Research

New Phytologist

Climate drives rhizosphere microbiome variation and divergent selection between geographically distant *Arabidopsis* populations

Paloma Durán[1,2,3]*, Thomas James Ellis[4,5]*, Thorsten Thiergart[1], Jon Ågren[4] and Stéphane Hacquard[1,2]

[1]Max Planck Institute for Plant Breeding Research, 50829, Cologne, Germany; [2]Cluster of Excellence on Plant Sciences (CEPLAS), Max Planck Institute for Plant Breeding Research, 50829, Cologne, Germany; [3]LIPME, Centre National de la Recherche Scientifique, Institut National de la Recherche Agronomique, Castanet-Tolosan 31326, France; [4]Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, SE-752 36, Uppsala, Sweden; [5]Gregor Mendel Institute of Molecular Plant Sciences, Austrian Academy of Sciences, Doktor-Bohr-Gasse 3, 1030, Vienna, Austria

Authors for correspondence:
Stéphane Hacquard
Email: hacquard@mpipz.mpg.de

Jon Ågren
Email: jon.agren@ebc.uu.se

Received: *9 June 2022*
Accepted: *29 June 2022*

New Phytologist (2022) **236:** 608–621
doi: 10.1111/nph.18357

Key words: *Arabidopsis thaliana*, climate, local adaptation, microbiome, plant fitness, soil.

Summary

- Disentangling the contribution of climatic and edaphic factors to microbiome variation and local adaptation in plants requires an experimental approach to uncouple their effects and test for causality.
- We used microbial inocula, soil matrices and plant genotypes derived from two natural *Arabidopsis thaliana* populations in northern and southern Europe in an experiment conducted in climatic chambers mimicking seasonal changes in temperature, day length and light intensity of the home sites of the two genotypes.
- The southern *A. thaliana* genotype outperformed the northern genotype in the southern climate chamber, whereas the opposite was true in the northern climate chamber. Recipient soil matrix, but not microbial composition, affected plant fitness, and effects did not differ between genotypes. Differences between chambers significantly affected rhizosphere microbiome assembly, although these effects were small in comparison with the shifts induced by physicochemical differences between soil matrices.
- The results suggest that differences in seasonal changes in temperature, day length and light intensity between northern and southern Europe have strongly influenced adaptive differentiation between the two *A. thaliana* populations, whereas effects of differences in soil factors have been weak. By contrast, below-ground differences in soil characteristics were more important than differences in climate for rhizosphere microbiome differentiation.

Introduction

The geographical distribution of plant species is determined by a number of biotic and abiotic factors, as well as the interactions between them that ultimately delineate species ranges worldwide. The same factors can act in concert to drive adaptive differentiation among populations belonging to the same plant species, a phenomenon known as local adaptation. A fitness advantage of local over nonlocal genotypes has been documented in many plant species (Leimu & Fischer, 2008; Hereford, 2009), including the model plant *Arabidopsis thaliana*. In *A. thaliana*, both reciprocal transplant experiments (Ågren & Schemske, 2012; Ågren *et al.*, 2013; Postma & Ågren, 2016; Thiergart *et al.*, 2020; Ellis *et al.*, 2021) and common-garden experiments (e.g. Fournier-Level *et al.*, 2011; Montesinos-Navarro *et al.*, 2011; Exposito-Alonso *et al.*, 2019) have found evidence of local adaptation across the native range in Europe. Correlations between genetically based variation in phenotype and environmental factors can suggest causes of divergent selection, but determining conclusively the respective contribution of abiotic and biotic factors such as climate, soil physicochemical properties and soil microbiome to differences in selection requires an experimental approach because these factors are typically correlated with one another. Specifically, it is difficult to uncouple the effect of soil physicochemical properties from the effect of the soil microbiome, and to identify the climatic variables that contribute the most to adaptive differentiation between populations. Recent evidence indicates that above-ground phenotypes and fitness in plants can be modulated by interactions with microbial root commensals (Friesen *et al.*, 2011; Lau & Lennon, 2012; Wagner *et al.*, 2014; Lu *et al.*, 2018; Hou *et al.*, 2021a; Van Nuland *et al.*, 2021). However, there is limited knowledge on the extent to which variation in soil microbiome can drive adaptive differentiation among plant populations. A recent report shows that interactions with microbes can affect estimates of plant local adaptation, although effects may vary among environments (Petipas *et al.*,

*Co-first authors.

2020). To understand the importance of microbe-mediated local adaptation, it is essential to disentangle how biotic and abiotic factors act as selective agents and affect the relative fitness of local and nonlocal populations (Petipas *et al.*, 2021).

Environmental conditions also drive geographical variation in below-ground soil microbial communities. Several studies identified a link between microbial community assembly and host distribution (Brundrett & Tedersoo, 2018; U'Ren *et al.*, 2019), suggesting that the evolutionary history between root symbionts and their host plants has shaped plant populations worldwide (Tedersoo *et al.*, 2020). Furthermore, relationships between microbial community assemblages and latitude (Vetrovsky *et al.*, 2019; Thiergart *et al.*, 2020) or soil physicochemical properties (Fierer & Jackson, 2006) have been reported. For example, climatic variables were shown to explain the global distribution of common soil fungi, as well as the composition and diversity of fungal communities, better than edaphic factors such as soil pH or bulk density (Tedersoo *et al.*, 2014; Vetrovsky *et al.*, 2019). By contrast, soil pH was repeatedly identified as the primary variable explaining bacterial community differentiation in soil at both small and large spatial scales (Fierer & Jackson, 2006; Rousk *et al.*, 2010; Karimi *et al.*, 2018). The large-scale sampling and substantial replication suggest that these associations are robust, but studies that subject predictions to experimental evaluation are lacking.

Here, we conducted an experiment under controlled conditions to examine the extent to which differences in climate and soil environment can explain divergent selection between two geographically distant and locally adapted populations of the model plant *A. thaliana*, from Italy and Sweden. In addition, we tested the effects of climate, soil matrix and plant genotype on the composition of the rhizosphere microbiome. A previous field experiment, in which plant genotypes and soil were reciprocally transplanted between the two source populations, indicated a strong effect of location but at most a weak effect of soil composition on the relative performance of the two genotypes (Thiergart *et al.*, 2020). However, that experiment could not distinguish between the effects of soil matrix and microbial composition on plant fitness, which is challenging to perform under field conditions owing to the difficulty of keeping soil microbial communities independent of one another. Moreover, although the two genotypes differ in tolerance to freezing (Oakley *et al.*, 2014), and there is a strong relationship between minimum temperature in winter and performance of the Italian genotype in Sweden (Ågren & Schemske, 2012), it is not clear whether differences in temperature, day length and light intensity are sufficient to explain the effect of location. In the present study, we experimentally examine: the independent and combined effects of soil matrix and soil microbiome on the relative fitness of the two genotypes in climate growth chambers that mimicked the seasonal changes in temperature, day length and light intensity at the two home sites; and the effects of soil matrix and plant genotype on the composition of the rhizosphere microbiome under the same chamber conditions (Fig. 1).

The results are consistent with the hypothesis that differences in climate between northern and southern Europe have driven much of the adaptive differentiation between the two *A. thaliana* populations, whereas differences in soil matrices and soil microbiome have been less important. By contrast, the results suggest that differences in below-ground soil physicochemical conditions, and to a lesser extent in above-ground climatic conditions, can explain differentiation in bacterial and fungal soil assemblages between the two sites.

Materials and Methods

A. thaliana source populations

We used genotypes derived from two populations of *A. thaliana* located close to the southern and northern range margins in Europe: one from central Italy (Castelnuovo di Porto, 42°07′N, 12°29′E), and one from north-central Sweden (Rödåsen, 64°48′N, 18°12′E) (Fig. 1a,b). Both populations grow on steep rocky slopes and are winter annuals. Seeds germinate in the autumn, overwinter as leaf rosettes, and flower in February/March (Italy) and in April/May (Sweden; for further details see Ågren & Schemske, 2012; Postma & Ågren, 2016). Reciprocal transplants have demonstrated strong local adaptation between the two populations (Ågren & Schemske, 2012; Ågren *et al.*, 2013; Postma & Ågren, 2016; Thiergart *et al.*, 2020; Ellis *et al.*, 2021). For the present experiment, we used two genotypes that have been phenotypically well characterized and that have served as parents for the production of a set of recombinant inbred lines used to explore the genetic basis of phenotypic differences between the two populations (Ågren & Schemske, 2012; Ågren *et al.*, 2013; Oakley *et al.*, 2014; Postma & Ågren, 2016, 2018; Thiergart *et al.*, 2020; Ellis *et al.*, 2021). The seeds used in the experiment had been produced in a common glasshouse environment to reduce variation in maternal effects.

Experimental treatments in climatic chambers

To disentangle effects of climate (conditions in the Italian and Swedish source populations), soil matrix (derived from the Italian vs Swedish site), and soil microbiome (from the Italian vs Swedish site) on the relative fitness of the Italian and Swedish genotypes, and effects of climate, soil matrix and plant genotype on the rhizosphere microbiome, we conducted a split-plot growth-chamber experiment (Fig. 1b,c). In this experiment, the two genotypes were grown in two chambers with different climatic regimes, on field-collected soil from either the Italian or Swedish site. Conditions in chambers were programmed to mimic seasonal changes between *A. thaliana* seedling establishment and fruit production at the Italian and Swedish site, respectively. Ideally, climatic conditions had been replicated in several chambers, but this was not logistically possible because of the duration of the experiment (> 6 months) and limited access to chambers with capacity for maintaining subzero temperatures. In each chamber, seedlings of the two genotypes were planted in blocks, where each block was subject to a given combination of soil (collected at the Italian or Swedish site) and microbial

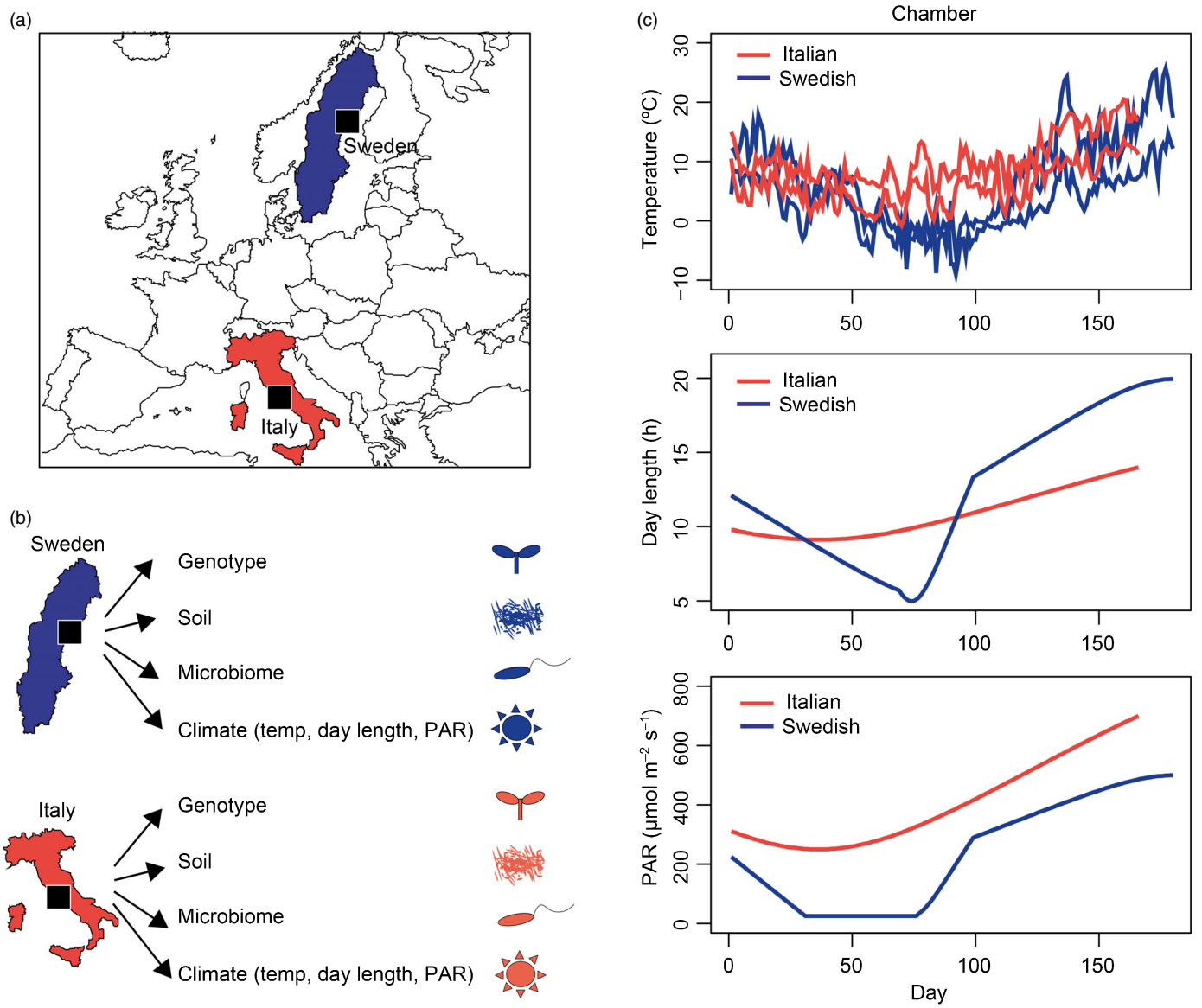

Fig. 1 Laboratory manipulation of local environmental conditions from two geographically distant sites. (a) Map indicating the locations of the two natural *Arabidopsis thaliana* populations in Sweden and Italy. (b) Deconstruction of local environmental conditions at each site (temp, temperature; PAR, photosynthetically active radiation). (c) Climatic conditions (temperature, day length and PAR) used in two climatic growth chambers mimicking day-to-day seasonal variation measured in the corresponding natural sites. In the uppermost graph, minimal and maximal temperatures are shown for each climatic chamber. Note that the winter period was reduced from 121 to 31 d in the climatic chamber mimicking Swedish climatic conditions (see the Materials and Methods section).

inoculate (extracted from soil collected at either the Italian or Swedish site). Chamber, soil and microbial inoculate were thus treatments applied at the block (= 'whole-plot') level, and genotype at the 'within-plot' level (cf. Quinn & Keough, 2002). To distinguish the effects of soil matrix and soil microbiome, the soil was sterilized and repopulated with a microbial inoculum extracted from soil collected at either the Italian or Swedish site before planting (Supporting Information Fig. S1). This factorial design allowed us to assess plant fitness and rhizosphere microbiome composition for all combinations of climate regime, recipient soil, inoculation treatment and plant genotype (referred to as 'main experiment', see main figures and tables). To determine the effect of sterilization on plant fitness and soil microbiome, we included four additional soil treatments: untreated soil (Italian or Swedish), and sterilized but not repopulated soil (Italian or Swedish) (Fig. S1).

Climatic conditions We defined day- and night-time temperatures based on the daily maximum and minimum soil temperatures recorded for each calendar day between 16 November 2005 and 15 April 2006 in Italy, and between 24 September 2005 and 15 June 2006 in Sweden at the sites where source populations were collected. For each site, this represents roughly the period from established seedling to fruit maturation in the local *A. thaliana* population. Soil temperature was recorded with temperature sensors placed 1 cm below the soil surface and connected

to a HOBO Pro Data Logger Series H08-031-08 (Onset Computer Corp., Bourne, MA, USA). The schedule for the Italian chamber ran for a total of 166 d. Since winter at the Swedish site is characterized by long periods of subzero temperatures, which strongly increases the risk that a growth chamber will fail, we shortened the 121 d between 1 December 2005 and 31 March 2006 to 31 d by using only every fourth day for the Swedish chamber (Dittmar *et al.*, 2014) (Fig. 1c), giving a programme with a total of 190 d. Although the durations of the two climate programmes are different, these durations were determined by the real seasonal differences and their association with the development from established seedling to fruit maturation at the two sites. We thus prioritized similarity in plant developmental stages over similarity in residence time for examining microbiome variation. We defined the transition from day to night as the times for sunrise and sunset recorded for Sundsvall (64 km south of the Swedish site) and Rome (24 km south of the Italian site) for each day in the schedules based on data taken from www.timeanddate.com (Fig. 1c). We set temperatures to increase from night to day temperatures over 4 h around sunrise, and to decrease to night temperatures for 4 h around sunset (Fig. 1c). The intensity of photosynthetically active radiation (PAR) in the chambers during daytime was set to vary with day length (range: 250–700 and 50–500 μmol m^{-2} s^{-1} in the Italian and Swedish chambers respectively; Fig. 1c) based on PAR measurements at the two sites (records taken every minute with a HOBO PAR sensor; Onset Computer).

Soil microbiome transplants To disentangle the effects of biotic and abiotic characteristics of the soil on plant fitness, we harvested soils from the two sites. The topsoil (5–10 cm) was removed and the layer between −5 and −30 cm was harvested, followed by an intermediate storage of < 2 wk at room temperature for drying and a subsequent storage at 4°C in the dark (see Bulgarelli *et al.*, 2012). Notably, bacterial and to a lesser extent fungal community composition in these stored soils resemble those from the native soils that were not stored (i.e. natural populations, Fig. S2; Thiergart *et al.*, 2020). As a first step, we sterilized Italian and Swedish soils by autoclaving them three times consecutively, with a resting time of 24 h before each autoclaving step and an additional incubation at 60°C overnight before the last autoclaving cycle. We checked the sterility of the soils by plating soil washes on Tryptic Soy Agar 50% medium and incubating them at 25°C for up to 7 d. Chemical analyses indicated that our sterilization procedure had only minor effects on the geochemical composition of the soils (Fig. S3). In a second step, we extracted microbial inocula from untreated Italian or Swedish donor soils. We extracted inocula from donor soil of a volume corresponding to 1/10 of that of the final recipient soil. For the extraction, we mixed donor soil with sterile 1× TE + 0.1% Triton X-100 (1 : 10 soil : detergent ratio) and shook vigorously by hand until the soil pellet was well mixed. We then further washed soil microbes from the soil at room temperature for 30 min at < 1 ***g*** in a tube rotator, and centrifuged the mixture briefly at 280 ***g*** for 1 min to pellet the large soil particles. We transferred the supernatant and centrifuged at 2000 ***g*** for 20 min to obtain a microbial pellet that was resuspended in the same initial volume of 10 mM $MgCl_2$ (Fig. S1). We then repopulated sterilized soils by pouring this microbial inoculum (referred to as input inoculum at T0) into the recipient soil and mixing thoroughly, resulting in four sterilized-and-repopulated soil conditions (Italian and Swedish recipient soils repopulated with either Italian or Swedish microbial inocula, Fig. S1). Given the quantity of soil needed for our randomized experimental design (see 'Experimental design' below), we mixed all soil treatments with sterile vermiculite in a 2 : 1 (soil : vermiculite) ratio to increase the total volume of the soil. Supplementation of soil with vermiculite did not alter the soil geochemical composition (Fig. S3). After repopulation of both soils with either of the two microbial inocula, we allowed microbial communities to acclimate for 2 wk at room temperature before transplanting seedlings (Fig. S1).

Experimental design We cut 160-well plug trays (DAN QPD160; Herkuplast Kubern GmbH, Ering, Germany) into 2 × 4-well blocks, sterilized by spraying with 95% ethanol and placed each into a separate plastic tub (SmartStore Förvaringslådor 2362001; Orthex Sweden AB, Tingsryd, Sweden) that had first been sterilized under UV light for 1 h. We filled each well with 18 cm^3 of soil; all wells in a block were filled with the same combination of recipient soil and microbial inoculum (i.e. with one of the eight soil treatments, Fig. S1). Soil treatments were replicated in eight blocks in each chamber for a total of 64 blocks per chamber. We germinated seeds from the Italian and Swedish genotypes on agar as described for field experiments (Ågren *et al.*, 2013; Thiergart *et al.*, 2020) and transplanted two seedlings to each well. Although seeds were surface-sterilized with bleach, we note that this experimental design cannot account for possible effects of seed endophytes at germination or transplantation. Four wells in each block received Italian seedlings and four wells Swedish seedlings. Due to a shortage of Italian seedlings, we were only able to transplant them into three wells for 58 blocks. We placed tubs in randomized positions in the growth chambers, and watered as necessary from below with sterile water. We randomized tub positions again at every watering.

Validation of soil microbiome transplantation

We assessed whether microbial communities of the untreated soils (i.e. used to prepare the microbial input inocula) resemble those of soils directly harvested from the original natural sites, and of soils used in an *in situ* field experiment (Thiergart *et al.*, 2020). For this, we retrieved demultiplexed sequencing data from soil samples of the former study and reanalysed them together with sequencing data of the untreated soil samples from the current study, following the pipeline described below for rhizosphere microbiome profiling (see 'Rhizosphere microbiome profiling and read processing' in the Materials and Methods section) (Fig. S2). To test the success of our microbial transplantation approach, we made two comparisons. First, we analysed microbial community diversity and composition in the two untreated soils and corresponding microbial inocula at preparation time (T0) and soils sterilized-and-repopulated with their indigenous

microbes after 8 wk of incubation (Fig. S4). Second, at the end of the climate schedule (i.e. at plant harvest), we analysed microbial diversity and community composition in native untreated soils, sterilized soils as well as in soils sterilized-and-repopulated with their indigenous microbial inocula (Fig. S5). We performed library preparation, 16S rRNA and ITS amplicon sequencing, as well as 16S rRNA and ITS read processing as described below (see 'Rhizosphere microbiome profiling and read processing' in the Materials and Methods section). To assess alpha diversity in inocula and soil samples, we calculated the Shannon index from amplicon sequence variant (ASV) tables, after rarefying the ASV tables to 1000 reads for both bacteria and fungi (using the *diversity* function within the VEGAN R package, and the *Rarefy* function within the GUNIFRAC R package, respectively). We tested for significant differences in alpha diversity across conditions using the Kruskal–Wallis test, with a Dunn's *post hoc* test ($P < 0.05$, Fig. S4a). We calculated Bray–Curtis distances between samples using the rarefied ASV tables (*vegdist* function within the VEGAN R package) and performed principal components analyses (PCoAs) using the *cmdscale* function (VEGAN R package) (Figs S4b, S5b). To quantify community differentiation between soil treatments (sterilized, sterilized-and-repopulated, untreated), we computed Bray–Curtis distances to untreated soil centroids, and then tested for significant differences using the Kruskal–Wallis test, with a Dunn's *post hoc* test ($P < 0.05$, Fig. S5c). To assess which ASVs were affected by the microbial transplantation procedure, we compared the relative abundances of total ASV counts in untreated soil and in soil sterilized-and-repopulated with its indigenous microbial inoculum using the R package DESEQ2 ($P < 0.05$, Love *et al.*, 2014) (Fig. S5d).

Plant fitness recording and data analysis

At the end of the climate schedule, we recorded the proportion of plants that had survived to flower, the number of fruits per flowering plant and, as an estimate of fitness, the mean number of fruits per seedling planted for each genotype in each block.

We analysed the effects of experimental treatments on fitness of the two plant genotypes with three different mixed-model analyses of variance, addressing separate questions. Since wells within the same block are not independent, all analyses of variation in fitness were conducted using genotype–block means, giving us eight replicates per genotype × soil matrix × inoculum × chamber treatment combination. All models included block (nested within chamber, soil matrix and inoculum) and its interaction with genotype as random factors. First, in the main analysis ('main experiment', Fig. 2; Table 1), restricted to treatment combinations including sterilized-and-repopulated soil, we examined whether the effect of genotype on plant fitness depended on the three other fixed factors included in the model: chamber (Italian vs Swedish climate), soil matrix (Italian vs Swedish origin) or microbial inoculum (Italian vs Swedish origin). In other words, we tested whether any interactions could be detected between these variables and genotype (Italian vs Swedish). Second, components of the soil microbiome may affect plant fitness positively, negatively or in nondetectable ways. To determine whether the net effect of the soil microbiome on plant fitness was negative or positive, and whether plant fitness on soil sterilized-and-repopulated with its indigenous microbiome was the same as that on corresponding untreated soil, we analysed a model that included chamber, genotype, recipient soil, soil treatment (untreated, sterilized or sterilized-and-repopulated) and their interactions as independent variables (referred to as 'sterile control comparison', Fig. S6; Table S1). Finally, to determine whether fitness variation among plants grown on untreated soil paralleled that observed when plants and soil were reciprocally transplanted in the field by Thiergart *et al.* (2020), we conducted an analysis restricted to plants grown on untreated soil (referred to as 'field comparison', Fig. S7a–d; Tables S2, S3). Models were fitted with restricted maximum likelihood (REML) with the software JMP 15.1 (SAS Inc., Cary, NC, USA).

Rhizosphere microbiome profiling and read processing

We harvested 167 rhizosphere samples at the end of the climate schedule, by sampling soils in wells in which plants were grown. Samples were snap-frozen in liquid nitrogen until further processing.

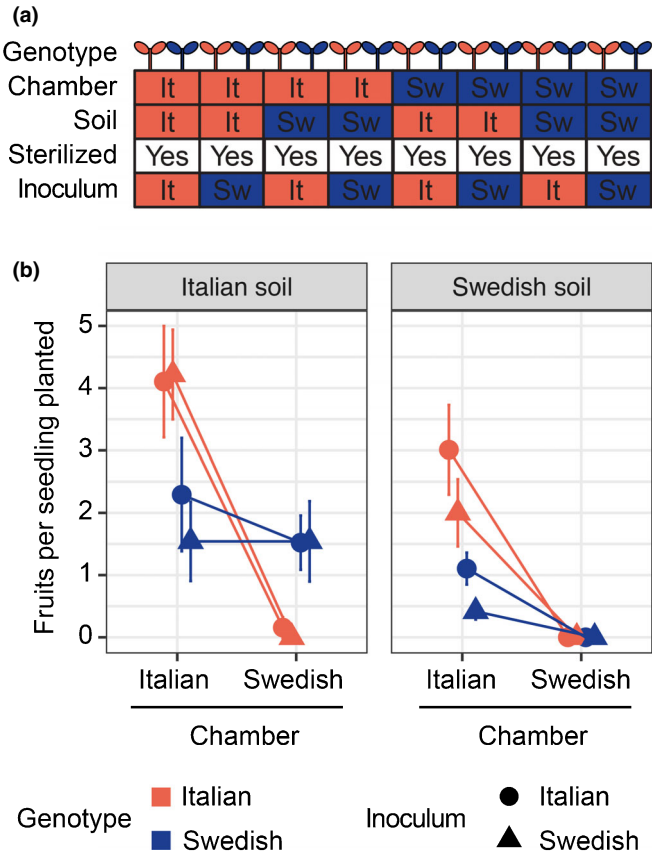

Fig. 2 Effect of plant genotype, microbial inoculum, soil matrix and climate chamber conditions on plant fitness. (a) Illustration of the 16 treatments being compared ($n = 8$ replicates (genotype–block means) per treatment, $n = 334$ plant individuals in total). (b) Interaction plots showing plant fitness in each treatment. Error bars indicate standard errors. The outcome of the statistical analysis is reported in Table 1.

Table 1 Mixed model analysis of variance in plant fitness on sterilized-and-repopulated soils.

	F	*P*
Chamber	54.288	**< 0.0001**
Soil	17.745	**< 0.0001**
Inoculum	1.383	0.2446
Chamber × Soil	1.314	0.2566
Chamber × Inoculum	1.096	0.2996
Soil × Inoculum	0.195	0.6605
Chamber × Soil × Inoculum	0.326	0.5706
Genotype	9.456	**0.0033**
Genotype × Chamber	43.541	**< 0.0001**
Genotype × Soil	1.335	0.2528
Genotype × Inoculum	0.049	0.8259
Genotype × Chamber × Soil	5.607	**0.0214**
Genotype × Chamber × Inoculum	0.190	0.6647
Genotype × Soil × Inoculum	0.375	0.5427
Genotype × Chamber × Soil × Inoculum	0.684	0.4116

Plant fitness was modelled as a function of climate chamber, plant genotype, soil matrix, microbial inoculum and their interactions (numerator df = 1, denominator df = 56; see Fig. 2a for treatments compared). Block (nested within chamber, soil matrix and microbial inoculum) and its interaction with genotype were included as random effects in the model. The analysis was conducted based on block means of each genotype. Statistically significant effects ($P < 0.05$) are indicated in bold type. The treatments that were compared are indicated in Fig. 2(a).

We extracted total DNA from rhizosphere samples using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA). We eluted DNA samples in 60 µl nuclease-free water (Agler *et al.*, 2016; Duran *et al.*, 2018; Thiergart *et al.*, 2020). We quantified DNA concentration using a fluorescence-based method, and diluted it to 3.5 ng μl^{-1}. A two-step PCR amplification protocol was used to amplify the V4V7 region of the bacterial 16S rRNA gene (799F – 1192R) and the fungal ITS1 fragment (ITS1F – ITS2) (Thiergart *et al.*, 2020). This two-step amplification protocol is detailed in Getzke & Hacquard (2022). We checked PCR quality by loading 5 µl of each reaction into a 1% agarose gel. We then combined the replicated reactions and purified them depending on the microbial amplicon: bacterial amplicons were loaded on a 1.5% agarose gel and run for 2 h at 80 V – bands with the correct size of *c.* 500 bp were cut out and purified using the QIAquick gel extraction kit (Qiagen); and fungal amplicons were purified using Agencourt AMPure XP beads (Beverly, MA, USA). We again determined DNA concentration using a fluorescence-based method, and pooled 30 ng DNA of each of the barcoded amplicons in one library per microbial group. We purified and reconcentrated each library twice with Agencourt AMPure XP beads, and pooled 100 ng of each library. We performed paired-end Illumina sequencing (2 × 300 bp) in-house using the MiSeq sequencer and custom sequencing primers (Thiergart *et al.*, 2020).

Sequencing reads were demultiplexed using QIIME2 (qiime demux emp_paired, Bolyen *et al.*, 2019) and merged using FLASH2 (Magoč & Salzberg, 2011). Reads were denoised and dereplicated using DADA2 (Callahan *et al.*, 2016), and remaining individual reads were denoted as ASVs. Chimeras were removed using QIIME2 (vsearch uchime-denovo). Taxonomic classification was done via the QIIME feature classifier using the silva_138 database for bacteria and the unite database for fungal sequences. For bacteria, sequences classified as mitochondrial or chloroplast were removed from the dataset. Remaining ASVs were included in count tables.

Rhizosphere microbiome data analysis

We analysed the effects of experimental treatments on the rhizosphere microbiome by asking two different questions. In a first main analysis, restricted to treatment combinations including sterilized-and-repopulated soil (i.e. 'main experiment', Fig. 3a), we examined the extent to which differentiation in rhizosphere microbiome composition was explained by chamber (Italian vs Swedish climate), soil matrix (Italian vs Swedish origin), microbial inoculum (Italian vs Swedish origin) and host genotype (Italian vs Swedish origin). We calculated Bray–Curtis dissimilarities between samples using the rarefied ASV tables (*vegdist* function within the VEGAN R package) and performed PCAs using the *cmdscale* function (VEGAN R package). To quantify the contribution of different variables and their interactions to the variance in pairwise Bray–Curtis dissimilarities, we analysed the Bray–Curtis distance matrix between pairs of samples with 999 iterations of a permutation-based test (permutational multivariate analysis of variance (PERMANOVA), *adonis* function, VEGAN R package) (Fig. 3b, c; Table 2). We further inspected the effects of genotype and climatic chamber using a constrained PCoA using the *capscale* function (VEGAN R package) (Fig. S8). In addition, and to ensure that different normalization methods would not provide different results to those obtained with rarefied ASV tables, we generated PCoAs and calculated the variance explained by different variables using either ASV tables with raw counts (not-normalized) or relative abundances (percentage of each ASV relative to the total read count in a given sample) (Fig. S9). To assess which microbial families were affected by the experimental treatments, we aggregated the ASV table at the family level, so that read counts of all ASVs in a given family were summed. The effect of experimental treatments on the abundance of these families was calculated with aggregated ASV counts using the R package DESEQ2 (Love *et al.*, 2014) (Fig. 3d; Table S4). In a second analysis (i.e. 'field comparison'), we determined whether rhizosphere microbiome variation of plants grown on untreated soil in chambers mimicking conditions at the Italian and Swedish sites paralleled that observed when plants and soil were reciprocally transplanted at the Italian and Swedish locations. We estimated the variance in pairwise Bray–Curtis distances explained by soil, plant genotype and chamber/location, and by their two- and three-way interactions with 999 iterations of a permutation-based test (PERMANOVA, *adonis* function, VEGAN R package) (Fig. S7a,b,e,f; Tables S5, S6).

We visualized the results using the GGPLOT2 R package (Wickham, 2016). All analyses were performed with R v.4.0.2 (R Core Team, 2021), except when stated otherwise.

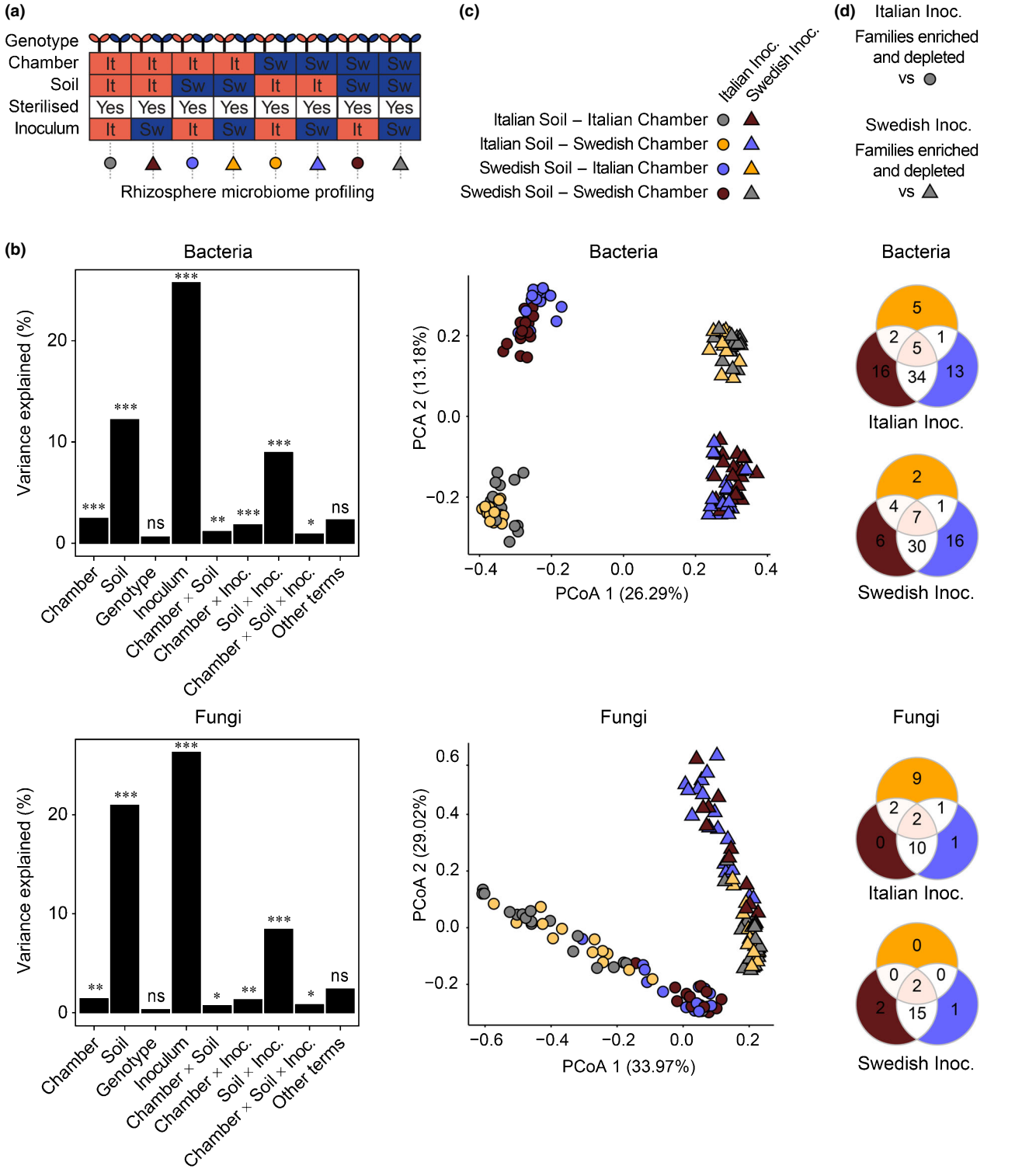

Fig. 3 Effect of plant genotype, microbial inoculum, soil matrix and climate chamber conditions on rhizosphere microbial community composition. (a) Illustration of the 16 treatments being compared ($n = 6$–9 replicates per treatment). Symbols, shape and colour below the treatment illustration refer to corresponding symbols in (c). Grey symbols reflect the two reference conditions. (b) Variance explained by different factors and their interactions on bacterial (upper panel) and fungal community composition (lower panel). Variance partitioning was calculated by PERMANOVA based on Bray–Curtis distance matrices (ns, statistically not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Nonsignificant terms are grouped as 'Other terms'. The complete statistical analysis is shown in Table 2. (c) Principal component analysis based on Bray–Curtis dissimilarities between all sample sets for bacterial ($n = 140$, upper panel) and fungal communities ($n = 131$, lower panel) in climatic chambers. (d) Venn diagrams representing the overlap of bacterial (two left panels) or fungal families (two right panels) that are significantly different (enriched or depleted, $P < 0.05$, FDR-corrected) compared to the reference condition (see also Supporting Information Table S4).

Table 2 Analysis of variance in rhizosphere microbial community composition on sterilized-and-repopulated soils.

	MS	df	*F*	*P*	R^2
Bacteria					
Chamber	1.198	1	6.933	**0.001**	0.02445
Soil	5.7843	1	34.577	**0.001**	0.12196
Genotype	0.2695	1	1.611	0.087	0.00568
Inoculum	12.1993	1	72.924	**0.001**	0.25721
Chamber × Soil	0.5389	1	3.222	**0.008**	0.01136
Chamber × Genotype	0.1918	1	1.147	0.281	0.00404
Soil × Genotype	0.1514	1	0.905	0.480	0.00319
Chamber × Inoculum	0.8512	1	5.088	**0.001**	0.01795
Soil × Inoculum	4.2476	1	25.391	**0.001**	0.08956
Genotype × Inoculum	0.1520	1	0.909	0.476	0.00321
Chamber × Soil × Genotype	0.1545	1	0.924	0.455	0.00326
Chamber × Soil × Inoculum	0.4549	1	2.719	**0.013**	0.00959
Chamber × Genotype × Inoculum	0.2012	1	1.202	0.246	0.00424
Soil × Genotype × Inoculum	0.1587	1	0.949	0.428	0.00335
Chamber × Soil × Genotype × Inoculum	0.1696	1	1.014	0.370	0.00358
Fungi					
Chamber	0.3134	1	4.358	**0.005**	0.01413
Soil	4.6466	1	64.613	**0.001**	0.20954
Genotype	0.0690	1	0.960	0.397	0.00311
Inoculum	5.8334	1	81.116	**0.001**	0.26305
Chamber × Soil	0.1517	1	2.109	0.053	0.00684
Chamber × Genotype	0.1164	1	1.619	0.143	0.00525
Soil × Genotype	0.0250	1	0.347	0.936	0.00113
Chamber × Inoculum	0.2934	1	4.080	**0.003**	0.01323
Soil × Inoculum	1.8691	1	25.991	**0.001**	0.08429
Genotype × Inoculum	0.0621	1	0.864	0.499	0.00280
Chamber × Soil × Genotype	0.0624	1	0.868	0.468	0.00282
Chamber × Soil × Inoculum	0.1946	1	2.706	**0.026**	0.00878
Chamber × Genotype × Inoculum	0.0861	1	1.197	0.289	0.00388
Soil × Genotype × Inoculum	0.0378	1	0.525	0.785	0.00170
Chamber × Soil × Genotype × Inoculum	0.1445	1	2.009	0.069	0.00651

Variance explained by different factors and their interactions on microbial community composition. Variance partitioning was calculated as a function of climate chamber, plant genotype, soil matrix, microbial inoculum, plus all two-, three- and four-way interactions by using a PERMANOVA using Bray–Curtis distance matrices. Statistically significant effects ($P < 0.05$) are indicated in bold type. The treatments that were compared are shown in Fig. 3(a).

Results

Validation of microbiome transplantation in recipient soils

We inspected whether microbial community composition of initial microbial inocula resembled those of the corresponding untreated Italian and Swedish soils and the extent to which microbial communities differed between untreated soils and corresponding sterilized-and-repopulated soils.

Bacterial and fungal diversity (alpha diversity, Shannon index) did not differ between microbial input inocula and their respective native soils (Fig. S4a). Similarly, only subtle shifts in microbial community composition (beta-diversity, Bray–Curtis distances) were observed between these two sample types (Fig. S4b). However, microbial diversity was significantly reduced and microbial composition differed between sterilized-and-repopulated soils (8 wk post-inoculation) and respective initial inocula (Fig. S4b,c), indicating that only a fraction of the microbes present in the input inocula actively repopulated the recipient soils, as previously observed (Tkacz *et al.*, 2015; Wubs *et al.*, 2016; Carini *et al.*, 2017). We cannot exclude the possibility that the transplant method that we used (i.e. with diluted microbial washes) also influenced these results (Howard *et al.*, 2017).

We then analysed soil microbial assemblages at the end of the climate schedule in untreated soils, sterilized soils, as well as in soils sterilized-and-repopulated with their indigenous microbes (Fig. S5a). Samples from both untreated and corresponding sterilized-and-repopulated soils showed qualitatively similar differentiation in microbial community composition, which was no longer observed in sterilized soils, thereby validating our microbiota reconstitution approach (Fig. S5b). Furthermore, calculation of average Bray–Curtis distances to the centroid of untreated soils validated that communities in sterilized-and-repopulated soils resemble those of corresponding untreated soils more than that of sterilized soil (Fig. S5c). Although microbiome composition of untreated soils can be largely recapitulated in recipient soils repopulated with their indigenous microbes (Fig. S5b,c), notable differences were observed. Inspection of relative abundance of microbial ASVs identified bacterial and fungal taxa primarily belonging to the phyla Proteobacteria and Ascomycota that were significantly altered between native and corresponding sterilized-and-repopulated soils (Fig. S5).

The results revealed that microbial communities in input microbial inocula resemble those of the respective field-collected soils and that differentiation in microbial community composition between the two untreated soils is qualitatively similar to that between the soils that were sterilized-and-repopulated with their native microbial inocula.

Differences in soil matrices affect plant fitness, but do not explain shifts in the direction of selection of the two plant genotypes

We asked whether differences in chamber and below-ground soil conditions (physicochemical properties of soil matrix and soil microbiome) influence the relative fitness of the two genotypes.

Analysis of fitness variation among plants grown on sterilized-and-repopulated soils ('main experiment', Fig. 2a) showed that both chamber and soil matrices affected the relative performance of the Italian and Swedish genotypes, reflected in significant chamber × genotype and chamber × soil matrix × genotype interactions (Table 1). In the chamber mimicking the Italian climate, the Italian genotype had 2.2-fold higher fitness than the Swedish genotype, when grown on the Italian recipient soil, and 3.3-fold higher fitness when grown on the Swedish recipient soil (Fig. 2b). By contrast, in the chamber mimicking the Swedish climate, the Swedish genotype had 19.6-fold higher fitness than the Italian genotype when grown on Italian recipient soil, whereas no plant survived on Swedish recipient soil (Fig. 2b). In the Italian climate, both genotypes had higher fitness when grown on Italian compared to on Swedish recipient soil, whereas in the Swedish climate this was true only for the Swedish genotype (the Italian genotype had very low survival on both soil matrices; Fig. 2b). Finally, the composition of the microbial inoculum (Italian or Swedish) did not affect plant fitness (no significant effect of inoculum, or of interactions involving inoculum; Table 1).

The results thus indicated a much stronger effect of the climatic conditions than of the soil variables on the relative fitness of the two genotypes, and also that the physicochemical composition of the soil matrix affected plant fitness more strongly than did the composition of the soil microbiome.

The net effect of the soil microbiome on plant fitness is negative

To determine the net effect of the soil microbiome on plant fitness, and whether plant fitness on soil sterilized-and-repopulated with native inoculum was the same as that on untreated soil, we analysed a mixed model that included climatic chamber, genotype, soil matrix, soil treatment (native, sterilized or sterilized-and-repopulated) and their interactions as fixed factors (Fig. S6a; see 'Sterile control comparison' in the Materials and Methods section).

In addition to the statistically significant chamber × genotype interaction, this analysis also detected a significant soil matrix × soil treatment interaction (Table S1). Soil sterilization tended to increase the fitness of both genotypes in both climatic chambers, and the effect was markedly stronger for plants grown on Italian soil (3.1-fold increase on average) compared to plants grown on Swedish soil (1.4-fold increase on average) (Fig. S6b). Plant fitness on sterilized-and-repopulated Italian soil was intermediate to that on untreated and sterilized Italian soil, whereas plant fitness was identical on untreated and sterilized-and-repopulated Swedish soil (Fig. S6b). All three soil matrix × soil treatment combinations where at least some plants survived in the Swedish chamber showed evidence of crossing reaction norms, with the Italian genotype outperforming the Swedish genotype in the Italian chamber, and the Swedish genotype outperforming the Italian genotype in the Swedish chamber (Fig. S6b). The weakest soil treatment effect was observed on Swedish soil in the Swedish chamber, that is the soil matrix × chamber combination in which very few plants survived.

The results indicate that the net effects of the soil microbiomes on plant fitness were negative, with no significant soil treatment × genotype interaction.

Relative fitness of the Italian and Swedish genotype grown on untreated soils in climatic chambers partly recapitulates results from field reciprocal transplants

To examine whether our manipulation of climatic and soil conditions in the chamber experiment had similar effects on the relative fitness of the two genotypes as had the reciprocal transplant of plant genotypes and soil in a previously published field experiment (Thiergart *et al.*, 2020; see 'Field comparison' in the Materials and Methods section), we analysed a data set that included treatment combinations that are directly comparable to those in the field experiment (two untreated soils × two plant genotypes × two chambers/locations; Fig. S7a,b).

The relative fitness of the Italian and Swedish genotypes grown on untreated soil differed between the two chambers (Fig. S7c). In the chamber mimicking conditions at the native Italian site, the Italian genotype had 3.3-fold higher fitness compared to the Swedish genotype when grown on Italian soil (Fig. S7c), and this advantage was not affected by soil category, as indicated by the statistically nonsignificant genotype × soil interaction (Table S2). This represents weaker selection against the Swedish genotype than observed in the field experiment (Fig. S7d), but is within the range of selection observed in previous transplants initiated with seedlings at this site (Ågren *et al.*, 2013). By contrast, in the chamber mimicking the Swedish climate, all plants died when grown on untreated soil (Fig. S7c). In the field experiment at the Swedish site, all plants of the Italian genotype also died, whereas survival of the Swedish genotype was 27% and 8% on Swedish and Italian soils, respectively (Thiergart *et al.*, 2020; Fig. S7d; Table S3). However, years with close to zero survival have been observed at the Swedish site. Across a 12-yr period, survival of the Swedish genotype at the Swedish site varied from < 1% to 99% (median 59%) and of the Italian genotype from 0% to 80% (median 6%) (J. Ågren,

unpublished). The results indicate that the conditions for plants growing on untreated soil in the chamber with Swedish climate were more stressful than those experienced by plants at the Swedish site in the field experiment.

We conclude that differences in temperature, day length and light intensity in our chamber experiment were sufficient to explain at least part of the divergent selection observed in the field.

Differences in soil matrices modulate rhizosphere microbiome composition more strongly than do differences in climatic conditions

We tested the extent to which chamber, soil matrices, inoculum and plant genotypes could explain rhizosphere microbiome differentiation at the end of the climate schedule ('Main experiment', Fig. 3a).

As expected, rhizosphere microbiome composition in sterilized-and-repopulated soils was primarily explained by the composition of the microbial inoculum (Italian vs Swedish, Fig. 3b,c; Table 2). Effects of the soil matrix and the interaction between soil matrix and inoculum were also highly significant and together explained 21% and 29% of the total variance in bacterial and fungal community composition, respectively (Fig. 3b; Table 2). This contrasts with a quantitatively weaker, yet significant effect of the climatic chamber on rhizosphere microbial assemblages, which explained < 3% of the total variance in microbial community composition based on both PERMANOVA (Fig. 3b; Table 2) and constrained PCoA (Fig. S8). Consistent with this, soil matrix affected the abundance of more bacterial and fungal families than did chamber conditions (Fig. 3d). No statistically significant effect of plant genotype on the composition of bacterial or fungal rhizosphere microbiomes was observed (Fig. 3b; Table 2). Effects of microbial inoculum, soil matrix, climate chamber and plant genotype on microbial community composition were robust, irrespective of the methods used to normalize the ASV count table (rarefied, nonrarefied, normalized, Fig. S9). Notably, the effect of the climatic chamber on microbial assemblages depended on the microbial inoculum (significant chamber × inoculum interaction, Fig. 3b; Table 2). The bacterial and fungal families whose abundances were affected by chamber conditions differed between soils inoculated with Italian and Swedish inoculum, respectively (Bacteria: Italian inoculum: 13 families, Swedish inoculum: 14, overlap: 1; Fungi: Italian inoculum: 14, Swedish inoculum: 2, overlap: 1, Table S4). The abundant bacterial and fungal families (relative abundance > 1%) that responded the most to climatic differences between chambers include bacterial families belonging to Nocardiaceae, Comamonadaceae and Solibacteracae and fungal families belonging to Nectriaceae, Teratosphaeriaceae and Hyaloscyphaceae (Table S4).

We conclude that trajectories in rhizosphere microbiome assemblages were more strongly affected by below-ground physicochemical differences in recipient soil matrices than by above-ground differences in temperature, day length and light intensity.

Compositional shifts in bacterial, but not fungal, rhizosphere microbiomes are consistent between chamber and field reciprocal transplants

We examined whether our manipulation of climatic and soil conditions in the chamber experiment had similar effects on rhizosphere microbiome assembly of untreated soil as had the reciprocal transplant of plant genotypes and soil in a previously published field experiment (Thiergart *et al.*, 2020; see 'Field comparison' in the Materials and Methods section; Fig. S7a,b).

Origin of the soil (i.e. untreated Italian vs Swedish soil) explained most of the variation in rhizosphere bacterial community composition in both the chamber and the field experiment, accounting for 37% and 35% of the total variance in rhizosphere microbiome composition, respectively (Fig. S7e,f; Tables S5, S6). The effect of 'Location' in the field and 'Chamber' in the current study had the second most important effect on bacterial assemblages, although differences between climatic chambers explained less variation than differences between locations in the field (4% vs 13%, Fig. S7e,f; Tables S5, S6). For both chamber and field experiments, a statistically significant soil × chamber/location interaction was also observed whereas no statistically significant effect of plant genotype was noted. In contrast to the high consistency observed for the bacterial microbiome between the two experiments, notable differences were observed for the fungal rhizosphere microbiome. Although location explained more variation in fungal assemblages than origin of the soil in the field (location: 19%, soil origin: 9%, Fig. S7f; Table S6), this was not the case in the chamber experiment, in which the effect of chamber was much weaker than the effect of soil origin (chamber: 4%, soil origin: 36%, Fig. S7e; Table S5).

The data suggest that differences in temperature, day length and light intensity in our chamber experiment were sufficient to recapitulate at least part of the location effect observed in the field and that environmental variables other than those tested here are probably important for variation in fungal rhizosphere assemblages in the field.

Discussion

Disentangling which environmental factors contribute the most to variation in selection in plant populations and in the composition of microbial communities across large spatial scales is critical for predictions of how global change will impact plant fitness, microbiome assembly, as well as the interaction between plants and their associated microbial communities (Ramirez *et al.*, 2019; Thiergart *et al.*, 2020; Petipas *et al.*, 2021; Hacquard *et al.*, 2022). The present results suggest that differences in seasonal changes in climatic conditions (temperature, day length and PAR) between the two *A. thaliana* source populations are sufficient to explain the fitness advantage of the local over the nonlocal genotypes observed in reciprocal transplants between the two populations (Ågren & Schemske, 2012; Postma & Ågren, 2016; Thiergart *et al.*, 2020; Ellis *et al.*, 2021), whereas differences in below-ground soil physicochemical composition and microbiome only weakly affect the relative fitness of the two genotypes. By

contrast, below-ground differences in soil physicochemical properties were more important than differences in above-ground climate for soil microbiome variation between the two populations. Therefore, although both above-ground plant populations and below-ground microbial communities are predicted to be affected by shifts in the climatic conditions tested here, the effect of climate change is likely to be particularly strong on selection acting in the plant populations.

Strong effect of climate, but not of soil matrix, on the relative fitness of the two genotypes

The growth chamber programme used to mimic the environments of the source sites in Italy and Sweden successfully reproduced the main patterns of selection observed in the field, but there was a notable difference regarding the effect of soil origin on the relative fitness of the two plant genotypes. Reciprocal transplants between the two source sites have demonstrated strong divergent selection (Ågren & Schemske, 2012; Ågren *et al.*, 2013; Thiergart *et al.*, 2020; Ellis *et al.*, 2021), and both the chamber experiment and the field experiment identify climate as a major contributor and soil conditions as a minor contributor to the difference between sites in the direction of selection. In the Italian chamber, the Italian genotype outperformed the Swedish genotype in all soil treatments, whereas in the Swedish chamber the Swedish genotype outperformed the Italian genotype in soil treatments where at least some plants survived. However, the chamber and field experiment differed in the effect of soil origin. In the field experiment, there were some weak signals of adaptation to the local soil: the Swedish genotype had higher fitness when grown in Swedish rather than Italian soil at the Swedish site, and at the Italian site the direction of the small, statistically nonsignificant differences in fitness when grown on Italian vs Swedish soil were for both genotypes consistent with adaptation to native soils (Thiergart *et al.*, 2020). By contrast, in the chamber experiment, both genotypes did better on Italian than on Swedish soil. The Italian soil should have greater water-holding capacity as it consists of finer particles ('loamy sand') than does the Swedish soil ('sand'; Thiergart *et al.*, 2020). This is likely to benefit both genotypes in the chamber environment since the chambers used in this study rely on moving air to regulate the temperature, which means that the plants are subject to a continuous wind, exacerbating desiccation stress to the plants. This effect is likely to have been particularly strong since it was not possible to simulate the effect of snow cover in the chambers. At the Swedish site, snow cover insulates plants from the lowest air temperatures, but also from desiccation in winter. These considerations suggest that interactions among the effects of climate and other environmental variables should be interpreted with caution.

No evidence for microbe-mediated local adaptation between the two plant genotypes

Although the Italian and Swedish genotypes studied are derived from two sites that differ considerably both in climatic conditions, soil and microbiome composition (Ågren & Schemske, 2012; Thiergart *et al.*, 2020), they showed no evidence of local adaptation or maladaptation to the soil microbiome. Microbe-mediated local adaptation occurs when plant genotypes display higher fitness at their home site due to genotype-specific interactions with associated microbes, and may have been overlooked in many systems (Petipas *et al.*, 2021). Common-garden experiments have demonstrated variation in the composition of the root microbiome among genotypes in *A. thaliana* (Lundberg *et al.*, 2012), including slight differences in root microbial assemblages between the Italian and Swedish genotypes used in the present study (Urbina *et al.*, 2018; Thiergart *et al.*, 2020). Nevertheless, although our results indicated strong effects of climatic conditions on the relative performance of the two genotypes, soil factors had relatively minor effects, which is consistent with the weak evidence of adaptation to local soil in the field reciprocal transplant (cf. Thiergart *et al.*, 2020). The present experiment further indicated that differences in soil matrix influence the fitness of the two plant genotypes more than do differences in the soil microbiome. The comparison of plants grown on untreated and on sterilized soil indicated that the net effect of the soil microbiome on plant fitness was negative, but there was no evidence that the local plant genotype was more tolerant to the local soil microbiome compared to the foreign genotype. The results suggest that between-site differences in the combination of temperature, day length and light intensity simulated in our growth chambers can explain much of divergent selection observed in reciprocal transplant experiments conducted between the two source populations. This is consistent with a central role for climate in driving local adaptation at the two sites.

Edaphic conditions primarily drive rhizosphere microbiome differentiation between the two sites

Our results suggest that the difference in microbial community composition observed between the two sites in Italy and Sweden (Thiergart *et al.*, 2020) is driven primarily by differences in edaphic conditions rather than by differences in temperature, day length and PAR. This result is consistent with the fact that there is an almost two-unit difference in soil pH between the Italian and Swedish recipient soils (Sweden: pH = 6.2, slightly acidic; Italy: pH = 7.9, moderately alkaline) and that soil pH has been repeatedly described as the primary factor driving bacterial community differentiation in soil (Fierer & Jackson, 2006; Kaiser *et al.*, 2016). We observed that the fungal rhizosphere microbiome was also affected by differences in edaphic factors more strongly than by chamber conditions and responded similarly to environmental change as the bacteria. Although the composition of fungal communities has been predicted to be strongly modulated by climate (Talbot *et al.*, 2014; Tedersoo *et al.*, 2014; Coleman-Derr *et al.*, 2016; Vetrovsky *et al.*, 2019), our results suggest that the differences in seasonal changes in temperature, day length and PAR in our chamber experiment were not sufficient to produce the extensive shifts in fungal community composition observed in the field reciprocal transplant (Thiergart *et al.*, 2020). This suggests that either other climatic variables acting in the field (humidity, precipitation, snow cover) are more important

for fungal community differentiation between the two sites than the three climatic variables that differed between the two chambers, or that other environmental factors, such as biotic interactions, contributed to the large 'location effect' observed for the composition of fungal communities in the field transplant (Thiergart *et al.*, 2020). Given that precipitation seasonality and temperature have been predicted as the most predominant drivers of fungal species distribution (Vetrovsky *et al.*, 2019), it is likely that differences in precipitation and soil moisture between Italian and Swedish sites are key for fungal microbiome differentiation between the two sites.

Climatic differences between the two sites influence the composition of below-ground microbial assemblages

The differences in seasonal changes in temperature, day length and PAR between chambers induced subtle, yet significant shifts in below-ground bacterial and fungal assemblages. These experiments in controlled laboratory conditions thus suggest a causal link between these above-ground climatic variables and below-ground microbial community composition. It is likely that temperature is a primary force driving this variation (cf. Campisano *et al.*, 2017), but we cannot exclude the possibility that the differences in above-ground light and day length also affected below-ground microbiome assemblages, as recently reported (Hubbard *et al.*, 2018; Hou *et al.*, 2021a,b; Zhao *et al.*, 2021). The family Commamonadaceae, which belongs to the core bacterial root microbiome (Lundberg *et al.*, 2012; Hacquard *et al.*, 2015), was one of the most strongly affected by climatic conditions. Given that members of the same families have been identified as critical for promoting plant performance in gnotobiotic systems (i.e. Duran *et al.*, 2018; Finkel *et al.*, 2020), it is conceivable that climate-induced change in the relative abundance of these taxa might influence plant fitness. However, our experimental design does not allow direct climate effects on plant fitness to be uncoupled from indirect effects through climate-induced shifts in microbial assemblages. Our results indicate that below-ground differences in soil physicochemical properties, combined with differences in above-ground temperature, day length and light intensity between northern and southern Europe, were probably important for rhizosphere microbiota variation observed between the two natural sites.

Despite some notable differences, variation in rhizosphere microbiome assemblages and relative fitness of the host plant observed in this experiment conducted in climatic growth chambers largely recapitulated variation documented in field reciprocal transplants. This indicates that reductionist approaches can provide insight into the complex interplay between climatic factors, edaphic factors and microbiome composition affecting plant fitness in nature, and can help disentangle effects of environmental factors that are strongly correlated in the field.

Acknowledgements

This work was supported by funds from a European Research Council starting grant (MICRORULES) to SH and grants from the Swedish Research Council to JÅ (2016-05435, 2020-04434). SH was also supported by funds from the Max Planck Society, the 'Priority Programme 2125 DECRyPT' funded by the Deutsche Forschungsgemeinschaft and the 'Cluster of Excellence on Plant Sciences' programme funded by the Deutsche Forschungsgemeinschaft. We thank Anna Lisa Roth and Brigitte Pickel who helped in processing microbiome samples for microbial community profiling. We thank Guillaume Chesneau for constructive comments on the manuscript. Open Access funding enabled and organized by Projekt DEAL.

Author contributions

SH and JÅ conceived the project. TJE set up the chamber experiment and PD prepared the soils. PD collected soil and rhizosphere samples and performed microbial community profiling. PD analysed microbial profiling data, with inputs from TT. TJE collected fitness data and analysed them, with inputs from JÅ. SH and JÅ supervised the project. SH and JÅ wrote the manuscript, with inputs from PD, TJE and TT. PD and TJE are co-first authors.

ORCID

Jon Ågren https://orcid.org/0000-0001-9573-2463
Stéphane Hacquard https://orcid.org/0000-0003-2293-3525

Data availability

Sequencing reads of microbiome samples (MiSeq 16S rRNA and ITS reads) have been deposited in the European Nucleotide Archive (ENA) under accession nos. ENA: PRJEB50402 (bacteria) and ENA: PRJEB50404 (fungi). Data and R scripts for computational analyses are available at https://github.com/duranpa/reciprocaltransplantsweita.git.

References

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biology* **14**: e1002352.

Ågren J, Oakley CG, McKay JK, Lovell JT, Schemske DW. 2013. Genetic mapping of adaptation reveals fitness tradeoffs in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **110**: 21077–21082.

Ågren J, Schemske DW. 2012. Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. *New Phytologist* **194**: 1112–1122.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F *et al.* 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* **37**: 852–857.

Brundrett MC, Tedersoo L. 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* **220**: 1108–1115.

Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E *et al.* 2012. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* **488**: 91–95.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Campisano A, Albanese D, Yousaf S, Pancher M, Donati C, Pertot I. 2017. Temperature drives the assembly of endophytic communities' seasonal succession. *Environmental Microbiology* **19**: 3353–3364.

Carini P, Marsden PJ, Leff J, Morgan EE, Strickland MS, Fierer N. 2017. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nature Microbiology* **2**: 16242.

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG. 2016. Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave* species. *New Phytologist* **209**: 798–811.

Dittmar EL, Oakley CG, Ågren J, Schemske DW. 2014. Flowering time QTL in natural populations of *Arabidopsis thaliana* and implications for their adaptive value. *Molecular Ecology* **23**: 4291–4303.

Duran P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S. 2018. Microbial interkingdom interactions in roots promote *Arabidopsis* survival. *Cell* **175**: 973–983.

Ellis TJ, Postma FM, Oakley CG, Ågren J. 2021. Life-history trade-offs and the genetic basis of fitness in *Arabidopsis thaliana*. *Molecular Ecology* **30**: 2846–2858.

Exposito-Alonso M, Burbano HA, Bossdorf O, Nielsen R, Weigel D, Team GFE. 2019. Natural selection on the *Arabidopsis thaliana* genome in present and future climates (vol 573, pg 126, 2019). *Nature* **574**: e16.

Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences, USA* **103**: 626–631.

Finkel OM, Salas-González I, Castrillo G, Conway JM, Law TF, Teixeira PJPL, Wilson ED, Fitzpatrick CR, Jones CD, Dangl JL. 2020. A single bacterial genus maintains root growth in a complex microbiome. *Nature* **587**: 103–108.

Fournier-Level A, Korte A, Cooper MD, Nordborg M, Schmitt J, Wilczek AM. 2011. A map of local adaptation in *Arabidopsis thaliana*. *Science* **334**: 86–89.

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. 2011. Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics* **42**: 23–46.

Getzke F, Hacquard S. 2022. High-throughput profiling of root-associated microbial communities. *Methods in Molecular Biology* **2494**: 325–337.

Hacquard S, Garrido-Oter R, Gonzalez A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R *et al.* 2015. Microbiota and host nutrition across plant and animal kingdoms. *Cell Host & Microbe* **17**: 603–616.

Hacquard S, Wang E, Slater H, Martin F. 2022. Impact of global change on the plant microbiome. *New Phytologist* **234**: 1907–1909.

Hereford J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *American Naturalist* **173**: 579–588.

Hou S, Thiergart T, Vannier N, Mesny F, Ziegler J, Pickel B, Hacquard S. 2021a. A microbiota-root-shoot circuit favours *Arabidopsis* growth over defence under suboptimal light. *Nature Plants* **7**: 1078–1092.

Hou S, Wolinska KW, Hacquard S. 2021b. Microbiota-root-shoot-environment axis and stress tolerance in plants. *Current Opinion in Plant Biology* **62**: 102028.

Howard MM, Bell TH, Kao-Kniffin J. 2017. Soil microbiome transfer method affects microbiome composition, including dominant microorganisms, in a novel environment. *FEMS Microbiology Letters* **364**: fnx092.

Hubbard CJ, Brock MT, van Diepen LT, Maignien L, Ewers BE, Weinig C. 2018. The plant circadian clock influences rhizosphere community structure and function. *ISME Journal* **12**: 400–410.

Kaiser K, Wemheuer B, Korolkow V, Wemheuer F, Nacke H, Schoning I, Schrumpf M, Daniel R. 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. *Scientific Reports* **6**: 33696.

Karimi B, Terrat S, Dequiedt S, Saby NPA, Horriguel W, Lelievre M, Nowak V, Jolivet C, Arrouays D, Wincker P *et al.* 2018. Biogeography of soil bacteria and archaea across France. *Science Advances* **4**: eaat1808.

Lau JA, Lennon JT. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences, USA* **109**: 14058–14062.

Leimu R, Fischer M. 2008. A meta-analysis of local adaptation in plants. *PLoS ONE* **3**: e4010.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550.

Lu T, Ke MJ, Lavoie M, Jin YJ, Fan XJ, Zhang ZY, Fu ZW, Sun LW, Gillings M, Penuelas J *et al.* 2018. Rhizosphere microorganisms can influence the timing of plant flowering. *Microbiome* **6**: 231.

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, del Rio TG *et al.* 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**: 86–90.

Magoč T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**: 2957–2963.

Montesinos-Navarro A, Wig J, Pico FX, Tonsor SJ. 2011. *Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude. *New Phytologist* **189**: 282–294.

Oakley CG, Ågren J, Atchison RA, Schemske DW. 2014. QTL mapping of freezing tolerance: links to fitness and adaptive trade-offs. *Molecular Ecology* **23**: 4304–4315.

Petipas RH, Geber MA, Lau JA. 2021. Microbe-mediated adaptation in plants. *Ecology Letters* **24**: 1302–1317.

Petipas RH, Wruck AC, Geber MA. 2020. Microbe-mediated local adaptation to limestone barrens is context dependent. *Ecology* **101**: e03092.

Postma FM, Ågren J. 2016. Early life stages contribute strongly to local adaptation in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **113**: 7590–7595.

Postma FM, Ågren J. 2018. Among-year variation in selection during early life stages and the genetic basis of fitness in *Arabidopsis thaliana*. *Molecular Ecology* **27**: 2498–2511.

Quinn GP, Keough MJ. 2002. *Experimental design and data analysis for biologists.* Cambridge, UK: Cambridge University Press.

R Core Team. 2021. *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL https://www.R-project.org [accessed 1 October 2020].

Ramirez KS, Snoek LB, Koorem K, Geisen S, Bloem LJ, ten Hooven F, Kostenko O, Krigas N, Manrubia M, Cakovic D *et al.* 2019. Range-expansion effects on the belowground plant microbiome. *Nature Ecology & Evolution* **3**: 604–611.

Rousk J, Baath E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME Journal* **4**: 1340–1351.

Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Liao HL, Smith ME *et al.* 2014. Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences, USA* **111**: 6341–6346.

Tedersoo L, Bahram M, Polme S, Koljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A *et al.* 2014. Global diversity and geography of soil fungi. *Science* **346**: 1256688.

Tedersoo L, Bahram M, Zobel M. 2020. How mycorrhizal associations drive plant population and community biology. *Science* **367**: eaba1223.

Thiergart T, Duran P, Ellis T, Vannier N, Garrido-Oter R, Kemen E, Roux F, Alonso-Blanco C, Ågren J, Schulze-Lefert P *et al.* 2020. Root microbiota assembly and adaptive differentiation among European *Arabidopsis* populations. *Nature Ecology & Evolution* **4**: 122–131.

Tkacz A, Cheema J, Chandra G, Grant A, Poole PS. 2015. Stability and succession of the rhizosphere microbiota depends upon plant type and soil composition. *ISME Journal* **9**: 2349–2359.

Urbina H, Breed MF, Zhao WZ, Gurrala KL, Andersson SGE, Ågren J, Baldauf S, Rosling A. 2018. Specificity in *Arabidopsis thaliana* recruitment of root fungal communities from soil and rhizosphere. *Fungal Biology* **122**: 231–240.

U'Ren JM, Lutzoni F, Miadlikowska J, Zimmerman NB, Carbone I, May G, Arnold AE. 2019. Host availability drives distributions of fungal endophytes in the imperilled boreal realm. *Nature Ecology & Evolution* **3**: 1430–1437.

Van Nuland ME, Ware IM, Schadt CW, Yang Z, Bailey JK, Schweitzer JA. 2021. Natural soil microbiome variation affects spring foliar phenology with consequences for plant productivity and climate-driven range shifts. *New Phytologist* **232**: 762–775.

Vetrovsky T, Kohout P, Kopecky M, Machac A, Man M, Bahnmann BD, Brabcova V, Choi J, Meszarosova L, Human ZR *et al.* 2019. A meta-analysis

of global fungal distribution reveals climate-driven patterns. *Nature Communications* **10**: 5142.

Wagner MR, Lundberg DS, Coleman-Derr D, Tringe SG, Dangl JL, Mitchell-Olds T. 2014. Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild *Arabidopsis* relative. *Ecology Letters* **17**: 717–726.

Wickham H. 2016. *GGPLOT2: elegant graphics for data analysis*. New York, NY, USA: Springer-Verlag. [WWW document] URL http://ggplot2.org [accessed 1 October 2020].

Wubs ERJ, van der Putten WH, Bosch M, Bezemer TM. 2016. Soil inoculation steers restoration of terrestrial ecosystems. *Nature Plants* **2**: 16107.

Zhao KK, Ma B, Xu Y, Stirling E, Xu JM. 2021. Light exposure mediates circadian rhythms of rhizosphere microbial communities. *ISME Journal* **15**: 2655–2664.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Preparation of microbial input inocula and recipient soils for soil microbiome transplantation.

Fig. S2 Comparative analysis of community composition in untreated soils across experiments.

Fig. S3 Biochemical analysis of soils utilized in this study before and after treatments.

Fig. S4 Similarity in microbiome assemblages between input microbial inocula and corresponding untreated soils, and sterilized-and-repopulated soils.

Fig. S5 Validation of soil repopulation with microbial inocula at the end of the experiment.

Fig. S6 Plant fitness on untreated, sterilized, and sterilized-and-repopulated soils.

Fig. S7 Comparison of results in chamber and field reciprocal transplants with untreated soils.

Fig. S8 Effect of chamber and genotype on rhizosphere microbial community composition.

Fig. S9 Influence of data normalization methods on the effect of climate chamber, plant genotype, soil matrix and microbial inoculum on rhizosphere microbial community composition.

Table S1 Mixed model analysis of variance in plant fitness on untreated, sterilized, and sterilized-and-repopulated soils.

Table S2 Mixed model analysis of variance in plant fitness on untreated soils in the chamber mimicking Italian climate.

Table S3 Mixed model analysis of variance of plant fitness on untreated soils in the field experiment presented by Thiergart *et al.* (2020).

Table S4 Log_2 fold-changes and FDR-corrected *P*-values of rhizosphere microbial families from Swedish or Italian origin (i.e. from soils sterilized-and-repopulated with either Swedish or Italian inocula) affected by a shift in chamber, soil matrix or both compared to their respective reference conditions.

Table S5 Analysis of variance in rhizosphere microbial community composition in untreated soils.

Table S6 Analysis of variance in rhizosphere microbial community composition in untreated soils in the field experiment presented by Thiergart *et al.* (2020).

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.