The impact of elevated temperature and drought on the ecology and evolution of plant–soil microbe interactions

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Abstract

1. Climate change is shifting the distribution of species, and may have a profound impact on the ecology and evolution of species interactions. However, we know little about the impact of increasing temperature and changing rainfall patterns on the interactions between plants and their beneficial and antagonistic root symbionts.

2. Here, we used a reciprocal multifactorial growth chamber experiment with seeds and soil microbial communities from three origins to investigate the impact of temperature and soil moisture on the growth, arbuscular mycorrhizal (AM) fungal colonization and root-associated fungal community of a perennial herb. Moreover, we tested whether plants and AM fungi performed better or worse when plants were grown with their local soil biota, for example, due to plant adaptation or changes in the genetic or species composition of the soil microbial community.

3. Temperature and soil moisture generally increased plant growth, whereas temperature but not soil moisture increased AM fungal colonization. The strength and direction of the plants’ response to temperature were dependent on soil moisture and differed among plant populations, and AM fungal colonization was further affected by the origin of the soil microbial community. The root-associated fungal community structure was impacted by temperature, soil moisture and the soil microbial origin, with interactive effects between the microbial origin and the abiotic environment. Plant biomass was lower when plants were grown with their local soil microbes, potentially due to intraspecific negative plant–soil feedbacks.

4. Synthesis. Our findings indicate that, beyond a relatively uniform increase of plant growth and arbuscular mycorrhizal (AM) fungal colonization with increasing temperature, plants and root-associated fungi of different origins will vary in their response to climate change (i.e. elevated temperature and shifts in rainfall). This may create pronounced, but difficult to predict, spatial and temporal variation in the ecology and evolution of plant–microbe interactions with a changing climate.

KEYWORDS
abiotic and biotic factors, arbuscular mycorrhizal fungi, local adaptation, Plantago lanceolata, plant–soil (below-ground) interactions, plant–soil feedback, root-associated fungi, soil moisture
1 | INTRODUCTION

Soil communities can have a large impact on plant community assembly, biodiversity and ecosystem functioning (Bardgett & van der Putten, 2014; van der Heijden, Bardgett, & van Straalen, 2008). Over the next century, we may expect to see elevated temperatures and changes in rainfall regimes (IPCC, 2014) changing the distribution of soil microbes and plants and modifying the outcome of plant–soil microbe interactions. Increasing our fundamental knowledge of the impact of climate on the ecology and evolution of plant–soil microbe interactions may allow us to predict the consequences of climate change for plant–microbe interactions and the surrounding ecosystem, and provide avenues to mitigate the consequences of climate change in natural and applied systems.

The distribution of plants and root-associated fungi, such as arbuscular mycorrhizal (AM) fungi and pathogens, is to a large part driven by spatial variation in abiotic factors, such as climate, soil physical and chemical properties and the biotic environment (e.g., Chaudhary, Lau, & Johnson, 2008; Rasmussen et al., 2018; Valýi, Mardhiah, Rillig, & Hempel, 2016). Spatial heterogeneity in the abiotic and biotic environment can also result in within-species spatial genetic structure and local adaptation, where genotypes are adapted to their local environment (Blanquart, Kaltz, Nuïsmer, & Gandon, 2013; Hoeksema & Forde, 2008). In this regard, plants are known to adapt both to the abiotic (Brady, Kruckeberg, & Bradshaw, 2005; Macel et al., 2007) and biotic soil environment (Crémieux et al., 2008; Johnson, Wilson, Bowker, Wilson, & Miller, 2010), and antagonistic and beneficial microbes are known to adapt to the local plant genotypes (Johnson et al., 2010; Tack, Thrall, Barrett, Burdon, & Laine, 2012). Plants may also perform better or worse in their local soil environment due to changes in the genetic and species composition of the local microbial community in response to the genetic or species composition of the local plant community (Kulmatiski, Beard, Stevens, & Cobbold, 2008; van der Putten et al., 2013). Such plant–soil feedbacks may be either positive, for example, leading to increased mutualist densities, or negative, for example, leading to increased pathogen densities (van der Putten et al., 2013).

The ecological outcome of species interactions, and patterns of local adaptation, may be impacted by climate change, both in the current distributional range and in the expanding range (Berg et al., 2010). Within the existing range, the ecological outcome of plant–soil microbe interactions depends not only on plant genotypes, microbial genes and microbial species composition but also on the environmental context (Hoeksema & Forde, 2008; Laine, 2009; Thompson, 2005). For example, soil moisture and temperature have been shown to alter fungal communities (Deveautour, Donn, Power, Bennett, & Powell, 2018; Rasmussen et al., 2018) and may thereby change feedbacks between plants and fungi, for example, if communities change from one more dominated by beneficial microbes to one more dominated by antagonistic microbes or vice versa. As such, environmental change can disrupt both plant local adaptation and plant maladaptation to the local soil microbial community within the present range. Climate change may also shift the ranges of species, which will result in new associations between plants and their soil microbes within the new range. If plants and soil microbes shift their range in space and time in a similar fashion, patterns of local (mal)adaptation may be re-established within a short time span. In contrast, if plants will associate with new microbial species or genotypes in the new range, the interaction may be fundamentally changed. As an example, if plants perform worse when growing with their local soil community in the existing range, plant performance may be higher than expected in the expanding range due to the absence of certain antagonistic microbes, the lack of time for adaptation of antagonistic microbes to the local plant genotypes or the adaptation to and by beneficial microbes.

We used a reciprocal multifactorial climate chamber experiment to investigate the impact of climate change (elevated temperature and drought) on the ecology and evolution of plant–soil microbe interactions. For this, we used Plantago lanceolata plants and soil microbial communities originating from three different locations (a coastal, forest and meadow site), planted in reciprocal combinations with two temperature and two soil moisture treatments. We then assessed plant growth, AM fungal colonization and the composition of the root-associated microbial community. We aimed to answer the following specific questions:

1. What is the impact of temperature, soil moisture, plant origin and soil microbial origin on plant growth, AM fungal colonization and the composition of the root-associated microbial community?
2. Do plants and AM fungi perform better or worse when plants are grown with their local soil biota? If there is evidence for local (mal) adaptation, is this pattern influenced by the abiotic environment?

We expected that increased temperature and high moisture levels would generally lead to an increase in plant growth, increase and decrease in AM fungal colonization, respectively, and alter the root-associated community composition (Augé, 2001; Compant, van der Heijden, & Sessitsch, 2010; Rustad et al., 2001), but that the response to the abiotic environment will differ among plant populations and soil biotic communities (e.g., Al-Karaki, McMichael, & Zak, 2004; An et al., 2010). For plant and fungal local adaptation, empirical studies have shown that P. lanceolata can both be locally adapted to its local soil community (Mursinoff & Tack, 2017) or perform worse in its local soil due to negative plant–soil feedbacks (Bever, 2002; Harrison & Bardgett, 2010). Therefore, we may expect to see either a positive or a negative response, depending on which soil microbial functional groups are dominating the response. Finally, Laine (2008) showed that temperature affected patterns of local adaptation of a foliar pathogen to P. lanceolata, and we therefore expect that the abiotic environment may likewise mediate patterns of local adaptation to the soil biota.

2 | MATERIALS AND METHODS

2.1 | Study system

Plantago lanceolata is a widespread perennial herb that occurs in a wide range of habitats, for example, dry grasslands, hayfields,
roadsides and disturbed areas (Cavers, Bassett, & Crompton, 1980). The plant produces rosettes and reproduces by seed production and clonal propagation through side rosettes (Cavers et al., 1980; Mook, Haeck, van der Toorn, & van Tienderen, 1992; Ross, 1973). *P. lanceolata* associates with many soil organisms (e.g. De Deyn, Raaijmakers, van Ruijven, Berendse, & van der Putten, 2004), including AM (e.g. Rasmussen et al., 2018) and other root-associated fungi, and has often been used in local adaptation studies (e.g. Crémiéux et al., 2008; Mursinoff & Tack, 2017) and studies on plant–soil feedbacks (e.g. Bever, 2002; Brandt, de Kroon, Reynolds, & Burns, 2013; Harrison & Bardgett, 2010). There is evidence for genetic variation in both plant (e.g. Azcon & Ocampo, 1981; Graham & Eissenstat, 1994; Hetrick, Wilson, & Cox, 1992) and mycorrhizal fungi (Burgess, Dell, & Malajczuk, 1994; Koch et al., 2004) for the outcome of the plant–mycorrhizal interaction, as measured by, for example, the number of fungal structures within plant roots or the percentage of AM fungal colonized plant roots. In this experiment, we used plant growth and AM fungal colonization as measures to assess plant and AM fungal performance.

### 2.2 Experimental design

To investigate how abiotic (temperature and soil moisture) and biotic (soil biota and plant population of origin) factors affect plant growth traits, AM fungal colonization, root-associated fungal community structure and adaptation, we used a reciprocal multifactorial growth chamber experiment. Seeds originating from three locations were reciprocally planted in a combination with a whole soil microbial inoculum or a sterile control. These 12 combinations of plant origin and soil microbial origin were then subjected to four abiotic environments: (a) low temperature and low soil moisture; (b) low temperature and high soil moisture; (c) high temperature and low soil moisture; and (d) high temperature and high soil moisture. These factors may represent some of the key abiotic factors affected by climate change. Each combination of factors was replicated four times.

#### 2.2.1 Plant origin

In order to investigate how plant genetic variation influences plant growth, AM fungal colonization, root-associated fungal community structure and local adaptation, we collected seeds (27–103 seeds per plant) from six plant individuals at each of three plant populations in central Sweden. These three Swedish plant populations are part of the global plant demographic network *PlantPopNet* (www.plantpopnet.com): Östra Ryd is a rocky seashore surrounded by forest (hereafter referred to as *coast site*), Tjuvstigen is a meadow-like roadside surrounded by forest (hereafter referred to as *forest site*), and Tullgarn näs is a semi-open meadow close to the seashore grazed by cattle (hereafter referred to as *meadow site*; see Table S1 for detailed abiotic and biotic measurements from these three populations). Seeds from the same mother plant were randomly allocated among treatment combinations, with two seeds from the same mother plant grown in each pot (with the exception of 10 pots, where only a single seed was planted). If both seeds germinated, one of the seedlings was removed.

To inform the abiotic treatments and allow comparison between the field and growth chamber, we also assessed the abiotic and biotic conditions at the *P. lanceolata* populations from which the seeds originated. More specifically, we recorded above-ground and below-ground temperature using iButton data loggers (DS1922L; Maxim Integrated, San Jose, CA, USA), measured soil moisture using a soil moisture meter (HH2, SM300; Delta-T, Cambridge, UK) and assessed plant growth traits and AM fungal colonization (see Table S1 for further details). Three soil samples were taken at each field site location and frozen at −20°C for later molecular identification of the fungal community.

#### 2.2.2 Soil microbial origin

To investigate the effect of the soil microbial communities on plant growth, AM fungal colonization, root-associated fungal communities and adaptation, we collected soil from the same locations as we collected seeds. Soil was collected at each location to a depth of 15 cm, pooled and passed through a 1 cm sieve and then homogenized thoroughly. Care was taken to sterilize surfaces and avoid cross contamination between soils throughout the experiment.

Our main aim was to investigate the impact of the biotic soil community. To isolate the effect of the three soil microbial communities from differences in the soil physical and chemical properties in which they were embedded, and at the same time reduce bias in response to a potential spike in nutrients due to soil sterilization (Troelstra, Wagenaar, Smant, & Peters, 2001), we took the following approach (as summarized visually in Figure S1). First, we filled 560 ml pots with c. 300 ml 3:1 mixture of sterile potting soil (Plugg- och sädjord, SW Horto, Hammenhög, Sweden) and sand (Specialsand, Rådasand, Lidköping, Sweden). Soil sterilization was conducted by double autoclaving the soil mixture at 121°C for 1 hr. We then inoculated the pots (except the control treatment) with a mix of 56 ml of soil from each soil origin (one live and two sterile). The sterile control treatment received 3 × 56 ml of sterile soil from each location. Lastly, the pots were topped with c. 50 ml of the sterile potting soil mixture. Taken together, this approach allowed us to isolate the effects of the soil microbial community from concurrent differences in the abiotic soil environment, such as soil pH, chemistry and physical structure. Using a commercial soil inoculated with microbes has frequently been used in a range of local adaptation (e.g. Hoeksema & Thompson, 2007; Lankau, Wheeler, Bennett, & Strauss, 2011) and plant–soil feedback studies (e.g. Callaway, Thelen, Rodriguez, & Holben, 2004; Packer & Clay, 2000), even though the method has potential caveats regarding the establishment and functioning of the experimental soil communities. To validate whether the soil communities at the field sites established themselves within the experimental setting, we compared the fungi in the soil at the original sites with the fungi that had colonized the roots in the end of the experiment.
2.2.3 | Temperature treatment

Plants were grown in climate chambers at either low or high temperature (15°C and 25°C) and a day length of 16 hr. The difference in temperature between the low and high treatments was based on approximate differences in soil temperature recorded in the field at the three focal plant populations (Table S1), and such temperature generally follows the long-term expectation in temperature increase of the worst-case emissions climate change scenario (IPCC, 2014). We randomized the placement of plants within each climate chamber every 2 weeks.

2.2.4 | Soil moisture treatment

For the first 2 weeks after seed sowing, pots in all treatments were given 100 ml water three times a week. For the following 3 weeks, the high soil moisture treatment continued to receive 100 ml, whereas the low soil moisture treatment received 50 ml water, three times a week. Hereafter soil moisture was maintained at a maximum of 40% and 10% water volume, as measured on a Type HH2 moisture meter with a SM300 sensor (Delta-T Devices Ltd, Cambridge, UK), for the high and low soil moisture treatment respectively. These values matched the variation in soil moisture between P. lanceolata populations at the field sites (Table S1) and follow the expectation of an increasing frequency of summer drought in Europe (IPCC, 2014). A pilot study showed that the low water treatment allowed plants to survive, but at the same time gave visual signs of drought stress.

2.3 | Measurements

We recorded seedling emergence for each pot. Every fortnight, starting 19 days after sowing, we recorded the number of leaves and the length and width of the longest leaf. From these measures, we calculated leaf size (leaf length × leaf width), total leaf area (leaf length × leaf width × number of leaves) and leaf allometry (leaf width/leaf length) (Rasmussen et al., 2017). We additionally measured plant rosette shape (flat or high) 74 days after sowing. Plants were harvested after 80 days, and leaves and roots were separated. Leaf biomass was assessed by weighing leaves before and after oven-drying at 60°C, while roots were washed, cut to 2 cm pieces, mixed thoroughly, frozen and then later freeze-dried and weighed. We also calculated the root to shoot ratio. The results for the full set of plant measures can be found in the Supplementary Information, while only key plant growth measures are presented in the main text.

To determine AM fungal root colonization, dried roots were transferred to tissue cassettes, cleared for 5 min in 3% KOH, acidified for 30 min in 2% HCl and stained for 20 min in 0.05% trypan blue solution (Koske & Gemma, 1989; Phillips & Hayman, 1970). Roots were then scored for AM fungal colonization using the gridline intersect method at 100 intersections per root (McDonigle, Miller, Evans, Fairchild, & Swan, 1990).

2.4 | Molecular methods and bioinformatics

To determine the fungal community composition within treatment roots (excluding the sterile soil treatment) and from soil taken at the field sites, DNA was extracted from c. 25 mg freeze-dried root material or from c. 250 mg frozen soil using NucleoSpin Plant and Soil kits (Macherey-Nagel, Düren, Germany). Extracted DNA was sent to McGill University and Génome Québec Innovation Centre, Montréal, Canada, where fungal DNA was sequenced on a MiSeq platform (Illumina Inc. San Diego, CA, USA) using primers fITS7 (Ihrmark et al., 2012) and ITS4 (White, Bruns, Lee, & Taylor, 1990), which target a 250–450 bp fragment encompassing the entire ITS2 region with flanking sequences in the 5.8 and LSU genes. These primers cover most of the root-associated fungal community and have been chosen as the universal DNA barcode for fungi (Schoch et al., 2012). It is worth noting though, that these primers do not pick up all fungal groups equally (e.g. Schadt & Rosling, 2015; Schoch et al., 2012). However, Lekberg et al. (2018) showed that while primers targeting the ITS or SSU region generated slightly different communities, the communities responded similar to the environmental parameters tested. Furthermore, insufficient genetic variability within the ITS regions means that species-level assignments can be unreliable or non-valid. As such, species-level identifications should be interpreted with caution.

Primers were removed using CutAdapt (Martin, 2011). We used the DADA2 ITS pipeline to filter the reads using the standard DADA2 filtering parameters (Callahan et al., 2016). The DADA2 algorithm uses a parametric error model which is trained on the entire dataset. This model is then applied to correct and group sequences into amplicon sequence variants (ASVs) (Callahan, McMurdie, & Holmes, 2017; Callahan et al., 2016). Chimeras were removed, and lastly, taxonomy was assigned using the UNITE database (Abarenkov et al., 2010; Kõljalg et al., 2005), where the DADA2 pipeline uses a native implementation of the naïve Bayesian classifier method for taxonomic assignment (Wang, Garrity, Tiedje, & Cole, 2007).

After removal of plant reads (c. 40%), 2,209,193 reads were obtained from the 116 root samples and 436,500 reads from the nine soil samples collected at the original sites. Species accumulation curves showed that the sequencing effort in most samples was sufficient (Figure S2). From these reads, a total of 3,580 fungal ASVs were recorded. For further analyses, we used the set of 200 most common ASVs making up 90% of the total number of reads, excluding one sample with too few reads (<500 reads), henceforth referred to as root-associated fungi. The set of most common ASVs did not include AM fungi, which were only found in c. half of the samples (n = 57). An AM fungal ASV table, which included 118 AM fungal ASVs making up 0.2% of the total amount of reads, was analysed separately and details on analysis and results can be found in the Supplementary Information (Analysis S1). DNA sequences have been deposited at NCBI under accession numbers PRJNA564044 and PRJNA564041 for root samples from the experiment and for the original soil samples respectively.
2.5 Statistical analyses

To investigate the effect of the abiotic and biotic environment on plant growth, AM fungal colonization, observed root-associated fungal richness, root-associated fungal Shannon diversity (Shannon, 1948) and local adaptation, we used generalized linear mixed effects models with normal distributions using the lme4 and car packages in R v. 3.4.2 (Bates, Maechler, Bolker, & Walker, 2014; Fox & Weisberg, 2011; R Core Team, 2017), while seedling emergence and rosette shape were analysed with a binomial distribution and logit link function. Non-significant three- and four-way interactions were excluded from the models. The significance of random effects was tested using the MASS package (Venables & Ripley, 2002), and significant plant and soil microbial origin main effects were assessed by post hoc Tukey tests.

To test the effect of the abiotic and biotic environment on the root-associated fungal community composition, we used PERMANOVA as implemented in the function adonis in the R-package vegan (Oksanen et al., 2015). CCA was used to visualize how fungal community treatments differed among treatment levels and how community composition at the original field sites overlapped with the treatment communities. We used both relative abundances and presence–absence data for all the statistical analyses. All community data were Hellinger pre-transformed (Legendre & Gallagher, 2001).

2.5.1 The effect of environmental factors on seedling emergence and plant growth

To investigate the impact of the abiotic and biotic environment on seedling emergence and plant growth, we modelled seedling emergence, leaf number, leaf length, leaf width, leaf size, total leaf area, leaf allometry, rosette shape, leaf fresh and dry weight, root dry weight and the root to shoot ratio as a function of plant origin, soil microbial origin, temperature, soil moisture and their interactions. To account for variation among siblings from different mother plants, we added mother plant nested within plant origin as a random effect. To achieve homogeneous residuals, diversity was ln + 1 transformed. We also tested whether plant biomass mediated the impact of the environmental factors on AM fungal colonization by adding it as a covariate in the model (Analysis S2).

To investigate whether root-associated fungal communities were influenced by the abiotic and biotic environment, we modelled fungal community composition as a function of plant origin, soil microbial origin, temperature, soil moisture and their interactions, in addition to mother plant nested within plant origin.

2.5.3 The effect of abiotic factors on plant and AM fungal adaptation

To investigate whether plants and AM fungi performed better or worse when grown in local or non-local combinations, and how the abiotic environment may influence patterns of local adaptation, we assessed seedling emergence, plant growth traits and AM fungal colonization in local (sympatric) and non-local (allopatric) combinations of plants and soil biota. Specifically, we modelled seedling emergence, plant growth and AM fungal colonization as a function of plant origin, soil microbial origin, temperature, soil moisture and sympatry (Blanquart et al., 2013; Laine, 2005; Mursinoff & Tack, 2017). Sympatry is categorized as either local or non-local combinations of plant and soil microbial origin, which captures the variation between local and non-local conditions after accounting for the main effects of plant and microbial origin (Blanquart et al., 2013) and the abiotic treatment levels. To test whether the abiotic environment might influence patterns of local adaptation of plants and AM fungi, we also added the interactions sympatry × temperature, sympatry × soil moisture and sympatry × soil moisture. For those plant growth traits that were measured every fortnight, we ran repeated measures analyses adding the effects of date and its interaction with sympatry, temperature and soil moisture as listed above. The random effect plant ID was also added to the models. As these models showed that the pattern of sympatry changed over time, we ran models for each individual time point. The sterile soil treatment was not included in these models.

3 RESULTS

3.1 The impact of climate on the ecology of plants and root-associated fungi

3.1.1 The effect of environmental factors on seedling emergence and plant growth

In most pots, either one or two seedlings emerged, except for 34 pots where no seedlings emerged. Emergence of seedlings was not influenced by the main effects investigated (Table 1). Instead seedling emergence was influenced by the interaction between plant origin and temperature, where seedling emergence was higher for
### TABLE 1  The impact of plant origin, soil microbial origin, temperature, soil moisture, their two-way interactions and mother plant on seedling emergence and plant growth traits of *Plantago lanceolata*

<table>
<thead>
<tr>
<th></th>
<th>Plant origin (P)</th>
<th>Soil microbial origin (Mic)</th>
<th>Temperature (T)</th>
<th>Soil moisture (Moi)</th>
<th>P × Mic</th>
<th>P × T</th>
<th>P × Moi</th>
<th>Mic × T</th>
<th>Mic × Moi</th>
<th>T × Moi</th>
<th>Mother plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling emergence (n = 192)</td>
<td>0.483</td>
<td>0.286</td>
<td>0.387</td>
<td>0.610</td>
<td>0.966</td>
<td>0.003</td>
<td>0.064</td>
<td>0.277</td>
<td>0.042</td>
<td>0.213</td>
<td>–</td>
</tr>
<tr>
<td>Leaf number (n = 948)</td>
<td>&lt;0.001</td>
<td>0.172</td>
<td>&lt;0.001</td>
<td>0.144</td>
<td>0.850</td>
<td>&lt;0.001</td>
<td>0.333</td>
<td>0.262</td>
<td>0.114</td>
<td>0.005</td>
<td>0.06</td>
</tr>
<tr>
<td>Leaf length (n = 948)</td>
<td>&lt;0.001</td>
<td>0.029</td>
<td>&lt;0.001</td>
<td>0.346</td>
<td>0.257</td>
<td>&lt;0.001</td>
<td>0.425</td>
<td>0.219</td>
<td>0.699</td>
<td>0.378</td>
<td>0.002</td>
</tr>
<tr>
<td>Leaf width (n = 948)</td>
<td>0.002</td>
<td>0.219</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.317</td>
<td>0.207</td>
<td>0.513</td>
<td>0.691</td>
<td>0.329</td>
<td>0.397</td>
<td>0.8</td>
</tr>
<tr>
<td>Leaf allometry (n = 948)</td>
<td>&lt;0.001</td>
<td>0.130</td>
<td>0.027</td>
<td>0.244</td>
<td>0.240</td>
<td>0.002</td>
<td>0.055</td>
<td>0.043</td>
<td>0.228</td>
<td>0.527</td>
<td>0.03</td>
</tr>
<tr>
<td>Shoot dw (n = 158)</td>
<td>0.464</td>
<td>0.108</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.143</td>
<td>0.966</td>
<td>0.811</td>
<td>0.854</td>
<td>0.253</td>
<td>&lt;0.001</td>
<td>0.4</td>
</tr>
<tr>
<td>Root dw (n = 158)</td>
<td>0.422</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.588</td>
<td>0.077</td>
<td>0.886</td>
<td>&lt;0.001</td>
<td>0.226</td>
<td>&lt;0.001</td>
<td>0.8</td>
</tr>
<tr>
<td>Root to shoot ratio (n = 158)</td>
<td>0.321</td>
<td>&lt;0.001</td>
<td>0.049</td>
<td>0.003</td>
<td>0.876</td>
<td>0.005</td>
<td>0.852</td>
<td>&lt;0.001</td>
<td>0.814</td>
<td>0.046</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Note: Shown are p-values, with significant values in bold.

For p-values when excluding the sterile soil treatment, see Table S2.

For p-values on all plant growth measures and p-values of date, and interactions between date, plant origin, soil microbial origin and soil moisture, see Table S3.

Abbreviations: fw = fresh weight, dw = dry weight.
3.1.2 | The effect of environmental factors on root-associated fungi

We detected a large diversity of fungi in all treatment combinations (Figure 3). The majority of fungi were present at relatively low abundances, with the exception for two particularly abundant fungal species (Figure 3). The plant pathogen complex *Olpidium brassicae*, likely *Olpidium virulentus* (Lay, Hamel, & St-Arnaud, 2018), had the highest relative abundance in all treatment combinations, whereas the second most abundant species, the yeast *Apiotrichum xylopini* was only abundant in the low temperature and low soil moisture treatments and in plants and soil originating from the coastal site (Figure 3).

The main drivers of the root-associated fungal community were temperature and the origin of the soil microbial community. AM fungal colonization was higher in the high temperature treatment, whereas root-associated fungal richness was lower at higher temperatures (Figure 4a, b, Tables 2 and S6). Temperature also impacted the composition of the fungal community (Table 2, Figure 5). AM fungal colonization and root-associated fungal richness were lowest when plants were grown with soil biota from the forest site.
**FIGURE 3** The relative proportion of root-associated fungal species in each treatment combination depending on plant origin, soil microbial origin, temperature and soil moisture. Plant = plant origin, Soil = soil microbial origin, Temp = temperature, Moisture = soil moisture, H = high, L = low and Coast, Forest and Meadow refer to the three sites.

**FIGURE 4** The impact of (a–b) temperature and (c–d) soil microbial origin on arbuscular mycorrhizal (AM) fungal colonization and root-associated fungal richness in the roots of *Plantago lanceolata* plants (n = 116). Shown are boxplots, where the thick horizontal line shows the median, boxes represent the first and third quantile and whiskers represent either the minimum and maximum value or 1.5 times the interquartile range of the data (whichever is smaller). Significant differences (p < .05) among soil microbial origins, based on post hoc Tukey tests, are indicated by different letters.
intermediate when plants were grown with soil biota from the coastal site and highest when plants were grown with soil biota from the meadow site (Figure 4c, d, Table 2 and Table S6). Soil microbial origin also strongly influenced root-associated fungal community composition (Figure 5, Table 2). Notably, the fungal community composition of soil from the coastal and meadow sites overlapped with the community composition at the original field locations, whereas the community composition of soil from the forest site differed from the soil at the original field location (Figure 5).

Soil moisture affected the root-associated fungal community composition both directly and interactively (i.e. as mediated by the origin of the microbial community and temperature; Table 2). Diversity and composition, but not richness, differed among plants from different origins (Table 2).

3.2 | The impact of climate on plant and AM fungal adaptation

Plant growth was reduced when plants were grown with their local compared to non-local soil microbial communities (Figure 6a, b; a significant effect of ‘sympatry’ in Table S7). Overall, the effect of sympatry was not (or only weakly) affected by the environmental conditions (Table S7), indicating that patterns of (mal)adaptation were not affected by climatic conditions.

AM fungal colonization levels were affected by the interaction between plant and soil microbial origin (Figure 6c, Table 2 and Table S2). However, we did not detect a difference in AM fungal colonization between plants grown with their local or non-local soil microbial community (Figure 6c, Table S7).

4 | DISCUSSION

We investigated the environmental drivers of plant growth, AM fungal colonization, fungal community structure and patterns of local adaptation. Plant growth was influenced by climate (temperature and soil moisture) as well as the origin of the plant and soil microbial community. As predicted, the response of plants to changes in temperature was conditional on plant origin, soil microbial community and soil moisture. AM fungal colonization and root-associated fungal community structure were strongly affected by temperature, soil microbial origin and soil moisture. Plants performed worse when grown with their local soil microbial community, which may be due to intraspecific negative plant-soil feedbacks. Our findings illustrate that genetic variation among plant populations and the microbial community will play a major role in the response of plant growth and plant-microbial interactions to a changing climate, but also that the local plant genotypes do – potentially due to negative plant-soil feedbacks – not always perform best when growing with their local soil microbial community. In contrast to our expectation, we found no or weak effects of climate on patterns of plant and AM fungal local adaptation. Given the variable responses of plants of different origin to
temperature, in combination with negative plant-soil feedbacks, we predict complex but profound effects of climate change on the ecology and evolution of plants and soil microbes in natural systems.

4.1 The impact of climate on the ecology of plants and root-associated fungi

4.1.1 The effect of environmental factors on seedling emergence and plant growth

Plant growth and relative investment in above-ground biomass was consistently higher in the warm (25°C) compared to the cold (15°C) temperature treatment, consistent with studies performed in both the lab and field, including studies on *Plantago lanceolata* (Clemmensen & Michelsen, 2006; Heinemeyer, Ineson, Ostle, & Fitter, 2006; Olsrud et al., 2004). Low soil moisture, mimicking low rainfall or drought conditions, led to reduced plant growth and higher below-ground biomass. As drought events increase and precipitation levels change as a consequence of global change (IPCC, 2014), we may expect to see a negative impact on plant growth and performance and higher investment below-ground. Interestingly, the increased root to shoot ratio in response to increasing drought events may be counteracted by a simultaneous decrease in the root to shoot ratio in response to increased temperature. Drought stress has previously been shown to increase root colonization by AM fungi (Jayne & Quigley, 2014), and AM fungi have therefore been proposed as an important mechanism for plants and ecosystems to alleviate the effects of drought (Mohan et al., 2014). However, in our study, we found no differences in AM fungal colonization between two strongly divergent moisture treatments. Moreover, there was no difference in plant responses to drought between plants grown

FIGURE 5 The impact of (a) temperature, (b) soil moisture, (c) plant origin and (d) soil microbial origin on the root-associated fungal community composition of *Plantago lanceolata* (n = 115) as based on canonical correspondence analysis (CCA). Coloured circles represent dispersion ellipses for each group using the standard deviation of point scores (using the ordiellipse function in the package vegan in R). The first two axes explained 2.5% and 2% of the variation respectively. Larger symbols in (d) represent the root-associated fungal communities found in the soil at the original locations.
in sterile or inoculated soil. One explanation for this discrepancy may lie in the fact that previous studies have focused on one or a few species of AM fungi (e.g. Al-Karaki et al., 2004; Porcel & Ruiz-Lozano, 2004; Wu & Xia, 2006), while we used a mixed field inoculum which contains a diverse mix of both beneficial and antagonistic soil microbes. Hence, the benefits of increased colonization levels by beneficial microbes in response to drought may be absent within the natural context. This highlights the importance of comparing the effect of beneficial microbes not only in isolation but also embedded within their natural microbial community, thereby obtaining a more realistic picture of the resilience of natural systems to global change. Interestingly, soil moisture frequently changed plant responses to temperature, illustrating that the effect of elevated temperature can be either exacerbated or alleviated by changes in rainfall patterns and drought.

Notably, the origin of the plants mediated the response of plants to temperature. Such dependency of the response of plants to the abiotic environment on plant genetics may be a common feature of natural systems (De Long et al., 2019; Franks, Weber, & Aitken, 2014; Jump & Penuelas, 2005). For example, Al-Karaki and Al-Raddad (1997) demonstrated that the response of wheat to drought differed among plant genotypes. Variable responses among plant populations to changes in climatic conditions may thereby play a major role in maintaining spatial and temporal heterogeneity in plant traits and demography.

4.1.2 | The effect of environmental factors on root-associated fungi

Root-associated fungal communities differed among the low and high temperature treatment, and AM fungal colonization increased with temperature while root-associated fungal richness decreased. Increased AM fungal colonization is frequently reported in observational and experimental studies in response to increased warming (e.g. Compant et al., 2010; Staddon, Heinemeyer, & Fitter, 2002); however, effects of warming on root-associated fungal richness are less clear, with reports of no (Fujimura, Egger, & Henry, 2008; Geml et al., 2015), increased (Geml et al., 2015) or decreased (Geml et al., 2015; Morgado et al., 2015) richness, likely depending on the taxonomic or functional group (Geml et al., 2015). Root-associated fungi can have many important ecosystem functions, such as carbon storage (Clemmensen et al., 2013) and improving soil structure (Rillig & Mummey, 2006). Given the strong dependence of root-associated fungal species richness and community composition on temperature, we may also see shifts in important ecosystem functions and services as the climate changes.

We found that AM fungal colonization was not significantly influenced by soil moisture. However, as predicted, the root-associated fungal community composition was affected both directly and interactively (as mediated by soil microbial origin and temperature) by soil moisture. Previous studies have similarly found that AM fungal colonization is not always affected by changes in precipitation (e.g. Hawkes et al., 2011), but that the community composition can be (Barnes, van der Gast, McNamara, Rowe, & Bending, 2018; Deveautour et al., 2018; Hawkes et al., 2011). Like in our study, Geml et al. (2015), investigating arctic fungi in Alaska, found an interactive effect of moisture and temperature, where the effect of temperature differed between dry and moist tussocks.

Perhaps not surprisingly, both AM fungal colonization and root-associated fungal community structure differed among
plants grown in soil originating from the three natural *P. lanceolata* populations. Other studies have similarly found that root-associated fungal communities differ among the same plant species grown at different locations (Ji et al., 2013; Rasmussen et al., 2018). Root-associated fungi were to some degree influenced by plant origin, matching the commonly reported effects of host identity and genetic variation on fungal community structure. For example, An et al. (2010) demonstrated that AM fungal colonization levels differed among maize plants of different genotypes, and Becklin, Hertweck, and Jumpponen (2012) showed that different alpine tree species hosted different AM and non-AM fungal communities.

### 4.2 | The impact of climate on plant and AM fungal adaptation

Plant performance was lower when plants were grown with their local soil microbial community, suggesting negative intraspecific plant-soil feedbacks, which may be due to an accumulation of locally adapted pathogenic microbes in the soil (Felker-Quinn, Bailey, & Schweitzer, 2011; Lankau et al., 2011; van der Putten et al., 2013; Wagg, Boller, Schneider, Widmer, & van der Heijden, 2015). This corresponds to findings of a strongly negative plant-soil feedback in *P. lanceolata* by Harrison and Bardgett (2010), who showed that plants performed worse when grown in soil conditioned by *P. lanceolata* than in soil previously conditioned by a mixed plant community. As such, our findings contrast to studies detecting local adaptation of plants to their local AM fungal community (Johnson et al., 2010; Rúa et al., 2016). Interestingly, negative intraspecific plant-soil feedbacks can also be caused by a change in the AM fungal community. As an example of negative feedback mediated by AM fungi, Bever (2002) found that AM fungi associated with *P. lanceolata* were poor growth promoters, and *P. lanceolata* plants grew better with AM fungi from soil previously occupied by another plant species. Notably, this pattern of negative plant-soil feedbacks was not affected by the abiotic environmental conditions (temperature and soil moisture). This suggests that climate change may not affect patterns of local adaptation within the current range, unless the individual species shift out of their present range. The overall negative impact of the local soil community in our study may indicate that temporal changes in pathogen community composition, or genetic changes within individual pathogen species, override changes in the genetics and community composition of beneficial soil microbes.

We detected no signal of AM fungal adaptation to local plant genotypes, as inferred by the lack of differences in root colonization between plants grown in their local and non-local soil. In contrast, Johnson et al. (2010) found that AM fungi produced more fungal structures when grown in their local compared to non-local soil, and that locally adapted mycorrhizas were more mutualistic when resources were limited. In accordance with this, Revillini, Gehring, and Johnson (2016) argue that local adaptation of soil mutualists happens when resources are scarce, thereby promoting plant-mutualist interactions in order to ameliorate resource limitation. When resources are plentiful, it promotes opportunistic plant pathogens relative to commensal and mutualist microbes. The patterns observed here for AM fungi could therefore be due to the lack of resource scarcity within the experiment, despite the use of a low-nutrient background soil, and suggest that with increased nutrient levels in natural environments, pathogens may become an increasingly dominant force compared to beneficial microbes in the structure and evolution of plant communities. As there is no clear consensus on how to measure fungal fitness (Bennett & Bever, 2009; Pringle & Taylor, 2002), future studies may include multiple potential components of fungal fitness, including, for example, number of arbuscules and amount of extraradical hyphae.

To disentangle the effects of the local soil abiotic environment from that of the local soil microbial community, we used a background soil inoculated with live and sterile inocula from each location, an approach frequently used in studies on local adaptation and plant-soil feedbacks (e.g., Callaway et al., 2004; Holah & Alexander, 1999; Lankau et al., 2011). We verified that inoculations were successful by assessing whether the fungal communities that established within the experiment were similar to those present in the original field sites. We found that while soil at the original coastal and meadow communities overlapped well with the communities found in treatment roots, the community composition from the original forest site differed in one dimension of the multivariate ordination space from that of the communities in the experiment. Not all soil fungi will establish in potting soil or colonize roots, and this may be behind the discrepancy between the fungal community composition in the roots of treatment plants and the soil at one of the original locations (as further supported by the higher species richness in the soil at the original locations). Overall, a comparison between the soil at the original sites and the fungi colonizing the roots in the experiment illustrates that the soil biota that establish within experimental settings may retain a high similarity to the soil biota in the original soil, but may also deviate from the original soil biota in some aspects. A frequently overlooked caveat of soil inoculations is whether established microbes retain their functioning within the experimental environment, like their role in the exchange of nutrients between plants and beneficial microbes, and the severity of attack by harmful microbes. While both local adaptation and plant-soil feedback studies rely on the fact that the functioning of microbes is retained in experimental settings, this assumption is rarely tested, and therefore would be an important area for future research.

### 5 | CONCLUSION

Our findings imply that climatic changes (elevated temperature and drought) may have a large impact on plant growth and root-associated fungal community structure, but that the direction and strength of the response will differ throughout the landscape due to spatial variation in plant genes and soil microbial
communities. This suggests that elevated temperatures may have variable outcomes on plant–soil microbe interactions in natural systems. Plants performed worse with their local soil microbial community, a pattern that may be driven by negative intraspecific plant–soil feedbacks. Interestingly, we detected no signs that climate (i.e. temperature or drought) will affect this pattern of (mal)adaptive interactions, indicating that future studies may need to focus on different aspects of local adaptation, such as plant performance variation in response to these treatments. For this, we may need to combine detailed growth chamber experiments focusing on the underlying mechanisms with experimental field manipulations to observe the realized outcome of the ecological and evolutionary dynamics in natural populations. Such knowledge may lead to advances in the effectiveness of restoration and sustainable management, as well as increase our ability to mitigate the consequences of anthropogenic environmental change on plants and soil microbes.

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AUTHORS’ CONTRIBUTIONS

P.U.R. and A.J.M.T. conceived and designed the experiment. P.U.R. conducted the empirical and molecular work and analysed the data. P.U.R. wrote the first draft, and P.U.R., A.E.B. and A.J.M.T. all contributed to the final manuscript.

DATA AVAILABILITY STATEMENT

Data associated with this study are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.dt7bh4b (Rasmussen, Bennett, & Tack, 2019). DNA sequences are deposited at NCBI under BioProjects PRJNA564044 and PRJNA564041.

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