Range-expansion effects on the belowground plant microbiome

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Abstract

Plant range expansion is occurring at a rapid pace, largely in response to human-induced climate warming. While the movement of plants along latitudinal and altitudinal gradients is well documented, effects on the belowground microbial communities remains largely unknown. Further, in range expansion not all plant species are equal: in a new range the relatedness between range-expanding plant species and native flora can influence plant-microbe interactions. Here we used a latitudinal gradient across Europe to examine bacterial and fungal communities in the rhizosphere and surrounding soils of range-expanding plant species. We selected range expanders with and without congeneric natives in the new range, and as a control, the congeneric natives, totaling 382 plant individuals collected across Europe. In general, a plant's status as range expander was a weak predictor of bacterial and fungal community composition. However, microbial communities of range-expanding plant species became more similar to each other farther from their original range. Range expanders unrelated to the native community also experienced a decrease in the ratio of plant pathogens to symbionts, giving weak support to the enemy release hypothesis. Even at a continental scale the effects of plant range expansion on the

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belowground microbiome are detectable, though changes to specific taxa remain difficult to decipher.

Keywords

bacteria; fungi; plant-soil interactions; climate change; microbial ecology

Species range expansion in response to climate change is recognized as a major uncertainty in predicting consequences of global warming for biodiversity and functioning of ecosystems[1,2]. Initially, attention was given to the ability of species to keep up with their shifting climate envelope; now research questions have expanded to include the consequences of range shifts for community interactions[3]. The disruption of plant range expansions on aboveground interactions have been well documented[4–6], including on aboveground herbivores and higher tropic levels[7,8]. While evidence suggests that introduced invasive species can alter soil communities[9–11], the effects of plant range expansion on belowground microbial communities remain ambiguous.

The relationships between plants and their associated microbes can influence plant establishment, fitness, and community assembly[12–14]. It has been proposed that plant range expanders will be successful in their new range because they lose their specialized soil pathogens[15–17]. At the same time, range expanders may also lose specialized mutualistic microbes[18–20]. Results of these studies lead to the similar expectation that the plant-associated microbial community in the rhizosphere and surrounding soil (named here the belowground plant microbiome) of range expanding plant species will associate less with the belowground microbiome in their new range than in their native range, and also when compared to native plant species. However, few studies have characterized or compared the microbiome community structure and diversity of range expanding plant species (however see[21]) and no study has made a direct comparison with related native plant species at a continental scale.

The soil and rhizosphere microbiome, made up largely of bacteria and fungi, is taxonomically and functionally diverse[22]. The community composition of the belowground microbiome is broadly structured by abiotic factors, yet effects differ between bacteria and fungi[23,24]. For example, while at large spatial scales bacterial communities are strongly influenced by soil pH[25,26], the composition of fungal communities are simultaneously affected by climate and nutrients[27–29]. At the same time, both the soil and rhizosphere microbiome are strongly controlled by biotic factors, including the composition of root exudates, plant species identities and plant traits[30–32]. Through these properties plant species can assemble species-specific microbiomes where microbial taxa are enriched or suppressed under some plants and not under others[14,33–36]. At the same time, phylogenetic relatedness of range-expanding plants with native flora can represent another potential effect of range expansion on microbial communities - where some research suggests that closely related plant species can harbor similar microbial taxa, especially pathogens[37,38]. Finally, plant-microbe interactions evolve over time, changing over years and even decades[39,40]; thus during range expansion, both distance from the original range and evolutionary history

between plants and microbes41 have potential to influence the belowground plant microbiome.

Here we analyze the microbiome of intra-continental range-expanding plant species along a latitudinal gradient to explore key hypotheses that have been previously proposed for exotic and invasive plants but may also apply to climate warming-induced range-expansions. To test for the influence of plant phylogeny on the belowground microbiome during range expansion, we selected range expanders that are either related or unrelated to the native flora (Fig. 1A). To test for the effects of range expansion on the belowground plant microbiome compared changes in community composition and pathogen relative abundance across the range expansion gradient (Fig.1B). We hypothesize that if plant range expansion influences the belowground plant microbiome, observed patterns will be stronger in the rhizosphere42 than in the bulk soil. Further, if range-expanding plants farther from their original range either lose the ability to interact with certain microbial taxa or preferentially promote the growth of a beneficial community, in the new range the microbiome or range-expanders will become more similar and alpha diversity with decrease. However, because plants more closely related to the native community may share microbes, this change will be less pronounced for range expanders that encounter congeneric natives in the new habitat. Finally, if *the enemy release hypothesis* common to invasive plant species is also applicable to range-expanders, we expect fewer belowground pathogens to be associated with range expanders unrelated to the native flora compared to related expanders and native.

In Europe, climate change induced range expansion is well documented; many plant species are expanding their range into higher latitudes and altitudes2,43. Here we used high-throughput Illumina sequencing to explore how the belowground microbiome of plant species changes when plants expand from their original range (in lower latitudes) to new ranges (in higher latitudes). We targeted the microbiome of three plant groups: *unrelated range-expanders* - plant species without native species from the same genus in their new range; *related range-expanders* - plant species that have native species from the same genus in their new range (Supplementary Table 1 and Supplementary Figure 1); and *native* plant species, which are congeneric to the related range expanders and native throughout the entire gradient. All range expanding plants either arrived to or had greatly expanded within the Netherlands in the late 20th and early 21st centuries44. In an effort to minimize variation in abiotic factors, we selected 11 plant species grown on similar parent soil (*see* methods). For each species, we sampled the microbiome in the rhizosphere and surrounding (bulk) soil of up to 9 plant individuals collected from up to 6 countries, spanning from Greece to the Netherlands, totaling 382 plant individuals (Figure 1 and Table 1). While some species were cosmopolitan45, others were quite rare and more difficult to find. Regardless of plant species dominance, here we have replicates not just in plant species, but in plant types (native, related and unrelated range expanders) and 382 bulk soil and rhizosphere samples in order to obtain a number that should be sufficient to capture large scale patterns in the microbial community26,28.

Results & Discussion

Overall, rhizosphere and bulk soil communities were significantly different from each other, both in community overlap as visualized by a PCA ($p < 0.001$ for both bacteria and fungi; Figure 2A and 2B), and in taxa overlap (Figure 2C and 2D). We found 47,704 bacterial phylotypes and 9,374 fungal phylotypes in soils, and 33,939 bacterial phylotypes and 6,438 fungal phylotypes in the rhizosphere. Further, there was low community overlap among plant individuals in both soil (averaging 4,092 (8%) unique bacterial taxa and 523 (5.5%) unique fungal phylotypes per sample) and the rhizosphere (averaging 1,932 (5.6%) unique bacterial phylotypes and 257 (4%) unique fungal phylotypes per sample). High microbiome diversity among 11 plant species is not a surprise, especially because the selected plants represent a range of phylogenetically and ecologically distinct species36,46,47.

Across the gradient, plant species was the strongest predictor of bacterial and fungal community composition in both soil and rhizosphere environments, explaining 7 to 14% of the variation (Figure 3; Supplementary Table 2), and plant genus a proxy of phylogenetic relatedness (Supplementary Figure 1) provided no additional prediction power. Conversely, the effects of plant grouping (unrelated range-expander, related range-expander and native) and latitude had a much smaller effect on microbial composition and explained a maximum of 2% of the variation in all cases. In general, soil abiotic factors also had a minor influence on variation, accounting for less than 1% of the variation for all factors (e.g. pH, N, C), except for soil bacterial communities where pH explained approximately 5% of the variation. The relatively minor effect of soil abiotics on microbial communities - compared to previous studies25 - can be explained by the small variation in soil factors across the gradient and between plants (Supplementary Figure 2), as was the goal of choosing plant species growing on the same parent soil material. In comparison, other studies have been more focused on elucidating patterns in microbial community composition relative to changes in abiotic factors26,28,48. Thus, the differences observed here are more likely due to plant species effects sensu47, such as plant ecology, relatedness with native flora, and life history traits45,49,50.

In support of our hypothesis, we found that range-expanders farther from their original range had more similar microbial communities to other plant individuals. Put another way, the variation in community composition decreased among individuals in the new range. Further, there were negative correlations between "range" (country samples were collected from) and community dissimilarity for all plant groups (Figure 4 and Supplementary Table 3); latitude and distance, gave equivalent results. This pattern was significant for bacterial communities in the soil and rhizosphere of all plant types (rho varied between -0.08 and -0.32 and $p < 0.05$ for all). However, for fungal communities, correlations were only observed in soils (rho varied from -0.10 to -0.13, p < 0.05 for all) and not in the rhizosphere. The negative correlation between range and community dissimilarity was strongest in unrelated range-expander species (Supplementary Table 3). We also found a significant difference in the degree of microbial community similarity by plant group yet there was an interaction of country in two scenarios (p< 0.0001 in all cases) (Supplementary Table 4). This suggests that controls on native and range-expanding plant microbiome community composition differs across the gradient. For instance, native plants microbiomes (and to a lesser extent

related range-expanders) may be more influenced by a long-term co-evolutionary history that would be consistent across this latitudinal gradient51,52, while unrelated range expander microbiome patterns might be more determined by more recent spatial effects and the native (neighbor) plant community53. Because we used a survey to explore changes to the belowground microbiome across a natural range expansion transect, we were unable to test for co-evolutionary history between microbes and plants. Still, our results suggest that future studies should be designed with this process in mind, particularly to identify the role of the microbial community for plant adaptions during climate change39,54.

While community structure became more similar across the gradient, changes in bacterial richness and fungal richness was much more variable (Figure 5; Supplementary Table 5). Under unrelated range-expanders, fungal alpha diversity in the rhizosphere of significantly increased with distance from the original range (rho = 0.36 p <0.001 in the rhizosphere, p>0.05 in soil). However, related range-expanders showed no relationship between fungal diversity and distance from original range (p > 0.05 for both soil and rhizosphere) in comparison to native plants which where fungal alpha diversity increased with latitude in *both* the rhizosphere (rho = 0.20, $p < 0.05$) and in the bulk soil (rho = 0.23 $p < 0.05$). The mechanisms behind increased fungal diversity in the rhizosphere of unrelated range-expanders remains in question. It could be that if range-expanding plants do not need to invest in belowground defense55,56 the rhizosphere becomes accessible for a larger proportion of microbes, though this varies by plant species57. Alternatively, it has been proposed that exotics and range-expanders promote high microbial diversity as part of a defense mechanism53,57. The later proposition, that range-expanding plants enrich their rhizosphere, is congruent with our findings that community composition becomes more similar among individuals in the northern part of the range (Figure 4), and that unrelated range-expanders had higher fungal and bacterial diversity in their rhizosphere and lower diversity in the associated soils (p < 0.0001 in all cases) (Supplementary Table 6). Overall, the inconsistency between the responses of the two expanders suggests that related and unrelated range-expanders have different controls on microbial diversity. Further, the variability in alpha diversity patterns indicates that alpha diversity and community similarity are affected by different mechanisms.

It has been proposed that in novel ecosystems plant success or failure is based on reduced exposure to soil-borne pathogens combined with continued association with symbionts58,59. We applied this concept here and used FunGuild60 to test how the abundance of potential fungal functional groups change as range-expanding plants move farther from their original range. Specifically, we examined potential plant pathogens and arbuscular mycorrhizal fungi (AMF), as these are the relevant mutualistic symbionts for most of our plant species, except for the crucifers. However, we could detect no significant change in the relative abundance in either of these groups under range expanding plant species (Supplementary Figure 3). Though there was a significant positive correlation in the ratio of plant pathogens to symbionts across the transect (rho = 0.31 $p < 0.001$) (Supplementary Table 7). On the other hand, under native plants the relative abundance of plant pathogens increased in both the soil and rhizosphere from south to north (rho = 0.23 for both). Contrary to previous studies, these results do not directly verify that range-expanders lose their specialist microbes58 or are released from specialist enemies61.

Instead, the results suggest that compared to natives, range expanders are exposed to fewer potential pathogens and symbionts in the new range, which has been predicted for range-expanding plant species[62] and demonstrated for introduced exotics in their new range[63,64]. At the same time, recent studies of plant succession [65,66] clearly demonstrate that plant success and nutrient cycling is tied to the microbial communities. Yet it remains unclear if the mechanisms underlying plant range-expansion are the same as those observed elsewhere.

Still, these results are not without caveats. The first being that the molecular methods used are not infallible- the DNA community analysis does not assess the active microbial community nor the true functional capabilities. Thus, potential functional groupings and relative abundances of taxa cannot indicate the expected pathogenicity of these fungi in the host plant's rhizospheres. Equally important is that for all plant groups the relative abundance of these functional groupings make up approximately 5% of the fungal community. Meaning, any changes in composition or diversity may overinflate or obscure true changes in these low abundance groups[67] and specific primers or culture work is necessary to explore functional changes more thoroughly. Our study exemplifies that high-throughput sequence data can be used to assess large-scale patterns in plant-soil associations, but future functional analyses (e.g. metagenomics and metatranscriptomics approaches) and experimental studies must be designed to take the low abundance of pathogen sequences into account.

Our study contributes initial steps of identifying the patterns of changes in the plant microbiome during plant range expansion. While, microbial community and diversity dynamics change across a range expansion gradient, clarifying the mechanisms behind the observed changes would require further experimental study. In the present study, we attempted to link the concepts from plant ecology to the microbiome by assuming that plant establishment outside the native range will result in altered exposure for soil microbes. Our results suggest that while terms like 'exotic', 'range-expander' and 'native' are helpful descriptors in plant ecology, it should not be assumed that these labels are equally relevant to describe the belowground microbial community of such plant species. Future research will require consideration of the ecological roles of both plants and microbes[26,36] but currently, the ecological roles on many microbial taxa still remain unknown. At the same time, we think that this large-scale biogeographical studies of plant-soil-microbe associations of native, related and unrelated range expanders along a latitudinal gradient is an essential step to understand how climate warming-induced range-expanding plant species may assemble a new microbiome in their novel range. This approach may also stand as a model for processes that take place belowground upon the introduction of exotic plant species in a new continent. Subsequent experimental work is needed in order to understand functional consequences for invasiveness and naturalization.

Almost 4% of extant global vascular flora have established outside their native range[68], and climate change induced range expansion is not expected to slow down[69]. Though soil microbes exert strong selective pressures on plant species and communities[70,71] our understanding of microbial community dynamics during range expansion remains limited. Range expansion offers an opportunity to explore how global change may alter the relationship between plants and their microbiome, but also how the belowground

microbiome changes across large geographic scales. Understanding the effect of range expansion on the belowground plant microbiome can provide baseline knowledge for predicting ecological consequences of current rapid climate warming, and it may also be used to enhance understanding of community responses to invasion scenarios for introduced exotic species.

Methods

Plant species and soil collection

In central Europe, rivers flow to the south and north away from the Alps, resulting in habitats with sediments from similar parent materials and soils that spread across a latitudinal gradient. Within these well-connected river habitats, and in response to climate change, many plant species are expanding their range with much more movement expected in the coming decades[1,74,75]. Within this latitudinal gradient, spanning from Greece in the south to the Netherlands in the north, we identified 7 range-expander species whose range has expanded north into Austria, Germany and the Netherlands over the last 50 years, approximately[76]. Range-expanders without native congenerics in the northern sites (here named *unrelated range-expanders*) include: *Dittrichia graveolens*, *Lactuca serriola* and *Rapistrum rugosum*. Range-expanders with native congenerics (related range-expanders) include: *Centaurea stoebe*, *Geranium pyrenaicum*, *Tragopogon pratensis* and *Rorippa austriaca.* As a control, we also included 4 native plant species that are congeneric with the related range-expanders: *Centaurea jacea*, *Geranium molle*, *Tragopogon dubious* and *Rorippa sylvestris. C. stoebe* and *R. austriaca* originated from Central and Eastern Europe, while all other range-expanders originated from southern Europe (www.gbif.org). Plant populations were sampled from 6 countries in Europe – Greece, Montenegro, Slovenia, Austria, Germany and the Netherlands - in the summer growing seasons of 2013 and 2014. All plants were flowering at the time of sampling. At each sampling site, environmental parameters, including weather conditions at sampling dates, were recorded. For each sampling location of a single species, 3 individuals of 3 distinct populations (in most cases at least 400m separation) were chosen, totaling 9 plant individuals for each location (see Supplementary Figure 1 for sample numbers). For collection of all samples, permissions were obtained from both nature reserves and government agencies responsible for the land.

To assess the soil and rhizosphere microbiomes of native and range-expanding plant species, soils and roots plus rhizosphere were collected from under individual plants. Briefly, the entire plant was dug up to a 10 cm radius around the plant and soil was shaken off the plant roots. Bulk soil was homogenized and 10 g was collected for microbial and chemical analyses. The fine plant roots plus rhizosphere soil were collected separately, hereon referred to as the rhizosphere community. All rhizosphere and soil samples were stored at 4°C until shipped, within 1 week, to the Netherlands Institute of Ecology (NIOO). At the NIOO soil and rhizosphere samples for DNA extraction were frozen at -80°C. A subset of soil was stored in the fridge at 4°C for chemical analyses.

Soil chemical analyses

For all soil samples collected in 2014 nutrients and pH were measured on fresh soil stored at 4°C (Supplementary data; Supplementary Figure 2). Gravimetric moisture (% water) was determined on soils oven dried at 105°C. Total soil C and N content was determined from these dried soils on an elemental analyzer (LECO, St Joseph, MI, USA). Extractable NO_3 and NH_4 were measure using the KCl extraction protocol. Briefly, soils were dried at 4°C, 10 g dry soil were then mixed with 1M potassium chloride (KCl) solution, shaken, and then the supernatant is used for analyses of NO_3 and NH_4. Soil pH was measured in an H_2O slurry solution using a bench-top pH meter following the ISO 10309 standard procedure.

Community level sequence analysis

To identify the bulk soil and rhizosphere microbiomes from native and range-expanding plants, DNA was extracted from 0.25 g of ground bulk soil and 0.35 g of ground rhizosphere material using the PowerSoil-htp 96 Well Soil DNA isolation kit (MO BIO Laboratories, Inc., California, USA) according to the manufacturer's instructions. Bacterial community composition was determined by targeting 16S rRNA amplicons using 515F/806R primers[77], and the fungal community composition by targeting the ITS region using primers ITS4/fITS978. To prevent the amplification of plant material[79], PNA Clamps© (PCR Blockers) (CGACACTGACACTGA-KK) were added at the PCR step for rhizosphere bacterial DNA. For all samples, DNA was PCR amplified in duplicate using barcoded primers[77]. PCR products were purified using the Agencourt AMPure XP magnetic bead system (Beckman Coulter Life Sciences, Indianapolis, Indiana, USA) and analyzed via the Standard Sensitivity NGS Fragment Analysis kit (1bp-6000bp). Pooled PCR amplicons were sequenced with the Illumina MiSeq platform at BGI Tech Solutions (HongKong) Co Limited.

MiSeq paired-end reads targeting 16s rRNA amplicon were merged and only reads which had minimum overlap of 150bp with PHRED score of 25 (estimated by RDP extension of PANDASeq[80]). Primer sequences were stripped using Flexbar version 2.58[1]. Sequences were then clustered to OTUs with VSEARCH v1.0.108[2], using the UPARSE strategy of de-replication, sorting by abundance and clustering using the UCLUST smallmem algorithm[83]. All singletons were removed, and potential chimeric sequences were removed using the UCHIME algorithm[84]. Taxonomic classification for each OTU was obtained by using the RDP Classifier version 2.10[85].

Likewise, MiSeq paired-end reads targeting ITS region were treated as described above with following adjustments: ITS primer sequences were stripped using ITSx 1.0.11[86] before clustering, and sequences were classified using the UNITE database[87]. All bioinformatics steps were implemented with a publicly available workflow made with Snakemake[88]. After samples were removed due to sampling error or falling below the rarified threshhold, 382 samples were included in downstream analyses of plant soil and rhizosphere microbiomes.

Community similarity was visualized with a PCA of the dissimilarity matrix based on Bray-Curtis distances. Plotted in Figure 1 is the centroid of each plant species community with lines representing connections to all other samples of that species. We quantified phylogenetic distances between all plant species used, but did not make a full analysis of

these distances with differences in microbiome composition, as plant genus or family-specific issues might interfere with pure phylogenetic distances (Supplementary Figure 1). To investigate how distance from the original range influences the microbiome for each plant species we tested within country dissimilarity of bacterial and fungal communities both in the rhizosphere and in soil. Briefly, pairwise Bray-Curtis dissimilarity was estimated between samples of each plant species within each country. Diversity of soil communities were analyzed using "vegan" package89 using the PERMANOVA test and visualized with "ggplot2" package. Correlation patterns were visualized with the LOESS smoothing function90. Because within country distance was so much smaller than between country distance diversity patterns were the same whether plotted by latitude, country or geographic distance, which here we refer to as "range". Spearman's Rank Correlations were run on latitude and plots show country name for clarity. FUNGUILD analyses were generated using the web interface and only taxa that received a 'highly probably' classification were included. When all taxa were included results remained the same. All other analyses were performed using R programming language (R Development Core Team 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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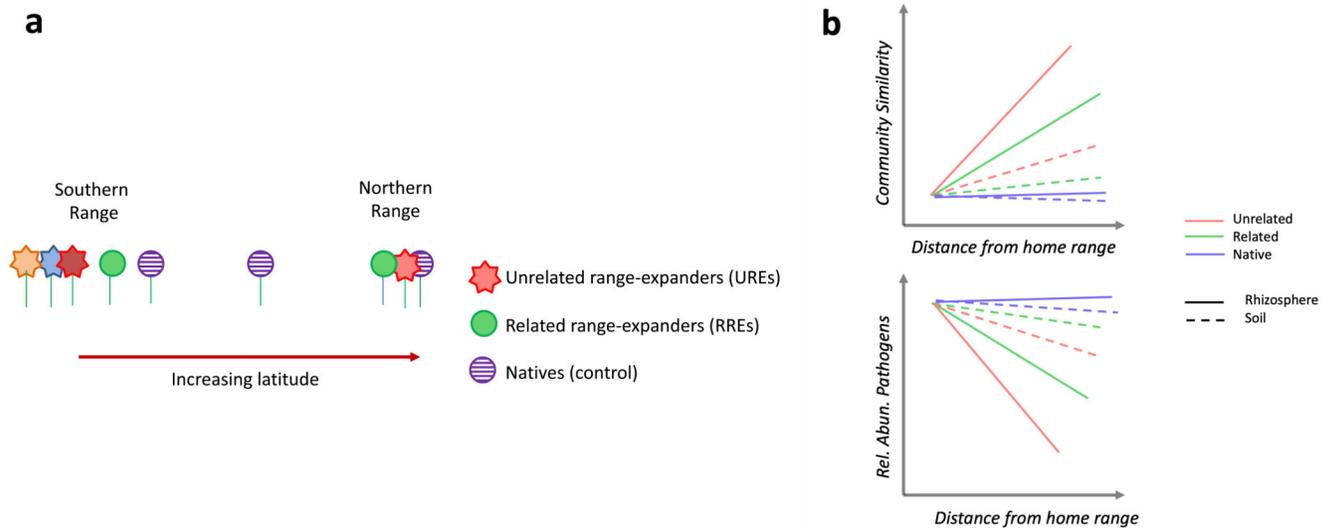
Figure 1. Changes in microbial community during plant range-expansions.
A. When plants move from the southern range to a new range, range expanders can be either related to the native flora (circles) or unrelated (stars). **B.** Hypothesized responses of microbial community similarity and pathogen relative abundance to range-expansion; where we expect observed patterns to be stronger in the rhizosphere (solid lines) than in the bulk soil (dashed lines), and that the relatedness of the range-expander to the native flora will affect the strength of the response.
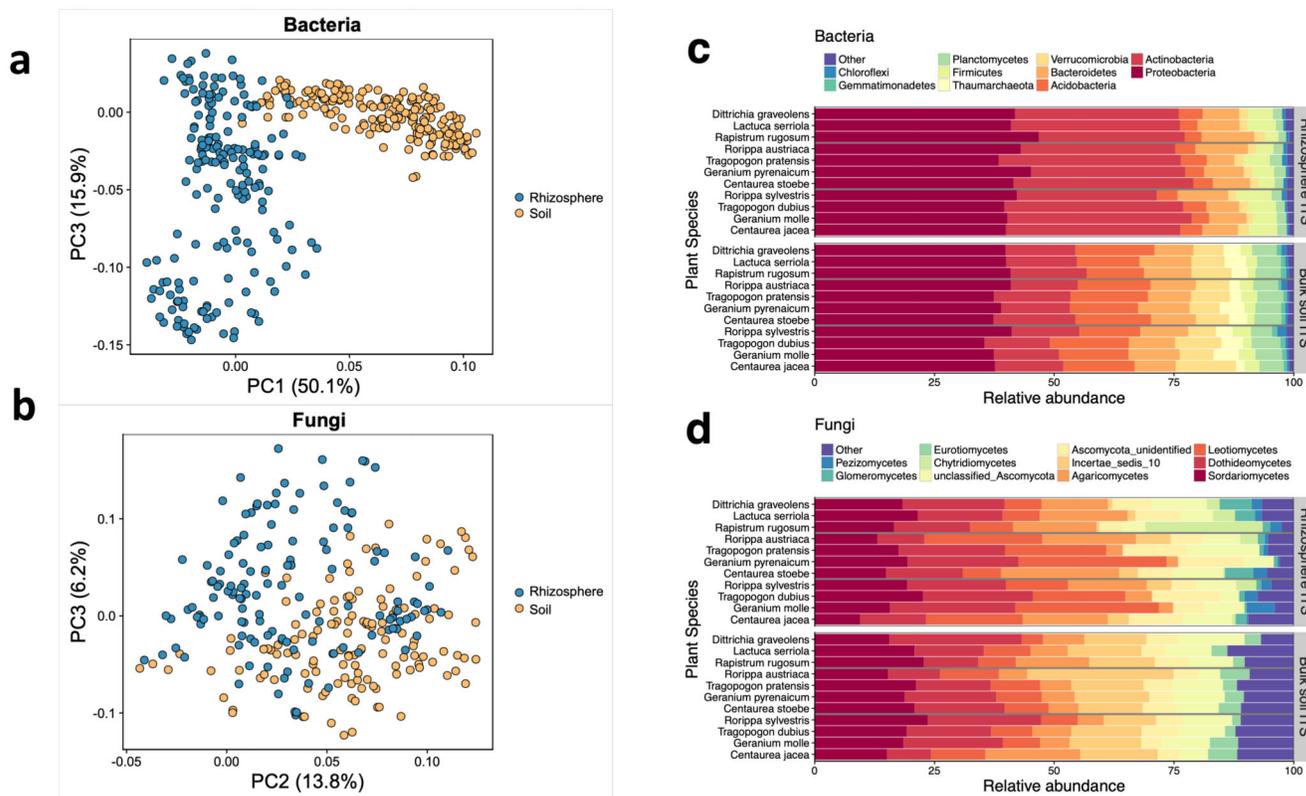
Figure 2. Rhizosphere and soils harbor different microbial communities.
Differences in bulk soil (yellow) and rhizosphere (blue) of **A** bacterial and **B** fungal communities, visualized by PCoA and differences determined by NMDS of Bray-Curtis differences (PERMANOVA: *p < 0.001* for both). Relative abundance of **C** bacterial and **D** fungal taxa from rhizosphere and soils.
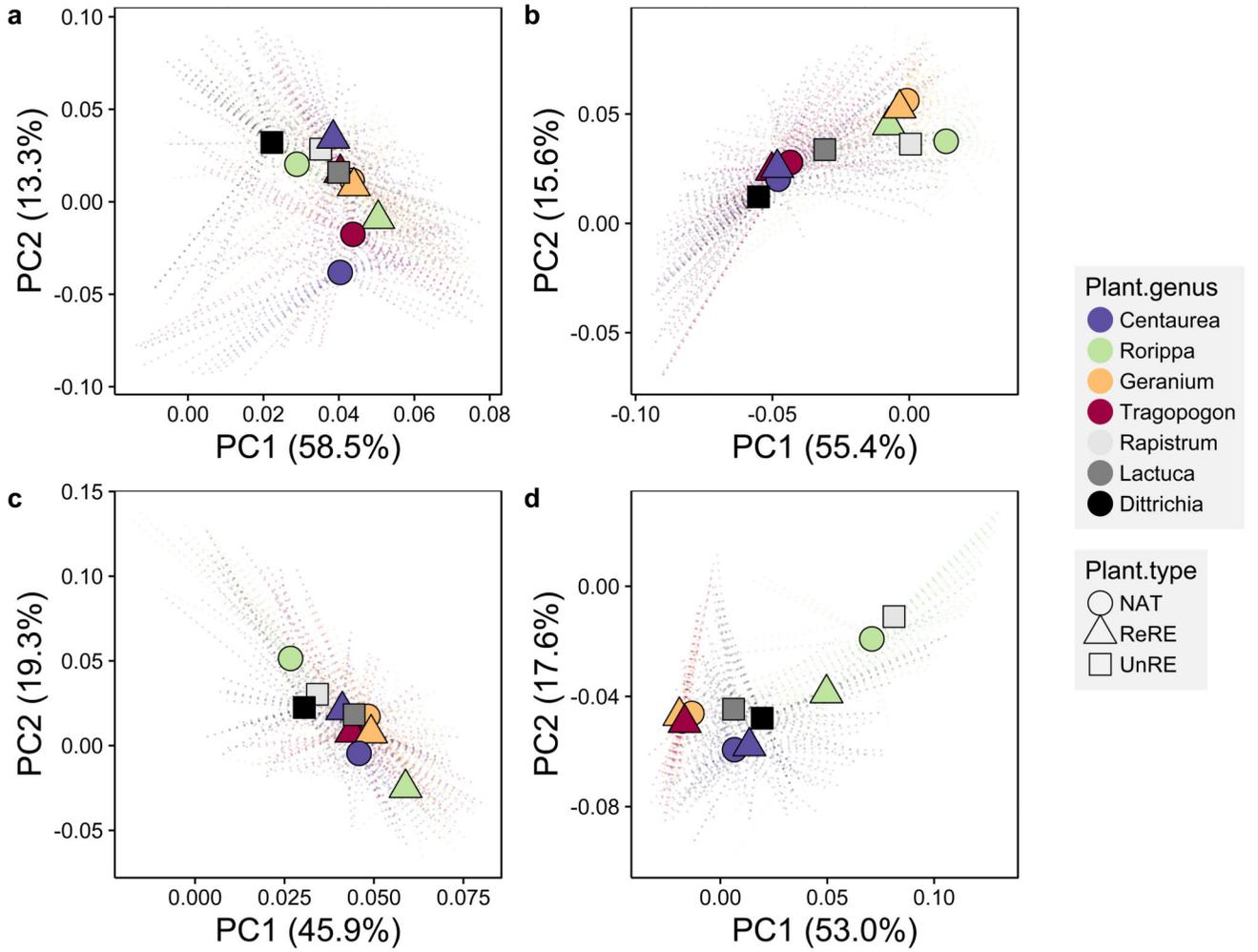
Figure 3. Plant species was the strongest predictor of bacterial (top) and fungal (bottom) community structure in both the soil (left) and the rhizosphere (right).

PCoA ordinations show the centroid of all individuals for each plant species with lines representing connections to individual samples (not plotted). Plant group (native, related range expander and unrelated range expander) is represented by shape, and plant genus by color.
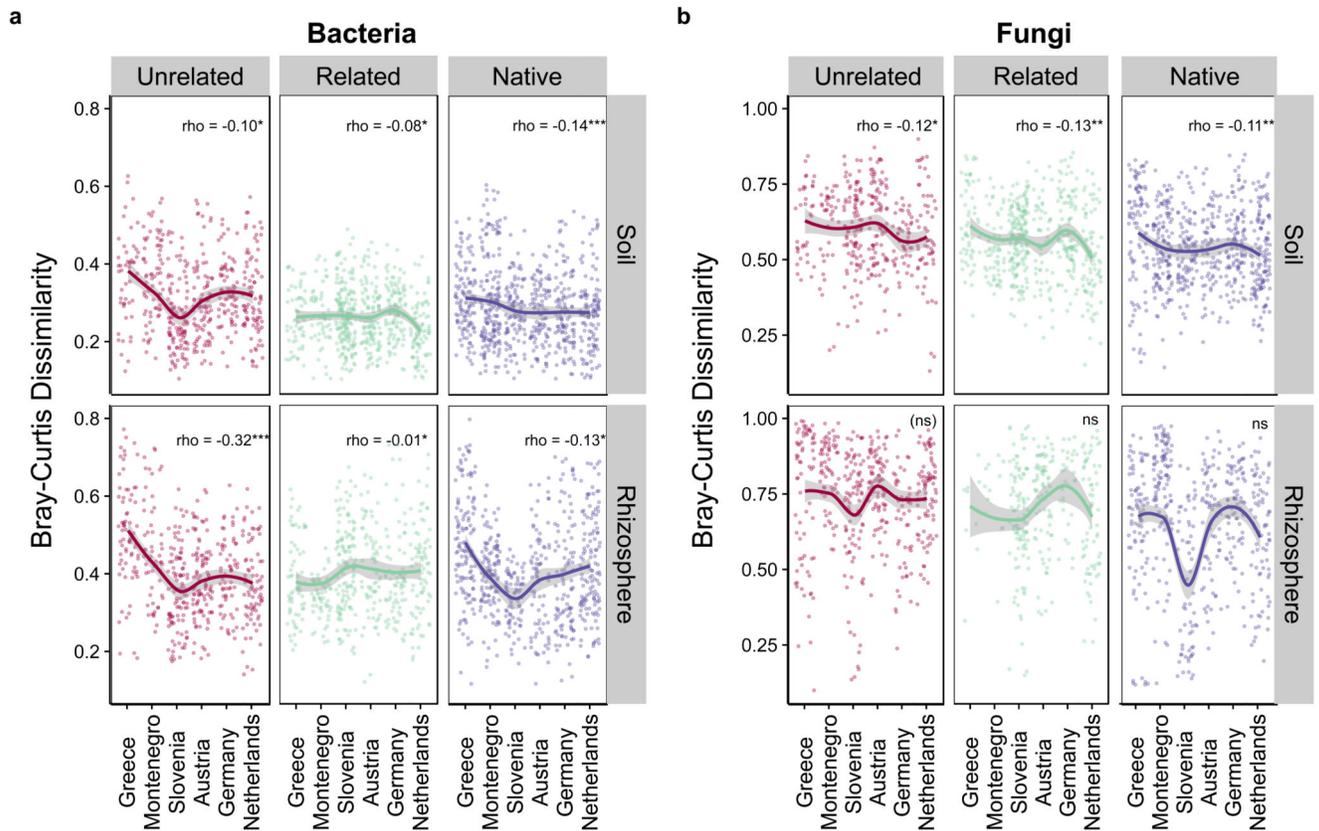
Figure 4. Changes in microbial community dissimilarly across the range expansion gradient.
A Both soil and rhizosphere bacterial communities become more similar under unrelated range-expanders (red) and to some extent native plants (purple) farther from the original range. Similar but weaker patterns were observed in related range-expanders (green). **B** Fungal communities showed a weaker response, and significant decreases were only observed in soils. (Spearman's Rank Correlation Coefficient: P < 0.05*; p <0.01**; p<<0.001***, SE shown in grey)
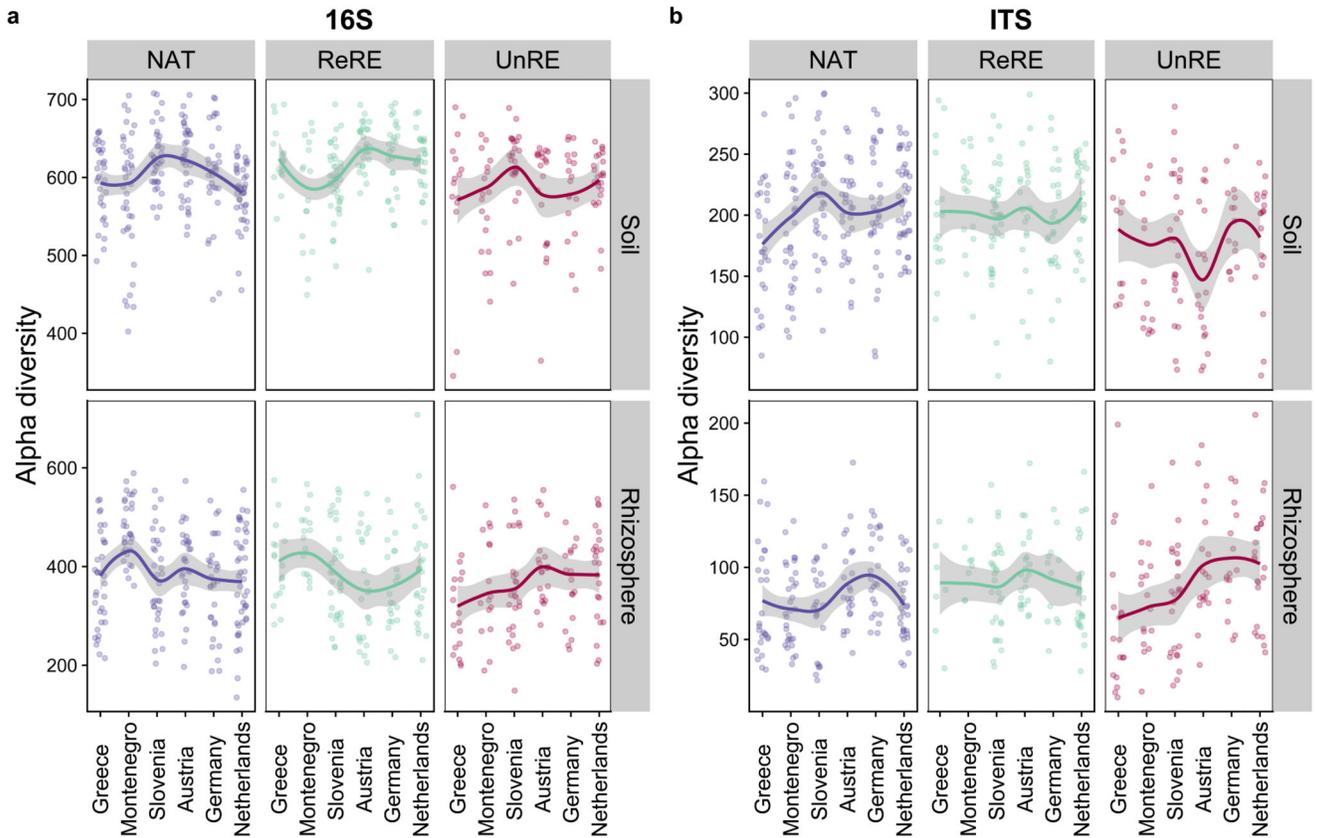
Figure 5. Changes in alpha diversity across the latitudinal gradient of range expansion differed between bacterial and fungal communities.

While **A** bacterial alpha diversity did not significantly change (ns in all cases), **B** fungal diversity increased in the rhizosphere of unrelated range expanders (red), to some extent native plant (purple), and no pattern was seen in related range expanders (green). (Spearman's Rank Correlation Coefficient: P < 0.05*; p <0.01**; p<<0.001***, SE shown in grey)