Range-expansion effects on the belowground plant microbiome

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Supplementary Data:

1. OTU tables (otuTables_Ramirez.zip)

2. *Environmental Factors by sample (SoilFactors_Ramirez.txt)*

Supplementary Table 1: Collection of belowground plant microbiome samples from 382 plant individuals across Europe (Greece, Montenegro, Slovenia, Austria, Germany and the Netherlands). Summary of 11 plant species and sample number. C. stoebe and R. austriaca originated from Central and Eastern Europe, while all other range-expanders originated from southern Europe.

Plant Type	Plant Species	Life Stage	AMF association	Total Sample #
Native	*Centaurea jacea*	perennial	yes	27
Native	*Geranium molle*	annual	yes	53
Native	*Rorippa sylvestris*	perennial	no	30
Native	*Tragopogon pratensis*	perennial	yes	60
Related RE	*Centaurea stoebe*	perennial	yes	24
Related RE	*Geranium pyrenaicum*	perennial	yes	54
Related RE	*Rorippa austriaca*	perennial	no	12
Related RE	*Tragopogon dubius*	annual	yes	45
Unrelated RE	*Lactuca serriola*	annual/bi-annual	yes	54
Unrelated RE	*Dittrichia graveolens*	annual	yes	48
Unrelated RE	*Rapistrum rugosum*	annual	no	24

Supplementary Table 2: Plant species had the strongest effect on community composition, yet no factor was particularly strong in predicting composition, as determined by the PERMANOVA model which included plant species, plant type, pH, Nitrogen, Carbon and Latitude.

	Plant Species	Plant Type	pH	Nitrogen	Carbon	Latitude
Rhizosphere - ITS	$R^2 = 0.14$***	ns	$R^2 = 0.05$***	$R^2 = 0.004$**	ns	ns
Soil-ITS	$R^2 = 0.07$***	$R^2 = 0.01$***	$R^2 = 0.005$***	ns	ns	ns
Rhizosphere – 16S	$R^2 = 0.08$***	$R^2 = 0.02$***	$R^2 = 0.01$***	$R^2 = 0.006$***	ns	ns

Soil-16S	$R^2 = 0.07$***	$R^2 = 0.02$***	$R^2 = 0.05$***	ns	ns	$R^2 = 0.013$***

Supplementary Table 3: Relationship between community composition and latitude across the gradient of range-expansion. Presented are the correlations between community dissimilarity and latitude for each individual species, and for each plant type (shaded rows). ($P < 0.05$*; $p < 0.01$**; $p << 0.001$***)

	Plant Species	Soil Fungi	Rhizosphere Fungi	Soil Bacteria	Rhizosphere Bacteria
Nat	*C. jacaea*	-0.40 (***)	-0.001 (ns)	-0.17 (*)	-0.39 (***)
Nat	*G. molle*	-0.44 (***)	-0.16 (ns)	-0.18 (**)	0.01 (ns)
Nat	*R. sylvestris*	0.19 (**)	0.13 (ns)	-0.11 (ns)	-0.22 (**)
Nat	*T. pratensis*	-0.18 (ns)	-0.07 (ns)	0.17 (*)	0.26 (**)
	Native	-0.11(**)	-0.4 (ns)	-0.14 (***)	-0.13 (*)
ReRE	*C. stobe*	-0.15 (ns)	0.12 ns*)	-0.06 (ns)	-0.04 (ns)
ReRE	*G. pyrenaicum*	0.12 (ns)	0.07 (ns)	-0.14 (ns)	0.00 (ns)
ReRE	*R. austriaca*	-0.31 (**)	-0.04 (ns)	-0.39 (***)	-0.29 (***)
ReRE	*T. dubius*	-0.24 (**)	0.41 (ns)	0.00 (ns)	0.39 (***)
	Related RE	-0.13 (**)	0.34 (ns)	-0.08 (*)	0.01 (*)
UnRE	*L. serriola*	0.01 (ns)	-0.24 (**)	-0.19 (***)	-0.47 (***)
UnRE	*D. graveolens*	-0.02 (ns)	-0.22 (**)	0.10 (ns)	0.12 (ns)
UnRE	*R. rugosum*	-0.40 (**)	-0.07 (ns)	-0.54 (***)	-0.33 (**)
	Unrelated RE	-0.12 (*)	-0.05 (ns)	-0.10 (*)	-0.32 (***)

Supplementary Table 4 Plant type had a significant effect on community composition, but there was an interaction with country for soil fungal communities. ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***)

	Nat	Related	Unrelated	Plant Type	Country
Rhizosphere fungi	a	a	b	F=30.878***	ns
Soil fungi	a	a	b	F=23.322***	F=22.135***
Rhizosphere bacteria	a	a	a	ns	F= 22.09***
Soil bacteria	a	a	b	F = 50.09***	ns

Supplementary Table 5: Relationship between Alpha Diversity and latitude across the gradient of range-expansion. Presented are the correlations between alpha diversity and latitude for each individual species, and for each plant type (shaded rows). (p < 0.05*; p < 0.01**; p < 0.001***)

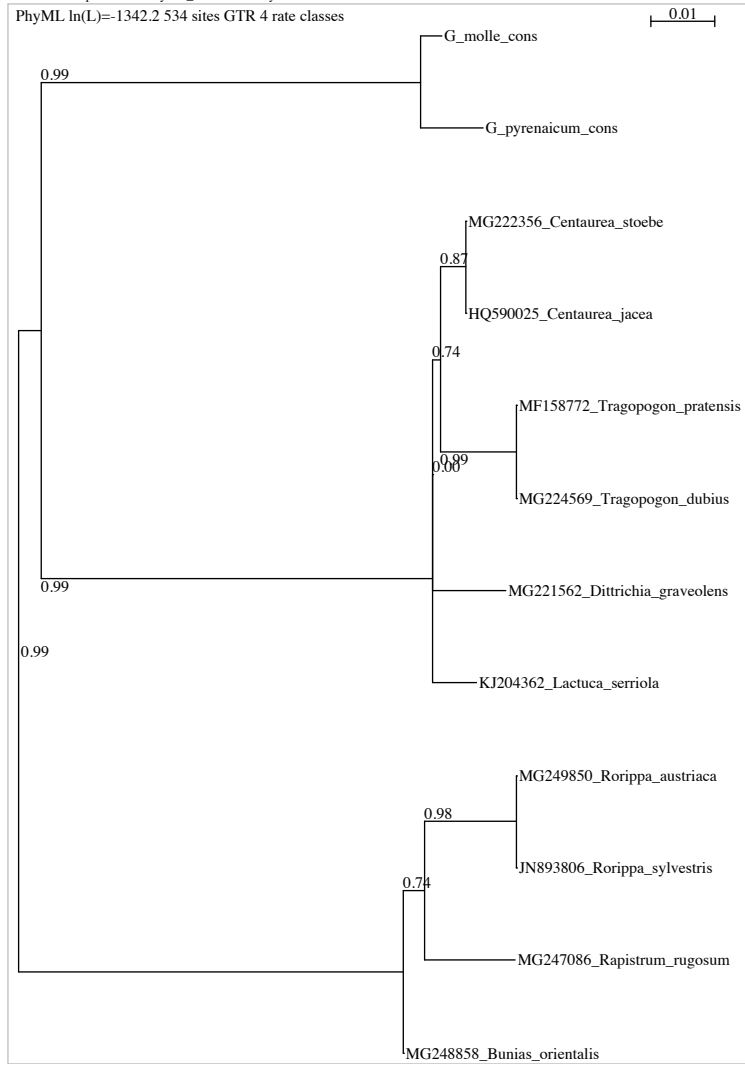|	Plant Species	Fungal Soil	Fungal Rhizosphere	Bacteria Soil	Bacteria Rhizosphere
Nat	*C. jacaea*	0.13 (ns)	-0.35 (*)	-0.05 (ns)	-0.05 (ns)
Nat	*G. molle*	0.43 (**)	0.21 (ns)	0.18 (ns)	0.18 (ns)
Nat	*R. sylvestris*	0.36 (**)	0.31 (*)	-0.14 (ns)	-0.15 (ns)
Nat	*T. pratensis*	0.17 (ns)	0.42 (*)	-0.10 (ns)	-0.10 (ns)
	Native	0.20 (*)	0.23 (*)	-0.06 (ns)	-0.09 (ns)
ReRE	*C. stobe*	0.11 (ns)	0.06 (ns)	0.72 (***)	0.73 (***)
ReRE	*G. pyrenaicum*	-0.22 (ns)	-0.19 (ns)	-0.11 (ns)	-0.11 (ns)
ReRE	*R. austriaca*	0.37 (ns)	-0.24 (ns)	-0.32 (ns)	-0.31 (ns)
ReRE	*T. dubius*	-0.04 (ns)	-0.58 (*)	0.12 (ns)	0.11 (ns)
	Related RE	-0.04 (ns)	0.12 (ns)	-0.05 (ns)	0.02 (ns)
UnRE	*L. serriola*	-0.19 (ns)	0.25 (ns)	-0.14 (ns)	-0.14 (ns)
UnRE	*D. graveolens*	0.42 (**)	0.24 (ns)	-0.02 (ns)	-0.02 (ns)
UnRE	*R. rugosum*	0.27 (ns)	-0.52 (*)	-0.36 (ns)	-0.36 (ns)
	Unrelated RE	(ns)	0.36 (***)	(ns)	(ns)

Supplementary Table 6 Unrelated range expanders had significantly lower microbial diversity than other plant types. Mean species richness of native, related range-expanding (RE) and unrelated range-expanding (RE) species. Significance of plant type and country determined by ANOVA.

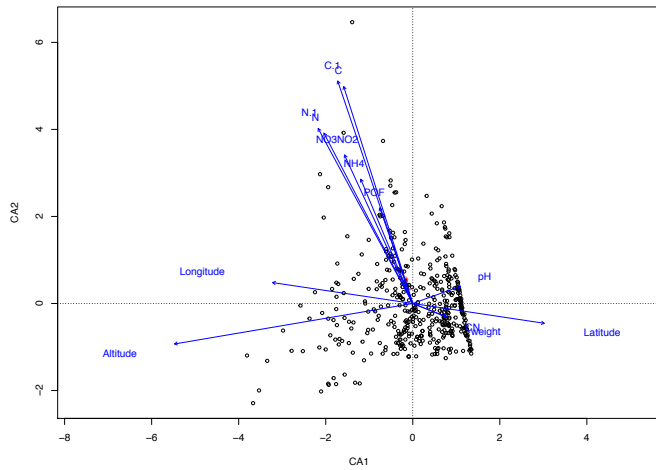|	Unrelated RE	Related RE	Native	Significance-Plant Type	Country
Fungal Rhizosphere	308 [b]	221 [a]	226 [a]	< 0.00001	< 0.00001
Fungal Soil	468 [b]	552 [a]	578 [a]	< 0.002	< 0.03
Bacterial Rhizosphere	2695 [b]	1230 [a]	1527 [a]	< 0.0001	<0.002
Bacterial soil	4078 [a]	4244 [a]	4228 [a]	ns	ns

Supplementary Table 7. The ratio of potential plant pathogens:symbionts and plant pathogens:AMF significantly increased from the south to the north in the rhizosphere of unrelated range expanders.

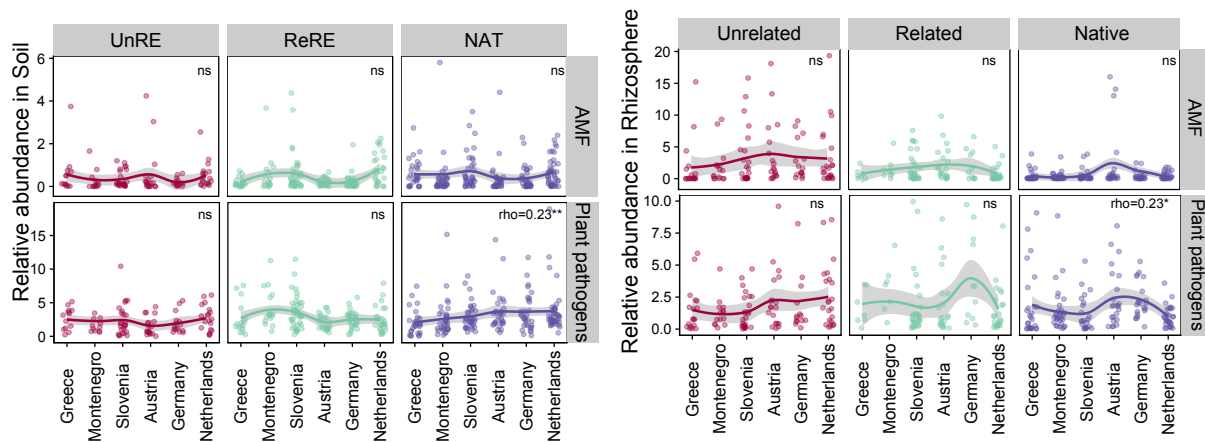Presented are the Pearson correlation values (p < 0.05*; p < 0.01**; p < 0.001***)

	Soil			Rhizosphere		
	Unrelated	Related	Native	Unrelated	Related	Native
Plant Pathogen:Sym	-0.07 (ns)	0.06 (ns)	0.11 (ns)	**-0.31(***)**	-0.5(ns)	0.17(ns)
Plant Pathogen:AMF	-0.05 (ns)	0.04 (ns)	0.13 (ns)	**-0.19 (*)**	0.06 (ns)	**-0.19(*)**

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Supplementary Figure 1: Phylogenetic relatedness of the 11 plant species collected.

Supplementary Figure 2: Canonical Correspondence Analysis (CCA) of soil edaphic factors.

Supplementary Figure 3: Relative abundance of potential AMF and plant pathogens across the latitudinal gradient of range expansion in the (A) soil and (B) rhizosphere. While no significant trends were observed under either unrelated (red) or related (green) range-expanders, native plants (purple) experienced an increase in plant pathogens.