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RESEARCH ARTICLE

Between- versus within-species variation in plant–soil feedback relates to different functional traits, but exudate variability is involved at both scales

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Abstract

1. Plant–soil feedback—feedback from plant-induced changes in soil properties to plant fitness—is increasingly shown to drive the maintenance of local plant diversity at both interspecific and intraspecific levels. A robust understanding of the relationships between plant–soil feedback and functional plant traits, which would improve our ability to generalize plant–soil feedback results beyond specific study systems, is, however, still lacking. This is especially true at the intraspecific plant level.
2. We assessed the relationship between plant–soil feedback and several functional traits in 13 co-occurring grassland species, including 20 genotypes of the dominant grass, *Festuca rubra*. The traits encompassed various aspects of growth, root properties and root exudate variability. Combining these traits into principal gradients of functional trait variation, we also tested the potential for the conservation and collaboration gradients to explain variation in PSF.
3. Between-species plant–soil feedback variation was explained by differences in biomass production and exudate composition, as well as contrasting strategies along the collaboration gradient. Within-species plant–soil feedback variation—that is between *Festuca rubra* genotypes—was associated with exudate variability, especially contrasting amounts of exuded phenols. Several traits had a significant effect on plant–soil feedback only via their interaction with exudate composition.
4. Overall, PSF was associated with different traits at between-species versus within-species levels. Root exudate variability was, however, involved at both diversity levels. Our results put forth the role of root exudation patterns as an important driver of variation in plant–soil feedback. Better integration between research on plant–soil feedback and on root exudation would therefore improve our understanding of the processes—both ecological and evolutionary—supporting the maintenance of plant diversity within grassland communities.

Youssef Yacine and Eliška Kuťáková shared first authorship.

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KEYWORDS
collaboration gradient, conservation gradient, grassland, interspecific, intraspecific, plant functional traits, plant–soil feedback, root exudation

1 | INTRODUCTION

Plants induce changes in soil biotic and abiotic properties. Examples include shifts in microbial community composition, or alterations in soil chemistry and nutrient levels (Ehrenfeld et al., 2005). These changes, in turn, influence the performance of plant individuals that grow in that soil, either positively or negatively. Such plant–soil feedback (Bever et al., 1997; van der Putten et al., 2013) may play an important role in the dynamics of plant communities across spatial and temporal scales, explaining patterns of species coexistence, abundance and succession (in 't Zandt et al., 2021; Kardol et al., 2006; Teste et al., 2017). Plant-induced changes in soil properties are for an important part determined by plant functional traits (Baxendale et al., 2014). Likewise, the response of a plant to soil changes depends on its traits (West et al., 2012). Plant functional traits thus offer a particularly relevant framework to investigate variation in plant–soil feedback (hereafter, PSF) across plant species and populations. Identifying functional traits associated with PSF variation will improve our ability to generalize PSF results beyond specific study systems (van der Putten et al., 2013) and provide new insights into the ecological and evolutionary processes that shape plant communities and ecosystem functioning.

Variation in direct PSF—that is PSF on conspecifics (Bever et al., 1997)—occurs at two levels: between species and between genotypes within species. While between-species variation has been extensively studied, only a few studies have investigated within-species PSF variation (Crawford et al., 2019), despite its potential to entail strong eco-evolutionary dynamics (Bolnick et al., 2011). Importantly, relationships between functional traits and PSF have almost exclusively been assessed at the interspecific level.

Several studies investigated the potential for principal gradients of functional trait variations to predict PSF (Cortois et al., 2016; Rutten & Allan, 2023; Semchenko et al., 2022). Two principal gradients associating with PSF have emerged: the conservation gradient and the collaboration gradient (Bergmann et al., 2020). The conservation gradient distinguishes between fast-growing species with high specific leaf area (SLA) and/or high specific root length (SRL), allowing rapid carbon gains from photosynthesis and nutrient acquisition from the soil, and slow-growing species, which are more resource-conservative (Simpson et al., 2020; Wright et al., 2004). Empirical studies have usually found faster-growing species to experience more negative PSF (Lemmermeyer et al., 2015; Semchenko et al., 2018; Xi et al., 2021) owing to a higher nutrient depletion, or a higher accumulation of antagonistic microorganisms to which they are also more vulnerable (growth-defence trade-off, Herms & Mattson, 1992). The second gradient, the collaboration gradient, classifies plant species according to their level of reliance on mutualistic fungal partners to acquire soil nutrients, a property that positively correlates with root diameter as coarse roots are needed to accommodate fungal partners (Brundrett, 2002). As a result, more collaborative species tend to display lower SRLs (Bergmann et al., 2020). A large number of studies (Cortois et al., 2016; Semchenko et al., 2018; Teste et al., 2017) have reported a positive relationship between PSF and collaboration, notably resulting from a higher accumulation of mutualists in their soil. Both of these gradients have, however, primarily been studied at the interspecific level, probably because the trade-offs from which they emerge are more pronounced at the interspecific level than at the intraspecific one (e.g. trade-offs constraining selection over longer evolutionary timescales). Their relevance to explain within-species PSF variation thus remains unknown.

Other traits, difficult to map into a conservation × collaboration framework, have also been found to explain PSF variation. Whole-organism biomass is a central biological trait that has been shown to scale positively with metabolic rate across a wide diversity of organisms, including plants (Brown et al., 2004). Consequently, larger individuals should induce soil changes of higher magnitudes. This pattern has been observed for both soil fungal community composition and chemical properties by Kuťáková et al. (2020), who accordingly found plant (conditioning) biomass to be the variable most frequently related to PSF variation across a set of several species. Theoretically, plant biomass should negatively affect PSF. More biomass production should induce a higher nutrient depletion (e.g. total *N*, Kuťáková et al., 2023). It should also increase the density and diversity of soil microbial communities as more resources are available (plant tissues, exudate amounts, e.g. Tückmantel et al., 2017). Assuming that the mutualistic and antagonistic soil compartments are equally affected, this should lead to more negative PSF owing to the higher functional redundancy in mutualistic versus antagonistic networks (Bascompte et al., 2003; Thébault & Fontaine, 2010). The latter indeed implies that plant fitness would increase less with a higher density/diversity of mutualists than it would decrease with a higher density/diversity of antagonists. This reasoning, which, however, remains speculative, should apply at both interspecific and intraspecific levels.

Root exudation is another key but largely overlooked mechanism affecting PSF (Ehrenfeld et al., 2005). Plants exude a large assortment of chemicals (Rovira, 1969), including a wide diversity of secondary metabolites such as alkaloids or phenolics. Compelling evidence indicates that exudates play a critical role in shaping rhizosphere microbial communities (Bais et al., 2006; Venturi & Keel, 2016), potentially driving the whole microbial community

assembly process (Zhalnina et al., 2018). Since plant–microbe interactions are central to PSF, exudation patterns are likely to induce variation in PSF, but evidence of such effects is still scarce (e.g. Steinauer et al., 2023). In particular, exudate composition appears as a good candidate to explain within-species PSF variation owing to its substantial variation at the intraspecific level (Jandová et al., 2015; Mueller et al., 2020).

While only a few studies have reported within-species variation in PSF (e.g. Bukowski & Petermann, 2014; Crawford & Hawkes, 2020; Dostálek et al., 2016; Kirchhoff et al., 2019), such variation is likely widespread as significant levels of soil microbiota differentiation have been documented between genotypes within populations (Micallef et al., 2009; Schweitzer et al., 2008). Contrasting changes in soil properties should therefore be expected, and accordingly contrasting signs and magnitudes of PSF between genotypes. The association between such variability and that of a heritable trait would then set the stage for the evolution of plant phenotypes under PSF-mediated selection (Crawford & Hawkes, 2020). Assessing such associations is critical to develop an evolutionary perspective on the role of PSF in shaping plant communities, as well as to understand the PSF-mediated consequences of novel selective pressures resulting from changing environmental conditions.

The aim of this study was to investigate the relationships between PSF and plant functional traits at both interspecific and intraspecific levels. Using a classical two-phase PSF experiment (Brinkman et al., 2010), we measured the PSF of 13 co-occurring grassland species, including 20 genotypes of the dominant grass, *Festuca rubra*. For this, we compared plant growth on soil previously conditioned by the same species/genotype versus soil conditioned by all 13 species. These species were taken from a species-rich mountain meadow where PSF plays a critical role in plant abundance fluctuations (in 't Zandt et al., 2021). For the intraspecific level, *Festuca rubra* was selected because of its high local trait variability, notably resulting from genetic differentiation (Skalova et al., 1997). Several traits capturing aspects of biomass production, growth, root morphology and chemistry and root exudation were also independently measured and used to test their potential to explain PSF variation. Specifically, we asked: (1) Which functional traits are associated with variation in PSF? (2) Do such patterns differ between interspecific and intraspecific levels?

2 | MATERIALS AND METHODS

2.1 | Biological material

All biological material used for the study was sampled from a yearly mown mountain grassland, which is approximately 300–400 years old, located in the Krkonoše Mountains in the Czech Republic (3.75 km ESE of the centre of Pec pod Sněžkou, 50°41′25″ N, 15°47′41″ E, 902 m a.s.l.). This grassland has a relatively stable species composition consisting almost exclusively of perennials, and a richness of approximately 32–36 species per m^2 (Herben et al., 2020). It is dominated by the grass *Festuca rubra* (represents ≈30% of above-ground biomass locally, Herben et al., 2003), which constitutes a particularly suitable study system for investigating the relationship between PSF and functional traits at the intraspecific level owing to a high genetic and phenotypic variability coupled with a large ability to reproduce clonally (Münzbergová et al., 2017; Skalova et al., 1997). The Krkonoše National Park Administration kindly permitted the soil and plant material sampling on their meadows (contract number OSML 38-4/2018).

In late June 2020, we collected 20 small *Festuca* tussocks located at least 5 m apart from each other to obtain 20 different Festuca rubra genotypes. The latter was confirmed by the subsequent sequencing of 86 microsatellite loci (unpublished data). After tussock growth, individual ramets were separated, resulting in 24 ramets per genotype used for the subsequent experiments. Throughout the summer of 2020, seeds of 12 other species covering a wide functional and phylogenetic diversity were also collected (Table 1), including three grasses (Poaceae), three legumes (Fabaceae), three Asteraceae and three other forbs. Seeds were dried and stored in paper bags at room temperature (20–22°C).

Prior to germination, some seeds were cold stratified (Table 1), and the seeds of *Lathyrus pratense* were scarified. Seeds were germinated on fine river sand (kept moist) in plastic bowls placed in a greenhouse in which the temperature was not allowed to drop below 12°C (germination ≈20 days, except *Achillea millefolium* and *Hypericum maculatum* ≈45 days).

In October 2020, we finally collected the top soil from six different spots located 10 m away from each other within our sampling grassland. At each spot, we removed the turf and sampled the soil to

TABLE 1 Studied species. Phylogeny provided in Figure S1. Cold stratification at 5°C prior to germination: *2 days, **7 days.

Dataset	Family	Species
Between-species	Poaceae	*Agrostis capillaris*
		Anthoxanthum odoratum
		Trisetum flavescens
	Fabaceae	*Lathyrus pratensis***
		Trifolium pratense
		*Trifolium repens**
	Asteraceae	*Achillea millefolium*
		*Crepis succisifolia***
		Leontodon hispidus
	Hypericaceae	*Hypericum maculatum*
	Ranunculaceae	*Ranunculus acris***
	Plantaginaceae	*Plantago lanceolata*
Within-species Between-genotypes	Poaceae	*Festuca rubra*

a depth of 20 cm. The soil from each spot was sieved through a 5 mm mesh, and kept separately at 6°C.

2.2 | Experimental design

Experimental work was carried out between November 2020 and November 2021 in the experimental facilities of the Institute of Botany of the Czech Academy of Sciences in Průhonice, Czech Republic (49°59′41.5″ N 14°33′59.2″ E). These facilities are located in a temperate climate zone at 320 m a.s.l. characterized by a mean annual temperature of 8.6°C and a mean annual precipitation of 610 mm. A classical two-phase plant–soil feedback experiment (Brinkman et al., 2010; Kulmatiski & Kardol, 2008) was carried out to measure specific plant–soil feedback (Figure 1). Independently, the plant species and *Festuca* genotypes were grown in sterilized sand to measure several functional traits potentially important to PSF.

2.2.1 | Plant–soil feedback experiment

2.2.1.1 | *Phase I: Conditioning (Figure 1A)*

In November 2020, the soil from each sampling spot was used to fill 32 individual pots (length×width×height: 7×7×8 cm; approx. 0.4 L in volume) as well as one mesocosm pot (61×30×7 cm; approx. 13 L). A geotextile was put on the bottom of each pot to prevent soil losses. Each individual pot was assigned to one of the 12 species or one of the 20 *Festuca* genotypes, and one young individual—an established seedling or ramet—of the assigned plant was planted into the pot in November/December (depending on seedling availability). The mesocosm pot was planted by one individual of each *Festuca* genotype and two individuals of each other species in a spatially structured randomized design (details in Appendix S1.I). Pots were located in a greenhouse in which temperature was not allowed to drop below 12°C, watered as necessary with tap water and subjected to 14 h of light per day. No fertilizer was used. This design was replicated for each sampling spot, leading to six replicates arranged in a block design, with each block corresponding to one soil sampling spot. After 6 months, at the beginning of June 2021, plant biomass from each pot was harvested—above- and below-ground separately—and dried to a constant weight at 60°C, then weighed. Total available nitrogen—that is sum of nitrate NO_3^- and ammonium NH_4^+, mass per mass unit of soil—was also assessed in each pot/mesocosm using a FLASH 2000 CHNS/O organic elemental analyzer (Thermo Fisher Scientific).

2.2.1.2 | *Phase II: Feedback (Figure 1B)*

The subsequent feedback phase started immediately after harvesting the conditioning phase, at the beginning of June 2021. The soil from each individual pot was placed into a new, smaller pot after plant extraction (5×5×7 cm; approx. 0.2 L). This resulted in 32 new pots—that is conditioned by each of the 12 species and 20 *Festuca* genotypes—for each block. We also filled 32 pots of the same size with soil from each mesocosm, which was thoroughly mixed beforehand. This resulted in 32 pots×2 soils (from individual pots or mesocosms)×6 blocks, that is 384 pots in total. A geotextile was again put on the pot bottoms to prevent soil losses. One established individual was planted into each of these pots, with each species/genotype planted into a pot with its own soil and a paired pot containing mesocosm soil from the same block. The pots were placed in an outdoor experimental garden and watered with tap water as necessary. No fertilizer was used. After 5 months, at the beginning of November 2021, the biomass of each pot was harvested, dried to a constant weight at 60°C and weighed. For each species or genotype, we obtained six measures of PSF—that is six replicates—by contrasting the total biomass produced in self- versus community-conditioned soil within each pair of feedback pots (Equation 1). A positive PSF (resp. negative) means that the focal plant produced more biomass (resp. less biomass) in its own-conditioned soil than in the community-conditioned 'away' soil.

$$\underset{\substack{\text{Species/Genotype}\\ \text{Block}}}{\text{PSF}} = \ln\left[\frac{\text{TotalBiomass}_{\text{SelfConditioned}}}{\text{TotalBiomass}_{\text{ControlMesocosm}}}\right]_{\substack{\text{Species/Genotype}\\ \text{Block}}} \quad (1)$$

2.2.2 | Plant functional traits

At the beginning of December 2020, six individual replicate pots (7×7×8 cm; approx. 0.4 L) filled with sterilized fine sand were assigned to each species and *Festuca* genotype, and one individual of the assigned plant was planted into it. Twenty-four such additional pots were left unplanted. The pots were placed in a greenhouse next to the conditioning phase pots and watered regularly with distilled water. Initially, as well as every 5 weeks, all pots were fertilized with 5 mL of Wuxal Super (AgroBio Opava, NPKCuMnZn) diluted 200 times in distilled water. After 5 months, at the beginning of May 2021, the biomass of each pot—above- and below-ground separately—was harvested.

Immediately after harvest, the rhizosphere sand from each individual was obtained by carefully brushing each individual root system, and 25 mL of distilled water was added to 50 g of sand after it was mixed to ensure homogeneity. Sand and root debris were then removed by vacuum filtration through a 0.45 µm PVDF membrane. The sand from unplanted control pots was similarly processed. These filtrates were used to determine exudate composition, as well as the amount of exuded phenols, as done by Rathore et al. (2023). They were stored at −20°C until further processing.

Relying on such an independent experiment in sterilized sand was mainly motivated by our intent to eliminate any effect of soil microbiota and adsorption to soil particles on exudate composition (Oburger & Jones, 2018; Rathore et al., 2023). An undamaging extraction of the complete root system is also much easier in sand. In order not to introduce any heterogeneity between functional trait measurements, we decided to rely on this same experiment—and not the PSF experiment—to determine total biomass production,

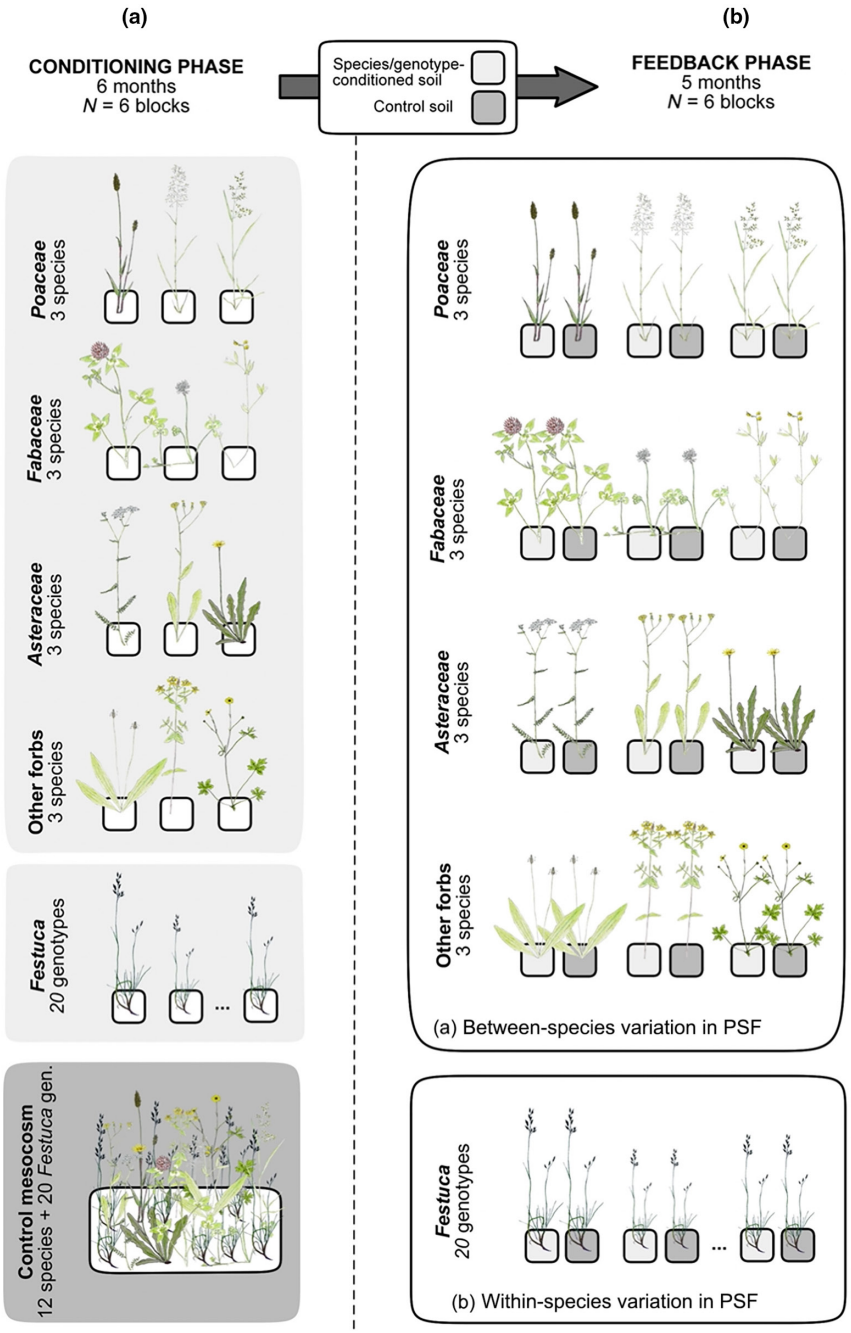


FIGURE 1 Design of the two-phase plant–soil feedback experiment. (A) Conditioning phase. In this first phase, plants were grown on soil collected from the grassland site either as a single individual or as a mixed plant community in control mesocosms. (B) Feedback phase. In this second phase, plants were grown on (1) their own species- or genotype-conditioned soil, and on (2) community-conditioned soil from the control mesocosm. PSF was then obtained by comparing biomass production in these two soils (light vs. dark grey; see Equation 1). Variation in PSF was investigated at two levels: (a) between 12 species corresponding to the between-species level, and (b) between 20 *Festuca* genotypes corresponding to the within-species level.

root: shoot ratio, specific leaf area, specific root length, root density and root C:N ratio. The functional traits tested to explain PSF were thus not measured in the same conditions as PSF itself (soil matrix, fertilizer use). While absolute trait values were certainly modified (phenotypic plasticity), relative trait values should have remained mostly unaffected, as a large number of studies indicate that the species/genotype factor explains much more variation in plant functional traits along environmental gradients than the species/genotype-by-environment interaction (e.g. Barker et al., 2019; Da Silveira Pontes et al., 2010; Mudrák et al., 2019; Pereira et al., 2017). Some studies directly report that species rankings based on a focal trait measured under different conditions are generally largely correlated (Garnier et al., 2001; Mudrák et al., 2019). Note that such a pattern was found here for total biomass (Spearman rank correlation, $r_s = 0.7$, $p = 0.01$), which is the only trait measured in both the PSF (as conditioning biomass) and the functional trait experiments. The (expected) consistency of trait-based rankings across experiments underpins the relevance of our experimental design. We hereafter detail how each functional trait was measured.

2.2.2.1 | *Total biomass & root: Shoot ratio*

Shoot and root biomass of each individual was dried at 70°C to constant weight, separately weighed and combined to obtain total biomass production and root to shoot biomass ratio (hereafter *Biomass* and *RootShoot*).

2.2.2.2 | *Specific leaf area*

Before drying, one randomly selected, fully developed leaf from each individual was cut and rinsed with distilled water. Non-grass species' leaves were scanned (Epson flatbed scanner system, 300 dpi) before measuring their area using ImageJ. For grass species, the width of a central—that is neither leaf tip nor basis—4 cm long part of the leaf was manually measured, and its area was calculated assuming a rectangle shape. The latter was more suitable for grass leaves, which were often rolled or folded, so that scanning was inappropriate. After drying, the leaf (or partial leaf) was separately weighed. Specific leaf area (*SLA*) was obtained by dividing the leaf area by the leaf dry weight. We wanted SLA to mainly reflect the level of investment towards photosynthetic capacity and, hence, minimize the variation resulting from structural differences. As a result, the species with large leafstalks—*Ranunculus acris*, *Trifolium pratense* and *Trifolium repens*—had their leaf area and weight measured after leafstalk removal.

2.2.2.3 | *Root morphology and chemistry: Specific root length, root density, C:N ratio*

Before drying, each individual root system was rinsed with water and cut into smaller fragments of around 2–3 cm. Roots were scanned (Epson flatbed scanner system, 300 dpi) after being evenly spread out in deionized water on a transparent tray while avoiding root segments to overlap (several subsamples per individual if needed). Scans were then processed by the WinRHIZO software to obtain the length and volume of each root system. Specific root length (*SRL*) was obtained by dividing the total root length by the root dry weight. Root density (*RTD*) was obtained by dividing the total root dry weight by the root volume. Root diameter was also measured, but as it displayed a strong negative correlation with *SRL* (means per (13) species: $r = -0.91, d_f = 11, p < 0.001$), it was not kept for further analyses.

After being weighed, the roots of each individual were ground into particles of less than 1 mm in diameter. Following Ehrenberger and Gorbach (1973), total carbon and nitrogen contents were measured using a FLASH 2000 CHNS/O organic elemental analyzer (Thermo Fisher Scientific), and the biomass ratio of carbon to nitrogen of each individual root system was calculated (henceforth *CtoN*).

2.2.2.4 | *Exudates: Amount of phenols and exudate composition*

The amount of total phenolic compounds was estimated by spectrophotometry using the Folin–Ciocalteu method (Ainsworth & Gillespie, 2007). The total phenolic amount was calculated as the concentration of gallic acid equivalent in the exudate solution (i.e. filtrate), with absorbance measured at 765 nm using a microplate reader (see Appendix S1.II for details). The amount of phenols exuded by each plant individual (henceforth *Phenols*) was then obtained by subtracting the mean amount of phenols measured across unplanted control samples (≈ 0.36 mg / L).

The metabolite composition of root exudates was determined in three randomly selected replicates (i.e. filtrates) for each species or *Festuca* genotype by LC–MS following the Agilent Technologies application note 5994-1492EN (Dai & Hsiao, 2019) for discovery metabolomics. The metabolite composition of 12 randomly selected soil solutions from the unplanted pots and 16 additional aliquots obtained from evenly mixing all analysed samples (quality controls) were similarly analysed. Eluting compounds were detected in negative ionization mode (scan range: 60–1600 mass to charge ratio (m/z)). After a first filtering by the Profinder 10.0 software (Agilent Technologies), the obtained dataset consisted of the relative intensities (peak areas) of 379 molecular features (unique retention time and m/z) in each of the 122 analysed samples (2 missing values, Table S1). The detailed protocol is provided in Appendix S1.III.1. Further analysis was then performed using the MetaboAnalyst 5.0 software (Pang et al., 2021).

First, missing values (below technical detection limit) were replaced by 1/5 of their minimal within-feature positive value. Second, 268 molecular features whose signal within quality controls was too variable—that is $\frac{SD}{mean} > 0.35$, indicative of low repeatability—were excluded. An additional set of 19 features, likely introduced unintendedly during the extraction/measurement process, were identified by comparing their intensities in unplanted controls versus planted samples, as detailed in Appendix S1.III.2. After their exclusion and the removal of unplanted controls, the final dataset consisted of 92 molecular features measured across 110 samples, including 16 quality controls. Prior to further analysis, intensities of molecular features were normalized according to their measured intensities in quality controls (PQN normalization, Dieterle et al., 2006), log-transformed and scaled to mean = 0 and SD = 1 (feature-wise). Kernel density plots allowed checking the normal distribution of processed data. Such extensive processing is necessary for metabolomics data to provide relevant biological information (van den Berg et al., 2006). Here, biological relevance was ascertained by quality controls, as well as species replicates of most species, clustering together in a dendrogram (Appendix S1.III.3).

A principal component analysis (PCA) was finally performed to identify the major axes of variation in exudate composition. Each of the first nine principal components (PC) explained significantly more variance than would have been expected by chance (R package *PCDimension* (Wang et al., 2018), *Rnd-Lambda* randomization, e.g. Peres-Neto et al., 2005). As the first two components explained substantially more variation than others (11.5 % & 10.6 % vs. < 6.7 %), coordinates along these two PCs were used as exudate composition traits for the analysis presented here (hereafter *ExComp*1 & *ExComp2*, see Figure AS1.4 in Appendix S1.III). The relationships between PSF and the other seven PCs of exudate composition were nevertheless tested (*ExComp3-9*, Figure S3).

2.3 | Statistical analysis

The two datasets—that is between-species with 12 species ($n = 65$ as 7 values were missing; see Table S1) versus within-species with 20 *Festuca* genotypes ($n = 120$)—were analysed independently

(Table 2). All statistical analyses were performed using the R software (R Core Team, 2022). We first assessed the level of between-species or within-species (i.e. between genotypes) variation in PSF by fitting a linear mixed-effects model with species or genotype as a fixed factor, and block (i.e. soil sampling spot, 6 levels) as a random factor using the *nlme* R package (Pinheiro et al., 2022; Pinheiro & Bates, 2000, function *lme*). Significant heterogeneity in residual variance per block was detected in both models (Bartlett test, df = 5, $p = 0.02$), and therefore allowed (*weight* parameter in *lme* function).

We then investigated the relationship between PSF and functional traits. Each trait was averaged over the six replicates (3 for exudate composition) per species/genotype and scaled. Averaging was necessary as there was no relevant way of pairing the replicates of our two independent experiments. It also impeded the few missing trait measurements from affecting sample sizes (Table S1).

First of all, we performed a PCA with our 13 species as individuals and nine traits as variables (*PCA* of R package *FactoMineR*, Lê et al., 2008) to obtain an overview of the variation of functional traits within our dataset. Note that this was the only analysis in which both between-species and within-species datasets were combined. Trait values for *Festuca rubra* were obtained as averages over genotypes so that *Festuca* would have the same weight as other species. The two first PCs were considered as two additional composite traits, and their values for each *Festuca* genotype were obtained by projecting their trait data on the two-dimensional PC space (i.e. supplementary individuals in *FactoMineR*::*PCA*; see Figure 3b). We then tested the potential for these composite traits to explain PSF variation by fitting linear mixed-effect models (*nlme*::*lme*).The species/genotype factor was considered as a random factor to account for the averaging of trait values per species (df = 12) or genotype (df = 20), and residual variance heterogeneity across block levels was allowed. Based on their correlations with functional traits but also AMF reliance data obtained from Akhmetzhanova et al. (2012) (details in Appendix S1.IV), these composite traits were interpreted (see Section 2) as capturing variability along the collaboration and conservation gradients (Bergmann et al., 2020).

Second, we tested the relationship between PSF and each original functional trait by fitting similar mixed-effects models. As we were particularly interested in the effect of exudate composition, we also tested all pairwise interactions with exudate composition traits, that is *ExComp1* and *ExComp2*, but only significant ones were reported. Such significant interactions could indicate that the effect of a trait on PSF is mediated by the composition of root exudates. All statistical models were fitted both with and without the biomass produced during conditioning in individual pots as a covariate (hereafter *CondBiomass*). This allows identifying the PSF–trait relationships that are mediated by, or independent from, such variation in conditioning biomass. Note finally that quantitative explanatory variables were scaled (mean = 0 & SD = 1) before statistical models were fitted, and model assumptions were checked with appropriate tests (Shapiro for normality and Bartlett for variance homogeneity).

3 | RESULTS

3.1 | Between-species versus within-species variation in plant–soil feedback

PSF was overall positive at both diversity scales (Figure 2a,b). On average, biomass production was two times higher in self-conditioned versus control mesocosm soil when averaged over the 12 species, and 1.5 times higher when averaged over *Festuca* genotypes. PSF was significantly positive (95% CI) for 9 species (Figure 2a) and 12 *Festuca* genotypes (Figure 2b), and neutral otherwise. The prevalence of positive PSF could result from a much higher biomass production per soil volume during conditioning in mesocosms than individual pots (Wilcoxon rank-sum: $W = 34, p < 0.001$, mesocosms vs. species/genotypes (means): 6.7 vs. [1.8, 4.9] $g.L^{-1}$).

PSF variation was significant both between species ($F_{11,49} = 2.4, p = 0.02$, Figure 2a) and between *Festuca* genotypes ($F_{19,95} = 2.29, p < 0.01$, Figure 2b). Conditioning biomass only affected between-species PSF variation (Figure 2c), although significant variation in conditioning biomass was found both between species ($F_{11,49} = 5.32, p < 0.001$), and between genotypes ($F_{19,95} = 4.93, p < 0.001$). Species that had produced more conditioning biomass experienced more negative PSF ($F_{1,59} = 18.3, p < 0.001$; with *Species* factor as covariate: $F_{1,48} = 4.41, p = 0.04$). Higher conditioning biomass was associated with lower levels of total available nitrogen in the soil after conditioning ($r = -0.84, \text{df} = 10, p < 0.001$), likely contributing to this result. For *Festuca* genotypes, no significant correlation between conditioning biomass and total available nitrogen was found ($r = -0.14, \text{df} = 18, p = 0.55$), potentially explaining the lack of association between conditioning biomass and PSF. Accounting for conditioning biomass led to marginally significant between-species PSF variation (Figure 2d, $F_{11,48} = 1.95, p = 0.06$), which indicates that a large amount of variation in PSF was due to differences in conditioning biomass between species.

TABLE 2 Replication statement.

Analysis		Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Between-species	PSF ~ species	Individual	Species	6 individuals per species
	PSF ~ traits	Individual	Species	12 species
Within-species	PSF ~ genotype	Individual	Genotype	6 individuals per genotype
	PSF ~ traits	Individual	Genotype	20 *Festuca rubra* genotypes

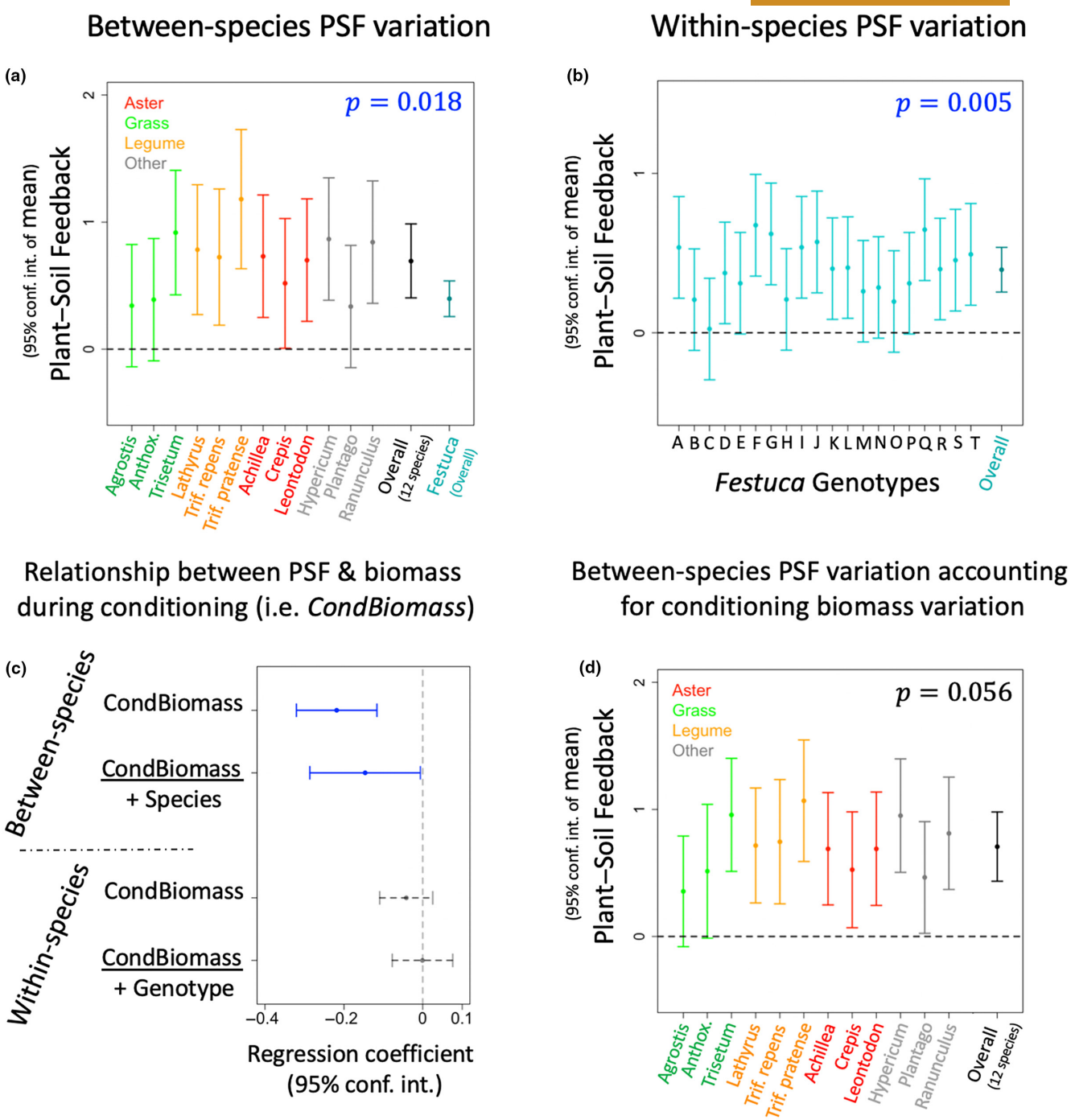


FIGURE 2 Between-species versus within-species plant–soil feedback (PSF) variation. Graphs show 95% confidence intervals of statistical model estimates for the fixed effect of interest. (a) Between-species PSF variation. Species as fixed factor. (b) Within-species PSF variation. Genotype as fixed factor. Measured PSF (raw data) at both levels can be seen in Figure S2. (c) Effect of conditioning biomass variability on PSF. Biomass during conditioning (*CondBiomass*) as fixed effect in both datasets, either alone or with species/genotype as covariate. (d) Between-species PSF variation when accounting for conditioning biomass. The model includes both species and *CondBiomass* as fixed terms, the species factor being the one of interest here. As conditioning biomass did not affect PSF at the within-species level (c), a graph similar to (d) at this later level is irrelevant.

3.2 | Plant–soil feedback and collaboration × conservation gradients

The first two dimensions obtained by principal component analysis captured 44.2% and 22% of functional trait variation respectively (Figure 3A). Root-related traits—C to N ratio, SRL & RTD—were the ones mainly contributing (=58%) to the first dimension (PC1 in Figure 3A), with a high negative correlation with SRL in particular ($r = -0.86$). This first dimension displayed a significant positive correlation with measured root diameters ($r = 0.77$, $p < 0.01$, $df = 11$),

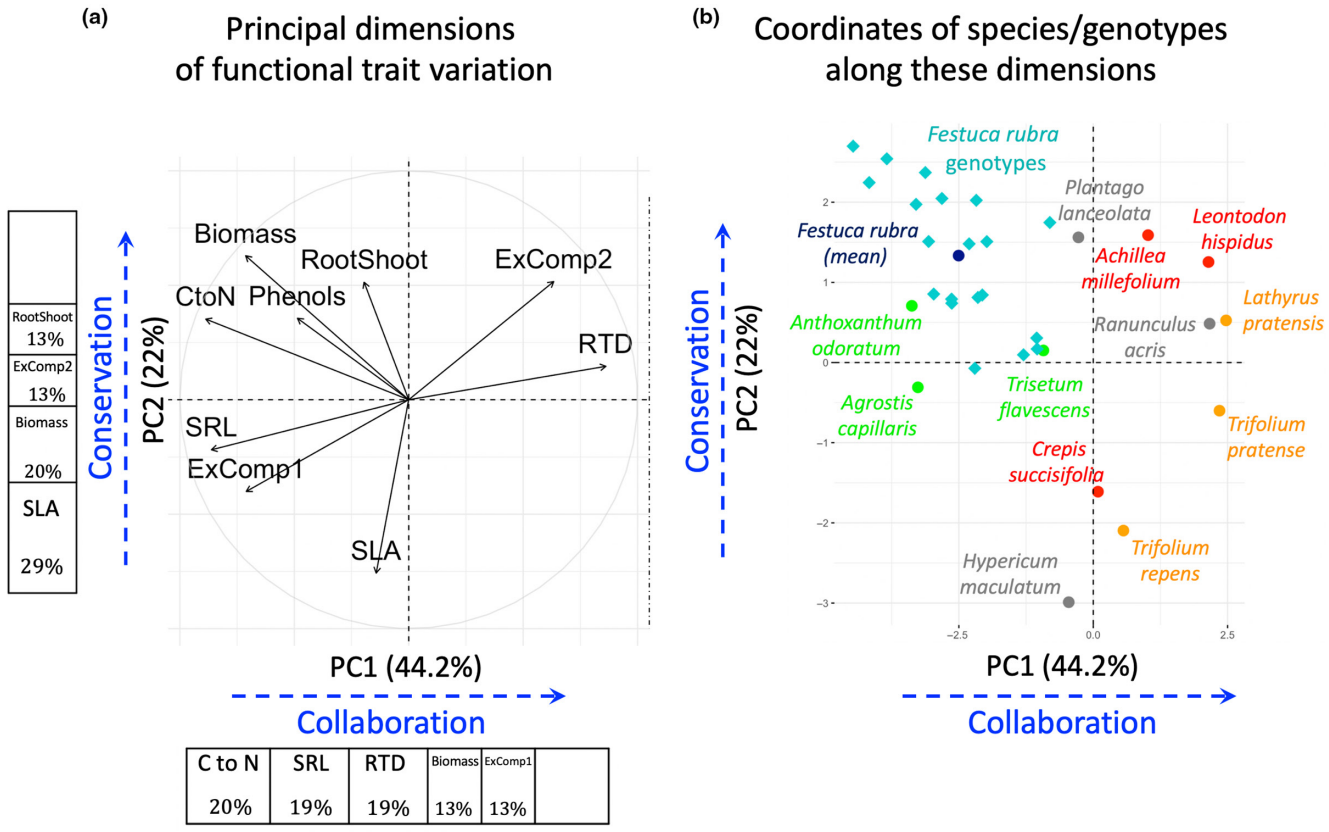


FIGURE 3 Principal component analysis (PCA) of functional trait variation. (a) Principal dimensions of functional trait variation. These two dimensions are interpreted as the collaboration and conservation gradients, respectively. The central graph depicts the projection of our focal nine traits on the first two PCA dimensions. Stacked bar charts indicate trait contributions to these dimensions (rounded). Only contributions higher than $1/9 \approx 11\%$ are shown. (b) Coordinates of studied 12 species and 20 *Festuca rubra* genotypes along principal dimensions of trait variation. The PCA is based on the trait data of the 13 species (averaged over genotypes in the case of *Festuca rubra*). After generating the PCA, *Festuca rubra* genotypes were projected over the multidimensional trait space obtained. Colours as in Figure 2a.

as well as a marginally significant positive correlation with quantitative AMF reliance data taken from Akhmetzhanova et al. (2012) ($r = 0.6$, $p = 0.053$, df = 9, data missing for two species, see Appendix A.IV). This correlation pattern suggests that the coordinates along this first PCA dimension capture the degree of fungal collaboration (collaboration gradient, Bergmann et al., 2020), which here mainly distinguishes between low collaborative grass species and more collaborative forb species, legumes in particular (Figure 3B).

The second principal dimension of trait variation (PC2 in Figure 3A) mainly depended on SLA and total biomass. As it negatively correlated with SLA ($r = -0.76$), which is usually associated with faster growth (Poorter & Remkes, 1990), the coordinates along this second dimension were considered as capturing aspects of plant differentiation along the conservation gradient (Bergmann et al., 2020). Importantly, this second dimension did neither correlate with root diameter data ($r = 0.33$, df = 11, $p = 0.27$) nor with AMF reliance data ($r = -0.18$, df = 9, $p = 0.6$).

At the between-species level, the collaboration gradient (PC1) consistently displayed a significant association with PSF variation (Figure 4A.a). More collaborative species experienced more positive PSF (alone: $F_{1,10} = 7.5$, $p = 0.02$; with PC2 as covariate: $F_{1,9} = 9.7$, $p = 0.012$). In contrast, the conservation gradient (PC2) related marginally to PSF, and only when tested with the collaboration gradient as a covariate (Figure 4A.a, $F_{1,9} = 3.7$, $p = 0.09$, otherwise $p = 0.39$). Overall, variation in PSF was significantly explained by the model with both gradients as fixed terms ($L_{ratio} = 9.2$, $p = 0.01$). The associations between gradients and PSF became less significant once accounting for conditioning biomass variation (Figure 4A.a vs. A.b). The increase in PSF with collaboration became marginally significant (Figure 4A.b, alone: $F_{1,10} = 3.45$, p = 0.09; with PC2 as covariate: $F_{1,9} = 4.4$, $p = 0.07$), while the conservation gradient did not relate any longer to PSF variation ($p > 0.24$).

More collaborative species produced significantly less biomass during conditioning in our experiment ($r = -0.6$, $df = 10$, $p = 0.04$). The negative effect of conditioning biomass on PSF (Figure 2c) could therefore partly underlie the positive relationship between collaboration and PSF, explaining the lower significance of PSF-gradient relationships once conditioning biomass is accounted for. No significant correlation between conservation and conditioning biomass was detected ($r = -0.29$, df = 10, $p = 0.36$). At the within-species level (Figure 4B), we found no significant relationship between the collaboration or conservation gradients and PSF (all $p_{values} > 0.1$).

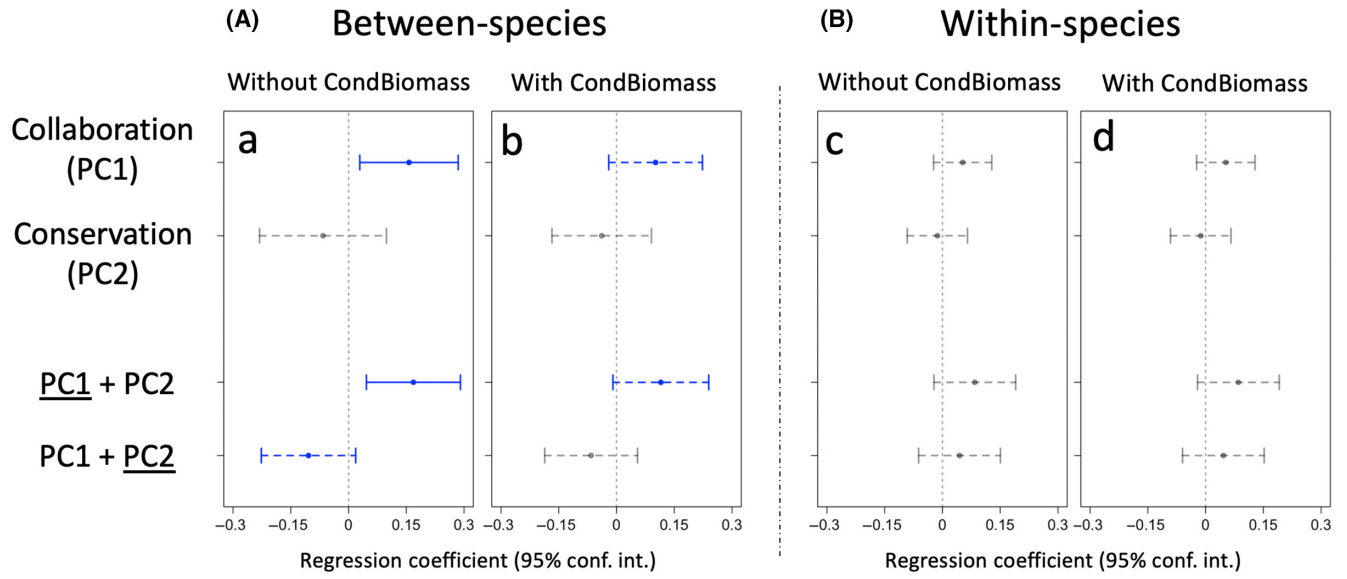


FIGURE 4 Relationships between plant–soil feedback and the gradients of collaboration and conservation at between-species level (a), and within-species level (b). The graphs provide the statistical model estimate associated with the gradient of interest. Each gradient was tested alone (first two lines) or with the other gradient as a covariate (last two lines). Conditioning biomass was ignored (a–c) or included as a covariate (b–d). Line type and colour indicate significance: solid & blue for $p < 0.05$, dotted & blue for $0.05 \leq p < 0.1$, dotted & grey for $p \geq 0.1$. The effect of *CondBiomass* on PSF was always significant and negative at between-species level (all $p < 0.01$), and never significant at within-species level (all $p > 0.75$).

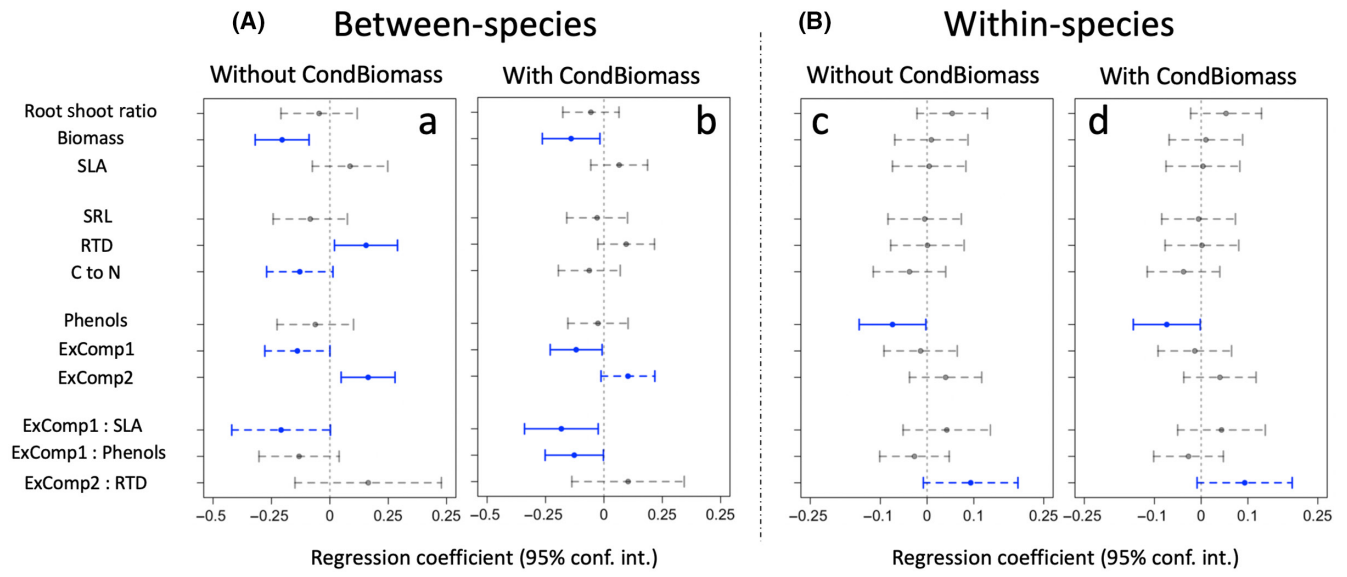


FIGURE 5 Relationships between plant–soil feedback and plant functional traits between-species level (A), and within-species level (B). The graphs provide the statistical model estimate associated with the trait or trait interaction of interest. Trait abbreviations: RTD, root density, SLA, specific leaf area, C to N ratio in roots (C to N), two first PCs of variation in exudate composition (ExComp1, ExComp2). Conditioning biomass was ignored (a–c) or included as a covariate (b–d). Line type and colour indicate significance: solid & blue for $p < 0.05$, dotted & blue for $0.05 \leq p < 0.1$, dotted & grey for $p \geq 0.1$. The effect of *CondBiomass* on PSF was always significant and negative at between-species level (all $p < 0.02$), and never significant at within-species level (all $p > 0.75$).

3.3 | Plant–soil feedback and functional traits

Species with higher root densities ($F_{1,10} = 6.5, p = 0.03$), and with lower C to N ratio in root tissues ($F_{1,10} = 4.1, p = 0.07$), experienced more positive PSF (Figure 5A.a). These relationships were no longer significant (all $p_{values} > 0.11$) when variation in conditioning biomass was considered (Figure 5A.b). We moreover found that species characterized by a higher biomass experienced more negative PSF ($F_{1,10} = 15.6, p < 0.01$, Figure 5A.a). These species had also produced more conditioning biomass ($r = 0.71, \text{df} = 10, p < 0.01$),

but the negative effect of (trait) biomass on PSF remained significant when accounting for conditioning biomass as a covariate ($F_{1,10} = 6.45, p = 0.03$, Figure 5A.b). The effect of conditioning biomass on PSF was also significant and negative. Between-species variation in PSF also related to exudate composition (Figure 5A,B). Specifically, *ExComp1* had a significant effect on PSF when conditioning biomass variation was also considered ($F_{1,10} = 5.7, p = 0.04$), while *ExComp2* significantly affected PSF when ignoring conditioning biomass ($F_{1,10} = 10, p = 0.01$). Marginally significant relationships were found otherwise, that is for *ExComp2* when considering conditioning biomass ($F_{1,10} = 3.9, p = 0.075$), and for *ExComp1* when not ($F_{1,10} = 4.9, p = 0.05$). Other traits, considered individually, were not related to between-species PSF variation (all $p_{values} > 0.25$).

At the within-species level, higher amounts of exuded phenols led to more negative plant–soil feedback, accounting or not for conditioning biomass variation (in both cases, $F_{1,18} = 4.7, p = 0.04$, Figure 5B). Exudate composition also affected PSF as we found the 8th PC of exudate composition variation (= 3.4% variance explained, *ExComp8* in Figure S3.B) to significantly relate to PSF (with & without *CondBiomass*: $F_{1,18} = 6.7, p = 0.02$). No other trait, considered individually, was related to within-species variation in PSF (all $p_{values} > 0.15$).

Finally, at both diversity scales, various traits had a significant effect on PSF via their interaction with exudate composition (Figure 4). A higher *SLA* led to more negative PSF among species scoring high on the first PC of exudate composition variation (i.e. *ExComp1*), but to more positive PSF among species scoring low on *ExComp1*, both without ($F_{1,10} = 4.9, p = 0.052$, Figure 4A.a) and with ($F_{1,10} = 6.6, p = 0.03$, Figure 4A.b) conditioning biomass as a covariate. Between-species PSF variation depended similarly on the interplay of exuded phenols and exudate composition, but only once accounting for conditioning biomass ($F_{1,10} = 5.1, p = 0.047$, Figure 4A.b). At the intraspecific level, a higher RTD led to more positive PSF among *Festuca* genotypes scoring high on ExComp2, but to more negative PSF among genotypes scoring low on *ExComp2*. These relationships were however marginally significant (Figure 4B, with & without *CondBiomass*: $F_{1,18} = 3.7, p = 0.07$).

4 | DISCUSSION

We found significant variation in PSF across both between-species (12 species) and within-species (20 *Festuca rubra* genotypes) levels. The plant traits explaining this variation differed between these levels. Differences in individual biomass, contrasting strategies along the collaboration gradient and variation in root exudate composition (*ExComp1*, *ExComp2*) mainly explained between-species PSF variation. Within-species variation was, on the other hand, explained by contrasting amounts of exuded phenols and differences in a distinct dimension of root exudate composition (*ExComp8*). The composition of root exudates was involved in both between-species and within-species PSF variation, both directly (*ExComp1* & *ExComp2* vs. *ExComp8*) and via interactions with other functional traits (*SLA* & *Phenols* vs. *RTD*). The effect of these latter traits on PSF became apparent only once accounting for their interaction with exudate composition. Our results thus especially highlight the role of exudate variability as a major driver of variation in PSF.

Plant–soil feedback was here measured for 13 species, including 20 *Festuca rubra* genotypes, all originating from the same species-rich mountain grassland in which they coexist (Herben et al., 2017). Most species and genotypes exhibited positive PSF, which seems surprising as negative direct PSF has often been reported for species within their native range (Kulmatiski et al., 2008). This might be due to the design of our 'away' soil (control mesocosms), that is conditioned by a diverse plant community consisting of all 12 species and 20 genotypes to mimic field conditions. In line with the well-established positive diversity–productivity relationship (Loreau & Hector, 2001; Tilman et al., 1996), much more biomass per soil volume was produced in the mesocosms than in the single pots, possibly because more diverse soil microbiota resulted in high nutrient availability for plants (e.g. higher C mineralization, Juarez et al., 2013). This would imply that lower nutrient amounts were left for plants to grow during the feedback phase in community- versus self-conditioned soil, explaining the prevalence of positive PSF.

Individual plant biomass—either measured as conditioning biomass or independently from the PSF experiment as a functional trait—influenced PSF only at the interspecific level. In particular, variation in conditioning biomass was found both between species and between genotypes, but only affected PSF at the interspecific level in which it correlated negatively with total available nitrogen. This indicates that biomass can be viewed as an effect trait (Goldberg, 1990; Suding et al., 2008) in terms of PSF: higher biomass production induces soil changes of higher magnitude, resulting in stronger feedback (e.g. Kuťáková et al., 2020). After accounting for conditioning biomass, total biomass was still negatively associated with PSF, suggesting that it can also be considered as a PSF response trait (Suding et al., 2008), that is a trait affecting the magnitude of the response to a given fixed change in soil properties. Assuming that nutrient needs increase with biomass, for instance, a similar reduction in nutrient availability would more negatively affect larger than smaller plant species.

At the interspecific level, PSF was strongly associated with the collaboration gradient, but rather weakly with the conservation gradient. Irrespective of the covariates included, more collaborative species consistently experienced more positive PSF, in line with previous investigations (Cortois et al., 2016; Semchenko et al., 2018; Teste et al., 2017). In addition to a higher accumulation of mutualists in their self-conditioned soil, a lower accumulation of antagonists might also be involved, as fungal partners often provide some level of defence against antagonistic microbiota (Jung et al., 2012). The resulting lower vulnerability of collaborative species to antagonists could as well be at play. In contrast, the conservation gradient related marginally to PSF, and only when also accounting for the collaboration gradient. Opposing the pattern expected from a growth-defence trade-off, the reported negative relationship between conservation and PSF could instead derive from

faster-growing species promoting faster nutrient cycling via changes in decomposer community composition promoting their own growth on self-conditioned soil (Baxendale et al., 2014).

Note finally that we cannot rule out the possibility for the first PC dimension of functional trait variability (PC1/collaboration, Figure 3A) to also capture some aspects of conservation as high SRLs and low RTDs can be associated with resource-acquisitive strategies (Roumet et al., 2016; Simpson et al., 2020). The latter could contribute to the weak association between PSF and the second PC dimension, which we interpreted as the conservation gradient. We, however, think that the significant positive correlation of PC1 with both the data of measured root diameters and the (external) data on AMF reliance compellingly indicate that (fungal) collaboration substantially underpins the association between PC1 and PSF, in line with our interpretation.

At both diversity levels, PSF was affected by the variability of exudate composition, but distinct aspects of such variability were involved at each level (*ExComp1* & *ExComp2* vs. *ExComp8*). This is an important result as, to our knowledge, only two studies have so far demonstrated a relationship between root exudate composition and PSF (Hu et al., 2018; Steinauer et al., 2023). In both cases, exudate-induced changes in the rhizosphere microbiota were involved. In line with our findings, Hu et al. (2018) also show that contrasting responses to exudate between genotypes might be responsible for within-species PSF variation. In addition to directly affecting PSF, exudate composition also mediated the effect of other traits, which related significantly to PSF only via their interaction with exudate composition. It was notably the case for SLA at the interspecific level. This may explain why several studies have found the effect of the conservation gradient on PSF to depend on the species (Münzbergová & Šurinová, 2015) or the functional group considered (grass vs. forb, Heinen et al., 2020), as significant differences in exudate composition have been reported both between species and functional groups (Dietz et al., 2020; Williams et al., 2022). The dependence of PSF–trait relationships on exudate composition could, in fact, indicate a dependence on soil microbial community composition, as exudate profiles are a strong determinant of the latter (Zhalnina et al., 2018). In line with this interpretation, the modelling work of Ke et al. (2015) shows how shifting the composition of soil microbiota alters the relative importance of several plant traits in explaining PSF variation. While the authors provide compelling empirical support for their results, further experimental assessments would probably consolidate these findings.

At the intraspecific level, higher amounts of exuded phenols were associated with more negative PSF. Based on the well-established role of phenols in plant defence against herbivores and pathogens (Levin, 1971; Vermerris & Nicholson, 2006; Zaynab et al., 2018), an opposite relationship might have been expected. However, phenols also have an allelopathic negative effect on competing plant individuals (John & Sarada, 2012), which may explain our result. The high amounts of phenols in the community-conditioned 'away' soil increased the PSF of lower phenol-exuding genotypes by decreasing their performance in away versus self-conditioned soil (low phenol amounts). In contrast, the PSF of higher phenol-exuding genotypes remained unaffected as (1) these genotypes experienced similarly high phenol amounts in away versus self-conditioned soil, and (2) their growth is probably not affected by phenols anyway due to a high evolved tolerance. As such, a negative association between PSF and phenol exudation emerges.

The association between within-species PSF variation and variation in exudates—both composition and amount of phenols here—have far-reaching implications. Exudates are involved in the ability of plant species to tolerate abiotic stress and cope with changing environmental conditions (Chai & Schachtman, 2022; Vives-Peris et al., 2020; Williams & de Vries, 2020). Reported examples notably include drought, elevated CO_2, extreme temperatures or nutrient shortages. Being partly genetically determined (Rengel, 2002), exudation patterns are likely evolving in response to the novel selective pressures arising from rapidly changing environmental conditions. According to our study, these changes might in turn affect PSF, potentially shifting the distribution of plant abundances and altering coexistence. Conversely, when PSF strongly impacts fitness, the (adaptive) evolution of exudation patterns might be altered by PSF-induced selection, that is facilitated, dampened or even prevented. In general, assessing the relationship between PSF and traits at the intraspecific level should improve our understanding of the evolutionary-mediated consequences of global change on plant communities (van der Putten et al., 2016).

5 | CONCLUSIONS

All in all, the traits that primarily explain PSF variation differed between diversity scales. This pattern is likely not due to contrasting trait variabilities, as we found the variability of traits relative to that of PSF to be fairly similar at interspecific and intraspecific levels (Table S2: $sd^{intra} = 0.5\, sd^{inter}$, except for *Phenols*). Whether such a pattern—that is scale-dependence of PSF–trait relationships—should be generally expected would require assessing within-species relationships for several coexisting species. Our finding nevertheless indicates that the relationships between PSF and functional plant traits fluctuate over evolutionary timescales, possibly as a result of the perpetual coevolution between plant species and their soil microbiome (Occhipinti, 2013; Schweitzer et al., 2008). Importantly, while different traits related to PSF at each diversity level, exudate composition was involved at both levels. Other traits significantly affected PSF only via their interaction with exudate composition, and this was again true at both diversity levels. Identifying and characterizing the specific exuded metabolites involved in such patterns would considerably strengthen our mechanistic comprehension of the interplay between functional traits, root exudation and PSF (e.g. Hu et al., 2018), but the latter was beyond the scope of this study. Overall, we are convinced that a better integration between PSF and root exudate research would benefit both research fields, and greatly improve our understanding of the processes—both

ecological and evolutionary—supporting the maintenance of diverse coexistence within plant communities.

AUTHOR CONTRIBUTIONS

Eliška Kuťáková and Zuzana Münzbergová designed the experiment, which was subsequently performed by Eliška Kuťáková and Věroslava Hadincová. Jaroslav Semerád and Tomáš Cajthaml processed root filtrates to provide exudate composition data. Data analysis was then performed by Youssef Yacine with input from Eliška Kuťáková, Dina in 't Zandt and Zuzana Münzbergová. Youssef Yacine, Eliška Kuťáková, Dina in 't Zandt and Zuzana Münzbergová all contributed to result interpretation. Youssef Yacine wrote the first draft of the manuscript, which has been subsequently reviewed and edited by Eliška Kuťáková, Dina in 't Zandt and Zuzana Münzbergová. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The empirical data and R workflow used for the analysis are available in Zenodo: https://doi.org/10.5281/zenodo.10669635.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Phylogeny of the 13 species of plant studied.

Table S1. Missing values for plant–soil feedback and functional traits.

Figure S2. Between-species versus within-species plant–soil feedback variation (raw data).

Figure S3. Relationships between PSF and additional dimensions of exudate composition.

Table S2. PSF and trait variability at interspecific versus intraspecific levels.

Appendix S1. Additional methodological details.

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