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Range-expansion effects on the belowground plant microbiome

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Plant range expansion is occurring at a rapid pace, largely in response to human-induced climate warming. Although the movement of plants along latitudinal and altitudinal gradients is well-documented, effects on belowground microbial communities remain largely unknown. Furthermore, for 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–microorganism interactions. Here we use a latitudinal gradient spanning 3,000 km across Europe to examine bacterial and fungal communities in the rhizosphere and surrounding soils of range-expanding plant species. We selected range-expanding plants with and without congeneric native species in the new range and, as a control, the congeneric native species, totalling 382 plant individuals collected across Europe. In general, the status of a plant as a range-expanding plant was a weak predictor of the composition of bacterial and fungal communities. However, microbial communities of range-expanding plant species became more similar to each other further from their original range. Range-expanding plants that were 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 belowground microbiome are detectable, although changes to specific taxa remain difficult to decipher.

Species range expansion in response to climate change is recognized as a major uncertainty in predicting the consequences of global warming for biodiversity and ecosystem functions[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]. Although 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 microorganisms can influence plant establishment, fitness and community assembly[12–14]. It has been proposed that range-expanding plants will be successful in their new range, because they lose their specialized soil pathogens[5,15,16]. At the same time, range-expanding plants may also lose specialized mutualistic microorganisms[17–19]. Results of these studies lead to the similar expectation that the plant-associated microbial community in the rhizosphere and surrounding soil (here called the belowground plant microbiome) of range-expanding plant species will associate less with the belowground microbiome in their new range compared to their native range, and compared to native plant species. However, few studies have characterized or compared the structure and diversity of the microbiome communities associated with range-expanding plant species (although see a previous study[20]), nor has a direct comparison been made 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[21]. The community composition of the belowground microbiome is broadly structured by abiotic factors, yet effects differ between bacteria and fungi[22,23]. For example, whereas at large spatial scales bacterial communities are strongly influenced by soil pH[24,25], the composition of fungal communities are simultaneously affected by climate and nutrients[26–28]. At the same time, both the soil and rhizosphere microbiomes are strongly controlled by biotic factors, including the composition of root exudates, plant species identities and plant traits[29–31]. Through these properties, plant species can assemble species-specific microbiomes in which microbial taxa are enriched or suppressed under some plants and not under others[14,32–35]. At the same time, phylogenetic relatedness of range-expanding plants with native flora can represent another potential effect of range expansion on microbial communities—for which some research suggests that closely related plant species can contain similar microbial taxa, especially pathogens[36,37]. Finally, plant–microorganism interactions evolve over time, changing over years and even decades[38,39]; therefore, during range expansion, both the distance from the original range and the evolutionary history between plants and microorganisms[40] have the potential to influence the belowground plant microbiome.

Here we analyse the microbiome of intra-continental range-expanding plant species along a latitudinal gradient to explore key

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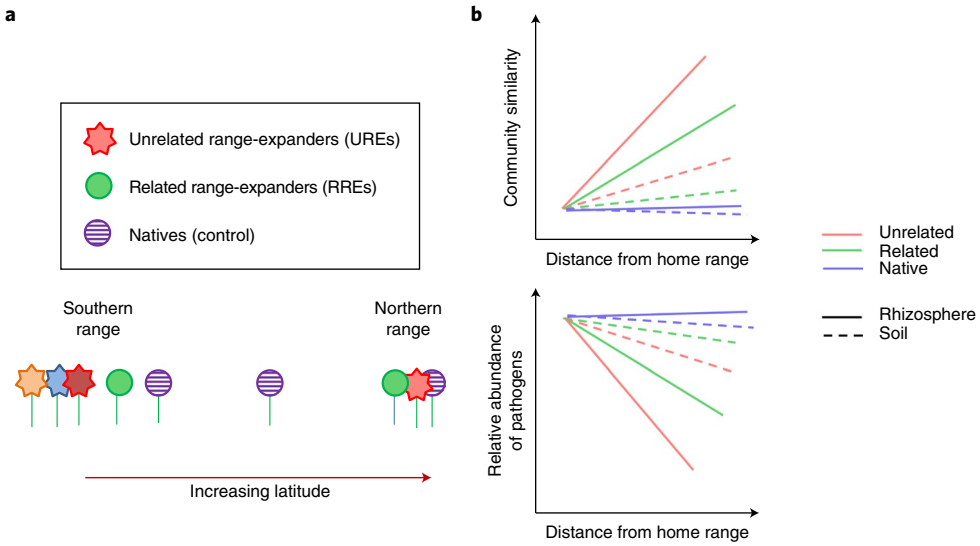

Fig. 1 | Changes in microbial community during plant range expansions. a, When plants move from the southern range to a new range, the range-expanding plants can either be related to the native flora (circles) or be unrelated to the native flora (stars). **b**, Hypothesized responses of the similarity of microbial communities and the relative abundances of pathogens to range expansion; we expect that observed patterns are stronger in the rhizosphere (solid lines) than in the bulk soil (dashed lines) and that the relatedness of the range-expanding plant to the native flora affects the strength of the response.

hypotheses that have been previously proposed for exotic and invasive plants, but that 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-expanding plants 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, we compared changes in community composition and the relative abundance of pathogens 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 rhizosphere[41] than in bulk soil. Furthermore, if range-expanding plants that are further from their original range either lose the ability to interact with certain microbial taxa or preferentially promote the growth of a beneficial community, the microbiome of the range-expanding plants will become more similar and alpha diversity of communities will decrease in the new range. However, because plants that are more closely related to the native community may share microorganisms, this change will be less pronounced for range-expanding plants that encounter congeneric native species in the new habitat. Finally, if the enemy release hypothesis common to invasive plant species is also applicable to range-expanding plants, we expect fewer belowground pathogens to be associated with range-expanding plants that are unrelated to the native flora compared to related expanding and native species.

In Europe, the range expansion of plants induced by climate change is well-documented; many plant species are expanding their range into higher latitudes and altitudes[2,42]. Here we use 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-expanding plants (species without native species from the same genus in their new range); related range-expanding plants (species that have native species from the same genus in their new range) (Supplementary Table 1 and Supplementary Fig. 1); and native plant species, which are congeneric to the related range-expanding plant species and native throughout the entire gradient. All range-expanding plants had either arrived or greatly expanded within the Netherlands in the late twentieth and early twenty-first centuries[43]. 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, totalling 382 plant individuals (Supplementary Table 1 and Supplementary Data 2). While some species were cosmopolitan[44], others were quite rare and more difficult to find. Here we included replicates not only for individual plant species, but also for each plant type (native, and related and unrelated range-expanding plant species), and we collected 382 bulk-soil and rhizosphere samples to obtain a number that should be sufficient to capture large-scale patterns in the microbial communities[25,27].

Results and discussion

Overall, rhizosphere and bulk-soil communities were significantly different from each other, both in community overlap—as visualized by principal component analysis (PCA) ($P<0.001$ for both bacteria and fungi; Fig. 2a,b)—and in taxa overlap (Fig. 2c,d). 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. Furthermore, there was little community overlap among plant individuals in both the 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 species[35,45,46].

Across the gradient, plant species was the strongest predictor of the composition of the bacterial and fungal communities in both soil and rhizosphere environments, explaining 7 to 14% of the variation (Fig. 3 and Supplementary Table 2) and plant genus as a proxy of phylogenetic relatedness (Supplementary Fig. 1) provided no additional predictive power. Conversely, the effects of plant grouping (unrelated range-expanding, related range-expanding and native

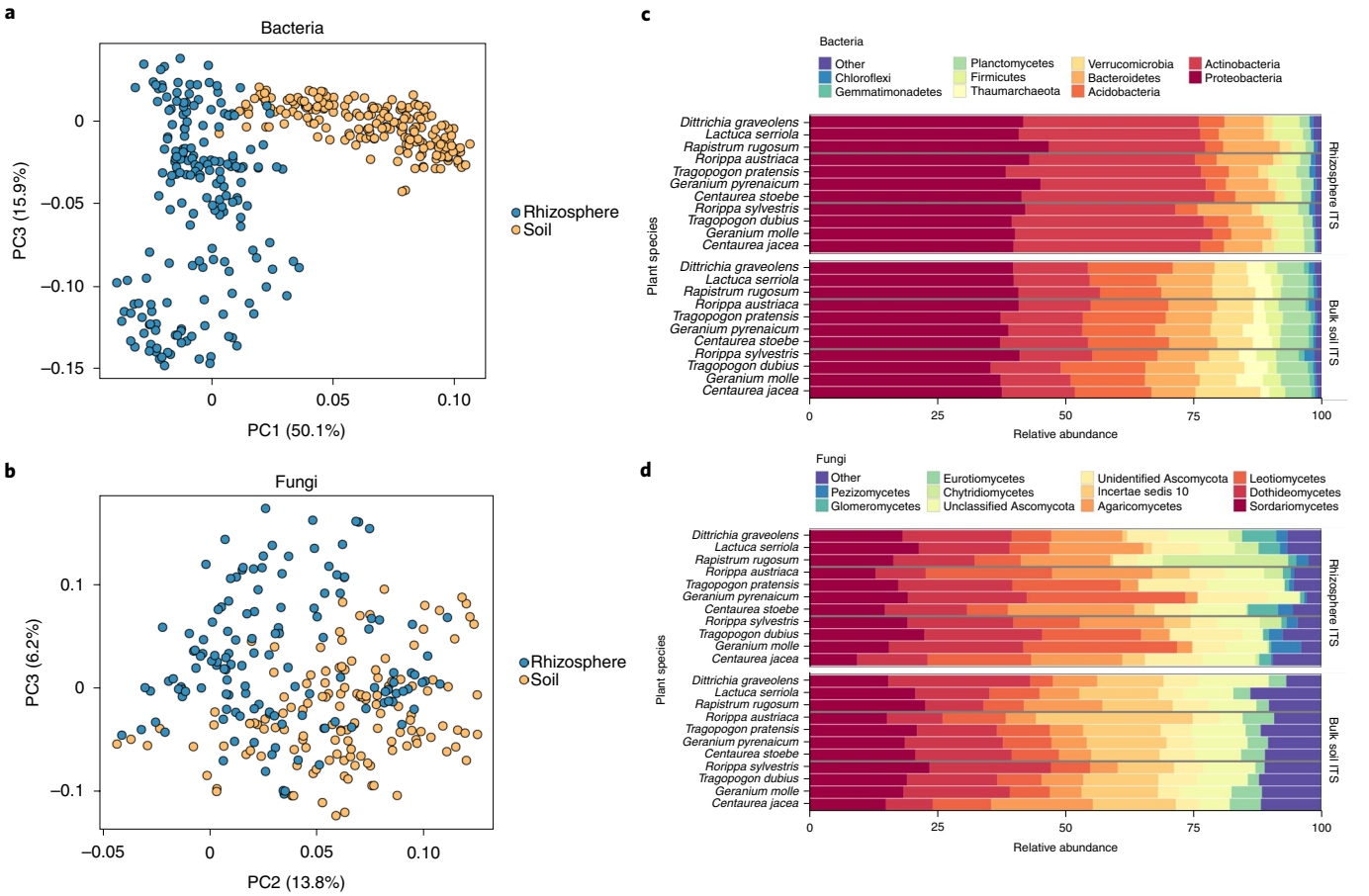

Fig. 2 | The rhizosphere and soil contain different microbial communities. a,**b**, Differences in bulk soil (yellow) and rhizosphere (blue) of bacterial (**a**) and fungal (**b**) communities, visualized by PCA and differences determined by non-metric multidimensional scaling (NMDS) of Bray–Curtis differences (permutational multivariate analysis of variance using distance matrices (PERMANOVA): $P < 0.001$ for both). PC, principal component. **c**,**d**, Relative abundances of bacterial (**c**) and fungal (**d**) taxa from the rhizosphere and soil.

plant species) 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 (for example pH, nitrogen and carbon), except for soil bacterial communities, for which pH explained approximately 5% of the variation. The relatively minor effect of soil abiotic factors on microbial communities—compared to previous studies[24]—can be explained by the small variation in soil factors across the gradient and between plants (Supplementary Fig. 2), as was the goal of choosing plant species that grow on the same parent soil material. In comparison, other studies have been more focused on elucidating patterns in the composition of the microbial community relative to changes in abiotic factors[25,27,47]. Thus, the observed differences are more likely to be due to the effects of the plant species themselves[46], such as plant ecology, relatedness with native flora and life-history traits[44,48,49].

In support of our hypothesis, we found that range-expanding plants that were further from their original range had microbial communities that were more similar to other plant individuals. Put another way, the variation in community composition decreased among individuals in the new range. Furthermore, there were negative correlations between 'range' (the country samples were collected from) and community dissimilarity for all plant groups (Fig. 4 and Supplementary Table 3); when analysed using latitude and distance, equivalent results were obtained. This pattern was significant for bacterial communities in the soil and rhizosphere of all plant types (ρ varied between −0.08 and −0.32 and $P < 0.05$ for all). However, for fungal communities, correlations were only observed in soils (ρ 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-expanding species (Supplementary Table 3). We also found a significant difference in the degree of microbial community similarity by plant group, although there was an interaction of country in two scenarios (soil fungi and rhizosphere bacteria) ($P < 0.0001$ in all cases) (Supplementary Table 4). This suggests that controls on the composition of microbiome communities of native and range-expanding plants differs across the gradient. For instance, the microbiomes of native plants (and to a lesser extent related range-expanding species) may be more influenced by a long-term co-evolutionary history that would be consistent across this latitudinal gradient[50,51], whereas microbiome patterns of unrelated range-expanding plants might be more determined by more recent spatial effects and the native (neighbour) plant community[52]. 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 microorganisms 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 change[38,53].

Whereas community structure became more similar across the gradient, changes in bacterial richness and fungal richness

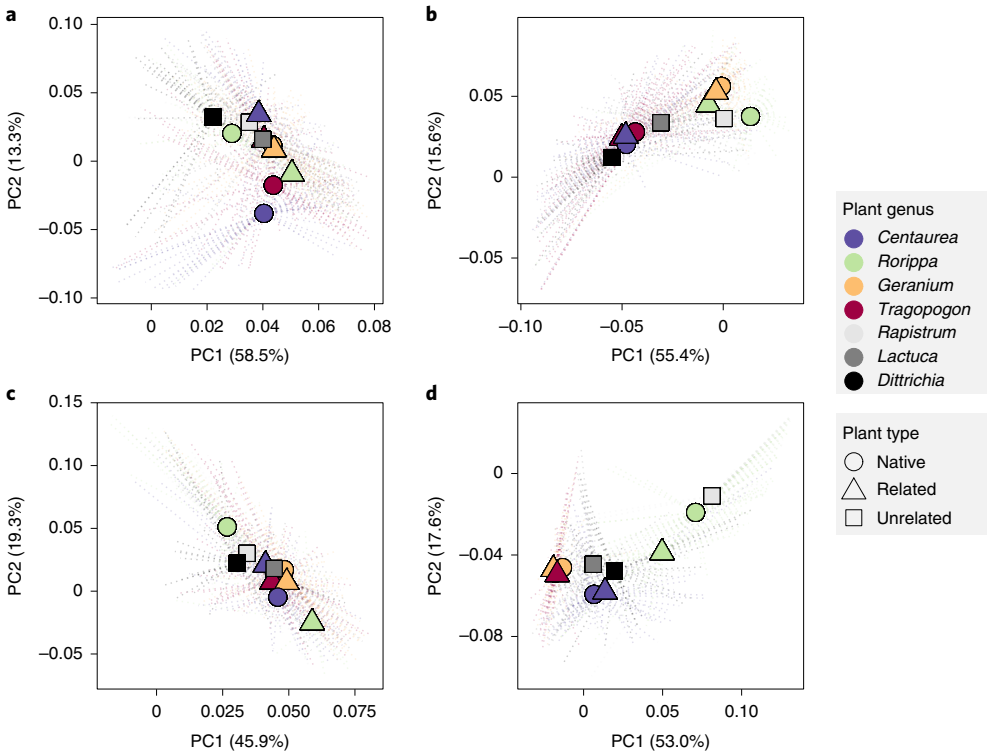

Fig. 3 | Plant species was the strongest predictor of bacterial and fungal community structure in both the soil and the rhizosphere. a–d, PCA ordinations show the centroid of all individuals for each plant species, with lines representing connections to individual samples (not plotted). **a**, Bacterial community structure in the soil. **b**, Bacterial community structure in the rhizosphere. **c**, Fungal community structure in the soil. **d**, Fungal community structure in the rhizosphere. Plant group (native: *Centaurea jacea*, *Geranium molle*, *Tragopogon dubious* and *Rorippa sylvestris*. *C. stoebe* and *R. austriaca*; related range expander (related): *Centaurea stoebe*, *Geranium pyrenaicum*, *Tragopogon pratensis* and *Rorippa austriaca*; and unrelated range expander (unrelated): *Dittrichia graveolens*, *Lactuca serriola* and *Rapistrum rugosum*) is represented by shape, and plant genus by colour.

was much more variable (Fig. 5 and Supplementary Table 5). Under unrelated range-expanding species, fungal alpha diversity in the rhizosphere 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-expanding plants showed no relationship between fungal diversity and distance from original range ($P > 0.05$ for both soil and rhizosphere) in comparison to native plants, for which fungal alpha diversity increased with latitude in both the rhizosphere ($\rho = 0.20$, $P < 0.05$) and the bulk soil ($\rho = 0.23$, $P < 0.05$). The mechanisms behind increased fungal diversity in the rhizosphere of unrelated range-expanding remain unclear. It could be that if range-expanding plants do not need to invest in belowground defence[54,55], the rhizosphere becomes accessible for a larger proportion of microorganisms, although this varies by plant species[56]. Alternatively, it has been proposed that exotic species and range-expanding plants promote high microbial diversity as part of a defence mechanism[52,56]. The latter 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 (Fig. 4), and that unrelated range-expanding plants had higher fungal and bacterial diversities in their rhizosphere and lower diversities in the associated soils ($P < 0.0001$ in all cases) (Supplementary Table 6). Overall, the inconsistency between the responses of the two types of range-expanding plant species suggests that related and unrelated range-expanding plants have different controls on microbial diversity. Furthermore, 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, the success or failure of a plant species is based on reduced exposure to soil-borne pathogens combined with continued association with symbionts[57,58]. We applied this concept here and used FunGuild[59] to test how the abundance of potential fungal functional groups changes as range-expanding plants move further from their original range. Specifically, we examined potential plant pathogens and arbuscular mycorrhizal fungi, as these are the relevant mutualistic symbionts for most of our plant species, except for the crucifers. However, we could not detect any significant changes in the relative abundance in either of these groups under range-expanding plant species (Supplementary Fig. 3). However, 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). By contrast, 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). In contrast to previous studies, these results do not directly verify that range-expanding plants lose their specialist microorganisms[57] or are released from specialist enemies[55]. Instead, the results suggest that compared to native species, range-expanding plants are exposed to fewer potential pathogens and symbionts in the new range, which has been predicted for range-expanding plant species[60] and demonstrated for introduced exotic species in their new range[61,62]. At the same time, recent studies of plant succession[63,64] clearly demonstrate that plant success and nutrient cycling is tied to the microbial communities. However, it remains unclear whether the mechanisms that underlie plant range expansion are the same as those observed elsewhere.

Still, these results are not without caveats. Notably, the molecular methods used are not infallible—the DNA community analysis

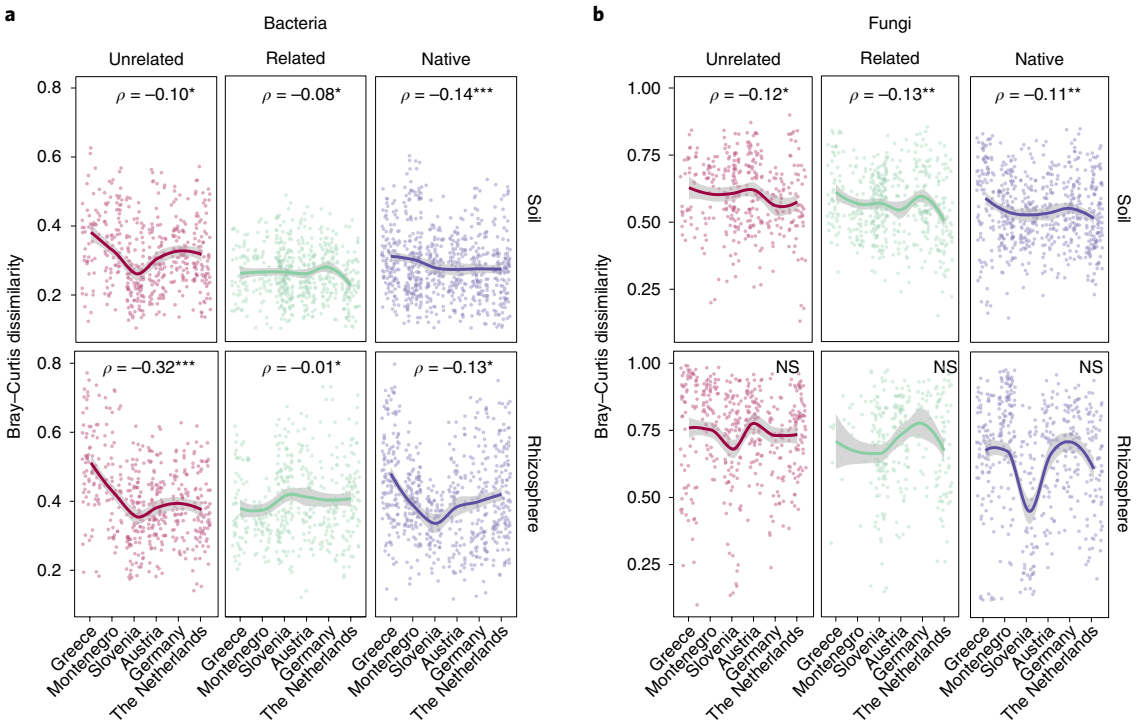

Fig. 4 | Changes in microbial community dissimilarly across the range-expansion gradient. a, Bacterial communities in both the soil and rhizosphere become more similar under unrelated range-expanding plants (red) and, to some extent, under native plants (purple) that are further from their original range. Similar but weaker patterns were observed in related range-expanding plants (green). **b**, For fungal communities, significant decreases were only observed in soils. Spearman rank correlation coefficients are shown; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P \ll 0.001$; NS, not significant. The lines indicate the mean, and grey shading indicates the s.e.m.

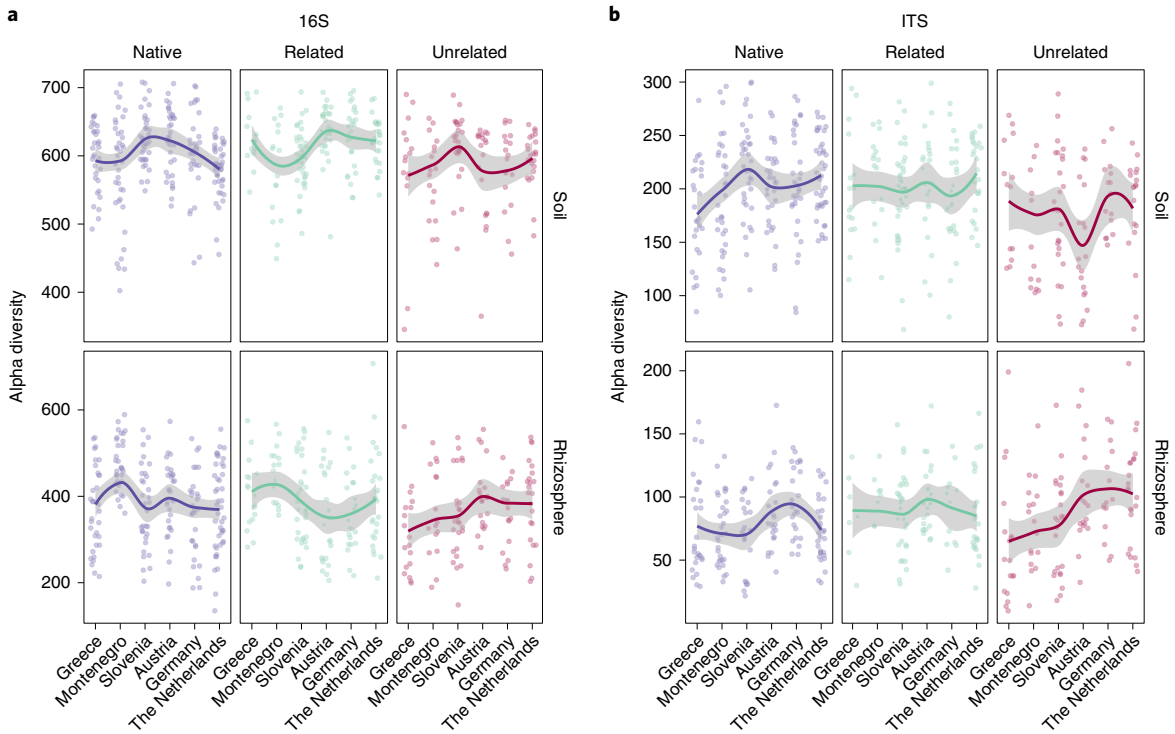

Fig. 5 | Changes in alpha diversity across the latitudinal gradient of range expansion differs between bacterial and fungal communities. a, Bacterial alpha diversity (operational taxonomic unit (OTU) count) did not change significantly (not significant in all cases). **b**, By contrast, fungal alpha diversity increased in the rhizosphere of unrelated range-expanding and, to some extent, native plants, although no pattern was seen in related range-expanding plants. The line and shading indicate mean ± s.e.m.

does not assess the active microbial community nor the true functional capabilities of the detected microorganisms. Thus, potential functional groupings and relative abundances of taxa cannot indicate the expected pathogenicity of these fungi in the rhizospheres of the host plant. Equally important is that, for all plant groups, the relative abundance of these functional groupings make up approximately 5% of the fungal community. This indicates that any changes in composition or diversity may overinflate or obscure true changes in these low-abundance groups[65] and specific primers or culture work is necessary to explore the functional changes more thoroughly. Our study exemplifies that high-throughput sequence data can be used to assess large-scale patterns in plant–soil associations; however, future functional analyses (for example, 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 for the identification of the patterns of the changes in the plant microbiome that occur during plant range expansion. Although we show that 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 results in altered exposure to soil microorganisms. Our results suggest that although terms such as 'exotic species', 'range-expanding species' and 'native species' 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 microorganisms[25,35]; however, the ecological roles of many microbial taxa currently remain unknown. At the same time, we think that this large-scale biogeographical study of plant–soil–microorganism associations of native, related and unrelated range-expanding plant species 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 after introduction of exotic plant species in a new continent. Subsequent experimental work is needed to understand the functional consequences of invasiveness and naturalization.

Almost 4% of extant global vascular flora have established outside their native range[66], and range expansion induced by climate change is not expected to slow down[67]. Although soil microorganisms exert strong selective pressures on plant species and communities[68,69], our understanding of microbial community dynamics during range expansion remains limited. Range expansion offers an opportunity to explore not only how global change may alter the relationship between plants and their microbiome, but also how the belowground microbiome changes across large geographical 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 our 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[1,70,71]. Within this latitudinal gradient, spanning almost 3,000 km from Greece in the south to the Netherlands in the north, we identified 7 range-expanding species for which the range has expanded north into Austria, Germany and the Netherlands over the last 50 years, approximately[72]. Range-expanding plants without native congeneric species in the northern sites (that is, unrelated range-expanding plants) include *Dittrichia graveolens*, *Lactuca serriola* and *Rapistrum rugosum*. Range-expanders with native congenerics (that is, related range-expanding plants) 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-expanding species, *Centaurea jacea*, *Geranium molle*, *Tragopogon dubious* and *Rorippa sylvestris*. *C. stoebe* and *R. austriaca* originated from central and eastern Europe, while all other range-expanding species 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 (Supplementary Data 2). For each sampling location of a single species, 3 individuals of 3 distinct populations (in most cases, with a separation of at least 400 m) were chosen, totalling 9 plant individuals for each location (see Supplementary Table 1 for sample numbers). For collection of all samples, permissions were obtained from both the nature reserves and government agencies that are responsible for the land.

To assess the soil and rhizosphere microbiomes of native and range-expanding plant species, soil and roots plus rhizosphere were collected from under individual plants. In brief, the entire plant was dug up within a 10-cm radius around the plant and bulk soil was shaken off the plant roots. Bulk soil was homogenized and 10 g was collected for microbial and chemical analyses. Separately from the bulk soil, the fine plant root and rhizosphere soil was then collected separately, which is 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 and Supplementary Fig. 2). Gravimetric moisture (percentage of water) was determined on soil samples that were oven-dried at 105 °C. Total soil carbon and nitrogen content was determined from these dried soils on an elemental analyser (LECO). Extractable NO_3 and NH_4 were measured using the KCl extraction protocol. In brief, soils were dried at 4 °C, 10 g dry soil was then mixed with 1 M KCl solution and shaken, after which the supernatant was 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 of 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) according to the manufacturer's instructions. Bacterial community composition was determined by targeting 16S rRNA amplicons using 515F/806R primers[73] and the fungal community composition was determined by targeting the ITS region using primers ITS4/fITS9[74]. To prevent the amplification of plant material[75], PNA Clamps (PCR Blockers) (CGACACTGACACTGA-KK) were added at the PCR step for rhizosphere bacterial DNA. For all samples, DNA was amplified by PCR in duplicate using barcoded primers[73]. PCR products were purified using the Agencourt AMPure XP magnetic bead system (Beckman Coulter Life Sciences) and analysed using the Standard Sensitivity NGS Fragment Analysis kit (1–6,000 bp). Pooled PCR amplicons were sequenced with the Illumina MiSeq platform at BGI Tech Solutions.

MiSeq paired-end reads targeting the 16S rRNA amplicon were merged and only reads that had a minimum overlap of 150 bp and a PHRED score of 25 (estimated using the RDP extension of PANDASeq[76]). Primer sequences were stripped using Flexbar version 2.5[77]. Sequences were then clustered to OTUs with VSEARCH version 1.0.10[78], using the UPARSE strategy of dereplication, sorting by abundance and clustering using the UCLUST smallmem algorithm[79]. All singletons were removed and potential chimeric sequences were removed using the UCHIME algorithm[80]. Taxonomic classification for each OTU was obtained using the RDP classifier version 2.10[81].

Similarly, MiSeq paired-end reads targeting the ITS region were treated as described above with the following adjustments: ITS primer sequences were stripped using ITSx version 1.0.11[82] before clustering, and sequences were classified using the UNITE database[83]. All bioinformatics steps were implemented with a publicly available workflow made with Snakemake[84]. 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 Fig. 3 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 Fig. 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 in both the rhizosphere and the soil. In brief, pairwise Bray–Curtis dissimilarity was estimated between samples of each plant species within each country. Diversity of soil communities were analysed using the 'vegan' package[85] using the PERMANOVA test and visualized with the 'ggplot2' package. Correlation patterns were visualized with the LOESS smoothing function[86]. Because within-country distance was much smaller than between-country distance, diversity patterns were the same whether plotted by latitude, country or geographical distance, which here we refer to as 'range'. Spearman 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 probable' classification were included. When all taxa were included results remained the same. All other analyses were performed using the R programming language (R Development Core Team).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information. Sequences have been deposited in the European Nucleotide Archive under accession numbers PRJEB25697, PRJEB25694, PRJEB25693 and PRJEB25692.

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Author contributions

W.H.v.d.P. conceived the idea of this study. Sample collection was completed W.H.v.d.P., K.S.R., K.K., S.G., L.J.B., F.t.H., O.K., N.K., M.M., D.C., M.A.T., B.V., T.Č., C.W. and R.A.W. Soil analyses and sequencing were completed by L.J.B., F.t.H., C.W., D.v.R. and K.S.R. Data analyses were completed by L.B.S. and K.S.R. The manuscript was written by K.S.R., with contributions from all co-authors.

Competing interests

The authors declare no competing interests.

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Research sample	The soil and rhizosphere microbes were characterized of 11 plant species were collected with their 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.
Sampling strategy	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).
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Timing and spatial scale	Collected in summer of 2013/2014
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Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
Volume censoring	*Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings	*Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*
Effect(s) tested	*Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	*Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*
Correction	*Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity	*Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*
Graph analysis	*Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.