RESEARCH PAPER

Origin makes a difference: Alternative responses of an AM-dependent plant to mycorrhizal inoculum from invaded and native soils under abiotic stress

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ABSTRACT

- The presence of invasive alien plants (IAPs) alters the composition of soil arbuscular mycorrhizal (AM) fungal communities. Although fundamental for plant development, plant responses to AM from invaded soils have not been widely explored, especially under environmental stress.
- We compared plant growth, P accumulation, root colonization and the photosynthetic responses of the native AM-dependent *Plantago lanceolata* growing in contact with AM fungi from communities invaded by *Acacia dealbata* Link (AM*inv*) or non-invaded communities (AM*nat*) exposed to water and light restriction (shade).
- Under optimal growing conditions, plants in contact with AM*nat* produced higher leaf biomass and accumulated more P. However, plant responses to different AM inocula varied as the level of stress increased. Inoculation with AM*inv* promoted plant growth and root length under light restriction. When plants grew in contact with AM*nat* under drought, leaf P increased under severe water restriction, and leaf and root P increased under intermediate water irrigation. Growing in contact with the AM*nat* inoculum promoted root P content in both full light and light restriction. Colonization rates of *P. lanceolata* roots were comparable between treatments, and plants maintained photosynthetic activity within similar ranges, regardless of the level of stress applied.
- Our results suggest that origin of the inoculum (native soils *versus* invaded soils) did not affect the ability of AM species therein to establish effective mutualistic associations with *P. lanceolata* roots but did influence plant responses depending on the type and level of the abiotic stress.

INTRODUCTION

One of the main consequences and characteristics of the Anthropocene is the unprecedented increase in biological invasions in response to large-scale phenomena, such as globalization, intensification of international trade and tourism and soil degradation. With consequences at the local, regional and global scale, the spread of invasive alien plants (IAPs) reduces and eliminates native species, limits plant biodiversity, modifies soil physicochemical parameters, nutrient cycling, water regimes and decreases the provision and quality of ecosystem services (Vilá *et al*. 2011; Pyšek *et al*. 2012; Simberloff *et al*. 2013; Banks *et al*. 2015). At the soil level, the presence of IAPs interferes with the structure and function of microbial communities, altering symbiotic relationships and the soil–plant exchange system (Van der Putten *et al*. 2007; Pringle *et al*. 2009; Vogelsang & Bever 2009; Tanner & Gange 2013; Inderjit & Cahill 2015).

When IAPs arrive in new territories they create novel or selective associations with soil microbial species (Richardson *et al*. 2000a; Moora *et al*. 2011; Nuñez & Dickie 2014; Kamutando *et al*. 2017; Le Roux *et al*. 2017) or bring their own mutualists (Correia *et al*. 2019; Kamutando *et al*. 2019). Soil microbiota and mycorrhiza in particular play a crucial role in determining the abundance and invasiveness of plant species (Levine *et al*. 2004). Arbuscular mycorrhizal (AM) fungi are located at the soil–root interface, expanding the range of plant influence and acting as intermediaries between plants and the soil matrix (Richardson *et al*. 2009). Through a symbiotic relationship established with more than 80% of terrestrial plants (Smith & Read 2010), AM fungi provide essential soil nutrients, mainly P and N, but also facilitate access and uptake of NH_4^+, NO_3^-, Zn, Cu and K (Mathur *et al*. 2019) in exchange for C compounds derived from photosynthesis. Besides their contribution to plant nutrition, AM fungi also serve as a first defence, increasing plant resistance to biotic and abiotic stresses (Hajiboland *et al*. 2019; Diagne *et al*. 2020).

Such AM communities are fundamental to maintaining forest soil balance as they can represent ecological barriers to limit the entry and spread of IAPs (Janos *et al*. 2013). Nevertheless,

their role in the invasion process can vary, acting as facilitators (positive effect), inhibitors (negative effect) or having a neutral effect (Klironomos 2003; Levine *et al.* 2004; Shah *et al.* 2009). Reciprocally, once established in the novel community, IAPs may, in turn, affect the structure of AM fungal communities (Cantor *et al.* 2011; Meinhardt & Gehring 2012; Guisande-Collazo *et al.* 2016), generally decreasing the abundance of native mycorrhiza and altering the structural composition of the soil fungal community (Grove *et al.* 2017).

The AM fungi are ubiquitous and widely distributed among almost all terrestrial environments, but interactions with plants become especially relevant under stress conditions. Multiple studies indicate that AM fungi help plants to deal with drought stress (Augé 2001; Augé & Moore 2005; Boomsma & Vyn 2008; Zhu *et al.* 2012; Endresz *et al.* 2015; Begum *et al.* 2019; Mathur *et al.* 2019). Drought stress reduces plant growth, mainly by reducing photosynthetic capacity (Teskey *et al.* 2015; Mathur *et al.* 2019). In this sense, the association with AM fungi alleviates drought stress by regulating hormone balance, increasing water absorption through hyphae (Augé *et al.* 2007), contributing to osmotic adjustment (Porcel & Ruiz-Lozano 2004), expanding antioxidant activity or increasing nutrient absorption (Fernández-Lizarazo & Moreno-Fonseca 2016). Plant association with AM fungi can also maintain photosynthetic capacity in shaded conditions (Shukla *et al.* 2009), improving responses to light deficiency (Liu *et al.* 2015; Koorem *et al.* 2017). On the other hand, light reduction limits C gain by the AM fungi due to a decrease in photosynthetic efficiency of host plants (Liu *et al.* 2015). Consequently, shady conditions can be further responsible for changes in AM community composition (Van Diepen *et al.* 2011; Shi *et al.* 2014; Liu *et al.* 2015).

Acacia dealbata Link is a N_2-fixing highly invasive species, native to Australia and Tasmania, that currently invades Mediterranean ecosystems in South Africa, South America and Europe (Fuentes-Ramirez *et al.* 2011; Richardson & Rejmanek 2011; Souza-Alonso *et al.* 2017). Here, *A. dealbata* severely impacts plant biodiversity, alters habitat conditions (including light intensity or water regime), soil physical-chemical properties and nutrient cycling (Fuentes-Ramírez *et al.* 2011; Lazzaro *et al.* 2014; Souza-Alonso *et al.* 2014; Kamutando *et al.* 2017; Lorenzo *et al.* 2017). Besides reducing aboveground plant diversity (Fuentes-Ramírez *et al.* 2011; Lorenzo *et al.* 2012; Lazzaro *et al.* 2014), the presence of *A. dealbata* also causes significant changes to the structure and function of the soil microbial community (Lorenzo *et al.* 2010; Souza-Alonso *et al.* 2015; Kamutando *et al.* 2017, 2019). Across geographical regions and nutritional levels, *A. dealbata* establishes relationships with different soil bacterial and fungal communities (Kamutando *et al.* 2017, 2019). In general, the *Acacia* genus associates with AM and ectomycorrhizal (EM) fungi (Brundrett 2009), but although both types of mycorrhizae have been identified in its rhizosphere (Kamutando *et al.* 2017), *A. dealbata* does not obtain clear benefits from the association with AM fungi (Crisóstomo 2012). However, even when their mycorrhizal dependence is low, IAPs generally succeed in competition with obligate mycorrhizal plants by disturbing local AM communities (Mummey and Rillig 2006; Vogelsang *et al.* 2006; Pringle *et al.* 2009; Vogelsang & Bever 2009).

The lower abundance of AM species (Kamutando *et al.* 2017) or changes in the community structure after *A. dealbata* invasion (Guisande-Collazo *et al.* 2016) represent direct consequences of the invasion process. Novel AM communities in soils invaded by *A. dealbata* reduced plant growth, P acquisition and physiological activity of AM-dependent plants (Guisande-Collazo *et al.* 2016). However, how these structural changes affect plant establishment under different environmental conditions has not been explored to date. In this work, we hypothesize that the origin of the AM community influences the growth and development of plants exposed to different environmental stresses. Therefore, we compared growth of the mycorrhizal-dependent *Plantago lanceolata* growing in contact with AM fungi obtained from areas invaded by *A. dealbata* with plants growing with inoculum from a local, non-invaded plant community, when plants were exposed to different environmental stresses.

MATERIAL AND METHODS

Experimental design: water and light stress experiments

The experiment was arranged in a fully randomized design. Seedlings of *P. lanceolata* were treated with two different inoculum sources (AM fungi from invaded and non-invaded soils) and exposed to different stress conditions (water and light stress) as independent variables. These variables were applied separately, and therefore the study was structured in two parts that were carried out at the same time. The water regime reduction was considered as Experiment 1 and light reduction as Experiment 2. Water limitation and light deficit were selected as treatments since both stresses are naturally observed in areas invaded by *A. dealbata* (de Neergard *et al.* 2005; Lorenzo *et al.* 2017).

In Experiment 1, three irrigation levels were applied to induce water stress: irrigation at field capacity (control level, W_{100}), half of field capacity (moderate stress, W_{50}) and a quarter of field capacity (high stress, W_{25}). The selected water regime was based on previous experiences in evaluating water limitation on the growth of AM-inoculated plants (Mathur *et al.* 2019). In Experiment 2, two light intensities were applied: full light and light reduction (shade). To simulate effective light reduction, *P. lanceolata* seedlings were placed inside a metallic structure ($2 \times 1 \times 1$ m length/width/height) covered by a shading net (polyethylene mesh, 1×2 mm) resulting in a reduction of light intensity similar to that observed under the canopy of *A. dealbata* (80% PAR reduction; Lorenzo *et al.* 2012). Ten replicates per treatment were established for Experiment 1 (2 inoculum sources $\times$ 3 levels of water reduction $\times$ 10 replicates, n = 60) and Experiment 2 (2 inoculum sources $\times$ 2 levels of light intensity $\times$ 10 replicates, n = 40).

The experimental inoculation was carried out using field-collected plant roots as the natural source of AM inoculum (Klironomos & Hart 2002; Gu *et al.* 2011; Hassan *et al.* 2013; Ba *et al.* 2018) as these are considered viable infection units (Smith & Read 2010). Thus, AM inoculum was created from roots obtained from two different sources: native inoculum (roots from a mixture of native shrub species, hereinafter AM*nat*) and invasive inoculum (roots of *A. dealbata*, AM*inv*). To create the inoculum, roots of native and invasive species were collected during June 2017 in two nearby locations that previously shown structural differences in the AM fungi community (Guisande-Collazo *et al.* 2016): (i) an area fully occupied by *A. dealbata* (Marcosende, Spain; 42°09'58.3" N,

8°41'23.1" W) and (ii) a native shrubland without the presence of *A. dealbata* (Morgadáns, Spain; 42°06'54.7" N, 8°43'15.9" W). Prior to *A. dealbata* invasion, both locations exhibited similar plant communities typical from Atlantic shrublands, composed of a mixture of annual grasses and perennial shrubs. At each location, small diameter (<0.5 cm) live roots from at least 25 different individuals were collected using a shovel and scissors and transported to the laboratory for preservation (4 °C). The AM*inv* inoculum was exclusively formed of fine roots obtained from *A. dealbata*, whereas the AM*nat* was formed of roots obtained from a selection of different native species (Table S1).

Experimental set-up

The soil used as substrate for the greenhouse assay was collected in June 2017 from an agricultural field (Ribadelouro, Spain; 42°06'12.1" N, 8°39'13.0" W). Soil from the top layer (upper 20 cm) was randomly collected at 30 different points, stored in polyethylene bags and transported to the laboratory for further processing. Once in the laboratory, the soil was air-dried for 1 week and sieved (0.5 cm mesh) to remove roots, small stones, coarse debris and other particles. Textural analysis classified the soil as sandy loam, and the following physical-chemical properties were obtained: pH $_{H2O}$ (1:2.5, w:v) = 5.7; electrical conductivity (EC, 20 g soil saturated with distilled water) <42 mS cm^{-1}; bulk density 1.21 g cm^{-3} (Carter & Gregorich 2007); organic matter 10.8%; total N 0.41% and available P 36 ppm (Olsen method; Page *et al.* 1982); assimilable K$^+$ 362 ppm and exchangeable Mg^{+2}, Ca^{+2}, Na$^+$, K$^+$ and Al^{3+} of 0.66, 2.40, 0.10, 0.90, 1.10 cmol$_{(+)}$ kg^{-1}, respectively (Page *et al.* 1982).

To prepare an adequate substrate for plant growth, the soil was mixed with perlite (2–6 mm; Gramme Flower, Germany) in a ratio of 1:2 (soil:perlite, v/v) to reduce compaction produced by sterilization and to improve plant growth (Guisande-Collazo *et al.* 2020). The mixture of soil and perlite was introduced into polyethylene bags (20 × 30 cm) and sterilized by autoclaving (121 °C, 20 min) for three consecutive days to inactivate the microbial community (Emam *et al.* 2014; Liao *et al.* 2015; Li *et al.* 2017). Roots of *A. dealbata* and of native species were then cut into small fragments (±1 cm) and homogeneously mixed with the autoclaved soil:perlite mixture in a 1:180 (root weight:soil weight) ratio. Then, plastic pots (5 × 5 × 15 cm), sterilized with 80% ethanol and UV light (30 min), were filled under sterile conditions (under laminar flow cabinet; FASTER, Italy).

Seeds of *P. lanceolata* were sterilized in a sodium hypochlorite solution (1%) and germinated in a growth chamber (72 h, 25 °C, dark conditions). After which two seedlings were planted in each pot and kept for 4 weeks under greenhouse conditions, without stress conditions, until the seedlings reached 5 cm (±1 cm) in height. After acclimatization, water restriction (Experiment 1) and light reduction (Experiment 2) were applied to *P. lanceolata* seedlings. Every 3 days, pots were watered with respective volumes of tap water to reach 100%, 50% and 25% of field capacity to maintain water regimes. Plants were maintained under semi-controlled conditions in a greenhouse at the University of Vigo for 12 weeks (average 19 °C, relative humidity 72%).

Plant harvest and biometric measurements, nutrient content and mycorrhizal colonization

The day before harvest, different fluorescence parameters were measured: the fraction of light energy captured by PSII used to synthesize ATP and NADPH (Φ_{II}), the fraction of energy lost through unregulated processes (a proxy to evaluate photosynthetic efficiency) (Φ_{NO}), loss of regulated energy in the form of heat (Φ_{NPQ}), maximum performance of PSII in the light-adapted state (F_v'/F_m') and electron flow of the antenna complexes (LEF). Fluorescence parameters have previously been used to evaluate plant physiological responses in AM inoculation experiments (Endresz *et al.* 2015; Guisande-Collazo *et al.* 2016; Mathur *et al.* 2019). Fluorescence parameters were individually measured in the first fully developed leaf using the Multispec Q fluorimeter (version 1.0; PhotosynQ platform).

Before harvesting, the number of fully developed leaves was counted, and plants were carefully removed from the pots. Aerial and root length were measured for all individuals, then plants were randomly separated into two groups of equal size for destructive measurements. One group was used to measure biomass and nutrient content (leaves and roots), while the other group was used to estimate root colonization and osmolarity. Total biomass was measured by drying plant material (70 °C) until constant weight. Specific leaf area (SLA) was calculated as the leaf length/leaf DW ratio, where DW represents leaf dry weight. Biomass partitioning was calculated as leaf mass fraction (LMF = leaf DW/total plant DW) and root mass fraction (RMF = root DW/total plant DW).

To evaluate AM fungi colonization, the roots of five plants were separated, washed under tap water and cut into small fragments (±1 cm). Root fragments were stained following the Phillips & Hayman (1970) method, modified by Koske & Gemma (1989). Initially, fragments were rinsed in 2.5% KOH (1 h, 90 °C), washed and acidified in 1% HCl overnight. Fragments were then stained in acidified Coomassie blue solution (1 h, 60 °C), rinsed and transferred to a solution of glycerol:distilled water:lactic acid (85%) at a ratio of 50:48:2 (v/v/v). Root colonization was evaluated using a modified gridline intersection method (McGonigle *et al.* 1990). Each root fragment was examined to find where intersections cut any arbuscules, vesicles and hyphae at 100 equidistant points on each root fragment. Thus, intersections were counted in the following categories: negative (no fungal presence), arbuscules, vesicles and hyphae. Presence of arbuscules, vesicles and hyphae was calculated by dividing the presence of each structure by the total number of intersections examined. Total colonization was calculated as the proportion of positive intersections.

Plant phosphorus (P) content was measured using 0.5 g dry plant material (leaves and roots), which was digested in an acidic solution of HCl:HNO$_3$ and subsequently calcined (3 h, 550 °C). After that, total P was quantified in the extract using ICP-OES (Perkin Elmer Optima 4300 DV; PerkinElmer Inc., Waltham, MA, USA). Throughout the text, the term *phosphorus content* will be used to refer the amount of P (in g kg^{-1}) of plant dry weight. Leaf fragments of *P. lanceolata* plants were used to measure osmolarity, calculated based on total osmolyte content. Fragments were placed in a disposable plastic syringe (10 ml) and frozen at −20 °C until analysis. The syringe content was then pressed and 100 µl of the extract and collected into an Eppendorf tube (2 ml). The osmolarity was then

measured in the solution using a Löser Type 6 cryoscopic osmometer.

Statistical analysis

Data normality and the homogeneity of variances were initially explored using the Kolmogorov-Smirnov (K-S) test and Levene's test, respectively. To identify the effect of the independent variables (stress level and inoculum origin) on the dependent variables (aerial and root length, biomass, leaves, SLA, LMF, RMF, total P, fluorescence parameters, osmolarity and root colonization), data were analysed with a two-way ANOVA, using Tukey's HSD or Dunnet's T3 (where variances were not homogeneous) as the *post-hoc* tests for treatment comparison. When interactions between independent variables were found, the effects were investigated through pairwise comparisons using Tukey's HSD test (water stress = 3 levels) or Student's *t*-test (light stress = 2 levels). The statistical analyses were performed using IBM SPSS Statistics version 23.0 (Chicago, IL, USA) software for Macintosh.

RESULTS

Experiment 1. Water stress

Fluorescence parameters
The results of the two-way ANOVA indicated that the irrigation level affected fluorescence parameters Φ_{II} ($P = 0.005$),

Φ_{NPQ} ($P = 0.001$) and F_v'/F_m' ($P = 0.002$). Also, LEF was affected by the interaction between the water regime and the inoculum ($P = 0.026$) (Table 1). *Plantago lanceolata* showed similar F_v'/F_m' values under the different water regimes when grown in contact with AM*nat*. In contrast, plants that were associated with AM*inv* showed a decrease in the F_v'/F_m' value after exposure to severe water reduction (W$_{25}$), compared to moderate reduction (W$_{50}$) and well-watered (W$_{100}$) plants (Table 2). Nevertheless, F_v'/F_m' values were generally maintained in an adequate range (>0.75). On the other hand, Φ_{II} values were similar in plants treated with AM*inv*, and significant differences were evidenced when AM*nat* inoculum was used: decreasing with low irrigation levels compared to the W$_{100}$ and W$_{50}$ (20%; $P = 0.03$). The effect of AM*inv* on plants grown with water limitation was evident in Φ_{NPQ} values, which increased significantly under severe water restriction (W$_{25}$). Finally, exposure to moderate and severe water restriction reduced LEF when plants grew in contact with AM*nat*. Under moderate irrigation, LEF values increased significantly in plants grown with AM*inv* compared to plants grown in contact with AM*nat*.

Biometric parameters, P content, osmolarity and root colonization
The two-way ANOVA indicated that some variables related to plant growth were affected by water restriction, such as the number of leaves, leaf length, leaf biomass, LMF, RMF and SLA (Table 1). Moreover, osmolarity and leaf P content were also

Table 1. Two-way ANOVA results for Experiment 1, including independent variables (water reduction and inoculum origin) and their interaction for the studied variables: fluorescence, plant growth parameters, P content and mycorrhizal colonization.

	Water level (W)		Inoculum (I)		W × I	
	$F_{(x,y)}$	P	$F_{(x,y)}$	P	$F_{(x,y)}$	P
Fluorescence parameters						
ϕ_{II}	5.905	**0.005**	0.323	0.573	2.596	0.086
ϕ_{NPQ}	11.138	**≤0.001**	0.944	0.337	1.143	0.329
ϕ_{NO}	0.061	0.941	1.768	0.191	2.735	0.076
F_v'/F_m'	7.118	**0.002**	1.782	0.189	1.270	0.291
LEF	7.697	**≤0.001**	1.648	0.206	4.002	**0.026**
Plant growth parameters						
Leaves (n)	37.244	**≤0.001**	0.675	0.415	0.071	0.931
Leaf length (cm)	21.042	**≤0.001**	1.669	0.202	1.313	0.278
Root length (cm)	0.062	0.940	0.614	0.437	0.092	0.912
Leaf biomass (g)	11.382	**≤0.001**	0.334	0.566	1.090	0.358
Root biomass (g)	2.246	0.116	0.000	0.992	0.238	0.789
LMF	8.617	**≤0.001**	0.113	0.738	0.049	0.952
RMF	8.617	**≤0.001**	0.113	0.738	0.049	0.952
SLA (m^2 kg^{-1})	9.201	**≤0.001**	0.059	0.809	0.039	0.962
Osmolarity (mosm kg^{-1} H$_2$O)	31.364	**≤0.001**	1.689	0.216	1.014	0.390
Phosphorus (P) content (g kg^{-1})						
Leaves	45.238	**≤0.001**	3.417	0.087	0.194	0.826
Roots	249.614	**≤0.001**	47.585	**≤0.001**	29.223	**≤0.001**
Mycorrhizal colonization (%)						
Colonization	0.103	0.958	1.171	0.682	1.979	0.140
Arbuscules	4.953	**0.007**	1.310	0.262	1.736	0.182
Hyphae	2.406	0.088	0.185	0.671	4.252	**0.014**
Vesicles	3.335	**0.020**	0.150	0.702	0.900	0.454

Values in bold indicate significant differences at $P < 0.05$. Quantum yield of PSII (Φ_{II}), quantum yield of non-photochemical quenching (ϕ_{NPQ}), quantum yield of other unregulated (non-photochemical) losses (ϕ_{NO}), maximum quantum yield of PSII primary photochemistry in the light-adapted state (F_v'/F_m'), Linear Electron Flux (LEF), specific leaf area (SLA, and P) content in leaves and roots, leaf mass fraction (LMF) and root mass fraction (RMF).

Table 2. Mean (±SE) of different fluorescence parameters: quantum yield of PSII (Φ_{II}), quantum yield of non-photochemical quenching (ϕ_{NPQ}), quantum yield of other unregulated (non-photochemical) losses (ϕ_{NO}), maximum quantum yield of PSII in light-adapted state (F_v'/F_m') and linear electron flux (LEF) in *P. lanceolata* plants under different levels of water reduction (W_{100} = field capacity, W_{50} = 50% field capacity, W_{25} = 25% field capacity) and light reduction (direct light, shade).

	W_{100}		W_{50}		W_{25}	
	AM*nat*	AM*inv*	AM*nat*	AM*inv*	AM*nat*	AM*inv*
Water reduction						
ϕ_{II}	0.48 (±0.02) a	0.49 (±0.019)	0.48 (±0.02) a	0.46 (±0.01)	0.40 (±0.03) b	0.45 (±0.02)
ϕ_{NPQ}	0.18 (±0.09)	0.14 (±0.01) B	0.13 (±0.0.02)	0.16 (±0.02) AB	0.20 (±0.05)	0.22 (±0.06) A
ϕ_{NO}	0.35 (±0.07)	0.36 (±0.06)	0.35 (±0.08)	0.38 (±0.04)	0.41 (±0.09)	0.33 (±0.04)
F_v'/F_m'	0.77 (±0.02)	0.78 (±0.01) A	0.78 (±0.01)	0.77 (±0.01) A	0.76 (±0.03)	0.75 (±0.03) B
LEF	67.20 (±12.6) a	61.10 (±12.4)	46.9 (±6.7) b	66.20 (±24.2)*	41.80 (±8.4) b	48.50 (±5.9)

	Light		Shade	
	AM*nat*	AM*inv*	AM*nat*	AM*inv*
Light reduction				
ϕ_{II}	0.48 (±0.02)	0.49 (±0.02)	0.43 (±0.02)**	0.50 (±0.015)
ϕ_{NPQ}	0.18 (±0.03)	0.14 (±0.005)	0.18 (±0.01)	0.15 (±0.01)
ϕ_{NO}	0.35 (±0.02)	0.36 (±0.02)	0.39 (±0.01)*	0.35 (±0.01)
F_v'/F_m'	0.77 (±0.006)	0.78 (±0.003)	0.77 (±0.004)	0.77 (±0.003)
LEF	67.23 (±4.23) a	61.07 (±4.14) A	41.20 (±1.7) b,***	31.30 (±1.48) B

Capital letters indicate significant differences between plants inoculated with AM*inv* and lowercase letters indicate significant differences in plants inoculated with AM*nat* after ANOVA using Tukey's HSD or Student's *t*-test at $P \leq 0.05$. Asterisks indicate significant differences between AM*nat* and AM*inv* plants within the same stress level at $*P \leq 0.05$, $**P \leq 0.01$ and $***P \leq 0.001$.

affected by the water regime. In the case of root P content, there was a significant interaction between factors. The reduction in water supply caused a significant decrease in leaf length and biomass of *P. lanceolata*, regardless of the inoculum origin (Fig. 1). However, the reduction in leaf length and biomass of plants growing in contact with AM*inv* was only evident when a severe water reduction was applied (W_{25}). The different irrigation levels did not affect root length and root biomass, regardless of the inoculum used.

Leaf mass fraction (LMF) showed a progressive decrease in response to water supply reduction, being more pronounced in plants treated with AM*inv*. In contrast, RMF increased as the water content decreased (Table 3), which also led to a reduction in the number of leaves and the SLA of plants under water restriction. Both parameters showed a differential reduction trend according to the water regime applied $W_{100} > W_{50} = W_{25}$ (for SLA) and $W_{100} = W_{50} > W_{25}$ (for number of leaves).

Total P showed similar trends in both leaves and roots of *P. lanceolata*, regardless of the inoculum applied (Fig. 2). In general, there was a progressive reduction in total P as the water restriction increased. However, leaf P content remained stable under intermediate and severe water regimes ($W_{50} = W_{25}$) when plants grew in contact with AM*nat*. In addition, differences in P content were evident between AM*inv* and AM*nat*: a significant P leaf reduction was observed when plants were exposed to W_{50} (20%; $P = 0.039$) and W_{25} (20%; $P < 0.001$), while root P was reduced even at optimal irrigation levels, W_{100} (22%; $P < 0.001$) and W_{50} (8%; $P < 0.001$). Osmolarity increased progressively as the water regime decreased (Fig. 3). However, plants associated with AM*nat* showed better responses in osmolyte accumulation ($W_{100} = W_{50} < W_{25}$) compared to plants grown with AM*inv* ($W_{100} < W_{50} < W_{25}$).

Although no significant differences in AM colonization were detected in roots of plants subjected to different water regimes, colonization percentages were, in all cases, >60% (Fig. 4). Under severe water restriction (W_{25}), plants grown in contact with AM*nat* had a significant increase in the presence of arbuscules (%) compared to plants under moderate water restriction (W_{50}) or no restriction (W_{100}). Despite a decreasing trend in hyphae and vesicles as the water stress level increased, no significant differences were observed (Fig. 4).

Experiment 2. Light stress

Fluorescence measurements
As in Experiment 1, the two-way ANOVA results indicated that sunlight reduction significantly affected the dependent variables (Table 4). Light reduction affected LEF ($P < 0.001$), whereas the origin of the inoculum influenced LEF ($P = 0.004$) and Φ_{II} ($P = 0.036$). The LEF decreased significantly in plants growing in the shade (Table 2) and, in turn, in those plants growing in contact with the AM*inv*. Additionally, under shade conditions, Φ_{II} values increased when *P. lanceolata* was grown with AM*inv*, while the opposite was observed for Φ_{NO}.

Biometric measurements, P content and root colonization
Leaf biomass, root biomass, LMF, RMF and leaf and root P content were affected by the independent factors (Table 4). Leaf length, SLA and root P were also affected by the interaction between both factors.

Shade conditions influenced *P. lanceolata* plants differently, depending on the origin of the inoculum. In contact with AM*inv*, leaf length (40%; $P < 0.001$) and biomass (48%; $P = 0.044$) increased significantly, while root length (31%;
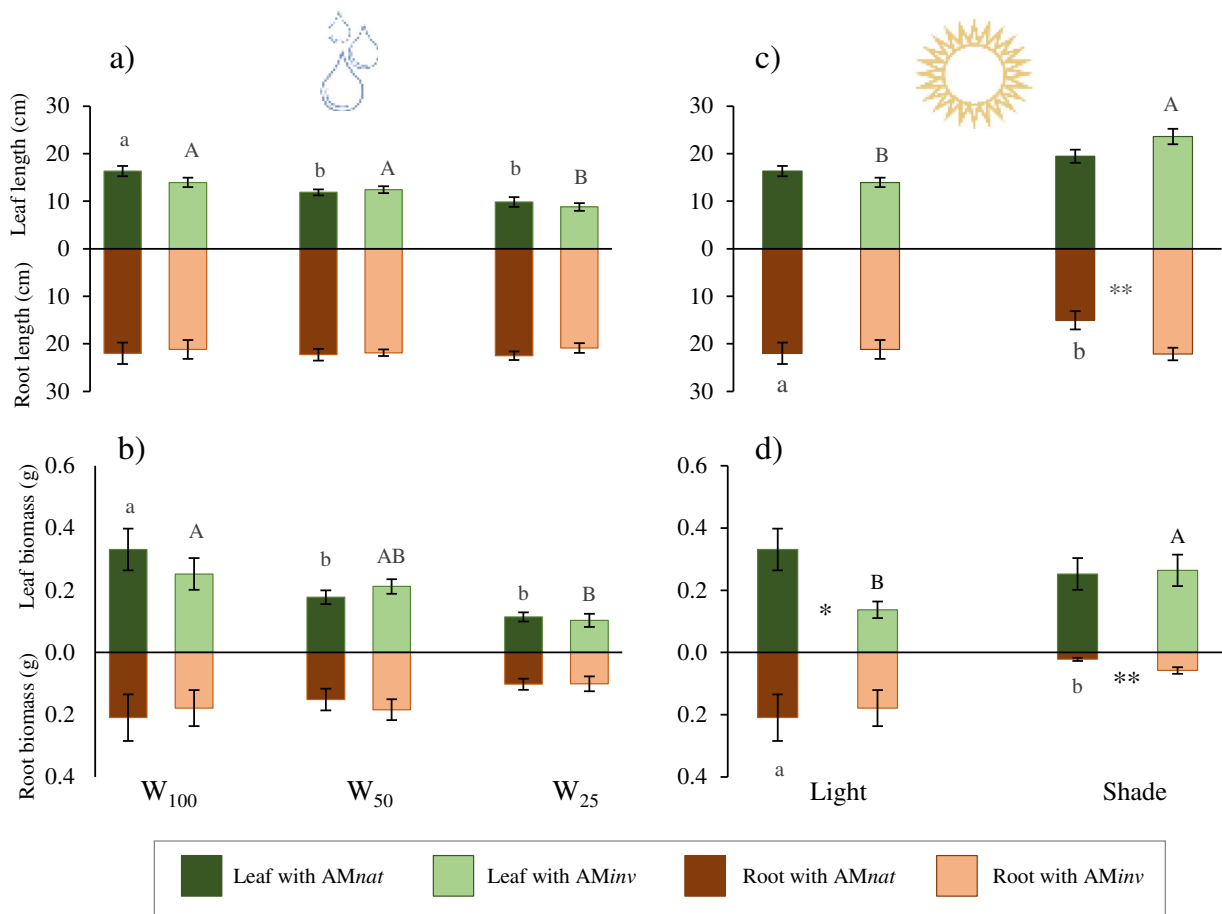
Fig. 1. Mean (±SE) of (a) leaf and root length, (b) leaf and root biomass under water reduction (W_{100} = field capacity, W_{50} = 50% field capacity, W_{25} = 25% field capacity) and (c) leaf and root length, (d) leaf and root biomass under light reduction (direct light or shade). Capital letters indicate significant differences between plants inoculated with AM*inv* and lowercase letters indicate significant differences in plants inoculated with AM*nat* after ANOVA using Tukey's HSD test or Student's *t*-test at $P \leq 0.05$. Asterisks indicate significant differences between AM*nat* and AM*inv* plants within the same stress level at *$P \leq 0.05$, **$P \leq 0.01$ and *** $P \leq 0.001$.

$P = 0.031$) and root biomass (90%; $P = 0.023$) were reduced in plants treated with AM*nat* (Fig. 1). Under direct light exposure, plants grown in contact with AM*nat* had higher leaf biomass (Fig. 1). In contrast, under shade conditions, root length and root biomass of plants growing with AM*inv* were significantly enhanced compared to those with AM*nat*. Sunlight reduction increased LMF and SLA while decreasing RMF in plants grown in contact with AM*nat* (Table 3).

Regardless of the level of light exposure, root P content in plants treated with AM*nat* was significantly higher than in plants treated with AM*inv*. Plants that grew in shade had decreased aerial P in contact with AM*inv* but, in contrast, had increased root P (Fig. 2). These plants did not show significant differences in root colonization, percentage of arbuscules, hyphae or vesicles, regardless of the inoculum origin (Fig. 4). As in Experiment 1, colonization percentages were, in all cases, >60%.

DISCUSSION

From a simplistic perspective, mycorrhizae are often perceived as mere appendages to obtain valuable resources: a symbiotic association to exchange part of the photosynthetic production

in return for essential nutrients. However, the role of mycorrhizae and mycorrhizal networks on ecosystem configuration is central by regulating competition, colonization, interplant resource transfers and cross-scale ecosystem interactions (Simard *et al.* 2012). The mycorrhizal contribution is particularly relevant for plant growth under environmental stress conditions (Brooker *et al.* 2008). Plant responses depend largely on their symbiotic relationships and, consequently, it would be expected that responses to inoculation with a novel AM consortium would vary depending on plant stress level (Bever 2002). Therefore, we aimed to address the mycorrhizal influence on a dependent plant under different water and light regimes, simulating limiting conditions of habitats transformed by *A. dealbata*. In general, we observed that both light and water restriction influenced plant growth and responses, but *P. lanceolata* was also affected, to some extent, by the origin of the AM inoculum.

Photosynthesis and plant growth

The establishment of mutualist relationships with different AM sources modulates plant physiological responses, *e.g.* by alleviating structural and functional damage to the PSII reaction

Table 3. Values (mean ± SE) of leaf mass fraction (LMF), root mass fraction (RMF), specific leaf area (SLA) and true leaves of *P. lanceolata* under different levels of water reduction (W$_{100}$ = field capacity, W$_{50}$ = 50% field capacity, W$_{25}$ = 25% field capacity) and light reduction (direct light, shade).

	Inoculum	W$_{100}$	W$_{50}$	W$_{25}$
Water Reduction				
LMF	Native	0.697 (±0.055) a	0.581 (±0.033) ab	0.548 (±0.029) b
	Invasive	0.695 (±0.05) A	0.557 (±0.029) B	0.543 (±0.03) B
RMF	Native	0.30 (±0.055) b	0.42 (±0.033) ab	0.45 (±0.029) a
	Invasive	0.30 (±0.05) B	0.44 (±0.029) AB	0.46 (±0.03) A
SLA (m^2 kg^{-1})	Native	29.49 (±7.82) a	19.25 (±2.04) b	14.41 (±0.49) b
	Invasive	31.49 (±4.2) A	19.33 (±1.59) B	14.61 (±0.83) B
Leaves (n)	Native	8.5 (±0.79) a	6.4 (±0.34) a	3.8 (±0.33) b
	Invasive	8.7 (±0.6) A	7.0 (±0.55) A	4.1 (±0.53) B

	Inoculum	Light	Shade
Light reduction			
LMF	Native	0.71 (±0.05) b	0.87 (±0.01) a,**
	Invasive	0.70 (±0.05)	0.77 (±0.03)
RMF	Native	0.29 (±0.05) a	0.13 (±0.01) b
	Invasive	0.3 (±0.05)	0.23 (±0.03)**
SLA (m^2 kg^{-1})	Native	22.04 (±2.64) b	42.5 (±1.21) a
	Invasive	31.49 (±4.21)	39.6 (±2.76)
Leaves (n)	Native	8.5 (±0.79)	6.9 (±0.5)
	Invasive	8.7 (±0.6)	8.0 (±0.22)

Capital letters indicate significant differences between plants inoculated with AM*inv* and lowercase letters indicate significant differences in plants inoculated with AM*nat* after ANOVA using Tukey's HSD or Student's *t*-test at $P \leq 0.05$. Asterisks indicate significant differences between AM*nat* and AM*inv* plants within the same stress level at * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$.
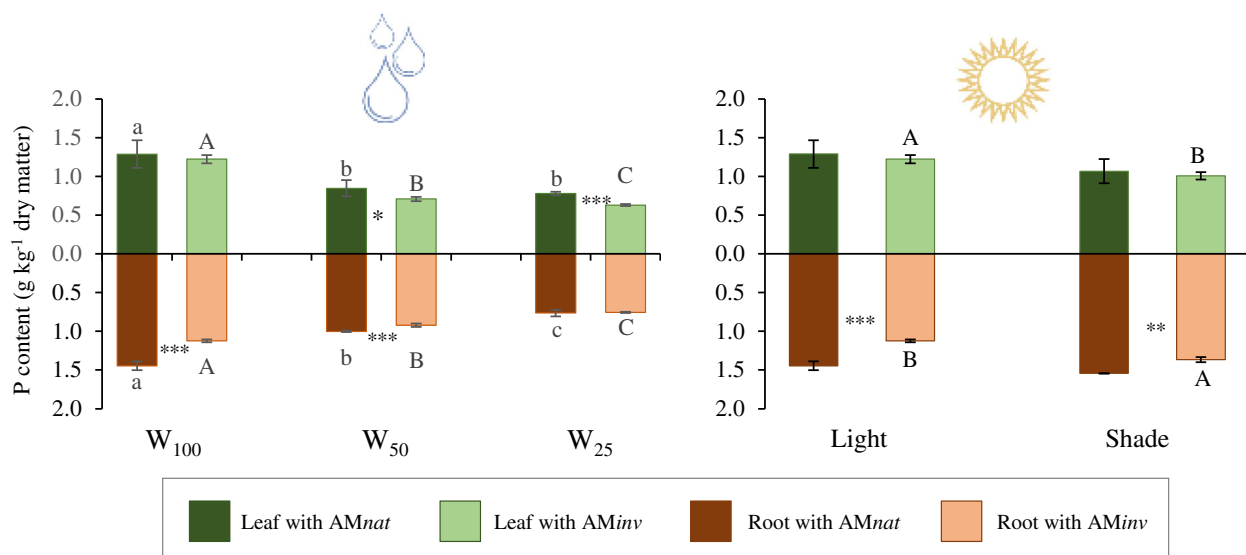
Fig. 2. Mean (±SE) of leaf and root P content (g kg^{-1}) under (a) water reduction (W$_{100}$ = field capacity, W$_{50}$ = 50% field capacity, W$_{25}$ = 25% field capacity) and (b) light reduction (direct light or shade). Capital letters indicate significant differences between plants inoculated with AM*inv* and lowercase letters indicate significant differences in plants inoculated with AM*nat* after ANOVA using Tukey's HSD test or Student's *t*-test at $P \leq 0.05$. Asterisks indicate significant differences between AM*nat* and AM*inv* plants within the same stress level at * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

centre and electron transport under drought stress (Mathur *et al.* 2019). However, it is important to note that different AM species/communities lead to variable plant photosynthetic responses during stress, such as water limitation (Augé 2001). In general, *P. lanceolata* maintained similar photosynthetic activity despite reduced water supply. Improving plant hydraulic conductivity through root expansion by AM fungi—the so-called *mycorrhizosphere*—alleviates drought stress (Augé *et al.* 2007; Mathur *et al.* 2019), also minimizing energy losses in the form of heat while protecting the electron flow of the antenna complexes (LEF) (Boomsma & Vyn 2008).

Commonly, plant photosynthetic activity decreases with drought stress; however, under mild or moderate water restriction it can remain temporarily stable or even increase (Morales
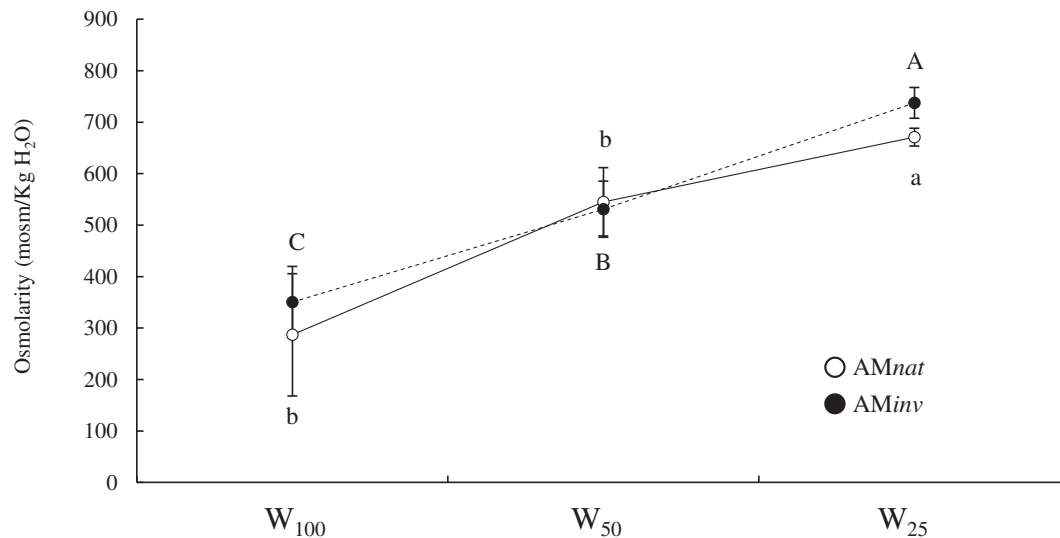
Fig. 3. Mean (±SE) of osmolarity (mosm kg^{-1} H_2O) in different water regimes (W_{100} = field capacity, W_{50} = 50% field capacity, W_{25} = 25% field capacity). Capital letters indicate significant differences between plants inoculated with AM*inv* and lowercase letters indicate significant differences in plants inoculated with AM*nat* after ANOVA using Tukey's HSD test or Student's *t*-test at $P \leq 0.05$.

et al. 2008), *e.g.* by increasing the rate of photorespiration (Massacci *et al.* 2008). Plants maintained similar photosynthetic activity across different water levels, regardless of the origin of the inoculum. In this sense, the different origin of the AM inoculum did not modify the photosynthetic response of *P. lanceolata*, since the photosynthetic efficiency (Φ_{II}) or F_v'/F_m'—indicators of damage to the photosynthetic apparatus—were maintained in ranges considered adequate. Nevertheless, the Φ_{II} value of plants inoculated with AM*nat* slightly decreased when plants were watered at 25% of field capacity. Nevertheless, plant growth and biomass production seemed to be more influenced by water availability (less availability, less growth) than by inoculum origin, since no differences were detected between plants treated with AM*nat* or AM*inv*.

Sunlight reduction generally decreases plant photosynthetic activity and photosynthesis rates (Ojanguren & Goulden 2013). Low light leads to insufficient ATP produced to allow C fixation and carbohydrate biosynthesis (Shao *et al.* 2014), reducing photoassimilate production and, consequently, plant growth and biomass (Mathur *et al.* 2018). As a consequence, limited C products constrain the mutualistic investment (Fellbaum *et al.* 2014) due to the significant energy requirements necessary to maintain the mutualist relationship, since AM receive up to 20% of primary plant production (Hobbie & Hobbie 2008; Smith & Read 2010). On the other hand, mycorrhization contributes, to some extent, to ameliorate photosynthetic responses (Zhu *et al.* 2012; Liu *et al.* 2015; Koorem *et al.* 2017; Mathur *et al.* 2019), and plants colonized by AM often show higher stress tolerance (Jung *et al.* 2012; Augé *et al.* 2015). Contrary to previous studies (Graham *et al.* 1982; Gehring 2003), the attenuation of natural irradiance did not reduce fungal colonization of *P. lanceolata* roots. Similar colonization rates, including essential organs for lipid storage in vesicles (Smith & Read 2010), suggests that *P. lanceolata* maintained the energy investment to preserve AM structures, even under restrictive treatments (W_{25}, shade).

Nevertheless, although the mycorrhization levels were similar, our results indicated that growth responses of *P. lanceolata* were, to some extent, influenced by light conditions. The photosynthetic capacity (ϕ_{II}) and efficiency (ϕ_{NO}) of plants growing in contact with AM*nat* decreased compared to AM*inv*, which performed better under reduced irradiance. The adaptive response of plants to light restriction was reflected in the aerial length and biomass, suggesting a more beneficial role of symbiosis under limiting conditions (Zhang *et al.* 2015), in line with the *stress-gradient* hypothesis (Brooker *et al.* 2008). Nevertheless, this effect also depends on the AM origin; plants in contact with AM*inv* could be more conservative in full light, prioritizing maintenance of the AM symbiosis over plant growth and the opposite under shade conditions. Growing in soils with AM*inv* allowed plants to maintain allocation patterns in shade, whereas plants in contact with AM*nat* relocated resources to the aerial parts, increasing LMF while reducing RMF. Although neither the quantum yield of PSII or the energy loss were affected, the allocation of plant resources to the aerial parts in AM*nat* plants, also the increase in SLA, would suggest an expansion of the available photosynthetic surface to compensate for the reduction in light.

Plant phosphorus content

From the plant point of view, the main advantage of investing in AM symbioses is the increased access to otherwise inaccessible soil nutrients, such as P or N, extending nutrient acquisition area beyond the limit of the root (Smith & Read 2010) and contributing up to 90% of the P obtained by the plant (Smith & Smith 2011). Phosphorus is taken up by extraradical hyphae, transported towards the root system and delivered to the plant *via* arbuscules (Smith & Read 2010), thus reducing plant dependence on soil environmental conditions for P uptake. In our study, water restriction caused a reduction in plant P content, which could be due to the decrease in P availability in water-deficient soils (Gahoonia *et al.* 1994; García
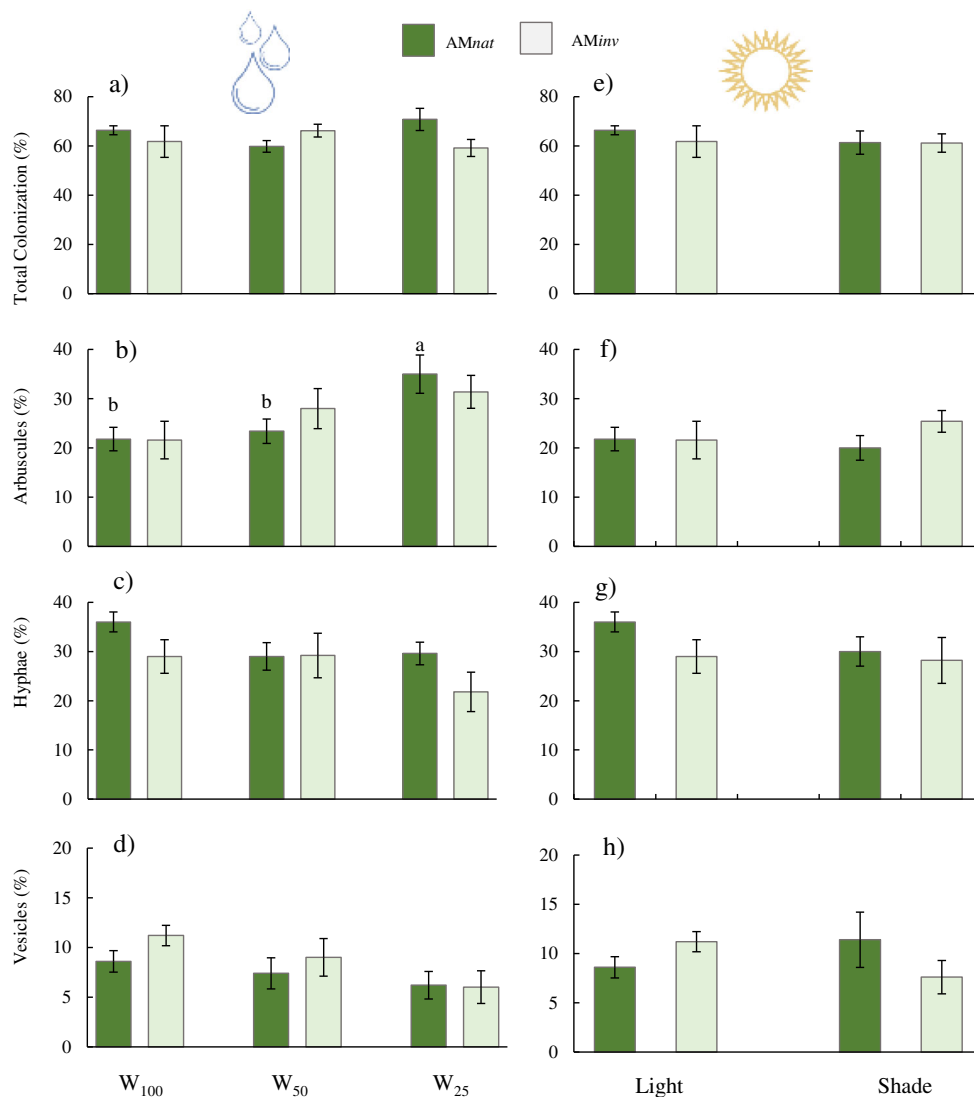
Fig. 4. Mean (±SE) of (a) colonization, (b) arbuscules, (c) hyphae and (d) vesicles in *P. lanceolata* roots under drought stress (W_{100} = field capacity, W_{50} = 50% field capacity, W_{25} = 25% field capacity) or (e), (f), (g) and (h) under light stress (direct light or shade). Lowercase letters indicate significant differences in plants inoculated with AM*nat* after ANOVA using Tukey's HSD test or Student's *t*-test at $P \leq 0.05$.

et al. 2008). However, plants in contact with AM*nat* tolerated water limitation better, maintaining root–stem transport, and therefore foliar P content. These plants had similar P levels under intermediate and severe water reduction ([P] at $W_{50} = W_{25}$), unlike plants growing with AM*inv* ([P] at $W_{50} > W_{25}$).

Although root colonization generally decreases under drought (García *et al.* 2008; Mathur *et al.* 2019), under light restriction (Koorem *et al.* 2017) or due to the contact with AM from invaded soils (Tanner & Gange 2013), in our case, the origin of the inoculum did not affect colonization, with similar infection levels and comparable AM structures regardless of the level of stress applied. As stated above, mycorrhizal relationships are maintained at a high metabolic cost, and the stress severity, *e.g.* drought level, influences the investment in mycorrhizal symbiosis (Augé 2001). Phosphorus solubility and availability decreases under water limitation and, therefore, increasing the number of arbuscules (75%) in W_{25} in plants

growing with AM*nat* would suggest an additional effort to obtain P, probably at a higher metabolic cost (Roth & Paszkowski 2017). This increase contrasts with previous results that reported a reduction in the presence of arbuscules under drought (García *et al.* 2008).

Despite the structural change in the AM community in soils invaded by *A. dealbata* (Guisande-Collazo *et al.* 2016), the level of mycorrhizal colonization observed suggests that *P. lanceolata* associates effectively with the AM community provided from the invader. Therefore, it could be argued that it was the origin of the inoculum (and the species within), rather than the root colonization level, that influenced the ability of *P. lanceolata* to obtain soil P under water limitation. Although AM species vary in the capacity to acquire and provide P to *P. lanceolata* (Pel *et al.* 2018), how a specific set of AM species would influence P acquisition under different stresses seems difficult to predict. Noteworthy, the increased P availability (x2) in shrublands invaded by *A.*

Table 4. Two-way ANOVA results for Experiment 2, including independent variables (light reduction and inoculum origin) and their interaction for the studied variables: fluorescence, plant growth parameters, P content and mycorrhizal colonization.

	Light (L)		Inoculum (I)		L × I	
	$F_{(x,y)}$	P	$F_{(x,y)}$	P	$F_{(x,y)}$	P
Fluorescence parameters						
Φ_{II}	1.690	0.203	4.779	**0.036**	2.756	0.107
Φ_{NPQ}	2.790	0.105	3.765	0.061	0.712	0.405
Φ_{NO}	0.091	0.765	1.570	0.219	2.183	0.149
F_v'/F_m'	1.477	0.233	1.188	0.284	0.014	0.905
LEF	82.790	**≤0.001**	9.620	**0.004**	0.018	0.894
Plant growth parameters						
Leaves (n)	3.196	0.083	1.021	0.320	0.489	0.489
Leaf length (cm)	19.835	**<0.001**	0.062	0.804	4.208	**0.048**
Root length (cm)	3.326	0.078	1.140	0.294	2.104	0.157
Leaf biomass (g)	0.005	0.945	4.892	**0.034**	1.956	0.172
Root biomass (g)	8.012	**0.008**	0.002	0.966	0.354	0.556
LMF	7.898	**0.008**	1.785	0.191	1.452	0.237
RMF	7.898	**0.008**	1.785	0.191	1.452	0.237
SLA (m^2 kg^{-1})	22.664	**≤0.001**	1.203	0.281	4.199	**0.048**
Phosphorus (P) content (g kg^{-1})						
Leaves	8.364	**0.015**	2.020	0.183	0.144	0.712
Roots	62.163	**≤0.001**	133.79	**≤0.001**	11.42	**0.006**
Mycorrhizal colonization (%)						
Colonization	0.185	0.833	0.000	0.992	0.382	0.688
Arbuscules	0.073	0.930	0.901	0.354	3.851	**0.038**
Hyphae	1.270	0.303	1.052	0.317	4.754	**0.020**
Vesicles	3.428	0.052	0.610	0.444	1.959	0.167

Values in bold indicate significant differences at $P < 0.05$. The quantum yield of PSII (Φ_{II}), quantum yield of non-photochemical quenching (ϕ_{NPQ}), quantum yield of other unregulated (non-photochemical) losses (ϕ_{NO}), maximum quantum yield of PS$_{II}$ in the light-adapted state (F_v'/F_m'), linear electron flux (LEF), specific leaf area (SLA) and P content in leaves and roots, leaf mass fraction (LMF) and root mass fraction (RMF).

dealbata across the region (Lorenzo *et al.* 2010; Souza-Alonso *et al.* 2014) could reduce the need to invest in specific mechanisms for P acquisition, and the mutualistic relationship could be focused on complementing other requirements (plant defence, abiotic stress, water uptake, etc.) (Jung *et al.* 2012; Hajiboland *et al.* 2019; Li *et al.* 2019; Diagne *et al.* 2020). Nevertheless, the costs and benefits of symbiotic exchanges are complex and depend on the relative resource availability and their balance between both symbiotic partners (Grman 2012).

In Experiment 2, plants growing in contact with AM*nat* did not show different responses to different light regimes, but in all cases accumulated more P in roots than plants inoculated with AM*inv*. In this case, plant response also varied between different irradiance conditions, showing different allocation patterns in the light (leaf P > root P) or shade (root P > leaf P). This trend was also observed for C resource allocation, where higher root length and biomass were observed under shade but not under full light. The interaction between light and soil nutrients can affect preferential bidirectional allocation patterns of C and P (Zheng *et al.* 2015). In this sense, leaf expansion and thus, increased photosynthetic surface, could be interpreted as a response of *P. lanceolata* when associated with AM*inv* to acquire more photosynthates under low-light conditions that can be further used to increase or maintain the bidirectional exchange with the AM.

The preservation of mycorrhizal structures in shade—and the associated energy cost—suggests that the stress level might not have been sufficiently intense (despite the 80% reduction in the natural irradiance) to affect plant growth or to produce noticeable changes. Considered globally, the benefit obtained in P acquisition was reduced when plants associated with the AM*inv* inoculum. Similar to observations in Experiment 1, it could be argued that fungal communities in AM*nat* and AM*inv* affected *P. lanceolata* differently under shade. Considering similar root infection levels, the association with AM*inv* favoured *P. lanceolata* growth, whereas AM*nat* was more effective in obtaining soil P.

Consequences of invasion and stress

In our study, *P. lanceolata* showed different responses to changes in water or light regime, probably due to differences in the type and intensity of the stress applied. In this sense, it is important to note that unidirectional negative consequences produced by the association with AM from invaded communities (Guisande-Collazo *et al.* 2016; Zubek *et al.* 2016) were not observed, at least in growing plants under different stress conditions. The presence of IAPs, such as *A. dealbata*, with low dependence on native mutualisms is expected to induce changes, decrease mutualist efficiency over time and affect mutualist-dependent species after disturbances (Vogelsang & Bever 2009). To some extent, plant responses were altered under stress, but instead of decreasing plant performance, we observed what can be considered an *adapted response*. Under optimal growth conditions, plants in contact

with AM*nat* were slightly favoured (leaf biomass, P content). However, the influence of AM*nat* and AM*inv* seemed to be related to the level of stress applied (water stress > light stress), providing slight advantages to plants growing in contact with AM*nat* under drought stress and to plants associated with AM*inv* under light reduction. Thus, as stated above, responses of *P. lanceolata* to cope with different environmental stresses would be conditioned by the origin of the AM inoculum. It is well established that AM fungi show interspecific functional diversity (Munkvold *et al.* 2004; Mensah *et al.* 2015), with differences, *e.g.* in soil exploration efficiency. The specific composition of inocula, and the intrinsic characteristics of species therein, might harbour different physiological attributes that produce different responses and benefits in mycorrhized plants according to the level and the type of stress applied (Augé 2001; Manoharan *et al.* 2017; Pel *et al.* 2018; Li *et al.* 2019) or also related to biotic factors (Kiers *et al.* 2011; Fellbaum *et al.* 2014).

The presence of IAPs that do not depend on mycorrhiza disrupts the fungal community structure, negatively influencing native species that depend on AM symbioses (Tanner & Gange 2013). It is generally considered that IAPs take advantage of associations with soil microorganisms—in our case the low mycorrhizal dependence of *A. dealbata*—changing species composition and decreasing the effectiveness of the native mutualists over time (Vogelsang & Bever 2009). As a result, mutualistic-dependent plants are adversely affected, especially under abiotic stress. In fact, the capacity of *A. dealbata* to modify its environment implies that changes occur on a much larger scale. It is not by chance that due to the ecosystem-level changes produced, this and other *Acacia* species (Souza-Alonso *et al.* 2017) are considered as transformer species (Richardson *et al.* 2000b). Nevertheless, the interpretation of the results and the ecological implications assumed should be considered with caution because of the limitations to the experimental design (limited number of target species, inoculum or sampling sites).

Our results indicated that the outcome of associating with AM*nat* or AM*inv* on plant performance is not unidirectional but is context dependent. Adapted responses of *P. lanceolata* could be related to its ability to associate with a wide range of AM species (Pel *et al.* 2018), mainly species of the genus *Glomeromycota* (Smith & Read 2010), and, at the same time, to the generalist character of AM species from different communities (invaded–native) to establish relationships with roots of different plants (Majewska *et al.* 2018). Here, the proportion, extent and number of AM structures, such as hyphae, vesicles or arbuscules, provide good insight into the plant–AM associative process. Considering that plant biomass and root mycorrhization are generally correlated (Zubek *et al.* 2016), our results separated plant performance from root colonization, suggesting that different sources of AM inocula and the level of stress applied do not limit the capacity of a generalist plant species such as *P. lanceolata* to associate with AM communities from areas invaded by *A. dealbata*.

CONCLUSIONS

Habitat transformation induced by *A. dealbata* lead to water and light limitation for native plants, but the association with AM fungi can alleviate these effects. Growing in contact with mycorrhiza from native and invaded soils, *P. lanceolata* responded differently to the reduction in water and light availability. Nevertheless, the source of the AM inoculum had different effects on the photosynthetic and growth responses of *P. lanceolata* under abiotic stresses, without noticeable effects on root colonization. Similar infection levels and fungal structures were evidenced across treatments, regardless of the type and level of stress. Hence, our results separated plant performance from root colonization, also suggesting that potential changes in the fungal community induced by *A. dealbata* and the level of stress applied did not affect the ability of the AM community of invaded areas to associate with AM-dependent plants, such as *P. lanceolata*.

However, plant responses were affected to some extent by the origin of the AM inoculum used. Both AM inocula led to similar plant responses to drought stress, but the association with AM*inv* slightly improved plant growth under reduced light. Interestingly, inoculum origin influenced the capacity of the plants to maintain P supply, reducing plant P content when roots were associated with AM fungi from areas invaded by *A. dealbata*. With similar root colonization levels between treatments, the origin of the inoculum was the main factor influencing the ability of *P. lanceolata* to obtain soil P under water limitation.

SUPPORTING INFORMATION

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

Table S1. List of the native species present in the native plant community with no presence of *A. dealbata*. Species composition is typical from Atlantic shrublands, mainly dominated by a mixed composition of perennial shrubs, and with the presence of different annual grasses occupying the basal layer.

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