**Abstract**

1. Our understanding of plant functional trait variation among populations and how this relates to local adaptation to environmental conditions is largely shaped by aboveground traits. However, we might expect belowground traits linked to resource acquisition and conservation to vary among populations that experience different environmental conditions. Alternatively, belowground traits might be highly plastic in response to growing conditions, such as availability of soil resources and association with symbiont arbuscular mycorrhizal fungi (AMF).
2. We assessed i) the strength of among-population variation in above- and belowground traits, ii) the effects of growing conditions on among-population variation, and iii) whether variation among populations is linked to source environment conditions, in a globally distributed perennial *Plantago lanceolata*. Using seeds from 14 populations across three continents, we grew plants in a common garden experiment and measured leaf and root traits linked to resource acquisition and water conservation. We included two sets of experimental treatments (high or low water availability; with and without AMF inoculation), which enabled us to assess trait responses to growing conditions.
3. Across treatments, the percentage of root trait variation explained by populations and continents was 9-26%, compared to 7-20% for leaf trait variation. From Principal Component Analysis (PCA), the first PC axis for both root and leaf traits largely reflected plant size, while the second PC broadly captured mass allocation. Root mass allocation (PC 2) was related to mean annual temperature and mean moisture index, indicating that populations from cooler, wetter environments had longer, thinner roots. However, we found little support for a relationship between source environment and leaf trait PCs, root system size (PC1) or individual traits. Water availability and AMF inoculation effects on size were consistent among populations, with larger plants under AMF inoculation, and less mass allocation to leaves under lower water availability.

4. *Plantago lanceolata* shows substantial population-level variation in a suite of root traits, but that variation is only partially linked to the source environmental variables studied. Despite considerable differences in source abiotic environments, geographically separated populations have retained a strong and similar capacity for phenotypic plasticity both above and belowground.

**Key-words.** Common garden, genetic differentiation, local adaptation, phenotype, plasticity, Ribwort plantain, roots, resource uptake

**Resumen**

1. La comprensión de la variación inter-poblacional en rasgos funcionales de plantas y cómo esto se relaciona con la adaptación local a las condiciones ambientales está en gran medida moldeada por rasgos aéreos. Sin embargo, cabe esperar que los rasgos subterráneos vinculados a la adquisición y conservación de recursos varíen entre poblaciones bajo diferentes condiciones ambientales. Alternativamente, los rasgos subterráneos podrían ser altamente plásticos en respuesta a las condiciones de crecimiento, como la disponibilidad de recursos del suelo y la asociación con hongos simbiontes micorrícicos arbusculares (HMA).
2. Analizamos i) la magnitud de la variación entre poblaciones en rasgos aéreos y subterráneos, ii) los efectos de las condiciones de crecimiento en la variación entre poblaciones y iii) si la variación entre poblaciones está vinculada a las condiciones ambientales de origen, en una planta perenne de distribución global, *Plantago lanceolata*. Utilizando semillas de 14 poblaciones de tres continentes, cultivamos plantas en un experimento de jardín común y medimos rasgos foliares y radiculares relacionados con la adquisición de recursos y la conservación de agua. Incluimos dos tipos de tratamientos experimentales (disponibilidad alta o baja de agua; con y sin inoculación de HMA), lo que nos permitió evaluar las respuestas de los rasgos a las condiciones de crecimiento.
3. Entre los tratamientos, el porcentaje de variación de rasgos radiculares explicado por las poblaciones y continentes fue del 9-26%, en comparación con el 7-20% para variación de rasgos foliares. A partir de Análisis de Componentes Principales (ACP, por sus siglas en inglés) tanto para rasgos radiculares como foliares, el primer eje de CP reflejaba principalmente el tamaño de la planta, mientras que el segundo CP capturaba en términos generales la asignación de biomasa. La asignación de biomasa

radicular (CP 2) estaba relacionado con la temperatura media anual y el índice medio de humedad, indicando que las poblaciones de entornos más frescos y húmedos tenían raíces más largas y delgadas. Sin embargo, encontramos poco respaldo para una relación entre el ambiente de origen y los CP de rasgos foliares, el tamaño del sistema radicular (CP1) o los rasgos individuales. La disponibilidad de agua y los efectos de la inoculación de HMA en el tamaño de planta fueron consistentes entre poblaciones, con plantas más grandes bajo la inoculación de HMA y menor asignación de biomasa a las hojas bajo una menor disponibilidad de agua.

1. *Plantago lanceolata* muestra una variación sustancial a nivel de población en una serie de rasgos radiculares, pero esa variación está solo parcialmente vinculada a las variables ambientales de origen estudiadas. A pesar de las diferencias considerables en las condiciones abióticas de origen, las poblaciones separadas geográficamente han conservado una capacidad fuerte y similar de plasticidad fenotípica tanto aérea como subterránea.

## Introduction

Plant populations often show strong signals of local adaptation among populations in response to prevailing abiotic or biotic environmental conditions, with differentiated functional traits linked to plant growth, reproduction and survival (Bischoff et al. 2006). There can be marked differences among geographically and environmentally separated populations in plant traits such as height, leaf shape and specific leaf area (Brandenburger et al. 2019). To date, population differentiation of belowground traits has received less attention than aboveground traits (Aoyama et al., 2022; Mao et al., 2023). The paucity of studies measuring root trait differentiation among populations is surprising given that multiple root traits are related to the ability of plants to acquire limiting resources (water and nutrients) from surrounding soil (e.g. Roumet et al. 2016). Belowground traits are just as important as aboveground traits in determining fitness, and this should result in a signal of root trait differentiation among populations that reflects differences in prevalent environmental conditions. We therefore expect the amount of among-population variation in root traits to be at least as large as leaf trait variation.

Root trait variation among species likely reflects at least two dimensions of 'strategy' in response to both abiotic and biotic environmental conditions in the habitats they occupy (Roumet et al. 2016; Valverde-Barrantes et al. 2017; Kong et al. 2019; Bergmann et al., 2020). Some traits reflect resource uptake ability and degree of reliance on mycorrhizal fungi for resource uptake (with greater root diameter and lower specific root length reflecting greater reliance; Bergmann et al. 2020). Other traits primarily reflect a resource acquisition-conservation continuum (lower root tissue density and high root N concentration reflect resource acquisition, while the opposite indicate resource conservation; Bergmann et al. 2020). Environmental conditions vary among populations of a species, and if variation in water availability exerts a selection pressure on plant genotypes, we would expect plants from drier, warmer source environments to have root traits that increase survival, reflecting a strategy of conservation and outsourcing to mutualists (shorter, thicker roots with less branching and lower specific root length, thus minimizing loss of water and expensive tissue; Fig. 1a). We might

also expect plants from warmer, drier environments to invest less biomass in leaves (due to risk of water loss) and relatively more in roots (higher root mass fraction; Funk & Larson, 2016).

Root traits might be highly plastic in response to low water availability, and this plasticity may itself be under selection in fluctuating environments where water availability varies strongly. Lozano et al. (2020) have shown that in response to varying water availability, root traits show greater plasticity than leaf traits among species. Within species, plastic responses of leaf or root traits to growing conditions such as water availability could be similar among populations, regardless of source environment (additive effect; Fig. 1b). Alternatively, plastic responses to altered growing conditions might also vary depending on the source environment (interaction effect; Fig. 1c). For example, plants from populations in more water-limited environments might already exhibit belowground traits that reduce water loss/enhance water acquisition, such as thicker roots. Therefore, these genotypes may not need to alter traits plastically in response to reduced water availability as much as genotypes from less water-limited environments (Fig. 1c). Experimental inoculation with arbuscular mycorrhizal fungi (AMF) can also alter root traits, including reduced root diameter (Basyal & Emery 2020), length and specific root length (Sun & Tang 2013). We therefore need to account for the possibility that AMF can modulate the effect of abiotic environmental conditions in AMF-associating species. Presence of AMF may simply benefit host plants through greater access to nutrients and water, resulting in less need for plants to alter root traits to increase resource uptake (Augé 2001). Thus, AMF inoculation may result in plastic root-trait shifts towards those expected in more resource-limited environments, even when water availability is not limited (Fig. 1b). Alternatively, plants from water-limited populations may already have root traits that maximise water uptake and minimise water loss, and expression of these may be fixed even with AMF inoculation (Fig. 1c).

While relationships between root traits and environmental gradients have been described within species for some systems using field-collected data (Liu et al. 2010; Cheng et al. 2016; Weemstra et al., 2022; Spitzer et al., 2023), understanding the strength of heritable root trait differentiation and plasticity among populations requires common garden experiments. To understand

how root traits and their plasticity vary among populations within a species in comparison to leaf traits, and whether this variation is related to source abiotic environmental conditions, we used 14 populations of the globally widespread herbaceous perennial, *Plantago lanceolata*, a species that forms strong AMF associations (Francis & Read, 1994). We conducted a glasshouse experiment under four treatments in common growing conditions to assess variation in leaf and root traits among populations, and whether this variation relates to three variables linked to water availability in the source population locations: mean annual temperature, mean and seasonality of soil moisture. We experimentally manipulated water availability and AMF during the growing period, to assess whether responses to biotic and abiotic growing conditions also vary among populations. Unlike recent work on populations of this species that focussed on aboveground traits (Villellas et al., 2021), we measured and analysed multiple root traits linked to resource capture, in addition to leaf traits.

We had three specific questions in our study, which we addressed with three distinct sets of models:

1) Do root traits vary among populations, and how does this variation compare to that of leaf traits?

2) Are plastic responses of traits to different growing conditions (high versus low water availability, AMF inoculation or not) consistent among populations?

3) Is variation in root and leaf traits related to source environment conditions, and does this relation depend on growing conditions (Fig 1a-c)?

## Materials & Methods

### *Study species*

*Plantago lanceolata* L. is a perennial herb native to Europe, which forms rosettes of leaves aboveground, and bears a main tap-root (short thick rhizome) and adventitious side roots closer to the soil surface (Sagar & Harper 1964; Soekarjo 1992). *Plantago lanceolata* is a very variable species and has established in a wider range of environmental conditions in the introduced than native range

153 (Sagar & Harper 1964). Population genetic diversity is higher in warmer and drier regions, and in
154 non-native range populations, most likely reflecting a history of repeated introductions and genetic
155 admixture in the non-native range (Smith et al. 2020). For this study, we used seeds collected from
156 individual *Plantago lanceolata* plants growing in 14 populations, distributed on three continents
157 representing the native and non-native range: Europe (Native), North America and Australasia (Table
158 1; Buckley et al. 2019). We used seeds collected from seven individual plants per population,
159 representing seed families from each individual parent plant.

160

161 *Experimental set-up*

162 A glasshouse experiment was set up on the 7$^{th}$ of June 2018 (Glasshouse location: Durham University,
163 UK; 54°45'52.81"N, 1°34'22.23"W). Glasshouse conditions included a constant temperature of 21°C,
164 and a natural photoperiod (i.e. no artificial lighting was used); the longer axis of the glasshouse is
165 oriented approximately E-NE to W-SW, with no obstruction to light on the south-facing side. Seven
166 replicate blocks were established on a single glasshouse bench, and each block was randomly
167 assigned one of the seven seed families from each population, so that all populations were present in
168 each block (Fig. S1). For each seed family in a block, five seeds were sown into each of four
169 individual pots (9 cm x 9 cm x 14 cm depth), containing a substrate mixture of 1-part sterile sand to 2-
170 parts vermiculite. A fine mesh (~2 mm) square of nylon net was placed at the bottom of each pot to
171 avoid loss of substrate. Within each block, pots were placed on upturned plastic trays with perforated
172 bases, to ensure free drainage and minimise cross-contamination of AMF between pots. Prior to seeds
173 being sown, pots were watered until the substrate was saturated, to ensure suitable conditions for seed
174 germination. After seeds were sown, pot positions within a block were fully randomised. In summary,
175 there were 56 pots per block and seven blocks, making an initial total 392 pots.

176 For each set of four pots representing a seed family, each pot was randomly assigned to one
177 of four, full-factorial treatment combinations: i) high water availability, inoculation with AMF spores
178 ii) low water availability, no AMF spore inoculation iii) high water availability, AMF spore

179 inoculation, and iv) low water availability, no AMF spore inoculation. The AMF inoculation
180 treatment involved adding a thin layer of 8 g of Symbiom® inoculant mixture Symbivit® (containing
181 six AMF species: *Glomus mosseae, G. intraradices, G. claroideum, G. microaggregatum, G.*
182 *caledonium* and *G. etunicatum*), consisting of lyophilized mycorrhizal roots containing sporocarps,
183 spores and hyphae of these fungi plus clay carrier substrate. To further minimise cross-contamination,
184 pots receiving AMF inoculation and non-inoculation procedural controls were prepared separately and
185 sequentially. In addition, the AMF inoculum was added approximately 2 cm below the top of the pots
186 and was then covered with sand/vermiculite substrate to the top of the pots. The pots with no AMF
187 inoculation had only the same volume of carrier substrate (supplied by Symbiom®) applied in the
188 same way. To facilitate germination and seedling survival, pots were sprayed with distilled water
189 every 2–3 days and covered by a transparent plastic sheet during the first 20 days after sowing.

190 After 20 days, we thinned the seedlings to the most centrally located one per pot. Seedlings
191 that germinated after day 20 were not included in the experiment. In total, there were plants in 352
192 pots (90%; Table 1). The watering treatments commenced on the 27$^{th}$ of June (Day 1); plants were
193 subsequently watered on days 1, 3, 6, 8, 10, 13, 17, 21, 25, 29 and 37. On these days, pots assigned to
194 the high-water availability treatment received 100 ml of distilled water, while low-water availability
195 pots received 50 ml of distilled water (applied to the substrate surface). To address concerns that
196 growth might be inhibited by the low nutrient concentration of the pot substrate, on day 8 we gave all
197 pots 25 ml of fertiliser solution (1g $L^{-1}$ concentration Universol® Green low-phosphate fertiliser, ICL:
198 23, 6 and 10 mg $ml^{-1}$ of N P and K respectively). On day 21 the pots within each block were re-
199 randomized. The experiment continued until the 5$^{th}$ of August 2018 (40 days after watering treatments
200 started), because roots were visible protruding through the pots' drainage holes. At the end of the
201 experiment, all leaves were harvested, and scanned per individual plant using a flatbed scanner
202 (Epson® Expression 11000XL; scanned images had a resolution of 600 dpi). Leaves were then dried
203 at 60°C for 72 hours and weighed per plant, giving total leaf mass per plant (g, to the nearest 0.00001
204 g). Leaf area was measured for each individual leaf and summed to give total leaf area per plant ($cm^2$)
205 using the program ImageJ (Rasband, 2018). Root systems were carefully washed free of substrate and

were subsequently stained for one hour in a Neutral Red dye solution (0.35 g Neutral Red dye, 5.25 g citric acid, 2.6 g NaOH per litre of distilled water) to heighten contrast. After staining, the roots were rinsed in water and then suspended in water within individual square petri dishes. We placed the dishes on top of the flatbed scanner, calibrated for use with the image analysis software WinRhizo$^{TM}$. Care was taken to spread out suspended root systems to increase measurement accuracy. Root images were scanned (600 dpi) and analysed using WinRhizo$^{TM}$; total root length (cm), average root diameter (mm), and the number of root forks were recorded. After scanning, we dried the root systems at 60°C for 72 hours and weighed them to obtain dry mass (g, to the nearest 0.0001 g).

To confirm mycorrhizal colonization of roots in inoculated treatments, we also assessed the proportion of root colonised by AMF by observing structures (arbuscules, vesicles, hyphae) in rehydrated, cleared and stained root using methods based on McGonigle et al. (1990). Full methods are provided in Methods S1, but briefly, 25 sections of root length were viewed per plant sample at x 100 magnification using a compound microscope. Roots from 55 plants were observed, representing all 14 populations and each of the four water availability:AMF inoculation treatment combinations, except one less for one population. Where possible, all the samples from each population were from the same seed family.

*Functional traits*

We analysed four aboveground traits: average leaf area ($cm^2$), average leaf mass (g), number of leaves, specific leaf area ($cm^2\ g^{-1}$) and leaf mass fraction (proportion of total mass that was leaf mass). We also analysed seven belowground traits: root length (cm), root mass (g), specific root length ($cm\ g^{-1}$), root branching intensity (number of forks $cm^{-1}$ root length), average root diameter (mm), fine root length fraction and root mass fraction (proportion of total mass that was root mass). Specific leaf area represents the amount of area deployed for photosynthesis per unit of mass invested. Specific root length represents the amount of resource-acquiring root length deployed per unit root mass investment. Root branching intensity is an architectural trait that represents the distribution of root

branching throughout the root system. A high branching intensity (more forks per unit length) reflects root branching and proliferation throughout the root system. Few forks per unit length indicate concentration of branching at points within the root system. The fraction of root length defined as fine roots indicates root length allocation to soil resource absorption; fine roots have traditionally been defined as those ≤2 mm, though it has been recommended to split this group into absorptive and transport root categories based on root order also (McCormack et al. 2015). We defined fine root length fraction as the proportion of total root length <0.5mm in diameter, because harvested root systems were still from young plants with 99% of root length being <2 mm for all plants. Using a diameter class definition was the only practical option to calculate fine root length fraction in our study given the number of samples processed. To describe how much biomass the plants allocate to roots, we calculated root mass fraction (proportion of total mass invested in root mass).

Leaf area and mass reflect plant investment in tissues for photosynthesis. Leaves with greater specific leaf area represent a greater pay-off for mass investment for photosynthesis, but also a greater risk of water loss through evapotranspiration of leaves (Wright et al. 2004). Root length and root mass give measures of plant size belowground, overall ability to acquire soil resources and root growth. Greater specific root length, branching intensity, fine root length fraction and smaller root diameter are thought to reflect a greater ability to acquire soil resources (Reich 2014; McCormack et al. 2015 Liese et al. 2017), while average root diameter may also be linked to AMF colonization, with thicker roots reflecting greater outsourcing of resource uptake to AMF (Kong et al. 2019; Bergmann et al., 2020). A higher root or leaf mass fraction reflects greater investment in roots or leaves (Larson & Funk 2016).

*Statistical analyses*

Leaf and root traits can correlate strongly within organs, and covarying traits reflect plants' locations within leaf and root economics spectra, syndromes of growth and resource allocation (Wright et al., 2004; Weemstra et al. 2023). We summarised trait correlation strength using Pearson's correlation

coefficient and captured covariation among traits using a Principal Components Analysis for leaf and root traits separately. Prior to this, traits were transformed to achieve normality (Table S1), centred on the mean and scaled (to 1 standard deviation). The first two principal components (PCs) for both root and leaf traits had eigenvalues >1, and following the Kaiser-Guttman Rule, they were extracted and used in subsequent analyses to represent the suite of root/leaf traits and their variation among plants. To simplify interpretation of results, we considered combinations of AMF inoculation and water availability as one treatment with four levels (Inoculation, high water availability; Inoculation low water availability; No inoculation, high water availability; No inoculation, low water availability). The one exception was the analysis of proportion of roots colonised by AMF: we used a binomial generalised linear mixed model (in the package 'lme4'; Bates et al. 2015) with AMF inoculation and water availability as fixed effects, and initially with an interaction term between the two. Population was a random effect.

To answer our three main questions, we used three different sets of models (hereafter referred to as Model Set 1, 2 and 3). To answer question 1 (Do root traits vary among populations, and how does this variation compare to that of leaf traits?), Model Set 1 included linear mixed effects models (restricted maximum likelihood; REML) with treatment as a fixed effect, and population nested within continent, and block as random effects. This allowed us to partition trait variance into five components: continent, population, experimental block, residual and among treatments. We ran models for each of the two PCs for leaf and root traits, and for each of the four leaf and seven root traits individually. To meet model assumptions of residual normality and variance homogeneity, some traits were transformed prior to analysis as they were in the PCA (Table S1). Models were fitted using the function lmer() in the R package lme4 (Bates et al. 2015). Variance components were calculated using the R package 'insight' (Lüdecke et al. 2019).

To answer question 2 (Are plastic responses of traits to different growing conditions consistent among populations?), Model Set 2 included the following five linear mixed effects models (maximum likelihood; ML) for each of the trait PCs (as a response): i) treatment + population + treatment:population; ii) treatment + population; iii) treatment alone; iv) population alone; v)

intercept-only model. Random effects included continent and block. We calculated sample size-corrected Akaike Information Criterion values (AICc) to assess relative support for each of the five models. Following Richards et al. (2010), we used a difference of 6 AICc units to distinguish between models. If the lowest-AICc model was >6 AICc units lower than the next best models, this was interpreted as strong evidence that the lowest-AICc model was the better model of the candidate set.

To answer question 3 (Is variation in root and leaf traits related to source environment conditions, and does this relation depend on growing conditions?), Model Set 3 included 11 mixed effect models (ML) per response variable (leaf/root trait PCs and individual traits), comprising all combinations of separate environmental variables and treatment as fixed effects, plus a random-intercept model (see Table S2). All models had population, continent and block as random effects. Source environment conditions were represented by variables linked to water availability: mean annual temperature (°C), annual mean moisture index (integrating data on rainfall and evaporation rate) and seasonality of moisture (coefficient of variation based on monthly index values), all obtained from the CliMond Archive v. 1.2. dataset at 10' resolution (Hutchinson et al. 2009; Kriticos et al. 2012; Kriticos et al. 2014). Mean moisture and seasonality of moisture correlated strongly (r = -0.64); correlations between temperature and the moisture variables were weaker (r = -0.45 for mean moisture; r = 0.33 for moisture seasonality). Experimental growth conditions (treatments) were the level of watering and addition of AMF in full factorial design, as described above. All variables were transformed as needed (Table S1).

For Model Set 3, we compared fitted models for each response variable using AICc, to assess relative support for each model in a candidate set. If a model was >6 AICc units lower than the next best models, we interpreted this as strong evidence that the lowest-AICc model was the better model of the candidate set. Large increases in AICc when any variable is excluded would indicate strong support for variable inclusion. For models within 6 AICc units' difference of the lowest-AICc model, if a simpler nested model has a lower AICc than a more complex nested model, we took parsimony into account, and considered the simpler nested model over the more complex one/s for inference. This follows recommendations by Richards et al. (2010) and in Grueber et al. (2011) and compensates

for the tendency of AIC(c) to include more complex models among the better-performing ones in a candidate set. The effect sizes and 95% confidence intervals for remaining models (using REML) were plotted for inference. More complex models with a lower AICc than simpler models but within 6 AICc units' difference were considered to have relatively weak support. We also calculated Akaike weights to provide an indication of certainty that a particular model is the 'best' one of a candidate set, with values closer to 1 indicating greater certainty. Marginal $R^2$ values (considering fixed effects only) were calculated for treatment-only and lowest-AICc models to understand the contribution made to explained variation by included source environment variables.

To interpret treatment effects in Model Set 3, we used the lowest-AICc REML model including treatment to calculate means and 95% confidence intervals using fixed effect errors. Where there was strong support for source environment effects, we plotted the fitted relationship (and 95% confidence envelope) between source environment and the response using the respective REML model and fixed effect errors.

## Results

### *Principal components and AMF colonisation*

As expected, the measured root and leaf traits often correlated strongly within organs (Fig. S2). Principal components analysis identified two principal component axes that represented 78% of leaf trait variation, and two axes representing 75% of root trait variation (Fig. 2). For leaf traits, the first principal component (PC1) most strongly represented variation in leaf mass, followed by leaf area and number of leaves (Fig. 2a; Table S3). The second component (PC2) most strongly represented variation in leaf mass fraction and specific leaf area (Fig. 2a; Table S3). For root traits, PC1 was most strongly associated with root mass, root length and branching intensity (Fig. 2b; Table S3). The PC2 for roots was most associated with root diameter, fine root length fraction but also total root length, with root diameter corresponding to root PC2 in the opposite direction to fine root length fraction and

specific root length (i.e. plants with thicker roots had lower specific root length and fine root length fraction, Fig. 2b; Table S3).

Overall, out of 675 root sections of AMF-inoculated plant root viewed, 283 contained at least one AMF structure type. This compared to only 50 root sections containing structures out of 700 viewed for inoculated plants. The interaction model explaining root colonisation by AMF had a greater AIC value (378.2) than the additive model (376.6), indicating limited support for an interaction between AMF inoculation and water availability. From the additive model (Table S4), AMF colonisation rate was estimated at 0.033 on average for roots from plants without AMF inoculation and with high water availability, while colonisation rate was 2.6 times higher for plants without inoculation and with low water availability (0.088; Fig. S2). For plants with AMF inoculation under high water availability, root colonisation rate was 0.185 on average, but more than doubled with AMF inoculation and low water availability 0.394; Fig. S3).

*Question 1) Do root traits vary among populations, and how does this variation compare to that of leaf traits?*

Across all traits and principal components in Model Set 1, the total amount of variation explained by treatment, population and continent combined ranged from 16 to 53% (Fig. 3). The percentage of root trait variation explained by populations and continents combined was 9-26%, compared to 7-20% for leaf trait variation (Fig. 3). For leaf traits, treatment effects explained more variation in traits associated with leaf PC1 (leaf area, leaf mass, and number of leaves; Fig. 2a) than population and continent did (leaf mass fraction, specific leaf area; Fig. 3a). In contrast, population and continent together explained more variation in leaf traits associated with leaf PC2 (leaf mass fraction, specific leaf area; Fig. 2a) than treatment did (Fig. 3a). For root traits, treatment effects explained no more than 24% (root mass) of total variation, and only 17% and 3% of total variation in root PC1 and PC2 respectively (Fig. 3b). Population and continent together accounted for more variation than treatment for all seven root traits; at least half of the explained variation was attributed to population and

continent for specific root length, branching intensity, root diameter and fine root leaf fraction (Fig. 3b). Consequently, population and continent were responsible for more than half of explained variation in root PC1 and PC2 (Fig. 3b).

*Question 2) Are plastic responses of traits to different growing conditions consistent among populations?*

For Model Set 2, there was little support for an interaction between treatment effect and populations (Table S5). Models including treatment and population as independent additive fixed effects had the most support (lowest AICc values) for both leaf traits (difference in AICc between the interaction and additive model of 36 for PC1 and 65.7 for PC2) and root traits (difference in AICc of 39.5 for PC1 and 38.3 for PC2).

*Question 3) Is variation in root and leaf traits related to source environment conditions, and does this relation depend on growing conditions?*

Model comparisons revealed that, for every response variable in Model Set 3, the model with the highest support always contained the experimental treatments (Table 2; Fig. S4; Table S6). In addition, the treatment-only model explaining PC variation was either the lowest-AICc model or within 6 units of the lowest-AICc model for leaf PC1 and PC2, and for root PC1 (Table 2). We only found support for a relationship with source environment conditions for root PC: the model including mean temperature had stronger support than the treatment-only model ($\Delta$AICc >+6; Table 2). The treatment + mean moisture index model had a marginally higher AICc value than the treatment + mean temperature model (Table 2). For root PC2, mean temperature and mean moisture index had effects of a similar magnitude to some of the treatment effects (Fig. S4d). Root PC2 values decreased with increasing mean temperature (Fig. 4a) and increased with increasing mean moisture index (Fig. 4b). The amount of root PC2 variation explained by treatments + mean temperature was 10% compared to <3% for the treatment-only model (Table S7).

For individual leaf and root traits, the lowest-AICc models also included a source environment variable for most traits except specific leaf area, leaf mass fraction, root mass and root mass fraction (Table S6). For these models, however, the increase in explained variation was only marginal compared to the treatment-only model, with the largest increases for number of leaves (19 vs 15%), root length (27 vs 21 %) and branching intensity (24 vs 18%; Table S7).

Plants showed marked responses to the experimental treatments (Fig. 5; Fig. S5 for untransformed population means). Plants under the low water availability treatment attained a total biomass that was on average 74% (SD=21) and 84% (SD=22) of the total biomass under high water availability (n= 14 population differences), without and with AMF inoculation respectively. Plants with AMF had greater leaf area, leaf mass, number of leaves, root length, root mass, branching intensity, and lower fine root length fraction compared to plants without AMF, regardless of water availability (Fig. 5a, b, e, f, g, i, and j respectively). Within AMF treatments, plants responded to lower water availability by decreasing leaf area (Fig. 5a), leaf mass fraction (Fig. 5d), and increasing root mass fraction (Fig. 5k). Some responses to low water availability were clearer in the absence than the presence of AMF; lower water availability without AMF resulted in lower specific leaf area (Fig. 5c), fewer leaves (Fig. 5e), lower specific root length (Fig. 5h), and thicker roots (Fig. 5j). These effects were reflected in trait PCs, with decreases in PC1 for both leaf and root traits in the presence of AMF (Fig. S4a, c; Fig. S6a, c), and decreases in PC2 of leaf and root traits with lower water availability in the absence of AMF (Fig. S4b, d; Fig. S6b, d).

## Discussion

Our first question asked if and how much root and leaf traits vary among globally widespread populations of *Plantago lanceolata*. We found that root traits tend to vary at least as much as leaf traits. Our second question asked whether plastic responses of traits to different growing conditions are consistent among populations, and we found no evidence of variation among populations in trait responses to water availability and AMF inoculation. Finally, our third question asked if trait variation

among populations is related to source environmental conditions, and if this relationship depends on growing conditions. We only found support for a relationship between a component of root trait variation and variables linked to water availability, and there was no evidence this relationship differed depending on growing conditions. These findings support scenario b) in Fig. 1, that genotypes across populations can plastically alter leaf and root traits to a similar degree in response to growing conditions. However, every trait was best explained by a model that included growing conditions as an explanatory variable (Table S6), and addition of a source environment variable only marginally increased explained variation in most cases (Table S7). Our results highlight that genotypes in very geographically and environmentally distant locations retain a strong ability to respond plastically to variable growing conditions, whereas any local adaptation may be subtle and limited. This is especially true for leaf traits, which were more dependent on experimental growing conditions than root traits. Here, we discuss our answers to our three questions in reverse, focusing on: 1) why the investigated source environments play at best a minor role in explaining root and leaf traits, 2) the functional implications of below- and aboveground plant responses to water availability and AMF association, and 3) what else might explain variation among populations in root (and leaf) traits.

*Why do source environments play a minor role in explaining root and leaf traits?*

In answer to our third question, the root trait differentiation among populations was only partly related the environment at the source populations. To be sure that this variation indeed results from local adaptation, we would have to carry out reciprocal transplant experiments, and we also cannot rule out maternal effects (Bischoff & Müller-Schärer 2010). Notwithstanding this, plants sourced from warmer, drier environments tended to have a suite of root traits reflecting greater resource conservatism or greater collaboration from resident AMF (thicker, shorter roots with lower fine root length fractions), while those from cooler, wetter environments had root traits reflecting resource acquisition or less reliance on AMF (Fig. 2b; Fig. 4). This result is in line with patterns detected across species in multiple studies, as well as in fewer studies focusing on within-species root

variability. However, we found no clear support for an interaction between source environment conditions and treatments affecting performance-related traits (leaf and root mass: Table S6, Table S7) suggesting that any adaptation to drier environments does not result in greater AMF collaboration when they are available. Instead, AMF colonisation of roots was greater under low water availability in samples drawn across populations, indicating a general shift to greater collaboration with AMF in water-limited conditions. However, we note that AMF present in the source environments could also vary in life history and collaboration preferences in different environments, and this needs further investigation.

Thicker roots reflect a conservative resource strategy, and they are typical for species and populations from warm and dry environments (Roumet et al., 2016; Laughlin et al., 2021). Among species, higher specific root length is thought to represent a greater ability to acquire water (Comas et al. 2012) but tends to be lower in species and populations from drier environments (Liu et al., 2010; Cheng et al., 2016). Within a species, Murren et al. (2020) also found evidence of selection against greater total root length in wild *Arabidopsis thaliana* in field sites with soils that had lower water-holding capacity. Roots represent an important carbon construction cost and require sufficient carbohydrate supply from the photosynthesising leaf tissue available (Eissenstat et al. 2000). In warmer environments, evaporation of water from soils and leaves may be too high for plants with highly branched, finer root systems to be worth investing in, while more resource-conservative plants with shorter, thicker roots may have a survival advantage.

We found little to no support for variation in leaf traits among source environments, while experimental treatment explained more leaf trait variation than populations did, in contrast to root traits, which had higher population-level variation. Among-population variation may be relatively greater in root than leaf traits because the belowground source environment is more variable than aboveground, and in ways that we have not been able to capture in our study. Glasshouse growing conditions in our study may have reduced air movement and the relative humidity gradient between the inside and outside of leaves, resulting in less pronounced expression of any source-environment differences in leaf traits linked to water conservation. When sampled in the field, specific leaf area

relationships with temperature can be positive, negative or neutral depending on the species (Liu et al., 2010; Rosbakh et al., 2015; Cheng et al., 2016; Liu et al., 2017), and root trait relationships with temperature and precipitation can diverge from those of leaf traits (Weemstra et al., 2022). Field observational data reflect plasticity as well as any underlying genetic differences, and it is likely that vegetative traits are more plastic in response to growing conditions (Villellas et al., 2021).

*Plastic trait responses to water availability and AMF inoculation*

We found no evidence that plant responses to growing conditions differed among the 14 populations of *Plantago lanceolata*. Instead, we found strong evidence that populations and treatments act independently in explaining trait variation (Table S5). Greater plasticity can evolve within a species in response to altered environmental conditions if the resulting selection pressure is strong enough (Dostal, 2022). In our study, we may have been unable to detect subtle differences in plastic responses among *Plantago* populations with our sample sizes. Alternatively, native and introduced populations of plants may show little sign of evolved differences in plasticity, and globally successful species like *Plantago lanceolata* may simply owe their success to a high inherent plasticity (Lamarque et al., 2013).

Plants can show responses to growing conditions primarily through growth and biomass accumulation, reflecting resource availability. Traits reflecting plant size (leaf area and mass, root length and mass, root branching) all showed marked increases with AMF inoculation under both levels of water availability, but especially the leaf traits (see Fig. 2 and 6). This highlights the importance of AMF for enhancement of growth through improved water and nutrient uptake (Rouhier & Read, 1998; e.g. Puy et al., 2022), which in turn allows greater photosynthesis and thus higher carbon provision for the AMF. Root colonisation by AMF was detected in the non-inoculated plants, showing that complete absence of AMF in this treatment was not achieved, but the greater root colonisation we observed under low water availability for even non-inoculated plants suggests an important collaborative role of AMF in water uptake for *Plantago*. However, while *Plantago* leaf area

and mass differed more between high and low water availability in the absence of AMF than in their presence, root length and mass responses were similar with and without AMF (Fig. 5). Thus, water limitation and relative AMF partner limitation combined may have forced plants to respond by constraining shoot proliferation per unit root length/mass deployed, thus avoiding excessive evapotranspiration.

As well as size, plants can respond to growing conditions through biomass allocation. A second dimension in leaf and root traits of *Plantago* seems to reflect allocation of mass (carbon) into resource uptake (specific leaf area, specific root length, root diameter, leaf and root mass fractions). These traits differed in a coordinated way under different water availabilities, with greater specific leaf area, specific root length, thinner roots and greater leaf mass (but lower root mass) fraction with high water availability, and particularly in the absence of AMF inoculation (Fig. 5). These plastic shifts in traits reflect results that have been observed in multiple species, both aboveground (Nicotra et al., 2010; Lozano et al. 2020) and belowground (Larson & Funk 2016; Zhou et al., 2018; Du et al. 2019; Lozano et al. 2020). Fine-root length fraction also tended to be greater in *Plantago* plants grown without AMF inoculation, and this could indicate a response from the plant to invest more in finer roots to increase nutrient or water uptake ability in the absence of the mutualists (as seen in Puy et al., 2022). Overall, while effects of AMF inoculation were similar across water availability treatments at least for leaf and root PC1, the effects of water availability on leaf and root trait PC2 appeared to be stronger without AMF inoculation, (Fig. S6b, d), and this likely reflects a shift towards a 'do-it-yourself' resource uptake strategy when fungal mutualist association is limited (Weemstra et al., 2023). These root trait results are supported by the lower proportions of root colonised by AMF that we observed under the high water availability treatment. Overall, while there is evidence among species (Kong et al., 2019) and within species (Weemstra et al., 2022) that root and leaf functional trait spectra do not simply mirror one another, we have demonstrated that plastic responses to resource availability and AMF can be tightly linked above and belowground within a species.

*What explains population-level root and leaf trait variation?*

Our study found that root traits vary among populations at least as much as leaf traits, but our ability to explain why this population-level variation exists has been limited. The environmental variables we used to describe the source environments were obtained from a global dataset and might not fully capture the finer-scale environmental variation experienced by individual populations. It is possible that our measures of temperature and soil moisture in the source environments do not sufficiently reflect the soil conditions experienced by plant roots. Other environmental variables such as soil nutrient concentrations (e.g. Wang et al., 2023), pathogen and mutualist communities (Dai et al., 2023) and pH (Robles-Aguilar et al., 2019; Wang et al., 2020) can impact on plant root traits, yet data on these are not readily available in the same way as climatic data and we thus could not test for their effect. Furthermore, the root traits we measured reflect resource uptake ability and plant size, but may also be correlated with important mechanical traits (e.g. tensile strength increases with root diameter; Mao et al., 2023) that could vary with different source environment conditions.

An alternative explanation for why source environment does not explain much variation in traits could be experimental. Water availability in our experiments could have been too high on average in comparison to natural conditions, so that plants may not have reached a point of drought stress that might be experienced in the source environments. As a result, differences in leaf or root traits among populations may not have been expressed. However, even though we could have subjected plants to lower water availability to the point of visible drought stress (i.e. wilting), the difference between high and low water availability was enough to detect sometimes substantial leaf and root trait responses to lower water availability (Fig. 5). These results suggest that plants under the low-water availability treatment were indeed water limited (as in Fig. 1b), and this is further supported by the difference in AMF colonisation. Finally, some variation among populations (and especially variation among continents) could be underpinned by neutral genetic diversity caused by admixture at least in the introduced ranges of North America and Australasia, which is not associated with environmental differences among populations (Smith et al. 2020).

## Conclusions

We revealed that the amount of variation in root traits can be at least as large as, if not larger than the amount of variation in leaf traits among populations of a globally widespread species. In addition, the effects of growing conditions on traits were similar for all populations, which indicates that populations have retained a strong capacity for phenotypic plasticity, while genotypic differences might still underpin trait variation among populations overall. However, the among-population variation in root traits was only partially due to variation in source environment variables, specifically temperature and soil moisture. Further research is needed to better understand what explains the root trait variation observed among populations, with a particular focus on root length, root diameter and fine-root deployment and how they link to association with AMF in source environments. Reciprocal transplants, consideration of other abiotic conditions and plant-soil interactions at population locations might yet reveal other drivers of differentiation and local adaptation in root traits in *Plantago lanceolata* and other globally successful plant species.

## Author Contributions

WD and YB conceived the ideas and designed methodology; WD set up the experiment and collected the data with help from YB and AF; WD analysed the data; WD and YB led the writing of the manuscript. All other authors contributed seeds for the experiment, contributed critically to the drafts and gave final approval for publication.

## Data Availability Statement

The data used in this study are available in the Zenodo data repository: https://doi.org/10.5281/zenodo.10473872 (Dawson et al. 2024).

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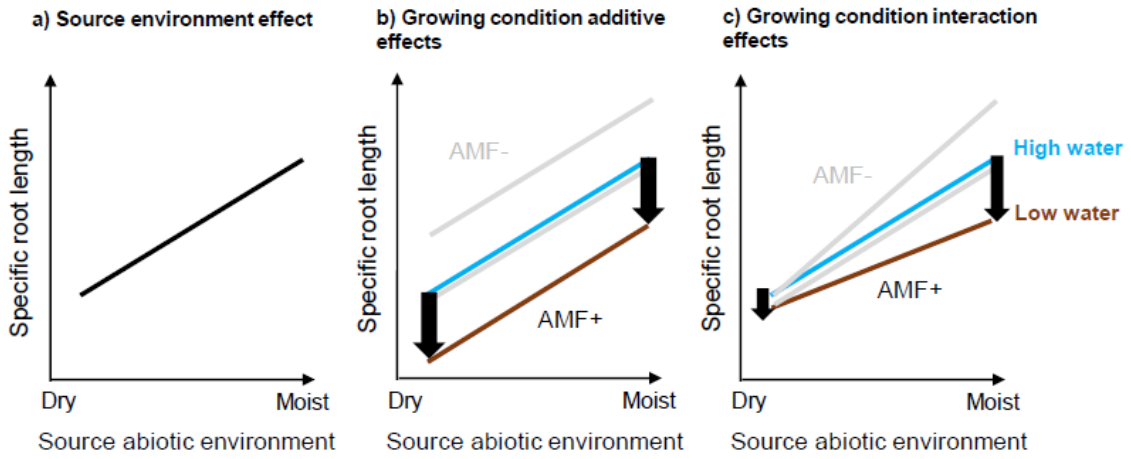
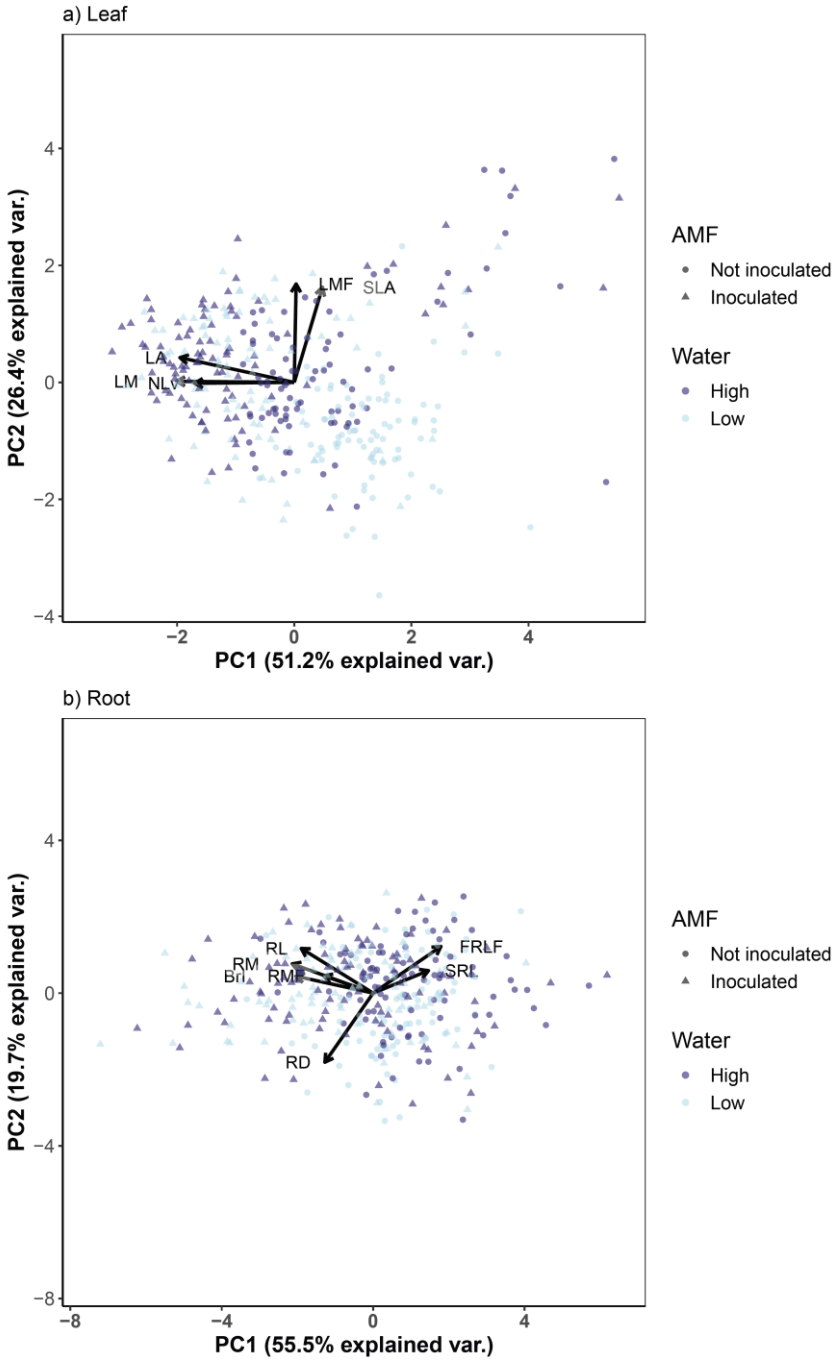



**Figure 1.** Hypothetical relationships between source environment conditions and root traits such as specific root length. a) If certain trait values are advantageous for plant growth and survival under source environment conditions, such as moisture, trait values might vary along the environmental gradient. b) Under experimental growing conditions, plant traits from all populations may respond similarly to changes in water availability (high versus low water availability) and to arbuscular mycorrhizal fungi (AMF) inoculation (coloured lines versus grey lines; AMF-); in other words, the effects of source environment, and treatment combinations are additive. c) Alternatively, traits of populations from drier environments might respond the least to AMF inoculation and/or higher water availability because their outsourcing or conservation strategy is fixed, whereas plants from more mesic environments respond more strongly to AMF presence and water availability (indicated by arrows). Note that the particular scenarios shown in a) to c) are not hypotheses that we are specifically testing. Instead, they serve to illustrate single variable, additive and interaction effects respectively.

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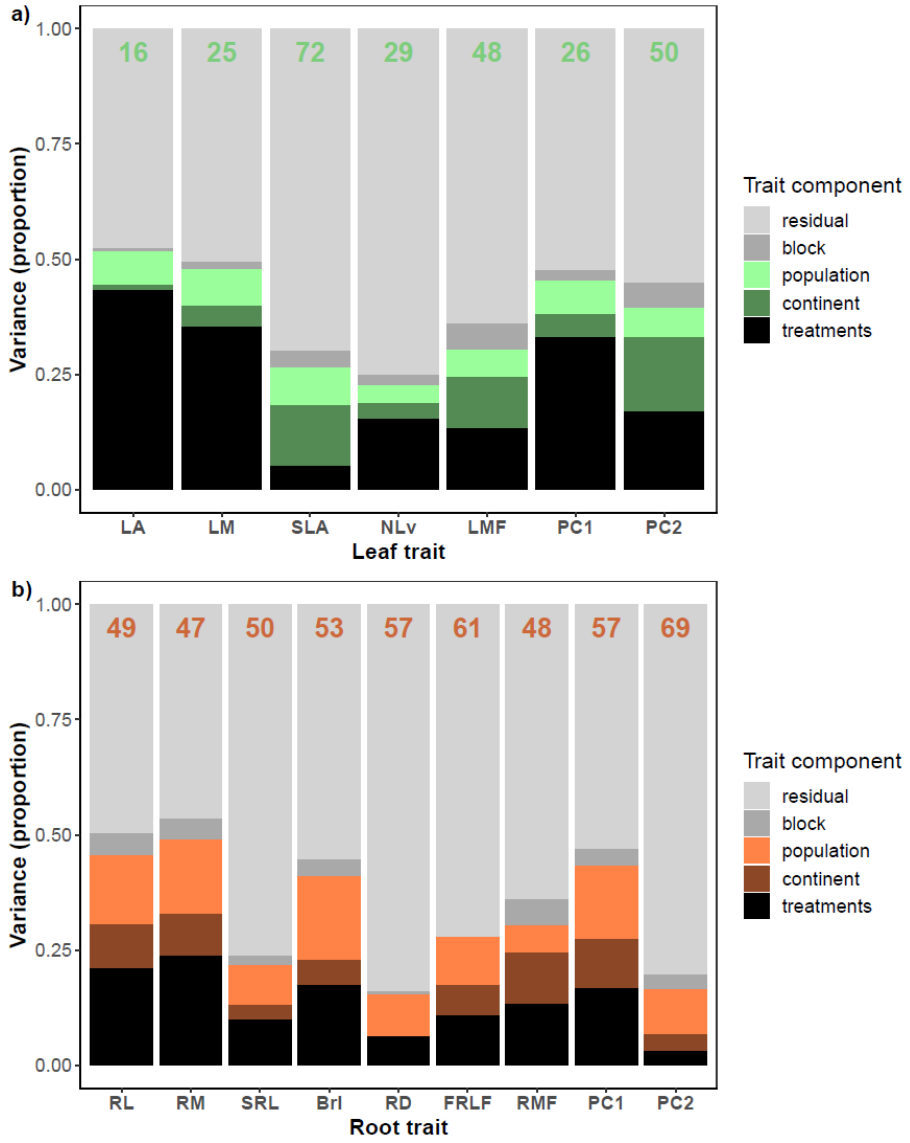
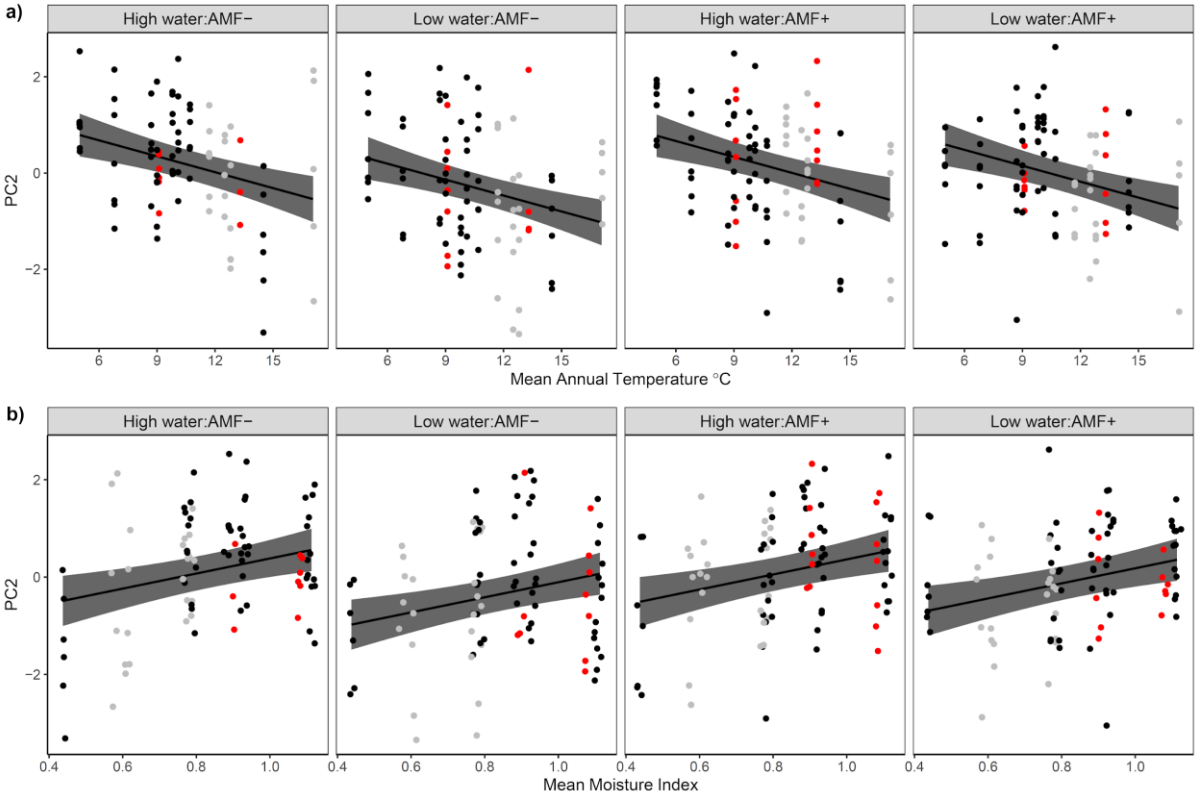
765 **Figure 2.** Biplots of first (PC1) and second (PC2) principal component analysis axes representing
766 variation (var.) in a) leaf and b) root traits. Arrows indicate trait variation in relation to each axis
767 (longer arrows = more trait variation explained by axes; arrow parallel to axis = trait solely
768 contributing to that axis). LA= ln(leaf area), LM= ln(leaf mass), SLA= ln(specific leaf area), NLv=
769 ln(number of leaves), LMF=logit(leaf mass fraction), RL= √(total root length), RM= √(root mass),
770 SRL= ln(specific root length), BrI=√(Root branching intensity), RD= Average root diameter,
771 RMF=logit(root mass fraction), FRLF= logit(fine root length fraction).



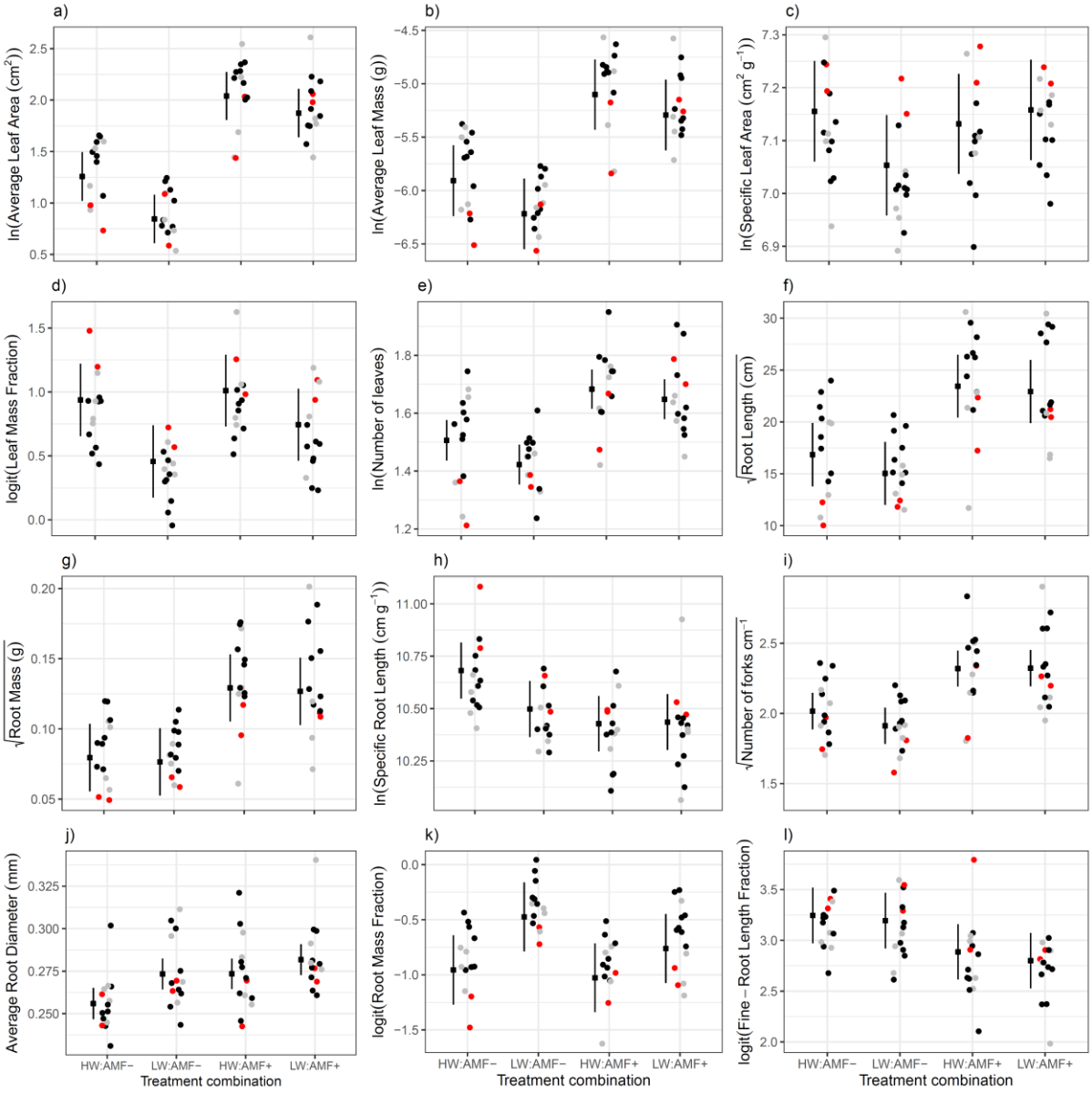

772

773 **Figure 3.** Variance components (expressed as proportion) from models in Model Set 1, explaining
774 *Plantago lanceolata* a) Leaf and b) root traits variation under different treatment combinations (high
775 /low water availability; with/without arbuscular mycorrhizal fungi inoculation). Variance components
776 include continent, population, experimental block, and residual variance. LA= ln(leaf area), LM=
777 ln(leaf mass), SLA= ln(specific leaf area), NLv= ln(number of leaves), LMF=logit(leaf mass
778 fraction), RL= √(total root length), RM= √(root mass), SRL= ln(specific root length), BrI=√(Root
779 branching intensity), RD= Average root diameter, FRLF= logit(fine root length fraction),
780 RMF=logit(root mass fraction), PC1= principal component 1, PC2= Principal component 2. Numbers
781 at the top of bars indicate the percentage of explained variation attributed to population and continent
782 combined.




783

784 **Figure 4.** The only relationships with source environment conditions from Model Set 3 that had
785 strong support: Principal Component 2 representing root traits and a) mean annual temperature, and b)
786 mean moisture index, per experimental treatment. Fitted lines and 95% confidence interval envelopes
787 (accounting for fixed effects uncertainty) are shown. Point colours represent continents of
788 populations: black= Europe; grey= Australasia; red= North America.




789

790 **Figure 5.** Trait values under each experimental treatment combination from Model Set 3 (High/Low
791 water availability: AMF presence/absence). Overall means (squares) with 95% confidence intervals,
792 and population means (circles) shown: black= European populations; red= North American
793 populations; and grey= Australian populations. See Table 1 for population sample sizes per treatment.
794 a) Average area per leaf, b) Average mass per leaf, c) Specific leaf area, d) Leaf mass fraction, e)
795 Number of leaves, f) Root length, g) Root mass, h) Specific root length, i) Root branching intensity, j)
796 Average root diameter, k) Root mass fraction, and l) Fine root length fraction.

797

798 **Table 1.** Information on populations of *Plantago lanceolata* used in the study, including sample sizes
799 (N) per experimental treatment: High Water No AMF inoculation; Low Water No AMF inoculation;
800 High Water AMF inoculation; Low Water AMF inoculation). MT= Mean annual temperature (°C),
801 MM= Mean Moisture index, SM= Seasonality of Moisture. Latitude and Longitude are in degrees.

| Location | Country | Continent | Latitude | Longitude | MT | MM | SM | N |
|---|---|---|---|---|---|---|---|---|
| Lincoln (BHU) | New Zealand | Australasia | -43.65 | 172.46 | 11.7 | 0.787 | 0.247 | 7, 7, 7, 5 |
| Toowoomba (TW) | Australia | Australasia | -27.58 | 151.99 | 17.1 | 0.577 | 0.157 | 5, 5, 6, 5 |
| Canberra (UC) | Australia | Australasia | -35.23 | 149.09 | 12.8 | 0.790 | 0.266 | 6, 5, 6, 6 |
| Urquhart (UR) | Australia | Australasia | -37.19 | 144.38 | 12.5 | 0.769 | 0.378 | 5, 6, 5, 6 |
| Coolclogh (CH) | Ireland | Europe | 52.14 | -8.95 | 9.8 | 1.107 | 0.085 | 7, 7, 7, 7 |
| Donegal (TNM) | Ireland | Europe | 55.25 | -7.62 | 9 | 1.114 | 0.060 | 7, 7, 6, 7 |
| Elva (EL) | Estonia | Europe | 58.26 | 26.35 | 5 | 0.884 | 0.171 | 6, 7, 7, 6 |
| Keszthely (HU) | Hungary | Europe | 46.75 | 17.24 | 10.7 | 0.773 | 0.215 | 6, 7, 7, 6 |
| Tjuvstigen (TJ) | Sweden | Europe | 58.98 | 17.56 | 6.8 | 0.790 | 0.266 | 7, 6, 7, 7 |
| Tübingen (TUE) | Germany | Europe | 48.54 | 9.04 | 8.7 | 0.928 | 0.100 | 6, 6, 7, 7 |
| Winchester (WIN) | UK | Europe | 51.04 | -1.31 | 10.1 | 0.933 | 0.193 | 7, 7, 7, 7 |
| Zaragoza (ZG) | Spain | Europe | 41.69 | -0.93 | 14.5 | 0.438 | 0.260 | 6, 6, 7, 7 |
| Rosedale (RO) | Canada | N America | 49.29 | -121.67 | 9.1 | 1.079 | 0.298 | 6, 7, 7, 6 |
| Virginia (VA) | USA | N America | 37.97 | -78.47 | 13.3 | 0.899 | 0.163 | 3, 4, 7, 6 |

802

803

**Table 2.** Comparison of Models in Model Set 3, explaining 1st and 2nd principal component axes of leaf and root traits of *Plantago lanceolata* plants from 14 populations. Differences in AICc between models and the lowest-AICc model (Δ = 0) are shown. Models in bold are within 6 AICc units of the lowest-AICc model (also in bold), excluding more complex models with a higher AICc than simpler nested models. Akaike weight (*w*) gives an indication of certainty that a given model is the best of the model set. Environmental conditions are: MT= Mean annual Temperature, MM= Mean Moisture index, SM= Seasonality of Moisture. Int.= intercept-only model (y~1). Tr= Treatment. All models included population nested within continent and replicate block as random effects (intercepts).

| Response | | *MT *Tr* | *MT +Tr* | *MT* | *MM *Tr* | *MM +Tr* | *MM* | *SM *Tr* | *SM +Tr* | *SM* | *Tr* | *Int.* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Leaf PC1* | Δ | **1.4** | **1.9** | 163.8 | 6.0 | **0** | 162.3 | 7.6 | **3.6** | 164.8 | **1.9** | 163.6 |
| | *w* | **0.20** | **0.16** | 0 | 0.02 | **0.40** | 0 | 0.01 | **0.07** | 0 | **0.15** | 0 |
| *Leaf PC2* | Δ | 5.2 | 1.9 | 89.4 | 2.0 | 1.95 | 89.4 | 1.9 | **0** | 87.4 | **0.05** | 87.5 |
| | *w* | 0.02 | 0.11 | 0 | 0.10 | 0.11 | 0 | 0.11 | **0.28** | 0 | **0.27** | 0 |
| *Root PC1* | Δ | 1.6 | 1.3 | 90.4 | 6.6 | 1.9 | 91.0 | 6.8 | 1.8 | 90.9 | **0** | 89.1 |
| | *w* | 0.16 | 0.19 | 0 | 0.01 | 0.14 | 0 | 0.01 | 0.14 | 0 | **0.35** | 0 |
| *Root PC2* | Δ | 2.5 | **0** | **5.2** | 5.94 | **1.7** | 6.9 | 12.7 | 6.4 | 11.4 | 8.2 | 13.3 |
| | *w* | 0.15 | **0.52** | **0.04** | 0.03 | **0.22** | 0.02 | 0.00 | 0.02 | 0.00 | 0.01 | 0.00 |