The ability of a host plant to associate with different symbiotic partners affects ectomycorrhizal functioning

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Abstract

Some plants that associate with ectomycorrhizal (ECM) fungi are also able to simultaneously establish symbiosis with other types of partners. The presence of alternative partners that may provide similar benefits may affect ECM functioning. Here we compared potential leucine-aminopeptidase (LA) and acid phosphatase (AP) enzyme activity (involved in N and P cycling, respectively) in ECM fungi of three hosts planted under the same conditions but differing in the type of partners: *Pinus* (ECM fungi only), *Eucalyptus* (ECM and arbuscular mycorrhizal -AM- fungi) and *Acacia* (ECM, AM fungi and rhizobial bacteria). We found that the ECM community on *Acacia* and *Eucalyptus* had higher potential AP activity than the *Pinus* community. The ECM community in *Acacia* also showed increased potential LA activity compared to *Pinus*. Morphotypes present in more than one host showed higher potential AP and LA activity when colonizing *Acacia* than when colonizing another host. Our results suggest that competition with AM fungi and rhizobial bacteria could promote increased ECM activity in *Eucalyptus* and *Acacia*. Alternatively, other host-related differences such as ECM community composition could also play a role. We found evidence for ECM physiological plasticity when colonizing different hosts, which might be key for adaptation to future climate scenarios.

Introduction

Ectomycorrhizal (ECM) fungi are an integral part of the belowground microbial community, playing a crucial role in forest ecosystem functioning (van der Heijden *et al.* 2015). ECM fungi improve water and nutrient uptake in host trees by increasing the absorptive surface area and by mobilizing mostly nitrogen (N) but also phosphorus (P) from soil organic matter (Smith and Read 2008). The knowledge of ecological processes driving ECM community composition has improved in recent years thanks to the use of molecular tools (Suz *et al.* 2014). However, the connection between community composition and function (i.e. the role of ECM fungi in ecosystem processes and biogeochemical cycling) is still a challenge in fungal ecology (Courty *et al.* 2016). In this sense, the use of functional traits should allow for a more mechanistic understanding of fungal ecology (Aguilar-Trigueros *et al.* 2015).

Host-related variables strongly influence ECM fungal communities (van der Linde *et al.* 2018). Functional characteristics of host plant species are important for the function of mycorrhizal associations (Hoeksema *et al.* 2010). Among those, an important selective factor may be the host's ability to establish symbiosis with other types of partners that might provide similar benefits to the host. In most woody plants, including the common genus *Pinus*, the symbiosis is bipartite (i.e. between ECM fungi and the host). However, some ECM hosts such as *Eucalyptus* can develop a tripartite symbiosis with ECM and arbuscular mycorrhizal (AM) fungi (Brundrett *et al.* 1996; Oliveira *et al.* 1997; Chen *et al.* 2000; Chilvers 2000; Lodge 2000; Giachini *et al.* 2004). Even more intriguingly, *Acacia* trees can establish tetrapartite symbioses with ECM, AM fungi and nitrogen (N)-fixing rhizobial bacteria (Ducousso 1991). While rhizobial bacteria provide N to the host plant, AM fungi mostly provide inorganic P (Smith *et al.* 2011). In addition, mycorhizospheric bacteria could be also contributing to phosphate mobilization (Margalef *et al.* 2018; Wagner *et al.* 2019).

It has been suggested that ECM communities on N-fixing hosts would be especially efficient in acquiring P (Chatarpaul *et al.* 1989; Molina *et al.* 1994; Horton *et al.* 2013). In this sense, different studies have demonstrated functional complementarity between fungal symbionts and N-fixing bacteria to promote host growth (Chatarpaul *et al.* 1989; Kaschuk *et al.* 2010; Larimer *et al.* 2010; Diagne *et al.* 2013). Walker *et al.* (2014) found that the ECM community developed on a host that also establishes symbiosis with N-fixing *Frankia* bacteria (*Alnus*) had increased potential P acquisition ability compared to that of the ECM community developed on a host with ECM fungi only (*Pseudotsuga menziesii*). This supports that the ECM community on a N-fixing host may be selected for a function other than N-acquisition. In contrast, it is less clear how the ECM community would respond to the presence of AM fungi on the same host given that both symbionts can access P but using different nutrient acquisition strategies, i.e. ECM are

able to access organic P while AM are not (Philips *et al.* 2013). It has been suggested that plants can shift their resource allocation to different root symbionts depending on nutrient availability, which could explain the observed shifts from AM to ECM fungi with increasing proportion of organic P during soil development (Albornoz *et al.* 2016). It has also been shown that plant growth can be larger in *Eucalyptus* seedlings simultaneously inoculated with ECM and AM fungi than in seedlings with only one symbiont type (Chen *et al.* 2000), suggesting functional complementarity among them.

Enzyme capabilities have been proposed as ecologically relevant fungal traits linked to different ecosystem processes (Mathieu *et al.* 2013; Aguilar-Trigueros *et al.* 2015). Measuring the potential activity of exoenzymes produced by molecularly identified ECM root tips allows the connection between ECM function and fungal identity (Pritsch *et al.* 2004; Courty *et al.* 2005). This approach has been used under a wide range of abiotic and biotic conditions (Buée *et al.* 2007; Courty *et al.* 2010; Jones *et al.* 2010; Rineau and Courty 2011; Herzog *et al.* 2013; Walker *et al.* 2014, 2016). Different ECM fungal species have been shown to differ in their activity profiles (Courty *et al.* 2005; Jones *et al.* 2012), based on soil conditions (Rineau and Garbaye 2009; Jones *et al.* 2010) or plant nutrient status (Courty *et al.* 2007; Walker *et al.* 2014). Both functional complementarity (lack of overlap among species in the trait of interest) and redundancy (overlap) among ECM fungal species that coexist in a given ecological niche have been reported (Jones *et al.* 2010; Rineau and Courty 2011). However, the relationship between the abundance of an ECM species and its potential enzyme activity is still little understood (Courty *et al.* 2016).

Here we characterized ECM community composition and functioning on three hosts differing in the number and type of symbiotic associations: *Pinus* (ECM fungi only), *Eucalyptus* (ECM and AM fungi) and *Acacia* (ECM, AM fungi and rhizobial bacteria). Our main objective was to assess how ECM communities associated with those hosts differed in their ability to process organic P and N by measuring acid phosphatase (AP) and leucine aminopeptidase (LA) enzyme activity, respectively. The fact that trees belonging to the three hosts were planted at the same time and under the same soil and microclimatic conditions, allowed us to test for differences among host species. In particular, we hypothesized that 1) the ECM community associated with a N-fixing host (*Acacia*) would have increased potential AP enzyme activity and reduced potential LA enzyme activity compared to the community associated with non-N-fixing hosts (*Eucalyptus* or *Pinus*); 2) enzyme capabilities would vary among ECM morphotypes colonizing a given host species (i.e. existence of functional complementarity), at least in some cases; and 3) a given morphotype would show differences in enzyme activity when colonizing different hosts. The use of functional traits in the present study allowed us to link diversity with functioning as well as to provide further insights on ECM physiology and ecological plasticity.

Materials and methods

Site description

The study was conducted at the Sefton Plantation (33°36′20.3″ S, 150°44′11.5″ E), a 1.3 ha forest plantation established in April 2000 (Sefton 2003) at the Hawkesbury Forest Experiment, a climate change research facility of the Western Sydney University (Richmond, NSW, Australia). The plantation is divided into six 40 x 48 m adjacent blocks, each of them consisting of 12 monodominant 10 x 16 m plots (see Supplementary Figure 1). Plots within each block are distributed randomly. Each plot consists of 20 individuals of one of the following tree species: eight eucalypt species (*Eucalyptus argophloia* Blakely., *E. camaldulensis* Dehnh., *E. dunnii* Maiden., *E. globulus* Labill. subsp. *maidenii* (F. Muell) Kirkpatr., *E. grandis* W. Hill ex Maiden., *E. occidentalis* Endl., *E. sideroxylon* Cunn. ex Woolls subsp. *sideroxylon* and *E. tereticornis* Smith.), three acacia species (*Acacia implexa* Benth., *A. mearnsii* De Wild. and *A. melanoxylon* R. Br.) or one pine species (*Pinus radiata* D. Don). The site elevation is 25 m a.s.l. with mean annual temperature of 17 °C and mean annual rainfall of 801 mm (Australian Government Bureau of Meteorology, Richmond-WSU Hawkesbury Station; http://www.bom.gov.au). Soil at the plantation is classified as a Blackendon sand, extending to 0.9 m depth and underlain by a clay hardpan.

Sampling and root tip collection

In February (summer) 2015, root sampling was performed in three of the six blocks of the Sefton Plantation (A, B and D blocks). In each block we sampled three different tree plots, corresponding to one species of each genus: *A. implexa*, *E. grandis* and *P. radiata*. Within a particular genus, the species looking the healthiest and having enough replication was chosen. From now on, we will refer to these species by genus name for clarity. Four trees from the central area of each plot (at a distance of at least 3 m from the edge of the plot) were selected for sampling. At each tree, we collected a 20 x 20 x 10 cm^3 (length, width, depth) sample of soil containing roots, after removing the top 5 cm. Each sample (soil with roots) was collected approximately one meter from the trunk and at least three meters away from adjacent sampled trees. A total of 36 samples were obtained (3 blocks x 3 tree species plots x 4 replicate trees). Each sample (soil with roots) was stored in a plastic bag and kept intact at 4 °C until the enzyme activity assay took place, which occurred within 72 h of sampling. Three samples were discarded due to insufficient quality and 33 out of the 36 collected samples were processed, 11 of each tree species. For each sample, roots were carefully picked and the surrounding soil was

retained for subsequent chemical analysis (see below). Then, root samples were gently washed and examined under a dissection microscope to separate root tips into ECM fungal morphotypes. The design of the plot, the sampling and processing strategy plus the morphological differences among roots of the different host species observed under the dissection microscope allowed us to be confident that root tips belonged to the target tree species. Separation into ECM fungal morphotypes was performed according to morphological differences. Three ECM root tips representing each morphological morphotype were excised under the dissection microscope and placed into 96 well microplates for enzyme activity assays. Once enzyme assays were carried out, all tips were frozen at -20 °C for subsequent molecular identification and only the tips that could be confidently assigned to a morhpotype were retained for analysis (see below). Molecular analysis allowed refining the initial separation in morphological morphotypes, i.e. confirming which morphotypes were distinct, which ones were actually the same and discarding any roots tips that could not be identified (see below). If necessary, assayed tips initially attributed to different morphological morphotypes were grouped together or reassigned to new morphotypes and any data analysis was performed with this corrected dataset (see below). We also collected any additional tips per each morphotype of each sample and stored them in 20 % ethanol for subsequent counting in order to further characterize the community composition of each sample.

Root tip enzyme assay

A total of 381 ECM root tips from the 33 samples were subjected to enzyme assays. We followed the methodology developed and improved by Pritsch *et al.* (2004) and Pritsch *et al.* (2011). ECM root tips were tested for the potential activity of the enzymes leucine aminopeptidase (LA) and acid phosphatase (AP), involved in N and P cycling, respectively. Substrates, standards and solutions were prepared according to protocols. Each of the three sampled root tips per morphotype and per sample was placed individually into 96 well filter plates. One column of the plate was left empty for control of background fluorescence and another one for the calculation of a standard curve. An incubation time of 1 h was optimal for LA, while 30 min was used for AP. Fluorescence was read using an EnSpire 2300 Multimode reader (PerkinElmer, Waltham, MA, USA) following manufacturer's instructions. Plates were then scanned and the surface area of each root tip was measured with ImageJ software (http://imagej.net/). Potential enzyme activity is subsequently expressed as a rate in pmol mm^{-2} min^{-1}.

Morphotype identification and community composition

ECM community composition was determined by counting the root tips of each morphotype in each sample. Relative abundance of each morphotype in each sample was calculated as the number of root tips colonized by a given morphotype divided by the total number of ECM root tips (i.e. colonized by any morphotype). A total of 9853 root tips were counted and assigned to morphotypes based on morphological identification and comparison to molecularly identified morphotypes. Root tips used for the enzyme assay were molecularly characterized. To do so, DNA of each root tip was extracted following the protocol of the Sigma Extract-N-Amp Plant PCR Kit (Sigma Aldrich, St. Louis, MO, USA). Then, the fungal Internal Transcribed Spacer (ITS) region of rDNA was amplified using the forward primer ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3', Gardes and Bruns 1993) and the reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3', White *et al.* 1990). The PCR reaction mixture included 2.5 µl of each primer at 10 µM, 25 µl of GoTaq master mix 2x (Promega, Madison, WI, USA), 18.5 µl of sterile water, and 1.5 µl of template DNA. Thermal cycler conditions were as follows: a 3-min initial denaturation at 94 ºC, followed by 35 cycles of 94 ºC for 1 min, 50 ºC for 1 min, 72 ºC for 1 min and a final 10-min extension at 72 ºC. Amplicons were visualized on a 2 % agarose gel, and those that provided single bands were purified with ExoSap purification kit (USB Corp., Cleveland, OH, USA). Sequencing of the amplicons was performed with forward primer ITS1F using the Big Dye Terminator Cycle Sequencing (Applied Biosystems INC, Foster City, CA, USA). Sequence chromatograms were manually analyzed and edited if necessary (BioEdit Sequence Alignment Editor v.7.0.9.0). Fungal sequences were identified by comparison with highly similar sequences deposited in UNITE database (Kõljalg *et al.* 2013).

Furthermore, the morphological characteristics of each proposed morphotypes were carefully contrasted according to Agerer (1987-2008) with descriptions of color, shape, abundance of hyphae, rhizomorphs and cystidia, among others. In the few cases that molecular identification was negative or doubtful, some tips were identified based on an unambiguous match of their morphological characteristics to those fungi molecularly identified. Root tips that could not be molecularly identified but had a clearly developed fungal mantle were retained for analyses and assigned as 'Unidentified' while the rest were discarded. A total of 272 root tips out of the 381 subjected to enzyme assays and molecular identification were assigned to morphotypes and retained for analysis (67 from *Acacia,* 106 from *Eucalyptus* and 99 from *Pinus*).

Soil chemical parameters

A 200 g subsample of the collected soil surrounding each root sample was oven-dried (48 h at 40 Cº). Dried soil was sieved to 2 mm and submitted for chemical analyses to the

Environmental Analysis Laboratory of Southern Cross University (Lismore, NSW, Australia) for analysis of pH, electrical conductivity (EC, 1:5 water) and contents of total carbon (C), N (LECO method), extractable nitrate (NO_3), ammonium (NH_4) (in KCl extraction), total acid extractable P and available P (Bray method) following standard protocols.

Data Analysis

For each enzyme (LA and AP), a representative community enzyme activity value was calculated per each root sample (i.e. tree) taking into account the abundance of each ECM fungal morphotype in a given tree and its mean activity (average activity of the tips belonging to that morphotype on that tree). This was accomplished with the following equation:

Community Enzyme Activity = $\Sigma(RA_i * X_i)$

(where RA: Relative Abundance of a given morphotype; X: mean enzyme activity of tips of that morphotype; i: number of distinct morphotypes per sample).

Differences in potential community enzyme activity among hosts were evaluated with general linear models (GLMs) that included host (*Acacia, Eucalyptus* and *Pinus*) as a fixed factor and block (A, B, D) as a random factor in an effort to account for any differences due to spatial location. The same statistical model was used to test for differences in soil chemical parameters. Differences in potential enzyme activity among the most abundant ECM fungal morphotypes within each host were assessed using GLMs with morphotype identity as factor, in order to test for complementarity or redundancy effects among fungi. A few morphotypes were detected in two or more hosts. For those, differences in enzyme activity of a given morphotype when colonizing different hosts were assessed using GLMs with host as factor. Normality and homoscedasticity assumptions were tested. Cube root transformation was used when needed. Post-hoc comparisons were performed using a Fisher LSD test. Relationships between community enzyme activity and soil chemical properties were analyzed with Pearson correlation tests. GLM results (F and p values) are shown in Supplementary Table 1. GLMs were also run on a per tip basis (without taking into account the abundance of morphotypes). Results were consistent with those based on the calculated community enzyme activity and are provided in Supplementary Table 2.

Similarity of the ECM community between hosts and blocks was analyzed with principal coordinates analysis (PCoA) using the Bray-Curtis similarity index after Hellinger data transformation, and differences among treatments were evaluated performing PERMANOVA with 9999 permutations. Differences among hosts were tested with post-hoc pair-wise analysis.

Analyses were performed in R version 2.15 (R Core Development Team, Vienna, Austria), either directly or using the interface implemented in InfoStat statistical software version 2017 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina). PCoA and PERMANOVA analyses were run in Past software (Hammer *et al.* 2001). Results are presented as mean values ± 1 SE throughout the text. Significance was established at $p<0.05$.

Results

Soil chemical properties

Overall, soils under the influence of each tree species differed in some chemical properties 15 years after the establishment of the plantation (Table 1). Soil pH, EC and C/N ratio were significantly lower under *Acacia* than under the other two hosts while the opposite was true for total N (i.e. total N was highest in *Acacia*). Available P was significantly lower in *Acacia* soils than in *Pinus* soils while values in *Eucalyptus* soils were intermediate. No differences were observed for organic matter, total C, extractable P, NO_3^- and NH_4^+.

Composition of the ECM community in the different tree hosts

Among the 33 collected samples, a total of 37 morphotypes were distinguished; of these, 21 were molecularly identified and 16 remained as 'Unidentified' (Table 2 and Supplementary Table 3). The six most abundant morphotypes on each tree host, with a mean relative abundance of at least 7 % (corresponding to the overall mean morphotype relative abundance), were Thelephoraceae 1, *Tomentella hjortstamiana*, *Tylospora* sp.1, *Laccaria* sp., *Tylospora* sp.2 and *Clavulina* sp. on *Acacia; Clavulina* sp., *Laccaria* sp., Thelephoraceae 2, *Scleroderma sp.*, Thelephoraceae 3 and *Tomentella* sp. on *Eucalyptus;* and *Rhizopogon* sp., *Tylospora* sp. 2, *Tylospora* sp. 1, *Wilcoxina mikolae*, Thelephoraceae 6 and Thelephoraceae 7 on *Pinus*.

Mycorrhizal community composition differed significantly among hosts ($F=2.13$, $p<0.001$). No differences among blocks ($F=0.68$, $p=0.69$) or interaction between host and block factors ($F=-0.18$, $p=0.09$) were detected. PCoA and pair-wise comparisons showed that community composition in *Acacia* and *Eucalyptus* was similar ($F=1.93$, $p=0.12$) while *Pinus* hosted a distinct ectomycorrhizal community ($F= 3.21$, $p<0.001$ for *Pinus* vs *Acacia*; $F= 2.65$, $p<0.001$ for *Pinus* vs *Eucalyptus*; Fig. 1).

Overall enzyme activity of the ECM community in the different tree hosts

The potential community LA activity (i.e. N-related) of ECM root tips in *Acacia* was significantly higher than that of the ECM community developed in *Pinus*, while the activity of ECM *Eucalyptus* tips was intermediate among them (i.e. not significantly different from either; Fig. 2a). The potential AP activity (P-related) of the ECM community on both *Acacia* and *Eucalyptus* was significantly higher than that of the ECM community on *Pinus* (Fig. 2b).

No correlations were found between either LA or AP activity and any of the measured soil chemical properties except for a negative correlation between soil EC and LA activity (see Supplementary Table 4). No correlations were found between the relative abundance of morphotypes in each sample and either their average LA or AP activity in that sample (Supplementary Table 4).

Enzyme activity of the most abundant morphotypes within each host

Differences in activity among the most abundant morphotypes within a host were found in *Acacia* and *Pinus* for LA (Fig 3a, c) and in *Pinus* for AP (Fig. 3f) while no differences were found in *Eucalyptus* (Fig. 3b, e).

In *Acacia*, Thelephoraceae 1, *Tomentella hjortstamiana* and *Tylospora* sp. 1 showed higher LA activity than *Clavulina* sp. (Fig. 3a). In *Pinus*, Thelephoraceae 6 and Thelephoraceae 7 had higher LA activity than *Tylospora* sp. 1 and *Tylospora* sp. 2 (Fig. 3c). Thelephoraceae 6 also had higher activity than *Rhizopogon* sp. (Fig. 3c). Also in *Pinus*, the AP activity of Thelephoraceae 6 was significantly higher than that of any of the other morphotypes (Fig. 3f). *Rhizopogon* sp., Thelephoraceae 7 and *Tylospora* sp. 1 also had higher AP activity than *Tylospora* sp. 2 (Fig. 3f).

Enzyme activity of a given morphotype in different hosts

Clavulina sp., *Laccaria* sp., Thelephoraceae 1 and *Tylospora* sp. 2 were found in two hosts while *Tylospora* sp. 1 was detected in the three hosts (Table 2). Potential activity was significantly higher when colonizing *Acacia* than when colonizing another host in Thelephoraceae 1 and *Tylospora* sp. 1 for both LA (Fig. 4a) and AP (Fig. 4b), in *Laccaria* sp. for LA (Fig. 4a) and in *Tylospora* sp. 2 for AP (Fig. 4b). No differences when colonizing different hosts were detected in *Clavulina* sp.

Discussion

We found differences in functioning among the ECM communities associated with the three target hosts (i.e. *Acacia*, *Eucalyptus* and *Pinus*). Both the ECM community in *Acacia* and in *Eucalyptus* showed higher potential AP enzyme activity (related to P acquisition ability) than the community in *Pinus*. In addition, the ECM community in *Acacia* also had increased potential LA activity (related to N acquisition ability) compared to *Pinus*. At the morphotype level, we found differences in enzyme activity among ECM morphotypes present on a host plant. More intriguingly, we found differences in enzyme activity for a given morphotype when colonizing different hosts.

Differences in overall potential enzyme activity among hosts

Our results only support our first hypothesis partially. As predicted, we detected increased AP enzyme activity in ECM fungi colonizing *Acacia* (a host that also associates with N-fixing bacteria) but also in ECM fungi colonizing *Eucalyptus* (a host that establishes symbiosis with AM fungi, which also provide P). Interestingly, and contrary to our hypothesis, *Acacia* ECM fungi also showed increased potential LA enzyme activity compared to ECM fungi colonizing *Pinus* (with no other symbiont) while the LA activity of *Eucalyptus* ECM fungi was intermediate. Some researchers have suggested that, given the potential overlap between ECM fungi and N-fixing bacteria, ectomycorrhizas in N-fixing hosts could be especially proficient at P acquisition, leaving most of the N acquisition to N-fixing organisms (Molina *et al.* 1994; Horton *et al.* 2013). In support of the above hypothesis, Walker *et al.* (2014) showed that the ECM community associated with *Alnus rubra* (with N-fixing *Frankia*) had larger potential AP activity and lower LA activity than the community developed in *Pseudotsuga menziesii*. On the contrary, in our study, both AP and LA activities were larger in the N-fixing host (*Acacia*) than in the host with ECM fungi only (*Pinus*), although results could have been different if we had also measured activity in the extraradical mycelium (see below).

One possible explanation for the high enzyme activity of ectomycorrhizas in *Eucalyptus* and, particularly, in *Acacia* could be that ECM fungi were competing with both AM fungi and N-fixing bacteria to gain access to host resources. Bueé *et al.* (2005) showed an increase of metabolic activity in ECM communities exposed to drought stress in European beech (*Fagus sylvatica*) trees, suggesting that enzyme production can respond to environmental conditions. In addition, it has been shown that competition in ECM fungi can promote changes in colonization, nutrient exchange dynamics with the host and RNA expression patterns (Hortal *et al.* 2017). In our study, both rhizobial nodules and AM fungi were observed but we did not measure colonization levels or enzyme activity for those symbionts. Therefore, although our

results suggest that competitive interactions could explain the observed patterns, we can't rule out the possibility that N-fixing bacteria or AM fungi were inactive or only present at low levels.

Alternatively, differences in enzyme activity among hosts could also be explained by the fact that the composition of the *Pinus* ECM community was different than that of the community associated with the other two hosts. We found that the host was key in defining ECM community composition, as the three plant species were established in the same site, at the same time, with similar soil conditions and, most likely, with the same initial fungal spore bank (although there could be differences in nursery inoculum). In addition, other differences among host species such as differential root vitality or C investment (Mosca *et al.* 2007), litter pH (Suz *et al.* 2017), their strategy of resource capture and use (Bauman *et al*. 2016) or the associated microbial mycorhizospheric community (Tarkka *et al.* 2018) could also have played a role in explaining the differences in ECM functioning. For instance, Bauman *et al.* (2016) found that host functional traits related to the 'leaf economics spectrum' may be important in explaining the variability of the ECM community and highlighted the need to test for belowground traits. In this sense, traits related to root architecture, morphology and defense chemistry have been shown to vary among co-existing woody species in North America (Comas *et al.* 2009).

Finally, it has been suggested that the functional response of ECM communities relates to resource availability in the surrounding soil (Buée *et al.* 2007; Rineau and Garbaye 2009; Jones *et al.* 2010, 2012). In our study, though, the soil was originally the same for the three tree species and therefore no large differences in soil properties were observed. Still, soils under *Acacia* had significantly lower available P content and lower pH than *Pinus* soils, which may have contributed to increased AP activity in *Acacia* soils. However, overall we found no correlations between enzyme activity and soil properties. Similar to our findings, Walker *et al.* (2016) showed that exoenzyme activity was more influenced by the composition of the ECM community than by soil chemistry.

Variation in potential enzyme activity among ECM morphotypes

We found evidence for both functional complementarity (lack of overlap in a trait) and redundancy (overlap) among the most abundant ECM morphotypes colonizing a given host species. Enzyme activity can vary considerably among individuals (Baldrian *et al.* 2012; Phillips *et al.* 2014). In this sense, and similarly to other studies (Courty *et al.* 2006; Walker *et al.* 2016), we detected functional complementarity among morphotypes in *Pinus* for both potential LA and AP activity and in *Acacia* for LA, since, in these cases, activity differed among morphotypes, as hypothesized. In contrast, no differences in activity were found for AP

in *Acacia* or for either enzyme in *Eucalyptus*, which suggests functional redundancy among morphotypes, similar to studies of other ECM fungal communities (Dahlberg 2001; Rineau and Courty 2011). These ecological trade-offs can co-exist: while functional complementarity may allow for different ECM benefits being provided to the host plant, functional redundancy can be a way to maintain ecosystem resilience (Pena and Polle 2014).

Interestingly, the most abundant morphotypes did not necessarily show the highest enzyme activity. In order to maximize host nutrient acquisition, it could be speculated that trees would prefer to associate with species that potentially have high enzyme activity. However, we found no correlation between morphotype abundance (in terms of root tips) and activity. In fact, Moeller and Peay (2016) found an inverse relationship between colonization levels and enzymatic activities in ECM fungi. The authors identified a trade-off between competition and function, perhaps mediated by the competing energetic demands associated with competitive interactions and enzymatic production. It has also been shown that ECM species with abundant soil mycelium are relatively more active in producing extracellular enzymes than species forming fewer amounts of mycelium (Tedersoo *et al.* 2012). However, this was not the case in our study. The most abundant morphotypes in *Eucalyptus* and *Acacia* (*Clavulina* sp. and Thelephoraceae 1, respectively) belonged to contact exploration types (Agerer 2001, 2006) with almost no mycelium but had higher activity than the most abundant morphotype in *Pinus* (*Rhizopogon* sp.), which was assigned to a long-distance exploration type forming an extensive mycelium net. Given that extraradical mycelium is a major site for nutrient absorption (Bending and Read 1995; Timonen and Sen 1998), it is possible that the overall enzyme activity measured directly on ECM tips of *Pinus* could have been underestimated. Further research should aim at measuring activity in soil extraradical mycelium, which is currently not feasible as hyphae of multiple species intermingle (Tedersoo *et al.* 2012).

ECM plasticity in enzyme activity when colonizing different hosts

Finally, we were able to show that ECM fungal morphotypes can display a substantial degree of physiological plasticity depending on which host plant species they associate with. This finding contributes to revealing the importance of belowground plasticity, which has been traditionally underappreciated (van der Linde *et al.* 2018). Specifically, we found that a given morphotype can show different levels of enzyme activity when colonizing different tree species, as hypothesized. In particular, four out of the five morphotypes that were found in more than one host showed higher enzymatic activity when colonizing *Acacia* than when colonizing another host. It has been shown that enzyme profiles of the same ECM fungal species can change depending on the soil niche, suggesting potential ability for adaptation to soil conditions

(Courty *et al.* 2005; Bueé *et al.* 2007). Here we show that, in addition to soil, host plant also has a clear influence on enzyme activity production by ECM fungi. In this sense, it has been found that, when given a choice of partners, the host plant is able to discriminate among them (Bogar *et al.* 2019) and limit colonization by the least cooperative fungus (Hortal *et al.* 2017). This may be regulated by changes in root C allocation (Bogar *et al.* 2019) or activation of defense compounds (Hortal *et al.* 2017) and, under some circumstances, may result in ECM fungi becoming more cooperative when competing with alternative partners (Hortal *et al.* 2017).

Conclusions

Overall, we found that the ECM community developed on *Acacia* had both higher LA and AP potential enzyme activity than the community associated with *Pinus*. Differences in enzyme activity could be due to the observed differences in ECM community composition among host species and/or differences in other host-related variables. This may suggest host preferential association patterns with ECM fungal species having different N and P acquisition abilities depending on host demands. However, the most abundant species were not necessarily the ones with the highest enzyme activity. Alternatively, competition among the different types of symbionts to gain access to the host could promote increased activity of ECM fungi in *Acacia*. Interestingly, the observed ECM physiological plasticity when colonizing different hosts supports substantial potential for adaptation in ECM fungi, which will be key under future climate scenarios.

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Conflicts of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1.

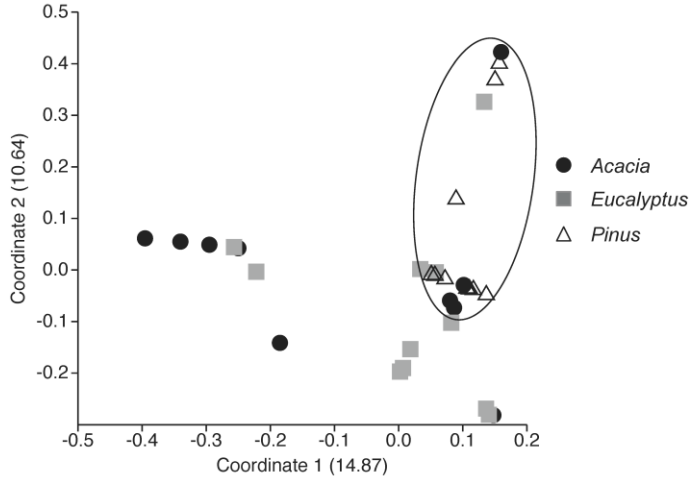

Figure 1. Ordination of ectomycorrhizal community composition by principal coordinates analysis (PCoA) using the Bray-Curtis similarity index. The composition of the community developed in *Pinus* is significantly different than that of the community in the other two hosts by PERMANOVA ($F=2.13$, $p<0.001$). Numbers in brackets indicate the percent of variation explained by each axis. Samples are coded by host, in particular: black filled circles = *Acacia*; grey filled squares = Eucalyptus; triangles = *Pinus*.

Figure 2.

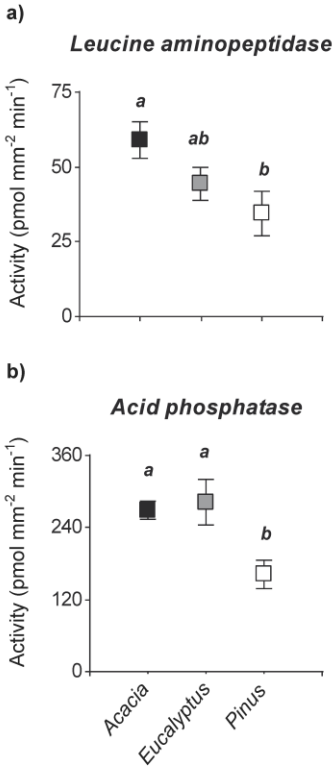

Figure 2. Overall potential community leucine aminopeptidase (a) and acid phosphatase (b) activity of the ectomycorrhizal community developed in roots of *Acacia*, *Eucalyptus* or *Pinus*. Different letters in a graph indicate significant differences ($p < 0.05$) among hosts by GLM and Fisher post-hoc comparison. Data are mean ± 1 SE.

Figure 3.

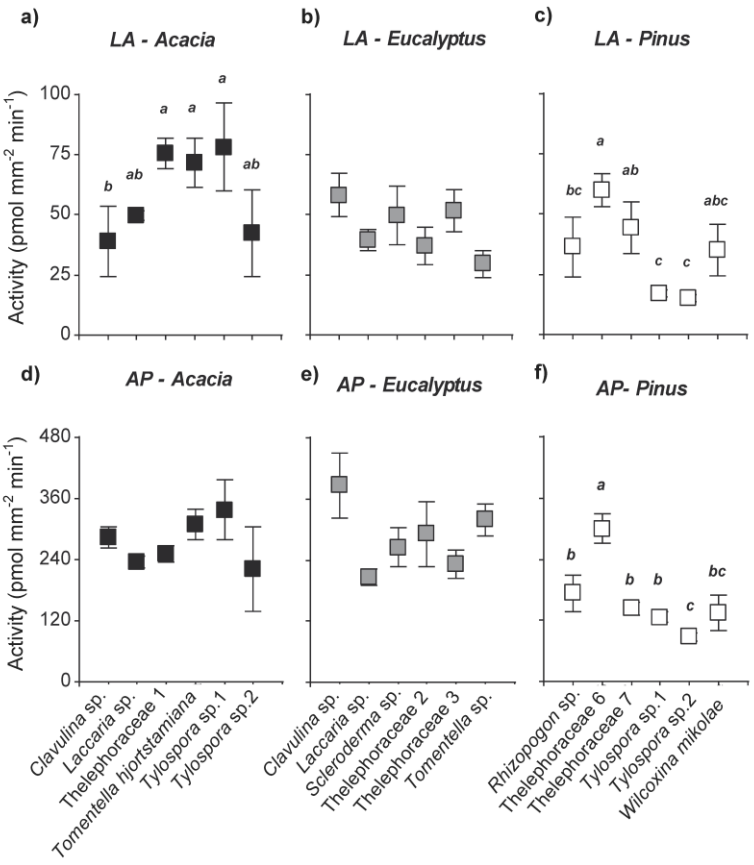

Figure 3. Mean potential leucine aminopeptidase (LA) and acid phosphatase (AP) activity of the most abundant ectomycorrhizal morphotypes in *Acacia* (a, d), *Eucalyptus* (b, e) and *Pinus* (c, f). Different letters in a graph indicate significant differences ($p < 0.05$) among morphotypes in each host by GLM and Fisher post-hoc comparison. Data are mean ± 1 SE.

Figure 4.

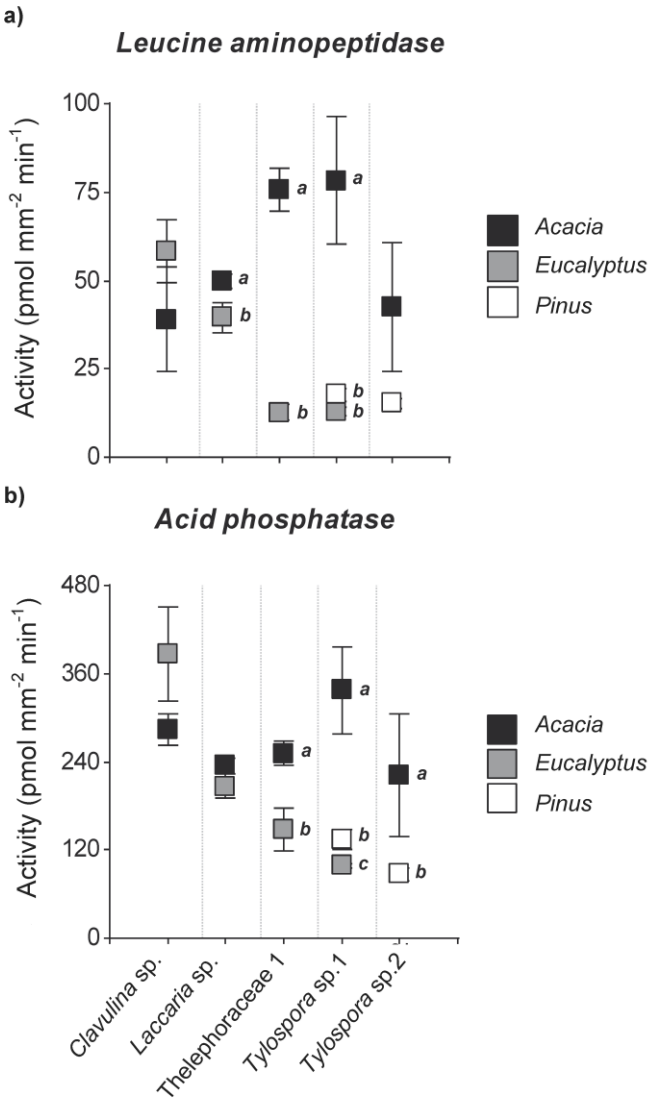

Figure 4. Mean potential leucine aminopeptidase (a) and acid phosphatase (b) activity of the morphotypes found in more than one host when colonizing each of them (*Acacia*, *Eucalyptus* or *Pinus*). Different letters in a graph indicate significant differences ($p < 0.05$) among hosts for a given morphotype by GLM and Fisher post-hoc comparison. Data are mean ± 1 SE.

Table 1. Mean values (± 1 SE) of the measured chemical properties in soil immediately surrounding the roots of the three different hosts. Differences among hosts were evaluated with a general linear model that included host as a fixed factor and block as a random factor. Different letters in a row indicate significant differences among hosts according to Fisher LSD post-hoc test ($p < 0.05$). Significant differences are marked in bold. EC: electrical conductivity; n = 11.

Soil parameter	***Acacia***	***Eucalyptus***	***Pinus***
pH	**5.63 ± 0.43 b**	**6.16 ± 0.06 a**	**6.09 ± 0.07 a**
EC (dS/m)	**0.03 ± 0.01 b**	**0.04 ± 0.01 a**	**0.04 ± 0.01 a**
Organic Matter (%)	2.15 ± 0.50 a	1.97 ± 0.01 a	1.99 ± 0.11 a
Total C (%)	1.24 ± 0.28 a	1.13 ± 0.06 a	1.14 ± 0.06 a
Total N (%)	**0.10 ± 0.02 a**	**0.08 ± 0.01 b**	**0.08 ± 0.01 b**
C/N	**11.65 ± 0.22 b**	**13.81 ± 0.39 a**	**13.63 ± 0.38 a**
Available P (mg kg^{-1})	**8.67 ± 1.58 b**	**15.05 ± 3.88 ab**	**21.11 ± 15.92 a**
Extractable P (mg kg^{-1})	171.55 ± 9.57 a	145.64 ± 5.20 a	162.82 ± 9.08 a
NO_3^- (mg kg^{-1})	1.76 ± 0.22 a	1.34 ± 0.24 a	1.50 ± 0.18 a
NH_4^+ (mg kg^{-1})	5.69 ± 1.66 a	5.68 ± 1.09 a	4.87 ± 0.42 a

Table 2. Mean relative abundance (%) of the different morphotypes found in each tree host. Values in the last row indicate the total number of morphotypes found in each host species. Species present in more than one host are marked in bold.

Assigned Morphotype Name	***Acacia***	***Eucalyptus***	***Pinus***
***Clavulina* sp.**	7.09	17.27	-
Inocybe sp.	0.73	-	-
Laccaria bicolor	-	3.82	-
***Laccaria* sp.**	8.09	14.36	-
Pseudotomentella rhizopunctata	-	-	1.82
Rhizopogon salebrosus	-	-	5.18
Rhizopogon sp.	-	-	17.73
Scleroderma sp.	-	10.27	-
Thelephoraceae 1	34.00	6.27	-
Thelephoraceae 2	-	11.64	-
Thelephoraceae 3	-	8.45	-
Thelephoraceae 4	-	4.55	-
Thelephoraceae 5	-	1.36	-
Thelephoraceae 6	-	-	9.36
Thelephoraceae 7	-	-	7.64
Tomentella hjortstamiana	14.18	-	-
Tomentella sp.	-	8.36	-
Tuber sp.	-	-	2.00
***Tylospora* sp. 1**	9.09	4.55	12.45

***Tylospora* sp. 2**	7.73	-	13.27
Wilcoxina mikolae	-	-	9.82
Unidentified 1	3.00	-	-
Unidentified 2	2.18	-	-
Unidentified 3	3.64	-	-
Unidentified 4	5.00	-	-
Unidentified 5	0.64	-	-
Unidentified 6	4.64	-	-
Unidentified 7	-	0.36	-
Unidentified 8	-	3.73	-
Unidentified 9	-	0.91	-
Unidentified 10	-	0.64	-
Unidentified 11	-	3.45	-
Unidentified 12	-	-	7.27
Unidentified 13	-	-	4.55
Unidentified 14	-	-	1.82
Unidentified 15	-	-	5.45
Unidentified 16	-	-	1.64
TOTAL RICHNESS	**13**	**16**	**14**