same strategy (Bennett et al., 2017;

Bliss, Jones, Mitchell, & Mou, 2002; Teste et al., 2017). A recent study even reported that plants in monocultures performed worse in spatially heterogeneous soils than predicted from their performance in homogeneously conditioned soils (Wubs & Bezemer, 2016).

When different plant species grow together in spatially homogeneous soils, interspecific competition generally enhances the plant-soil feedback effects (e.g. Crawford & Knight, 2016; Jing, Bezemer, & van der Putten, 2015; Kardol, Cornips, van Kempen, Bakx-Schotman, & van der Putten, 2007; Petermann, Fergus, Turnbull, & Schmid, 2008; van der Putten & Peters, 1997). Similar to what is observed when soil resources are distributed heterogeneously, in soils with spatially heterogeneous plant-soil feedbacks, plants growing in "own" soil patches will experience a competitive disadvantage and inferior competitors may benefit in these patches (Burns, Brandt, Murphy, Kaczowka, & Burke, 2017; Day, John, & Hutchings, 2003; Hendriks et al., 2015a; Hutchings, John, & Wijesinghe, 2003). Hence, competing species may all preferentially forage in "foreign" patches and this may reduce competitive imbalances between species.

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

In the present study, we examine how plant-soil feedback heterogeneity influences the performance and competitive interactions between two grassland plant species, and how these effects depend on soil fertility. We grew the grass *Anthoxanthum odoratum* and the forb *Centaurea jacea* in field plots in monocultures in either high nutrient or low nutrient soil. After three years, we collected soil from these monocultures and tested the performance of *A. odoratum* and *C. jacea* in monocultures and in 1:1 mixtures in a greenhouse experiment in homogeneous mixtures of "own" and "foreign" soil, and in spatially heterogeneous soils with distinct patches of "own" and "foreign" soil. The experiment was carried out with live and sterilized soil to test the impact of soil biota on the response of the two plant species to spatial plant-soil feedback heterogeneity. We tested four hypotheses: (1) in monocultures (intraspecific competition) plants will produce similar amounts of biomass in pots with two conditioned soils placed in discrete patches (heterogeneous soil) as in evenly mixed soil (homogenous soil) as, at the pot level, on average the biotic and abiotic composition of both soils are identical. However, there will be more variation in biomass among the soil patches within heterogeneous soils than within homogeneous soils. (2) In plant mixtures (interspecific competition), at the pot level, the difference in growth between the two competing species will be smaller in heterogeneous soils than in homogeneous soils, as each of the competing species will produce more biomass in "foreign" soil patches than in "own" soil patches within the heterogeneous soils. (3) Effects of plant-soil feedback heterogeneity in the test phase will be stronger when the soil was initially nutrient-poor than when the soil was initially nutrient-rich during conditioning, as plant-soil feedback effects generally diminish with increasing soil fertility. (4) Plant-soil feedback heterogeneity effects will disappear in sterile soils.

Materials and methods

Plant species

We used a grass species, *Anthoxanthum odoratum* L. (Poaceae), and an herb, *Centaurea jacea* L. (Asteraceae). Both species can reproduce by seeds and vegetative growth (Hartemink, Jongejans, & de Kroon, 2004). *Anthoxanthum odoratum* produces closely connected ramets while *C. jacea* forms extensive branches underground (Jongejans & de Kroon, 2005). Both species are native in western Europe and commonly coexist in meadows (van Ruijven & Berendse, 2003). Both plant species experience negative conspecific plant-soil feedbacks (supporting information: Fig. S1B, D: less root biomass in "own" than "foreign" live soils for *A. odoratum*, and less root and shoot biomass for *C. jacea*).

Soil conditioning in monoculture field plots

In an outdoor experimental garden (from April 2013 to September 2015), we planted monocultures (144 seedlings/plot) of *A. odoratum* and *C. jacea* in plots filled with either high nutrient soil ($N\text{-}NH_4$: 3.31 mg/kg; $P\text{-}PO_4$: 1.88 mg/kg; $N\text{-}NO_3$: 41.10 mg/kg) or low nutrient soil ($N\text{-}NH_4$: 2.44 mg/kg; $P\text{-}PO_4$: 0.36 mg/kg; $N\text{-}NO_3$: 0.09 mg/kg). There were 20 plots (2 levels of nutrient availability × 2 plant species × 5 replicate plots) of 1 m^2 each distributed over five replicated blocks in a randomized block design. Weeds were regularly removed during the experiment. In September 2015, all plants in the central 60 × 60 cm^2 of each plot were clipped at a height of 1 cm. Aboveground biomass in each plot was determined after being oven-dried to constant weight. Productivity of both plant species in high nutrient and low nutrient soils is shown in the supporting information (Fig. S2). In February 2016, we collected all topsoil (20 cm deep) from the central area of 60 × 60 cm^2 in each experimental plot and kept soil from different plots in different sealed bags. Then, soil collected from each plot was sieved (1.5 cm mesh) and further separated into two parts both kept in separate

151 sealed bags. One of the two bags from each plot was sterilized by γ-irradiation (minimum
152 25KGray, Isotron, Ede, the Netherlands). Hence, there were 40 different conditioned soils (2
153 nutrient levels × 2 plant species × 5 replicate plots × 2 sterilization treatments). In the
154 greenhouse experiment, for each of the two nutrient levels and for sterile and non-sterile soil,
155 we created two levels of PSF heterogeneity (spatially homogeneous PSF and spatially
156 heterogeneous PSF) using soils conditioned by *A. odoratum* and *C. jacea* from the same field
157 block (Fig. 1). A total of 120 pots (2 nutrient levels × 2 sterilization treatments × 2 PSF
158 heterogeneity treatments (described below) × 3 planting treatments (described below) × 5
159 replicates) of 4.6 L each were used in the greenhouse experiment.

160

161 *Greenhouse experiment*

162 In the greenhouse experiment, two levels of PSF heterogeneity (spatially homogeneous PSF
163 and spatially heterogeneous PSF) were created using soil conditioned by *A. odoratum* and *C.*
164 *jacea* from the same field block (Fig. 1). In the heterogeneous soil treatments, each pot was
165 equally divided into 4 patches using a metal grid and each patch was alternately filled with 1.4
166 kg soil conditioned by monocultures of *A. odoratum* or *C. jacea*. In the homogeneous soil
167 treatments, each pot was filled with 5.6 kg of a 1:1 (w:w) homogenized mixture of soil
168 conditioned by monocultures of *A. odoratum* and *C. jacea* (Fig. 1). In this way, there were
169 pots that differed in spatial variation in plant-soil feedbacks while the abiotic and biotic soil
170 conditions in the homogenous and heterogeneous soils were kept constant. We allocated pots
171 filled with soils originated from the same field block in the same block in the greenhouse
172 experiment so that there were five blocks. Pots of different treatments were randomized
173 within each block. Holes were made in the bottom of each pot to allow vertical movement of
174 water. To prevent soil from passing through holes, a piece of filter paper (15 cm in diameter)

was placed at the bottom of each pot before filling the pot with soil. Each pot was placed on a tray to prevent possible contamination through leachate. The metal grid was removed after each pot was filled so that plants could grow freely across different patches. We randomly selected three field blocks, and collected subsamples from the soil of each plot in those blocks for soil chemical analysis. We measured soil organic matter content, nutrient content (NH_4, NO_3 and PO_4), water content and pH (Table S1). The amount of NH_4, NO_3 and PO_4 (mg/kg dry soil) were determined by adding 30.0 ml of 0.01 mol/L $CaCl_2$ solution to soil samples (3.0 g), shaking mechanically for at least 2 h at room temperature (20 ℃), filtering the solution and analyzing the nutrients in the soil extracts in a flow analyzer (SKALAR SAN plus system). Soil pH-H_2O was determined by adding 25.0 ml demi-water to soil samples (volume 5.0 ml), shaking for 5 min and measuring 2 h later. Soil organic matter was determined by measuring the difference between weights of the oven-dried (105 ℃) soil samples (5.0-10.0 g) before and after being heated in a furnace at 550 ℃. The weight of each sample was determined after cooling it down in the air to handwarm temperature and further cooling it for at least 45 min in a desiccator. Soil moisture content was determined by measuring the difference between the weights of each soil samples before and after oven-drying (105 ℃).

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

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

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

In this experiment, we analysed the effects of spatial plant-soil feedback heterogeneity by comparing spatially heterogeneous soils with homogeneously mixed soils that have the same origin. Hence, each pot consisted of the same initial nutritional and microbial composition. For completeness, in the experimental design we also included the two pure soil treatments (pure Ao soils and pure Cj soils; Fig. 1). In these two pure soil treatments, each pot was filled with 5.6 kg of soil conditioned by monocultures of *A. odoratum* (pure Ao soil treatment) or *C. jacea* (pure Cj soil treatment) growing in either high or low nutrient soil and originating from the same field block. The data of root and shoot biomass in these pure soils are presented in the supplementary information (Table S2; Fig. S1).

Harvest measurements

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

Data analysis

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

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

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

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

265 For mixed plant communities, at the pot level, we first combined the growth of the two
266 species in 1:1 mixtures in each pot by calculating the growth difference (*D*) to evaluate the
267 effects of spatial plant-soil feedback heterogeneity on the competition between the two
268 species. The *D*-value was calculated as the log-ratio of aboveground biomass of *A. odoratum*
269 and *C. jacea* in mixtures. The *D*-value will be equal to zero if the two species perform equally
270 well in mixtures; it will be positive if the biomass of *A. odoratum* is higher than *C. jacea*, and
271 negative if *C. jacea* biomass is higher. We used three-way ANOVA to test the effects of

nutrient availability, sterilization, soil heterogeneity and their interactions on *D*, block was included as a random factor. A one-sample *t*-test was used to test whether *D* for each combination of nutrient availability, sterilization and soil heterogeneity differed from zero. A significant soil heterogeneity effect or a significant interaction with nutrient and/or sterilization would suggest that the difference in the growth between the two competing species in the 1:1 mixture is different in heterogeneous and homogenous soils. We also analysed the plot-level aboveground biomass (total aboveground biomass of a species in one pot divided by the number of seedling in the pot) separately for each of the two species grown in the 1:1 mixture using a mixed-effect three-way ANOVA with nutrient availability, sterilization, soil heterogeneity and their interactions as fixed factors, and block as a random factor.

At the patch level, we tested whether the two species in the 1:1 mixtures produced more biomass in "foreign" soil patches than in "own" soil patches within the heterogeneous soils. We analysed the patch-level aboveground biomass (total aboveground biomass of a species in one patch divided by the number of seedlings in the patch) separately for each of the two species grown in the 1:1 mixture, using a mixed-effect three-way ANOVA. Nutrient availability, sterilization, soil heterogeneity and their interactions were included as fixed factors, and soil type nested in pot, and pot nested in block (block/pot/soil type) as a random factor.

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

Results

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

In monocultures, *A. odoratum* overall produced less aboveground biomass in heterogeneous soils than in homogeneous soils (Table S3A; Fig. 2A), but there was no significant difference in the aboveground biomass of *C. jacea* between the two soils (Table S3A; Fig. 2C). These results suggest that heterogeneity in PSFs did influence the aboveground biomass of *A. odoratum* but not of *C. jacea*. Both species produced much more aboveground biomass in sterile soil than in live soil (Table S3A; Fig. 2A, C), indicating that soil biota inhibited plant growth of both species.

PSF heterogeneity also influenced belowground biomass but the effect varied between the two species and soil sterilization. *A. odoratum* produced similar amounts of belowground biomass in heterogeneous and homogeneous soils (Table S3B; Fig. 2B). *C. jacea* produced less belowground biomass in live heterogeneous than in live homogeneous soils, but in sterilized soil there was no difference between these heterogeneity treatments (Table S3B: significant sterilization × heterogeneity effect; Fig. 2D). These results suggest that heterogeneity in PSFs influenced the belowground biomass of *C. jacea* but not of *A. odoratum*. Belowground biomass per soil core of both species was significantly greater in sterile soil than in live soil (Table S3B; Fig. 2B, D).

Soil heterogeneity and the interaction with nutrient and/or sterilization did not affect the CV of aboveground biomass of either *A. odoratum* or *C. jacea* (Table S4A; Fig. 3A, C). CVs of belowground biomass of both plant species were significantly greater in live heterogeneous soil than in live homogeneous soil. In sterilized soil there was no difference between the two heterogeneity treatments (Table S4B: significant and marginally significant sterilization × heterogeneity effect for *A. odoratum* and *C. jacea*, respectively; Fig. 3B, D). Hence, PSF

heterogeneity increased spatial variation in root growth in live soil but not when soil biota were excluded.

In monocultures, in pots with spatially heterogeneous soil, *A. odoratum* produced more aboveground biomass in live "foreign" soil patches than in live "own" soil patches when soil nutrient is low, but no difference was found between these two patches in high nutrient soil or in sterile soils (Table S5A: significant nutrient × sterilization × soil interaction effect; Fig. S3A). *C. jacea* produced more aboveground biomass in live "foreign" soil patches than in live "own" soil patches but there was no difference between the two soil patches in sterile soils (Table S5A: significant sterilization × soil interaction effect; Fig. S3C). The same pattern was found for the belowground biomass of *A. odoratum*, while *C. jacea* overall produced less belowground biomass in "foreign" soil patches than in "own" soil patches (Table S5B; Fig. S3B, D). These results suggest that plant monocultures showed different responses to spatially heterogeneous PSFs.

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

In mixtures, the growth difference between the two species tended to be smaller in heterogeneous soils than in homogeneous soils (Table S6: marginally significant heterogeneity effect; Fig. 4), indicating that the growth inequality between the two competing species was reduced in heterogeneous soils. The growth difference index (D) was generally negative in live soil but positive in sterile soil, i.e. *C. jacea* was superior to *A. odoratum* in live soil while the reverse was true in sterile soil (Table S6; Fig. 4). The aboveground biomass of both species grown in mixtures is presented in the supporting information (Table S3C; Fig. S4).

343 In mixtures, in pots with spatially heterogeneous soil, *A. odoratum* produced more
344 aboveground biomass in "foreign" soil patches than in "own" soil patches (Table S5C; Fig.
345 5A). A similar trend was observed for *C. jacea* but this was not significant (Table S5C; Fig.
346 5B). This result suggests that both plant species selectively grew in "foreign" soil patches in
347 spatially heterogeneous soils.

348

349 **Discussion**

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

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

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

In monocultures, plant growth varied more among the four patches within the heterogeneous soils than within the homogeneous soils, indicating that spatially heterogeneous PSFs promote growth divergence. This may be explained by the greater variety of microsites within the heterogeneous soils, i.e. there were two conditioned soils placed in discrete patches within the heterogeneous soils but the two conditioned soils were evenly mixed within the homogeneous soils. Hence, plants can avoid contact with their enemies by placing more shoots/roots in the "foreign" soil patches (Fig. S3; Hendriks et al. 2015b) in the heterogeneous soils, which increases the growth variations among these patches. Importantly, we only found such difference in live soil but not in sterile soil, indicating that soil biota were likely involved in the responses of plant monocultures to spatially heterogeneous PSFs. Further studies should

aim to disentangle the role of the microbial community in creating spatial heterogeneity effects on plant growth.

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

As expected, sterilizing the soil increased plant growth. Our results show that soil biota in our system have a negative effect on plant growth, i.e. there are more pathogenic or harmful microbes than beneficial ones present in conditioned soil. However, it is important to note sterilization of soils also increased the soil nutrient availability (Table S1), and this obviously promotes the growth of plant species. Unfortunately, we cannot distinguish to what extent the exclusion of soil biota and release of soil nutrients may have promoted the growth of the plant in sterilized soil, yet it must be a net effect of elimination of soil biota and an increase in soil nutrients (Brinkman, van der Putten, Bakker, & Verhoeven, 2010). Remarkably, sterilization of soils changed the competition hierarchy of the two competing species, i.e., *C. jacea* is superior to *A. odoratum* in live soil while the reverse is true in sterile soil. One possible explanation is that *C. jacea* has a greater association with mycorrhizal fungi than *A. odoratum* under poor soil conditions as indicated by previous studies (the mycorrhizal fungi dependency of *C. jacea* and *A. odoratum* is about 64% and 35%, respectively; Grime, Mackey, Hillier, & Read, 1987; Tawaraya, 2003; van der Heijden, Bardgett, & van Straalen, 2008). Another

possible explanation may be related to the competition for different resources. *Anthoxanthum odoratum* profits from the higher nutrient supply in the sterile soil treatments. In nutrient-rich environments, competition for light is important, thus species that can produce more leaves have a competitive advantage (Aerts 1999). *Anthoxanthum odoratum* is a species that can produce dense tillers rapidly (Humphrey and Pyke 1998; Lovett-Doust 1981) and they were taller than *C. jacea* plants in the greenhouse experiment (W. Xue, *pers. obs.*). This may explain why *A. odoratum* was the stronger competitor in sterile soil. In nutrient-poor environments (live soils in the present study), competition for nutrients prevails, and hence, species with larger rooting systems may have a competitive advantage (Aerts, 1999; Grime, 2006). *C. jacea* has a deeper root system than *A. odoratum*, thus most underground space was occupied by *C. jacea*, which may explain its competitive advantage in nutrient poor conditions.

We hypothesized that PSF heterogeneity effects in the test phase would be stronger when the soil was originally nutrient poor during conditioning, as PSF effects generally diminish with increasing soil fertility (De Deyn, Raaijmakers, & van der Putten, 2004; van der Putten & Peters, 1997). In contrast to our hypothesis, the effects of PSF heterogeneity did not differ between the two soil fertility levels as indicated by the absence of significant nutrient × heterogeneity effects. At the end of the conditioning period in the field, the amount of organic matter was higher in high nutrient than in low nutrient soils, but there were no differences in other soil chemical properties between the two soil nutrient treatments (Table S1). This may explain why we did not observe stronger conditioning effects on PSF heterogeneity effects in low nutrient soils. More studies are needed to examine the role of spatial plant-soil feedback heterogeneity on plant performance and competition along a gradient of soil nutrient availability.

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

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Author contributions

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

Data Accessibility

Data deposited in the Dryad Digital Repository: https://datadryad.org/doi: XXXX (Xue, Berendse & Bezemer XXXX).

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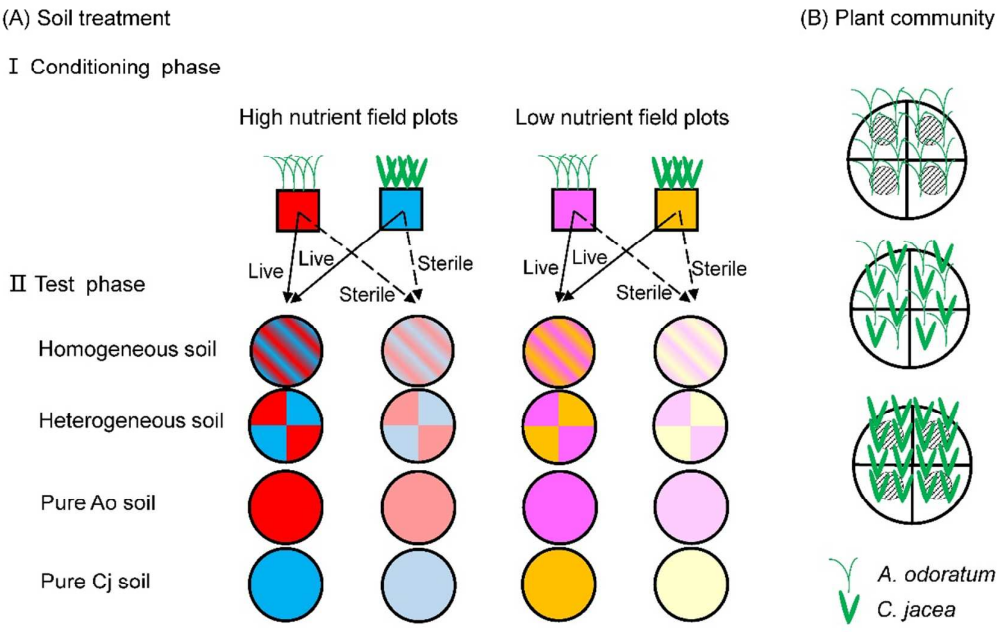
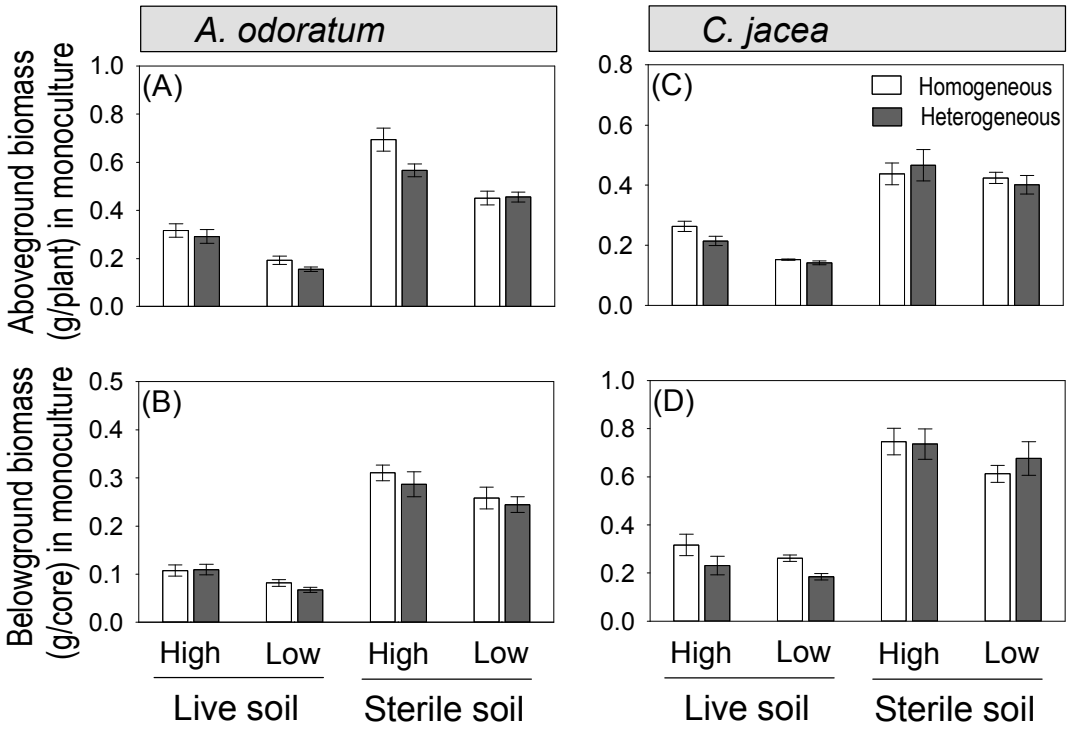
672

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



686

687 **Fig. 2.** Aboveground biomass per plant (A and C) and belowground biomass per soil core (B
688 and D) of *A. odoratum* (A and B) and *C. jacea* (C and D) in plant monocultures in
689 homogeneous and heterogeneous soils at the pot level. "High" and "Low" refer to high
690 nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil"
691 indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1
692 SE) are presented. See Table S3A-B for statistic results.



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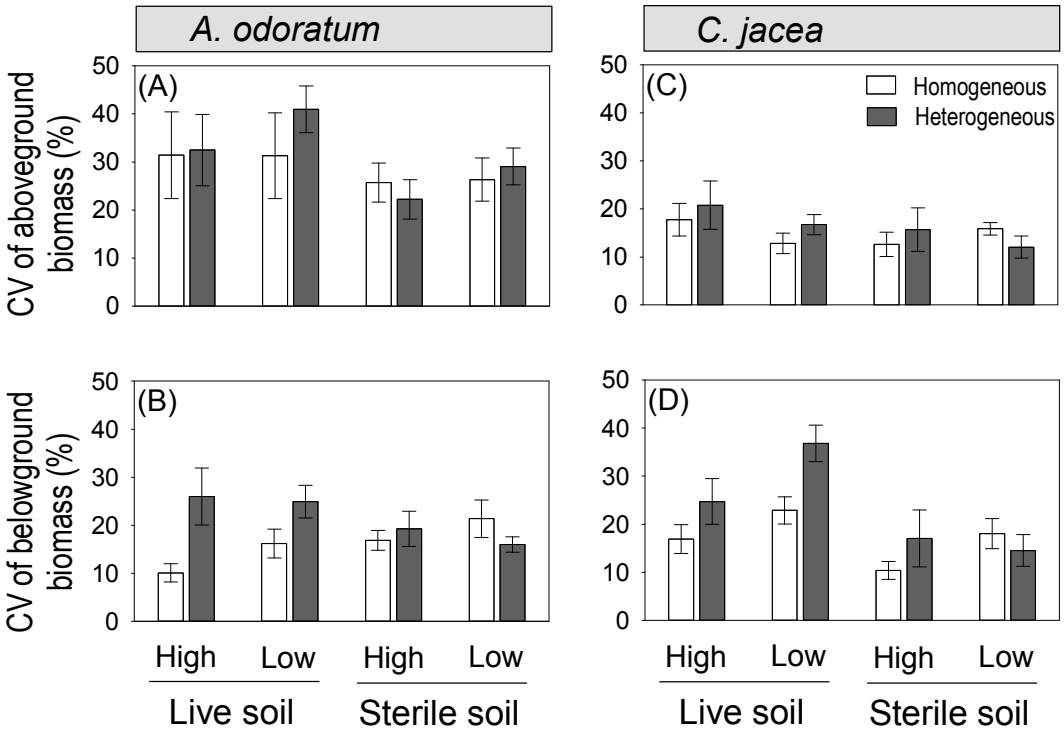
694

695 **Fig. 3** Coefficients of variation (CV) of aboveground biomass (A and C) and CV of
696 belowground biomass (B and D) of *A. odoratum* (A and B) and *C. jacea* (C and D) in plant
697 monocultures among the four patches within homogeneous and heterogeneous soils. "High"
698 and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase.
699 "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil,
700 respectively. Mean values (± 1 SE) are presented. See Table S4 for statistic results.



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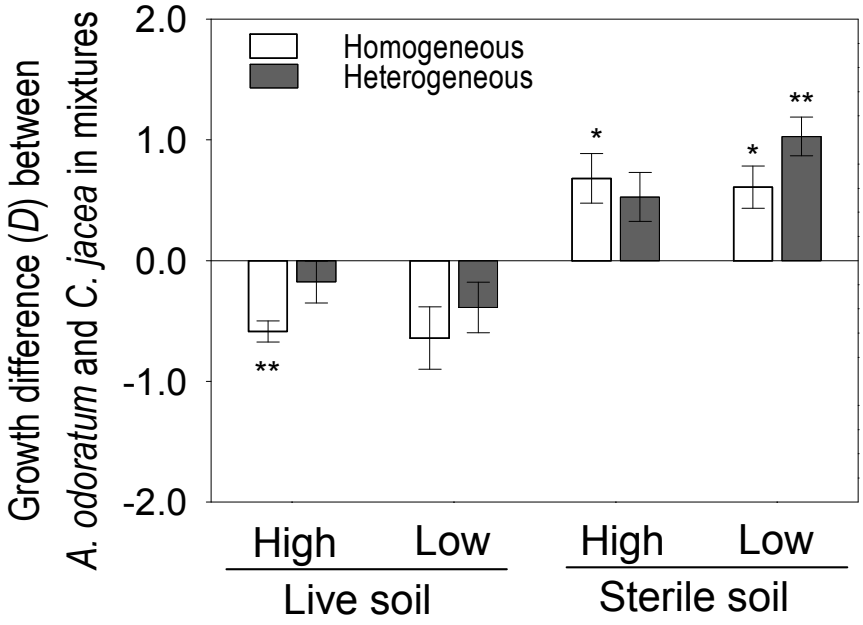
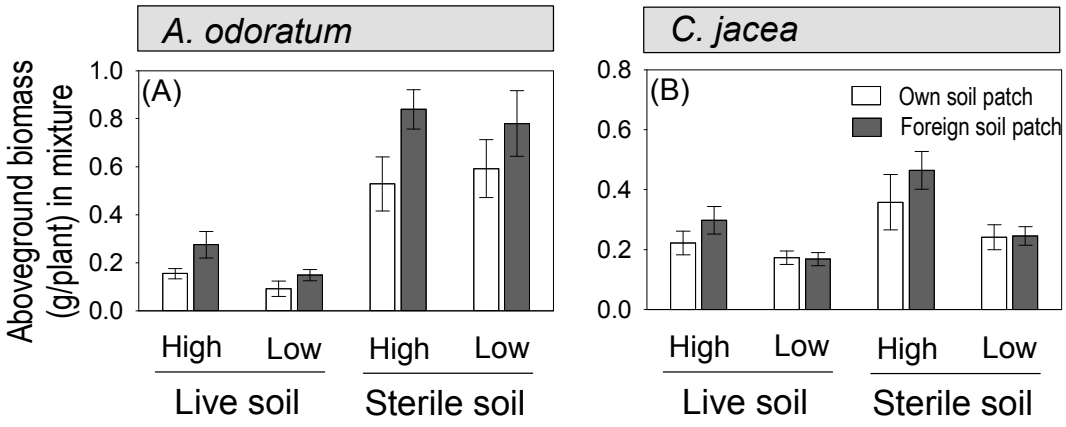
703 **Fig. 4** Growth difference (*D*, log-ratio of aboveground biomass of *A. odoratum* and *C. jacea*
704 in plant mixtures) in homogeneous and heterogeneous soils. Positive values indicate the
705 biomass of *A. odoratum* is higher than *C. jacea* and negative values indicate the reverse is true.
706 "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning
707 phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected
708 soil, respectively. Mean values (± 1 SE) are presented. See Table S6 for statistic results. Stars
709 at the end of bars indicate which means differed from zero (one-sample *t*-test). Symbols give
710 *P*: *** $P<0.001$, ** $P<0.01$ and * $P<0.05$.



711

712

713 **Fig. 5** Aboveground biomass per plant per patch of *A. odoratum* (A) and *C. jacea* (B) in plant
714 mixtures in "own" and "foreign" soil patches for pots with heterogeneous soils. "High" and
715 "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live
716 soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil,
717 respectively. Mean values (± 1 SE) are presented. See Table S5C for statistic results.



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Supporting information

Table S1. Soil chemical analysis of different soils after three-years of conditioning in the field. Means (±SE), *F*- and *P*-values of three-way ANOVA are given. Tukey post-hoc tests were made among these soils, mean values sharing the same superscript (a-c) are not significantly different among the twelve soils. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase; "pure Ao soil" and "pure Cj soil" represent soils conditioned by monocultures of *A. odoratum* and *C. jacea*, respectively; "homogeneous soil" represents the 1:1 (v:v) mixture of pure Ao soils and pure Cj soil. Data of $N\text{-}NO_3$ was ln-transformed before analysis and the value of $N\text{-}NO_3$ in the live, high nutrient pure Ao soil is based on only one soil sample. Symbols give: $^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$

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

Table S2. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile) and soil type (pure Ao soil vs. pure Cj soil) on plot-level aboveground biomass (A and C) and belowground biomass (B) of *A. odoratum* and *C. jacea* in monocultures (A and B) and in 1:1 mixtures (C) in the pure soils. Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

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

[1] Data were ln-transformed

Table S3. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile) and PSF heterogeneity (homogeneous soil vs. heterogeneous soil) on plot-level aboveground biomass (A and C) and belowground biomass (B) of *A. odoratum* and *C. jacea* in monocultures (A and B) and in 1:1 mixtures (C). Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

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

[1] Data were ln-transformed

Table S4. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile), PSF heterogeneity (homogeneous soil vs. heterogeneous soil) on CV (coefficients of variation) of aboveground biomass (A) and belowground biomass (B) of *A. odoratum* and *C. jacea* in monocultures. Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

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

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

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

[1] Data were ln-transformed

Table S6. Results of linear mixed-effects ANOVA testing the effects of nutrient availability (high vs. low), sterilization (live vs. sterile), PSF heterogeneity (homogeneous soil vs. heterogeneous soil) on growth difference (*D*) between *A. odoratum* and *C. jacea* in 1:1 mixtures. Degrees of freedom (DF, denDF), *F*- and *P*-values of are presented.

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

Fig. S1. Aboveground biomass per plant (upper and bottom panels) and belowground biomass per soil core (middle panels) of *A. odoratum* and *C. jacea* in monocultures and mixtures in pure Ao soil and pure Cj soils. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Plants were grown in monocultures and in 1:1 mixtures. Mean values (± 1 SE) are presented. See Table S2 for statistic results.

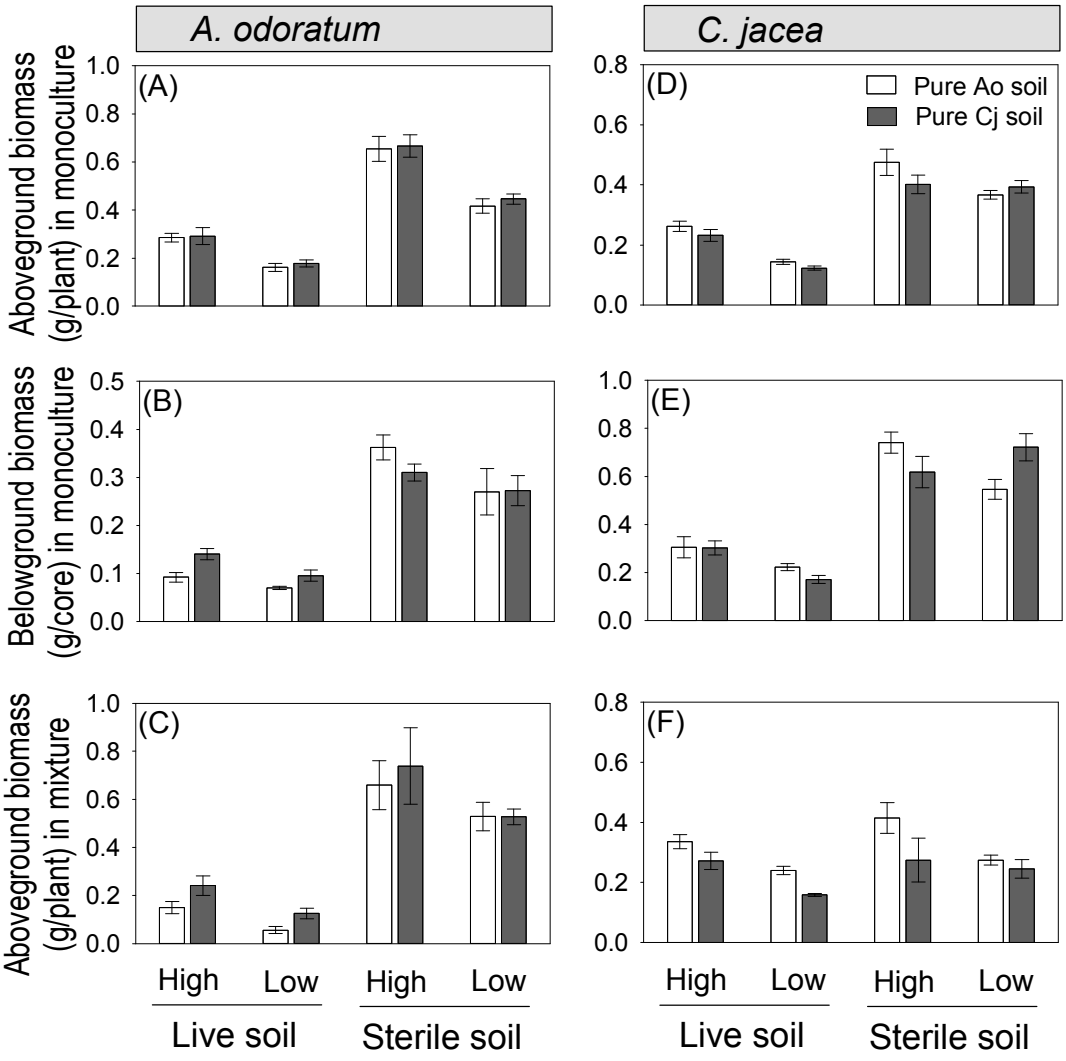

Fig. S2. Aboveground biomass in the field plots at the end of the conditioning period for *A. odoratum* and *C. jacea* monocultures in high nutrient and low nutrient soils. Mean dry weight (± 1 SE), *F*- and *P*-values of a two-way ANOVA with species (S), nutrient (N) and the interaction (S × N) are also presented: ** $P<0.01$. Bars sharing the same superscript (a-b) are not significantly different based on a Tukey post-hoc test.

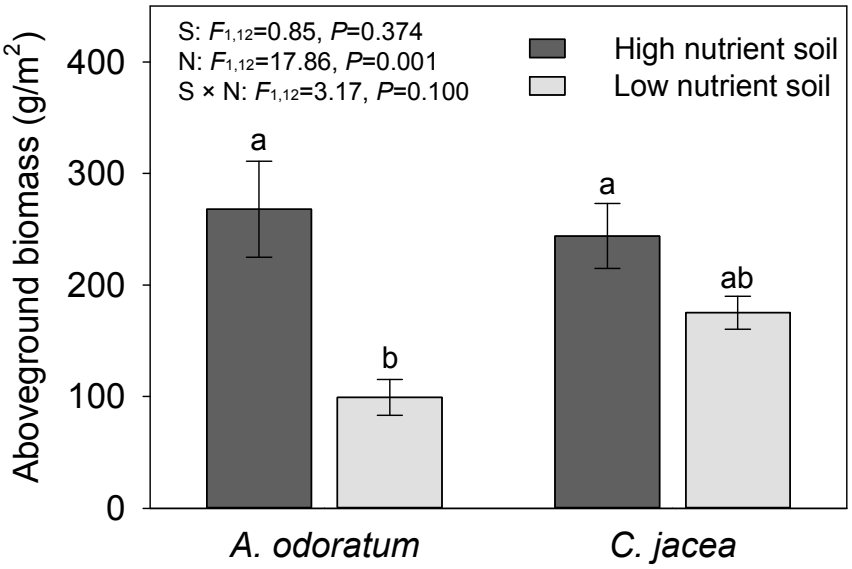

Fig. S3. Aboveground biomass per plant per patch (A and C) and belowground biomass per soil core per patch (B and D) of *A. odoratum* and *C. jacea* in the greenhouse experiment. Data are for monocultures with heterogeneous soils. "Own" and "foreign" soil patches refer to conspecific and heterospecific soil patches respectively. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1 SE) are presented. See Table S5A-B for statistic results.

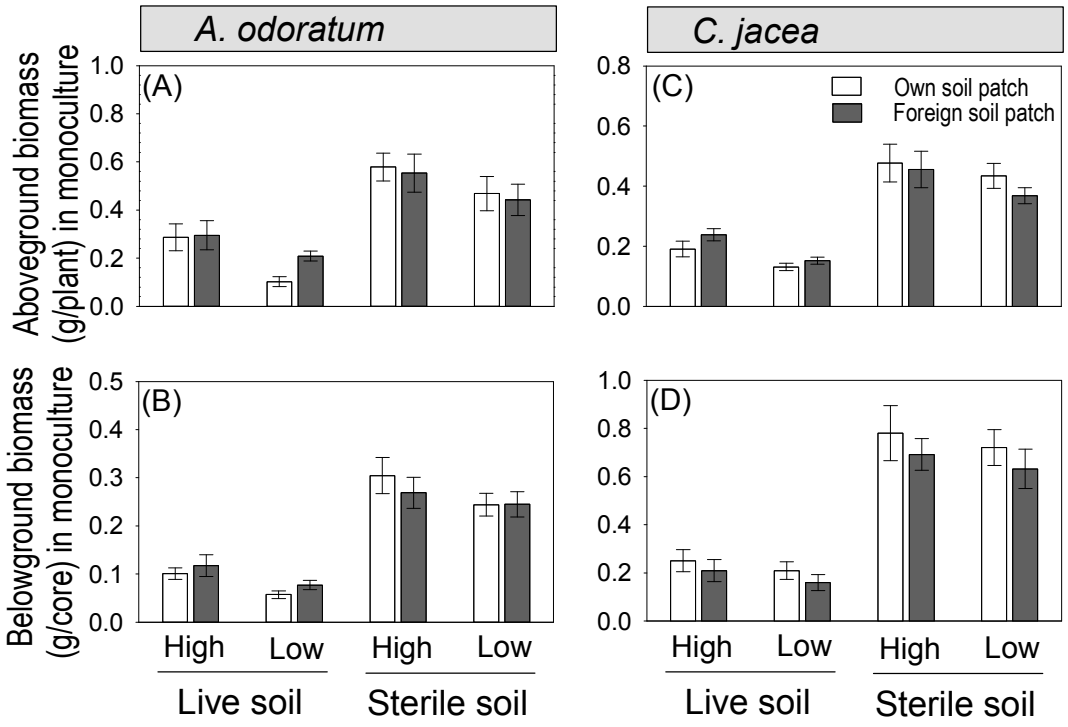

Fig. S4. Aboveground biomass per plant of *A. odoratum* (A) and *C. jacea* (B) in 1:1 mixtures in homogeneous and heterogeneous soils at the pot level. "High" and "Low" refer to high nutrient soil and low nutrient soil used in the conditioning phase. "Live soil" and "Sterile soil" indicate field-collected soil and sterilized field-collected soil, respectively. Mean values (± 1 SE) are presented. See Table S3C for statistic results.