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Belowground mutualists and the invasive ability of *Acacia longifolia* in coastal dunes of Portugal

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Abstract The ability to form symbiotic associations with soil microorganisms and the consequences for plant growth were studied for three woody legumes grown in five different soils of a Portuguese coastal dune system. Seedlings of the invasive *Acacia longifolia* and the natives *Ulex europaeus* and *Cytisus grandiflorus* were planted in the five soil types in which at least one of these species appear in the studied coastal dune system. We found significant differences between the three woody legumes in the number of nodules produced, final plant biomass and shoot ^{15}N content. The number of nodules produced by *A. longifolia* was more than five times higher than the number of nodules produced by the native legumes. The obtained ^{15}N values suggest that both *A. longifolia* and *U. europaeus* incorporated more biologically-fixed nitrogen than *C. grandiflorus* which is also the species with the smallest distribution. Finally, differences were also found between the three species in the allocation of biomass in the different studied soils. *Acacia longifolia* displayed a lower phenotypic plasticity than the two native legumes which resulted in a greater allocation to aboveground biomass in the soils with lower nutrient content. We conclude that the invasive success of *A. longifolia* in the studied coastal sand dune system is correlated to its capacity to nodulate profusely and to use the biologically-fixed nitrogen to enhance aboveground growth in soils with low N content.

Keywords AMF · Broom · Gorse · Nitrogen · Rhizobia

Introduction

The effect of net soil communities on plant growth can explain vegetation patchiness in an ecosystem and the progression of plant invasion (Janzen 1970; Connell 1971; Mills and Bever 1998; Bever 2003; Reinhart et al. 2003). This statement is particularly accurate for leguminous species which largely depend on the symbiosis with nitrogen-fixing bacteria (collectively known as rhizobia) to establish and grow successfully. Biological nitrogen fixation is also a P-demanding process, so most legumes are also highly dependent on P-supply by arbuscular mycorrhizal fungi (AMF) in natural ecosystems (Azcón et al. 1991). Nevertheless, legumes are more likely to suffer from a scarcity in compatible rhizobia partners because this interaction is more specific than the mycorrhizal symbiosis.

Although some leguminous species are among the most aggressive invaders worldwide (Cronk and Fuller 1995), legumes in general are poorer

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colonizers than most flowering plants (Parker 2001). The access to compatible rhizobia is probably the most critical factor determining the colonizing ability of legumes (Parker 2001; Parker et al. 2006). Therefore, specific differences that affect the symbiosis with rhizobia might explain why some legumes become invasive in new areas. Promiscuous legumes are more likely to find compatible symbionts in new areas, and consequently, grow successfully in the introduced sites. In fact, several highly invasive woody legumes are able to nodulate with a wide range of rhizobial partners (Richardson et al. 2000; Ulrich and Zaspel 2000; Rodríguez-Echeverría et al. 2003; Parker et al. 2007). Also, legumes that can nodulate at lower rhizobial densities will clearly have an advantage in establishing symbiosis in new areas where compatible rhizobia are initially rare (Parker 2001). A different factor that can affect the availability of compatible rhizobia and the ability of legumes to nodulate is the presence of indigenous legumes phylogenetically close to the introduced species. In this case, the native legumes could provide rhizobia that are more likely to be compatible with the introduced species. This can explain the ability to nodulate of European *Lathyrus*, *Lotus*, *Trifolium* and *Vicia* species introduced in California (Parker 2001).

In spite of all this, the ability of invasive legumes to recruit belowground mutualists in new areas is not very well understood. A few studies have dealt with the identity and source of the rhizobia associated with exotic invasive legumes (Weir et al. 2004; Stepkowski et al. 2005; Parker et al. 2006; Parker et al. 2007; Rodríguez-Echeverría et al. 2007), but there are many gaps in our knowledge about the nodulation ability of legumes and their invasive potential. This work was designed to address this question taking advantage of the co-existence of three woody legumes with different invasive status in a Mediterranean coastal dune system.

The Natural Reserve of São Jacinto (NRSJ) is a protected coastal area in Portugal with a high environmental value for migratory birds. Nevertheless, several reforestation projects for dune stabilisation and timber production have been undertaken in this site since the beginning of the twentieth century. The main species introduced in the plantations were *Pinus pinaster* Aiton (maritime pine) and

Acacia longifolia (Andrews) Willd. (Sydney golden wattle). As a result of the plantations and the quick establishment of *A. longifolia*, the inter-dune slack and the secondary dunes are nowadays primarily covered by this exotic legume. Other native spontaneous woody species co-occur with the introduced plants in different parts in the stabilised secondary dunes. Of special interest for this study are the leguminous woody species *Cytisus grandiflorus* (Brot.) DC. and *Ulex europaeus* L.

Acacia longifolia is the most widespread of the three species in the studied site, covering about 50% of the area of the NRSJ. This species forms thick monospecific stands from the first dune ridge to the secondary stabilised dunes, grows in the understorey of pine trees patches and is starting to colonize the back of the foredunes where, otherwise, there is little vegetation cover. *Ulex europaeus* also forms monospecific stands and can be found occasionally in the understorey of the pine forest. In contrast, the native *C. grandiflorus* only occurs in monospecific stands in open areas of the secondary dunes. Monospecific patches of the three species can be found closely associated in the secondary dunes but they rarely form mixed stands. At a global level, *A. longifolia* and *U. europaeus* are considered aggressive invasive species in different areas of the world. *Acacia longifolia,* originally from South Western Australia, has an invasive status in Portugal (Marchante et al. 2003), New Zealand (Parsons et al. 1998) and South Africa (Cronk and Fuller 1995). *Ulex europaeus* is native to Western Europe, and invasive in other continents and islands where it has been introduced (Cronk and Fuller 1995). *Cytisus grandiflorus,* on the other hand, is a native species to the Iberian Peninsula and North Morocco (Castroviejo et al. 1999), and has not been reported as invasive anywhere.

Since belowground mutualisms are crucial for the establishment and growth of legumes in natural systems, this study was designed to examine the effect of soils collected from the different sites occupied by these three species in the NRSJ on plant growth. We aimed at elucidating if soil properties and biota can have a significant effect on the distribution and invasive ability of the three studied legumes. Our hypothesis was that the most abundant species will be also the most efficient at establishing mutualistic associations with soil microorganisms.

Materials and methods

Soil

Soil was collected from five different habitats that represent the distribution areas of the studied legumes within the Natural Reserve of São Jacinto (NRSJ), Portugal. Bulk soil samples were collected from well established stands ($\geq$15 years old) of the three studied legumes, *A. longifolia* (wattle soil)*, C. grandiflorus* (broom soil) and *U. europaeus* (gorse soil); from a stand ($\geq$15 years old) of *P. pinaster* (pine soil) and from the back of the foredunes (foredune soil). Soil from the five sites was collected 1 week before the experimental set-up, thoroughly mixed (for each site) and used to fill 1-l pots.

Soil analyses for pH, organic matter, total nitrogen, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, available phosphorus and potassium were performed following standard protocols after soils were air-dried and sieved through a 2-mm sieve. Soil pH was measured in soil suspensions in distilled water (LQARS 1977). Soil organic matter was estimated after combustion of the samples at 550°C (Rossell et al. 2001). Total nitrogen was estimated following the Kjeldahl method (Bremner and Mulvaney 1982). $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ were extracted using $CaCl_2$ and measured using a molecular absorption spectrophotometer following a modified protocol of Keeney and Nelson (1982). Available potassium and phosphorus were extracted using ammonium lactate and acetic acid and measured using an atomic absorption spectrophotometer for potassium (Balbino 1968) and a colorimetric method for phosphorus (Watanabe and Olsen 1965).

Plants

Seeds of *A. longifolia, U. europaeus* and *C. grandiflorus* were collected from adult plants in the NRSJ and kept in paper bags at 18°C in the dark before the experimental set-up. Seeds of *A. longifolia* were mechanically scarified and surface disinfected by sequential immersion in ethanol 96% for 30 s, commercial bleach at 4% for 2 min and six washes in autoclaved water. Seeds of *C. grandiflorus* and *U. europaeus* were soaked in concentrated sulphuric acid (36 N) for 40 and 180 min respectively and subsequently rinsed with autoclaved water. All seeds were placed in wet autoclaved sand inside Petri dishes for germination. After the emergence of radicle and cotyledons the seedlings were individually transferred to 1-l pots filled with each soil type.

Experimental set-up

Nine seedlings of each plant species were planted individually in pots containing each of the five soils. All pots were covered with aluminium foil to prevent desiccation and contamination with air-borne organisms. Pots were placed in the greenhouse using a randomised design and watered every 3 days with distilled water. Plants were kept in the greenhouse for 14 weeks under natural photoperiod and temperature from April to July 2006.

Data collection

At harvest all plants were carefully removed from the pots. The number of root nodules and the fresh weight of shoots and roots were recorded for each plant. A portion of each root was weighed and stained using acid fuchsin for assessment of intra-radical organisms such as mycorrhizal fungi or nematodes (Baker and Gowen 1996). Using a compound microscope, root infection by AMF was estimated as the percentage of 1-cm root fragments containing mycorrhizal structures (arbuscules, vesicles or hyphae). After taking the root fraction for assessing colonization, the remaining plant material was dried at 65°C for 48 h to estimate plant biomass. The root/shoot ratio was calculated based on values of dry biomass. Subsequently, shoots were ground using an electric mill (Restch, Zürich, Switzerland). Nitrogen and ^{15}N contents were measured by combustion using an automatic elemental analyser FlashEA 1112 coupled with gas chromatographic (GC) separation and thermal conductivity detection (TCD) systems (ThermoFinnigan, CA, USA). Phosphorus content was analysed by inductively coupled plasma mass spectrometry after acidic digestion of plant material. Some plants could not be analysed for P, N and isotopic composition because their biomass was lower than the required sample weight for the analytical procedure.

Statistical analyses

Two-way ANOVA using plant species and soil type as factors was used to test if those two factors had any

effect on final plant biomass, number of nodules, root colonization by AMF, shoot nitrogen content and shoot δ^{15}N values. Tukey's test was used for post hoc overall comparisons. Data were transformed when necessary to meet the ANOVA assumptions. Normality and homogeneity of variances could not be achieved for shoot phosphorus content. Therefore, the Kruskall–Wallis test was used to compare phosphorus content within each soil type and also within each plant species. The SNK test was used for overall comparisons after Kruskall–Wallis. The root/shoot ratio data were compared within each plant species to check for specific responses to the different soil types. One-way ANOVA was used for the data of *A. longifolia* and *U. europeaus*. Normality and homogeneity of variances could not be achieved for the data of *C. grandiflorus* which were analysed with Kruskall–Wallis and SNK tests.

The Pearson correlation was used to check for correlations between (a) AMF colonization and (b) the number of nodules produced by each plant, and either plant biomass, shoot N content or δ^{15}N.

All statistical analyses were performed using STATISTICA v6.0 for Windows.

Results

The wattle soil—collected from the stands of *A. longifolia*—had the highest values of organic matter, total nitrogen, nitrate and ammonia (Table 1). The soil from the foredune had the lowest values of organic matter, available potassium, ammonium and total nitrogen. The foredune soil also had the highest value of available phosphorus. Soil pH in the foredune was about 7. The other four soils had more acidic pH with values around 5.

The two-way ANOVA found a significant effect of soil type and plant species on the number of nodules produced, but there was no interaction between these factors (Table 2). The post hoc comparisons showed that the overall number of nodules produced in the foredune soil was significantly lower than that on the other four soils. The highest mean number of nodules in the foredune soil was about seven nodules per plant for *A. longifolia*, while for the two native species this value was less than 1 nodule per plant (Fig. 1). No significant differences were found between the other four soils. Significant differences were found between the three plant species. The exotic species, *A. longifolia*, produced a significantly higher number of nodules than the two native species in all soils (Fig. 1). In the soils from pine trees, wattles, gorses and brooms, *A. longifolia* had an average of 40–70 nodules per plant and *U. europaeus* of 8–10 nodules per plant. *Cytisus grandiflorus* was the species that produced fewer nodules with the highest mean value (5.6) in the gorse soil.

The two-way ANOVA showed that shoot nitrogen content was affected by soil type, plant species and the interaction between these two factors (Table 2). Shoot nitrogen content was, in general, higher in the wattle, gorse and broom soils than in the foredune and pine soils. Within each soil type, significant differences between plant species were found only in the wattle and broom soils (Fig. 2). In the wattle soil, the shoot nitrogen content in *U. europaeus* plants was significantly lower than in the other two species. In the broom soil, the value of shoot N content for *C. grandiflorus* plants was significantly lower than the N content in *U. europaeus* plants (Fig. 2).

The values obtained for shoot δ^{15}N were significantly affected by soil type and plant species, but an interaction between these two factors was not detected (Table 2). Values of δ^{15}N negative or around zero were found in all soils excepting the wattle soil. Pooling the data within each soil, shoot

Table 1 Chemical characteristics of the five soils used in this study

	Foredune soil	Pine soil	Wattle soil	Gorse soil	Broom soil
pH	**7.6**	5.2	5.1	5.3	5.2
O. M. (%)	n.d.	0.32	**1.07**	0.79	0.57
P_2O_5 (mg/kg)	**12**	8	4	8	5
K_2O (mg/kg)	4	7	**13**	**13**	8
$N\text{-}NO_3^-$ (mg/kg)	1.26	0.22	**41.22**	12.41	15.49
$N\text{-}NH_4^+$ (mg/kg)	2.45	5.52	**6.34**	4.53	3.50
N total (%)	0.004	0.011	**0.044**	0.023	0.024

The maximum values are marked in bold
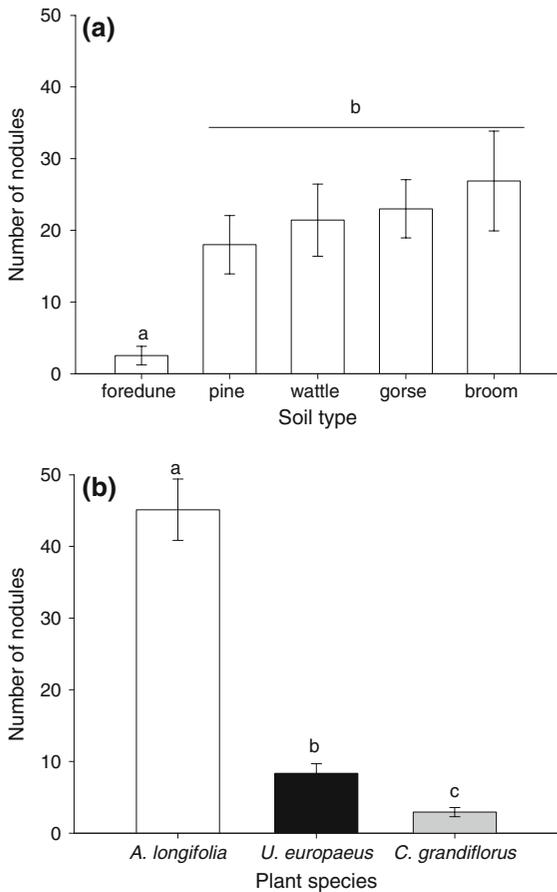
n.d.: not detectable

Fig. 1 Number of nodules per plant (mean ± SE) pooling the data within each soil (a), or within each plant species (b). Different letters mean significant differences after ANOVA and Tukey's test

$\delta^{15}N$ values were significantly higher in the wattle soil than in the remaining soils. Although the three plant species had similar values of shoot N content, values of $\delta^{15}N$ tended to be higher for *C. grandiflorus* than for the other two species (Fig. 2). In fact, $\delta^{15}N$

values close to or below zero for *C. grandiflorus* were only found in the foredune soil. *Acacia longifolia* and *U. europaeus* had values around or below zero in all soils excepting the wattle soil (Fig. 2). Overall significant differences ($P < 0.001$) were found between the three plant species, with *A. longifolia* having the lowest overall $\delta^{15}N$ value (0.09) and *C. grandiflorus* the highest (2.75). Shoot $\delta^{15}N$ was significantly lower ($P < 0.05$) in *A. longifolia* in the wattle soil. Significant differences ($P < 0.05$) were also found between *C. grandiflorus* and the other two species in the gorse and broom soils (Fig. 2).

Root colonization by AMF was significantly affected by soil type and the interaction between this factor and plant species (Table 2). Plant species by itself was only marginally significant ($P = 0.05$). The post hoc comparison showed that root colonization by AMF was significantly lower in the pine soil and significantly higher in the broom soil. The values of root colonization in the pine soil were lower than 5% for the three plant species (Fig. 3). Root colonization by AMF in the broom soil, was about 70% for *A. longifolia* and *U. europaeus* and about 40% for *C. grandiflorus*. When comparing plant species within each soil, significant differences were only found in the foredune soil and in the broom soil. *Ulex europaeus* had a significantly lower value of root colonization than the other two species in the foredune soil (5% vs. 20–25%). In the broom soil significant differences were found between *C. grandiflorus* and the other two plant species (Fig. 3).

Shoot phosphorus content was only measured in plants grown in wattle, gorse and broom soils. Plants grown in the foredune and pine soils did not reach the minimum sample size required for phosphorus analysis. For *C. grandiflorus*, phosphorus analyses were only possible for the plants grown in the wattle soil.

Table 2 Two-way ANOVA results for plant biomass, number of nodules and root colonization by AMF, shoot nitrogen content and $\delta^{15}N$

	d.f.	Nodules		Shoot N[a]		Shoot $\delta^{15}N$[a]		AMF		Plant biomass	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Soil	4	7.778	<0.001	5.291	<0.01	23.468	<0.001	25.622	<0.001	84.34	<0.001
Plant	2	74.281	<0.001	9.744	<0.001	55.155	<0.001	3.072	0.050	46.801	<0.001
Soil × plant	8	1.762	0.096	3.202	<0.01	1.747	0.129	2.440	<0.05	3.345	<0.01

[a] Data from foredune soil not included because of the limited sample for *U. europaeus* and *C. grandiflorus* (d.f. soil = 3, soil × plant = 6)

Fig. 2 Shoot nitrogen (%) and δ^{15}N content (mean ± SE) for *A. longifolia* (A), *U. europaeus* (U) and *C. grandiflorus* (C) in the five soils used in this study. Different letters mean significant differences between plant species within each soil type after ANOVA and Tukey's test
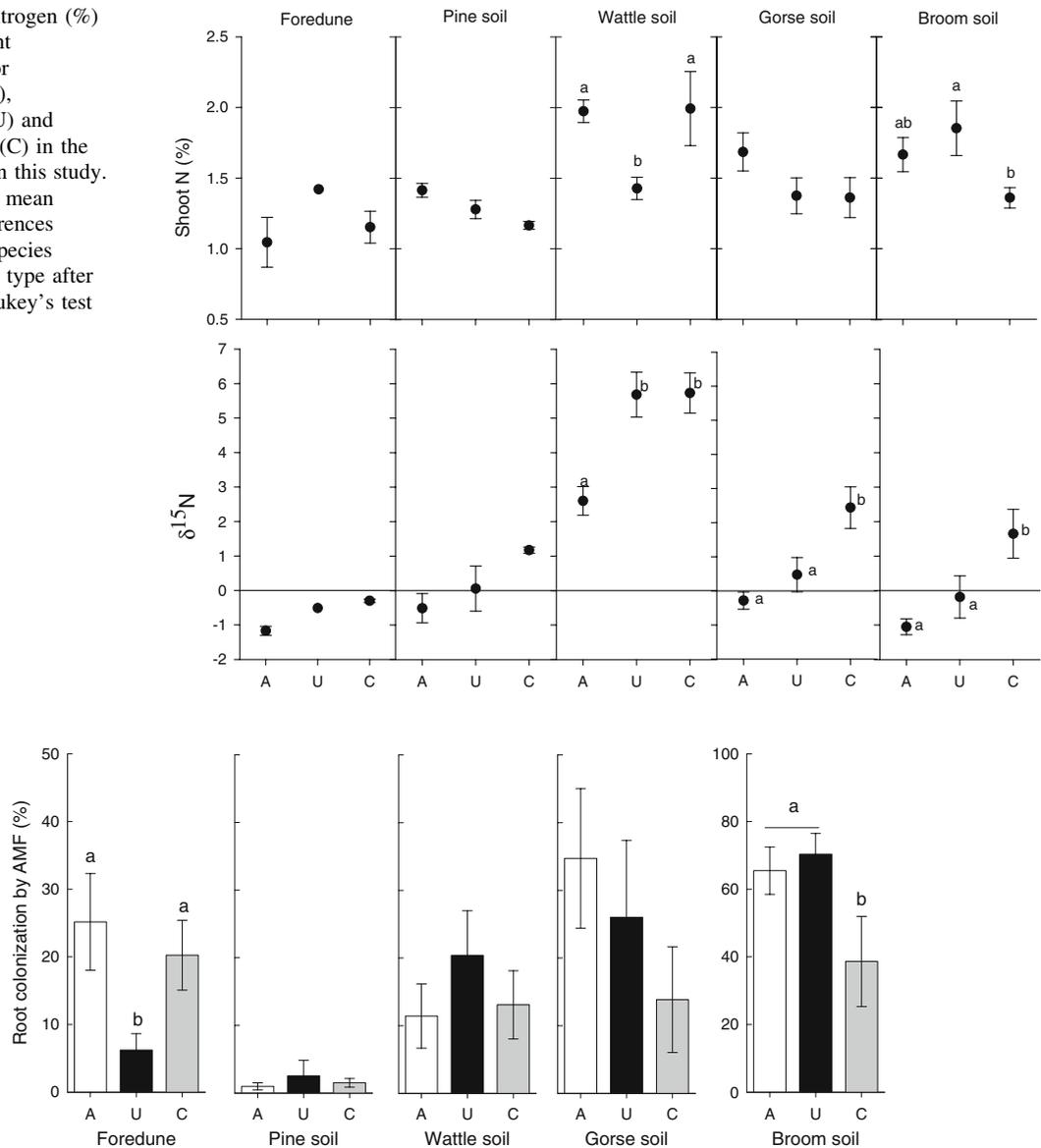

Fig. 3 Root colonization by AMF (%, mean ± SE) for *A. longifolia* (A), *U. europaeus* (U) and *C. grandiflorus* (C) in the five soils used in this study. Different letters above the bars mean significant differences between plant species within each soil type after ANOVA and Tukey's test. Note different scale in the last graph (broom soil)

Shoot phosphorus content ranged between 0.69 mg/g and 1.21 mg/g (Table 3). Significant differences in the shoot phosphorus content of the three plant species were not detected (wattle soil: $F_{2,27} = 1.262$, $P = 0.321$; gorse soil: $F_{2,27} = 0.269$, $P = 0.771$; broom soil $F_{2,27} = 3.150$, $P = 0.116$).

Soil type and plant species had a significant effect on total biomass, according to the two-way ANOVA (Table 2). There was also a significant interaction

Table 3 Shoot P content (mg/g, mean ± SE) of *A. longifolia* (A), *U. europaeus* (U) and *C. grandiflorus* (C) in wattle, gorse and broom soils

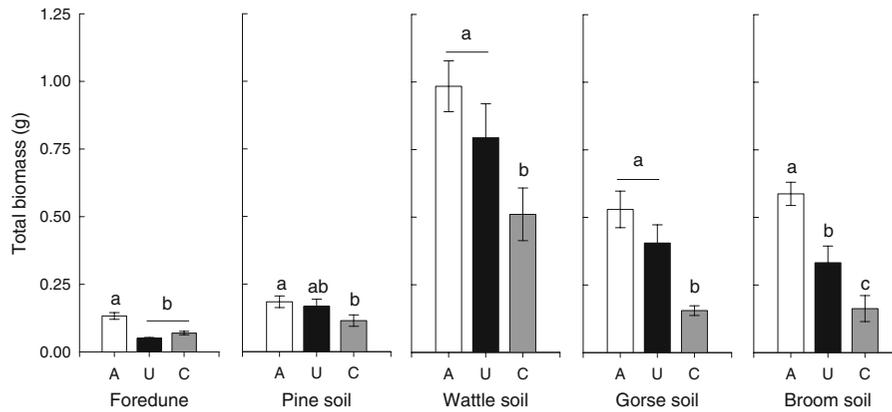|	Wattle soil	Gorse soil	Broom soil
A. longifolia	0.69 ± 0.06	0.74 ± 0.07	0.74 ± 0.01
U. europaeus	0.85 ± 0.09	1.21 ± 0.05	0.83 ± 0.05
C. grandiflorus	0.78 ± 0.05	n.a.	n.a.

n.a.: not available

Fig. 4 Total plant biomass (g, mean ± SE) for *A. longifolia* (A), *U. europaeus* (U) and *C. grandiflorus* (C) in the five soils used in this study. Different letters above the bars mean significant differences between plant species within each soil type after ANOVA and Tukey's test

between these two factors. The highest value of biomass corresponded to *A. longifolia* and the lowest to *C. grandiflorus*. The highest values of biomass, 0.98 g for *A. longifolia*; 0.79 g for *U. europaeus* and 0.51 g for *C. grandiflorus*, were found in the wattle soil (Fig. 4). The lowest values of biomass, recorded in the foredune soil, were 0.14 g for *A. longifolia*, 0.05 g for *U. europaeus* and 0.07 g for *C. grandiflorus*. Analysing the results within each soil type, *A. longifolia* produced a significantly higher biomass than the native species in the foredune soil and the broom soil (Fig. 4). No differences were found between *A. longifolia* and *U. europaeus* in the soils from pine trees, wattles and gorses. Plant biomass in the soils from the two native species was around 0.53 g for *A. longifolia*, 0.16 g for *C. grandiflorus,* and between 0.33 g and 0.45 g for *U. europaeus*.

There was a significant correlation between total plant biomass and the number of nodules ($R = 0.578$, $P < 0.01$) or AMF colonization ($R = 0.541, P < 0.01$) in the foredune soil. There was also a significant correlation between plant biomass and the number of nodules in the wattle ($R = 0.438$, $P < 0.05$), gorse ($R = 0.587$, $P < 0.01$) and broom ($R = 0.702$, $P < 0.001$) soils. No correlation between number of nodules or AMF colonization and plant biomass was found in the pine soil. The number of nodules was also correlated with shoot $\delta^{15}N$ content in the wattle ($R = -0.676$, $P < 0.01$), gorse ($R = -0.636$, $P = 0.01$) and broom ($R = -0535$, $P < 0.05$) soils.

The root/shoot ratio was analysed for each plant species separately since this is a specific trait that

Table 4 Values of root/shoot ratio for each plant species in each of the five studied soils

	A. longifolia	*U. europaeus*	*C. grandiflorus*
Foredune	0.69 ± 0.05	1.15 ± 0.11a	1.25 ± 0.11a
Pine soil	0.61 ± 0.07	0.53 ± 0.07b	0.90 ± 0.11b
Wattle soil	0.60 ± 0.04	0.57 ± 0.08b	0.64 ± 0.05b
Gorse soil	0.47 ± 0.03	0.65 ± 0.06b	1.43 ± 0.51ab
Broom soil	0.62 ± 0.08	0.66 ± 0.06b	1.39 ± 0.66ab

Different letters mean significant differences between the values within each plant species

changes in response to resource availability. There were no significant differences between the *A. longifolia* plants grown in the five different soils (Table 4, $P = 0.121$). The plants of *U. europaeus* grown on the foredune soil had a significantly higher value than the plants grown on the other four soils (Table 4, $P < 0.001$). For *C. grandiflorus* plants, the Kruskal–Wallis test showed significant differences between the five soils ($X^2 = 0.017$). The SNK test showed that the root/shoot ratio of *C. grandiflorus* plants from the foredune soils was significantly higher that the values found in the pine and wattle soils (Table 4).

Discussion

Although the three legumes established mutualistic associations with belowground organisms in the five studied soils, *A. longifolia* developed a significantly higher number of nodules than the European species

in all the tested soils. For each soil, *A. longifolia* produced a number of nodules between four and seven times higher than *U. europaeus* and about ten times higher than *C. grandiflorus*. In fact, this result reflects the invasive ability and distribution range in the NRSJ of the three species: the highest number of nodules was found in *A. longifolia,* followed by *U. europaeus* and then by *C. grandiflorus*. Previous studies have suggested that nodulation is essential for the successful colonization of new soils by legumes (Parker 2001; Richardson 2004). Our results indicate that the invasive ability of the three woody legumes selected for the study, and in particular of *A. longifolia*, are indeed intimately linked to the ability to nodulate in different soils. Because large, vigorous plants tend to develop more nodules, other physiological mechanisms, such as pathogen resistance or higher relative growth rates in *A. longifolia*, could also produce the correlations we observed. However, in the absence of other soil biota, *A. longifolia* young seedlings develop a greater number of nodules than similar size seedlings of other species (Rodríguez-Echeverría, unpublished data). Therefore, we suggest that the ability of *A. longifolia* to nodulate profusely is a functional trait that makes this plant a successful colonizer and invader species. Whether the differences found in the number of nodules produced by the three legumes occur because of different degrees of symbiotic promiscuity and/or differences in the minimum size of a rhizobial population needed to nodulate is currently under study.

The contribution of nitrogen fixation in root nodules to shoot nitrogen can be estimated by measuring shoot ^{15}N (Lajtha and Marshall 1994). The index δ^{15}N compares the ^{15}N content of the sample against that of atmospheric N_2. Soil nitrogen is usually richer in ^{15}N than is the atmospheric N_2 (Lajtha and Marshall 1994). Therefore, δ^{15}N values close to zero or negative (because of fractionation during fixation) are usually indicative of nitrogen fixation. According to the obtained δ^{15}N values, nitrogen fixation seems to be an important source of nitrogen for *A. longifolia* and *U. europaeus* in all soils excepting the rich wattle soil. Nitrogen-fixation can be prevented in nitrogen-rich soils because of the high cost of this process (Stephens and Neyra 1983). The results on plant nitrogen content and δ^{15}N grown in the wattle soil show that the amount of soil nitrogen was enough to meet the plant demands and

nitrogen-fixation was not an important source of nitrogen in this soil. It is important to note the differences observed between *C. grandiflorus* and the other two legumes in the remaining soils. The shoot δ^{15}N values for the non-invasive *C. grandiflorus* indicated that N-fixation was only important in the foredune soil, which had a very low amount of nitrogen. The limited nodulation or a low nitrogen fixation rate could explain the results obtained for *C. grandiflorus*. Also, they can be related to the smaller growth and distribution of this species. It is also remarkable that the δ^{15}N values found in *U. europaeus* were very similar to those of *A. longifolia* in spite of the lower number of nodules produced by the European species. This result agrees with previous data showing that *U. europaeus* has a high N-fixation rate (McQueen et al. 2006), and suggests that this rate could be higher than in *A. longifolia*. An alternative explanation is that *A. longifolia* developed a higher proportion of nodules with non-effective rhizobia which do not contribute to plant nitrogen nutrition (Pérez-Fernández and Lamont 2003; Rodríguez-Echeverría et al. 2003).

While the number of nodules produced by each plant depended on plant species and soil type, root colonization by AMF was mainly affected by soil type, i.e. by the availability of AMF and by soil nutrients. The lowest values of AMF colonization were found in the pine soil, probably because of the lack of AMF species in this soil. Pine trees are ectomycorrhizal and do not establish mutualisms with AMF (Smith and Read 1997), which could lead to a significant decline of these fungi in the soil. The level of AMF colonization for *U. europaeus* was also quite low in the foredune soil. It has been previously shown that co-existing plants can harbour different AMF (Vandenkoornhuyse et al. 2003). Therefore, it is possible that the AMF existing in the foredune soil failed at colonizing *U. europaeus*. An alternative explanation for this result could be that the plant prevented root infection by AMF for physiological reasons since soil P content was quite high in this soil (Koske and Polson 1984). Further research about the physiology and AMF specificity of *U. europaeus* is needed to explain this result. Root colonization by AMF was about two times higher in the broom soil than in the remaining soils which could be explained by the lower P content of this soil (Barea and Jeffries 1995). Interestingly, both *A. longifolia* and

U. europaeus had significantly higher root colonization rates than *C. grandiflorus* in its own soil. This result suggests that *A. longifolia* and *U. europaeus* are more efficient than the non-invasive legume at establishing mycorrhizal associations under limiting conditions.

The positive correlation between AMF colonization and plant growth can be a result of the improved nutrient uptake and soil stabilization provided by the mycobiont (Koske and Polson 1984; Jakobsen et al. 2002). AMF can also contribute to plant growth by offering protection against pathogens, parasites or herbivores (Azcón-Aguilar and Barea 1996; de la Peña et al. 2006). Root pathogenic fungi were rarely observed during the examination of the stained roots. We also detected very few endoparasitic nematodes in the roots of the three species, with higher percentage of colonization in the pine soil (data not shown, the highest value was 0.08% for *C. grandiflorus*). Therefore, it is possible that AMF could be playing an important role protecting the seedlings of these three species against harmful organisms.

Legumes can increase soil fertility through the decomposition of nitrogen-rich litter and the release of nitrogen from roots and nodules. Actually, nitrogen enrichment is one of the consequences of the invasion by exotic legumes and can profoundly transform the native communities (Fogarty and Facelli 1999; McQueen et al. 2006; Yelenik et al. 2007). The elevated nitrogen content of the areas invaded by *A. longifolia* can be explained by the large amount of litter produced by this species (Marchante et al. 2004). All three plant species grew better in the wattle soil, probably because of the high values of nitrogen and organic matter content present in this soil. Invasive plant species can change soil conditions to facilitate further invasion and hinder the growth of native plants (Jordan et al. 2007). In this case, the modifications in the soil occupied by *A. longifolia* do not seem to prevent the growth of at least these two native legumes which could, therefore, be used in restoration projects. In fact, plant biomass for *U. europaeus* and *A. longifolia* seedlings was not significantly different in the wattle soil. The reason why *U. europaeus* do not colonize *A. longifolia* patches in the field remains unclear, but it might be related to the poor light conditions in the understorey of *A. longifolia* and the dense litter layer produced by this plant (Marchante et al. 2004).

Plants with a high phenotypic plasticity can alter the root/shoot ratio, i.e. plant biomass allocation, in response to abiotic factors such as light, CO_2 or soil nutrients (Poorter and Nagel 2000). Both *U. europaeus* and *C. grandiflorus* allocated more biomass belowground when growing in the less fertile soil but no significant variation in the root/shoot ratio was found for *A. longifolia*. This low plastic response by *A. longifolia* allowed this species to maintain a higher shoot elongation in the less fertile soils and could confer a competitive advantage in the sand dunes (Peperkorn et al. 2005). Instead of investing in belowground growth, *A. longifolia* might rely on mutualisms with AMF and rhizobia to maintain nutrient uptake and aboveground growth. In this way, *A. longifolia* seedlings can allocate more biomass aboveground and overshade competing neighbouring species.

The results presented here show that the different distribution ranges and invasive ability of the three studied woody legumes can be greatly determined by the interactions with soil mutualists, mainly with symbiotic nitrogen-fixing bacteria. We suggest that the invasive ability of *A. longifolia* is primarily determined by the capacity to nodulate profusely in different soils. This conclusion is supported by the results of a previous study about the relative growth rate of these three species in sterilized soil and provided with sterile nutrient media (Crisóstomo et al. 2007). In those conditions, *A. longifolia* plants were much smaller than *U. europaeus* and similar in size to *C. grandiflorus* (Crisóstomo et al. 2007). Therefore, it seems that the symbiosis with soil mutualists is more beneficial for *A. longifolia* than for the other two species and can affect biomass allocation making of *A. longifolia* an aggressively competitive invader in poor soils.

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