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RESEARCH ARTICLE

Functional traits of plant roots and Collembola determine their tri-trophic interactions with soil microbes

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Abstract

1. Traditionally, leaf litter has been recognized as the main driver of the soil food web, but more recently roots have been shown to play an important role in fueling soil organisms. Root functional traits were shown to have direct effects on microbes and Nematoda, but many knowledge gaps remain such as the effects of root traits on Collembola.
2. Here, in a microcosm experiment, we studied the tri-trophic interactions between roots, microbes and Collembola in relation to 10 plant species individually. Eleven root traits were measured to test whether they have an influence on Collembola and microbe community structure and Collembola functional structure based on six traits. The interactions between microbes and Collembola were also tested.
3. Our results show that plant species identity significantly influences the structure of Collembola communities, and this variation is primarily explained by root traits and microbial communities. Collembola feeding traits based on mandibular morphology were useful to identify top-down control on microbial communities. Our study also suggests that root traits such as fine root length and root diameter modify Collembola–microbe interactions, hypothetically by modifying soil porosity.
4. Overall, we obtained better results by looking at the whole system, rather than looking at bi-trophic interactions. This illustrates the importance of a holistic approach when studying biotic interactions in soil ecosystems.

KEYWORDS

bacteria, feeding traits, fungi, soil food web

1 | INTRODUCTION

Above-ground–below-ground interactions have been shown to be important drivers of the composition and dynamics of plant, animal and microbial communities. Plants and soil organisms interact in complex feedback loops, which influence key ecosystem processes such as primary productivity, organic matter decomposition and nutrient cycling (Bardgett & Wardle, 2010; Winck

et al., 2020). Recent studies have shown that above-ground and below-ground community compositions are correlated, suggesting a high degree of connectivity between soil fauna and plants (Forey et al., 2021; Wubs et al., 2019). Traditionally, leaf litter has been recognized as the main driver of the soil food web, but recently, plant roots have also shown to play a major role (Pollierer et al., 2012; Scheunemann et al., 2015). Following this idea, it has been shown that certain root functional traits, such as chemical composition

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and dry matter content, offer mechanistic insights into above- and below-ground interactions (Otfinowski & Coffey, 2020; Spitzer et al., 2021). However, little research has been conducted on this topic and many knowledge gaps remain. In particular, the effect of root traits on Collembola, a dominant soil microarthropod group, has been poorly studied.

The plant economic spectrum classifies roots from conservative to acquisitive as a function of their resource management strategy (Reich, 2014). Plants with a conservative strategy (e.g. roots that require greater investment, but live longer) tend to have low specific root length (SRL), high root tissue density (RTD) and a high C/N ratio, and vice versa for plants with an acquisitive strategy (e.g. roots cheaply constructed, but rapidly efficient) (Bardgett et al., 2014). Recently, the root economics space was expanded to include a collaboration dimension (Bergmann et al., 2020). This dimension includes a gradient from high root diameter favourable to symbiotic fungi, to roots with a high SRL acquiring nutrients mostly by themselves. It was shown to be advantageous to rely on broad root strategies when studying large schemes, such as the variation in the bacteria/fungi ratio or soil fauna abundance (Spitzer et al., 2021; Zhang et al., 2020). However, it may be more useful to rely on individual root traits when studying more taxonomically resolved communities, as different taxa can respond differently to different trait values (Spitzer et al., 2021; Wan et al., 2021).

Recently, some studies have shown interest in relating root traits to microbial communities, allowing to identify some relationships. Root chemistry is an important determinant of microbial communities (Legay et al., 2014). In particular, high C content is favourable for fungal development, while high N content is favourable for bacterial development (Bardgett et al., 2014). Roots also produce exudates comprising molecules such as sugars, amino acids and organic acids (Zieger et al., 2017), which can act as attractants for beneficial microbes or as a defence against pathogens, thus influencing soil microbial abundance and community structure (Huang et al., 2014). Some physical root traits have also been shown to play a role in structuring microbial communities. Root forks per root length (RFRL) was shown to be favourable for mycorrhizal integration (Spitzer et al., 2021), while SRL and RTD were correlated with high levels of exudation, and bacterial growth (Wan et al., 2021). Root dry matter content (RDMC) was also shown to influence microbial community structure, but the mechanism was not identified (Legay et al., 2014; Spitzer et al., 2021). Bacteria and fungi, in turn, represent an intermediate for root-derived C and N between plants and soil invertebrates (Eissfeller et al., 2013; Pollierer et al., 2007). Therefore, it is expected that plants have an indirect effect on Collembola communities via microbes.

The direct influence of root traits on Collembola community structure is less well understood, and only a few hypotheses have been tested. One exception is the presence of chemical compounds such as phenolics and tannins, which have been shown to negatively affect Collembola abundance (Abgrall et al., 2018; Tsunoda & van Dam, 2017). However, additional traits can be

proposed as potential drivers of Collembola community structure based on ecological factors. Results of Salamon et al. (2004) suggest that fine root biomass has a positive effect on the abundance of some euedaphic species, likely due to its favourable effect on microbes. It can also be hypothesized that root architecture, by modifying soil structure and porosity, may influence the ability of Collembola to move in the soil (Erktan et al., 2020). For example, fine root biomass and complexity may homogenize soil pores to a smaller diameter (Bodner et al., 2014), which may impede Collembola movement in the soil (Larsen et al., 2004). Similarly, larger roots may reduce soil porosity by compressing the soil near the root while growing (Bardgett et al., 2014).

As microbes can influence Collembola population dynamics and community structure (Biryol et al., 2024; Rumble et al., 2022), Collembola can also influence microbes by selective grazing (Coulibaly et al., 2019; Seastedt, 1984). Although the top-down impact of microbivory has been poorly studied, it is thought to be important for how microbial communities are structured (Thakur & Geisen, 2019). Their grazing may also be beneficial for fungi by inducing compensatory growth (Bengtsson et al., 1993).

Integrating the functional aspect of Collembola could help to better understand their tri-trophic interactions with roots and microbes. Even if the functional trait approach is well established in Collembola studies (Brousseau et al., 2018), the set of traits traditionally used is hardly relatable to root systems or their trophic interactions with the microbes. Mandibular type is sometimes used to reflect resource selection (fungi, bacteria or plant material) (Austruy et al., 2016; Ross et al., 2022; Santorufo et al., 2015), but it provides only a very broad view of the Collembola feeding interactions. Recently, a new set of traits related to the structure of the Collembola mandibles was developed, which may help to introduce the dimension of feeding interactions in functional trait studies (Raymond-Léonard et al., 2019). Similar mandibular traits have been shown to be useful in determining the distribution of other arthropod groups in relation to their resources (Ang et al., 2023; Brousseau et al., 2019; Le Provost et al., 2017) and could play a similar role for Collembola.

The objective of this study was to determine how root traits can influence microbial and Collembola community structure and to identify the mechanisms involved, using a functional traits approach. To this end, we grew 10 herbaceous plant species with contrasting root traits in microcosms and investigated their effect on soil community assemblages. We hypothesized that plant species with contrasting root traits would drive distinct soil assemblages of both microbes and Collembola. Leaf litter and macrofauna were removed from the microcosms, so the roots were the sole primary source of nutrients and the Collembola were almost free of predators. Otherwise, the microbes, the microfauna and the mesofauna were not manipulated and represented natural communities at the start of the experiment. In this context, we assumed that the forces influencing the Collembola community structure came from a bottom-up constraint starting at the root level, and from root-induced changes in soil chemistry and structure. Similarly, we assumed that

the microbes would be subject to a bottom-up pressure from the roots, and a top-down pressure from the Collembola.

2 | MATERIALS AND METHODS

2.1 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Microcosms	Treatments	10 of each 11 treatments

2.2 | Soil and plant collection

The soil was collected in Mont-St-Aignan, Normandy, France (49.4583° N, 1.07746° W) on 1 July 2019, in a permanent grassland at the edge of a wooded area situated less than 1 km away from the laboratory. The soil was sieved at 5 mm in the field to remove coarse debris and macroinvertebrates. On the following days, the soil was gently sieved at 2 mm in the laboratory and stored at 18°C until the start of the experiment.

We selected 10 plant species representing grasses (*Holcus lanatus*, *Poa annua*, *Poa pratensis*), forbs (*Achillea millefolium*, *Daucus carota*, *Plantago lanceolata*, *Rumex crispus*, *Veronica persica*) and legumes (*Medicago lupulina*, *Trifolium pratense*). These plants were selected to maximize the diversity in root traits based on available data in the TRY database (Kattge et al., 2020). All seeds were obtained from the botanical Garden of Caen (Normandy), except for *M. lupulina* which were purchased from a local producer.

2.3 | Experimental setup

We had 11 treatments including the 10 plant species and a control with no plants. The microcosms were established in 4-L flower pots (21 cm diameter and 17 cm high) filled with 750 g of sieved soil. The bottom of each microcosm was lined with a 250 µm mesh to prevent the escape of microarthropods. One seedling was transplanted into each microcosm (except the controls) on July 8. The seedlings of *D. carota* and *V. persica* were transplanted 10 days later (July 18) due to delayed germination. If a seedling died during the first 2 weeks, it was replaced. A total of 110 microcosms were prepared: 11 treatments x 10 replicates. Our experiment did not require ethics approval.

The microcosms were kept outside in a shaded area in the backyard of the University of Rouen. During hot and dry periods, the microcosms were manually moistened every 2 or 3 days with 50–75 mL of tap water. The microcosms were regularly monitored to remove any spontaneous germination. The experiment was stopped on September 25 for *R. crispus*, that is 1 week earlier than planned, due to a massive aphid invasion on some individuals of this species. Only

minor herbivory events were observed on the other plants. The experiment ended between 3 and 14 October and for the other microcosms (i.e. ~13 weeks after the beginning).

At the end of the experiment, the roots were gently but thoroughly cleaned with tap water to remove all soil particles and were kept apart for the measurements of functional traits. The soil was placed in a plastic bag and stored in a freezer at 4°C until further processing. Less than 5 days later, ~200 g equivalent dry soil was placed in Berlèse-Tullgren devices over a 10-day period to extract Collembola, which were kept in 70% ethanol until identification. The remaining soil was used for the chemical and the microbial analyses.

2.4 | Root traits

Root traits were chosen for their potential direct or indirect interactions with soil organisms (Table 1). The traits were measured on all individual plants at the end of the experiment. Less than 12 h after uprooting, the volume of the root was measured by immersing it in a graduated cylinder filled with water. The roots were then scanned and digitally analysed using the WinRhizo software (Regent Instruments, Canada). The fresh roots were weighed before being dried at 35°C for 72 h to obtain dry root biomass.

All structural root traits (Table 1), but the fine root length, were measured based on standardized protocols (Pérez-Harguindeguy et al., 2013). The fine root length was measured as the combined length of all roots with a diameter less than 1 mm. Total C and N of each plant sample were measured by gas chromatography with a CHN pyrolysis micro-analyser (Flash 2000 Series, CHNS/O Analysers Thermo Scientific, France). The total phenolic concentration was measured by colorimetry following the protocol described in Santonja et al. (2015) using gallic acid as standard (expressed as equivalent gallic acid). A 250 mg sample of roots reduced to dust was extracted in 20 mL of a 70% aqueous methanol solution, shaken for 1 h and then filtered (0.45 µm filter); 50 µL of filtered extract was mixed with 200 µL of saturated aqueous Na₂CO₃ (to stabilize the colour reaction), 1650 µL of distilled water and 100 µL Folin-Ciocalteu reagent. After 30 min, the reaction was completed, and the concentration of phenolics was measured at 765 nm on a UV/Vis spectrophotometer (Thermo Scientific®, Waltham, MA, USA).

2.5 | Soil microbial community

The phospholipid fatty acids (PLFA) are essential components of all living cells and are used as biomarkers of soil microbial communities (Tollefson & McKercher, 1983). The PLFA extraction followed the protocol described by Aupic-Samain et al. (2021). Four millilitres of Bligh–Dyer extractant containing 4 µL of 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (C19:0; Avanti® Polar lipids, Inc.) as internal standard was added to 0.5 g of samples. Lipid separation was performed by solid-phase extraction (SPE) on Phenomenex® (Strata SI-1 with 50 mg of silica, 55 µm, 70 Å).

Root traits	Unit	Function
Structural		
Mean diameter	mm	Related to resource acquisition strategy and influences the spatial configuration of the roots. May affect organism movement in the soil
Root biomass	g	May influence the overall impact of roots on soil characteristics
Root dry matter content (RDMC)	%	Roots with low RDMC produce less exudation which can be consumed by microbes and Collembola
Fine root length	mm	Influences the spatial configuration of the roots. May affect organism movement in the soil
Root forks per root length		Influences the spatial configuration of the roots. May affect organism movement in the soil
Root tissue density	g cm ⁻³	Related to plant conservation. May be less palatable to herbivores. Also associated with a high level of exudation
Specific root length (SRL)	g cm ⁻³	Related to resource uptake and to soil exploration strategy ability; high SRL may result in lower microbial abundance
Chemical		
Carbon (C) content	%	Nutrient intake; influences soil chemistry
Nitrate (N) content	%	Nutrient intake; influences soil chemistry
C/N ratio		Influences palatability
Defence		
Phenol	mg g ⁻¹	May act as a deterrent to microbes and Collembola

Trait	Type	Function
Body shape	Continuous	Movement. May be easier to move in thick fine roots with a slender body
Post antennal organ	Categoric	Chemical receptor. Could be sensitive to root exudates
Mechanical advantage	Continuous	Related to food used (prey, fungi or plants)
Molar plate length	Continuous	Related to food used. High value associated with fungivory
Deployment capacity	Continuous	Food processing. Scrapping (high value) to cutting (low value) movement
Apical development	Continuous	Food processing. High value associated with mechanical processing (grinding, cutting or scrapping)

TABLE 1 List of selected root traits and their hypothetical function in plant interactions with microbes and Collembola.

TABLE 2 List of selected Collembola traits with their hypothetical function in Collembola interactions with microbes and plants.

The resulting fatty acid methyl esters (FAMES) were analysed by gas chromatography/mass spectrometry (GC-MS) on an Agilent 7890 system equipped with an MSD5977A Network mass detector, an ALS7693 automatic injector and an HP5-MS apolar column (30 m × 0.25 mm × 0.25 μm; J&W Agilent Technologies) and MassHunter software. Qualitative analysis of FAMES resulted from retention time comparison via FAME mixture (range between C4 to C24). Biomasses were expressed in μg g⁻¹ dw of soil. Among the 45 PLFAs identified in the samples, 41 microbial-specific PLFAs were analysed (Frostegård & Bååth, 1996). The complete list of used fatty acids and their corresponding microbial groups is found in Table S1.

2.6 | Soil Collembola community and traits

Collembola individuals were counted and identified to species level under a microscope (630×) using the identification key in Hopkin (2007). The traits were selected to represent their dispersal capacity through the soil (body shape), their resource management (mandibular traits) and their interaction with the environment (post-antennal organ [PAO]) (Table 2). Body shape is defined as the mean body width (measured at the level of the mesothorax, first and fourth abdominal segments)/body length. PAO was derived from the literature (Hopkin, 2007) and included three potential values: 0=none, 1=simple, 2=complex. Finally, mandibular traits were measured

following Raymond-Léonard et al. (2019). We included traits such as deployment capacity and apical development, which are directly related to food acquisition, and the molar plate length and mechanical advantage, which are related to food processing (Raymond-Léonard et al., 2019). For each species, the traits were measured on 6–10 specimens in good shape, randomly selected across the treatments.

2.7 | Statistical analysis

Within nine microcosms out of 100, plants did not survive to the end of the experiment and they were totally removed from the analyses (Table S2). One control was also removed due to the high number of Isopoda found in the microcosms. Some plants have comparatively small roots at the end of the experiment; therefore, all analyses were performed with all plant individuals and only individuals with a root volume $>0.5\text{ mm}^3$. As only one *V. persica* individual respected this criterion, the species was completely excluded from this part of the analyses. Overall, 30 plants were removed from the analyses. Comparison of the two analysis methods revealed only a slight increase in statistical power when plants with low root volumes were removed. Therefore, only these results are presented. The abundance of Collembola species was standardized for each microcosm to represent the number of individuals in 200 g of dry soil.

The analyses were separated into two parts. First, we used descriptive analysis to compare the microcosms to see how root traits, Collembola and microbial communities were distributed across microcosms. Second, we tested whether variation in Collembola and microbial communities could be explained by an effect of root traits and/or by an interaction between Collembola and microbes. All analyses were performed using R version 4.0.2 (R Core Team, 2021).

The descriptive part of the analyses was based on principal component analysis (PCA) performed with the function *rda* of the *vegan* library v. 2.5–7 (Oksanen et al., 2020). A first PCA was performed on the standardized root traits to describe the variation between the different plant species and plant individuals. Three further PCAs were performed to account for the taxonomic and functional structure of the Collembola community, and the structure of the microbial community. The PCA on the Collembola taxonomic structure was conducted on a log-transformed species matrix, after checking that it did not lead to a horseshoe effect (Legendre & Legendre, 2012). The PCA on the Collembola functional structure was conducted on the standardized community weight mean (CWM) of Collembola traits, while the PCA on the microbial community was performed on the standardized PLFA abundances. General linear models (GLMs) were performed on the multivariate data of the three PCAs to test whether the structure varied significantly between treatments. The GLMs were performed with and without the control treatment. For the microbial and the Collembola taxonomic structure, we used a negative binomial model, as the mean variance of the abundance data followed a quadratic relationship, while Poisson's model was used for the Collembola functional structure (Warton et al., 2012). Residual

permutation was used as the resampling method to calculate the *p*-values. GLMs were performed using the *manyglm* function of the *mvabund* library (Wang et al., 2012).

The analyses of variance (ANOVAs) were used to test whether the treatments had an effect on the abundance of Collembola, bacteria and fungi, and the diversity (Shannon's entropy index) of Collembola. Type III ANOVAs were used due to the unequal number of microcosms of each treatment at the end of the experiment. It was combined with Tukey's test to find a significant difference between treatments. The normality of each variable was tested using a Shapiro test. Variables with a non-normal distribution were log-transformed. The mycorrhiza was excluded from the fungi abundance; otherwise, the variable was not normal even after the log transformation.

The next analytical step was to relate the three trophic levels in order to analyse their interactions. To do this, we used the partial Mantel test and Procrustes analysis as complementary analyses. While the partial Mantel test allows the calculation of the direct relationship between two trophic levels, the Procrustes analysis allows the analysis of the pairwise collinearity between variables.

A partial Mantel test was used to determine the relationship between two trophic levels while taking into account the effect of the third. The test was performed for each pair of trophic level interactions (Collembola, microbes and plants), using the third level as a conditional variable. For Collembola, community structure and the traits were taken into account, so six partial Mantel tests were performed. All the datasets were transformed into an alternative Gower distance matrix, and the Pearson correlation coefficient was measured based on 9999 permutations. The analyses were performed using the *partial.mantel* function of the *vegan* library (Oksanen et al., 2020).

Procrustes analysis was used to relate the root and soil variables to the variation in Collembola and microbial community structure between treatments based on their ordination (PCA). Procrustes analysis allows the comparison of two ordinations, by determining how much stress must be applied to fit them together (Peres-Neto & Jackson, 2001). To lower stress it requires, the higher the correlation is between the components of these two ordinations. Procrustes are mainly to compare the structure of different component of a community: for example plants versus soil chemistry (Lisboa et al., 2013) or functional traits of soil arthropods versus traits of their resources (Brousseau et al., 2019).

Here, we also wanted to determine which variables had the strongest influence on the structure of the studied communities, requiring a more complex approach. This implies to determine the contribution to the covariation of each variable when the effect of the other variables is removed. Doing this in a single step, considering all variables at once, could lead to spurious results, as a variable with little impacts overall, but with no correlation with other variables, could get a better score than an important variable, but correlated to another variable. Therefore, the comparison was made considering several subsets of variables (see bullet points for details). The exercise was carried out for four datasets: Collembola community structure, Collembola functional traits (CWM), Collembola feeding traits (CWM) and microbial

community structure. The Collembola feeding traits' matrix is a subset containing only the four mandibular traits. The analysis was conducted in three steps:

- *Collinearity between the variables*: the collinearity between the variables was tested using Spearman correlation. If two variables from the same category (root structural traits, chemical, etc.) (Table 1) have a score ≥ 0.6 (Figure S1), they are never used together in a model.
- *For all potential trio of variables*: The Procrustes R^2 (covariation strength) was calculated for each trio and compared with the Procrustes R^2 of the model when one variable is removed. It was done for each variable alternatively.
- *Contribution of each variable*: It was calculated as the mean value of the difference between the Procrustes R^2 of the precedent step. This is the equivalent of measuring the contribution of a variable when the effect of the other variable is removed in variation partitioning (Peres-Neto et al., 2006). Note that in Procrustes analysis, unlike in regression, the addition of a variable may increase or decrease the Procrustes R^2 ; thus, the resulting contribution may be negative or positive. A positive result represents the unique contribution of the variable. A negative result implies that the variable explains less than a random variable and has zero contribution to the model. The mean contribution value was calculated for all models and the results were standardized on a scale from 0 to 100 for comparison purposes.

The variables used in collinearity with the Collembola community included the root traits, soil chemistry variables and five variables describing the microbial communities: fungal and bacterial abundances, fungal/bacterial ratio and microbial community structure expressed as the first two axes of the PCA on microbial PLFA. The same variables were used for the microbial community structure, but the Collembola feeding traits were added, and the Collembola community structure expressed as the first two axes of the PCA on Collembola species were used. The number of dimensions analysed in the Procrustes in this section was always set to two.

The final analytic step was to determine the model explaining the highest covariation in the community/functional structure of each trophic level based on Procrustes analysis. Procrustes analyses were performed on all combinations of variables, excluding models with correlated variables, and we kept the significant model with the highest Procrustes R^2 . As the number of PCA dimensions analysed in the Procrustes can affect the results, we varied the number of dimensions to obtain the best fit (Lisboa et al., 2014).

3 | RESULTS

3.1 | Plant root traits

The PCA on root traits showed a clear division of plant species into three groups (Figure 1a). The first group is formed by the three Poaceae species and is characterized by high C concentration, SRL

and fine root length. The second group includes the forbs and legumes with high N concentration, low phenolic concentration and low RDMC and biomass. Finally, *R. crispus* is separated from other plant species and is characterized by higher RDMC, biomass and phenolic concentration.

3.2 | Microbial community

The soil microbial communities show a random distribution, except for the controls which are closely clustered in the PCA (Figure 1b). The lack of significant results in the GLM when the control plots are removed confirms this observation (Table 3). However, many microbial communities are different from the controls, showing an impact of the presence of a plant, which could not be directly related to the plant identity. Also, the average abundance of bacteria and fungi was lower in the control microcosms compared with those containing plants, though this difference was only statistically significant for *A. millefolium*, *P. annua*, and *R. crispus* (both bacteria and fungi) and *M. lupina* (fungi) (Figure 2A,B).

3.3 | Collembola communities

A total of 4961 individuals and 23 species of Collembola were identified and used in the analyses (Table S3). The PCA on Collembola species abundance showed that taxonomic community structure varied between treatments (Figure 3a), which was confirmed by GLM analysis (Table 3). Communities of some plant species (such as *R. crispus*, *P. lanceolata* and *T. pratense*) separated from control communities on the first axis ($\lambda=0.36$). The second axis ($\lambda=0.12$) separated Poaceae species and *P. lanceolata* from *R. crispus* and *M. lupulina* (although two out of eight individuals of this species were similar to Poaceae communities).

The GLM on the Collembola functional traits showed a variation between treatments (Table 3), but the separation was not as clear as for the species (Figure 3b). The variation in functional trait value is low in the communities from control microcosms, compared with those from plant species such as *P. lanceolata* and *R. crispus*. The ANOVAs reveal that Collembola abundance is higher in control microcosms than in microcosms with three Poaceae species, but the average diversity is lower in controls than in association with any plant species, although the difference is significant only for *P. annua* and *A. millefolium* (Figure 2C,D).

3.4 | Link between microbes, plants and Collembola

The six partial Mantel tests were not significant with a Mantel r approaching 0 (Table 4). Procrustes analyses show that the three aspects of Collembola community structure (species, functional traits and feeding traits) were mainly influenced by the root structural traits and their phenolic content (Table 5). The root RDMC and root biomass

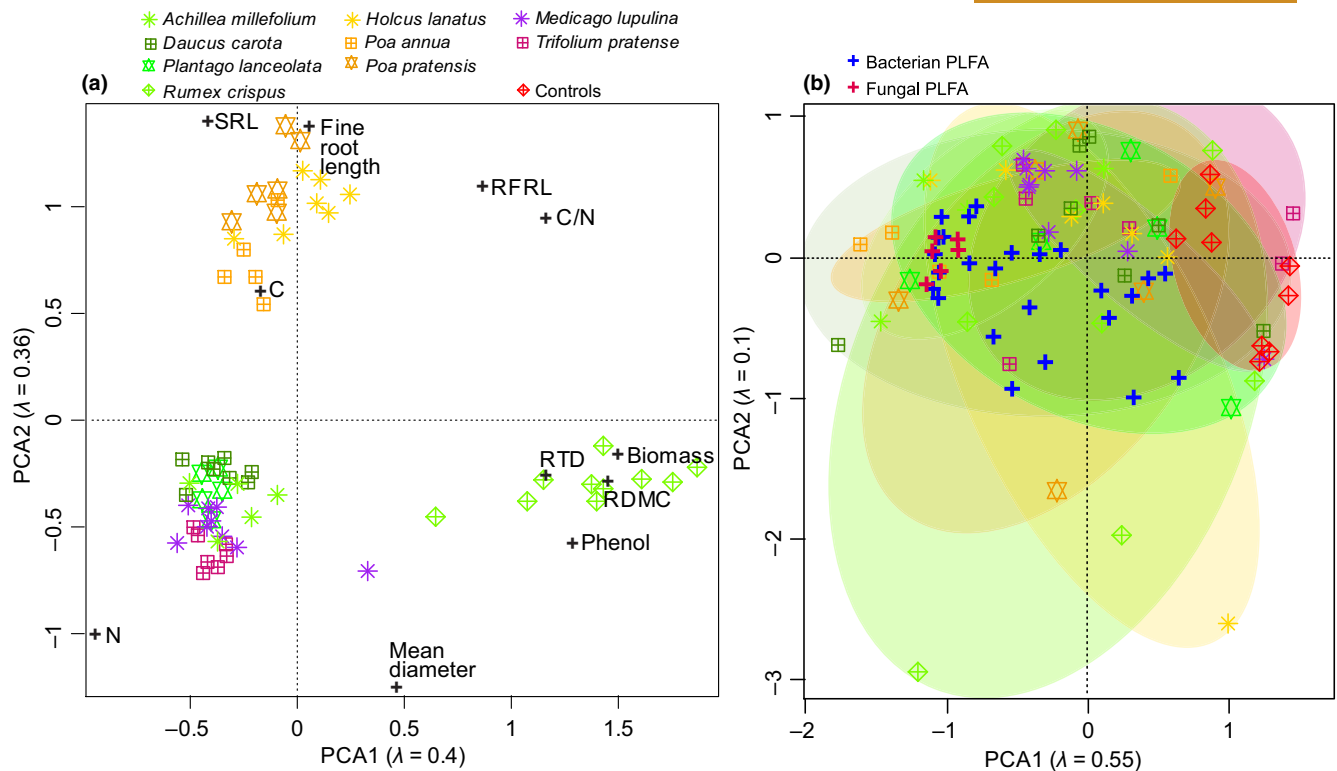


FIGURE 1 Principal component analysis (PCA) showing (a) the distribution of individual plants based on their root functional traits and (b) the abundance of microbial phospholipid fatty acids (PLFA). Different shapes and colours represent different treatments (plant species or control). See Table 1 for trait description and abbreviation. Ellipses are included for visual clarity but do not indicate any statistical significance. RDMC, root dry matter content; RFRL, root forks per root length; RTD, root tissue density; SRL, specific root length.

TABLE 3 Results of general linear models testing the effects of nine plant species and no-plant controls on the community structure of Collembola (at the taxonomic and functional levels) and the community structure of microbes. The results are presented with and without the controls.

	With controls		Without controls	
	df	F	df	F
Collembola: taxonomic	61	323.2**	53	279.7**
Collembola: functional	61	1945.6**	53	1971.6**
Microbes	61	8.2*	53	38.2

* $p < 0.01$; ** $p \leq 0.001$.

ranked in the top five for their contribution to the Procrustean models for the three aspects of Collembola community structure. Root chemistry had a higher ranking than soil chemistry at the species level, but the contrary was observed at the functional and feeding trait levels. Microbial abundance ranked at or near zero at the Collembola species level, but ranked higher than 70 for the feeding trait level.

The microbial community was mainly influenced by root C content and Collembola mandibular traits. Fine root length, and RTD and phenolic content were the root traits with the highest ranking. Other structural traits and chemical aspects had a low ranking under 30.

The models with the highest Procrustes R^2 for the three aspects of Collembola community structure all included the root mean diameter as a variable and at least one soil chemical and one microbe-related variable (Table 6). The Procrustes R^2 was higher when analysing the taxonomic structure (0.53) than the functional (0.43) and feeding traits (0.41) of Collembola communities. The model for the feeding traits was the most complex, including seven variables and three dimensions instead of two as the others. The best model for microbial communities had a Procrustes $R^2 = 0.33$ and included two structural traits, a root chemical trait, the phenolic content and the mechanical advantage of Collembola mandibles.

The visual representation of the Procrustes with all variables and Collembola traits shows that three mandibular traits (molar plate, mechanical advantage and deployment) covary positively with bacteria and fungi (Figure 4). The apical development of mandibles covaries negatively with the fungi/bacteria ratio and positively with the soil C content. The body shape covaries negatively with the fine root length, while the presence of PAO covaries negatively with the root phenolic content.

4 | DISCUSSION

Our results show that plant species can drive variations in Collembola community structure, and these variations are well

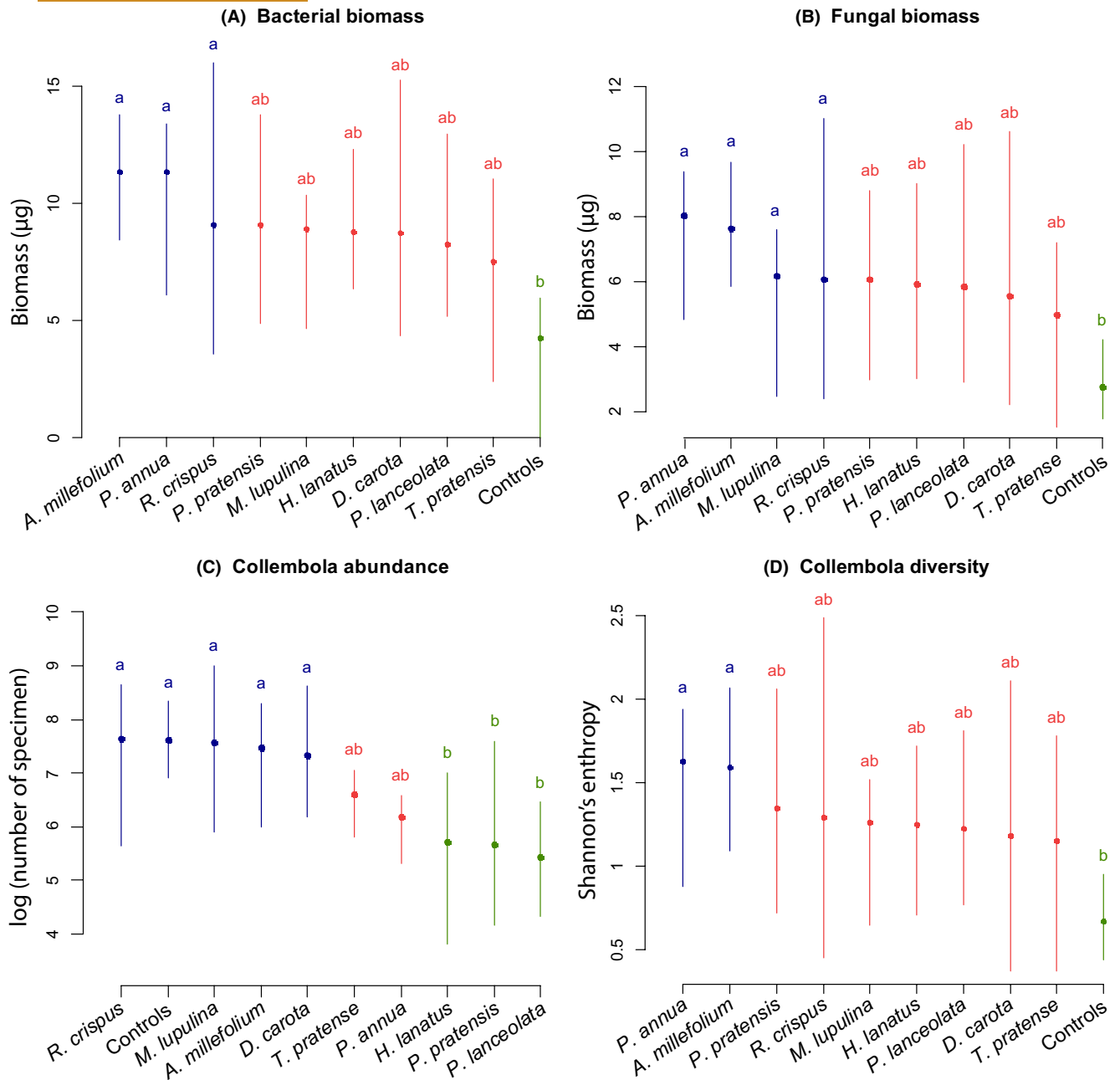


FIGURE 2 Boxplots comparing the difference in the biomass ($\mu\text{g g}^{-1}$) of (A) bacteria (derived from phospholipid fatty acid [PLFA]), (B) fungi (derived from PLFA) and (C) Collembola abundance (log), and (D) diversity based on the Shannon's entropy index between 10 treatments (nine plant species and no-plant controls). Different letters indicate significant differences with $a > b$ (post hoc Tukey tests).

explained by root traits and microbial communities. We hypothesized that plant species would drive both Collembola and microbial communities, but this was only confirmed for the Collembola. While some significant variations were observed in Collembola community structure across plant species, none was observed for microbes. Nevertheless, the Procrustes analyses revealed a three-level interaction, translated as a bottom-up structuring from plant root traits towards microbial and Collembola communities, and a feedback loop between these two groups, confirming our mechanistic hypothesis.

The non-significant Mantel test results suggest that, in our simplified system, roots, microbes and Collembola do not exhibit direct bi-trophic interactions. This means that the correlations observed in the Procrustes analyses are due to a tri-trophic interaction. This illustrates the importance of considering a holistic approach when studying interactions in soil ecosystems and their food webs (Potapov, 2022). Two-level interactions may be more difficult to detect in the soil than in the above-ground food web due to its particular structure. The soil food web differs from the above-ground by being physically structured by the leaf layer and the soil porosity,

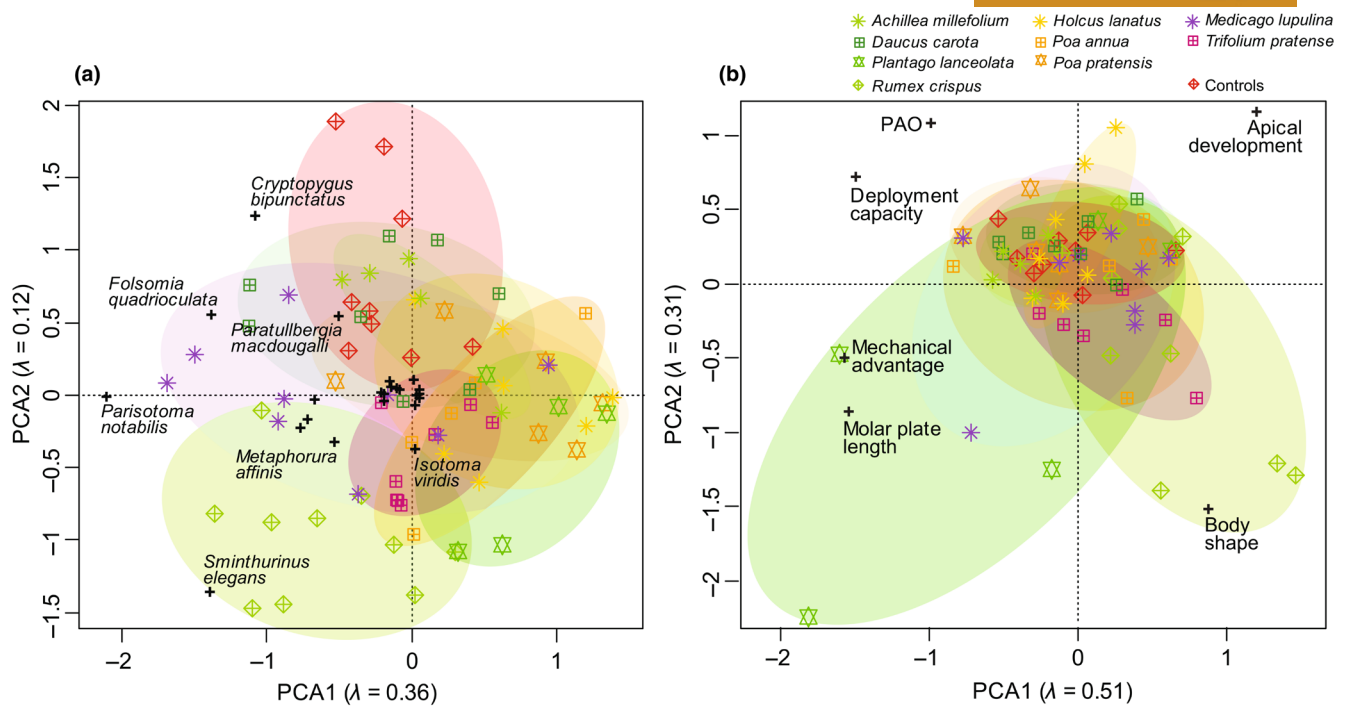


FIGURE 3 Principal component analysis (PCA) of the Collembola community structure at (a) the taxonomic level, and (b) the functional level (community weight mean). The Collembola were caught in relationship with nine plant species and a no-plant control. Ellipses are included for visual clarity but do not indicate any statistical significance. PAO, post-antennal organ.

TABLE 4 Relationship between two trophic levels when the effect of the third (conditional) level is removed based on partial Mantel tests. Pearson's correlation coefficient (Mantel r) is presented based on 9999 permutations.

Correlated trophic levels	Conditional	Mantel r
Collembola × plant traits	Phospholipid fatty acid (PLFA)	-0.03
Collembola × PLFA	Plant traits	-0.03
PLFA × plant traits	Collembola	-0.02
Collembola traits × plant traits	PLFA	0.03
Collembola traits × PLFA	Plant traits	0.01
PLFA × plant traits	Collembola traits	-0.02

which affect the interactions that are realized (Erktan et al., 2020). Furthermore, soil organisms are more generalist than above-ground organisms (Digel et al., 2014), allowing them to switch resources when the availability or accessibility of their preferred food decreases (Endlweber et al., 2009).

4.1 | Microbial communities

Our results showed that the microbes were influenced by both bottom-up root control and top-down Collembola pressure. Root carbon content was the most influential root trait affecting microbial communities. This is unsurprising, as carbon content is a

well-established factor in driving bacterial growth from the bottom up (Bardgett et al., 2014). More surprising is that both soil and root N content, which is generally considered as an important factor influencing fungal communities (Morrison et al., 2016; Zhou et al., 2016), have a very low ranking. Nonetheless, soil N content was one of the five variables included in the best model to fit microbial communities. This could mean that the effect of N varied in function of the composition of the microbial community. It could also be explained by a lower diversity of fungi than bacteria, rendering the effect of N harder to detect. Furthermore, while we measured C and N contents of the roots themselves, it is highly probable that rhizodeposits released from the roots and preferentially consumed by microbes may have a different C/N ratio.

Three other root traits had a strong influence on the microbial communities: RTD, fine root length and phenolic content. RTD is associated with high exudation (Guyonnet et al., 2018), which can stimulate fast-growing bacteria (Wan et al., 2021). The other two traits are not expected to have a direct bottom-up effect, but may still modify microbe/Collembola interactions. Fine root length can affect microbivory interactions by altering soil structure (Bodner et al., 2014; Erktan et al., 2020). In our experiment, fine root length ranked higher in importance for the feeding trait of Collembola, than for their overall functional traits and species composition. In turn, mandibular traits ranked the highest for the microbes (rank 100 and 86), much higher than the fine roots (rank 38). This means that fine root length had a strong effect on Collembola feeding traits, which in turn, had a strong effect on microbial communities. This could be consistent with an effect on feeding interactions based on soil

TABLE 5 Contribution of each variable (in column) to three aspects of the Collembola community (species level, functional traits and feeding traits) and microbial community (see Section 2 for how the contribution was calculated).

Variables	Collembola			Microbes
	Species	Traits	Feeding traits	
Structural root traits				
Mean diameter	82	100	72	28
Fine roots length	62	57	91	31
Roots dry matter content	94	93	98	19
Root forks per root length	51	44	69	15
Roots biomass	94	100	100	26
Roots tissue density	68	27	21	33
Specific root length	76	52	15	23
Roots defence				
Phenol	100	83	75	31
Chemical (roots and soil)				
Root C/N ratio	45	26	36	16
Root C content	38	2	54	75
Root N content	40	14	15	13
Soil C/N ratio	22	44	93	0
Soil C content	22	41	51	24
Soil N content	31	0	15	5
Microbial community				
Bacterial biomass	1	27	76	—
Fungal biomass	0	29	73	—
Fungi/Bacteria ratio	31	16	0	—
Community structure (Axis 1)	20	22	29	—
Community structure (Axis 2)	24	6	2	—
Collembola community and traits				
Apical development	—	—	—	10
Deployment	—	—	—	76
Mechanical advantage	—	—	—	100
Molar plate	—	—	—	51
Community structure (Axis 1)	—	—	—	17
Community structure (Axis 2)	—	—	—	24

compression and a reduction in soil pores (Erktan et al., 2020), rather than a direct effect on Collembola and microbes, but more data would be required to confirm this interpretation. Finally, the effect

of phenolics on microbial communities was shown to depend on their composition and concentration (Qu & Wang, 2008; Zwetsloot et al., 2020). The phenolics can also have an indirect effect by reducing the microbivory by the Collembola (Abgrall et al., 2018). However, it is difficult to determine the exact mechanism by which the phenolic content acted without identifying the composition of each plant and testing them individually.

Three Collembola mandibular traits were also identified as important in the top-down control of the microbes: mechanical advantage, deployment capacity and size of the molar plate. The mechanical advantage of the mandibles is related to their strength and speed of closure, and has a direct effect on the characteristics of the consumed food (speed and toughness). In Collembola, it is hypothesized that species with low mechanical advantage (i.e. fast closing movements) are predators, while those with higher mechanical advantage (i.e. slow, but strong) consume recalcitrant food such as plant material (Perdomo et al., 2012; Raymond-Léonard et al., 2019). The deployment capacity refers to the length of the mandible that can be extended from the head to acquire food. A high deployment capacity is hypothesized to be related to scrapping and bacterivory (Raymond-Léonard et al., 2019). Finally, the molar plate of Collembola is used to brush and scrape food, such as fungal hyphae, so long molar plates are associated with fungivory (Goto, 1972). Although our data do not allow us to determine how these traits modified the microbial community, they suggest that the mandibular traits of Collembola can be used to assess top-down control.

The relationship we observed between microbial communities and root traits is consistent with observations in sub-arctic ecosystems (Spitzer et al., 2021). However, in contrast to other studies (Leff et al., 2018; Spitzer et al., 2021), we did not observe clear differences between plant species. This could be due to the fact we sampled microbes from the entire soil in the microcosms, not just the rhizosphere. Looking at the PCA of the microbes, we see that the communities are randomly distributed, except for the control plots, which are quite similar. So, the communities were modified by the presence of plants, but without a clear pattern, which could be related to the soil used at the start of the experiment. The soil used came from a natural meadow where the studied plants were absent, so the microbial community probably contained mostly generalist taxa, and secondary colonization was unlikely to happen. Thus, the low abundance of microbes specifically associated with the studied plants might have reduced the probability to obtain plant-specific communities as might be expected in a natural environment.

4.2 | Collembola communities

Collembola community structure is known to be influenced by soil properties (Santorufu et al., 2015) and plant diversity (Henneron et al., 2017; Salamon et al., 2004), as well as by functional traits (Abgrall et al., 2017; Forey et al., 2021). The exact mechanisms involved have not been clearly identified, but roots are expected to play an important role (Fujii et al., 2014). Our results are consistent

TABLE 6 Best model (i.e. with the highest significant Procrustes R^2) for three levels of Collembola community structure (species, functional traits and feeding traits) and microbial phospholipid fatty acids with the number of principal component analysis dimensions used in the Procrustes analysis.

Community	Model	Dimension	R^2
Collembola			
Species	Mean diameter+RFRL+Phenols+C (roots)+Fungi/Bacteria ratio	2	0.53**
Functional	Mean diameter+RDMC+RFRL+C/N (soil)+Bacteria	2	0.41**
Feeding traits	Mean diameter+Biomass+Fine roots length+C/N (soil)+Fungi	3	0.40**
Microbes	Mean diameter+Fine roots length+Phenol+N (soil)+Mechanical advantage (Collembola mandibles)	5	0.33*

Abbreviations: RDMC, root dry matter content; RFRL, root forks per root length.

* $p < 0.01$; ** $p < 0.001$.

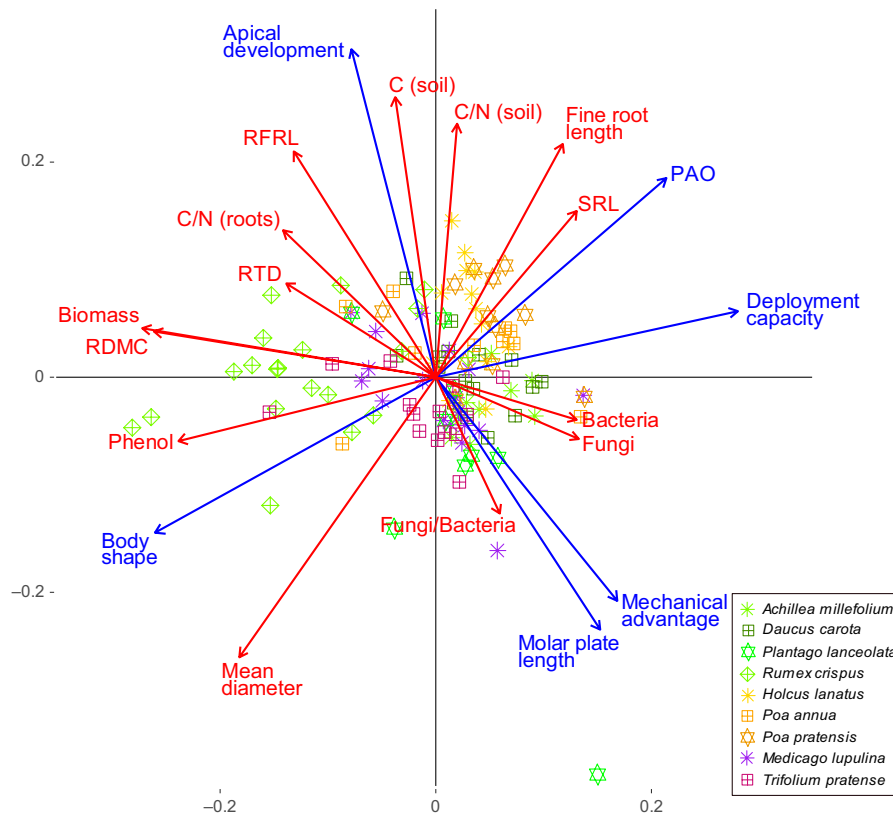


FIGURE 4 Visual representation of the covariation between a set of Collembola traits (blue arrows) and the traits of plant roots, microbe community and soil chemistry (red arrows) based on Procrustes analysis. PAO, post-antennal organ; RDMC, root dry matter content; RFRL, root forks per root length; RTD, root tissue density; SRL, specific root length.

with this idea, showing that root traits were important in structuring Collembola communities. Some root traits, such as mean diameter, RDMC, biomass and phenolic content, were highly ranked for each level of Collembola community aspects studied (species, functional and feeding traits), but some important variations were also observed. In particular, bacterial and fungal abundances ranked high for the feeding traits (76 and 73, respectively), but very low for the species community structure (1 and 0, respectively). This suggests that the selected feeding traits are indeed related to the consumption of microbes. Similarly, RTD was ranked high at the species level, but low at the trait level. Thus, while the analyses at the trait level

allow the identification of some mechanisms, the results at the species level suggest that some traits are missing to identify all the mechanisms relating the RTD to the Collembola communities.

The visual representation of the Procrustes analysis (Figure 3) reveals interesting correlations. Principally, the mechanical advantage and the size of the molar plate are positively correlated with the fungi/bacteria ratio. On the contrary, the apical development of the mandibles is negatively correlated with this ratio. This could indicate a separation of the fungivorous and bacterivorous Collembola. Indeed, high mechanical advantage and large molar plate are associated with fungivory and recalcitrant food, while apical development

is associated with bacterivory (Goto, 1972; Perdomo et al., 2012; Raymond-Léonard et al., 2019). Root phenolic content was also negatively correlated to the presence of postantennal organs. As the postantennal organ is a chemical receptor (Hopkin, 1997), this suggests that Collembola with PAO are more likely to avoid plants that secrete potentially harmful phenolics (Abgrall et al., 2018; Tsunoda & van Dam, 2017). Finally, body shape is negatively correlated with fine root length and positively correlated to mean root diameter. Both root traits were hypothesized to favour thinner Collembola, so this dichotomy is unexpected. It could be that in our experiment, the roots of the plants with thick roots occupied less space than the roots of plants with long fine roots. In this perspective, a root trait describing the space occupied by the roots could potentially give a better result. Also, for an unknown reason, the two plant species with thicker roots (*M. lupina* and *R. crispus*) have a higher abundance of Symphypleona than the other plant species. Symphypleona are generally epigeic (Hopkin, 1997) and are not expected to be affected by root traits in the same way as euedaphic species.

Collembola species community structure varied significantly between plant species, but the variation is only clear for a few plant species such as *R. crispus* versus *D. carota* and *P. pratense*. For other plant species, trends were observed, but with some outliers. The microcosms were designed to minimize initial variation in Collembola communities and to lower the likelihood of secondary colonization during the experiment. Furthermore, the experiment was relatively short (~13 weeks), whereas it has been shown that plant-soil biota interactions can be stronger in the long term than in the short term (Steinauer et al., 2017; Wubs et al., 2019). Therefore, our results should be interpreted as the short-term effects of root traits on Collembola and microbial communities.

4.3 | Controls versus plants

All PCA figures show that the nine controls tend to cluster (Figures 1a and 3), with the microcosms containing a plant more or less diverging from them. This seems to demonstrate that the presence of roots changes the conditions in the microcosm and this is reflected by a modification in the microbial and Collembola communities. Interestingly, when we look at the results including all plants (Figures S3B and S4), that is including those with small roots, more microcosms with plants are close to the cluster made up of the nine controls. In particular, the microcosms with the species *V. persica* with all but one microcosm having a root volume <0.5 mm³. Therefore, root size may be the main factor determining whether an effect is measurable, and other traits such as phenolic content and RDMC may determine the direction of the modification.

At the microbial level, we observed that all of the fungi and most of the bacteria were in the opposite direction from the control plots, suggesting that most microbes are favoured by the presence of roots, regardless of the plant species considered. This is also reflected in the lower biomass of both fungi and bacteria in the controls than in relation to any plant species, including *V. persica* (Figure S2). The

response of microbes to the presence of a plant is clearer than any aspect of the Collembola community and suggests a faster response, mainly due to shorter generation times.

The control was the treatment with the second highest abundance of Collembola, but also the lowest species diversity. The common species *Folsomia quadrioculata* was dominant in the control plots representing 72% of the total abundance. An extraction from 300g of soil before the experimentation shows that *F. quadrioculata* was already the dominant species in the initial stage, representing 72% of all Collembola (result not shown). Therefore, the average dominance of this species in the control did not change during the time of the experiment. *F. quadrioculata* is the dominant species in all treatments, but its relative abundance varies from 57% (*P. annua* and *R. crispus*) to 80% (*A. millefolium*). Therefore, it seems that in our microcosms, growing roots from a single plant tend to reduce the dominance of *F. quadrioculata*, favouring the development of new microbial and Collembola communities that interact with each other, most probably due to a change in the available resources provided by the roots (exudates). Several species were not found in the controls (e.g. *Isotoma viridis* and *Isotomodes productus*), and some common species are very rare in the controls. For example, 50 specimens of *Kalaphorura burmeisteri* were found overall, but only two in the controls. On the contrary, only one species with a single specimen was found uniquely in the controls. It seems that the presence of some plant species had a positive effect on the abundance of some Collembola species. *M. lupulina* and *R. crispus* had the strongest positive impact on the abundance of species such as *Metaphorura affinis* and *Sminthurinus elegans*. Both species have different ecological niches, as *M. affinis* is a euedaphic species living deep in the soil, while *S. elegans* lives rather close to the soil surface and therefore harbours different traits.

Indeed, the impact on the Collembola functional trait values are harder to address, but the controls are again clustered together, this time near the centre of the graph (Figure 3b). All plant species had some microcosms closed to the controls, but generally expressed a higher variability in Collembola traits. The microcosms outside of the control clustering zone tend to have lower proportions of *F. quadrioculata* and sometimes have a different dominant species. *F. quadrioculata* is generally an average species in terms of trait values, except that it is the second thinnest species (body shape) and has the longest mandibular apical development; the only mandibular traits with low impact on microbial community structure (Table 5). Thus, changes in the Collembola community structure can shift the CWM of most traits in one direction or another. The duration of our experiment was rather short and it can be hypothesized that a longer experiment would have produced a clearer separation between the plants and the controls and between the different plant species.

5 | CONCLUSION

Our controlled microcosm experiment was designed to facilitate the identification of the mechanisms driving the relationship between

the roots, microbes and Collembola. In this respect, we were able to identify some root traits that influence the microbial and Collembola communities. Our results also demonstrate the importance of Collembola mandibular traits in considering their feeding interactions, an aspect that has been largely neglected so far. Feeding traits are interesting as they represent a dynamic link between the community structure and ecosystem function (Gravel et al., 2016), but are still not fully understood for the Collembola as they were made available to the trait toolbox only recently (Raymond-Léonard et al., 2019). Here, the observed interactions between the Collembola mandibular traits, the microbe community structure and root traits suggest an impact on soil porosity of traits such as fine root traits which, in turn, can impede Collembola/microbe interactions. The importance of soil porosity in trophic interactions is already known (Erktan et al., 2020), but it is rarely considered in functional trait studies and could deserve some more attention. A longer experiment and a more detailed characterization of the microbial community by metagenomics or transcriptomics could help to better identify the mechanisms involved in the relationships between roots, microbes and Collembola.

We observed that a holistic analysis of our microcosms, considering all three trophic levels simultaneously, provides clearer results than examining bi-trophic interactions in isolation. This principle has already been demonstrated for several aspects of soil ecology (Potapov, 2022). For example, although Collembola are generally considered to be good decomposers of plant material (Hanisch et al., 2022; Seastedt, 1984), their impact is mainly determined by their grazing on fungi (Hopkin, 1997) and their efficiency related to the nutrient concentration in the soil (Hanlon, 1981). Thus, the impact of Collembola on decomposition should be better assessed by considering litter quality, soil substrate and microbial communities. Predators can also affect Collembola (Santonja et al., 2018; Schneider & Maraun, 2009), with consequences cascading down to the microbial community level (Aupic-Samain et al., 2019; Lucas et al., 2020). Our ability to integrate all these levels of complexity is critical to successfully predict the ecological consequences of environmental perturbations, such as climate change.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception of the experimentation. The microcosms were established by Pierre-Marc Brousseau, Estelle Forey et Matthieu Chauvat; the Collembola and the physical trait data were collected by Pierre-Marc Brousseau and the microbial and chemical traits data were collected in the laboratory of Mathieu Santonja. Pierre-Marc Brousseau did the analysis and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data are available on Mendeley Data: <https://doi.org/10.17632/8ymkfw2tgn.1> (Brousseau et al., 2024).

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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. Spearman correlations between all variables used in Procrustes analysis including root traits, soil chemistry, microbial communities, and Collembola traits and communities.

Figure S2. Boxplots comparing the difference in the biomass ($\mu\text{g g}^{-1}$) of (A) bacteria (derived from PLFA), (B) fungi (derived from PLFA), and (C) Collembola abundance (log), and (D) diversity based on the Shannon's entropy index between 11 treatments (ten plant species and no-plant controls).

Figure S3. Principal component analysis showing (A) the distribution of individual plants based on their root functional traits and (B) the abundance of microbe phospholipid fatty acids.

Figure S4. Principal component analysis of the Collembola community structure at (A) the taxonomic level, and (B) the functional level (community weight mean).

Table S1. List of measured 41 fatty acids and their microbial group correspondence.

Table S2. Number of individuals of each species of plant alive at the end of the experiment and used in the analyses.

Table S3. List of Collembola species and their abundance in association with nine plant species growth in microcosms or in no-plant control microcosms.

Table S4. Mean root traits value of nine plant species growth individually in microcosms ~13 weeks after their emergence.

Table S5. Mean functional traits value of 23 species of Collembola.

Table S6. Results of general linear models testing the effects of ten plant species and no-plant controls on the community structure of Collembola (at the taxonomic and functional levels) and the community structure of microbes.

Table S7. Relationship between two trophic levels when the effect of the third (conditional) level is removed based on partial Mantel tests.

Table S8. Correlation between three levels of community structure (Collembola species, Collembola functional traits and microbe fatty acids) and root functional traits.

Table S9. Contribution of each variable (in column) to three aspects of the Collembola community (species level, functional traits and feeding traits) and microbial community.

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