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Root traits and belowground herbivores relate to plant-soil feedback variation among congeners

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Abstract

Plant-soil feedbacks contribute to vegetation dynamics by species-specific interactions between plants and soil biota. Variation in plant-soil feedbacks can be predicted by root traits, successional position and plant nativeness. However, it is unknown whether closely related plant species develop more similar plant-soil feedbacks than more distantly related species. Where previous comparisons included plant species from distant phylogenetic positions, we studied plant-soil feedbacks of congeneric species. Using eight intra-continentally range-expanding and native *Geranium* species, we tested relations between phylogenetic distances, chemical and structural root traits, root microbiomes and plant-soil feedbacks. We show that root chemistry and specific root length better predict bacterial and fungal community composition than phylogenetic distance. Negative plant-soil feedback strength correlates with root-feeding nematode numbers, whereas microbiome dissimilarity, nativeness or phylogeny does not predict plant-soil feedbacks. We conclude that root microbiome variation among congeners is best explained by root traits, and that root-feeding nematode abundances predict plant-soil feedbacks.

Introduction

It is increasingly acknowledged that soil biota contribute to the control of plant species diversity by causing positive or negative plant-soil feedbacks[1-4]. These feedback effects are caused by the accumulation of antagonistic and mutualistic-symbiotic soil organisms by plants, which thereby reduce or promote themselves, their neighbours, or their successors[3,5]. Plant species differentially shape soil communities by a number of mechanisms, including the production of unique combinations of root exudates and volatiles[6,7]. However, the resulting changes in soil communities that feedback to plant performance often remain unknown and we therefore largely lack an understanding of the exact biotic drivers of plant-soil feedbacks.

Soil communities in the rhizospheres of plant species are expected to vary with phylogenetic distance between plant species, as phylogenetically closely related plant species are more likely to share structural and chemical traits than distantly related species[8,9]. In turn, closely related plant species might also be similarly affected by their conditioned soil communities, as they often have comparable natural enemies[10,11] and mutualistic symbionts[12,13]. As plant-soil feedbacks are net effects of positive and negative interactions between plants and soil biota[14], it may be assumed that plant-soil feedback is, at least in part, phylogenetically determined.

So far, studies have found mixed support for a predictive relationship between phylogeny and plant-soil feedbacks[15-17]. In most of these studies hetero-specific plant-soil feedback was examined: the authors tested whether the phylogenetic distance between conditioning plant species predicts the feedback to other plant species[16,18-20]. The effect of plant relatedness on plant-soil feedback effects on conspecifics has so far only been tested along a wide phylogenetic gradient[17]. However, in these types of comparisons deeply conserved traits, which show variation on higher taxonomic levels such as plant families, will likely determine the presence of a phylogenetic signal. Therefore, such studies may not be suitable to test whether phylogenetic distances can be used to understand variation in plant-soil feedbacks among closely related congeneric species. Here, we specifically determined whether closely

related plant species have a more comparable plant-soil feedback than more distantly related plant species by using plant species from the same genus. We examined how rhizosphere microbiome variation related to phylogenetic distance within this genus, as the microbiome is expected to underlie plant-soil feedback effects[12]. So far, only few studies have examined rhizosphere microbiome variation in relation to plant phylogeny[21]. Instead, most studies only examined single groups of rhizosphere organisms[22,23], whereas we included the full soil rhizosphere community.

Plant-soil feedbacks can strongly differ between native plant species and introduced exotics, which may contribute to the success of non-native plant species in their new range[4,24,25]. Reduced negative plant-soil feedbacks of non-native plant species in their new range is not found for invasive exotics[25], but also for plant species that expand their range within the same continent, as a consequence of climate warming[26-28]. However, the biotic interactions potentially underlying plant-soil feedback differences between non-natives and natives may be explained by differences in root chemistry[29]. Therefore, variation in plant-soil feedback outcomes between non-natives and natives might be predicted with phylogenetic distance, when root traits underlying these biotic interactions are phylogenetically conserved[8,9]. However, it is unknown whether plant origin, in addition to phylogenetic distance, may influence plant-soil interactions of non-native plant species in their new range. For example, introduced exotics or range-expanding plant species can become released from natural soil-borne pathogens, even in the presence of a closely related plant species[30,31].

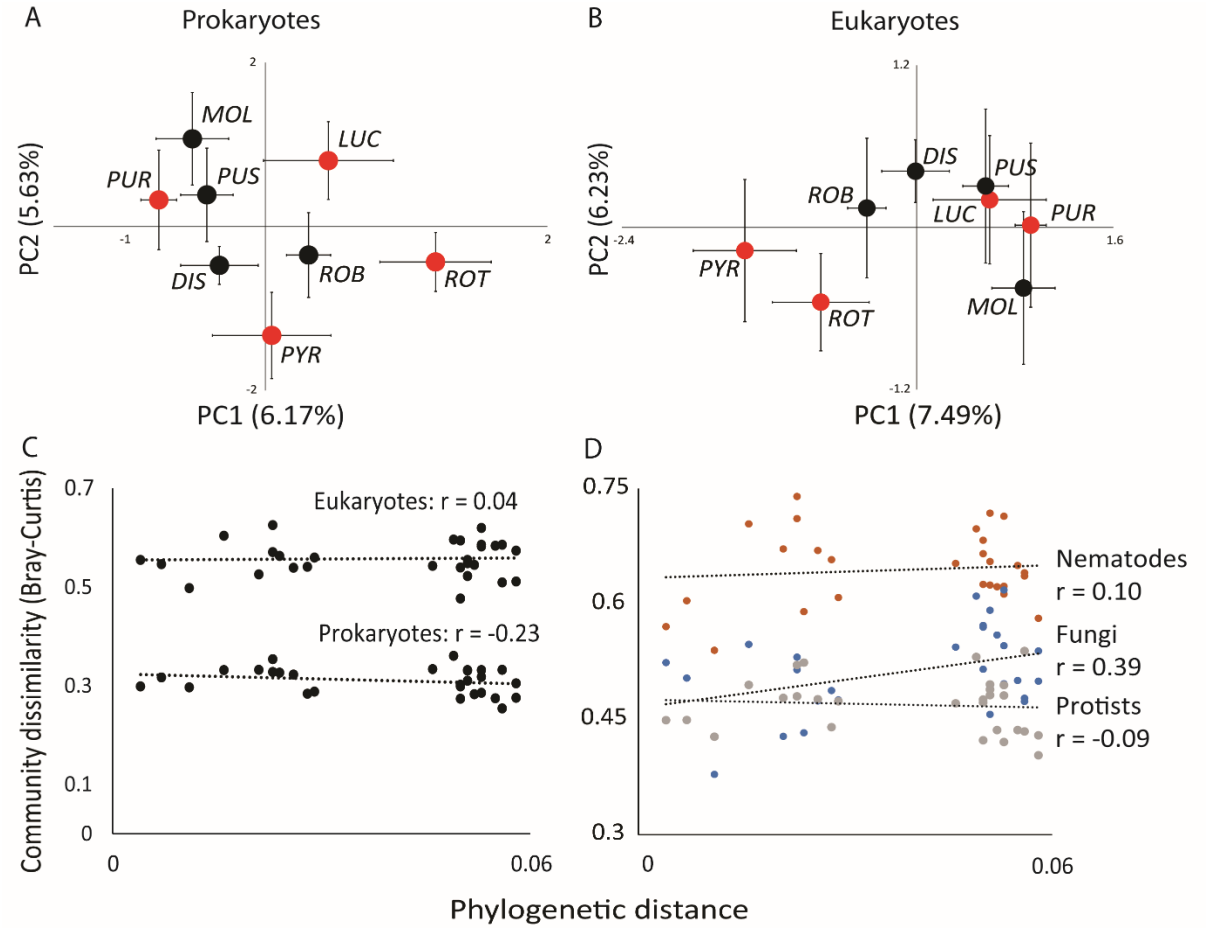
Here, we test the hypothesis that differences in rhizosphere community composition and plant-soil feedback among range-expanding and congeneric native plant species, and the traits underlying this variation, are explained by their phylogenetic distances. We test our hypothesis using eight congeneric *Geranium* species that all occur in north-western Europe. Four of these species are native, whereas the other four recently have become established, most likely as a consequence of recent climate warming. We are able to test plant origin effects irrespective of phylogenetic distance, because native and range-expanding plant species are not phylogenetically clustered (Supplementary Figure 1). We

combine a plant-soil feedback experiment with sequencing of prokaryotic and eukaryotic communities conditioned by the different plant species, and perform analyses of root chemical profiles and structural root traits. Thereby we relate plant-soil feedback effects to variations in soil community composition and belowground plant traits, and determine whether closely related plant species have a more comparable plant-soil feedback than more distantly related plant species.

Results

Rhizosphere community composition

The conditioned prokaryotic (16S rRNA gene reads) and eukaryotic (18S rRNA gene reads) rhizosphere communities varied significantly between the eight plant species (Fig. 1A,B; Supplementary Table 1). The composition of the three distinct taxonomic groups composing the 18s rDNA, fungi, protists and nematodes, also differed between the plant species (Supplementary Table 1, Supplementary Figure 2). Compositional differences in any of these communities were not explained by plant origin, indicating that soil communities in general were not differently conditioned by natives than by related range expanders (Supplementary Table 1). Between-species dissimilarity of the full prokaryotic and eukaryotic rhizosphere communities did not correlate with the phylogenetic distance between the plant species (Fig. 1C, Supplementary Table 1). However, distantly related plant species had more dissimilar fungal communities than closely related plant species (Mantel test: $r = 0.39$, $p < 0.05$; Fig. 1D). Rarefaction curves showed that we did not fully sample all biodiversity as no plateau in the rarefaction curves was reached (Supplementary Figure 3). This indicates that we likely have missed members of the rare biosphere. However, the intend of our study was to assess the importance of the major diversity and functional groups within the studied soil biota.



121

122 **Figure 1: Community composition of prokaryotes and eukaryotes**

123 Compositional variation in rhizosphere communities of A) prokaryotes (16S rRNA gene reads) and B) eukaryotes
124 (18S rRNA gene reads) among native (black dots: *G. dissectum* (*DIS*), *G. molle* (*MOL*), *G. pusillum* (PUS) and
125 *G.robertianum* (*ROB*)) and range-expanding plant species (red dots: *G. lucidum* (*LUC*), *G. pyrenaicum* (*PYR*), *G.*
126 *purpureum* (*PUR*) and *G. rotundifolium* (*ROT*)), based on 5 independent replicate soils. Error bars represent
127 standard errors of PCA coordinates. Correlations of pairwise phylogenetic distance with community dissimilarity
128 of prokaryotes and eukaryotes (C) and eukaryotic groups nematodes (orange), fungi (blue) and protists (grey)
129 (D). The Pearson coefficient r for each correlation is shown.

130 Further analyses showed that variation in fungal community composition correlated with differences
131 in specific root length (Mantel test: $r = 0.41$, $p < 0.05$; Fig. 2, Supplementary Figure 4), whereas
132 differences in bacterial community composition tended to correspond with variation in root metabolic
133 profiles (Mantel test: $r = 0.52$, $p = 0.08$; Fig. 2, Supplementary Figure 4). Average root diameter did not

explain variation in any of the groups in the rhizosphere community (Supplementary Figure 4). Between-species variation in root chemical profiles could not be explained by phylogenetic distance, whereas differences in specific root length and average root diameter marginally significantly correlated with phylogenetic distance (Supplementary Figure 5). Between plant species, protist and nematode communities co-varied with the community composition of bacteria, whereas the composition of nematode communities also co-varied with the composition of fungal communities (Fig. 2; Supplementary Figure 6).

A structural equation model was constructed based on the correlational links between root traits and rhizosphere community dissimilarity (Fig. 2). This model confirmed the effect of root chemical profile and specific root length on the bacterial and fungal community dissimilarity, respectively (Supplementary Figure 7). Moreover, it showed that protist community dissimilarity could be well-predicted by bacterial community dissimilarity (Supplementary Figure 7).

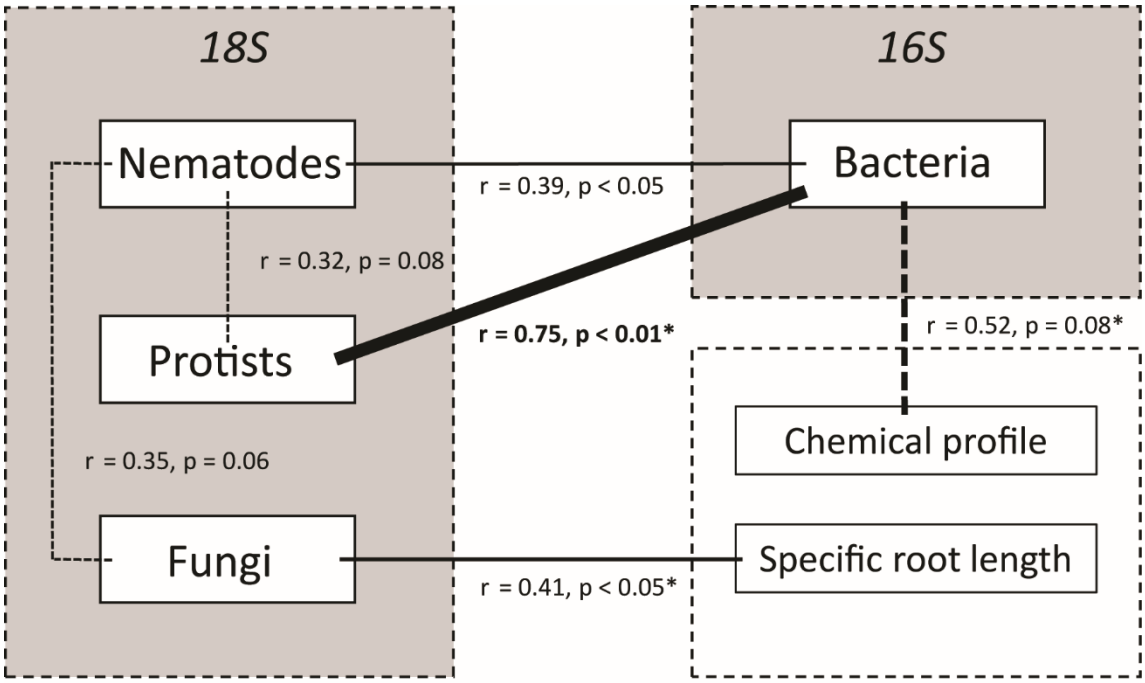


Figure 2: Correlational links in the rhizosphere

Overview of correlational links (all correlations with $p < 0.1$ are shown) between rhizosphere community dissimilarities of bacteria, nematodes, protists and fungi (all based on OTUs; 4-5 replicates per species) and

between root trait variation (Root chemical profile (4 replicates per species), Specific root length (3 replicates per species)) and dissimilarities of rhizosphere communities. Significant correlations (based on Mantel-tests) are depicted with solid lines, whereas trends ($p > 0.05$, <0.10) are depicted with dashed lines. Line thickness represents the relative strength of the correlational link based on r-values. Links that are significant based on a structural equation model (Supplementary Figure 7) are represented with asterisks (*).Source data are provided as a Source Data file.

Plant-soil feedback

There was an overall effect of soil conditioning (general linear model: $F_{1,60} = 435.6$, $p < 0.001$) on plant performance in the feedback phase, and all species grew equally poor in soil conditioned by their conspecifics (Fig. 3A). However, the species differed profoundly in their proportional loss of biomass in response to soil conditioning and thus in their plant-soil feedback responses (general linear model: conditioning*species: $F_{7,60} = 6.20$, $p < 0.001$). On average, range-expanding plant species responded more negatively to soil conditioning than natives (general linear model: contrast range-expanders-natives: F: 13.81, $p < 0.001$), which was mainly due to the greater biomass of the range-expander *G. purpureum* in unconditioned soils and its relatively low biomass in conditioned soils (Fig. 3A). Pairwise comparisons of plant-soil feedback strength did not reveal that plant-soil feedback is phylogenetically determined in this group of plant species (Mantel test: $r = -0.03$, $p = 0.49$; Fig. 3B).

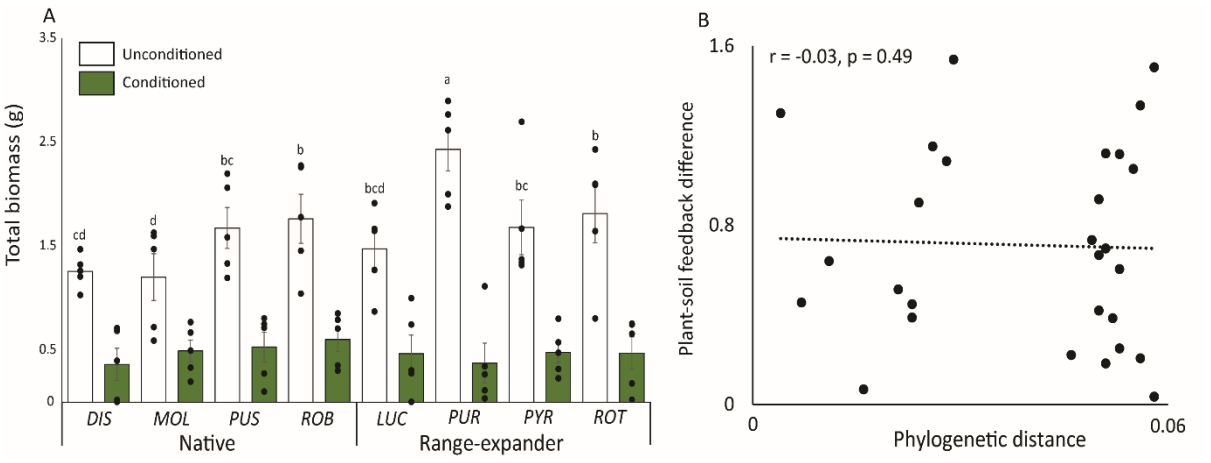


Figure 3: Plant-soil feedback

A) Plant biomass of eight *Geranium* species in soils conditioned by conspecifics (green) or in unconditioned soils (white) (N = 5 per species). Native plant species are *G. dissectum* (*DIS*), *G. molle* (*MOL*), *G. pusillum* (PUS) and *G. robertianum* (*ROB*) and range-expanding plant species are *G. lucidum* (*LUC*), *G. pyrenaicum* (*PYR*), *G. purpureum* (*PUR*) and *G. rotundifolium* (*ROT*). Bars and whiskers represent average biomass ± standard errors as examined using a general linear model. Small letters show post-hoc test results between plant species in unconditioned soils. B) Absolute between-species differences in average plant-soil feedback ($Ln(biomass_{conditioned}/biomass_{control})$) do not correlate with pairwise phylogenetic distance as examined using a Mantel test. Source data are provided as a Source Data file.

Plant-soil feedback variation was neither correlated with dissimilarity in complete 16S and 18S communities, nor with the dissimilarity in fungal, nematode or protist communities (Supplementary Figure 8). These results motivated us to explore the relationship between specific organismal groups of soil biota and the strength of plant-soil feedbacks. We tested the correlation between the relative abundances of potential mutualistic, arbuscular mycorrhizal fungi (AMF) and eukaryotic plant pathogens and root-feeding nematodes (see methods). Relative abundances of root-feeding nematodes correlated with plant-soil feedback strength (Mantel test: $r = 0.82$, $p < 0.05$; Fig. 4, Supplementary Figure 9). There was a weak trend that also the relative abundance of plant pathogens correlated with the strength of negative plant-soil feedback, whereas there was no correlation between plant-soil feedback and AMF abundance (Fig. 4, Supplementary Figure 9). Relative abundances of root-feeding nematodes did not correlate with plant biomass in the conditioning phase, indicating that root traits other than biomass determine the accumulation of these organisms (Supplementary Figure 10).

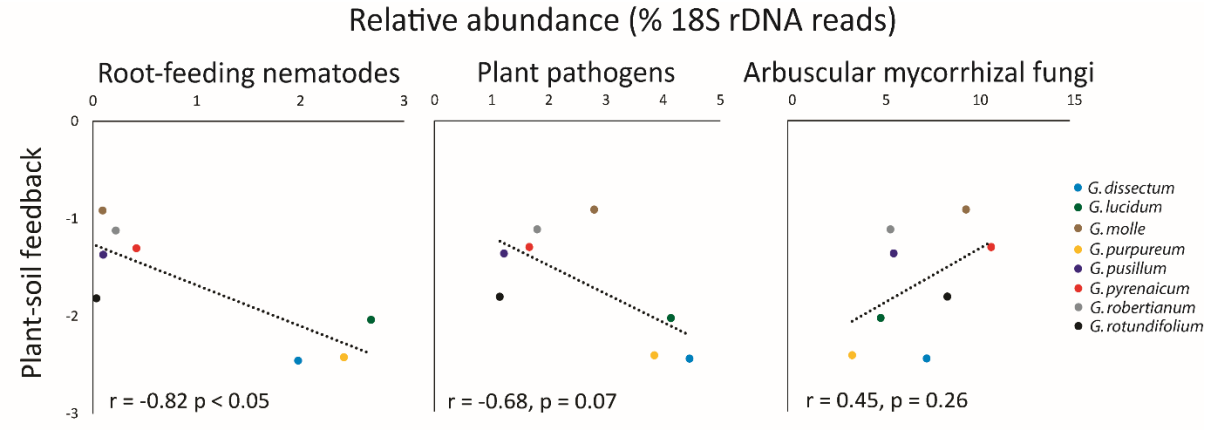


Figure 4: Predictors of plant-soil feedback

Correlations between plant-soil feedback ($\ln(biomass_{conditioned}/biomass_{control})$; average of five independent replicate soils) of eight *Geranium* species and the relative abundances (% 18S rRNA gene reads) of root-feeding nematodes, plant pathogens (see Methods) and arbuscular mycorrhizal fungi (AMF) in the conditioned soils. Statistical differences were examined using a Mantel test. Pearson correlation coefficients r and p-values of Pearson correlation tests are shown. Source data are provided as a Source Data file.

Of all root-feeding nematode taxa the genus *Meloidogyne* was most abundant in the conditioned soils (68% of root-feeding nematodes reads per sample). To test whether plant-soil feedback is also related to absolute root-feeding nematode abundance, we correlated plant-soil feedback with the reproduction of the widespread species *Meloidogyne hapla* on all eight plant species, which was examined in a separate experiment. *Meloidogyne hapla* numbers depended on plant species identity (generalized linear model: $X^2 = 134.38$, $p < 0.0001$; Fig. 5A) and plant species that developed the most negative plant-soil feedbacks indeed were the best hosts for *Meloidogyne* ($r = -0.72$, $p < 0.05$; Fig. 5B).

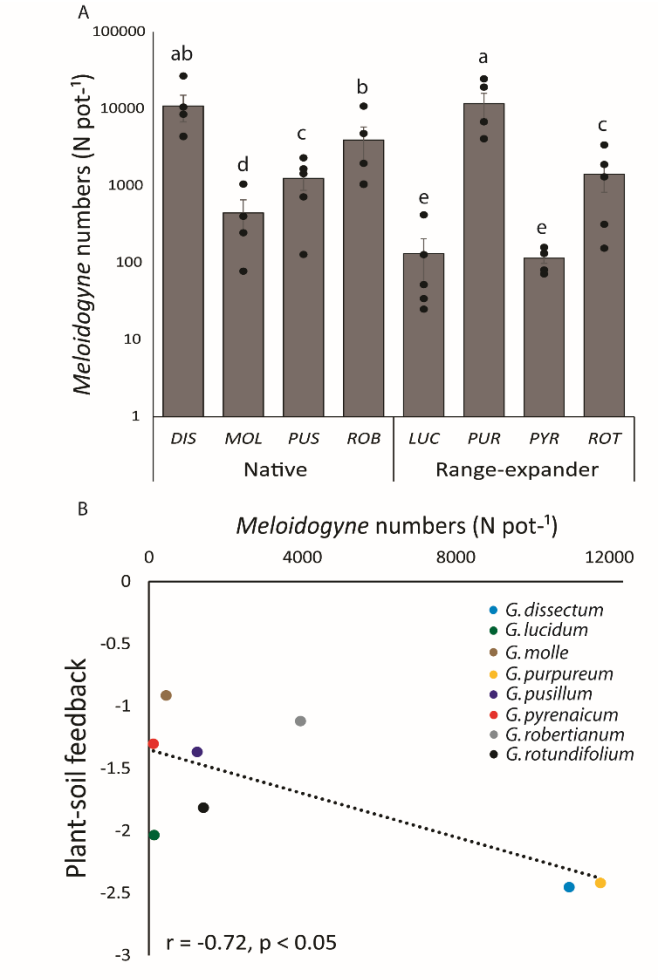


Figure 5: Nematode reproduction

A) Abundances of root-feeding nematode *Meloidogyne hapla* on native (black) and range-expanding (red) *Geranium* species in a nematode reproduction experiment (N = 5 per species). Bars represent average nematode numbers ± standard errors. Letters indicate the significant differences based on negative binomial GLM and post-hoc Wald tests. Note that the x-axis has a logarithmic scale. B) Correlation between *Meloidogyne* abundances and plant-soil feedback ($\ln(biomass_{conditioned}/biomass_{control})$). A Mantel test was applied to evaluate the correlation. Source data are provided as a Source Data file.

Discussion

Our results show that variation in rhizosphere community composition among eight congeneric native and climate warming-driven range-expanding plant species could be explained most strongly by

variation in their root chemical profiles and morphological root traits. Only differences in fungal community composition could, at least in part, be explained by phylogenetic distance, possibly underlain by the phylogenetic signal in specific root length. Communities of fungi varied with specific root length, which is possibly explained by plant interactions with root-associated fungi, such as AMF[32]. The link between root chemistry and bacterial community composition is in line with previous research[33]. Interestingly, there was evident co-variation in the composition of the different rhizosphere groups among plant species, especially between bacteria and protists. This is likely explained by feeding relationships between these two groups[34,35], and suggests that protist feed species-specifically on bacteria.

Variation in plant-soil feedbacks could not be directly linked to the composition of the rhizosphere community, but rather to the abundances of groups of antagonistic soil organisms, especially root-feeding nematodes. These nematode communities were dominated by *Meloidogyne* species, which are among the most detrimental nematode pests[36], and plant-soil feedback correlated to the reproduction of a generalist root knot nematode, *Meloidogyne hapla.* This suggests that generalistic belowground natural enemies drive plant soil feedbacks in this study, rather than specialists that are expected to underlie the Janzen-Connell dynamics, which have been shown to correlate with phylogenetic distance[37].

While root-feeding nematodes have been widely acknowledged as major agricultural pests[38], we here show that they also likely affect plant performance in natural systems[39] Further research might consider interactions between plant species and soil type, increased numbers of replicates to account for the variation, and inoculation trials in order to experimentally verify the role of plant-feeding nematodes in plant growth reduction. Furthermore, deeper sequencing, the use of nematode-specific primers, or longer-read sequencing in future studies should be considered to increase taxon sampling and the taxonomic resolution focusing on root-feeding nematodes as well as other soil-borne

241 pathogens in order to potentially identify taxa that are specifically hosted by the different plant
242 species.

243 Interestingly, the highest relative abundances of potential plant pathogens were found in the plants
244 that hosted highest nematode numbers and that experienced strongest negative plant-soil feedbacks.
245 This suggests that these plants are generally less well defended against a wide array of natural enemies
246 in the soil. Apart from the biotic actors, the observed negative plant-soil feedbacks may to some
247 degree have been caused by differences in nutrient availability between the control and conditioned
248 pots. While we added Hoagland solution to compensate for the lack of macro-nutrients, it cannot be
249 excluded that plants in the feedback phase still suffered from shortage of certain micronutrients.

250 Our experiment revealed no plant origin effect on rhizosphere community composition, unlike
251 previously assumed[40]. In contrast to previous work[26], we found that range-expanding plant species on
252 average developed a more negative plant-soil feedback than native species. However, the plant-soil
253 feedback effect in the present study was likely mainly driven by one single range-expanding plant
254 species (*Geranium purpureum*; Fig. 3A), and plant-soil feedback outcomes differed within both native
255 and range-expanding plant species. Moreover, the previous studies showed plant-soil feedback values
256 across plant families, whereas the present comparison was made within only one plant genus. Finally,
257 information on the plant species used in the present study suggests that the negative plant-soil
258 feedbacks of some of the range-expanding *Geranium* species most likely have not hampered their
259 successful establishment in the new range[41].

260 Phylogenetic distance has been successfully used as a measure of ecological (dis)similarity and as a
261 predictor of biotic interaction outcomes in studies that included plant species from multiple
262 families[11,17]. It is likely that ecological differences in such studies may be determined by deeply
263 conserved traits that can vary between families. Our study shows that among a group of congenerics,
264 even the most closely related species (e.g., *G. robertianum* and *G. purpureum*) can have stronger
265 differences in rhizosphere communities and plant-soil feedback effects than less closely related

266 species. Therefore, our study challenges the use of phylogenetic distance as a measure to explain
267 plant-soil interaction patterns of closely related plant species. Instead, we show that non-
268 phylogenetically conserved root traits may be more effective to predict plant-soil interactions. Thus,
269 in order to fully understand plant-soil feedback variation among more closely and more distantly
270 related plant species, a combination of phylogenetically conserved traits and un-conserved traits is
271 needed.

272 Our results raise the question under which conditions phylogenetic distance will explain variations in
273 plant-soil feedbacks among closely related species. The examined *Geranium* species differ in their
274 preferred abiotic conditions[42] (Supplementary Table 4), and do not all co-occur under the same field
275 conditions. This suggests that their root traits have been selected in the presence of different soil
276 communities, which may co-vary with abiotic soil conditions[43,44]. Congeneric plant species that co-
277 occur under the same abiotic soil conditions may face more similar selection pressures [11] and in turn
278 may have root traits that more strongly resemble their phylogenetic history. Moreover, plant species
279 from different genera with comparable root traits[45] or with similar life histories[3] might have more
280 comparable plant-soil feedback effects than closely related plant species from contrasting
281 environments, as have been compared in our study.

282 We conclude that within the same plant genus, closely related plant species did not have a more
283 comparable microbiomes and plant-soil feedbacks than more distantly related plant species. Enemy-
284 release is likely not underlying the success of *Geranium* species to expand their range as the
285 performance of native and range-expanding plant species was similar. Root traits were the best
286 predictors of rhizosphere community composition and root-feeding nematode numbers – and not
287 rhizosphere community dissimilarity - best predicted the strength of plant-soil feedback.

288

289 **Methods**

Plant species and germination

We collected seeds of native *Geranium* species *G. dissectum* L., *G. molle* L., *G. pusillum* L. and *G. robertianum* L. and range-expanding species *G. lucidum* L., *G. purpureum* Vill., *G. pyrenaicum* Burm.f. and *G. rotundifolium* L. from natural populations in The Netherlands (Supplementary Table 2). We identified Ellenberg indicator values for each of those species (Supplementary Table 3). All natives naturally occur in The Netherlands, whereas the range-expanders established populations in north-western Europe in the late 20th century (*G. lucidum* and *G. purpureum*), or were already present in restricted areas and strongly expanded their range in the last decades of the 20th century (The Netherlands: http://verspreidingsatlas.nl[41], United Kingdom: https://www.brc.ac.uk/plantatlas). For each experiment, seeds were surface-sterilized by washing them for 3 min in a 10% bleach solution, followed by rinsing with demineralized water, after which they were germinated on glass beads.

Phylogeny reconstruction

We concatenated three barcoding regions commonly used to infer plant phylogenies: the genes rbcL[46] and trnL [47] as well as the intergeneric spacer trnL-trnF[48]. Due to multiple sequences for rbcL present in GenBank we decided to re-amplify the rbcL gene for all plants used in our experiment. For this, root DNA was extracted from all *Geranium* species using the PowerSoil DNA Isolation kit (Qiagen, USA), which was adjusted by using iron beads to increase physical impact. We amplified the large chain of the ribulose bisphosphate carboxylase (rbcL) using the primers 1F [46] and the newly designed primer 1361rMod (5'-TATCCGTAAGGCTTGCAAGTGGAGT-3') modified from a previously described primer[49], with PCR cycling conditions as follows: initiation for 5 min at 95 °C, followed by 30 cycles of 30 sec at 95 °C, 1 min at 59 °C and 75 sec at 72° with a final elongation for 5 min at 72 °C). DNA sequencing was performed by LGC Limited (Middlesex, United Kingdom). Obtained sequence chromatograms were manually curated in Chromas Lite v 2.11 (http://chromas-lite.software.informer.com/2.1/; Technelysium, Queensland, Australia). Curated sequences were uploaded to NCBI under the accession numbers 2195399. Sequences of the new rbcL gene reads as well as the trnL and the intergeneric

spacer trnL-trnF were aligned using MAFFT[50] and visualized in Seaview v4.6.3[51]. Maximum likelihood analyses were run directly in Seaview using PhyML using the GTR model with four rate categories based on 2251 nucleotide sites. The stability of the branches in the resulting phylogenetic tree was assessed based on 1000 bootstrap replicates. Pairwise phylogenetic distance was then estimated with the ratio of divergent nucleotides.

Soil conditioning experiment

The plant-soil feedback experiment consisted of two phases. For the conditioning phase, we prepared a common background soil by homogenizing sandy clay soil from a former agricultural field (Beneden-Leeuwen, Netherlands; N51° 53.952, E05° 33.670) with sand, after which it was sterilized using gamma-sterilization (25 KGray, Syngenta bv, Ede, Netherlands). To establish independent replicates, we collected field soil from five different sites in the same river valley (Supplementary Table 4), each with 4 different sub-samples. These subsamples were pooled, sieved and homogenized, after which the mixtures were kept separate throughout the experiment as 5 replicate soils. Per replicate soil, 16 2.5 l pots were filled with a mixture of 1.8 kg of sterilized background soil and 200 g of sieved (1 cm) alive field soil. For each replicate soil, 8 pots were planted with one of the eight different *Geranium* species, while the other pots were left unplanted. All pots were then positioned in a randomized block design with 5 replicate blocks in a climatised greenhouse (16/8 h light/dark and 20/15°C). For the next 14 weeks the pots were watered twice per week and kept at the same soil moisture content (~15%). Thereafter, shoots were clipped, dried and weighed, while roots were washed, dried and weighed. Soils from each pot were collected and kept separate and a sub-sample was stored at -4 ˚C for DNA-extraction.

Feedback experiment

In the second phase, soils from each of the first phase pots were individually transferred to 1 l pots, which were filled with 830 g soil (moisture content ~15%) and put in the same randomized block design as in the first experimental phase. Soils that were conditioned by a plant in the first phase, were

340 planted with a seedling of the same species in the second phase. Each pot with unconditioned soil was
341 also planted with one of the eight species. The same watering regime was applied as in the first phase.
342 To compensate for differences in nutrient availability originating from the conditioning phase, all pots
343 each week received 10 ml of 25% Hoagland solution from the second week onwards, so that all plants
344 had ample available nutrients. After seven weeks of plant growth, shoots and roots were harvested
345 as described above.

346 *Soil DNA extraction*

347 For each pot with conditioned soil, DNA was isolated on the principle of the MoBio PowerSoil DNA
348 isolation kit. Briefly, 1 g of soil was added to 3 ml bead solution and 0.24 mL of an SDS based lysis
349 buffer. Eight iron spheres (ø 3mm) were added to increase extraction efficiency. Tubes were shaken
350 for 6 minutes at 580 RPM in a paint shaker (COROB™ SIMPLEshake). After centrifuging, the
351 supernatant was abstracted from humic acids using 0.8 mL of an ammonium aluminium sulphate
352 dodecahydrate solution. A vacuum manifold with a 96 well plate containing a silica membrane
353 (PALL8032) was used in order to purify DNA. DNA quantity was measured with nanodrop.

354 *16S and 18S rRNA gene amplicon sequencing*

355 The community structure of prokaryotes (bacteria and archaea) was determined using the prokaryote-
356 wide primers 515F/806R targeting the V4 region of the 16S rRNA gene[52]. The eukaryotic community
357 structure was assessed using the general eukaryotic primers 3NDf[53] and 1132rmod[54] targeting the
358 most variable V4 region of the 18S rRNA gene[55]. All primers were pre-tagged with Illumina adapters,
359 a 12 bp long barcode to allow demultiplexing of the reads after sequencing, a primer linker and the
360 sequencing primers. PCRs were conducted using in 96 well plates containing 25 µl mixes. For
361 prokaryotes, these mixes included 11.75 µl milliQ water, 10 µl 5 Prime Hot 2,5x mastermix
362 (QuantaBio), 1.25 µl BSA 4ug/ul, 0.5 µl of each of the primers and 1 µl of DNA template. PCR conditions
363 consisted of an initial denaturation step at 94 °C for 5 min followed by 35 cycles of 94 °C for 45 sec, 50
364 °C for 60 sec and 72 °C for 90 sec with a final elongation at 72 °C for 10 min. For eukaryotes, these

mixes included 15.3 µl milliQ water, 1 µl dNTPs, 1.25 µl BSA 4ug/ul, 2.8 µl $MgCL_2$, 0.5 µl of each of the primers, 10 µl buffer with 0.15 µl Taq polymerase (Fast start, Roche) and 1 µl of DNA template. PCR conditions were identical to those targeting prokaryotes with only the annealing temperature increased to 54 °C. All PCRs were performed in duplicates, before quality assessment on 1.5% agarose gel. PCR duplicates were pooled and cleaned using Agencourt AMPure XP magnetic beads (Beckman Coulter). DNA concentrations were assessed with a fragment analyser (Advanced Analytical), pooled in equimolar ratios and sent for sequencing to BGI, China.

Bioinformatics

The obtained raw 16S and 18S rRNA gene sequence reads were curated in the Hydra pipeline (nioo-knaw/hydra (Version 1.3.3)) implemented in Snakemake[56]; in short, after filtering contaminants and removing barcodes, 16S rDNA reads were merged with the fastq_mergepairs option of vsearch[57], while for the 18S rRNA gene data the forward reads were used. Thereafter, for both 16S and 18S rRNA gene reads VSEARCH was used to cluster all reads into operational taxonomic units (OTUs) using the UPARSE strategy by de-replication followed by sequence-sorting by abundance (singletons were removed) and clustering using the UCLUST smallmem algorithm[58]. Chimeric sequences were removed using UCHIME[59], implemented in VSEARCH. To create an OTU table, all reads were mapped to OTUs using the usearch_global method (VSEARCH). OTUs obtained from 16S rRNA gene sequences were taxonomically assigned by aligning them to the SILVA database[60], 18S rRNA gene sequences were aligned to the PR2 database[61]. Reference sequences were first trimmed with forward and reverse primer using cutadapt[62]. We constructed rarefaction curves to estimate sampling saturation, separately for bacteria, protists, nematodes and fungi using Past statistics (Supplementary Figure 3). Prior to the analyses, we deleted all OTUs present in less than 25% of the samples. Moreover, we removed samples with fewer than 3,000 18S rRNA gene reads from further analyses (See Supplementary Table 5). While a small number of these removed OTUs had high abundance in some samples, none of them was consistently abundant in any of the treatments. All 16S rRNA gene read

samples contained at least 17,000 reads and therefore none was discarded from further analyses. We then recalculated read numbers to relative abundances of the OTUs. OTUs were then manually assigned into the functional groups (Supplementary Table 6), allowing estimates of relative abundances of root-feeding nematodes[63], arbuscular mycorrhizal fungi (Glomeromycota), and plant pathogens (Plasmodiophorida, Oomycetes and *Rhizoctonia* sp.).

Structural root traits

For each plant species, three seedlings were grown individually in sterilized soil as described above. After four weeks of growth, all plants were stored at 4 °C until root trait analyses. Prior to this analysis, shoots were clipped and dried at 70 °C until constant weight, whereas root systems were carefully washed. Individual root systems then were fragmented and scanned using an Epson Perfection V850 Pro scanner (Epson America, Inc). Scans were subsequently analysed using WINRHIZO Pro v.2005b[64] for total root lengths and mean diameters. After scanning, root systems were dried until constant weight and weighed, after which the root/shoot ratio was determined.

Root chemistry analysis

For all plant species, four 5-week old plants were harvested from sterilized soil, after which their root systems were carefully washed. Thereafter, we used Direct Analysis in Real Time mass spectrometry (DART-HRMS) to determine the root chemical profile of all plant species. The DART mass spectrometry set-up consists of a DART ion source (model DART-SVP, IonSence, Saugus, USA) coupled with Q Exactive Focus high-resolution mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The mass spectrometer was calibrated prior to the samples measurements. The Xcalibur software (v.3.0) was used for instrument control and data acquisition. The distance between mass inlet and the DART outlet was kept at ~3cm. To standardize the measurements, root samples were placed on glass plates, and automatically moved (0.4 mm/s) along the ion source. DART settings were: Helium as ionizing gas, fixed flow of ~3.5L/min; gas beam temperature set at 450 ^{o}C; grid electrode voltage +350V. The

415 resolution was set at ultrahigh and a scan rate of 1Hz was used. The mass spectra were recorded in
416 the *m/z* range 100-1500 at acquisition rate of 2 spectra s^{-1}.

417 *Mass spectrometry data processing*

418 The DART-MS spectra were acquired and converted from their respective raw data formats to open-
419 source mzXML file format using MSConvertGUI (64-bit) available from ProteoWizard [65]. For further
420 mass spectral data processing, the open-source software package MZmine 2.20 [66] was used. Acquired
421 mass spectrometry data from the samples was imported in MZmine 2.20 and the total ion current
422 (TIC) chromatographic data was evaluated. Based on the evaluation, mass detection, chromatogram
423 building and chromatogram deconvolution was performed in a step-wise manner using the available
424 functionalities in the software. The detected and deconvoluted peaklists containing mass features for
425 each sample were aligned using the RANSAC aligner available in MZmine. The aligned peaklists were
426 exported in .csv format for subsequent chemometric analysis.

427 Chemometric analysis was performed using MetaboAnalyst 3.0[67]. Prior to applying chemometrics, the
428 uploaded data was filtered and normalized. Thereafter, differences of ion abundances within the
429 samples were investigated by applying Partial Least Square Discriminate Analysis (PLS-DA). To visualize
430 the degree of relatedness amongst different samples, hierarchical clustering was performed using
431 complete linkage and Euclidean distance. Dendrograms were constructed using the *stats* package in
432 R[68] (version 3.3.3). To generate the distance matrix and dendrogram, the resulting peaklists exported
433 from MZmine were averaged over the four replicates for each sample, giving an average peaklist per
434 sample.

435 *Nematode reproduction experiment*

436 Sterilized background soil was prepared as described above. Forty 1l pots were filled with 830 g of
437 sterilized background soil, and were assigned to one of the eight plant species. After planting of single
438 seedlings per pots, the pots were placed in a randomized block design under the same greenhouse

conditions as described above. After two weeks of plant growth, a suspension containing approximately 400 *Meloidogyne hapla* juveniles was inoculated near the main root of each of the plants. The same watering regime was applied as in the feedback experiment. After twelve weeks, shoots were clipped and dried and root systems were carefully separated from the soil. All soil from each pot was individually bagged and stored at 4 ˚C until nematode extraction. Nematodes were subsequently extracted using an Oostenbrink elutriator[69] and concentrated to 10 ml. Subsequently, we extracted nematodes from the roots. For this, roots from all plants were separated in two parts, which both were weighed fresh. One part of the roots then was dried at 70°C until constant weight, while the other half was cut into pieces of 1–2 cm and placed in a mistifier for 4 weeks to extract nematodes from the inside of the roots[69]. Nematode suspensions were harvested from the mistifier after 2 and 4 weeks, combined, and concentrated to 10 ml. Both nematode samples were then counted using an inverse light microscope (200x; Olympus CK40)). Using the total fresh weight and the dry-fresh root weight ratio, total nematode numbers inside the roots were estimated.

Statistical analyses

Variations in prokaryotic and eukaryotic communities were explored by running separate PCA analyses in Canoco 5[70], comparing the communities between plant origins and plant species, while including soil replicate as a covariate. We then performed partial RDA-analyses to individually test the effect of plant origin and plant species on variation in prokaryotic and eukaryotic communities, while partialling out the variation caused by the different soils. Similar analyses were performed to test plant origin and species effects on variation in the major subgroups of the eukaryotic communities: fungi, protists and nematodes. We then used vegdist in the R *vegan* package[71] to calculate pairwise community dissimilarities of prokaryotes and all eukaryotes and the eukaryotic subgroups fungi, protists and nematodes between all eight plant species in each independent soil. Overall pairwise

463 community dissimilarities were calculated by averaging the pairwise dissimilarities in the five
464 independent soil replicates.

465 We examined the phylogenetic effects on community composition by testing the correlation between
466 pairwise phylogenetic distances and community dissimilarities using two-tailed Mantel tests in *vegan*,
467 with correlation method pearson and 999 permutations. To determine whether closely related species
468 had more similar root traits than distantly related species, we similarly tested the correlations
469 between pairwise phylogenetic distances and absolute differences in specific root length, average root
470 diameter and chemical dissimilarity based on the DART-analysis. Subsequently, also the correlations
471 between rhizosphere community dissimilarities and trait dissimilarities were tested in a similar way.
472 Based on these correlation tests, we constructed a structural equation model (piecewiseSEM in R)
473 testing the predictive effects of specific root length and root chemistry dissimilarities on community
474 dissimilarities of bacteria, fungi, protists and nematodes, and the predictive effects of bacterial and
475 fungal community dissimilarities on community dissimilarity of protists and nematodes.

476 Plant-soil feedback variation among plant species was tested by modelling the biomass response in a
477 general linear model including fixed factors block, soil treatment and plant species, and the plant
478 species*treatment interaction (lm in R). A significant plant species*treatment interaction would
479 indicate that plant species differ in their biomass response to soil conditioning. Overall feedback
480 differences between native and range-expanding plant species were tested by the specification of a
481 contrast. Significant differences in biomass in the conditioning and control treatments were tested
482 using *lsmeans* (package). Average plant-soil feedback values per plant species were calculated by
483 averaging the feedback value ($\ln(biomass_{conditioned}/biomass_{control})$) in each of the five independent soil
484 replicates. To test whether feedback differences were stronger between more distantly related
485 species than between closely related species, we tested the correlation between pairwise
486 phylogenetic distance and pairwise feedback differences using a Mantel test (correlation method
487 pearson, 999 permutations, two-tailed). Moreover, correlations between plant-soil feedback outcome

and the relative abundance of root-feeding nematodes (genera), plant pathogens (genera/families) and arbuscular mycorrhizal fungi were tested to examine whether these groups may have determined the observed plant-soil feedback patterns.

The reproduction of *Meloidogyne hapla* was modelled using a generalized linear model with a negative binomial distribution[72] that included the fixed factors species and soil replicate (glm.nb in *mass*[73]). Between-species differences were tested using post-hoc Wald tests with the package *phia*[74]. Finally, we tested the correlation between *Meloidogyne* numbers and plant-soil feedback using a Pearson correlation test (two-tailed).

Data availability

The data that support the findings of this study are available from the corresponding author upon request. Newly sequenced rbcl gene information is available at NCBI under the accession number 2195399. rDNA sequence data of 16S and 18S rRNA gene reads is uploaded to https://www.ebi.ac.uk/ena/data/view/PRJEB29769. Source data for figure 2, 3, 4 and 5 are provided with the paper.

Statement of authorship

RAW, SG and WHvdP designed the study. RAW, WK and SG performed the greenhouse experiments. HM, PH, SG and RAW performed the molecular lab work. PG and PK analysed the metabolomics data. RAW performed the statistical analyses. The manuscript was written by RAW, SG and WHvdP and all authors commented on the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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