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RESEARCH ARTICLE

Inter- and intraspecific plant-soil feedbacks of grass species

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Abstract

Background and aims Plants continuously interact with soil microbiota. These plant-soil feedbacks (PSFs) are considered a driving force in plant community dynamics. However, most PSF information comes from inter-family studies, with limited information on possible causes. We studied the variation of PSFs between and within grass species and identified the soil microbes that are associated with the observed PSFs effects.

Methods We grew monocultures of ten cultivars of three grass species (*Lolium perenne, Poa pratensis, Schedonorus arundinaceus*) using a two-phase PSF experiment. We measured plant total biomass to determine PSFs between and within species and correlated it with sequenced rhizosphere bacteria and fungi.

Results In the soil conditioning phase, grass species developed microbial legacies that affected the performance of other grass species in the feedback phase. We detected overall negative interspecific PSFs. While we show that *L. perenne* and *P. pratensis* increased their performance respectively in conspecific and heterospecific soils, *S. arundinaceus* was not strongly affected by the legacies of the previous plant species. Contrary to our expectation, we found no evidence for intraspecific variation in PSFs. Bacterial taxa associated with PSFs included members of *Proteobacteria*, *Firmicutes, Verrucomicrobia* and *Planctomycetes* whereas fungal taxa included members of *Ascomycota.*

Conclusion Our results suggest differences in PSF effects between grass species, but not between cultivars within species. Thus, in the studied grass species, there

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might be limited potential for breeding on plant traits mediated by PSFs. Furthermore, we point out potential microbial candidates that might be driving the observed PSF effects that could be further explored.

Keywords Specificity of plant-soil feedbacks · Intraspecific variation · Interspecific variation · Soil biota · Rhizosphere · Grass species

Introduction

Interactions between plants and soil microorganisms are important determinants of plant performance. The influence that plants exert on microorganisms generates microbial legacies in the soil that, in turn, affect the performance of other plants growing later in that soil, which is called plant-soil feedback (PSF) (Bever et al. 1997; Ehrenfeld et al. 2005; Kulmatiski and Kardol 2008; van der Putten et al. 2013). Plant-soil feedbacks can be considered positive when plant growth is promoted, negative when plant growth is reduced, and neutral when there is no impact on plant growth. Plant-soil feedback effects are net effects, and their direction and strength depend on the ratio between pathogenic and beneficial microorganisms and the physicochemical properties of the soil, including nutrient availability, soil moisture level, and soil structure (Cong et al. 2015; Cavagnaro 2016; De Deyn et al. 2011; Metcalfe et al. 2011; van der Putten et al. 2013). Negative PSFs play key roles in plant community dynamics such as maintaining plant species diversity and rarity by reducing the abundance of dominant and subordinate plant species (Klironomos 2002). The specificity of PSF effects is most often studied at the species level, comparing how soil microorganisms associated with one plant species affect growth of the same (conspecific) or of different (heterospecific) species. However, PSFs may also be influenced by the genetic variation within species, named intraspecific variation (Lau and Lennon 2011; Wagg et al. 2015; Allen et al. 2017; Bukowski and Petermann 2014).

Intraspecific variation in PSFs may drive the selection of microbiome-mediated plant traits and determine consequent adaptation of natural populations (Maron et al. 2011; Bolnick et al. 2011; Maron et al. 2016). Furthermore, these interactions may be of agronomic interest and utilized for selective breeding. For instance, negative feedbacks caused by a build-up of soil pathogens, could lead to selection of traits that diminish negative feedbacks, such as the promotion of mutualistic associations with soil organisms or resistance to soil-borne pathogens. Interest in PSFs within species has recently increased and a growing body of literature has shown intra-specific differences in PSFs effects. For instance, studies on the model species *Arabidopsis thaliana* showed that negative PSF effects depended on which accession had previously occupied the soil (Aguilera et al. 2011; Bukowski and Petermann 2014). Differences in PSFs within species have been documented also in *Plantago lanceolata* (Kirchhoff et al. 2019) and *Trifolium pratense* (Wagg et al. 2015). However, studies have mainly focused on forbs, and the intraspecific variation for plant-soil feedbacks in other plant taxonomic groups remain still unresolved.

Similar to PSFs between species, the degree of variation within species may differ among plant taxonomic groups (Bukowski and Petermann 2014; Cortois et al. 2016; Heinen et al. 2018; De Long et al. 2021). Thus, the patterns observed in forbs might very well differ from, for example, grasses. So far, little is known about the specificity of PSFs within grass species. Recent studies have investigated this in wetland grasses. For instance, studies on *Phragmites australis* showed that invasive plants may have an advantage over other plant species driven by soil legacies (Allen et al. 2017, 2018, 2020). A better understanding of the role of plant genetic diversity in grass-soil-microbiome interactions might help to slow down the accumulation of soil-borne pathogens since grasses, especially in current livestock farming systems, are often grown in monocultures.

A significant challenge in studying the specificity of PSFs, is to identify the microbial taxa that are responsible for the feedback effects. High-throughput amplicon sequencing has enabled the characterization of the composition of microbial communities and derived functional approaches such as FUNGuild can be used to classify microbial species into functional categories (Nguyen et al. 2016; Zanne et al. 2020). Moreover, studying individual microbial taxa may also be relevant to identify which microbes may contribute most to the observed effects (Cortois and De Deyn 2012; Putten et al. 2016). Most of this research

has focused on differences in microbial community composition *between* plant species, whereas little is known on differences *within* plant species. To our knowledge, there are hardly any studies that have focused on differences within terrestrial grasses with respect to their effects on soil microbial community composition.

We measured the strength and direction of PSFs between and within three grass species: *Lolium perenne, Poa pratensis*, and *Schedonorus arundinaceus*. These are all perennial grass species that grow in a wide range of habitats and are known to engage in interactions with beneficial microorganisms (Saikkonen et al. 2016). All three species are widely used by grass breeding companies as forage and turf grasses due to their high nutritional properties, high productivity, and tolerance to abiotic stressors. To examine variations in PSFs within each species, we conducted a PSF experiment using multiple commercial cultivars of each grass species, and correlated the variation in PSFs to variations in the soil microbes based on sequencing. We then tested: (1) whether rhizosphere bacterial and fungal community composition differ between grass species and intraspecific cultivars of each of the species (2) whether there are differences in PSFs between species and between cultivars of the same species and in what direction (3) what are the microbes that are associated with the observed PSFs effects.

Materials and methods

Plant material and germination

We used ten commercial cultivars used for turf for each of the three perennial grass species (*Lolium perenne, Poa pratensis*, and *Schedonorus arundinaceus*). The plant material was provided by the Barenbrug grass seed company research facility (Wolfheze, The Netherlands) (Table S1). The cultivars used for *L. perenne* and *S. arundinaceus* show some level of segregating genetic variation within cultivars whereas cultivars used for *P. pratensis* are single genotypes, owing to apomictic reproduction of *P. pratensis*. Seeds were surface-sterilized by washing them for one min in commercial bleach (< 5%) and 0.1% Tween-20 solution. After rinsing three times for one minute with demi-water, seeds were germinated on sterilised glass beads in a germination cabinet at 20 °C (*L. perenne* and *S. arundinaceus*) and 15 °C (*P. pratensis*). One week after germination, the seedlings were stored at 4 °C for a week under continuous light conditions until the start of the experiment.

Experimental design

The PSF experiment consisted of two phases, conditioning, and feedback phase (Fig. 1). The conditioning phase was started from a mixture of 90% sterilised background soil and 10% live inoculum soil. The background soil was sandy loam soil collected from a former agricultural field abandoned in 1995 close to Barenbrug research facility (Mossel, The Netherlands; N 52.06141, E 5° 75.266). It was sterilised by gamma-sterilization (25 KGray, Syngenta by Ede, The Netherlands). The inoculum soil was collected from field plots where the same species and 30 cultivars were growing in monocultures at the Barenbrug grass seed company research facility (Wolfheze, The Netherlands). To capture all microbial diversity, the sampling consisted of 150 sub-samples (5 soil samples for each of 30 cultivars monocultures) that were all pooled together and homogenised to generate a single inoculum soil mixture. The conditioning phase followed a randomised block design with five replicated blocks (each block has 1 replicate per variety). Per replicate block, 38 pots of 4L were filled with a mixture of 3.78 kg of sterilised background soil and 0.42 kg of sieved (1 cm diameter) of inoculum soil. In each pot, 15 plants were planted in monoculture, and for each replicate block, eight pots were left unplanted to be used as controls, (called hereafter "unconditioned soil"). The experiment was performed in a climatised greenhouse at 16/8 h light/dark and 20/15 °C day/night conditions. During the last four weeks, all pots received a weekly amount of 10 ml 5% Hoagland solution to avoid nutrient deficiency. Pots were weighted two times per week to adjust soil moisture to 15% (w/w). After 20 weeks of growth, the above-ground biomass was clipped, dried at 60 °C until constant weight, and weighed, whereas roots were chopped in ~ 2 cm pieces and homogenised with the soil and thus used as a source of microbial inoculum.

In the feedback phase, soil from each conditioning phase pot was individually transferred to four 1L pots filled with 920 g soil on a dry weight basis.

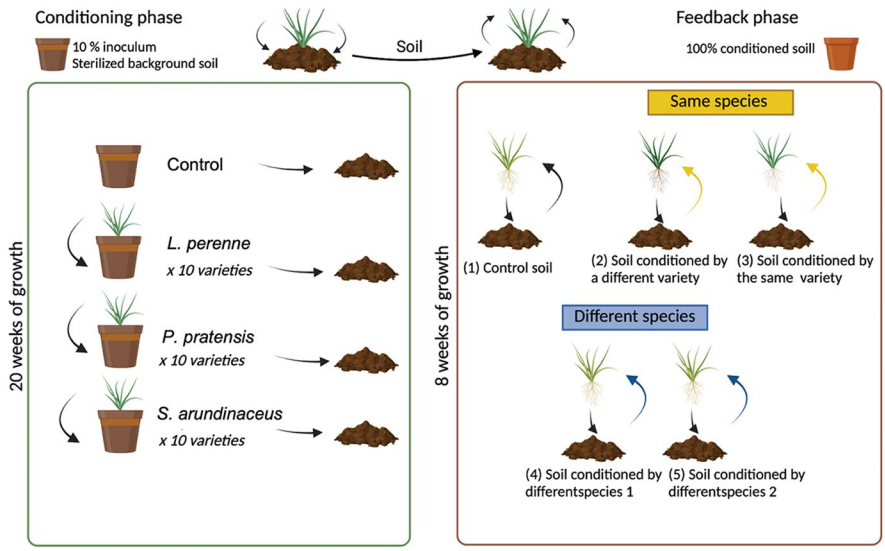

Fig. 1 Experimental design. In the conditioning phase, 10 cultivars of each of *L. perenne*, *P. pratensis* and *S. arundinaceus* species were grown in soil composed of 90% sterilized background and 10% live inoculum soil. In the control treatment, pots were filled with the soil mixture, but no seedlings were planted. After 20 weeks, rhizosphere samples were collected for the sequencing of bacterial and fungal communities. The aboveground biomass was collected whereas the roots were left in the soil after chopping. In the feedback phase, new seedlings were planted into unconditioned (control) soil (1), soil conditioned by different cultivars of the same species (2), soil conditioned by the same cultivars of the same species (3), soil conditioned by a different species 1 (4) and, soil conditioned by different species 2 (5). After 8 weeks of growth, total biomass was determined and used to calculate feedback effects

Every pot, irrespective of having conditioned or unconditioned soil, was planted with three cultivars of one of the three species in monoculture following a partial factorial design in a replicated block design with five replicates. Every cultivar was tested on the following soil treatments: (1) unconditioned soil (conditioning phase control soil), (2) conditioned by a different cultivar of the same species, (3) conditioned by the same cultivar, (4 and 5) conditioned by a cultivar of either one or the other grass species. For treatments 2, 4, and 5, we tested each cultivar on only one of the available cultivar-conditioned soils and not on all possible soils. For instance: to test the growth of a cultivar in soil that was conditioned by another cultivar of the same species, nine possible soils could be used; we choose only one of those (Figure S1). The design that we used for pairing conditioned soils to feedback plants ensured that all 30 cultivars contributed equally to soil feedback effects in the overall experiment. The experiment was performed in a climatised greenhouse at 16/8 h light/dark and 20/15 °C day/night conditions, and the same watering regime was applied as in the conditioning phase to maintain a moisture content of maximally ~ 15% (w/w). Every week, all pots received 10 ml of 5% Hoagland. After eight weeks of plant growth, shoots were clipped, dried at 60 °C until constant weight, and weighed, whereas roots were first washed and then dried at 60 °C, and weighed to determine biomass.

Microbial DNA extraction and sequencing

DNA was extracted from 0.25 g of rhizosphere soil collected at the end of the conditioning phase using the Power Soil DNA extraction kit (Qiagen, Hilden, Germany). Bacterial and fungal DNA was amplified using respectively the primers 515F/806R targeting the V4 region of the 16S rRNA gene (Caporaso et al. 2012; Apprill et al. 2015). For fungi, ITS4ngs and ITS3mix targeting the ITS2 region of fungi were used (Tedersoo et al. 2015). Preparation of libraries and sequencing (Illumina MIseq PE 250) were performed at McGill University and the Génome Québec Innovation Centre (Canada).

Data analysis

The variation of PSFs was analysed by subjecting the biomass data from the feedback phase to a two-way ANOVA. To fulfil requirements of normality, plant biomass was log-transformed prior to analyses. To test if soil legacies generated in the conditioning phase affected the responding plants in the feedback phase, we used a linear mixed model [lmer function in R—lme4 package (Bates et al. 2015)] to model the effects of plant species, plant cultivars, soil treatments (unconditioned soil, conditioned by a different cultivar, conditioned by the same cultivar, conditioned by different species 1 and conditioned by different species 2), block, and the interactions between soil treatments and cultivars and soil treatment and plant species on plant biomass. Because a single pot in the conditioning phase contributed soil for four pots in the feedback phase, conditioning phase pot was included in the model as a random factor. The factors soil treatments, cultivar, their interactions, and block were considered fixed effects. Tukey's HSD post hoc tests were performed to guide interpretation of significant main effects and interactions. To address how soil microbial legacies generated in the conditioning phase affected the responding grass species in the feedback phase, we excluded the unconditioned soil and used a linear mixed model per each species (lmer in R) to model the effects of plant cultivars, soil treatments, block, conditioning phase pot, interactions between soil treatments (conditioned by a different cultivar, conditioned by the same cultivar, conditioned by different species 1 and conditioned by different species 2) and cultivars on plant biomass. The aboveground biomass produced in the conditioning phase was used as a cofactor to account for possible effects of conditioning plant size differences on conditioned soils, which might have contributed to nutrient depletion. Tukey's HSD post hoc tests were performed for biomass data and soil treatments to highlight significant differences between treatments.

The raw 16S and ITS sequence reads were analysed using Dada2 (v. 1.12) (Callahan et al. 2016) and Pipits (v. 2.3) pipelines (Gweon et al. 2015). The SILVA (v.132) database was used to classify bacteria whereas the UNITE (v. 8.0;) database (Abarenkov et al. 2010) was used for the identification of fungi, and the ITSx extractor was used to extract fungal ITS regions. FUNGuild (Nguyen et al. 2016) was used to classify fungal operational taxonomic units (OTUs) into potential functions, and the assignment was further curated using an in-house database (Hannula et al. 2017). The OTUs were grouped into saprotrophs, plant pathogens, plant endophytes, arbuscular mycorrhizal (AMF), and others (i.e. fungal/animal-plant pathogens). In the case of uncertain fungal guilds, combination assignments (such as saprotroph – plant pathogen) were done. All reads not belonging to bacterial or fungal kingdoms were excluded from the datasets. To normalize our data we followed a compositional approach (Gloor et al. 2017) using the Total-Sum Scaling (TSS). To test the central hypothesis of the effects of plant species and the plant cultivar on soil microbial community structure, a PERMANOVA model was constructed using Bray–Curtis distances [vegan package in R (Oksanen et al. 2013)]. To assess the total variation explained by a variable in the model, we used the R^2 values derived from the model. Non-metric multidimensional scaling (NMDS) ordination was used to visualise the effects of plant species and cultivar within species on microbial community structure. To explore possible effects of relative abundance of microbial taxa on variation in PSFs effects, linear mixed models (lmer in R) were used to predict total plant biomass at the end of the feedback experiment from the relative abundance of each fungal and bacterial family quantified at the end of the conditioning phase. The model was built for each grass species where soil treatments, cultivar and block were set as fixed factors, whereas conditioning phase pot was used as a random factor and the relative abundance of each fungal and bacterial family as a covariate. To achieve normality of residuals, a Hellinger transformation was used. To maintain a low chance of making type I errors and therefore avoid false discoveries, we implemented the false discovery rate (FDR) approach with an FDR threshold for significance of 0.05 (pdjust function with FDR method in R) (Benjamini and Hochberg 1995; Verhoeven et al. 2005).

Results

Rhizosphere community composition between and within grass species

NMDS visualised whether the three grass species accumulated different bacterial (16S rRNA) and fungal (ITS) rhizosphere communities. The structure of both

bacterial and fungal communities was shaped by plant species that explained approximately 4% of the variation in the bacterial community and 6% of the variation in fungal community structure. When only fungi assigned to major guilds were included, 9% of the variation was explained (Fig. 2). However, plant cultivar did not significantly explain compositional differences of bacteria ($R^2=0.19$; $p>0.05$ in *L. perenne*, $R^2=0.27$, $p>0.05$ in *P. pratensis*, $R^2=0.22$, $p>0.05$ in *S. arundinaceus*), and fungi ($R^2=0.19$; $p>0.05$ in *L. perenne*, $R^2=0.28$, $p>0.05$ in *P. pratens,* $R^2=0.19$, $p>0.05$ in *S. arundinaceus*) (Figure S5). Therefore, overall soil microbial communities in general were not differently conditioned by individual cultivars of the same grass species.

Interspecific plant- soil feedbacks

To test whether soil legacies generated in the conditioning phase affected the responding plants in the feedback phase, we compared plant biomass generated in conditioned soils versus unconditioned soils. We detected overall negative PSFs (Figure S2). Specifically, biomass production was dramatically decreased when growing in conditioned soils compared to unconditioned soils both when plants were tested on soil conditioned by the same or by a different species. To address how soil microbial legacies generated in the conditioning phase affected the responding grass species in the feedback phase, we compared plant biomass production in conditioned soils and detected an overall significant soil treatment effect on plant biomass (Table S2). Further study indicated that these effects are not caused by intraspecific soil differences (no significant differences between soil conditioned by different or same cultivar in any of the species; see Fig. 3). Instead, the strongest effects were caused by interspecific PSFs. *Lolium perenne*

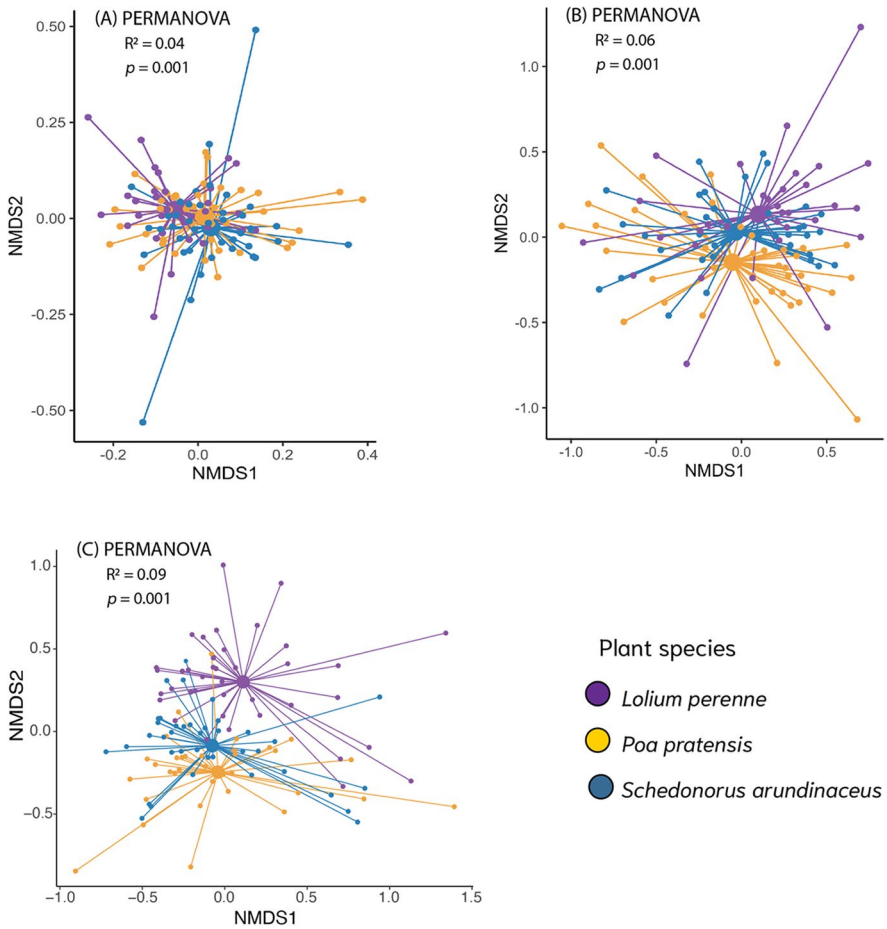

Fig. 2 NMDS based on Bray Curtis distances visualize the effects of plant species on (**A**) bacterial and (**B**) fungal community structure, and (**C**) on fungal guilds. Centroids are shown as large dots and the individual plants are displayed with small dots

plants produced more biomass in conspecific soils compared to heterospecific soils (Fig. 3A). The pattern of enhanced performance in conspecific soils was not observed in *P. pratensis* and *S. arundinaceus.* In fact, *P. pratensis* produced most biomass in heterospecific soils and particularly in soil conditioned by *L. perenne* (Fig. 3B) whereas *S. arundinaceus* did not show a strong preference between conspecific and heterospecific soils (Fig. 3C).

Intraspecific plant-soil feedbacks

Within species, different cultivars did not cause significant differences on each other's biomass production through soil conditioning (soil treatments x plant cultivar $p > 0.05$; Table S2). To test if the disproportionate biomass in the unconditioned soil might have influenced findings of the treatment comparisons, we also did our tests without the unconditioned soils. Nevertheless, also when using a statistical model per each species where the unconditioned soil had been excluded, no soil treatments x cultivar effect was detected (Table S3). Thus, we have no evidence for intraspecific specificity in PSFs.

Correlations between plant performance and microbial taxa

We explored the relationship between the relative abundances of individual microbial taxa at the end of the conditioning phase and plant growth in the feedback phase. When examining bacteria, we found significant correlations only in *P. pratensis*, and identified mainly positive correlations between plant biomass and relative abundance of the *Opituraceae* family (*Verrucomicrobia* phylum), CPla.3_termite_group family (*Planctomycetes* phylum), *Rhodospirillaceae* and *Steroidobacteraceae* families *(Proteobacteria* phylum). We observed a negative correlation between plant biomass and relative abundance of *Planococcaceae* family (*Firmicutes* phylum) (Figure S3). When examining fungi, we found significant

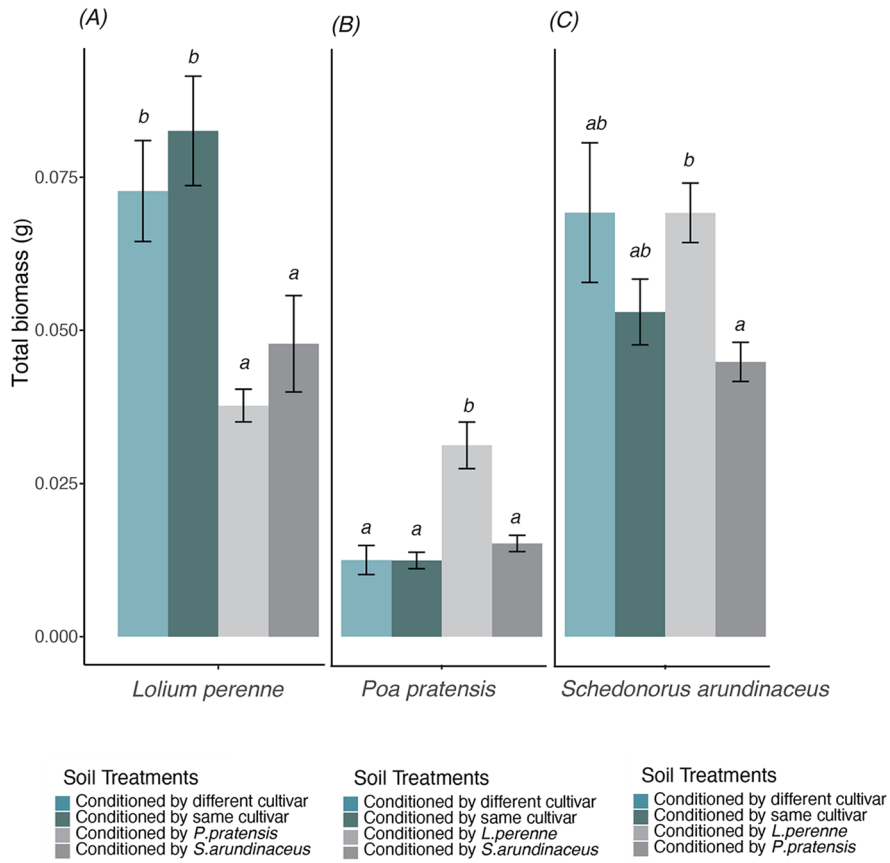

Fig. 3 Total biomass (g) in the feedback phase of (**A**) *Lolium perenne*, (**B**) *Poa pratensis*, and (**C**) *Schedonorus arundinaceus* grass species when grown on conditioned soils: conditioned by a different cultivar of the same species (light blue), conditioned by same cultivar (dark blue), conditioned by different species 1 (light grey), conditioned by different species 2 (dark grey). Bars and whiskers represent $\log_{10}$ transformed biomass + SE using a linear mixed model. Tukey's HSD post hoc tests are performed for biomass data of each species and soil treatments

correlations between plant total biomass of each grass species and the relative abundance of families belonging to the *Ascomycota* phylum. Specifically, in *L. perenne* we found a negative correlation with the relative abundance of *Nectriaceae* fungal family, in *P. pratensis* we found a positive correlation with the relative abundance of the fungal family *Magnaportaceae,* and in *S. arundinaceus* the relative abundance of the *Didymosphaeriaceae* and *Leptosphaeriaceae* families was positively correlated with plant biomass.

Discussion

Our results confirm that grass species generate microbial legacies in the soil that, in turn, affect the performance of other grass species growing later in these soils. Thus, plant-soil feedbacks (PSFs) occur among members of the grass family. We detected strong negative PSF effects between species. Furthermore, while comparing the effects of conditioned soils on plant biomass production in each species, we demonstrate that *L. perenne* enhanced its performance on conspecific soils, *P. pratensis* produced most biomass in heterospecific soils, whereas *S. arundinaceus* was not strongly impacted by the legacies of the previous plant species. Within species, the composition of the microbiome did not vary across cultivars during the conditioning phase, and we did not find evidence for intraspecific variation in PSFs.

Lolium perenne, P. pratensis and, S. arundinaceus created specific rhizosphere microbiomes that contributed to variations in the performance of the plants in the feedback phase. Specifically, we showed that the presence of live soil communities led to strong negative feedbacks in each of the three grass species compared to the unconditioned soils. Plant biomass was substantially reduced in conditioned compared to the unconditioned soil, irrespective of which plant species had conditioned the soil. These results suggest that negative interactions may have affected plant performance more strongly than positive interactions. This is in line with previous research that has shown that in similar grassland ecosystems negative interactions dominated by pathogenic microorganisms drive plant community dynamics (Heinen et al. 2020; Hannula et al. 2021). However, in our approach the substantial difference between the unconditioned and the conditioned soils overwhelmed all other effects. Therefore, the question of whether soil microbes or nutrients might have caused the negative effect when compared to the unconditioned treatment cannot be answered unequivocally. While we aimed to minimize different availabilities of nutrients by providing external nutrients during plant growth in the conditioning phase, it cannot be excluded that nutrient depletion of the soils in the conditioning phase has contributed to a negative growth effect of conditioned soils relative to unconditioned soils, which received nutrients as well. Therefore, it appears that differences between unconditioned and conditioned soils will have been caused by a combination of nutrient limitation and negative biotic interactions. However, when using the plant biomass of the conditioning phase as covariate in our statistical model, we observe no significant effect suggesting that nutrient depletion might not be a dominant factor.

The consequences for the performance of a specific plant species may depend on whether the soil has a predominant legacy of its own (conspecific), or of other (heterospecific) plant species. There is increasing awareness that most plant species show enhanced performance when growing in soil with a legacy of heterospecific plants relative to soil with a legacy of conspecific plants (Kulmatiski and Kardol 2008). This appears to be especially true for grass species. However, in the present study, the biomass production of *P. pratensis* was promoted only by the soil of *L. perenne* (heterospecific), whereas biomass production was negatively affected by conspecific soils. This suggest that within a grassland community, *P. pratensis* may be advantaged not only by PSFs from the other grasses (positive heterospecific feedback), but also by generating negative feedbacks towards other grass species (negative heterospecific feedback). Nevertheless, *P. pratensis* was less productive than the other species because it created soils that decreased its own growth (negative conspecific feedback), which might not be suitable for breeding purposes. While negative conspecific feedbacks maintain plant diversity in grassland ecosystems, they could also reshuffle plant spatial distribution (in't Zandt et al. 2021). In fact, it had been shown that some grass species may escape soil-borne pathogens overcoming negative effects on plant growth by occupying different soil patches overtime (Vincenot et al. 2017; Real and McElhany 1996; Thakur et al. 2021).

Interestingly, while the feedbacks observed in *P. pratensis* support previous findings on other grass species, *L. perenne* species did not support this general pattern, as it produced most biomass in conspecific soils. This might suggest that in *L. perenne*, beneficial microorganisms such as mutualists or plant growth-promoting rhizobacteria had a relatively greater impact on plant performance than pathogens. These findings suggest that the PSFs might allow *L. perenne* to thrive in monoculture. However, we cannot exclude that *L. perenne* could be disadvantaged in a diverse community as its microbiome might enhance biomass production of other grass species. In order to assess consequences of PSFs for grass community dynamics in more detail, further studies are needed comparing plant-soil community effects to individual effects (Van der Putten and Peters 1997). Similar studies may also help to test consequences for plant community overyielding in grass mixtures compared to a single grass species (Maron et al. 2011).

Schedonorus arundinaceus did not exhibit a strong preference towards conspecific or heterospecific soils, suggesting potential neutral PSFs. As a result of neutral PSF, *S. arundinaceus* species may be effective competitors in grassland ecosystems, which may eventually lead to a plant's tolerance towards a wide range of biotic environmental factors, such as damage by aboveground herbivores (Fraser and Grime 1998). However, when comparing heterospecific soils, it matters what species had previously conditioned the soil. In fact, *S. arundinaceus* produced more biomass in soil conditioned by L. *perenne* than in soil conditioned by *P. pratensis.*

To explore the potential underlying causes, we correlated the relative abundances of individual microbial taxa at the end of the conditioning phase with the plant biomass production of the feedback phase. Such correlations between relative abundance and plant biomass might identify microbial taxa that are candidates for driving the observed PSF effects. We demonstrated that the relative abundance of the *Necritriaceae* fungal family, which include well known plant pathogens, is negatively correlated with plant biomass production of *L. perenne* but not with the other two species. Possibly, *Necritriaceae* could be specialized pathogens of *L. perenne* that hardly interfere with plant growth of other plant species. In the other two grass species we observed positive correlations between the relative abundance of specific fungal families and plant biomass production. Some of the families, such as the *Magnaporthaceae* family, have been identified in previous studies (Hannula et al. 2021). Although *Magnaporthaceae* include many pathogenic members it is possible that non-target pathogens have relatively positive effects on plant growth (Cortois et al. 2016), or that the plants were protected by symbionts which allowed the pathogens to multiply without harming the plants.

We also observed positive correlations between bacterial members of *Proteobacteria* phylum and the biomass of *P. pratensis*. The *Rhodospirillaceae* family, for example, include members with the ability to colonize the roots and promote plant growth and development (Chabot et al. 1996; Antoun et al. 1998). We identified members of the *Firmicutes* phylum which are known to be part of the plant growth promoting rhizobacteria (PGPR) community and although several studies show their involvement in promoting plant growth, we found a negative correlation with the plant biomass production. We cannot exclude that these bacterial families include members that could indirectly generate negative effects on plant performance. We acknowledge that correlations do not imply causality and therefore further tests with the bacterial and fungal taxa identified are needed to tease biological effects apart from nutrient depletion and unravel further potential microbial candidate of PSFs.

Grass cultivars did not create a clear distinct rhizosphere microbiome and consistently, the soil communities generated by different grass cultivars at the end of the conditioning phase did not differentially affect the growth of the cultivars in the feedback phase. This is in contrast with previous studies that showed, for instance in the model species *A. thaliana*, that genotypes differed in the direction and strength of feedback due to genotype-specific soil communities. Here, we used ten cultivars per each species, and despite this being a relatively high number compared to other studies, the genetic differences between the cultivars might not have been large enough to cause differences in plant growth. To minimize possible bias in assessing PSF effects within species relative to the PSF effects between species, we did not select the cultivars based on previous existing knowledge of growth difference between cultivars.

One factor that might have affected our results is that commercial grass cultivars are not always genotypes; in fact, cultivars of *S. arundinaceus* and *L. perenne* are a mix of different genotypes and therefore they contain substantial amounts of segregating

variation which makes it difficult to pinpoint genotypes-level effects. However, *P. pratensis* had a much narrower genetic profile than the other two species, yet we did not find evidence for intraspecific variation in PSFs. Nevertheless, even when PSFs within species might exist in these species, the effects were clearly not strong enough to be expressed in our experimental design. Therefore, our results suggest that in the studied grass species, there may be limited scope for breeding on plant traits that are mediated by PSFs.

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Author contribution P.R., K.J.F.V., J.K AND W.H.v.d.P. designed the study; P.R. and F.C.H conducted the greenhouse study and laboratory work; S.E.H. conducted the fungal annotations, P.R., S.E.H, J.K.V., J.K, and W.H.v.d.P. discussed the data analysis; P.R. lead the writing of the manuscript; all authors contributed critically to the drafts and gave final approval for publication.

Data availability The raw sequencing data generated in this work can be accessed through the European Nucleotide Archive (ENA) under project PRJEB59473. Plant biomass data are deposited into Zenodo (https://doi.org/10.5281/zenodo.7575226).

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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