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# Plant–soil feedback in European grasslands is phylogenetically independent but affected by plant species origin

Julia Dieskau[1,2,*], Isabell Hensen[1,2], Nico Eisenhauer[2,3], Susanne Lachmuth[1,4] and
Harald Auge[2,5]

[1]Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Halle (Saale) 06108, Germany, [2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig 04103, Germany, [3]Institute of Biology, Leipzig University, Leipzig 04103, Germany, [4]Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg 15374, Germany, [5]Department of Community Ecology, Helmholtz Centre for Environmental Research – UFZ, Halle 06120, Germany

*Corresponding author. E-mail: julia.dieskau@botanik.uni-halle.de

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## Abstract

Interspecific plant–soil feedback (PSF)—the influence of soil conditioned by one plant species on another—is key to ecosystem processes but remains challenging to predict due to complex factors like species origin and phylogenetic relatedness. These aspects are underexplored, limiting our understanding of the mechanisms driving PSFs and their broader implications for ecosystem functioning and species coexistence. To shed light on the role of plant species origin and phylogenetic distance in interspecific PSFs, we conducted a greenhouse experiment with 10 native responding species and soils conditioned by 10 native and 10 exotic species resulting in 20 species pairs. These pairs represented a range of phylogenetic distances between both species, spanning up to 270 million years of evolutionary history since their last common ancestor. Conditioning by both native and exotic species reduced biomass production, with stronger inhibition observed for native-conditioned soils. Native-conditioned soils also exhibited lower phosphorus levels, higher basal and specific respiration, and greater cation exchange capacity, base saturation, and magnesium content compared to exotic-conditioned soils. Contrary to expectations, phylogenetic distance did not influence PSFs, regardless of conditioning species origin. Our findings suggest that co-evolution drives native plants to foster microbial communities with low carbon-use efficiency, highlighting soil biota's critical role in PSFs. This advances our understanding of interactions between plant species origin and microbial communities and underlines the importance of microbial management for promoting native species and controlling invasives. The lack of phylogenetic distance effects aligns with prior studies, indicating evolutionary relatedness alone does not reliably predict PSF outcomes.

***Keywords:*** co-evolution, enemy release hypothesis, greenhouse experiment, microbial respiration, native *vs*. exotic, plant–soil interactions, soil microorganisms

## 欧洲草地植物-土壤反馈关系受物种起源的影响而与系统发育无关

摘要：植物-土壤反馈(interspecific plant–soil feedback, PSF)，即一种植物通过改变土壤条件影响另一种植物，在调控生态系统过程中起着关键作用。然而，植物物种起源和系统发育关系如何影响PSF及其相对重要性，还缺乏系统研究。为此，本研究利用温室盆栽实验，将10种本地植物分别种植于由10种本地和10种外来植物接种后的土壤，探究物种起源和系统发育距离在影响种间PSF的作用。本研究选择的植物的系统发育距离从共同祖先开始横跨2.7亿年，具有较远的进化距离。研究结果表明，本地植物和外来植物处理后的土壤均都降低了植物生物量，其中本地植物处理的土壤对生物量的抑制作用更强。这可能是因为与外来植物处理的土壤相比，本地植物处理的土壤具有较低的磷含量、较高的呼吸速率、阳离子交换能力、盐基饱和度和镁含量。然而，不论用于处理土壤的植物物种起源如何，系统发育距离均未对PSF产生显著影响，可能是因为共同进化致使本地植物驯化产生低碳利用效率的微生物群落，这一结果突出了土壤生物群在调节PSF中的关键作用。上述研究加深了学术界对植物物种起源与微生物群落交互作用的理解，并强调了微生物管理在促进本地物种繁殖和控制外来物种入侵中的重要性。此外，本研究中系统发育距离的作用较弱与先前研究一致，表明仅凭植物进化关系无法预测植物–土壤反馈关系的方向和强度。

关键词：共同进化，天敌释放假说，温室实验，微生物呼吸，本地与外来物种，植物–土壤相互作用，土壤微生物

## INTRODUCTION

Interactions between plants and the soil system are crucial for ecosystem functioning (Wardle *et al.* 2004). For example, they play a critical role in the invasion success of exotic plant species (Inderjit and van der Putten 2010), the succession of plant communities (Kardol *et al.* 2006), as well as the diversity and productivity of terrestrial ecosystems (van der Heijden *et al.* 2008). The effect of soil influenced by an early arriving species (hereafter referred to as conditioning species) on the performance of a later-arriving species (hereafter referred to as responding species) is also known as interspecific or heterospecific plant–soil feedback (hereafter referred to as interspecific PSF; van der Putten *et al.* 2013). Their strength and direction depend on various biotic and abiotic factors, which can act simultaneously, leading to either a positive or negative net effect or potentially cancelling each other out, resulting in a neutral outcome. Underlying mechanisms may include the accumulation of pathogenic or mutualistic soil biota (Bever 2003), the release of allelopathic substances (Hierro and Callaway 2003), and other soil chemical changes—such as in pH, soil moisture and nutrient levels (Ehrenfeld *et al.* 2005)—induced by the conditioning species. Since the interactions between plants and soil properties seem to depend on various factors, predicting the outcome of interspecific PSFs remains a challenge.

Interactions between plants and soil can depend, for example, on the origin of the species involved. Native and exotic species often differ in their resource-use strategies, resulting in altered nutrient cycling and availability (Vilà *et al.* 2011), and may thus differ in their PSFs on the responding species. Furthermore, plants can release allelopathic substances to which coexisting species in their native range are adapted. Conversely, species in new distribution areas may not yet be adapted to these substances and therefore may be more strongly negatively affected (Callaway and Aschehoug 2000). In invasion biology, this phenomenon is known as the novel weapons hypothesis (Callaway and Ridenour 2004). Based on this hypothesis, we would expect that the interspecific PSFs of exotic species on native species are more negative than those of native species. Moreover, compared to native species, exotic species may form different associations with soil mutualists, such as mycorrhizal fungi (Reinhart and Callaway 2006), or accumulate fewer specialized soil pathogens and herbivores (Mitchell and Power 2003). According to the enemy release hypothesis (ERH; Brian and Catford 2023; Keane and Crawley 2002; Mitchell and Power 2003; Wolfe 2002), exotic conditioning species are expected to accumulate significantly fewer soil-borne pathogens and herbivores compared to native ones. This is proposed to result in a less negative or even positive net PSF on native responding species compared to native conditioning species. However, research investigating the ERH has predominantly focussed on interactions occurring aboveground (e.g. Liu and Stiling 2006; Memmott *et al.* 2000; Wolfe 2002) and there is only a limited number of experimental studies examining the ERH from a belowground perspective (but see Andonian *et al.* 2012; Broadbent *et al.* 2018; Dieskau *et al.* 2020; Gundale *et al.* 2014; Müller *et al.* 2016; Nuske *et al.* 2021). Due to the variety of mechanisms influenced by the origin of species, we assume that the interspecific PSF differs between native and exotic species. However, because these mechanisms can have opposing effects, predicting the direction of the net effect can be challenging. Therefore, more research is needed to clarify which processes are predominant under different conditions.

The identity of the involved plant species and their relatedness can also play a significant role, as plants induce species-specific modifications in soils and react to these changes in a species-specific way (Bever 1994; Ehrenfeld *et al.* 2005). Many studies found that the PSF of a species on itself is often negative (Bever 1994; Mangan *et al.* 2010). Assuming that ecologically relevant plant traits are highly conserved (Senior *et al.* 2018) and correlate with phylogenetic distance, we can expect this effect to decrease with increasing phylogenetic distance between the involved plant species. This decrease is likely due to fewer shared specialized soil-borne pathogens (Gilbert and Webb 2007) and a reduction in the overlap in resource requirements (Godoy *et al.* 2014). Research indicating that closely related plant species can indeed share (specialized) natural enemies and resources (Gilbert and Webb 2007) supports the assumption that plants tend to perform less well in soils previously cultivated by closely related species. However, despite the significantly increased interest in belowground processes over the past decades, only a limited number of studies have explicitly tested whether phylogenetic relatedness is a useful predictor for PSFs, and these studies have produced contradictory results. While Liu *et al.* (2012), Anacker *et al.* (2014), Münzbergová and Šurinová (2015) and Kempel *et al.* (2018) found evidence supporting a relationship between phylogenetic relatedness and the strength of PSFs, Mehrabi and Tuck (2015) did not.

To address these research gaps in PSFs, we conducted a greenhouse experiment to investigate the role of origin and phylogenetic distance in interspecific PSFs. We analysed interactions within 20 plant species pairs, consisting of 10 responding species native to Germany, paired with 10 other native and 10 exotic conditioning species. These pairs comprised biennial and perennial European grassland species from various families and functional groups, covering a range of phylogenetic distances between conditioning and responding species. Based on the knowledge gaps mentioned above, this experiment aims to investigate the following questions: Firstly, does the origin of the conditioning species (native/exotic) influence differences in abiotic and biotic soil conditions and the strength and direction of the interspecific PSF on native responding species. Secondly, does the strength of interspecific PSF decrease as the phylogenetic distance between conditioning and responding species increases? Thirdly, is the phylogenetic signal weaker for exotic conditioning species compared to native ones, possibly due to their release from specialized soil biota?

## MATERIALS AND METHODS

### Design and species selection

To investigate the role of origin and phylogenetic distance in interspecific PSFs, we conducted a multispecies greenhouse experiment at the UFZ experimental station in Bad Lauchstädt. We analysed the growth of 10 native plant species from central German grassland communities, following an approach by Germain *et al.* (2016) that we recently applied in another study (Dieskau *et al.* 2024). Each of these 10 species was paired with one additional native and one exotic conditioning species (most of our exotic species were introduced to Germany around the 19th century. See Supplementary Table S1 for a complete list of the native and exotic species used, their taxonomic affiliation, life span and minimum residence time). This resulted in 20 plant species pairs (either native-native or exotic-native), comprising a total of 30 different species, including forbs, legumes and grasses. These species pairs represented a range of phylogenetic distances between the conditioning and the responding species, spanning up to 270 million years of evolutionary history since their last common ancestor (see Supplementary Table S2 for phylogenetic distance and shared community types among paired species). This design enabled us to explore the interactive effects of origin and phylogenetic distance. To ensure comparability of origins, we aimed to select conditioning species with similar distances to their respective native or exotic counterparts. Moreover, to prevent systematic bias and ensure phylogenetic independence

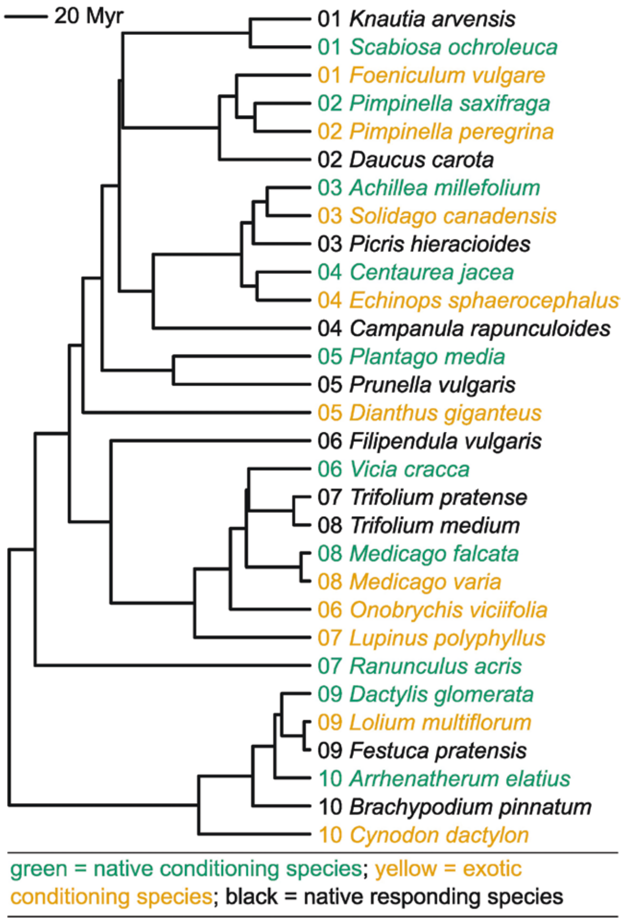

**Figure 1:** Phylogenetic tree of all experimental species with their species pair number (01–10) and origin (modified after Dieskau *et al.* 2024). Phylogenetic distances were extracted from the Daphne data set (Durka and Michalski, 2012).

among species pairs, we made efforts to minimize overlapping branches (Germain *et al.* 2016). In the few cases where this was not completely feasible, we minimized the lengths of the overlapping branches (Fig. 1). Phylogenetic distances were extracted from the Daphne data set (Durka and Michalski 2012). One species (*Dianthus giganteus*) was missing in the data set and was substituted by the most closely related congener (*Dianthus carthusianorum*) to construct the phylogeny. We purchased seeds for most species from local seed suppliers and collected seeds for *D. giganteus* and *Pimpinella peregrina* ourselves in the summer of 2016 in central Germany.

## Experimental setup

### *Conditioning phase*

The experiment started in December 2017 with the soil conditioning phase. For this purpose, 100 seeds of each of the 10 native and 10 exotic conditioning species were sown in 10 L boxes (L: 27, W: 17, H: 22 cm) with two replicates per species, resulting in 40 boxes. The boxes were equipped with drainage holes at the bottom to prevent waterlogging. To meet the specific germination requirements (previously tested in a separate germination experiment, data not shown), *Lupinus polyphyllus* seeds were covered with 1–2 cm of soil, whereas the seeds of the other species were sown on the soil surface. To accelerate germination, we covered the boxes with transparent foil until germination reached 25% (5 to 28 days, depending on the species). Since potting soil is often sterilized and less representative in terms of chemical, physical, and soil biotic properties, we decided to use the top layer of sieved unsterilized chernozem soil. Chernozem is the predominant soil type in Central German dry regions and thus representative of the grasslands in the region where seeds had been collected (for more detailed information regarding soil conditions, see Altermann *et al.* 2005). The soil used was sourced from a site at the UFZ experimental station in Bad Lauchstädt (51.3917° N, 11.8779° E), which had been cultivated as arable land for over 125 years, including regular weed control, ensuring that none of our experimental species had previously established there. Before the experiment, the soil was thoroughly mixed to ensure uniform distribution across all boxes. Additionally, we prepared two control boxes per responding species with soil that was not conditioned, using the same soil and keeping them free of any spontaneously occurring seedlings, resulting in an additional 20 boxes. Thus, we had a total of 60 boxes. These were placed into two greenhouse cabins, each of them representing one replicate. Within the greenhouse cabin, boxes were randomly assigned to two greenhouse benches. Throughout the experimental period, plants were exposed to additional light from 7 am to 7 pm to standardize light conditions within the cabins. The temperatures ranged from 15 °C at night to 20 °C during the day. Each box was watered from the top approximately every one to four days, depending on its individual requirements. The requirements varied among plant species due to their different biomasses and were assessed by estimating soil moisture by touching the substrate. After two months of growth, the plants were trimmed 4 cm above the soil surface with scissors to simulate mowing, a common management practice in central European grasslands. An additional two months were added to maximize the time during which the plants could condition the soil. This extended period facilitated the establishment of dense stands of mature plants, including some flowering individuals.

### Assessment of abiotic and biotic soil conditions

Four months after sowing, three soil samples per box were taken to gain insight into some of the abiotic and biotic soil changes caused by the different species. Samples were taken in 0–5cm depth using a spoon, sieved using a 2 mm sieve to remove small stones and plant roots and afterwards divided into two plastic bags. One sample was stored for a few days at 8 °C for analysis of the response of soil microorganisms, including basal respiration (μL $O_2$ $h^{-1}$ $g^{-1}$ soil dry weight), microbial biomass (μg Cmic $g^{-1}$ soil dry weight) and specific respiration (μL $O_2$ mg $cmic^{-1}$ $h^{-1}$). For this purpose, we used an automated $O_2$-uptake method involving the addition of glucose as a carbon substrate to measure the respiratory response of soil microorganisms, as done before (Eisenhauer *et al.* 2010; Sünnemann *et al.* 2021; for details of the method, see Scheu 1992). The other sample was air-dried and stored at room temperature. We measured the pH following Blakemore (1987) and analysed the phosphorous content (following the Egner–Riehm (DL) method). The remaining soil was dried for 72 h at 80 °C and subsequently ground in an oscillating mill (MM 400, Retsch, Haan, Germany) until they became a homogeneous powder. Five milligrams of the soil powder were used to measure soil C and N gas-chromatographically with the Dumas method (Vario EL Cube, Elementar Analysensysteme, Langenselbold, Germany), from which we further calculated the carbon to nitrogen ratio (soil C:N ratio). Furthermore, we used atomic absorption spectroscopy (AAS vario6, Analytik Jena) to determine the content of soil phosphorus, calcium, potassium, iron, and magnesium (soil P, Ca, K, Fe, Mg), cation exchange capacity and base saturation.

### Response phase

The soil from the two replicates of each conditioning species and the controls was pooled and transferred into 1-L pots. Each responding species was allocated to one pot with soil conditioned by a native species, to one pot with soil conditioned by an exotic species and to one control pot with unconditioned soil. This setup was replicated four times, resulting in a total of 120 pots. Pots were distributed across four greenhouse cabins, each cabin representing a block containing one replicate. Within each cabin, pots were randomized across two greenhouse benches. The responding species were sown with 40 seeds per pot. To avoid competition, seedlings were thinned to one individual after two weeks, and any subsequently emerging seedlings were removed every two days. To prevent the exchange of soil biota between the pots and the leaching of nutrients from top watering, pots were positioned on saucers and watered from beneath as needed every one to five days. Three months after sowing, the responding plants reached the adult stage and were partially flowering. Subsequently, aboveground biomass of responding plants was harvested and dried (72 h at 70 °C) for the calculation of the aboveground biomass for each pot.

### Statistical analysis

To quantify the magnitude and direction of interspecific PSFs, we related the aboveground biomass of adult responding plants grown in conditioned pots to those in control pots. We used log response ratios (LRR) based on mean values across the four replicate pots for these comparisons (Hedges *et al.* 1999). The use of LRR provides a statistically robust measure to determine the strength and direction of PSFs relative to unconditioned soil. Positive LRR values indicate a facilitative PSF (higher aboveground biomass of responding plants in conditioned soil), while negative values indicate an inhibitory PSF (higher aboveground biomass of responding plants in control pots).

To test the effects of origin and phylogenetic distance on the PSF, we fitted a linear mixed-effects model that contained the origin of the conditioning species (native or exotic), phylogenetic distance between conditioning and responding species (PD; centred and scaled) and all possible interactions as explanatory variables. LRRs of the aboveground biomass of responding species were used as response variable (using the package lmerTest in R, version 4.2.0, Kuznetsova *et al.* 2017). Since each responding species was sown into soil conditioned by a native and into soil conditioned by an exotic species, the responding species were treated as a random effect in the model. The significance of fixed effects was tested using the Wald type III test. In addition, we used the emmeans command from the emmeans package (Lenth *et al.* 2022) with the above model to assess whether the mean values per origin significantly deviated from zero.

Subsequently, we compared soils conditioned by native and exotic species in terms of their abiotic and biotic properties. To reduce the complexity of our soil data and account for correlations among abiotic soil characteristics, we conducted a principal component analysis (PCA) using the vegan package (Oksanen *et al.* 2001) in R (version 3.4.0, R Development Core Team 2013). This analysis included C:N ratio, pH value, base saturation, cation exchange capacity, microbial biomass, and the content of nitrogen, carbon, magnesium and phosphorus. To compare the effects of native and exotic species on soil properties, we fitted four different models that contained origin of the conditioning species as explanatory variable and either the first PCA axis (PC1), the second PCA axis (PC2), basal respiration, microbial biomass or specific respiration as responding variables.

## RESULTS

The mixed-effects models investigating the effect of origin and phylogenetic distance revealed a significant influence of plant species origin on the interspecific PSF. Conditioning by species of both origins showed an inhibitory PSF on the aboveground biomass of the responding species compared to the unconditioned control. This inhibitory effect was significantly stronger when the soil was conditioned by native species compared to exotic species (Fig. 2a; Table 1). Furthermore, the LRR of the responding species' aboveground biomass showed no correlation with phylogenetic distance (Fig. 2b), nor was there a significant interaction between phylogenetic distance and origin (Table 1). Fig. 2b shows greater data point dispersion at small phylogenetic distances, suggesting higher variability in aboveground biomass LRR among closely related species compared to distantly related ones. However, we did not statistically test this relationship.

The first two PCA axes of soil abiotic conditions collectively explained 56.6% of the variance in soil abiotic properties. The first axis (PC1, 33.5% of variation) correlated positively with base saturation, cation exchange capacity and magnesium content, and negatively with phosphorus content; the second axis (PC2, 23.1% of variation) correlated positively with C:N ratio and pH (Fig. 3). Native species conditioning led to higher values for PC1 compared to exotic species (Fig. 4a; marginally significant), while PC2 showed no difference between the two origins (Table 2). Basal respiration (Fig. 4b) and specific respiration (Fig. 4c) attained significantly lower values for soil conditioned by exotic species compared to soil conditioned by native species (Table 2). Microbial biomass did not differ between native and exotic species.

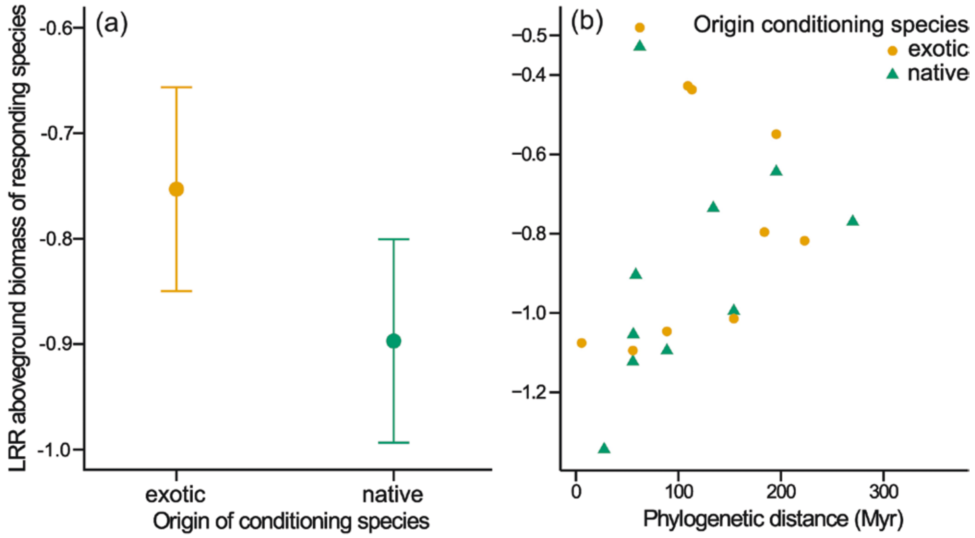

**Figure 2:** LRR for the aboveground biomass of responding species as a measure of the PSF in response to (a) soil conditioned by native and exotic species, and (b) soil conditioned by native and exotic species of different phylogenetic distance to the native responding species. It should be noted that the effect of phylogenetic distance and the interaction between phylogenetic distance and origin are not significant. Negative LRR values indicate an inhibitory PSF of conditioned soil compared to unconditioned soil, while positive values indicate a facilitative effect. The LRRs in response to native ($t = -9.529$, $df = 11.5$, $P = <0.001$) and exotic ($t = -7.988$, $df = 11.5$, $P = <0.001$) conditioning species were both significantly negative.

**Table 1:** Results of the mixed-effects model analysis assessing the impact of conditioning species origin, phylogenetic distance between conditioning and responding species and their interactions on the LRR of aboveground biomass of responding species as a measure of the PSF

| | **Log response ratio (LRR) for aboveground biomass** | | |
|---|---|---|---|
| | **Chi$^2$** | ***df*** | ***P*** |
| (Intercept) | 60.8 | 1 | <0.001 |
| Origin | 4.0 | 1 | **0.046** |
| Phylogenetic distance | 0.1 | 1 | 0.791 |
| Origin × Phylogenetic distance | 0.4 | 1 | 0.549 |

Bold *P*-values indicate significant main effects or interactions ($P < 0.05$).

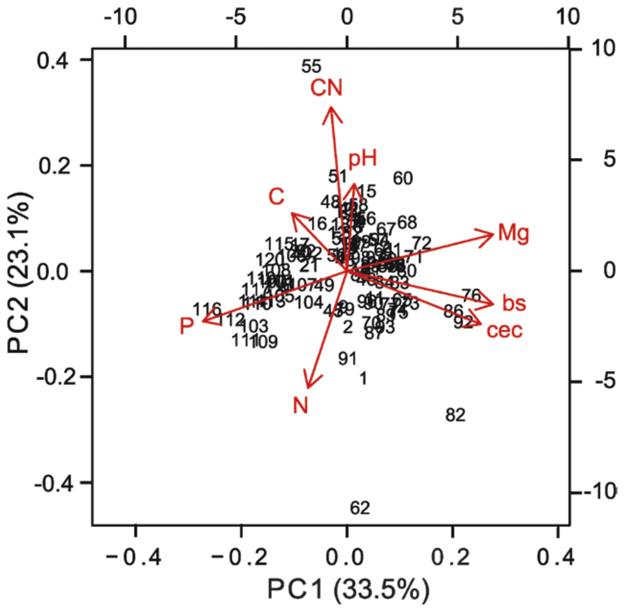

**Figure 3:** PCA ordination diagram of 1st and 2nd axis. Letters refer to soil characteristics (N = nitrogen content, C = carbon content, CN = CN ratio, Mg = magnesium content, P = phosphorus content, pH = pH value, bs = base saturation, cec = cation exchange capacity). Numbers and arrows refer to recorded variables. The first and second principal components (PC1 and PC2) accounted for 33.5% and 23.1% of the total variation, respectively.

## DISCUSSION

We investigated the significance of origin and phylogenetic distance between conditioning and responding species for interspecific PSFs. In general, soil conditioning by native and exotic species had an inhibitory effect on the productivity of the responding species. This effect was stronger for native conditionin, species. Furthermore, the effec, of nativ, and exotic conditioning species on abiotic and biotic soil properties differed. Soil conditioned by native species exhibited higher basal respiration and greater specific respiration. Additionally, it showed higher values on the first principal component axis (PC1), indicating lower phosphorus content but higher base saturation, cation exchange capacity and magnesium content compared to soil conditioned by exotic species. Contrary to our expectation, phylogenetic distance appeared to have no significant influence on the interspecific PSF, irrespective of the origin of the conditioning species.

### PSFs of exotic conditioning species are less inhibitive

The results of our study indicate that the effect of soil conditioning by both native and exotic species had an inhibitory impact on the production of aboveground biomass of the responding species. Our finding is consistent with other studies that have observed interspecific PSFs, particularly in grasslands, to have negative impacts on plant growth (Maron *et al.* 2016; Petermann *et al.* 2008). This negative net effect suggests that the impact of inhibitory mechanisms, such as those mediated by soil pathogens or the release of allelopathic substances, outweighs potential positive effects, such as those arising from soil mutualists. In our experiment, it is also quite possible that nutrient depletion contributed to the inhibitory net effects. Although the chernozem soil we used is relatively nutrient-rich, it is possible that nutrient limitation became increasingly important over the 7-month experimental period, as no additional fertilizer was applied and the PSF of the conditioning species was calculated in relation to empty control boxes that showed no plant-related changes in soil nutrient content.

As expected, the net effect differed between native and exotic conditioning species. Conditioning by native

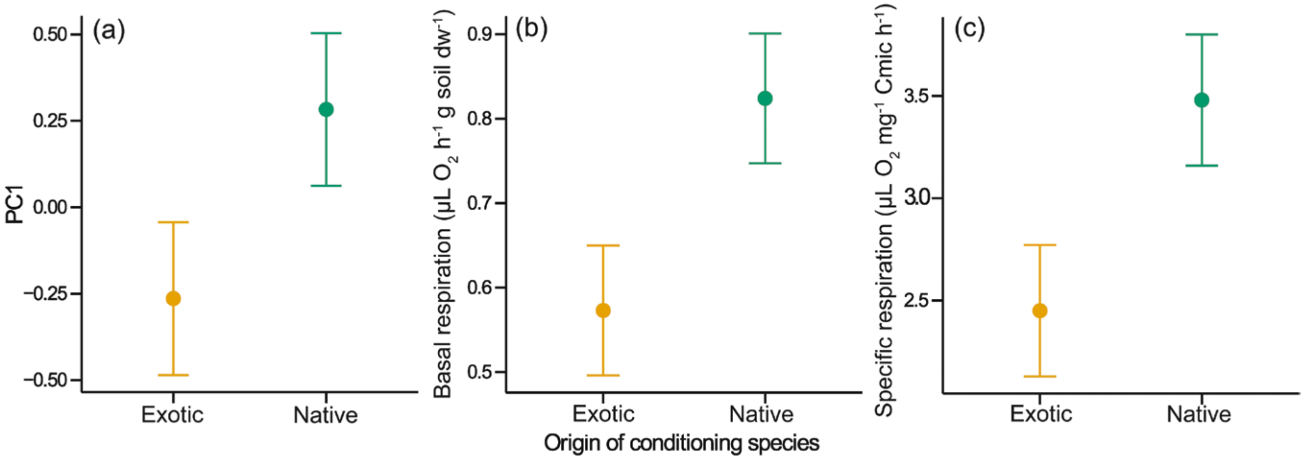

**Figure 4:** Effects of soil conditioned by native and exotic species on (a) the first axis (PC1) of a PCA of abiotic soil characteristics (see Fig. 3 for the PCA ordination diagram), (b) the basal respiration, and (c) the specific respiration. PC1 explains 33.5% of the variation in soil abiotic properties and correlates negatively with phosphorus content and positively with base saturation, cation exchange capacity and magnesium content.

**Table 2:** Results of the mixed-effects model analysis assessing the impact of the conditioning species origin on abiotic, including (a) first (PC1) and (b) second (PC2) axes of a PCA and biotic soil conditions, including (c) basal respiration, (d) microbial biomass and (e) specific respiration

| | *df* | *F* | *P* |
|---|---|---|---|
| (a) PC1 | | | |
| (Intercept) | 1 | 1.43 | 0.248 |
| Origin | 1 | 3.06 | 0.097 |
| Residuals | 18 | | |
| (b) PC2 | | | |
| (Intercept) | 1 | 0.01 | 0.925 |
| Origin | 1 | 0.1 | 0.752 |
| Residuals | 18 | | |
| (c) Basal respiration | | | |
| (Intercept) | 1 | 55.63 | <0.001 |
| Origin | 1 | 5.32 | **0.033** |
| Residuals | 18 | | |
| (d) Microbial biomass | | | |
| (Intercept) | 1 | 117.19 | <0.001 |
| Origin | 1 | 0.01 | 0.906 |
| Residuals | 18 | | |
| (e) Specific respiration | | | |
| (Intercept) | 1 | 58.41 | <0.001 |
| Origin | 1 | 5.05 | **0.037** |
| Residuals | 18 | | |

Bold *P*-values indicate significant main effects ($P < 0.05$).

species had a more pronounced inhibitory effect on the biomass production of responding species compared to exotic species. One possible explanation is that the microbial activity and community composition may vary depending on the origin of the conditioning species. This assumption is supported by our finding of a higher basal respiration and specific respiration in soils conditioned by native species compared to those conditioned by exotic species. This suggests that co-evolutionary processes may have caused native plant species to foster microbial communities more strongly dominated by fast-growing, high-metabolism microbes exhibiting elevated respiration rates, in contrast to those associated with exotic species. While a high basal respiration indicates higher microbial activity, a high specific respiration value suggests that microbes are allocating a larger proportion of carbon resources to maintenance respiration rather than biomass production (e.g. Eisenhauer *et al.* 2013). When both values are increased, this indicates that plant species promote higher overall microbial activity but under conditions that hinder efficient microbial carbon utilization. However, it is important to note that other factors may have also contributed to the observed differences. Stress, such as nutrient imbalances and microbial nutrient limitations, could have increased both basal respiration (due to higher metabolic demands) and specific respiration, as microbes redirect energy to maintain cellular functions rather than growth and reproduction (Eisenhauer *et al.* 2010). Furthermore, native plant species may have produced root exudates or litter with high-quality carbon substrates, which could stimulate microbial activity and elevate basal respiration.

Our results are consistent with other studies that have also demonstrated differences in microbial communities in soils conditioned by native and exotic plant species (Kourtev *et al.* 2002; Upton *et al.* 2020). Checinska Sielaff *et al.* (2018), for instance, found that native and exotic grasslands primarily differed in the structure and function of soil fungal community composition, and these differences were correlated with varying phosphorus mineralization rates. This aligns with our finding that soils conditioned by native species exhibited marginally significantly lower levels of plant-available phosphorus compared to those conditioned by exotic species. This difference may have contributed to the stronger suppressive PSF observed in soils conditioned by native species in our study. While other studies have found that not only native but also exotic plant species can accumulate generalist pathogens shared with native species (e.g. Waller *et al.* 2021), our data are more in line with the ERH (Brian and Catford 2023; Keane and Crawley 2002) and suggest that co-evolutionary processes may have driven a stronger specialization of soil pathogens toward plant species of the same origin. This could result in the accumulation of not only distinct specialized soil pathogens but also varying degrees of generalist soil pathogens as generalist soil organisms can also influence variations in PSFs when their effects and accumulation rates differ between plant species (Cortois *et al.* 2016; Semchenko *et al.* 2022; Wilschut *et al.* 2023). However, it must be considered that the observed differences may not necessarily result from a less inhibitory effect of exotic conditioning species, but from promoting mechanisms. As our focus was on capturing the overall net interspecific PSF, we are unable to conclusively determine the processes underlying the differing interspecific PSFs of native and exotic conditioning species in our experiment.

## PSF independent of phylogenetic distance

Contrary to our hypothesis and the findings of Liu *et al.* (2012), Anacker *et al.* (2014), Münzbergová and Šurinová (2015) and Kempel *et al.* (2018), the phylogenetic distance did not influence the strength of the interspecific PSF in our experiment, regardless of the origin of the conditioning species. Although there appears to be a trend aligning with our expectation of a stronger positive correlation between the performance of the responding species and phylogenetic distance for native conditioning species, this relationship was not statistically significant. We suggest that effects not accounted for by phylogeny may have overridden any phylogenetic effects, as the PSF varied largely even at small phylogenetic distances. Our results therefore suggest that phylogenetic distance does not have any decisive influence on how strongly the performance of the responding species is affected by soil conditioning. A possible explanation for this could be that the traits critical for PSFs are highly plasticity and are not sufficiently phylogenetically conserved. This interpretation is also supported by a meta-analysis by Mehrabi and Tuck (2015) of all available pairwise PSF experiments conducted over two decades, involving 133 plant species in 329 pairwise interactions. They found that the sign and magnitude of PSFs were not explained by the phylogenetic distance between interacting species. This result was consistent across different life forms, life cycles, provenances and phylogenetic scales.

We still caution against concluding that phylogenetic distance generally does not play a role in interspecific PSFs. We do not assume that the conditioning phase was too short to produce effects of phylogeny, as it was evidently sufficient to generate differences between native and exotic species. However, it is possible that opposing effects may cancel each other out. For example, closely related responding species might be more inhibited by pathogens accumulated by conditioning species, yet simultaneously possess better defences against allelopathic effects. Depending on which mechanism is more pronounced, there could be varying degrees of deviation in either direction, which would align with our observation of higher variability at smaller phylogenetic distances. For a clearer distinction between these mechanisms, studies involving inoculation and sterilization would be necessary. In addition, plant–microbe interactions are influenced by various environmental factors, making them highly context-dependent. These factors can include soil type, moisture levels, temperature, nutrient availability (van der Putten *et al.* 2013). Our findings support the assumption that the phylogenetic distance between conditioning and responding species may not be a reliable predictor of interspecific PSFs across different environmental contexts.

## CONCLUSIONS

We investigated whether native and exotic conditioning species differ in their interspecific PSFs on native responding species. Our findings reveal that PSFs were consistently negative, with the effect being significantly stronger for native conditioning species than for exotic ones. This stronger negative impact of native species likely stems from the activity and accumulation of local soil microorganisms, further emphasizing the importance of soil biota for interspecific PSFs. The marginally lower phosphorus availability in soils conditioned by native species also supports the hypothesis that native plants may exert stronger nutrient depletion effects, possibly through differential interactions with soil microorganisms involved in nutrient cycling or competition for limited resources. These findings have important implications for strategies aimed at promoting native plant species and controlling invasive species. For instance, during the restoration of degraded ecosystems, soil microorganisms could be deliberately utilized to enhance the growth of native species while suppressing that of invasive species. However, since nutrient limitations tend to be more pronounced in greenhouse settings without additional fertilizer than in natural field conditions, it should be noted that these results are only partially transferable. Future studies should therefore test the ERH with a focus on belowground processes, conducting more detailed investigations to differentiate the underlying mechanisms. The absence of an influence of phylogenetic distance on the PSF in our study and others suggests that analysing phylogenetic relationships among the involved plant species is insufficient to predict the direction and strength of interspecific PSFs.

### *Supplementary Material*

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Species list with triplet number (T01–T10), role in the experiment (NR = native responding species, NC = native conditioning species, EC = exotic conditioning species), family, lifespan, invasion status, minimum residence time (MRT) for exotic species.

Table S2: Species pairs and the respective triplet according to experimental design, phylogenetic distance within species pair, and shared community types according to biolflor.de.

### *Authors' Contributions*

Julia Dieskau (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing—original draft, Writing—review & editing), Isabell Hensen (Conceptualization, Resources, Supervision, Writing—review & editing), Nico Eisenhauer (Conceptualization, Funding acquisition, Methodology, Resources, Writing—review & editing), Susanne Lachmuth (Formal analysis, Writing—review & editing), and Harald Auge (Conceptualization, Methodology, Resources, Supervision, Validation, Writing—review & editing)

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*Conflict of interest statement*. The authors declare that they have no conflict of interest.

### *Data Availability*

Raw data and metadata used to generate tables and figures are publicly available at the iDiv data repository: https://doi.org/10.25829/idiv.3583-p235q9 (Dieskau *et al.* 2025).

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