

SUPPLEMENTARY INFORMATION

Root traits vary as much as leaf traits and have consistent phenotypic plasticity among 14 populations of a globally widespread herb

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Methods S1.

We assessed the level of root colonisation by arbuscular mycorrhizal fungi, using a method based on McGonigle et al. (1990). Briefly, we rehydrated, cleared and stained 55 dried root samples (one sample= one plant) drawn across treatments and populations, to assess the level of root colonisation by AMF (one root sample per population for each of the four water availability:AMF inoculation treatment combinations, except one less for one population). Roots were rehydrated in distilled water for approximately 24 hours, after which we destained them with 10% KOH solution, in Falcon® tubes placed within a water bath at 80°C for 2.5 minutes. After rinsing with distilled water, we stained the roots in a solution of 5% acetic acid containing 57 ml per L of Parker® Quink® Black ink. Eight sections of root (2-4 cm in length) per sample were mounted on a microscope slide, one section parallel to the next, and 25 views of the roots per sample were observed at x 100 magnification with a compound microscope (with x 400 used for verification where needed), moving through the sample from one view to the next in a systematic manner. In each view, we recorded whether the view contained arbuscules, vesicles, hyphae, or one of the 4 possible combinations of the three structures, or no structures. Per sample, we then calculated and analyse the proportion of views containing AMF structures. We used binomial generalised linear mixed model (using the R package ‘lme4’) to analyse proportion root colonisation by AMF, with AMF inoculation and water availability as fixed effects plus an interaction term initially. Population was a random effect. The interaction model had a greater AIC value than the simpler additive model, so used the additive model for further inference.

Reference:

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495-501.

Table S1. Leaf and root traits measured on *Plantago lanceolata* plants, abbreviations and statistical transformations used in analyses throughout the study.

Trait	Abbreviation	Transformation
Average leaf area (cm ² leaf ⁻¹)	LA	ln
Average leaf mass (g leaf ⁻¹)	LM	ln
Specific leaf area (cm ² g ⁻¹)	SLA	ln
Number of leaves	NLv	ln
Leaf mass fraction (LM/Total biomass)	LMF	logit
Root length (cm)	RL	√
Root mass (g)	RM	√
Specific root length (cm g ⁻¹)	SRL	ln
Branching intensity (Number of forks cm ⁻¹)	BrI	√
Average root diameter (mm)	RD	
Root mass fraction (RM/Total biomass)	RMF	logit
Fine root length fraction (root length <0.5mm in diameter/RL)	FRLF	logit

Table S2. The fixed effects of eleven models fitted to explain *Plantago lanceolata* root and leaf trait PCs, and which were compared using small sample-size corrected Akaike Information Criterion. Treatment= four-level factor, representing the four combinations of AMF inoculation/water availability. 1 signifies intercept.

Model Fixed Effects
~ Treatment + Mean Annual Temperature+ Treatment:Mean Annual Temperature
~ Treatment + Mean Soil Moisture+ Treatment:Mean Soil Moisture
~ Treatment + Soil Moisture Seasonality+ Treatment:Soil Moisture Seasonality
~ Treatment + Mean Annual Temperature
~ Treatment + Mean Soil Moisture
~ Treatment + Soil Moisture Seasonality
~ Treatment
~ Mean Annual Temperature
~ Mean Soil Moisture
~ Soil Moisture Seasonality
~1

Table S3. Eigenvalues and trait loadings for first and second principal components representation leaf and root traits of *Plantago lanceolata* plants (n=352) representing 14 populations and grown under experimental conditions (No AMF inoculation/AMF inoculation; High water/Low water availability)

	PC 1	PC 2
Leaf traits: Eigenvalue	1.600	1.148
ln(Leaf Area)	-0.59	0.18
ln(Leaf Mass)	-0.61	0.01
ln(Specific Leaf Area)	0.15	0.68
ln(Number of Leaves)	-0.51	-0.01
logit(Leaf Mass Fraction)	0.01	0.71
Root traits: Eigenvalue	1.971	1.175
$\sqrt{\text{Root Length}}$	-0.41	0.43
$\sqrt{\text{Root Mass}}$	-0.47	0.28
ln(Specific Root Length)	0.32	0.21
Branching Intensity	-0.45	0.16
Average Diameter	-0.28	-0.66
logit(Fine Root Length Fraction)	0.39	0.45
logit(Root Mass Fraction)	-0.28	0.17

Table S4. Estimates (\pm SE) from binomial generalised linear mixed model explaining proportion root colonisation by arbusculae mycorrhizal fungi as a function of AMF inoculation and water availability. Random effect standard deviation also shown.

Variable	Estimate (SE)
Intercept	-3.389 (0.225)
AMF Inoculated	1.908 (0.180)
Low Water Availability	1.105 (0.161)
Population	SD= 0.4037

Table S5. AICc values from compared models in Model Set 2, explaining leaf and root trait principal components as a function of: experimental treatments interacting with population; treatments and population independently; treatments alone; population alone; and intercept only (no fixed effects). Lowest AICc values in bold.

Model	Leaf traits		Root traits	
	PC 1	PC 2	PC 1	PC 2
Treatments*Population	1189.4	1026.4	1362.0	1118.5
Treatments+Population	1153.4	960.75	1322.5	1080.2
Treatments	1195.7	1020.47	1418.2	1111.1
Population	1323.5	1050.7	1416.0	1085.4
Intercept	1334.2	1094.2	1479.2	1114.7

Table S6. Comparison of Models in Model Set 3, explaining individual leaf and root traits of *Plantago lanceolata* plants from 14 populations subjected to one of four treatments. Differences in AICc between models and the lowest-AICc model ($\Delta = 0$) are shown. Models in bold are within 6 AICc units of the lowest-AICc model (also in bold) and exclude 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. Models compared included an intercept model (no fixed effects), one of three source environment variables alone, as an additive effect with experimental treatments or as an interaction with treatments. Environmental conditions are: MT= Mean annual Temperature, MM= Mean Moisture index, SM= Seasonality of Moisture. Int.= intercept-only model ($y \sim 1$), Tr = experimental treatment. All models included population nested within continent and replicate block as random effects (intercepts). LA= ln(leaf area), LM= ln(leaf mass), SLA= ln(specific leaf area), NLv= ln(number of leaves), LMF=logit(leaf mass fraction), RL= $\sqrt{\text{total root length}}$, RM= $\sqrt{\text{root mass}}$, SRL= ln(specific root length), BrI= $\sqrt{\text{Root branching intensity}}$, RD= Average root diameter, FRLF= logit(fine root length fraction), RMF=logit(root mass fraction).

Response		MT *Tr	MT +Tr	MT	MM *Tr	MM +Tr	MM	SM *Tr	SM +Tr	SM	Tr	Int.
Leaves: LA	Δ	2.8	0.9	217.8	5.78	0	216.5	5.9	1.8	217.4	5.9	217.8
	w	0.08	0.22	0	0.02	0.35	0	0.02	0.14	0	0.02	0
LM	Δ	2.9	1.5	178.5	5.6	0	177.4	7.2	2.0	178.2	0.7	177.5
	w	0.08	0.16	0	0.02	0.35	0	0.02	0.13	0	0.24	0
SLA	Δ	4.1	0.9	18.9	3.0	1.9	19.8	6.0	1.5	19.4	0	17.9
	w	0.05	0.22	0	0.08	0.13	0	0.02	0.16	0	0.35	0
NLv	Δ	0	0.9	59.1	7.7	2.1	60.5	9.3	7.0	65.0	5.0	63.1
	w	0.47	0.30	0	0.01	0.16	0	0.00	0.01	0	0.04	0
LMF	Δ	6.5	1.5	60.8	5.2	2.1	61.5	4.4	0.9	60.2	0	59.4
	w	0.02	0.18	0	0.03	0.13	0	0.04	0.24	0	0.37	0
Roots: RL	Δ	0	0.3	115.7	6.1	1.5	117.0	10.4	4.2	119.4	2.1	115.7
	w	0.35	0.30	0	0.02	0.17	0	0.00	0.04	0	0.12	0
RM	Δ	1.1	0.5	136.9	5.1	0.6	137.0	7.6	2.1	138.2	0	136.3
	w	0.16	0.22	0	0.02	0.21	0	0.01	0.10	0	0.28	0
SRL	Δ	1.6	0	36.0	2.2	1.2	37.2	4.3	2.1	38.2	0.0	36.1
	w	0.12	0.27	0	0.09	0.14	0	0.03	0.09	0	0.26	0
BrI	Δ	3.6	0	94.4	9.6	3.5	97.8	10.0	4.6	98.9	2.5	96.8
	w	0.10	0.57	0	0.01	0.10	0	0.00	0.06	0	0.17	0
RD	Δ	4.4	1.5	20.2	6.5	0.3	19.1	6.4	0	18.9	1.3	20.3
	w	0.04	0.16	0	0.01	0.28	0	0.01	0.33	0	0.17	0
FRLF	Δ	4.0	2.2	44.1	5.6	1.5	43.2	5.9	0	42.0	0.2	42.1
	w	0.05	0.11	0	0.02	0.16	0	0.02	0.34	0	0.31	0
RMF	Δ	6.5	1.5	60.8	5.2	2.1	61.5	4.4	0.9	60.2	0	59.4
	w	0.02	0.18	0	0.03	0.13	0	0.04	0.24	0	0.37	0

Table S7. Marginal R-squared values for models in Model Set 3, explaining leaf and root traits/principal components, for the lowest-AICc and treatment-only models. Environmental conditions are: MT= Mean annual Temperature, MM= Mean Moisture index, SM= Seasonality of Moisture. Int.= intercept-only model ($y \sim 1$). All models included population nested within continent and replicate block as random effects (intercepts). LA= ln(leaf area), LM= ln(leaf mass), SLA= ln(specific leaf area), NLv= ln(number of leaves), LMF=logit(leaf mass fraction), RL= $\sqrt{}$ (total root length), RM= $\sqrt{}$ (root mass), SRL= ln(specific root length), BrI= $\sqrt{}$ (Root branching intensity), RD= Average root diameter, FRLF= logit(fine root length fraction), RMF=logit(root mass fraction), PC1= Principal component 1, PC2= Principal component 2.

Response	Lowest-AICc model	Treatment-only model
Leaves: LA	MM+Treatment: 0.444	0.433
LM	MM+Treatment: 0.357	0.353
SLA	Treatment: 0.049	
NLv	MT*Treatment: 0.196	0.152
LMF	Treatment: 0.131	
PC1	MM+Treatment: 0.339	0.329
PC2	SM+Treatment: 0.176	0.169
Roots: RL	MT*Treatment: 0.269	0.209
RM	Treatment: 0.237	
SRL	MT+Treatment: 0.123	0.098
BrI	MT+Treatment: 0.244	0.183
RD	SM+Treatment: 0.087	0.063
FRLF	SM+Treatment: 0.105	0.107
RMF	Treatment: 0.131	
PC1	Treatment: 0.167	
PC2	MT+Treatment: 0.103	0.026

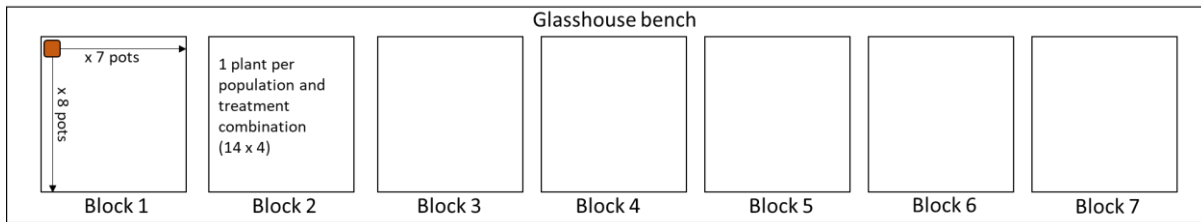


Figure S1. Schematic of experimental set-up, comprising seven blocks on a glasshouse bench, with 56 *Plantago lanceolata* plants in pots per block. The 56 plants in a block comprised four plants per population, one for each treatment combination. Plants representing a population in a specific block were from a single seed family.

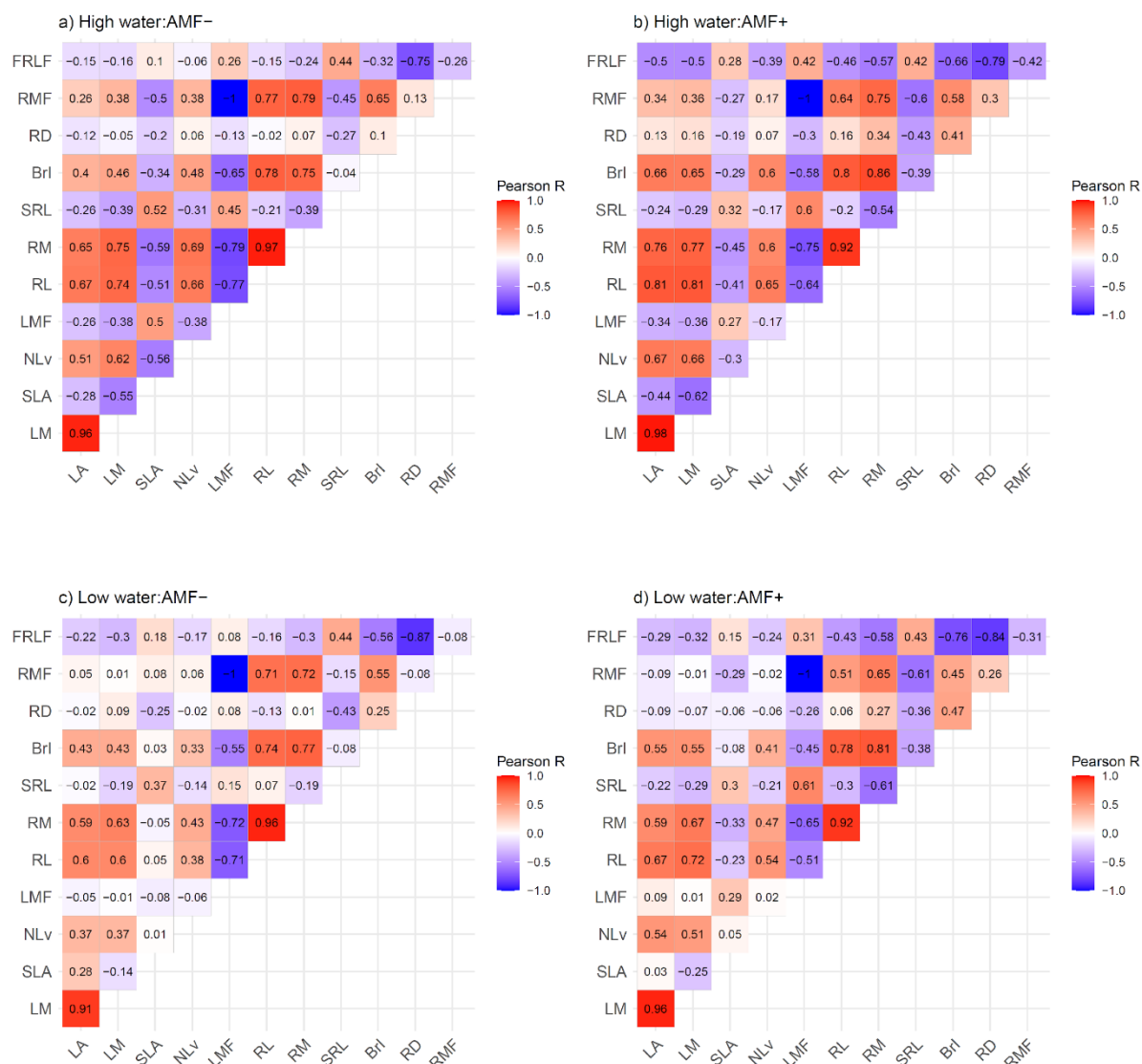


Figure S2. Pearson's correlation coefficients between pairs of leaf and root traits of *Plantago lanceolata* plants within each of the four experimental treatment combinations. LA= ln(leaf area), LM= ln(leaf mass), SLA= ln(specific leaf area), NLv= ln(number of leaves), LMF=logit(leaf mass fraction), RL= $\sqrt{\text{(total root length)}}$, RM= $\sqrt{\text{(root mass)}}$, SRL= ln(specific root length), BrI= $\sqrt{\text{(Root branching intensity)}}$, RD= Average root diameter, FRLF= logit(fine root length fraction), RMF=logit(root mass fraction). The strongest pairwise trait correlation within organs (leaves or roots) were between leaf mass and leaf area ($r \geq 0.91$ for plants in each experimental treatment), and between root mass and root length ($r \geq 0.92$ for plants in each experimental treatment). Specific leaf area was negatively correlated with leaf area and leaf mass under high water treatment ($r= -0.28$ to -

0.62; a, b), but these correlations were weaker or even positive under lower water treatments ($r = 0.28$ to -0.25 ; c, d). Specific root length was negatively correlated with both root mass and root length in all treatments ($r = -0.20$ to -0.61) except low water and without AMF ($r = 0.07$ with root length). Roots with a greater branching intensity also tended to be thinner, but this positive correlation was stronger under high water treatments. Correlations between root and leaf traits were variable, but leaf area and mass, and root length and mass were always positively correlated ($r > 0.5$ for each trait pair and experimental treatment). Specific leaf area and specific root length were always positively correlated ($r = 0.30-0.52$).

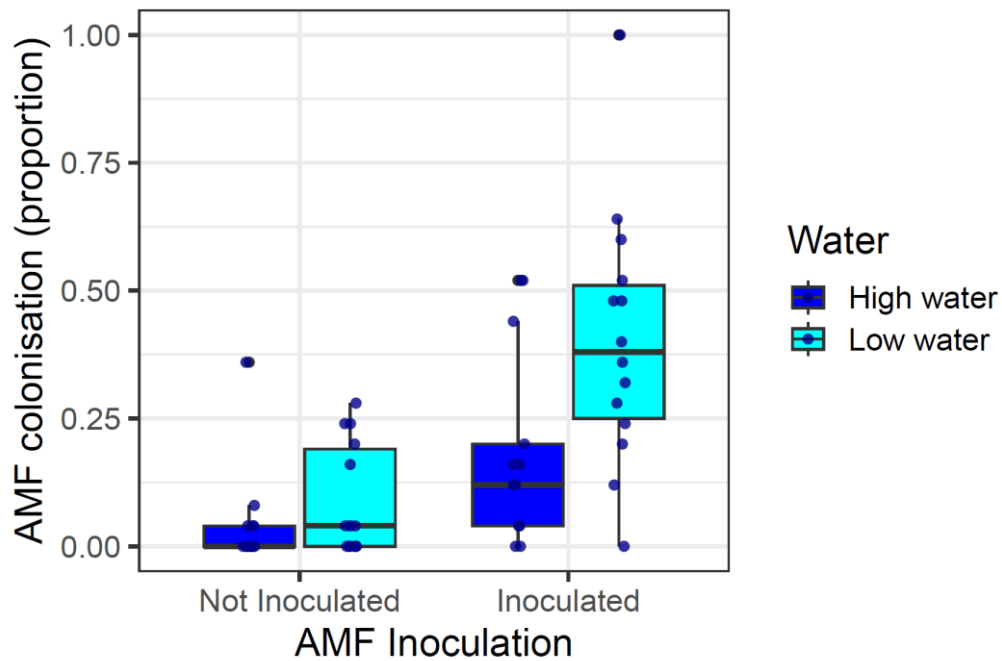


Figure S3. Proportion of root colonised by arbuscular mycorrhizal fungi in roots of *Plantago lanceolata* plants not inoculated or inoculated with AMF, and with high or low water availability. Root colonisation was confirmed with presence of arbuscules, hyphae, vesicles or some combination of structures, within each section of root sample viewed (25 views per sample). N= 14, 14, 13 and 14 for non-AMF:high water, non-AMF:low water, AMF:high water and AMF:low water, respectively. Every population was represented by one sample per treatment combination, with the exception of TNM (missing an AMF:high water sample).

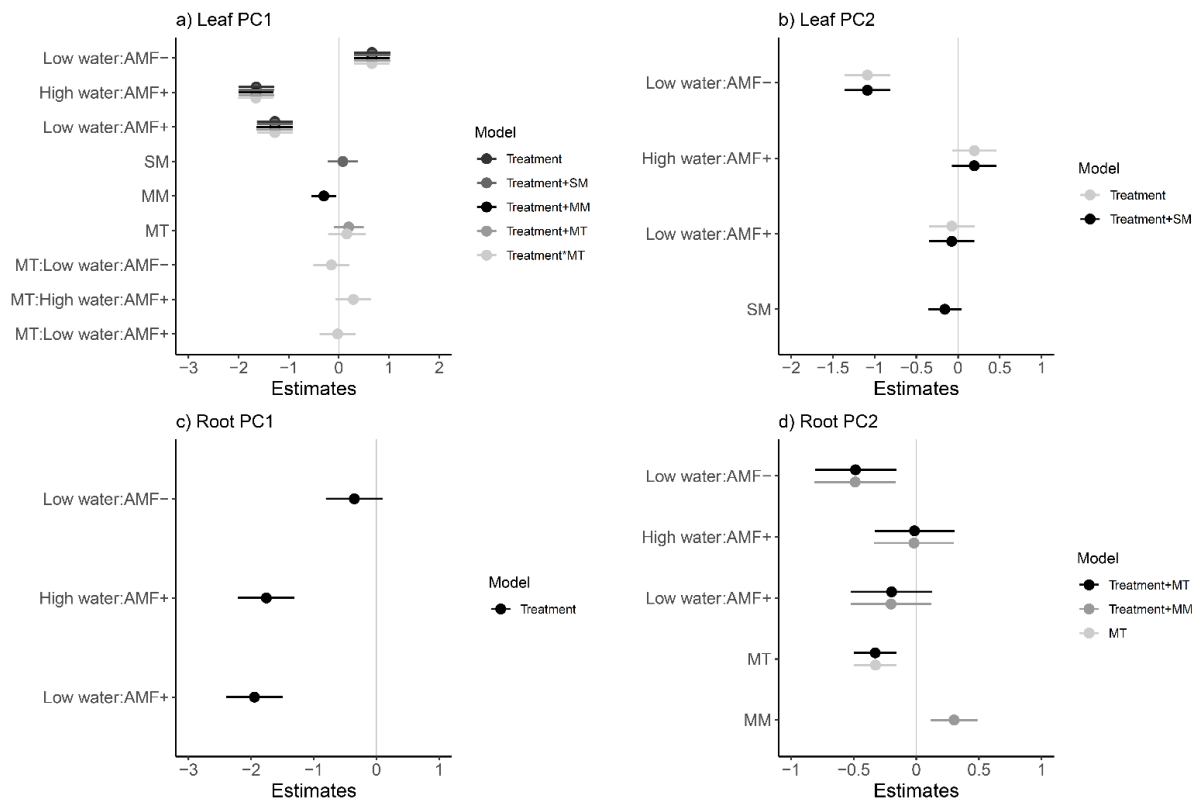


Figure S4. Parameter estimates from the sets of parsimonious models within 6 AICc units of the lowest-AICc model (black circles), explaining principal components 1 (a) and 2 (b) for leaf traits, and principal components 1 (c) and 2 (d) for root traits. Experimental treatment effects are contrasts with plants in the High-water: No AMF inoculation treatment (intercept group). Trait values and source environment conditions are centred on means and scaled to 1 standard deviation to aid effect size comparison. MT= Mean annual temperature, MM= Mean moisture index, SM= Seasonality of moisture. Error bars are 95% confidence intervals.

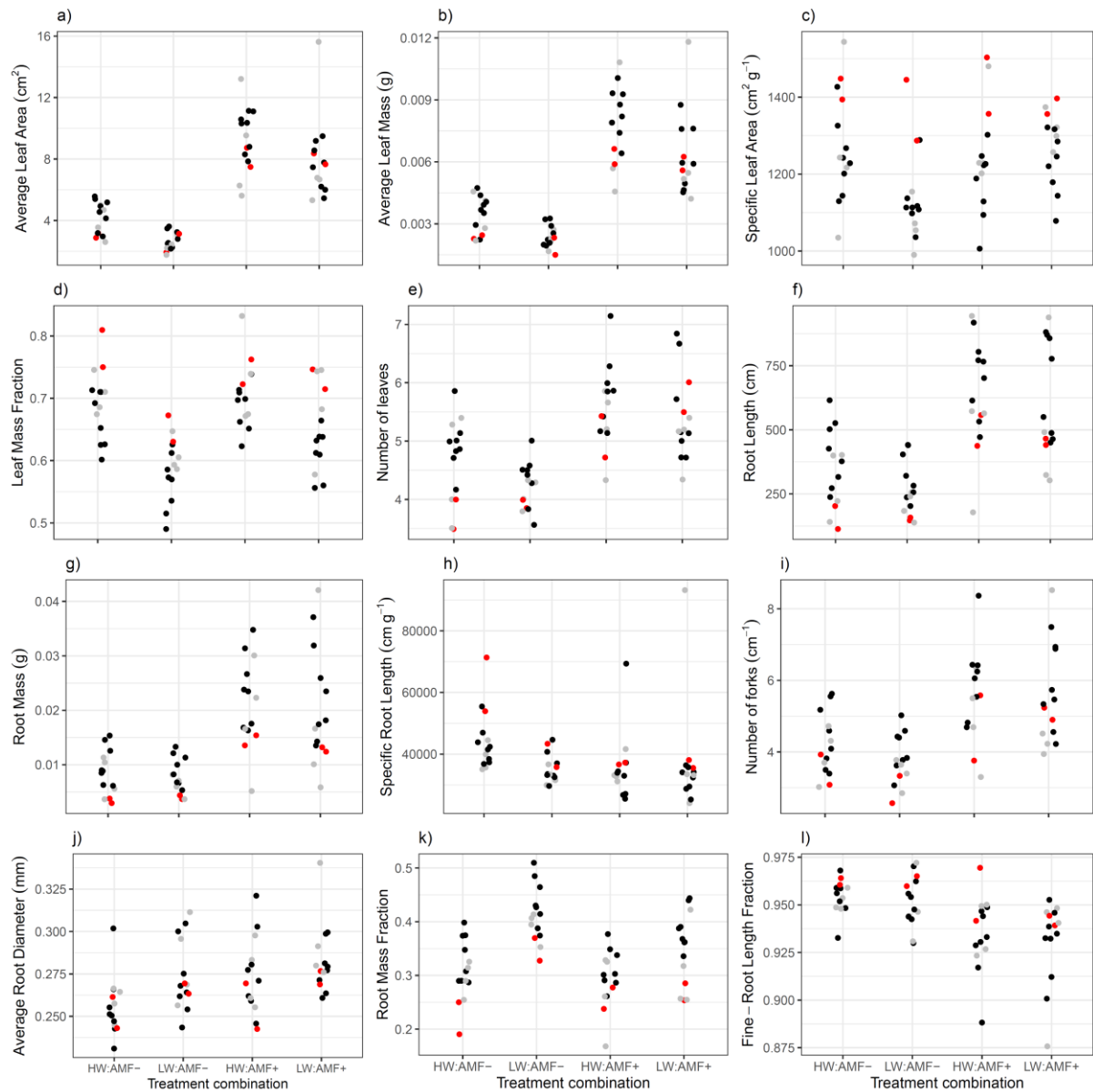


Figure S5. Population mean trait values (untransformed) under each experimental treatment combination (High/Low water availability: AMF presence/absence). Black= European populations; red= North American populations; and grey= Australian populations. See Table 1 for population sample sizes per treatment. a) Average area per leaf, b) Average mass per leaf, c) Specific leaf area, d) Leaf mass fraction, e) Number of leaves, f) Root length, g) Root mass, h) Specific root length, i) Root branching intensity, j) Average root diameter, k) Root mass fraction, and l) Fine root length fraction.

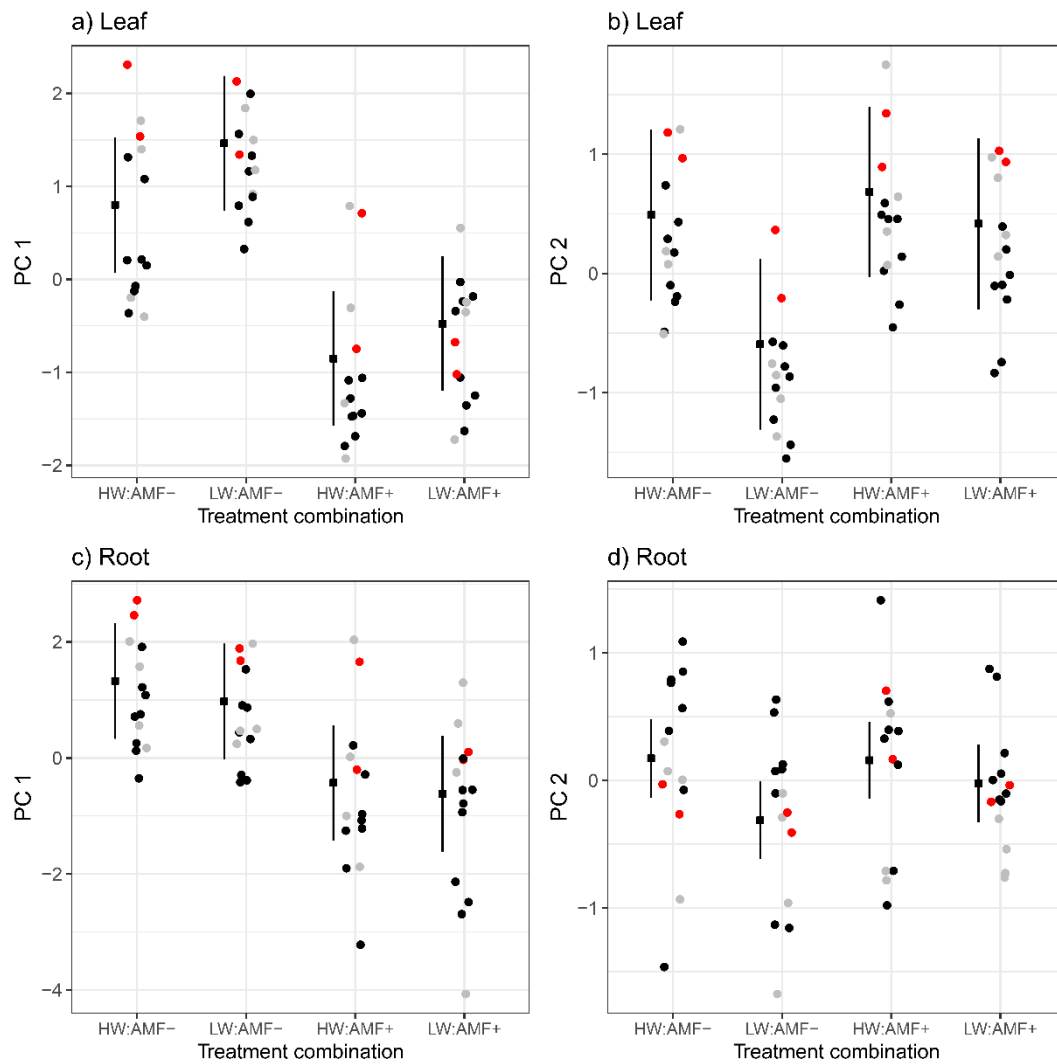


Figure S6. Treatment means and 95% confidence intervals for each of the two principal components representing leaf (a, b) and root (c, d) traits. Treatment combinations include: High water availability (HW), Low water availability (LW), AMF absent (AMF-), AMF present (AMF+). Population means (circles) shown: black= European populations; red= North American populations; and grey= Australian populations. See Table 1 for population sample sizes per treatment.