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Intraspecific and intergenerational differences in plant–soil feedbacks

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Interactions between plant and soil communities are known to play an integral role in shaping ecosystems. Plants influence the composition of soil communities and soil communities in turn influence plant performance. Such a plant–soil feedback may incur selection pressure on plants and the associating soil community. However, the evolutionary consequences of these above–belowground feedback interactions remain largely speculative. Here we assess whether plant–soil feedback effects differ between intraspecific plant populations and between generations within the same plant population. We used two populations of *Trifolium pratense* and assessed their performance when grown in association with their home versus away soil biota. Both populations were colonized by distinct microbial communities and performed better with their own home soil communities than with the soil community from the other intraspecific population, demonstrating intraspecific positive feedback effects of home soil. In one of the two populations, we found that plant performance and the root associated microbiota community differed between parental and progeny plants when inoculated with their own home soil. Differences in root associated community characteristics could explain more than 80% of the variation in performance among the progeny and parental plants. Our results highlight that intraspecific differences in both plant and associated soil communities shape plant–soil feedback effects, and consequently indicate that plant–soil feedback can influence the direction of selection between intraspecific plant populations.

Plants and their associated soil biota are entangled in a relationship where the performance and abundance of organisms in either the above- or belowground community is influenced by the performance and abundance of the other. For instance, the structure and performance of plant communities are frequently affected by the abundance and diversity of soil organisms, such as plant beneficial arbuscular mycorrhizal fungi and soil pathogens (van der Heijden et al. 1998, Petermann et al. 2008, Schnitzer et al. 2011,Wagg et al. 2014). Conversely, the structure of soil communities and the root-associated microbiome is shaped by the identity of the plant species (De Deyn et al. 2010, Bezemer et al. 2010, Bulgarelli et al. 2013). This bidirectional relationship between plants and soil biota often results in a feedback effect where a build-up of beneficial and antagonistic organisms associated with a particular plant species determines the establishment and performance of a succeeding plant (Bever et al. 1997, Bever 2003, Reynolds et al. 2003).

This plant–soil feedback mechanism has been known to drive key ecological processes, such as plant community succession (van der Putten et al. 1993, Kardol et al. 2006, 2007), plant species invasions (Callaway et al. 2004, Reinhart and Callaway 2006), the maintenance of plant diversity (Petermann et al. 2008), as well as drive plant species rarity (Klironomos 2002). Negative feedback can arise from the accumulation of plant species specific pathogens that negatively affect succeeding plants, or through supporting mutualistic organisms that benefit the competitive ability of neighboring interspecific plants. These mechanisms limit the ability of a particular plant species to become dominant within a community and are a primary mechanism in the maintenance of plant diversity within a community (Bever et al. 1997, Klironomos 2002, Bever 2003, Petermann et al. 2008). Based on these observations it is has been previously proposed that plant–soil feedback interactions may also function as a mechanism for natural selection (Bever et al. 1997, van Breeman and Finzi 1998, Brundrett 2002, Lau and Lennon 2011). For instance, strong negative feedback effects could incur selection on a plant population for traits that reduce negative feedback, such as through increased pathogen resistance or traits that promote increased mutualism with soil organisms.

Changes in the magnitude and direction of the feedback effects coupled with changes in the associating soil community over generations may lead to co-evolutionary relationships between plants and belowground organisms. Thus, the plant and associated soil communities may be exposed to local selective pressures induced by the other that result in a specific soil community – plant performance outcome (Hoeksema 2010, Lau and Lennon 2011). Moreover since different intraspecific plant populations inhabit different local environments, they may also experience varying degrees in the strength of a plant–soil feedback since environmental characteristics shape the strength of mutualist and antagonist interactions between plants and associating soil organisms (Johnson et al. 1997, Thrall et al. 2006, Heath and Tiffin 2007).

Previous studies have observed plant genotype and cultivar specific influences on associating soil bacterial and fungal communities (Westover et al. 1997, Easton et al. 2001, Schweitzer et al. 2004, Kiers et al. 2007, Silfver et al. 2007). In conjunction with this, it has been shown that different plant genotypes are differentially influenced by specific rhizobia and mycorrhizal fungi (Heath and Tiffin 2007, Kiers et. al. 2007, Johnson et al. 2010). Together this provides evidence that intraspecific differences in plant–soil feedback effects may occur. For instance, it has recently been demonstrated that different *Arabidopsis thaliana* genotypes exhibit intraspecific variation in plant–soil feedback effects (Bukowski and Petermann 2014). Such variation in intraspecific plant–soil feedbacks can potentially play an important role in the divergence in plant population traits over generations (Schweitzer et al. 2014). However, the role of intraspecific plant–soil feedback in shaping evolutionary processes in plants is still largely unexplored.

Here we assess intraspecific plant–soil feedback effects in two different populations of red clover *Trifolium pratense*. Each *T. pratense* population was grown in pots inoculated with soil collected from the plot where the population was grown as a monoculture (home soil) and soil from plots where the other population was grown as a monoculture (away soil). This allowed us to test the hypothesis 1) that plant–soil feedback effects are dependent on intraspecific plant populations. In addition to this we assessed plant–soil feedback between generations by comparing the performance of parent plants and progeny plants of the same population when grown in their own home soil. This allowed us to assess the hypothesis 2) that plant–soil feedback effects may incur selection across plant generations.

Methods

Seed production and soil conditioning

We chose *Trifolium pratense* for its broad use and value in agriculture (Boller et al. 2010) and since *Trifolium* sp. are known to depend heavily on soil microbial symbiotic interactions with rhizobia and AM fungi for the uptake of N and P (Boller and Nösberger 1987, Feng et al. 2003). Additionally, it has been demonstrated that differing legume cultivars can vary in their efficiency in mycorrhizal and rhizobial symbiotic associations (Mytton 1975, Kiers et al. 2007). Legumes, are thus, sensitive to change in soil community characteristics, which makes them ideal candidates to assess the role of plant–soil feedbacks in evolutionary processes.

The two populations of *T. pratense* (a tetraploid 4x and a diploid 2x population) differ in breeding history. The 4x *T. pratense* seeds were originally created by colchicine induced genome duplication from the variety Formica (Boller 1996), followed by five generations of propagation. Diploid *T. pratense* seed originated from intercrossing the diploid varieties Milvus (Boller and Nüesch 1995) and Corvus (Boller 2000) followed by four generations of propagation. For simplicity we refer to the two populations as 4x and 2x. The seeds of these two populations were collected from the final generation of propagation in 2007 and stored at 10°C in the dark until sown into the field as large monocultures in the spring of 2008. The 4x population was sown as a monoculture located near the Reckenholz research station of Agroscope (47°42′55″N, 008°54′02″E) and the 2x population 30 km away near the Tänikon research station of Agroscope (047°47′54″N, 008°90′96″E).

After three years of propagation in the autumn of 2010 seeds were harvested from both populations and used for this experiment. At the same time, soil cores 2.5 cm in diameter, with a 10–12 cm depth were collected from below parent *T. pratense* plants at both the sites where the 2x and 4x populations were grown. In addition, soil cores were also taken from *Lolium* sp. monoculture plots that had been cultivated at each of the two sites during the same time as the cultivation of the *Trifolium* populations. This was done as a control for site-specific inocula effects resulting in a total of four plots (two plots per site) in which soils were collected. In each of the four plots, soil cores were taken approximately every 0.5 m along four transects across each plot, such that roughly 5 kg of soil was collected per plot. Soil cores were pooled and homogenized for each plot by sieving through a 5 mm mesh. Half of each of the four soil inocula were sterilized by autoclaving for 60 min at 121°C. This resulted in a total of eight soil inocula treatments: two soil origins (2x or 4x site) two plant treatments (*Trifolium* or *Lolium*-conditioned) two soil sterilization treatments (sterilized or unsterilized).

Intraspecific feedback

We tested for intraspecific plant–soil feedback by inoculating the two *T. pratense* progeny populations with each of the eight inocula treatments. Each of these 16 combinations were replicated 10 times in a complete factorial design for a total of 160 pots. The experiment was performed in 1-l pots filled with 800 g of a standardized substrate consisting of a 1:1 (by weight) quartz sand – natural grassland soil mix that was sterilized by autoclaving for 60 min at 121°C. The grassland soil for the standardized substrate originated from a natural grassland (Wagg et al. 2011). Each pot then received 90 g (approximately 7.5 % of total substrate by weight) of one of the eight soil inocula treatments. The inocula was mixed throughout the substrate. Pots were subsequently topped up to 1200 g with additional sterilized sand–soil substrate to avoid cross-contamination of soil inocula between pots and treatments following protocols used for research with AM fungi (Wagg et al. 2011). The substrate characteristics of each of the eight inocula

treatments were analyzed using two soil samples per treatment (Supplementary material Appendix 1).

Intergenerational feedback

To assess intergeneration feedback effects we used seeds of the population that was initially sown in 2008 into the fields as monocultures (e.g. the parental seed population) and seeds produced by the plants after three growing seasons at these locations and collected in 2010 (the progeny population). By using these two seeds sources we could test for intergenerational differences in plant–soil feedback effects. In order to do this, 2x and 4x parental plants were grown with sterilized or unsterilized *Trifolium* conditioned inocula from the home 2x and 4x plots respectively. These treatments (two parental plant populations × two soil treatments) were replicated 10 times for an additional 40 pots and used for comparison to their progeny plants grown with the same 'home' inocula mentioned above. Combined with the 160 pots to assess 'home' and 'away' intraspecific effects this yielded a total of 200 pots. The pots, substrate and inoculation procedures were the same as mentioned above.

It was also tested whether parental and progeny seed populations had similar weights to verify that maternal environmental effects did not influence the results. A sample of 100 seeds from each parental and progeny seed population was weighted and compared by t-test. Seed mass did not vary between progeny and parental populations in both the 2x population (progeny = 2.11 mg, SE = 0.29 and parental = 2.10 mg, SE = 0.35; p = 0.98) and 4x population (progeny = 2.80 mg, SE = 0.39 and parental = 2.87 mg, SE = 0.37; p = 0.18) ruling out potential maternal environmental effects on seed quality.

Growing conditions

Prior to planting all seeds were surface sterilized by slow agitation in 50 % household bleach (roughly 2.5% sodium hypochlorite) with a few drops of Tween 20 for 5 min. Seeds were rinsed with dH_2O and allowed to germinate on 1% water agar for three days. Six *T. pratense* seedlings of a single population (4x or 2x) were transplanted into each pot. Seedlings that did not survive the transplanting were replaced within a week of initial planting.

All 200 pots were randomly distributed within the greenhouse and grown under natural light conditions subsidized by 400-ver' high-pressure sodium lamps to maintain a light level above 300 W m^{-2}. This maintained growth conditions at 16 h / 25°C days and 8 h/16°C nights. Soil moisture was maintained between 20–30% by weight. Plants were harvested 14 weeks post initial planting and a census of mortality per pot recorded (Supplementary material Appendix 2). Aboveground biomass was dried at 60°C and their biomass recorded. Roots were washed clean of soil and frozen at −20°C until they could be processed further.

Processing of roots

Roots were thawed, fresh weight recorded and cut into small pieces, 1–2 cm in length in cold water. A random sample of 0.5 g of fresh root was selected and fixed in 50% ethanol for scoring AM fungal root colonization. These roots were then cleared and stained with 0.05% (w/v) trypan blue in lactoglycerol (Brundrett et al. 1994). Stained roots were mounted on slides and scored for the presence of AM fungal structures (arbuscules, vesicles, hyphae) following the transect method outlined in McGonigle et al. (1990) for 100 intersects. Colonization by non-AM fungal structures was also scored in the same manner (such as dark septate hyphae, microscleroita, as well as fine or hyaline hyphae and spores) as an indication of colonisation by saprophytic and pathogenic fungi. Pots receiving sterilized inocula were also checked for nodulation and AM fungal colonization. Plants in nine pots receiving sterilized inocula exhibited AM fungal colonization and or root nodulation and were excluded from the data set (see Supplementary material Appendix 2 for the number of replicates per treatment after excluding those exhibiting contamination).

All plants grown with unsterilized soil inocula exhibited root nodulation. Three root systems were randomly selected per pot and 10 root nodules per root system were removed and lyophilized for a total of 30 root nodules per pot. These were weighed and used for molecular assessment to ensure DNA extracted from roots included DNA of nodule forming microbes. The remaining root system was lyophilized and 20 mg of lyophilized roots was added to the lypholized nodules for DNA extraction using the Qiagen DNeasy plant mini kit following the manufacturer recommendations for the purification of total DNA from plant tissue.

Molecular analysis of root associated microbial communities

Root associated bacterial community profiles were assessed using ribosomal internal spacer analysis (RISA). RISA was performed using extracted DNA diluted to 10 ng μl^{-1}; which was previously quantified using PicoGreen on a fluorescence spectrophotometer. Primers targeting bacterial communities were bRISArev and bRISAfor and using PCR reagent concentrations and cycling conditions that are provided in Hartmann et al. (2005).

In short, in order to assess the variation in the size of the amplified fragments from the bacterial PCR reactions, 2 µl of the PCR product was mixed with 12 µl HiDi-Formamid and 0.2 µl MapMarker 1000 as the size standard and subject to fragment analysis using a genetic analyzer. Run conditions were set to 7 kV and 60 C with a run time of 3000 s. Relative migration units were then assessed using GenMarker 1.5 genotyping software and in each profile unambiguous fragment peaks were used as operational taxonomic units (OTU). Peak intensities of the OTUs were scored as relative florescence units with a threshold value of 50 units. Bacterial OTU peak intensities were converted to proportions of the total peak intensity sum in each sample in order to reduce the amount of variation due to variation in PCR amplification among samples following Hartmann et al. (2005). Bacterial OTU matricies were then standardied by z-score transformations of each OTU column (mean = 0, SD = 1).

Data analysis

All mentioned statistics and calculations were carried out using the software R ver. 3.0.0. Since not all plants survived

until harvest (Supplementary material Appendix 2) resulting in differences in the number of plants per pot plant mortality was added as a covariate in all ANOVAs to counteract plant density dependent effects.

To assess intraspecific differences in plant–soil feedback effects, aboveground biomass was assessed by ANOVA with plant population (4x or 2x), inocula origin (2x or 4x site), plant history (*Trifolium* or *Lolium*-conditioned), soil sterilization treatment (sterilized or unsterilized) as well as all interactions, as sources of variation. We also assessed the growth response to soil biota by calculating the mean difference in biomass when grown with unsterile soil inocula and the biomass produced with the same inocula only sterilized. Root associated community characteristics (bacterial richness, AM fungal and non-AM fungal root colonization) were each assessed by ANOVA using the plant population (4x or 2x), inocula origin (2x or 4x site), plant history (*Trifolium* or *Lolium*-conditioned) as well as all interactions, as sources of variation. Tukey HSD was then used to determine differences in biomass and root community characteristics among the plant history and site origin combinations of the soil inocula for each plant population and soil sterilization treatment using the least square means package 'lsmeans' for R (Lenth 2014). Bacterial community structure was assessed using a permutational multivariate ANOVA with the Euclidean distance matricies using the function 'adonis' with 200 permuitations to determine the level significance (Oksanen et al. 2011) in bacterial OTU community dissilimarity using the same sources of variation as mentioned above for the other root community characteristics. Differences in bacterial community structure were further assessed and visualized by principal component analyses (PCA) using the same soil and and plant combinations as used in the permutational multivariate ANOVAs.

To elucidate the importance of the root associated soil community characteristics in explaining the differences in the performance of plants when inoculated with the various soil communities (unsterilized inocula treatments), we first assessed the variation in plant performance as a result of the soil inocula origin (2x or 4x site) and plant history (*Trifolium* or *Lolium* conditioned soil) combinations by ANOVA. The root community characteristics were then included in the model ahead of the soil inocula factor, in order by which they explained the greatest amount of variation. The change in the variation in plant performance explained by the inocula treatments prior to and after the addition of all root community characteristics, indicates how much the difference among inocula treatments is explained by root community characteristics.

Differences in plant biomass between generations when inoculated with their own home soil was assessed for variation among the plant population (4x or 2x), plant generation (parent or progeny) and soil sterilization treatment (sterilized or unsterilized) as well as all interactions. The response to soil biota was assess as the mean difference between unsterilized and sterilized inocula as mentioned above for assessing intraspecific plant responses. AM fungal colonization, non-AM fungal colonization and bacterial richness were all assessed by ANOVA with the plant population (4x or 2x), plant generation (parent or progeny) as sources of variation. Permutational multivariate ANOVA using Euclidean distance was then used to assess the bacterial community composition among plant population (4x or 2x) and plant generation (parent or progeny) combinations and results were visualized by PCA. Additionally, we assessed the variation between parental and progeny plants in each population when inoculated with their own home soil before and after fitting all root community characteristics ahead of the generation factor in order by which they explain the most variation. As mentioned above this allows for determining how much the root community characteristics explain the differences between the generations.

Results

Intraspecific differences

Plant biomass varied among soil inocula treatments depending on the plant population, the inocula origin (2x or 4x site), the plant history of the soil (*Trifolium* or *Lolium*-conditioned) and the soil sterilization treatment (sterilized or unsterilized) resulting in a four-way interaction (Table 1). Both the 4x and 2x plants obtained the greater biomass with their home soil inocula compared to soil inocula conditioned by the other *Trifolium* population (Fig. 1a–b). Importantly, this was not found with soil inocula from the adjacent *Lolium* monocultures at each site. Thus, this demonstrates that intraspecific differences are not a result of site effects (Fig. 1a–b). The response of the plants to inoculation (the mean difference from the same soil inocula only sterilized) were all greater than 0, demonstrating that the plants were all heavily dependent on the presence of soil biota for biomass production (Fig. 1c–d). Specifically the response in biomass of the 4x plants showed that they benefited most from their own home soil inocula compared to all other

Table 1. ANOVA results for the assessment of the response of the plant populations to intra- and interspecific effects their home and away soil biota. The effects of inoculation with sterile soil (sterilized or unsterilized), the origin of the inocula (4x site or 2x site), the history of the inocula (*Lolium* or *Trifolium* monoculture history), the plant population (2x or 4x) and all possible interactions on the total plant biomass per pot are shown.

Source of variation	DF	MS	F
Inocula sterilization (S)	1	1108.61	4469.14 ***
Inocula origin (O)	1	29.56	119.16 ***
Inocula history (H)	1	1.06	4.29 *
Plant population (P)	1	12.03	48.50 ***
S × O	1	0.00	0.01
S × H	1	0.64	2.57
O × H	1	2.16	8.71 **
S × P	1	0.02	0.06
O × P	1	0.16	0.66
S × P	1	< 0.01	< 0.01
S × O × H	1	0.01	0.06
S × O × P	1	13.13	52.93 ***
S × H × P	1	8.95	36.08 ***
O × H × P	1	18.44	74.35 ***
S × O × H × P	1	3.22	13.00 ***
Residuals	136	0.25	

*$p < 0.05$, **$p > 0.01$, ***$p < 0.001$, DF = degrees of freedom, MS = mean squares

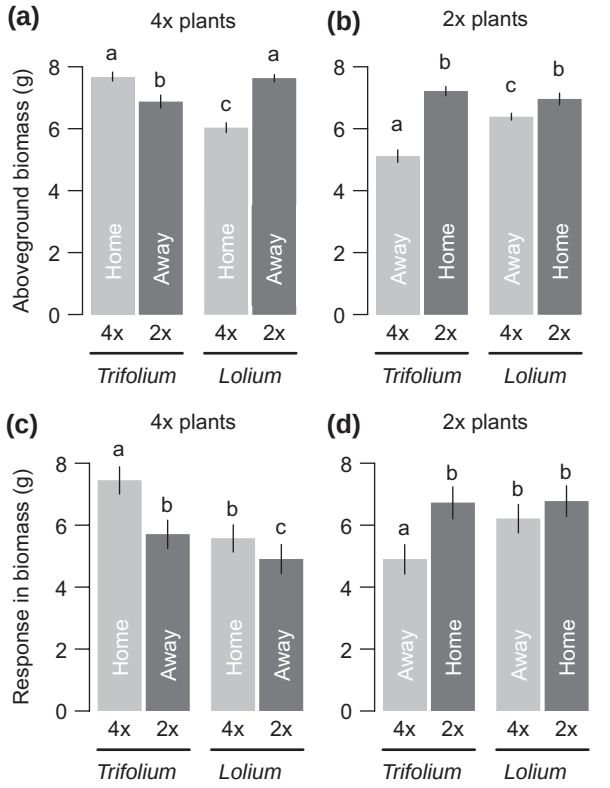

Figure 1. Means and standard errors of aboveground *Trifolium* biomass for the (a) the 4x plants and (b) the 2x plants when inoculated with soils from the 4x site (light grey) and 2x site (dark grey). Soil inocula originate from the plots where the parental *Trifolium* plants were grown as a monoculture and the adjacent plots conditioned by the *Lolium* monoculture at each site (4x site or 2x site) are indicated on the x-axis. The biomass response of plants to soil biota (difference from inoculation with the same soil sterilized) is shown for (c) the 4x plants and (d) the 2x plants. In (c) and (d) error bars represent 95 % confidence intervals for a difference from 0 (no difference between sterile and unsterilized soil inocula). Plant biomass produced with sterile inocula is shown in Supplementary Appendix 3. Different letters represent differences among means (Tukey $p < 0.05$). Inocula originating from the plant populations 'home' or 'away' site is indicated in each bar.

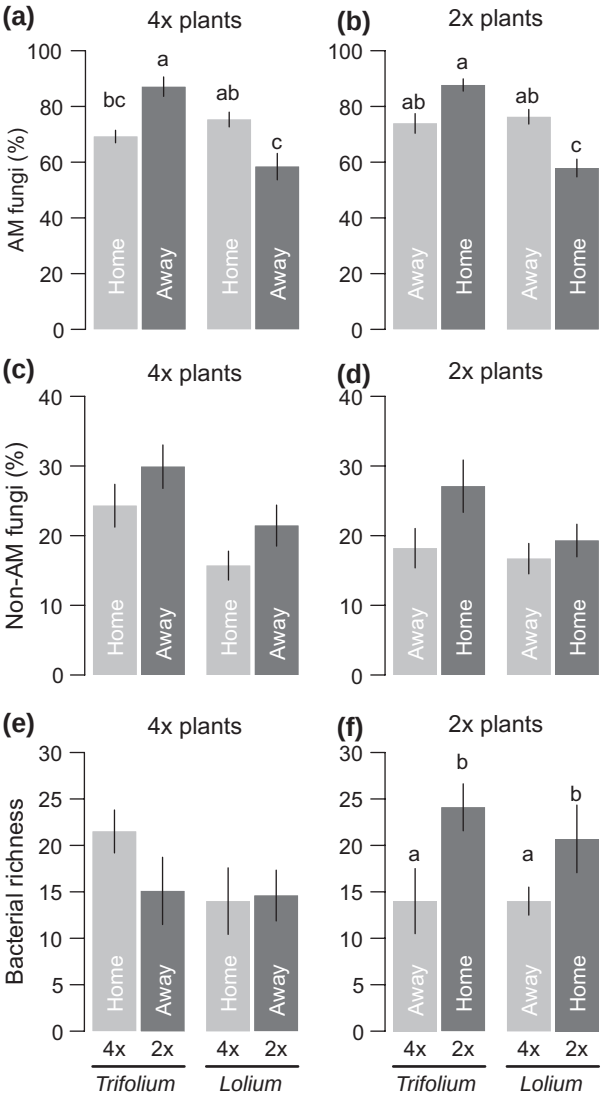

Figure 2. Means and standard errors for root colonization by AM fungi (a, b), non-AM fungi (c, d) and bacterial richness of root associated bacteria (e, f) are shown for both 4x plants and 2x plants when inoculated with soil from the 4x site (light grey) and 2x site (dark grey). Soil inocula originating from the plots where the parental *Trifolium* plants were grown as a monoculture and the adjacent plots conditioned by the *Lolium* monoculture at each site (4x site or 2x site) is indicated on the x-axis. Different letters represent differences among means within each panel (Tukey $p < 0.05$).

inocula treatments (Fig. 1c), while the 2x plants benefited the least from the soil inocula conditioned by the 4x plant population (Fig. 1c).

The extent of root colonization by AM fungi was dependent upon the site from which the soil originated in combination with plant history or the soil (e.g. soil conditioned by *Trifolium* or *Lolium*; two-way interaction $F_{1,71} = 54.9$, $p < 0.0001$). Only when inoculated with soil originating from the 2x site, did inoculation with the *Trifolium* soil result in greater AM fungal root colonization than with the *Lolium* conditioned soil in both plant populations (Fig. 2a–b). The colonization of roots by non-AM fungal structures was overall greater in soils originating from the 2x site than in the 4x site ($F_{1,71} = 8.04$, $p = 0.006$, Fig. 2c–d) and was also greater in the soil conditioned by the *Lolium* monoculture than in the *Trifolium* monoculture ($F_{1,71} = 9.92$, $p = 0.002$, Fig. 2c–d). In general both plant populations showed similar levels of AM fungal and non-AM fungal colonization of their roots when inoculated with the same soil (Fig. 2a–d).

The bacterial richness differed between the two populations resulting in a site origin by plant population interaction term ($F_{1,71} = 6.88$, $p = 0.01$). Specifically, while the 4x population had the highest richness of bacterial OTUs when inoculated with its home *Trifolium* conditioned soil there was little difference in the bacterial richness of the 4x plants inoculated with the various inocula treatments (Fig. 2e). Conversely, the 2x population was found to have a greater richness of bacterial OTUs when inoculated with soil from its home site than soil from the 4x site (Fig. 2f).

The composition of bacterial communities varied depending on inocula site origin, the plant monoculture history of the soil inocula and the identity of the plant population, revealed by a significant three-way interaction term (mul-

tivariate permutational ANOVA $F_{1,71} = 1.49$, $p = 0.02$). Both 4x and 2x plants showed distinct bacterial community compositions when associating with soil from their home site that was conditioned by the *Trifolium* monoculture of their previous generation (Fig. 3). Additionally, when inoculated with the soil from the *Lolium* monocultures the two plant populations were found to associate with similar bacterial communities (Fig. 3). This demonstrates that the two plant populations have an affinity with a specific root-associated bacterial community in the soils in which the plant population and soil communities share a history. Full ANOVA results for assessing intraspecific effects of all root community characteristics are presented in Supplementary material Appendix 4.

The variation in the biomass response of both the 4x and 2x plants to inoculation with the four different soils (shown in Fig. 1c and d respectively) was best explained by the principal component axes 1 and 2 (PCA 1 and PCA 2); which together explained 67.8% of the total variation in the response of the 4x plants and 40.0% of the total variation in the 2x plants (Table 2). Prior to fitting all root community characteristics the four inocula treatments (shown in Fig. 1) explained 77.0% of the total variation in the biomass response of the 4x plants ($F_{3,36} = 40.2$, $p < 0.0001$), while after fitting all root community characteristics, these treatments only explained 10.0% (Table 2a). This indicates that 87% of the difference in the 4x plant responses among the soil inocula treatments could be explained by the root community characteristics. This was similar for the variation in the response of the 2x plants. Prior to fitting the root community characteristics to the model the soil inocula treatments explained 70.0% of the total variation in the response of the 2x plants ($F_{3,36} = 28.0$, $p < 0.0001$), while after including the root community characteristics, these treatments only explained 30.1% (Table 2b). This indicates that the 57% of the difference among the inocula treatments in the response of the 2x plants can be attributed to the variation in the root associated community characteristics.

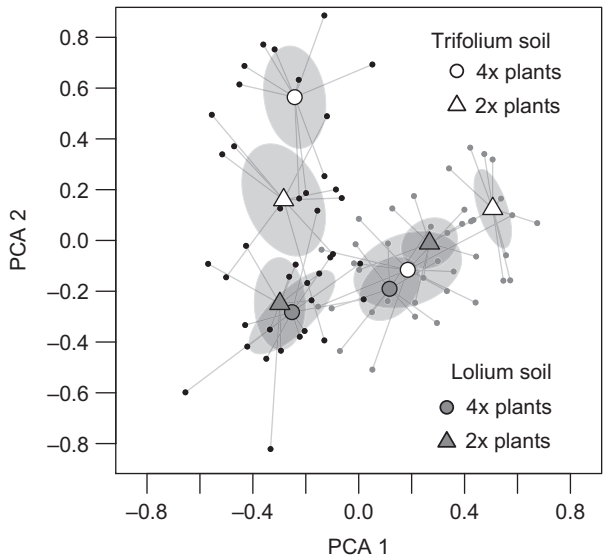

Figure 3. Ordination axes 1 and 2 of the PCA on the plant root associated bacterial community with data from the 2x site (grey points) and 4x site (black points). Centroids for each plant population are shown for 2x plants (triangles) and 4x plants (circles). Centroid points highlight plants grown with soil conditioned by the home parent *Trifolium* population (white centroid points) or by the adjacent *Lolium* population (grey centroid points). Lines connect the points (individual community observations) to the centroids in each plant and soil combination. Grey ellipses represent 95 % confidence around each centroid.

Table 2. ANOVA results for variation in the plant biomass response of (a) the 4x plants and (b) the 2x plants shown in Fig. 1c and d respectively. The root community characteristics PCA 1, PCA 2, root colonization by AM fungi, bacterial history, and root colonization by non-AM fungi were all included as sources of variation in the model ahead of the inocula treatment term to assess the proportion of variation (% SS) explained by each. The PCA 1 and 2 refer to axes in Fig. 3 and represent bacterial community composition.

(a) 4× plants	DF	MS	F	% SS
PCA 2	1	24.47	86.02 ***	57.5
PCA 1	1	4.36	15.32 ***	10.3
AM fungi	1	0.45	1.60	1.1
Bact. richness	1	0.46	1.62	1.1
Non-AM fungi	1	< 0.01	< 0.01	< 0.01
Inocula treatment	3	1.42	4.98 **	10.0
Residuals	30	0.28		
(b) 2× plants	DF	MS	F	% SS
PCA 1	1	9.56	34.22 ***	29.2
PCA 2	1	3.54	12.66 **	10.8
AM fungi	1	0.83	2.96 †	2.5
Bact. richness	1	0.16	0.58	0.5
Non-AM fungi	1	0.15	0.54	0.5
Inocula treatment	3	3.29	11.76 ***	30.1
Residuals	30	0.28		

†$p < 0.1$, *$p < 0.05$, **$p > 0.01$, ***$p < 0.001$, DF = degrees of freedom, MS = mean squares, % SS = percent of total sum of squares

Intergenerational differences

Parental and progeny generations differed depending on the plant population (4x or 2x), and inocula sterilization treatment (sterilized or unsterilized) resulting in a three-way population by generation by sterilization interaction term ($F_{1,67} = 78.0$, $p < 0.0001$, see Supplementary Appendix 5 for full ANOVA results). Specifically, we found the progeny plants of the 4x population outperformed their parental plants when inoculated with the same home soil that the population had conditioned, while the 2x progeny plants performed slightly less than their parental population (Fig. 4a). Importantly, these differences between parent and progeny plants were not detected in either population when using the sterilized soil inocula (Supplementary material Appendix 6).

The parental 4x plants had greater AM fungal colonization (Fig. 5a), greater non-AM fungal colonization (Fig. 5b) and greater bacterial richness (Fig. 5c) associated with their roots than the 4x progeny plants, while the 2x parental and progeny plants were similar in all cases. This resulted in a population by generation opulation interaction term for the level of root colonization by AM fungi (Fig. 5a, $F_{1,35} = 6.02$, $p = 0.02$), non-AM fungal structures (Fig. 5b, $F_{1,35} = 5.02$, $p = 0.03$) and the bacterial richness

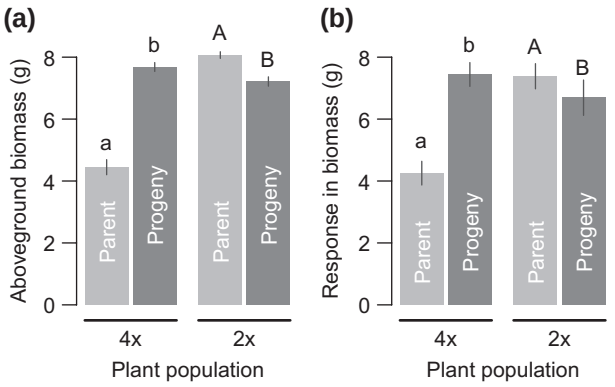

Figure 4. Means and standard errors for the (a) aboveground biomass and (b) the biomass response (difference from inoculation with the same soil sterilized) are shown for the parental plants (light grey) and the progeny plants (dark grey) for the 4x population and the 2x population when inoculated with their own home soil. In (b) error bars represent 95% confidence intervals for a difference from 0 (no difference between sterile and unsterilized soil inocula). Differences between progeny and parental plants are indicated by lowercase letters for the 4x population and by capital letters for the 2x population ($p < 0.05$).

associated with the roots (Fig. 5c, $F_{1,71} = 3.94$, $p = 0.05$). The bacterial community composition associated with the roots depended on plant generation and population (multivariate permutational ANOVA population by generation interaction term $F_{1,35} = 2.29$, $p = 0.005$). Specifically, the bacterial community composition associated with 4x plants differed greatly between generations while there was no difference between the 2x generations (Fig. 6). Full ANOVA results for assessing root community characteristics between generation and populations are presented in Supplementary material Appendix 7.

The bacterial community composition (PCA 2 in Fig. 6) explained a large proportion of the variation in the response of the 4x parental and progeny plants to inoculation with their own home soil, followed by colonization of non-AM fungi, bacterial richness and slightly by AM fungal colonization (Table 3a). Before fitting the root community characteristics to the model, the generation of the 4x plants explained 87.6 % ($F_{1,18} = 50.8$, $p < 0.0001$) of the total variation, while after explaining the variation in root community characteristics, only 2.7% was explained by plant generation. This indicates that 97.0% of the difference in the performance between the parental and progeny 4x plants when inoculated with the same soil can be attributed to their differences in root community characteristics. Conversely, the variation among the 2x parental and progeny plants inoculated with their own home soil was poorly explained by all root community characteristics (Table 3b). Prior to fitting all root community characteristics the generation of the plants explained 37.2% of the overall variation ($F_{1,18} = 10.6$, $p = 0.004$), while after explaining the variation in all root community characteristics it still could explain 35.9 % of the total variation in the 2x parental and progeny plants (Table 3b). Thus, the root community characteristics only accounted for 3.4% of the difference between the 2x parental and progeny plants.

Discussion

Our results show that plants from both populations performed better with their home soil communities than with the soil community conditioned by the other intraspecific population. Moreover, both plant populations harbored specific root associated bacterial communities. This demonstrates that plant–soil-feedback occurs at the intraspecific population level, in addition to earlier findings that plant–soil-feedback varies among plant species (Bever et al. 1997, Bever 2003, Kardol et al. 2007, Reinhart and Callaway 2006, Petermann et. al. 2008). In both plant populations we could explain a large proportion of the variation in the performance of the plants inoculated with home and away soils by the variation in the root associated bacterial community composition. This suggests that the degree to which the plants benefited from the soil communities with which they associated with was dependent on the composition of the root associated bacterial community. This supports our hypothesis 1) that there are plant population specific plant–soil feedback effects and it suggests a co-adaptation between the plants and their soil biota with which they have a history of interaction.

We observed that progeny plants of the 4x population outperformed their parental plants and we found that the

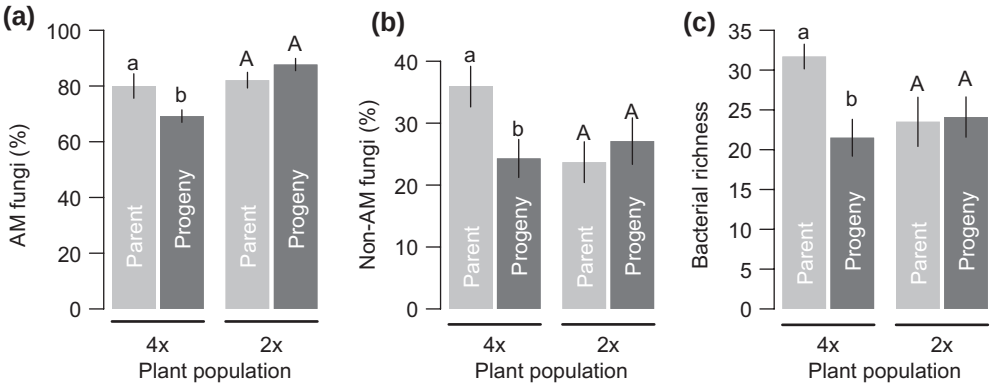

Figure 5. Means and standard errors for root associated community characteristics are shown for the parental plants (light grey) and the progeny plants (dark grey) for each population (4x and 2x) when inoculated with their own home soil. Differences between progeny and parental plants are indicated by lowercase letters for the 4x population and by capital letters for the 2x population ($p < 0.05$).

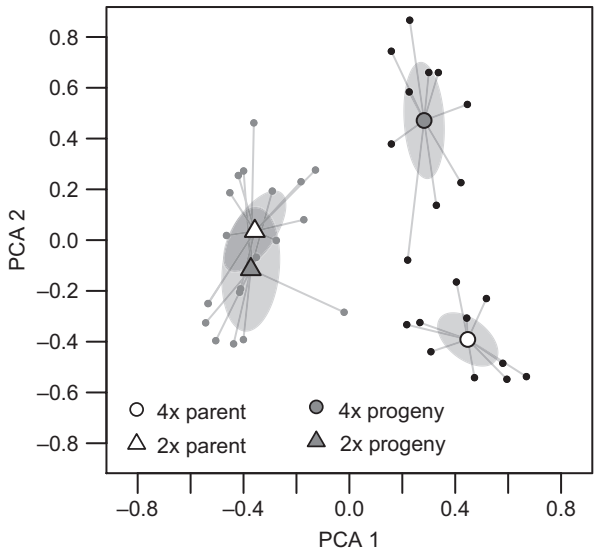

Figure 6. Ordination axes 1 and 2 of the PCA on the plant root associated bacterial community with the parental and progeny plants when inoculated with their own soil biota. Centroid points highlight the parental (white) and progeny (grey) plants of the two populations; 2x plants = grey dots, 4x plants = black dots. Lines connect the points (individual community observations) to the centroids in each plant and soil combination. Grey ellipses represent 95% confidence around each centroid.

progeny plants associated with a unique bacterial community and were generally less colonized by endophytic fungi than their parental plants. The differences in the root community characteristics associated with the poorer performance of the parental plants relative to their progeny in this population suggests that the parental seed population initially sown into the field likely experienced selection pressure by the local soil biota resulting in the production of progeny with altered interactions with the local soil community. These results provide some support for our hypothesis 2) that soil feedback effects can incur selection pressure resulting in more locally adapted progeny plants to their soil communities. This result provides support for previous notions that negative plant–soil feedback as an agent of natural selection (Bever et al. 1997, Lau and Lennon 2011, Schweitzer et al. 2014). Contrastingly however, similar evidence for intergenerational changes in feedback effects was not found for the 2x population. Therefore this population may not have experienced similar selective pressure in its local environment. Whether our findings that plant–soil feedbacks can change across generations are a general occurrence in nature and the role of the local environment requires further empirical studies.

Table 3. ANOVA results for variation in the plant biomass response between the parental and progeny plants of (a) the 4x population and (b) the 2x population when inoculated with their own home soil (shown in Fig. 4b). The root community characteristics PCA 1, PCA 2, root colonization by AM fungi, bacterial history, and root colonization by non-AM fungi were all included as sources of variation in the model ahead of the Generation term to assess the proportion of variation (% SS) explained by each. The PCA 1 and 2 refer to axes in Fig. 6 and represent bacterial community composition.

(a) 4 × plants	DF	MS	F	% SS
PCA 2	1	42.88	120.80***	74.0
Non-AM fungi	1	3.87	10.91**	6.7
Bact. richness	1	3.44	9.69**	5.9
AM fungi	1	1.54	4.32†	2.7
PCA 1	1	0.09	0.24	0.2
Generation	1	1.54	4.34 †	2.7
Residuals	13	0.36		
(b) 2 × plants	DF	MS	F	% SS
Non-AM fungi	1	0.15	0.75	3.3
PCA 1	1	0.07	0.35	1.5
PCA 2	1	0.06	0.31	1.4
Bact. richness	1	0.02	0.09	0.4
AM fungi	1	< 0.01	0.02	0.1
Generation	1	1.63	8.12*	35.9
Residuals	13	0.20		

† $p < 0.1$, * $p < 0.05$, ** $p > 0.01$, *** $p < 0.001$, DF = degrees of freedom, MS = mean squares, % SS = percent of total sum of squares

Intraspecific feedback effects

Plants encounter both beneficial and antagonistic soil organisms and their combined feedback effect determines plant performance (Morris et al. 2007). Negative feedback effects are frequently observed in natural systems and are generally associated with increased localized abundance of pathogens associated with a particular plant species (van der Putten et al. 1993, Westover and Bever 2001, Morris et al. 2007, Petermann et al. 2008, Liu et al. 2012). It is possible that both of the investigated plant populations in our study had built up a unique community of soil pathogens that contributed to their performance. However, all plants performed extremely poor when inoculated with sterilized soil inoculum demonstrating that plant beneficial interactions were overall more critical to plant performance than antagonistic interactions. This overall positive response to soil biota is a common observation in legumes as a result of their well-known dependence on symbiotic associations with AM fungi and rhizobia that enhance nutrient acquisition and performance of *Trifolium* (Boller and Nösberger 1987, Feng et al. 2003, Wagg et al. 2011). Moreover our findings show that both plant populations benefited more from their own soil biota than soil that was conditioned by the other (away) intraspecific population indicating they are better adapted to their own home soil biota. This parallels similar findings by Johnson et al. 2010 who found different ecotypes of the grass *Andropogon gerardii* to have increased symbiotic efficiency with their home AM fungi and Pregitzer et al. (2010) who similarly found greater benefits of home soil relative to intraspecific away soils in *Populus angustifolia* seedlings.

In our study the home advantage of the soil biota could have arisen from selection for greater symbiotic efficiency as theory suggests legumes may select for more efficient rhizobia and mycorrhizal symbioses (Denison and Kiers 2004a, b, Oono et al. 2009, Hoeksema 2010). In conjunction with this we also found that the plants grown in their home soil associated with a unique bacterial community composition. Moreover, we found the composition of the bacterial communities associated with the roots of the plants explained a large proportion of the performance of the plant populations when inoculated with home and away soils. Such a specific

home site plant-root community composition is suggestive of their co-adaptation. This is supported by previous observations that plant genotypes and ecotypes perform better when interacting with soil organisms and soil community compositions with which they have shared a history of association, thus suggesting their co-adaptation within their local environment (Heath and Tiffin 2007, Johnson et al. 2010, Pregitzer et al. 2010, Lau and Lennon 2011).

The home soil bacterial composition and benefit to plant performance corresponds with recent findings that show that locally adapted bacterial community composition can beneficially influence plant performance and alters the selective pressure on plant traits (Lau and Lennon 2011, 2012). Additionally our results parallel the concept that mutualistic interactions between plants and soil biota, such as in the legume–rhizobia relationship, may become locally adapted to each other for their specific environment and result in the divergence of plant–soil biota interactions among differing environments (Parker 1999, Garau et al. 2005, Heath and Tiffin 2007, Johnson et al. 2010). Although AM fungi may likely have also played a role in our results, we may not have detected their importance since root colonization is not necessarily related to nutrient supply and mycorrhizal efficiency (Facelli et al. 2009).

Intergenerational effects

It is commonly observed that seedling survivorship and performance is improved with greater distance from maternal plants in other systems as a result of negative feedbacks (Janzen 1970, Connell 1971, Packer and Clay 2000, Hille Ris Lambers et al. 2002, Mangan et al. 2010). Our findings expand on such past studies and provide evidence that plant–soil feedback effects can change between plant generations. Specifically, 4x progeny plants performed better, showed reduced non-AM fungal colonization of roots, reduced bacterial richness, and associated with a unique bacterial community relative to their parental plants. These differences between progeny and parental plants were strongly linked to their difference in performance. Such performance differences between the two generations when grown with a common soil environment indicates a genetic basis for the differences between the two generations. This points to a selection for plant genotypes that are better able to benefit from their associating soil biota. Selection for increased positive feedback has been thought to be the mechanism for a home soil advantage in other systems (Pregitzer et al. 2010). However, similar differences could not be detected between the 2x parental and progeny plants and their performance was poorly attributed to the root associated community characteristics. This indicates that selection through plant–soil feedback in this 2x population may not have occurred and future empirical studies are needed to assess the generality of negative feedback selection pressure.

Aside from the selective pressure of the local environment for reduced negative feedback, the intergenerational differences in the response to soil communities between our two populations may be attributed to genetic differences in ploidy and breeding history. In particular, plants with higher ploidy are thought to be favoured in the co-evolution of host–parasite interactions (Nuismer and Otto 2004) as the increase in the dosage of resistant genes in polyploid plants can result in improved resistance to pathogens (Levin 1983, Flagel and Wendel 2009). Moreover, many previous studies report improved pathogen and pest resistance in *T. pratense* tetraploids compared to diploid progenitors (Metha and Swaminathan 1957, Vestad 1960, Arseniuk 1989). Hence, the increased performance of the 4x population relative to the 2x population across generations might be related to selection for pathogen resistance. Additionally, genetic variation in the 4x population was likely higher as a result of polyploidy and may have also aided in more rapid adaption through selection in the 4x population (Parisod et al. 2010). However, since we cannot separate the effect of plant pedigree from the effect of ploidy, as 4x and 2x populations did not originate from the same plant population, both factors could have contributed to the observed differences between our populations. Future work is needed to assess the potential of genetic diversity and ploidy as mechanisms that reduce negative feedback.

Conclusion

Our study demonstrates that feedback effects between plant and soil communities are not only relevant for interspecific plant–soil interactions, but can vary between intraspecific plant populations. This supports our hypothesis that local selection on plant performance through plant–soil feedback effects can occur and may vary among intraspecific populations. Moreover, our results indicate that intraspecific genotypic differences, such as ploidy and breeding history, may also play a role in shaping how plant–soil feedback influences the performance of progeny plants. Future research with many more plant populations is needed to generalize our findings and demonstrate the ecological importance of plant–soil feedback for plant population dynamics and diversification.

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Supplementary material (available online as Appendix oik.01743 at <www.oikosjournal.org/readers/appendix>). Appendix 1–7.

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