



The temporal development of plant-soil feedback is contingent on competition and nutrient availability contexts

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Abstract

Strength and direction of plant-soil feedback (PSF), the reciprocal interactions between plants and soil, can change over time and have distinct effects on different life stages. PSF and its temporal development can also be modified by external biotic and abiotic factors such as competition and resource availability, yet most PSF research is conducted in simple experimental settings without considering temporal changes. Here I have studied the effect of different competitive settings (intraspecific, interspecific, and no competition) and nutrient addition on the magnitude and direction of biomass-based PSF (performance in conspecific relative to heterospecific inoculum) across 46 grassland species, estimated at the 4th, 10th, and 13th month of the response phase. I also examined whether conspecific inoculum had a long-term effect on plant survival at the 36th month, and whether biomass-based PSF may predict survival-based PSF effects. PSF pooled across all treatments and time points was negative, but a significant overall temporal trend or differences among competitive settings were missing. PSF developed unimodally for interspecific competition across the three time points, whereas it declined gradually in case of intraspecific and no competition. Nutrient addition attenuated negative biomass-based PSF and eliminated negative effects of conspecific inoculum on survival. Interspecific differences in biomass-based PSF were related to survival-based PSF, but only after nutrient addition. This study demonstrates that PSF is dynamic and modulated by external abiotic and biotic factors. PSF research should consider the temporal dynamics of focal communities to properly estimate how PSF contributes to community changes, preferably directly in the field.

Keywords Multispecies experiment · Nutrient addition · Plant competition · Plant-soil feedback · Temporal variation

Introduction

Understanding the mechanisms driving variation in plant species' abundance, their coexistence or exclusion, and changes in their distribution ranges are the principal research topics of current plant ecology research. Mounting evidence supports the view that the above patterns and processes are to a large extent driven by reciprocal effects of plants on biotic and abiotic soil characteristics, a phenomenon termed plant-soil feedback (PSF; Bever et al. 1997; van der Putten et al. 2013; but see Reinhart et al. 2021). The booming PSF research is typically based on conditioning and response

experimental phases that use conspecific and heterospecific individuals to condition the soil, and then study the plants' response to this trained soil (Bever et al. 2010). Most studies conclude that species are more suppressed by conspecific-trained soil relative to heterospecific-trained soil (resulting in a negative PSF), thereby preventing species from becoming monodominant and thus maintaining diversity of plant species communities (Bever 2003; Kulmatiski et al. 2008; Petermann et al. 2008).

Despite overwhelming evidence for negative PSF, Kardol et al. (2013) and other researchers (Hawkes et al. 2013; Bezemer et al. 2018; Dudenhöffer et al. 2018) have questioned this pattern. They assumed that because most PSF studies are short, they are biased toward detecting a negative PSF. Kardol et al. (2013) in their review study have shown that studies with longer response phases (12 months and longer) are less likely to detect negative PSF, possibly because the effect of species used in the soil conditioning phase diminishes over time. Another issue raised is that a

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single endpoint assessment of PSF based on biomass harvest cannot capture the dynamic nature of reciprocal effects of plants on soil, and thus cannot predict long-term plant community dynamics. Indeed, Hawkes et al. (2013), who assessed strength and direction of PSF over a period of 19 months during four harvests, found that PSF became progressively more negative in native grasses, but switched from neutral to positive in non-native grasses. Dudenhöffer et al. (2018) then assessed the PSF across different life stages and found that self-trained soil had positive effects on juvenile life stages, but became neutral and ultimately negative at more advanced life stages (see also Aldorfová et al. 2020). We still poorly understand the long-term effects of PSF on plant performance, and this needs to be improved by exploring PSF over longer time periods and across different life-history stages of plants (Kulmatiski et al. 2017; Kulmatiski 2019).

External biotic and abiotic factors such as competition or nutrient availability can significantly modify PSF (Smith-Ramesh and Reynolds 2017; Lekberg et al. 2018; De Long et al. 2019; Klinerová and Dostál 2020). Competition can exacerbate the negative PSF effects (Lekberg et al. 2018) as performance of weak competitors can further decrease in self-trained soil when they compete against strong heterospecific competitors (Bezemer et al. 2018). Likely, not only presence, but also type of competitors can control the strength of PSF. Casper and Castelli (2007) and Maron et al. (2016) have argued that intraspecific rather than interspecific competitors will exacerbate the negative PSF by culturing and thus increasing the abundance and effects of specialized soil pathogens present in self-trained soil, though evidence for this assumption remains mixed (Casper and Castelli 2007; Kardol et al. 2007; Petermann et al. 2008).

Similarly, it remains unclear how soil nutrient availability affects the strength of PSF (but see Manning et al. 2008 or McCarthy-Neumann and Kobe 2019). High nutrient supply is assumed to promote pathogens rather than mutualists (Johnson et al. 1997; van der Putten et al. 2016), resulting in more negative PSF, but the opposite effect is expected if more nutrients boost plant immunity and thus defense against pathogens (Smith-Ramesh and Reynolds 2017). We still know little about competition and nutrients, and their interactive effects on PSF, as most previous research was conducted on plants grown in isolation and at a single nutrient level (but see De Deyn et al. 2004). More complex experimental settings are thus needed to study PSF, while explicitly considering the temporal context of competition and nutrient effects on PSF, as both factors can differentially and dynamically influence plant performance and biotic interactions over time (Bezemer et al. 2018; Trinder et al. 2012).

Here, I present results of an extensive and long-term garden study that investigated temporal development of

PSF across 46 grassland plant species. PSF was investigated in a complex experimental setting by including or omitting competition (both interspecific and intraspecific) and nutrient addition. Whereas a previous study (Klinerová and Dostál 2020) based on the same experiment compared relative strength of competition, PSF, and their joint effect at a single time point, here I want to investigate the temporal development of PSF across three time points of the response phase (at 4, 10, and 13 months). I hypothesized that the strength of the PSF would decline with the duration of the response phase (Kardol et al. 2013). However, I expected that the temporal development of PSF would differ between plants grown in isolation and those grown in the presence of competitors. Specifically, I expected that PSF would be increasingly more negative in the presence of intraspecific competitors, because of accumulation of specialized soil pathogens cultured by conspecific competitors (Casper and Castelli 2007; Maron et al. 2016). With respect to nutrient addition, previous studies showed that higher nutrient supply weakened the strength of PSF in the case of plants grown in isolation (Gustafson and Casper 2004; Petermann et al. 2008; Wubs and Bezemer 2018; Klinerová and Dostál 2020). I expected a different effect of nutrients on PSF of plants grown in competition, as more available nutrients may alter competitive interactions among species (Rajaniemi 2002; DeMalach et al. 2016), and thus indirectly also the strength of PSF.

In the second part of this study, I analyzed the long-term legacy of soil conditioning and nutrient addition on plant persistence. Specifically, I checked survival of the experimental plants at 36 months of the response phase growing in different types of soil inoculum (conspecific and heterospecific inoculum) and distinct competitive settings (no, intra- and interspecific competition), with and without nutrients added 25 months prior to survival check. I was interested in whether inoculum and nutrient treatments might have a long-lasting effect on plant persistence, and whether their effects depended on competitive settings. Finally, I asked whether interspecific variation in short-term, biomass-based PSF estimates could serve as a reliable proxy for the variation in the long-term PSF effects on plant survival.

By doing so, I wanted to answer these research questions:

1. Does PSF strength change over time, and does this temporal change depend on competition and nutrient availability?
2. Does addition of conspecific inoculum have a long-term effect on survival of conspecific individuals, and is this effect modulated by competition and nutrient availability?
3. Is interspecific variation in biomass-based PSF measured in early response phases related to the variation

in survival-based PSF measured in a more advanced response phase?

Materials and methods

This study is based on data from a large garden experiment (Klinerová et al. 2018; Dostál et al. 2019; Klinerová and Dostál 2020) conducted at the experimental garden of the Institute of Botany CAS in Průhonice, Czech Republic (50°00' N, 14°34' E). This experiment consisted of two phases (Online Resource 1, Fig. S1) and involved 46 herbaceous perennial plant species from semi-dry to mesic grasslands of Central Europe (see Online Resource 1, Table S1 for their overview). In the first (conditioning) phase, monocultures of each species were grown from June 2015 to May 2016 in 2.75-L pots ($n = 6$ pots per species) filled with a mixture of field-collected topsoil (obtained at a mesic grassland), sand, and compost (2:2:1). Monoculture pots were prepared by planting 12 conspecific seedlings per pot. However, fewer seedlings per pot were planted for approximately half of the species due to poor germination (Klinerová and Dostál 2020). At the end of May 2016 (i.e., after one year of the soil conditioning), above-ground biomass was harvested, and main roots removed from the pots. The soil conditioned by the same species was then pooled and used in the second (feedback) phase. Due to a large number of pots in the feedback phase (described further), it was not feasible to use independent soil samples (Reinhart and Rinella 2016) from the conditioning phase. Further, I was interested in the mean effects of conspecific—relative to heterospecific-trained soil across the study species, rather than in differences in the inocula effects among the species. This justifies pooling of the conditioned soil (Cahill et al. 2017).

In the feedback (response) phase initiated in June 2016, 0.15 l of living soil inoculum from the first phase was added to 2.75 l pots filled with steam-sterilized soil mixture as used in the conditioning phase (sterilized at 150 °C for 12 h). Two types of soil inoculum were used: one conditioned by the same species (conspecific inoculum) and the other one prepared by mixing first-phase soils of all species with equal volume proportions of the species-specific soils (heterospecific inoculum). The pots with conspecific and heterospecific inoculum were used for establishment of three community types for each study species (Online Resource 1, Table S2): (i) growth without competition, where a single seedling was planted; (ii) growth in intraspecific competition, where a seedling was planted together with eight seedlings of the same species, and (iii) growth in interspecific competition, where a seedling of a study species was planted in the pot center and was surrounded by eight seedlings of a heterospecific competitor. The two heterospecific competitors used, *Arrhenatherum elatius* and *Origanum vulgare*, were never

mixed in the planted communities. Both species are widespread and locally abundant (Chytrý and Rafajová 2003), so they are likely to interact with the study species under field conditions. *A. elatius* is a grass from nutrient-rich grasslands, whereas *O. vulgare* is a forb from unproductive environments, and thus the two are likely to represent distinct competition strategies.

For each study species, 36 communities were prepared (i.e., pots; Online Resource 1, Table S2): 12 pots with plants grown without competition, 8 pots with plants grown in intraspecific competition and 16 pots with plants grown in interspecific competition (with *A. elatius* in half of the pots and *O. vulgare* in the other half). The soil inoculum treatment was factorially crossed with the nutrient addition treatment. Nutrient addition was conducted in August 2016 and again in May 2017, and included fertilization by 6 g slow release fertilizer pellets (N:P:K 15:9:12 + 2.5MgO + trace elements, longevity 5–6 months; Osmocote© Extract Standard, Everris, Geldermalsen, NL). An overview of treatment combinations together with number of replicates ($N = 2$ or $N = 3$ per species and respective treatment combinations) is provided in Online Resource 1, Table S2.

Target and competitor biomass production from the response phase was measured in October 2016, April 2017, and July 2017, i.e., 4, 10, and 13 months after the second (response) phase was initiated. In April 2017, the biomass was estimated non-destructively by counting the number of ramets multiplied by dry weight of a single ramet that was harvested from each of 12 communities/species grown without competitor. Data from the conditioning phase and from the April 2017 to July 2017 response phases have been used in previous studies (Klinerová et al. 2018; Dostál et al. 2019; Klinerová and Dostál 2020). These previous studies, however, asked different research questions and did not analyze temporal development of plant-soil feedback that is the focus of the present study.

After the July 2017 harvest, pots were kept in the experimental garden and were maintained by weeding (i.e., by removal of non-planted species from the pots) on a monthly basis. Further, in October 2017, June 2018, and October 2018, above-ground biomass was cut at a height of 10 cm above the pots and removed. In June 2019, each pot was checked for survival of target species, and the experiment was terminated. Data of survival have not been used in any previous study. During both experimental phases, potted plants were placed outside in beds of the experimental garden and maintained by spray irrigation on a regular basis. The beds were covered with a polyethylene non-woven fabric to prevent plants from rooting outside the pots. Granulated molluscicide (Limanish, Nohel Garden, Czech Republic) spread between the pots was used to deter slugs. Caterpillars, detected feeding occasionally on experimental plants, were removed manually. Despite these measures, herbivory was

occasionally observed though its extent was always small (less than 5%).

Statistical analyses

Does the strength of PSF change over time, and does the temporal change depend on competition and nutrient availability?

I used biomass of target species grown in the two types of soil inoculum to express the strength of PSF. Specifically, PSF was calculated as the natural log of the response ratio (lnRR; in the metafor package of R; Viechtbauer 2010), $PSF = \ln(B_{con}/B_{het})$ where B_{con} is the mean biomass of plants grown in conspecific inoculum, and B_{het} is the mean biomass of plants grown in heterospecific inoculum. I calculated lnRR separately for each species, treatment combination and harvest time. Negative lnRR values indicate inferior plant performance in conspecific compared to heterospecific inoculum.

In the first analysis, I used a linear mixed effects model in the nlme package (Pinheiro et al. 2020) to examine effects of competition (inter-, intra-, and no competition), time (4, 10, and 13 months of the response phase; entered as a categorical variable) and nutrient addition (with and without added fertilizer) and their interactions on the strength and direction of the PSF. Species identity was entered as a random factor. The inverse of the variance of the lnRRs was used as weighing factor. I prepared full model and used stepwise backward model selection via likelihood ratio tests to test for the significance of main effect and interactions (similarly as, for example, Müller et al. 2016 did). For significant effects, I performed multiple pairwise comparisons to test for differences among factor levels using the glht function in the multcomp package of R (Hothorn et al. 2008) (with P values adjusted for multiple testing). I checked visually homogeneity of variance by plotting standardized residuals against fitted values. I used the QQ-normal plot to check normality of the residuals.

In the second modified analysis, I used identity of heterospecific competitor (*Arrhenatherum elatius* and *Origanum vulgare*) instead of their pooled effect in interspecific competition. Finally, in the third analysis, I analyzed the temporal development in PSF separately for forbs ($N = 35$ species) and grasses ($N = 11$ species), but with identical predictors as in the first analysis.

Does addition of conspecific inoculum have a long-term effect on survival of conspecific individuals, and is this effect modulated by competition and nutrient addition?

I analyzed whether persistence of target species' populations as of June 2019 depended on addition of conspecific or

heterospecific inoculum in June 2016, i.e., I tested whether the inoculum effect was still present 36 months after the response phase had been established. In the same model, I also searched for the effect of nutrient addition (the last nutrients had been added in May 2017, i.e., 25 months prior to the survival check), competition type, and of interaction of the three factors on the survival.

In the analysis I included pots with target species' individuals still alive as of July 2017. I also restricted the analysis to species with survivors in at least 3 pots by June 2019, resulting in 39 target species that were included in the analysis (instead of 46 species included in the first study part; Online Resource 1, Table S1). To test the effect of inoculum, nutrient addition, competition and their interactions, I used the glmer function in the lme4 package (Bates et al. 2015) of R with binomial family. Species was entered as a random factor. Significance of the effects and differences among factor levels were estimated as described for the lnRR.

Is interspecific variation in biomass-based PSF measured in the early response phase related to the variation in survival-based PSF measured in a more advanced response phase?

To answer this research question, I calculated PSF as the standardized mean difference (SMD) between mean biomass of plants grown in conspecific and heterospecific inoculum, respectively. I calculated SMD (using the metafor package of R) separately for each species, harvest time (4, 10, and 13 months since the start of the response phase), and nutrient treatment (but always across all competition treatments). I further calculated PSF as the standardized mean difference (SMD) between proportion of plants surviving in conspecific and heterospecific inoculum, respectively. SMD was chosen as it allows analyzing effect sizes based on the responses with distinct distributions (Viechtbauer 2010), which was the case for this analysis. Finally, I correlated biomass-based PSF from individual biomass harvest times with survival-based PSF using Spearman's ρ . R software v.4.0.2 (R Development Core Team 2020) was used to perform all analyses of this study.

Results

Does the strength of PSF change over time, and does this temporal change depend on competition and nutrient availability?

When pooled across all treatments and time points, plant performance was significantly reduced in pots with conspecific inoculum (effect size estimate \pm standard error (SE): -0.206 ± 0.055 ; t value = -3.743 , P value < 0.001).

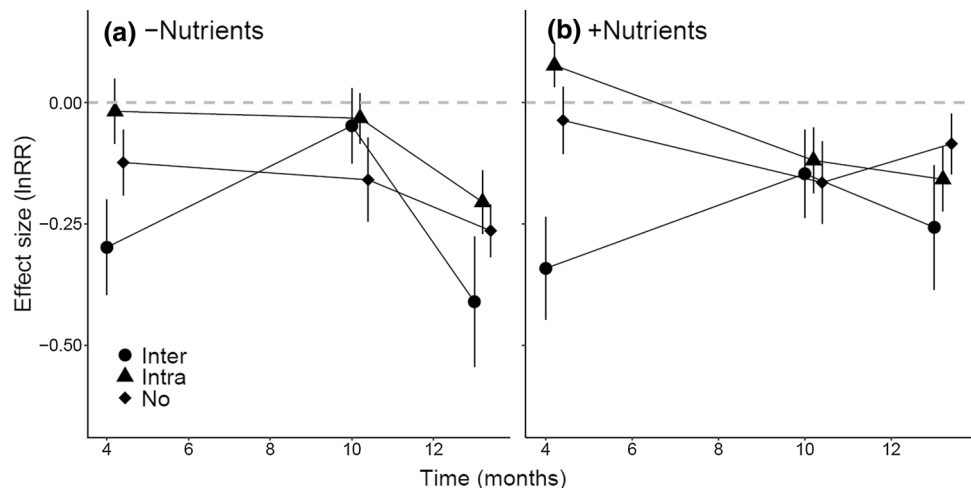
From the main effects, only nutrient addition had a significant effect on the strength of the PSF (Table 1). Specifically, PSF was less negative in fertilized than control pots, though the effect was not very strong (estimate ± SE in control pots: -0.221 ± 0.060 ; in pots with fertilizer: -0.191 ± 0.060). In addition, I detected a significant interaction between competition type and the time of PSF measurements (Table 1, Fig. 1). Specifically, plants grown with interspecific competitors developed the most negative PSF already after 4 months compared to other competition types (-0.466 ± 0.099), but this effect weakened after 10 months (-0.029 ± 0.115) to turn more negative again after 13 months (-0.219 ± 0.092 ; differences were significant or weakly significant between all three time points). In case of intraspecific competition, I observed PSF to become more negative over time (4 months: -0.002 ± 0.077 ; 10 months: -0.147 ± 0.070 ; 13 months: -0.260 ± 0.068), though the differences were not significant at $P \leq 0.05$. For plants grown without competition, the temporal trend was similar to that of intraspecific competition, without significant differences between time points (Fig. 1).

Table 1 Results of likelihood ratio tests examining the effects of competition, time, nutrients and their interactions on the strength of biomass-based plant-soil feedback of 46 species (Residual standard error = 0.265, $N = 780$)

	χ^2	<i>P</i> value
Competition	0.329	0.848
Time	0.198	0.906
Nutrients	3.881	0.049
Competition × Time	16.277	0.003
Competition × Nutrients	3.699	0.157
Time × Nutrients	4.466	0.107
Competition × Time × Nutrients	0.609	0.962

See Fig. 1 for the PSF estimates within the treatments

Fig. 1 Strength of plant-soil feedback, PSF (log response ratio, lnRR, of plant biomass from conspecific and heterospecific inoculum) pooled across 46 species grown in inter-, intraspecific and no competition, without (a) and with (b) added nutrients (mean ± SE). The PSF was estimated at the 4th, 10th, and 13th month of the response phase. Nutrient addition and interaction between competition type and time were the only significant predictors of variation in PSF (at $P \leq 0.05$)



When I used an identity of the heterospecific competitor (instead of effect of interspecific competition), I found time to be the only significant predictor of the PSF variation (Table S3 and Fig. S2). After splitting the target species into forbs and grasses, variation in PSF of forbs was significantly (at $P \leq 0.05$) related to the interactive effect of time and competition type only (Table S4; Fig. S3). In grasses, the PSF was significantly less negative after nutrient addition, but most pronounced under interspecific competition (Table S5; Fig. S4).

Does addition of conspecific inoculum have a long-term effect on survival of conspecific individuals, and is this effect modulated by competition and nutrient addition?

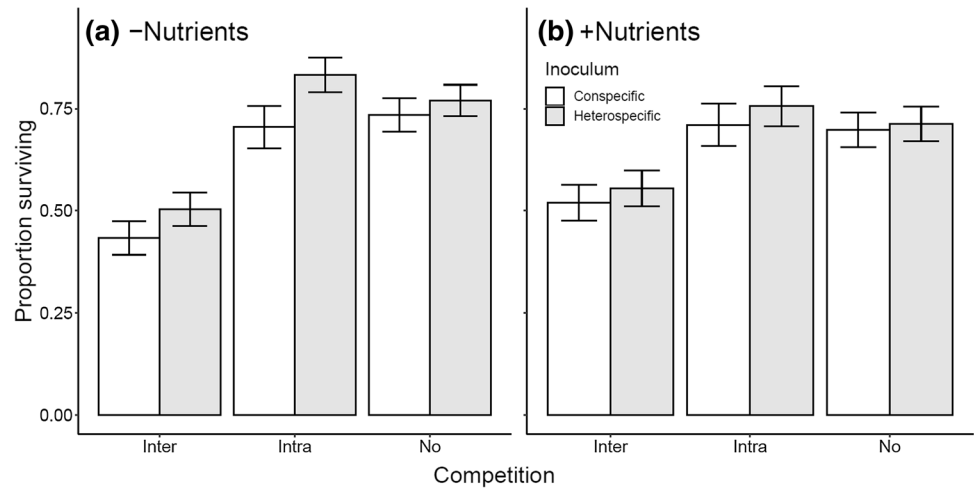
On average, 63.9 (± 1.3 SE)% individuals of those that were alive in July 2017 survived till June 2019. Plants grown in heterospecific inoculum had, however, a 5.3% higher chance of survival than plants grown in conspecific inoculum (Table 2, Fig. 2). Differences in persistence were also detected among competition types: whereas 50.0 (± 2.1

Table 2 Results of likelihood ratio tests examining the effects of competition, inoculum, nutrients and their interactions on survival probability of 39 species by June 2019 (Residual standard error = 1.612 on 1340 observations)

	χ^2	<i>P</i> value
Competition	114.310	<0.001
Inoculum	5.801	0.016
Nutrients	0.176	0.675
Competition × Inoculum	1.206	0.547
Competition × Nutrients	3.435	0.180
Inoculum × Nutrients	0.979	0.322
Competition × Inoculum × Nutrients	0.405	0.817

See Fig. 2 for the survival within the treatments

Fig. 2 Proportion of surviving individuals (mean \pm SE) of 39 species at the 36th month of the response phase shown separately for pots (a) without and (b) with added nutrients under conspecific and heterospecific inoculum treatments. Statistical significance of the differences among treatments is in Table 2. Competition type and inoculum were the only significant predictors of differences in survival (at $P \leq 0.05$)



SE)% individuals survived in interspecific competition, significantly more plants survived if grown in intraspecific and without competition, respectively [75.1 (± 1.8 SE)% and 73.9 (± 1.9 SE)%] (Table 2, Fig. 2). The effect of nutrient addition on survival was not significant, nor were its interactions significant with inoculum or competition type (Table 2). However, PSF expressed as the standardized mean difference (SMD) between proportion of plants surviving in conspecific and heterospecific inoculum indicated nutrient addition to modulate the PSF effect size. Specifically, whereas SMD pooled across all species was significantly negative in control conditions (z value = -2.452 ; P value = 0.014), nutrient addition eliminated this effect (z value = -0.824 ; P value = 0.409 ; Fig. 3).

Is interspecific variation in biomass-based PSF measured in early response phases related to the variation in survival-based PSF measured in a more advanced response phase?

Interspecific differences in biomass-based PSF were a poor predictor of variation in survival-based PSF in the absence of nutrient addition, irrespective of the time of PSF measurement (Fig. 3a). After nutrient addition, however, the relationship between the two variables proved to be significant and grew closer for later time points of PSF assessment (Fig. 3b).

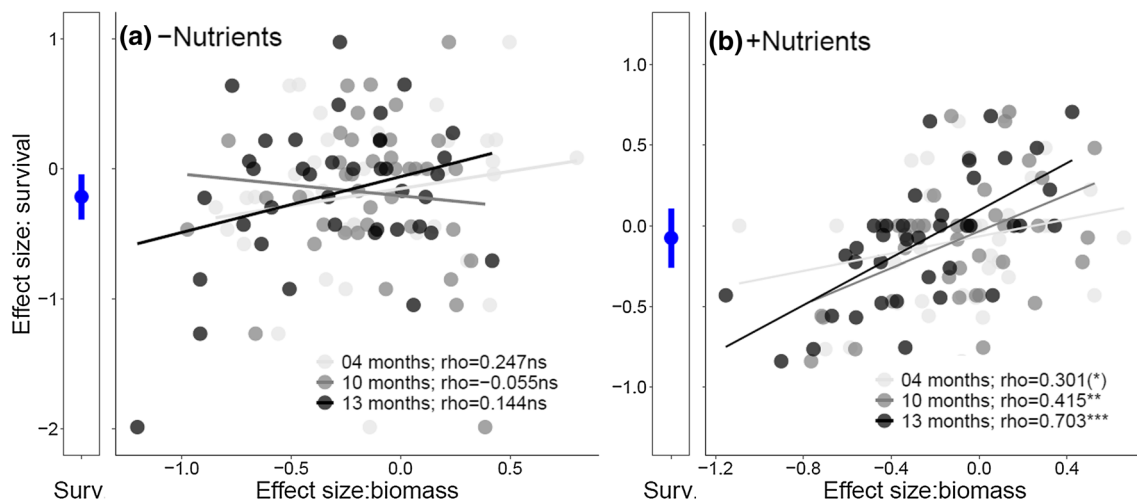


Fig. 3 Association between the strength of plant-soil feedback (PSF) effects based on biomass (the 4th, 10th, and 13th month of the response phase), and PSF effects based on survival (36th month; with Spearman's ρ of the associations) for pots (a) without and (b) with added nutrients. Effect size is expressed as a standardized mean dif-

ference calculated separately for each of 39 species. Overall effect size for survival (Surv) pooled across all species is in blue (estimate \pm 95% confidence intervals), and is provided on the left of each panel (control: $P = 0.014$; nutrient addition: $P = 0.409$). Please note the different y-axis ranges in (a) and (b)

Discussion

Here I explored how the strength of plant-soil feedback, expressed as plant performance in soil trained by conspecific versus performance in soil conditioned by heterospecific individuals, changes over time, and how the temporal development is related to competition and nutrient addition contexts. In addition, I studied whether the conspecific inoculum had a long-term effect on survival of conspecific individuals. By measuring PSF for 46 species at three time points (4, 10, and 13 months), I found that conspecific inoculum has a significantly negative effect on plant performance. However, development of PSF over time was context-dependent, and was related to competitive settings. I also found conspecific inoculum to have a long-term negative effect on plant survival, but this effect was attenuated by nutrient addition.

Though I expected that negative PSF effects would diminish over time (Kardol et al. 2013), I found no overall temporal trend in the strength of PSF. The temporal development was rather contingent on presence and type of competitors. Whereas in case of plants grown alone and in plants grown with intraspecific competitors, the PSF became more negative over time, for plants grown in interspecific competition I rather observed a unimodal change in the PSF strength, which was more negative in the 4th and 13th month of the response phase (Fig. 1). So far, the mechanisms underlying both competition and PSF temporal dynamics are poorly understood (Trinder et al. 2013), which makes it difficult to interpret the temporal PSF patterns observed for individual competitive settings. Nevertheless, I failed to find support for the assumption that PSF would be most negative for plants grown in intraspecific competition compared to no and interspecific competition, and that such an effect would intensify over time. I had assumed so as high concentration of conspecific plants was expected to increase abundance and thus also the effects of specialized soil pathogens (Casper and Castelli 2007; Maron et al. 2016). However, the overall effect of competition type on the strength of PSF was not significant (Table 1), and therefore the present study does not clarify whether competition (by inter- or intraspecific neighbors) exacerbates or rather attenuates negative PSF effects (Kardol et al. 2007; Maron et al. 2016; Petermann et al. 2008; Xue et al. 2018).

I further found that addition of conspecific inoculum had a significant long-term effect on plant persistence. Specifically, plants treated with conspecific inoculum were 5.3% less likely to survive until the 36th month of the response phase. The long-term negative effect of self-trained soil on plant persistence can decrease species' dominance and thus maintain community diversity. It has

been shown that PSF can modify competitive interactions to reduce fitness differences between interacting species (Petermann et al. 2008; Maron et al. 2016; Klinerová and Dostál 2020 but see Kandlikar et al. 2020) but PSF can also have a direct effect on plant performance. A classic example of the direct effects is reduced juvenile recruitment close to adults that harbor host-specific enemies (i.e., the Janzen-Connell Hypothesis; Janzen 1970; Connell 1971). Here, I have shown that self-trained soil, compared to heterospecific-trained soil, has a direct negative effect on survival of established adult plants, likely allowing their earlier replacement. A modeling approach is however needed to better quantify how PSF contributes directly (by affecting individual plant life stages; Dudenhöffer et al. 2018) and indirectly (by altering species' competitive ability; Kandlikar et al. 2020) to plant species coexistence or exclusion.

Importantly, nutrient addition eliminated the long-term legacy of self-trained soil on adult plants' persistence (when based on standardized mean difference; Fig. 3). Plants may thus persist longer in a patch that they have previously occupied, likely leading to dominance of stronger competitors and diversity loss in fertilized communities. Nutrient addition can reduce negative effects of PSF (but of competition as well) to a variable degree in different species as has been found in a previous study by Klinerová and Dostál (2020). Specifically, it has been shown that nutrient-demanding species (expressed by nutrient affinity of the species using Ellenberg indicator values for nutrients; Chytrý et al. 2018) benefited more from nutrient addition in terms of reduced negative biotic interactions (of competition and PSF effects) compared to species from nutrient-poor habitats (Klinerová and Dostál 2020). Here, I did not, however, observe that nutrient addition reduced negative PSF effects on survival to a larger extent in nutrient-demanding species compared to the other group of species (Online Resource 1, Table S6, Fig. S5).

In my final research question, I asked whether short-term, biomass-based estimates of PSF could predict long-term, survival-based indicators of PSF. I showed that PSF measured during early phases of the response phase (up to 13 months) poorly predicts long-term PSF effects on survival in non-fertilized conditions. Nutrient addition, however, much improved the match between the two, and even PSF differences measured as early as the 4th month were related (though weakly significantly only) to variation in survival-based PSF (Fig. 3). It seems that nutrient addition predictably structures species' variation in their PSF, and these differences persist even after fertilization stops. Nutrient context, together with the duration of the response phase, should thus be considered when exploring the PSF variation among species, and linking this variation to species' functional traits.

This study demonstrated context-dependent temporal variation in the PSF strength and its long-term legacy, namely with respect to nutrient availability and competition type, but several methodologic limitations should be borne in mind. First, the density of seedlings planted during the conditioning phase was not equal across all species (typically twelve seedlings per pot, but fewer seedlings for half of the species), which might have increased initial heterogeneity in the PSF strength among species (Klinerová and Dostál 2020) and might have also influenced temporal development of PSF. Second, I mixed the soil cultivated during the first (conditioning) phase. Such an approach can result in falsely precise or inflated estimates of the soil microbial community's effect on plant performance (Reinhart and Rinella 2016), despite some previous studies that did not confirm this confounding effect (Gundale et al. 2019; Kandlikar et al. 2020). Third, results of the study could have been affected by including conspecific-conditioned soil biota in heterospecific inoculum (similarly as Lemmermeyer et al. 2015 or Cortois et al. 2016 did). Though strongly diluted, the difference between conspecific and heterospecific effects on plant performance could have been larger had the conspecific-conditioned soil been entirely devoid of heterospecific inoculum. Finally, I cannot rule out the seasonality effects on the observed temporal development in biomass-based PSF: whereas sampling at the 10th month of the response phase was done in April, i.e., during early growing season, sampling at the 13th month was done in July, i.e., during vegetation peak possibly with a greater soil microbial activity.

Given the temporal variation in the strength and direction of PSF found in this study, what is the optimal time frame for studying it then? Hawkes et al. (2013) suggested that it depends on both the time scale over which PSF varies and the life stages at which feedback is most important to plant fitness. Temporal variation in the strength and direction of PSF is likely to influence temporal development and output of competition among heterospecific individuals (as it has been suggested that transient dynamics determine output of asymmetric competition for light; Herben 2016), and the PSF variation itself should thus not be ignored (Bezemer et al. 2018). Optimal timing for measuring PSF can also depend on the community in question. In some communities such as abandoned old fields, species abundance and composition may change very fast whereas more mature communities such as dry grasslands may remain relatively stable over the same time (Klironomos 2002; Maron et al. 2016; Chung et al. 2019). Shorter-term PSF may thus be relevant for the former but not the latter types of communities. PSF research should therefore reflect the temporal framework of the focal community dynamics to properly estimate how PSF contributes to the dynamics and consider the real-world complexity such as heterogeneity in resource availability (Bennett and Klironomos 2019).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00442-021-04919-6>.

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Author contribution statement PD designed the study, performed the experiment, analyzed the data and wrote the manuscript.

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Data availability The data used in this study are available from the author upon request.

Declarations

Conflict of interest The author declares that he has no conflict of interest.

Ethics approval Not applicable.

References

- Aldorfová A, Knobová P, Münzbergová Z (2020) Plant–soil feedback contributes to predicting plant invasiveness of 68 alien plant species differing in invasive status. *Oikos* 129:1257–1270. <https://doi.org/10.1111/oik.07186>
- Bates D, Mächler M, Bolker B, Walke S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bennett JA, Klironomos J (2019) Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. *New Phytol* 222:91–96. <https://doi.org/10.1111/nph.15603>
- Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytol* 157:465–473. <https://doi.org/10.1046/j.1469-8137.2003.00714.x>
- Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *J Ecol* 85:561–573. <https://doi.org/10.2307/2960528>
- Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M (2010) Rooting theories of plant community ecology in microbial interactions. *Trends Ecol Evol* 25:468–478. <https://doi.org/10.1016/j.tree.2010.05.004>
- Bezemer TM, Jing J, Bakx-Schotman JMT, Bijleveld E-J (2018) Plant competition alters the temporal dynamics of plant–soil feedbacks. *J Ecol* 106:2287–2300. <https://doi.org/10.1111/1365-2745.12999>
- Cahill JF, Cale JA, Karst J, Bao T, Pec GJ, Erbilgin N (2017) No silver bullet: different soil handling techniques are useful for different research questions, exhibit differential type I and II error rates, and are sensitive to sampling intensity. *New Phytol* 216:11–14. <https://doi.org/10.1111/nph.14141>
- Casper BB, Castelli JP (2007) Evaluating plant–soil feedback together with competition in a serpentine grassland. *Ecol Lett* 10:394–400. <https://doi.org/10.1111/j.1461-0248.2007.01030.x>

- Chung YA, Collins SL, Rudgers JA (2019) Connecting plant–soil feedbacks to long-term stability in a desert grassland. *Ecology* 100:e02756. <https://doi.org/10.1002/ecy.2756>
- Chytrý M, Rafajová M (2003) Czech national phytosociological database: basic statistics of the available vegetation-plot data. *Preslia* 75:1–15
- Chytrý M, Tichý L, Dřevojan P, Sádlo J, Zelený D (2018) Ellenberg-type indicator values for the Czech flora. *Preslia* 90:83–103. <https://doi.org/10.23855/preslia.2018.083>
- Connell JH (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: den Boer PJ, Gradwell GR (eds) *Dynamics of populations*. Center for Agricultural Publishing and Documentation, pp 298–312
- Cortois R, Schröder-Georgi T, Weigelt A, van der Putten WH, De Deyn GB (2016) Plant–soil feedbacks: role of plant functional group and plant traits. *J Ecol* 104:1608–1617. <https://doi.org/10.1111/1365-2745.12643>
- De Deyn GB, Raaijmakers CE, van der Putten WH (2004) Plant community development is affected by nutrients and soil biota. *J Ecol* 92:824–834. <https://doi.org/10.1111/j.0022-0477.2004.00924.x>
- De Long JR, Fry EL, Veen GF, Kardol P (2019) Why are plant–soil feedbacks so unpredictable, and what to do about it? *Funct Ecol* 33:118–128. <https://doi.org/10.1111/1365-2435.13232>
- DeMalach N, Zaady E, Weiner J, Kadmon R (2016) Size asymmetry of resource competition and the structure of plant communities. *J Ecol* 104:899–910. <https://doi.org/10.1111/1365-2745.12557>
- Dostál P, Tasevová K, Klinerová T (2019) Linking species abundance and overyielding from experimental communities with niche and fitness characteristics. *J Ecol* 107:178–189. <https://doi.org/10.1111/1365-2745.13005>
- Dudenhöffer J-H, Ebeling A, Klein A-M, Wagg C (2018) Beyond biomass: soil feedbacks are transient over plant life stages and alter fitness. *J Ecol* 106:230–241. <https://doi.org/10.1111/1365-2745.12870>
- Gundale MJ, Wardle DA, Kardol P, Nilsson M-C (2019) Comparison of plant–soil feedback experimental approaches for testing soil biotic interactions among ecosystems. *New Phytol* 221:577–587. <https://doi.org/10.1111/nph.15367>
- Gustafson DJ, Casper BB (2004) Nutrient addition affects AM fungal performance and expression of plant/fungal feedback in three serpentine grasses. *Plant Soil* 259:9–17. <https://doi.org/10.1023/B:PLSO.0000020936.56786.a4>
- Hawkes CV, Kivlin SN, Du J, Eviner VT (2013) The temporal development and additivity of plant–soil feedback in perennial grasses. *Plant Soil* 369:141–150. <https://doi.org/10.1007/s11104-012-1557-0>
- Herben T (2016) Size asymmetry of resource competition and the structure of plant communities: commentary on DeMalach et al. 2016. *J Ecol* 104:911–912. <https://doi.org/10.1111/1365-2745.12591>
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical J* 50:346–363. <https://doi.org/10.1002/bimj.200810425>
- Janzen DH (1970) Herbivores and the number of tree species in tropical forests. *Am Nat* 104:501–508
- Johnson NC, Graham J-H, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytol* 135:575–586. <https://doi.org/10.1046/j.1469-8137.1997.00729.x>
- Kandlikar G, Yan X, Levine JM, Kraft NJB (2020) Quantifying microbially mediated fitness differences reveals the tendency for plant–soil feedbacks to drive species exclusion among California annual plants. *bioRxiv* 3:2020.02.13.948679. <https://doi.org/10.1101/2020.02.13.948679>
- Kardol P, Cornips NJ, van Kempen MML, Bakx-Schotman JMT, van der Putten WH (2007) Microbe-mediated plant–soil feedbacks causes historical contingency effects in plant community assembly. *Ecol Monogr* 77:147–162. <https://doi.org/10.1890/06-0502>
- Kardol P, De Deyn GB, Laliberté E, Mariotte P, Hawkes CV (2013) Biotic plant–soil feedbacks across temporal scales. *J Ecol* 101:309–315. <https://doi.org/10.1111/1365-2745.12046>
- Klinerová T, Dostál P (2020) Nutrient-demanding species face less negative competition and plant–soil feedback effects in a nutrient-rich environment. *New Phytol* 225:1343–1354. <https://doi.org/10.1111/nph.16227>
- Klinerová T, Tasevová K, Dostál P (2018) Large generative and vegetative reproduction independently increases global success of perennial plants from Central Europe. *J Biogeogr* 45:1550–1559. <https://doi.org/10.1111/jbi.13236>
- Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417:67–70. <https://doi.org/10.1038/417067a>
- Kulmatiski A (2019) Plant–soil feedbacks predict native but not non-native plant community composition: a 7-year common-garden experiment. *Fron Ecol Evol* 7:326. <https://doi.org/10.3389/fevo.2019.00326>
- Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant–soil feedbacks: a meta-analytical review. *Ecol Lett* 11:980–992. <https://doi.org/10.1111/j.1461-0248.2008.01209.x>
- Kulmatiski A, Beard KH, Norton JM, Heavilin JE, Forero LE, Grenzer J (2017) Live long and prosper: plant–soil feedback, lifespan, and landscape abundance covary. *Ecology* 98:3063–3073. <https://doi.org/10.1002/ecy.2011>
- Lekberg Y, Bever JD, Bunn RA, Callaway RM, Hart MM, Kivlin SN, Klironomos J, Larkin BG, Maron JL, Reinhart KO, Remke M, van der Putten WH (2018) Relative importance of competition and plant–soil feedback, their synergy, context dependency and implications for coexistence. *Ecol Lett* 21:1268–1281. <https://doi.org/10.1111/ele.13093>
- Lemmermeyer S, Lörcher L, van Kleunen M, Dawson W (2015) Testing the plant growth–defense hypothesis belowground: do faster-growing herbaceous plant species suffer more negative effects from soil biota than slower-growing ones? *Am Nat* 186:264–271. <https://doi.org/10.1086/682005>
- Manning P, Morrison SA, Bonkowski M, Bardgett RD (2008) Nitrogen enrichment modifies plant community structure via changes to plant–soil feedback. *Oecologia* 157:661–673. <https://doi.org/10.1007/s00442-008-1104-0>
- Maron JL, Laney Smith A, Ortega YK, Pearson DE, Callaway RM (2016) Negative plant–soil feedbacks increase with plant abundance, and are unchanged by competition. *Ecology* 97:2055–2063. <https://doi.org/10.1002/ecy.1431>
- McCarthy-Neumann S, Kobe RK (2019) Site soil-fertility and light availability influence plant–soil feedback. *Front Ecol Evol* 7:383. <https://doi.org/10.3389/fevo.2019.00383>
- Müller G, van Kleunen M, Dawson W (2016) Commonness and rarity of alien and native plant species – the relative roles of intraspecific competition and plant–soil feedback. *Oikos* 125:1458–1466. <https://doi.org/10.1111/oik.02770>
- Petermann JS, Fergus AJF, Turnbull LA, Schmid B (2008) Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* 89:2399–2406. <https://doi.org/10.1890/07-2056.1>
- Pinheiro J, Bates D, DebRoy S, Sarkar D; R Core Team (2020) Linear and nonlinear mixed effects models. R package version 3.1-151. Retrieved from <https://CRAN.R-project.org/package=nlme>.
- R Development Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing

- Rajaniemi TK (2002) Why does fertilization reduce plant species diversity? Testing three competition-based hypotheses. *J Ecol* 90:316–324. <https://doi.org/10.1046/j.1365-2745.2001.00662.x>
- Reinhart KO, Rinella MJ (2016) A common soil handling technique can generate incorrect estimates of soil biota effects on plants. *New Phytol* 210:786–789. <https://doi.org/10.1111/nph.13822>
- Reinhart KO, Bauer JT, McCarthy-Neumann S, MacDougall AS, Hierro JL, Chiuffo MC, Heinze J, Bergmann J, Joshi J, Duncan RP, Diez JM, Kardol P, Rutten G, Fischer M, van der Putten WH, Bezemer TM, Klironomos J (2021) Globally, plant-soil feedbacks are weak predictors of plant abundance. *Ecol Evol*. <https://doi.org/10.1002/ece3.7167>
- Smith-Ramesh LM, Reynolds HL (2017) The next frontier of plant-soil feedback research: unraveling context dependence across biotic and abiotic gradients. *J Veg Sci* 28:484–494. <https://doi.org/10.1111/jvs.12519>
- Trinder C, Brooker R, Davidson H, Robinson D (2012) Dynamic trajectories of growth and nitrogen capture by competing plants. *New Phytol* 193:948–958. <https://doi.org/10.1111/j.1469-8137.2011.04020.x>
- Trinder CJ, Brooker RW, Robinson D (2013) Plant ecology's guilty little secret: understanding the dynamics of plant competition. *Funct Ecol* 27:918–929. <https://doi.org/10.1111/1365-2435.12078>
- van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, Van de Voorde TFJ, Wardle DA (2013) Plant-soil feedbacks: the past, the present and future challenges. *J Ecol* 101:265–276. <https://doi.org/10.1111/1365-2745.12054>
- van der Putten WH, Bradford MA, Pernilla Brinkman E, van de Voorde TFJ, Veen GF (2016) Where, when and how plant–soil feedback matters in a changing world. *Funct Ecol* 30:1109–1121. <https://doi.org/10.1111/1365-2435.12657>
- Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. *J Stat Softw* 36:1–48. <https://doi.org/10.18637/jss.v036.i03>
- Wubs ERJ, Bezemer TM (2018) Temporal carry-over effects in sequential plant–soil feedbacks. *Oikos* 127:220–229. <https://doi.org/10.1111/oik.04526>
- Xue W, Bezemer TM, Berendse F (2018) Density-dependency and plant-soil feedback: former plant abundance influences competitive interactions between two grassland plant species through plant-soil feedbacks. *Plant Soil* 428:441–452. <https://doi.org/10.1007/s11104-018-3690-x>