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# Functional outcomes of fungal community shifts driven by tree genotype and spatial-temporal factors in Mediterranean pine forests

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**Running Head:** Outcomes of fungal shifts in Mediterranean forests

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**"Originality-Significance Statement"**

Among microbial communities of forest ecosystems, fungi are key actors implied in main ecosystem services such as organic matter decomposition and nutrient cycling. Hence, there is an increasing interest in elucidating their interactive intricate relationships with the surrounding environment, which is indeed one of the major challenges in fungal ecology at present. Here, we approach this topical subject by studying the effect of biotic (i.e. tree genotype) and abiotic (i.e. season, site) factors on forest soil fungal communities, going beyond by questioning whether changes in the structure of these microbial communities may trigger functional responses affecting key ecosystem services. We demonstrate that, together with spatial-temporal factors, the tree genotype strongly structures fungal communities, that variations in fungal diversity affect carbon turnover and nutrient mobilization, and that all this can differ depending on whether the fungi involved are ectomycorrhizal or saprotrophic. Moreover, we propose an innovative mechanistic model providing an integrative view of these complex interrelations between ecosystem functions, fungal diversity, trees productivity and edaphic variables. The knowledge about how plant-fungus-environment interact and the mechanisms underlying ecosystem functioning will allow us making predictions to tackle future climate change scenarios in Mediterranean forests, helping to foster the sustainable management of these particularly vulnerable ecosystems.

**Summary**

Fungi provide relevant ecosystem services contributing to primary productivity and the cycling of nutrients in forests. These fungal inputs can be decisive for the resilience of Mediterranean forests under global change scenarios, making necessary an in-deep knowledge about how fungal communities operate in these ecosystems. By using high-throughput sequencing and enzymatic approaches, we studied the fungal communities associated with three genotypic variants of *Pinus pinaster* trees, in 45-yr-old common garden plantations. We aimed to determine the impact of biotic (i.e. tree genotype) and abiotic (i.e. season, site) factors on the fungal community structure, and to explore whether structural shifts triggered functional responses affecting relevant ecosystem processes. Tree genotype and spatial-temporal factors were pivotal structuring fungal communities, mainly by influencing their assemblage and selecting certain fungi. Diversity variations of total fungal community and of that of specific fungal guilds, together with edaphic properties and tree's productivity, explained relevant ecosystem services such as processes involved in carbon turnover and phosphorous mobilization. A mechanistic model integrating relations of these variables and ecosystem functional outcomes is provided. Our results highlight the importance of structural shifts in fungal communities because they may have functional consequences for key ecosystem processes in Mediterranean forests.

**Keywords**: fungal community, ectomycorrhizas, seasonality, *Pinus pinaster*, tree genotype, enzymes

## Introduction

Fungal communities are key components of forest ecosystems involved in the biogeochemical cycling of nutrients and the productivity of trees. Saprotrophic fungi are primary decomposers, and ectomycorrhizal (ECM) fungi play main roles in decomposition and mobilization of nutrients (Lindahl *et al.*, 2007; Rineau *et al.*, 2013). Trees can invest up to a third of its primary production to maintain their associated ECM fungi (Smith and Read, 2008) in exchange for water and nutrients, fungal traits that can be especially important under harsh environmental conditions. Fungi decompose the organic matter by the production of a wide set of extracellular enzymes capable of degrading complex cell wall biopolymers (Baldrian, 2014; Shah *et al.*, 2015). Fungal decomposition processes fluctuate seasonally in forest soils, parallel to shifts in substrate availability and temperature and moisture variations (Baldrian *et al.*, 2013). Seasonal effects can be particularly pronounced in warm and water limited forests such as the Mediterranean ones (Scarascia-Mugnozza *et al.*, 2000). Trees are main drivers of seasonality in resource availability for fungi via litter fall in autumn and belowground carbon exudation and uptake of nutrients in spring (Kaiser *et al.*, 2010; Voříšková *et al.*, 2014). Substrate supply in turn, stimulates the production of extracellular enzymes by fungi (Hernández and Hobbie, 2010; Navrátilová *et al.*, 2016), which can display distinct enzymatic traits depending on the environmental conditions and the fungal species (Courty *et al.*, 2005; Buée *et al.*, 2007; Bödeker *et al.*, 2009).

Microbial communities have been considered the extended phenotype of plant individuals, i.e. a heritable trait of a foundation tree species whose variation can impact the entire ecosystem (Whitham *et al.*, 2003; van der Heijden *et al.*, 2015). This allows interpreting certainly at present the plant and its microbiota as a unique holobiont system (Vandenkoornhuyse *et al.*, 2015; Hacquard, 2016). The characteristics of the dominant tree species in a site may delimit the fungal communities in soil through microclimatic variations and the organic inputs provided (Priha *et al.*, 1999; Kernaghan *et al.*, 2003), with potential effects on the ecosystem functioning. Within this context, for instance the

poplar genotype determined the degree of colonization of different ectomycorrhizal fungal isolates (Tagu *et al.*, 2005), or the enzymatic activity of *Laccaria bicolor* ectomycorrhizas (Courty *et al.*, 2011). Other studies have revealed the tree host genotype as crucial structuring their associated fungi (Korkama *et al.*, 2006; Sthultz *et al.*, 2009; Courty *et al.*, 2011; Velmala *et al.*, 2013; Lamit *et al.*, 2016). Given the heterogeneous spatial-temporal distribution patterns of fungal communities, their dependence on the edaphic-climatic characteristics, the plant community composition and/or the tree host, assessing their interactive responses to biotic and abiotic factors is currently a major challenge in fungal ecology (van der Heijden *et al.*, 2015).

*Pinus pinaster* Ait. is a representative species in the Mediterranean Basin, covering approximately 1800000 ha in Spain (Villanueva, 2005). Three main geographic provenances, i.e. Atlantic, Mediterranean and African, with a clear genetic differentiation have been described (Baradat and Marpeau, 1988; Alía and Moro, 1996; Rodriguez-Quilon *et al.*, 2016). These different genotypes display a great phenotypic variability in traits such as cold, fire and drought tolerance, pest resistance, or growth and biomass production (Alía and Moro, 1996). We examined trees from the three main *P. pinaster* genotypes established in replicated long-term common garden plantations with the aim to (i) study the impact of biotic (i.e. tree genotype) and abiotic (i.e. season and site) factors on the diversity and assemblage of their associated fungal communities, and to (ii) explore whether structural shifts in fungal communities trigger functional responses affecting relevant ecosystem processes. Due to the heterotrophic nature of fungi, we predicted that under rather similar environmental conditions, tree genotypes differing in their productivity would support different taxonomic and functional fungal assemblages. Since carbon inputs are tightly linked to the phenology of trees (Buée *et al.*, 2005; Koide *et al.*, 2007) and the influence of roots (Cheng and Gershenson, 2007), fungal responses to the tree genotype would be dependent on the season, particularly affecting obligate biotrophic fungal guilds such as the ectomycorrhizal one. Expected structural shifts in fungal communities were further predicted to entail functional consequences related with the cycling of nutrients.

## Results

*Sequencing yields and identification of fungi*

A total of 1412 MOTUs were obtained (Fig. 1a; Table S1). Almost half of MOTUs were shared by the three tree genotypes, while close to 9 % were common to each two genotypes, or exclusively found under one tree genotype (Fig. 1a). The 65.7 % of MOTUs were present at both seasons, and the 15.5 % and the 18.8 % found in spring and autumn, respectively. Almost a third of MOTUs was found in all sites (Fig. 1a; Table S1).

Sequencing and MOTUs yields per sample were quite homogeneous across treatments (Table S1). The 81.7 % of MOTUs, representing approximately the 99 % of reads, were assigned to phylum, e.g. 38 % Basidiomycota, 37.7 % Ascomycota, and 5.5 % Zygomycota. The 60.7 % of MOTUs was ascribed to family, the 50.4 % to genus, and the 27.5 % identified down to the species level. The life style of near the 60 % of MOTUs, representing the 93 % of reads, was inferred, most of which were saprotrophic (SAP, 47.4 %) and ectomycorrhizal (ECM, 44.6 %).

Among the 20 most abundant fungi, the ECM predominated together with two saprotrophic *Mortierella* sp. (Table 1). Certain MOTUs were preferentially associated with a tree genotype, while others were indicators of each season (Fig. 1b; Table S2). Among the tree genotypes, the Atlantic showed the most divergent indicator species profile, while the Mediterranean and African were relatively similar (Fig. 1b). Additionally, the tree genotype preferentially associated with certain fungi depending on the season and the site (i.e. genotype × season, genotype × site) (Table S2).

*Fungal community structure*

The tree genotype significantly affected the α-diversity of basidiomycetes (i.e. less α-diverse under the Atlantic trees), but not that of the overall community, or the rest of fungal guilds (Fig. 2a). The season clearly affected the total fungal α-diversity (i.e. higher in autumn than spring) (Fig. 2b); ascomycetes and zygomycetes kept this pattern, whereas basidiomycetes were equally α-diverse in

both seasons (Fig. 2b). By life style, the ECM fungi were more α-diverse in spring than autumn, whereas the saprotrophic fungi displayed the opposite pattern (Fig. 2b). All factors, i.e. tree genotype, season and site, significantly structured local soil fungal assemblages, with particularly strong spatial-temporal effects (Table 2a; Fig. S3). As drawn by NMDS, many edaphic variables and fungal functions significantly correlated with the local assemblage of fungi (Fig. S3).

Concerning the regional species pool, total fungal β-diversity was unaffected by the tree genotype or the season, while a strong site effect interacting with the rest of factors was observed (Table 2b; Fig. S4). The β-diversity of ascomycetes, zygomycetes, and saprotrophic fungi were more β-diverse (i.e. more heterogeneous) in autumn than spring, while the ECM guild showed the opposite pattern (Table 2b; Fig. S4). Except for ECM, a significant strong site effect was observed for all fungal guilds, generally with lower β-diversity (i.e. more homogeneous) in Cabañeros site (Table 2b; Fig. S4).

Focusing on lower taxonomic levels and consistently across sites, the tree genotype selectively affected the α-diversity of certain representative ectomycorrhizal fungal families, while others did not respond (e.g. *Russulaceae*, *Thelephoraceae*) (Table 3). For example, under the Atlantic trees, *Atheliaceae* and *Entolomataceae* were less α-diverse compared with the other tree genotypes, and with the Mediterranean one in the case of *Bankeraceae, Sebacinaceae* and *Tuberaceae* (Table 3); contrarily, *Amanitaceae, Inocybaceae* and *Pyronemataceae* were more α-diverse under the Atlantic than the African trees (Table 3). As previously pointed out by overall diversity results, the season had a significant effect that was specifically revealed on certain ectomycorrhizal families, generally more α-diverse in spring (Table 3), and on numerous saprotrophic families that peaked up in autumn (Table 3).

The assemblage of MOTUs within families did mainly vary with the site, interacting with the tree genotype (e.g. *Atheliaceae*, *Russulaceae*, *Sebacinaceae*, *Thelephoraceae*) and the season (e.g.

166 *Amanitaceae*, *Herpotrichiellaceae*, *Mortierellaceae*, *Tricholomataceae*), depending on the family
167 (Table S3).
168
169 *Linking fungal diversity, abiotic and biotic environment, and ecosystem functioning*
170 When modelled as a function of abiotic variables, higher fungal α-diversity was explained by greater
171 values of relative humidity, pH, EC and N, or by lower values of K and C:N (Table 4), although
172 variations were observed depending on the fungal guild. For example, high organic matter explained
173 low α-diversity of ascomycetes and zygomycetes, and oppositely high α-diversity of basidiomycetes
174 and ECM (Table 4). The α-diversity of basidiomycetes and ectomycorrhizal fungi did not vary with
175 soil pH or C:N ratio, opposite to the rest of fungi. Furthermore, the productivity of trees particularly
176 affected the ECM fungi, for which higher tree productivity explained lower α-diversity (Table 4).
177 The tree genotype mainly influenced the glucuronidase activity (Table 5), while most C-cycle related
178 enzymes varied with the season, especially for ascomycetes, zygomycetes and the saprotrophic guild,
179 (Table 5). In most cases, fungal α-diversity significantly explained ecosystem functions related with
180 the degradation of hemicellulose (i.e. xylosidase, glucuronidase) and recalcitrant C compounds (i.e.
181 laccase) (Table 5). For example, the α-diversity of basidiomycetes was negatively related with
182 almost all C-cycle processes (Table 5). Furthermore, high α-diversity of saprotrophs explained high
183 C turnover (Table 5), whereas contrarily high ectomycorrhizal α-diversity explained low C-cycling
184 (Table 5).
185 The structural-equation model provided a good fit for all enzymatic activities, with non-significant f
186 value ($\chi^2 = 4.90$; P = 0.672) and with goodness-of-fit indices (RMSEA < 0.001, NFI and GFI > 0.97).
187 Significant effects differed depending on the enzymatic set (Fig. 3). In all cases, the tree productivity
188 marginally and positively affected the P content in soil, on which pH had a strong negative effect
189 (Fig. 3). Edaphic variables had positive (i.e. pH, soil humidity, P) or negative (i.e. OM) effects on
190 overall fungal diversity (Shannon). The productivity of trees exerted a positive and direct effect over

cellulose degrading-enzymes and a marginal effect over hemicellulose degrading ones (Fig. 3). Hemicellulose degrading-enzymes and laccase activities were positively affected by soil humidity, and laccase also by pH (Fig. 3). By contrast, the soil humidity and pH negatively affected N-cycle enzymes (Fig. 3). Phosphatase and N-cycle enzymes were significantly more active with increased OM (Fig. 3). Total fungal diversity was negatively related with hemicellulose degrading-enzymes and phosphatase activity (Fig. 3).

**Discussion**

In addition to recognized abiotic features such as soil moisture, organic matter content, and acidity, our study reveals biotic (i.e. tree genotype) and spatial-temporal (i.e. site, season) factors as key agents structuring fungal communities in Mediterranean forests, and brings out mechanistic patterns linking fungal diversity and environmental conditions with functional traits.

We found high fungal diversity associated with *P. pinaster*, similar to that previously reported for this tree species (Rincón *et al.*, 2014; Buscardo *et al.*, 2015). As predicted, the tree genotype was an important agent structuring the fungal communities associated with *P. pinaster*, mainly through influencing their assemblage and the diversity of certain groups, such as basidiomycetes and representative fungal families. Under semi-arid conditions, (Gehring and Whitham, 1991) observed a much higher negative effect of herbivory on ectomycorrhizal fungi under susceptible than resistant pinyon pines. They also detected more diversity of basidiomycetes under the resistant trees, similar to what we observed under the Mediterranean and African genotypes, probably in relation with a high representation of ectomycorrhizal fungi within this phylum and/or the quantity/quality of the carbon inputs delivered by trees. In this sense, fast and low growing spruce clones have been shown to associate different ECM fungi both in greenhouse (Velmala *et al.*, 2013) and field plantations (Korkama *et al.*, 2006), similar to results reported by Sousa *et al.* (2012) for *P. pinaster* clones, the same tree species of the present study. In our study, when fungal diversity was responsive to the tree

genotype, main differences were found only under one of the two less productive trees i.e. Atlantic, indicating the importance of additional factors as for example the quality of tree organic inputs. However, it should be additionally considered that a single tree may associate multiple fungal genotypes and each interacting organism (i.e. plant-fungus-fungus) can differently respond to the same environmental constraints or/and stimuli (Bahram *et al.*, 2011; Johnson *et al.*, 2012), which greatly complicate interpreting interaction outcomes.

As expectable in a Mediterranean ecosystem with contrasted annual climatic variation, the season exerted a great influence on the structure of fungal communities. Both α and β-diversity of the total fungal community were generally higher in autumn than spring, when peaks of spore dispersion, as well as of mycelium and sporocarp production occur (Boddy *et al.*, 2014). However, when analyzed by life-style, the strategy changed from ectomycorrhizal-dominated communities in spring to saprotrophic ones in autumn, probably in relation with the preference and/or availability of resources (i.e. belowground carbon exudation or litter fall). Together with the season effects, the site was a strong filter at local and regional scales for all fungal guilds, and unequivocal signs of fungal site dependent responses were observed emphasizing the importance of local environment and processes, as recently underlined (Tedersoo *et al.*, 2016). This spatial-temporal habitat filtering leaded in all cases to more heterogeneous communities probably by increasing the competition of species (Olden *et al.*, 2004; Flores-Rentería *et al.*, 2016). Spatial-temporal scale fungal shifts are tightly linked to the environmental conditions and the phenology of trees, with the light, soil pH, soil nutrients, temperature and moisture as main abiotic drivers (Cooke *et al.*, 1993; Buée *et al.*, 2005; Coince *et al.*, 2014; Rincón *et al.*, 2015), many of them higly related with the assemblage and diversity of fungi in our study.

*Are soil ECM fungal communities particularly responsive to the tree host?*

Although the α-diversity of ectomycorrhizal fungi was quite independent of the tree genotype, our initial hypothesis was partially supported by the response of representative ectomycorrhizal families (i.e. usually less α-diverse under the Atlantic trees), and by β-diversity results (i.e. significant interactions of tree genotype with site and season). A potential host filtering effect was supported by the indicator species associated with each tree genotype that were mainly ectomycorrhizal and more similar between the Mediterranean and African trees. Besides, these results indicated that, in some cases, fungal host preference was dependent on the particular seasonal and site conditions (i.e. environmental filtering). All these findings support that the tree genotype may select their associated fungi, particularly the ectomycorrhizal ones, and that this is likely to be context dependent, suggesting that the plant can modulate its associated microbial community for a dynamical adjustment to the environment (Vandenkoornhuyse *et al.*, 2015). The productivity of trees did not influence total fungal diversity, but it negatively impacted that of ECM fungi, probably indicating a stronger host filtering effect on fungi with which establishing an exchange partnership (Johnson *et al.*, 2010). This could be related with preferential host plant photosyntate allocation to more beneficial (Bever *et al.*, 2009), or less carbon demanding fungi (Gehring *et al.*, 2014) observed within spatially structured mycorrhizal fungal communities, which has been interpreted as a mechanism for mutualism stabilization (Kiers *et al.*, 2011). In our study, together with strong seasonal effects, the tree genotype was implicated in the response of some ecosystem functions to variations in fungal α-diversity (e.g. hemicellulose degradation). In concordance with previous studies (Bending and Read, 1996; Bailey *et al.*, 2005; Velmala *et al.*, 2013; Lamit *et al.*, 2016), altogether our results give evidences to support that differences on photosynthetic productivity (quantity/quality) of the tree genotypes may be at the origin of their dissimilar structural and functional associated fungal communities, especially the ectomycorrhizal ones.

*Establishing links between fungal diversity, environment, and functional traits*

Our results revealed that relevant ecosystem services involved in C turnover were explained not only by variations in total fungal α-diversity but also in that of specific fungal guilds. Ectomycorrhizal fungal α-diversity was negatively related with most C-cycle processes, while that of saprotrophs displayed a positive relation, according to the divergent life history of these two major fungal guilds, and possibly reflecting competitive interactions (Fernandez and Kennedy, 2016; Martin *et al.*, 2016). Results relating α-diversity and C-cycle activities mirrored a possible predominance of functional guilds within taxonomic ones and vice versa (i.e. basidiomycetes and cellulose-degrading ascomycetes could be mostly ectomycorrhizal, and hemicellulose and cellulose-degrading ascomycetes and zygomycetes mostly saprotrophic), results which would deserve further phylogenetic examination. These findings could also reflect separated main mechanisms (i.e. hydrolytic *vs* oxidative) of saprotrophs and ectomycorrhizal fungi for organic matter decomposition (Shah *et al.*, 2015; Fernandez and Kennedy, 2016).

Structural equation models gave a mechanistic integrative view linking fungal diversity, edaphic conditions and functional traits. The productivity of trees directly influenced the cycling of carbon trough triggering cellulose and hemicellulose degrading enzymes, in agreement with the "priming effect" (i.e. increased carbon inputs stimulate microbial decomposition, Phillips *et al.* (2012)). According to Lindahl *et al.* (2002), this could imply the removal of C with retention of N, as nitrogenous compounds are delivered from complex polyphenolic substrates. This could be supported by the direct and positive relation observed between organic matter and N-related enzymes in our study. Laccase, which degrades recalcitrant compounds, was not related to tree's productivity or organic matter, probably because a more subtle interrelation based on the quality and not the quantity of C inputs occurs, though this would merit further analysis. Soil pH and moisture negatively affected N cycle related enzymes, as observed by Sinsabaugh *et al.* (2008) for pH and chitinase activity. Tree productivity and soil pH controlled phosphorous availability. Together with nitrogen, phosphorous is usually deficient in Mediterranean soils characterized by fast

decomposition and extremely thin litter layers (Sardans *et al.*, 2004), a nutritional limitation that may severely reduce the productivity of trees (Plassard *et al.*, 2011). Phosphorus availability increased fungal diversity, which in turn predicted lower phosphatase activity in soil. Plants can increase C allocation to roots and their mycorrhizal associates to alleviate P deficiency (van der Heijden, 2001; Kiers *et al.*, 2011), although the reduced phosphatase activity and its activation by organic matter suggest that other mechanisms could be operating, e.g. the production of organic acids or chelators, and/or bacterial inputs (Plassard and Dell, 2010; Clarholm *et al.*, 2015). Contrarily to P, high organic matter explained reduced fungal diversity, which in turn predicted higher hemicellulose degrading activity pointing out to the possible dominance of certain fungi more competitive under these conditions.

In conclusion, our results show that the intricate relations between aboveground tree individuals and spatial-temporal variants drive structural shifts in fungal communities with functional consequences that affect relevant ecosystem processes i.e. C turnover, phosphorous mobilization. According to Bardgett *et al.* (2005), we highlight the need of experimental field designs recovering spatial and temporal variability for better predicting the consequences of tree-soil feedbacks. Our results suggest that the tree genotype is able to modulate its associated fungal community to adapt better to the environment by selecting certain fungal consortia, which may influence the functioning of the entire ecosystem.

## Experimental procedures

### *Study sites and sampling*

The study was conducted in common gardens established by the Spanish Forest Patrimony of State in 1967 with *P. pinaster* trees from different geographic origins (Alía and Moro, 1996). Three sites with rather similar environmental characteristics were located in central Spain: Cabañeros (39º 22'N, 4º 24'W), Riofrío (39º 8'N, 4º 32'W), and Espinoso del Rey (39º 36'N, 4º 48'W) (Fig. S1a). In all

sites, the climate is Mediterranean, with cold wet winters and hot dry summers, mean annual temperature between 12-13.4 ºC and precipitation of 716-800 mm. The plant community is dominated by *P. pinaster*, with scattered *Quercus suber* L., *Quercus pyrenaica* Willd., and the understory composed of dispersed evergreen shrubs (e.g. *Erica arborea* L., *Cystus* sp., *Arcthostaphyllus uva-ursi* (L.) Spreng, *Lavandula stoechas* L., *Hallimiumum bellatum* (L.) Spach.).

Originally, all common garden plantations were settled in a completely randomized block design with four blocks and *P. pinaster* of different provenances (named "genotype" from herein), with 16 trees per each, separated of 2.5 m (Alía and Moro, 1996). Trees of contrasted geographic provenances, i.e. Atlantic (Galicia, Spain), Mediterranean (Valencia, Spain), and African (Jbel Tassali, Morocco), were selected for this study (Fig. S1a). These tree genotypes have been previously demonstrated to diverge genotypically and phenotypically (Alía and Moro, 1996; Rodriguez-Quilon *et al.*, 2016). The three selected tree genotypes showed different productivity i.e. diameter at breast height, consistently across sites (Fig. S1b).

At each site, three trees per genotype and block were sampled in spring and autumn of 2012 (3 sites × 3 tree genotypes × 4 blocks × 3 trees × 2 seasons). Because a firewall created at one site (Espinoso del Rey) 6 trees lacked for a complete factorial design, and a total of 102 trees were sampled each season. Under each tree, litter was removed 1 m far from the trunk and subsamples were obtained by excavating 10 x 10 x 20 cm, at N, S, E and W orientations. The four subsamples per tree were joined in a single soil sample and kept at 4 ºC until processing. Once in the lab, soil samples were homogenized, sieved at 2 mm, and aliquots stored at -20 ºC for further molecular analyses. Remaining soil was air-dried for chemical analyses.

*Soil analyses and enzymatic tests*

Soil samples were pooled by tree genotype per site and experimental block into single composite replicates for chemical analyses (n = 35, per season). The relative humidity (RH) of soils was

determined by drying at 65 ºC for 48 h. Other soil variables were measured, such as pH (1:5, w:v in $H_2O$), electrical conductivity (1:5, w:v in $H_2O$), organic matter (OM) and total carbon (C), total nitrogen (N) (Kjeldahl method), and extractable phosphorus (P) and potassium (K) determined by inductively coupled plasma spectrometry (Optima 4300DV, Perkin-Elmer).

Fungal community functioning was evaluated by measuring soil activities of eight hydrolytic and oxidative exoenzymes secreted by fungi, following the methodology adapted from Mathieu *et al.* (2013). Seven enzymatic tests targeting different nutrient cycling processes were performed e.g. β-glucosidase (EC 3.2.1.3), cellobiohydrolase (EC 3.2.1.91), implicated in cellulose degradation; xylosidase (EC 3.2.1.37), and β-glucuronidase (EC 3.2.1.31), involved in hemi-cellulose degradation; laccase (1.10.3.2) involved in the oxidation of recalcitrant substrates such as phenols or lignin; phosphatase acid (EC 3.1.3.2) mobilizing phosphorous; and chitinase (EC 3.2.1.14) and L-leucineaminopeptidase (3.4.11.1) involved in the mobilization of nitrogen. The Lacasse activity was determined by a photometric assay based on ABTS substrate (2,2'-Azino-bis (3-ethylbenzo-thiazolin-6-sulfonic acid) as described by Mathieu *et al.* (2013). The rest of tests were based on fluorogenic substrate release, i.e. methylumbelliferone (MU) or methylcoumarine (AMC) (for L-leucineaminopeptidase). Measurements were carried out in a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA), with 355/460 nm excitation/emission wavelengths for the fluorogenic assays and 415 nm for laccase. At each season, enzymatic analyses were performed on single soil samples (n = 102), and data were thereafter pooled into composite replicates (n =35), as previously explained. All enzymatic activities were expressed in pmol $min^{-1}$ mg of $soil^{-1}$.

*DNA extraction, PCR and 454-pyrosequencing*

Genomic DNA was extracted from 0.5 g of soil with the PowerSoil kit (MoBio, Carlsbad, CA, USA). The internal transcribed spacer region ITS-1 of the nuclear ribosomal DNA was amplified with the primer pair ITS1F-ITS2 (Gardes and Bruns, 1993) adapted for pyrosequencing as described by Buée

365 *et al.* (2009). PCR amplifications (3 min 94 ºC, 30 cycles of 1 min 94 ºC, 30 s 53 ºC and 45 s 72 ºC,
366 with a final step of 10 min 72 ºC) were conducted in a Verity Thermal Cycler (Life Technologies),
367 and each sample amplified in three independent 20 µl reactions, each containing 2 µl of 10x
368 polymerase buffer, 2.4 µl of 25 mM $MgCl_2$, 1.12 µl of 10 mg $ml^{-1}$ BSA, 0.4 µl of 10 mM nucleotide
369 Mix, 0.4 µl of 10 mM forward/reverse primers (adaptor A-tag-ITS1F/adaptor B-ITS2), and 0.2 µl of
370 AmpliTaqGold polymerase (5 U $ml^{-1}$) (Applied Biosystems, Carlsbad, CA, USA). Negative controls
371 without DNA were included in all runs to detect possible contaminations. Independent reactions
372 were combined per sample, and each PCR product was purified (UltraClean PCR clean-up kit of
373 MoBio, Carlsbad, CA, USA), quantified (PicoGreen, Life Technologies, Carlsbad, CA, USA) and
374 pooled in equimolar libraries (one per season) containing 35 uniquely tagged replicates, each
375 resulting of pooling three samples by each tree genotype per site and experimental block.
376 Pyrosequencing was carried out in a GsFLX-454 system (Roche Applied Biosystems, USA) in an
377 external service (Parque Científico de Madrid, Spain).
378
379 *Bioinformatic analyses*
380 Sequences were de-multiplexed according to their tags, filtered and trimmed using the *fastq_filter*
381 command and *fastq_truncqual* option of USEARCH v7.0.1001 (Edgar, 2013) and quality scores less
382 or equal than 10 were eliminated. The ITS1 was extracted with the Fungal ITSx v1.0.3 (Bengtsson-
383 Palme *et al.*, 2013) and partial ITS sequences shorter than 100 bp were discarded. De-replication of
384 extracted ITS sequences was performed with the *derep_fulllength* USEARCH command. De-
385 replicated sequences were then sorted by decreasing abundance, and singletons discarded with the
386 *sortbysize* USEARCH command. The 92.3 % (166927) of the initial set of sequences (180921) was
387 retained. Molecular operational taxonomic units (MOTUs) were generated from abundance-sorted
388 sequences using the *cluster_otus* USEARCH command with a 97 % similarity threshold. Extracted
389 ITS sequences, including singletons, were then mapped against the MOTU representative sequences

using the *usearch_global* USEARCH command. Taxonomic assignation of representative sequences for each MOTU was done by using the Basic Local Alignment Search Tool (BLAST) algorithm v 2.2.23 (Altschul *et al.*, 1990) against the UNITE database release 7.1 (Kõljalg *et al.*, 2013). Once taxonomic identification was achieved, fungal MOTUs were classed by their life style i.e. ectomycorrhizal, saprotrophic, endomycorrhizal, parasite, pathogen, lichen or unknown according to Tedersoo *et al.* (2014). The 454 .sff files and raw data were deposited in the Sequence Read Archive (SRA-NCBI, http://www.ncbi.nlm nih.gov/sra) as SRP076022.

*Statistical analyses*

All variables were verified for normality and homoscedasticity, and relations among them tested by Spearman correlation analysis ($p<0.05$). Alpha-diversity (i.e. number of MOTUs) of total and fungal guilds (i.e. life style, fungal phyla, families) was modelled by Generalized Linear Mixed Models (GLMM) with the tree genotype and season as fix factors, and the site as random factor, considering the number of reads as covariate. Relationships between fungal alpha-diversity (total and by fungal guilds i.e. different phyla and life styles) with soil properties and enzymatic variables were also tested by GLMM (Pinheiro *et al.*, 2014).

To identify fungal MOTUs significantly more represented across the different treatments, the Indicator Species Analysis (with MOTUs >100 reads) was carried out ($p<0.05$) (Cáceres *et al.*, 2013). Bray-Curtis distance matrices of fungal species were calculated based on the abundance matrix of MOTUs, previously normalized (i.e. DESeq variance stabilization; McMurdie and Holmes 2014) (Anders and Huber, 2012). Over this matrix, fungal Beta-diversity was calculated (Anderson *et al.*, 2006; Oksanen *et al.*, 2015), considering the factors genotype, season, site and their interactions. Fungal community assemblage was analyzed by multivariate analysis of variance (PERMANOVAs) and nonmetric multidimensional scaling (NMDS) analysis (Oksanen *et al.*, 2015).

All statistical analyses were carried out with the R software v3.0.2 (R Core Team, 2014).

*Structural Equation Models*

To get an integrative outline of the relationships among fungal diversity, function and edaphic properties, structural equation modelling (SEMs) was performed. An aprioristic model explicitly including the causal relationships among variables was built based on literature (Flores-Rentería *et al.*, 2016) (Fig. S2). Our sample size was relatively small (n = 70) and the predictors included in the model were restricted, as recommended (Shipley, 2002). Enzymatic activities, representative of different nutrient cycles (i.e. glucosidase, cellobiohydrolase, xylosidase, glucuronidase, laccase for C; leucine and chitinase for N; acid phosphatase for P), were analyzed in separated models, and the Shannon index, which integrates frequency and abundance, was chosen as fungal diversity variable. It was hypothesized that fungal diversity, as well as the tree productivity (represented by the diameter at breast height, DBH), and edaphic conditions (e.g. RH, pH, C/N, OM and P) would determine the ecosystem functioning (Fig. S2). Causal relations and correlations among biotic and abiotic variables were included in the model, and all direct and indirect relations between exogenous and endogenous variables tested. Several models including all explicative variables were run, and the best fitted chosen according to the setting between the covariance in observed and expected data (i.e. goodness-of-fit $\chi 2$). Standardized path coefficients were estimated by using the maximum likelihood algorithm (Shipley, 2002). Model fit to data was evaluated by root mean square error of approximation (RMSEA) and the goodness-of-fit index (GFI) and the Bentler and Bonett's normed-fit index (NFI). SEMs were built with AMOS v.20.0 software (IBM Corporation Software Group, Somers, NY).

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444 **References**

445 Alía, R. and Moro, J. (1996) Comportamiento de procedencias de *Pinus pinaster* en el centro de
446 España. *For. Syst.* **5**: 57–75.
447 Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic Local Alignment
448 Search Tool. *J. Mol. Biol.* **215**: 403–410.
449 Anders, S. and Huber, W. (2012) Differential expression of RNA-Seq data at the gene level–the
450 DESeq package Heidelberg, Germany: European Molecular Biology Laboratory (EMBL).
451 Anderson, M., Ellingsen, K., and McArdle, B. (2006) Multivariate dispersion as a measure of beta
452 diversity. *Ecol. Lett.* **9**: 683–693.
453 Bahram, M., Põlme, S., Kõljalg, U., and Tedersoo, L. (2011) A single European aspen (*Populus*
454 *tremula*) tree individual may potentially harbour dozens of Cenococcum geophilum ITS
455 genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiol. Ecol.* **75**: 313–
456 320.
457 Bailey, J.K., Deckert, R., Schweitzer, J.A., Rehill, B.J., Lindroth, R.L., Gehring, C., and Whitham,
458 T.G. (2005) Host plant genetics affect hidden ecological players: links among *Populus* ,
459 condensed tannins, and fungal endophyte infection. *Can. J. Bot.* **83**: 356–361.
460 Baldrian, P. (2014) Distribution of Extracellular Enzymes in Soils: Spatial Heterogeneity and
461 Determining Factors at Various Scales. *Soil Sci. Soc. Am. J.* **78**: 11.
462 Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T., and Valášková, V. (2013)
463 Responses of the extracellular enzyme activities in hardwood forest to soil temperature and
464 seasonality and the potential effects of climate change. *Soil Biol. Biochem.* **56**: 60–68.
465 Baradat, P. and Marpeau, A. (1988) Le pin maritime Pinus pinaster Ait. Biologie et génétique des
466 terpènes pour la conaissance et l'amélioration de l'espèce Université Bordeaux I, Bordeaux,
467 France.
468 Bardgett, R.D., Bowman, W.D., Kaufmann, R., and Schmidt, S.K. (2005) A temporal approach to

linking aboveground and belowground ecology. *Trends Ecol. Evol.* **20**: 634–641.

Bending, G.D. and Read, D.J. (1996) Effects of the soluble polyphenol tannic acid on the activities of ericoid and ectomycorrhizal fungi. *Soil Biol. Biochem.* **28**: 1595–1602.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., et al. (2013) Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods Ecol. Evol.* **4**: 914–919.

Bever, J.D., Richardson, S.C., Lawrence, B.M., Holmes, J., and Watson, M. (2009) Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecol. Lett.* **12**: 13–21.

Boddy, L., Büntgen, U., Egli, S., Gange, A.C., Heegaard, E., Kirk, P.M., et al. (2014) Climate variation effects on fungal fruiting. *Fungal Ecol.* **10**: 20–33.

Bödeker, I.T.M., Nygren, C.M.R., Taylor, A.F.S., Olson, Å., and Lindahl, B.D. (2009) ClassII peroxidase-encoding genes are present in a phylogenetically wide range of ectomycorrhizal fungi. *ISME J.* **3**: 1387–1395.

Buée, M., Courty, P.E., Mignot, D., and Garbaye, J. (2007) Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. *Soil Biol. Biochem.* **39**: 1947–1955.

Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S., and Martin, F. (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytol.* **184**: 449–456.

Buée, M., Vairelles, D., and Garbaye, J. (2005) Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus silvatica*) forest subjected to two thinning regimes. *Mycorrhiza* **15**: 235–245.

Buscardo, E., Rodriguez-Echeverria, S., Freitas, H., De Angelis, P., Pereira, J.S., and Muller, L.A.H. (2015) Contrasting soil fungal communities in Mediterranean pine forests subjected to different

494 wildfire frequencies. *Fungal Divers.* **70**: 85–99.
495 Cáceres, M.D., Legendre, P., and He, F. (2013) Dissimilarity measurements and the size structure of
496 ecological communities. *Methods Ecol. Evol.* **4**: 1167–1177.
497 Cheng, W. and Gershenson, A. (2007) Carbon Fluxes in the Rhizosphere. In, Cardon,Z.G. and
498 Whitbeck,J.L. (eds), *The Rhizosphere. An Ecological Perspective*. Elsevier Inc., London,UK, pp.
499 31–56.
500 Clarholm, M., Skyllberg, U., and Rosling, A. (2015) Organic acid induced release of nutrients from
501 metal-stabilized soil organic matter - The unbutton model. *Soil Biol. Biochem.* **84**: 168–176.
502 Coince, A., Cordier, T., Lengellé, J., Defossez, E., Vacher, C., Robin, C., et al. (2014) Leaf and root-
503 associated fungal assemblages do not follow similar elevational diversity patterns. *PLoS One* **9**:
504 e100668.
505 Cooke, R.C., Rayner, A.D.M., and Whipps, J. (1993) Ecophysiology of fungi Oxford, UK:
506 Blackwell Science Publications.
507 Courty, P.E., Labbé, J., Kohler, A., Marçais, B., Bastien, C., Churin, J., et al. (2011) Effect of poplar
508 genotypes on mycorrhizal infection and secreted enzyme activities in mycorrhizal and non-
509 mycorrhizal roots. *J. Exp. Bot.* **62**: 249–260.
510 Courty, P.E., Pritsch, K., Schloter, M., Hartmann, A., and Garbaye, J. (2005) Activity profiling of
511 ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New Phytol.*
512 **167**: 309–319.
513 Edgar, R. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. **10**:
514 996–998.
515 Fernandez, C. and Kennedy, P. (2016) Revisiting the ' Gadgil effect ': do interguild fungal
516 interactions control carbon cycling in forest soils ? Tansley review Revisiting the ' Gadgil
517 effect ': do interguild fungal interactions control carbon cycling in forest soils ? *New Phytol.*
518 **209**: 1382–1394.

519 Flores-Rentería, D., Rincón, A., Valladares, F., and Curiel Yuste, J. (2016) Agricultural matrix
520 affects differently the alpha and beta structural and functional diversity of soil microbial
521 communities in a fragmented Mediterranean holm oak forest. *Soil Biol. Biochem.* **92**: 79–90.
522 Gardes, M. and Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes,
523 application to the identification of mycorrihiza and rusts. *Mol. Ecol.* **2**: 113–118.
524 Gehring, C.A., Mueller, R.C., Haskins, K.E., Rubow, T.K., and Whitham, T.G. (2014) Convergence
525 in mycorrhizal fungal communities due to drought, plant competition, parasitism, and
526 susceptibility to herbivory: Consequences for fungi and host plants. *Front. Microbiol.* **5**: 1–9.
527 Gehring, C.A. and Whitham, T.G. (1991) Herbivore-driven mycorrhizal mutualism in insect-
528 susceptible pinyon pine. *Nature* **353**: 556–557.
529 Hacquard, S. (2016) Disentangling the factors shaping microbiota composition across the plant
530 holobiont. *New Phytol.* **209**: 454–457.
531 van der Heijden, E.W. (2001) Differential benefits of arbuscular mycorrhizal and ectomycorrhizal
532 infection of Salix repens. *Mycorrhiza* **10**: 185–193.
533 van der Heijden, M.G.A., Martin, F.M., Selosse, M.A., and Sanders, I.R. (2015) Mycorrhizal
534 ecology and evolution: The past, the present, and the future. *New Phytol.* **205**: 1406–1423.
535 Hernández, D.L. and Hobbie, S.E. (2010) The effects of substrate composition, quantity, and
536 diversity on microbial activity. *Plant Soil* **335**: 397–411.
537 Johnson, D., Martin, F., Cairney, J.W.G., and Anderson, I.C. (2012) The importance of individuals:
538 Intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New Phytol.* **194**: 614–
539 628.
540 Johnson, N.C., Wilson, G.W.T., Bowker, M. a, Wilson, J. a, and Miller, R.M. (2010) Resource
541 limitation is a driver of local adaptation in mycorrhizal symbioses. *Proc. Natl. Acad. Sci. U. S.*
542 *A.* **107**: 2093–8.
543 Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., et al. (2010)

544 Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme
545 activities by altering microbial community composition in a beech forest soil. *New Phytol.* **187**:
546 843–58.
547 Kernaghan, G., Widden, P., Bergeron, Y., Legare, S., and Pare, D. (2003) Biotic and abiotic factors
548 affecting ectomycorrhizal diversity in boreal mixed-woods. *Oikos* **102**: 497–504.
549 Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., et al. (2011)
550 Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. *Science (80-. ).* **333**:
551 880–882.
552 Koide, R.T., Courty, P.E., and Garbaye, J. (2007) An evolving host for mycorrhizal research. *New*
553 *Phytol.* **174**: 225–228.
554 Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., et al. (2013)
555 Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* **22**: 5271–
556 5277.
557 Korkama, T., Pakkanen, A., and Pennanen, T. (2006) Ectomycorrhizal community structure varies
558 among Norway spruce (*Picea abies*) clones. *New Phytol.* **171**: 815–824.
559 Lamit, L.J., Holeski, L.M., Flores-Rentería, L., Whitham, T.G., and Gehring, C.A. (2016) Tree
560 genotype influences ectomycorrhizal fungal community structure: Ecological and evolutionary
561 implications. *Fungal Ecol.* 1–11.
562 Lindahl, B.O., Ihrmark, K., Boberg, J., Trumbore, S., Högberg, P., Stenlid, J., and Finlay, R.D.
563 (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal
564 forest. *New Phytol.* **173**: 611–620.
565 Lindahl, B.O., Taylor, A.F.S., and Finlay, R.D. (2002) Defining nutritional constraints on carbon
566 cycling in boreal forests - Towards a less “phytocentric” perspective. *Plant Soil* **242**: 123–135.
567 Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., and Hibbett, D.S. (2016) Unearthing the
568 roots of ectomycorrhizal symbioses. *Nat. Rev. Microbiol.* **14**: 760–773.

569 Mathieu, Y., Galhaye, E., Dumarçay, S., Gérardin, P., Harvendt, L., and Buée, M. (2013) Selection
570 and validation of enzymatic activities as functional markers in wood biotechnology and fungal
571 ecology. *J. Microbiol. Methods* **92**: 157–163.
572 McMurdie, P.J. and Holmes, S. (2014) Waste not, want not: why rarefying microbiome data is
573 inadmissible. *PLoS Comput. Biol.* **10**: e1003531.
574 Navrátilová, D., Větrovský, T., and Baldrian, P. (2016) Spatial heterogeneity of cellulolytic activity
575 and fungal communities within individual decomposing Quercus petraea leaves. *Fungal Ecol.*
576 1–9.
577 Oksanen, A.J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., Hara, R.B.O., et al. (2015)
578 Vegan: Community Ecology Package R package version 2.3-1. http://CRAN.R-
579 project.org/package=vegan.
580 Olden, J.D., Poff, N.L., Douglas, M.R., Douglas, M.E., and Fausch, K.D. (2004) Ecological and
581 evolutionary consequences of biotic homogenization. *Trends Ecol. Evol.* **19**: 18–24.
582 Phillips, R.P., Meier, I.C., Bernhardt, E.S., Grandy, A.S., Wickings, K., and Finzi, A.C. (2012) Roots
583 and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO 2. *Ecol. Lett.*
584 **15**: 1042–1049.
585 Pinheiro, J., Bates, D., DebRoy, S., and Sarkar, D. (2014) R Core Team (2014). nlme: linear and
586 nonlinear mixed effects models R package version 3.1–117. URL: http://cran. r-project.
587 org/web/packages/nlme/index. html.
588 Plassard, C. and Dell, B. (2010) Phosphorus nutrition of mycorrhizal trees. *Tree Physiol.* **30**: 1129–
589 1139.
590 Plassard, C., Louche, J., Ali, M.A., Duchemin, M., Legname, E., and Cloutier-Hurteau, B. (2011)
591 Diversity in phosphorus mobilisation and uptake in ectomycorrhizal fungi. *Ann. For. Sci.* **68**:
592 33–43.
593 Priha, O., Grayston, S., Pennanen, T., and Smolander, A. (1999) Microbial activities related to C and

N cycling and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil. *FEMS Microbiol. Ecol.* **30**: 187–199.

R Core Team (2014) R Core Team. *R a Lang. Environ. Stat. Comput. Vienna, Austria R Found. Stat. Comput. https//www.r-project.org/*.

Rincón, A., Santamaría-Pérez, B., Ocaña, L., and Verdú, M. (2014) Structure and phylogenetic diversity of post-fire ectomycorrhizal communities of maritime pine. *Mycorrhiza* **24**: 131–141.

Rincón, A., Santamaría-Pérez, B., Rabasa, S.G., Coince, A., Marçais, B., and Buée, M. (2015) Compartmentalized and contrasted response of ectomycorrhizal and soil fungal communities of Scots pine forests along elevation gradients in France and Spain. *Environ. Microbiol.* **17**: 3009–3024.

Rineau, F., Shah, F., Smits, M.M., Persson, P., Johansson, T., Carleer, R., et al. (2013) Carbon availability triggers the decomposition of plant litter and assimilation of nitrogen by an ectomycorrhizal fungus. *ISME J.* **7**: 2010–22.

Rodriguez-Quilon, I., Santos-del-Blanco, L., Serra-Varela, M.J., Koskela, J., Gonzalez-Martinez, S.., and Alia, R. (2016) Capturing neutral and adaptive genetic diversity for conservation in a highly structured tree species. *Ecol. Appl.* **26**: 2254–2266.

Sardans, J., Rodà, F., and Peñuelas, J. (2004) Phosphorus limitation and competitive capacities of Pinus halepensis and Quercus ilex subsp rotundifolia on different soils. *Plant Ecol.* **174**: 305–317.

Scarascia-Mugnozza, G., Oswald, H., Piussi, P., and Radoglou, K. (2000) Forests of the Mediterranean region: Gaps in knowledge and research needs. *For. Ecol. Manage.* **132**: 97–109.

Shah, F., Nicolás, C., Bentzer, J., Ellström, M., Smits, M., Rineau, F., et al. (2015) Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytol.* **209**: 1705–1719.

619 Shipley, B. (2002) Cause and correlation in biology: a user's guide to path analysis, structural
620 equations and causal inference Cambridge University Press.
621 Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., et al.
622 (2008) Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* **11**: 1252–64.
623 Smith, S. and Read, D. eds. (2008) Mycorrhizal Symbiosis Academic Press: London.
624 Sousa, N.R., Ramos, M.A., Franco, A.R., Oliveira, R.S., and Castro, P.M.L. (2012) Mycorrhizal
625 symbiosis affected by different genotypes of *Pinus pinaster*. *Plant Soil* **359**: 245–253.
626 Sthultz, C.M., Whitham, T.G., Kennedy, K., Deckert, R., and Gehring, C.A. (2009) Genetically
627 based susceptibility to herbivory influences the ectomycorrhizal fungal communities of a
628 foundation tree species. *New Phytol.* **184**: 657–67.
629 Tagu, D., Bastien, C., Faivre-Rampant, P., Garbaye, J., Vion, P., Villar, M., and Martin, F. (2005)
630 Genetic analysis of phenotypic variation for ectomycorrhiza formation in an interspecific F1
631 poplar full-sib family. *Mycorrhiza* **91**: 15–87.
632 Tedersoo, L., Bahram, M., Cajthaml, T., Põlme, S., Hiiesalu, I., Anslan, S., et al. (2016) Tree
633 diversity and species identity effects on soil fungi, protists and animals are context dependent.
634 *ISME J.* **10**: 346–362.
635 Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N.S., Wijesundera, R., et al. (2014) Global
636 diversity and geography of soil fungi. *Science (80-. ).* **346**: 1256688.
637 Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A. (2015) The
638 importance of the microbiome of the plant holobiont. *New Phytol.* **206**: 1196–1206.
639 Velmala, S.M., Rajala, T., Haapanen, M., Taylor, A.F.S., and Pennanen, T. (2013) Genetic host-tree
640 effects on the ectomycorrhizal community and root characteristics of Norway spruce.
641 *Mycorrhiza* **23**: 21–33.
642 Villanueva, J. (ed) (2005) Tercer Inventario Forestal Nacional (1997–2007). Ministerio de Medio
643 Ambiente,M. (ed).

644 Voříšková, J., Brabcová, V., Cajthaml, T., and Baldrian, P. (2014) Seasonal dynamics of fungal
645 communities in a temperate oak forest soil. *New Phytol.* **201**: 269–278.
646 Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S.M., et al.
647 (2003) Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology*
648 **84**: 559–573.
649
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**Table 1.** The 20-most abundant fungal MOTUs found in *Pinus pinaster* Ait. forests under different tree genotypes: Atl = Atlantic. Med = Mediterranean. Afr = African, and at different seasons: Sp = spring. Au = autumn. *= not in the top-20 list of the respective treatment. ECM = ectomycorrhizal; SAP = saprotrophic. ¥ = number of reads.

| Tentative identification | Life style | NCBI / UNITE / RDP | BLAST ID | % id | E-value | Total Sequences | Genotype¥ | | | Season¥ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Atl | Med | Afr | Sp | Au |
| *Russula amethystina* | ECM | UDB000303 | *R. amethystina* | 100 | 1E-84 | 8413 | 4072 | 2184 | 2157 | 5492 | 2921 |
| *Amphinema* sp. | ECM | SH210842 | *Amphinema* | 100 | 5E-85 | 7874 | 3259 | 2699 | 1916 | 5645 | 2229 |
| *Mortierella* sp. | SAP | SH214832 | *Mortierella* | 100 | 8E-69 | 6232 | 2327 | 2074 | 1831 | 1443 | 4789 |
| *Tylospora* sp. | ECM | FJ013075 | *Tylospora* | 100 | 4E-79 | 5780 | 1637 | 1932 | 2211 | 3369 | 2411 |
| *Russula cessans* | ECM | UDB015971 | *R. cessans* | 100 | 1E-92 | 4739 | 764 | 2130 | 1845 | 3437 | 1302 |
| *Hydnellum ferrugineum* | ECM | KC571730 | *H. ferrugineum* | 89 | 3E-77 | 4506 | 232* | 2138 | 2136 | 2351 | 2155 |
| *Sebacina* sp. | ECM | SH231619 | *Sebacina* | 100 | 3E-82 | 3845 | 829 | 1267 | 1749 | 2648 | 1197 |
| *Russula torulosa* | ECM | UDB011110 | *R.torulosa* | 100 | 4E-93 | 3315 | 1259 | 1392 | 664 | 2593 | 722* |
| *Mortierella* sp. | SAP | DQ093726 | *Mortierella* | 100 | 5E-83 | 3069 | 1061 | 1091 | 917 | 1228 | 1841 |
| *Cenococcum geophilum* | ECM | KC967408 | *C. geophilum* | 98 | 2E-60 | 2825 | 1323 | 788 | 714 | 1752 | 1073 |
| *Amphinema* sp. | ECM | SH210842 | *Amphinema* | 99 | 3E-81 | 2675 | 1295 | 784 | 596 | 2115 | 560* |
| *Russula amethystina* | ECM | KF850402 | *R. amethystina* | 98 | 2E-73 | 2487 | 868 | 1195 | 424* | 1291 | 1196 |
| *Clavulina* sp. | ECM | SH220805 | *Clavulina* | 100 | 3E-102 | 2225 | 319* | 1099 | 807 | 1180 | 1045 |
| *Inocybe* sp. | ECM | SH231190 | *Inocybe* | 100 | 2E-77 | 2084 | 1218 | 234* | 632 | 1469 | 615* |
| *Inocybe posterula* | ECM | JF908152 | *I. posterula* | 99 | 3E-121 | 1847 | 212* | 1097 | 538* | 1421 | 426* |
| *Inocybe* sp. | ECM | JF908227 | *Inocybe* | 99 | 4E-111 | 1764 | 331* | 858 | 575 | 813* | 951 |
| *Tricholoma portentosum* | ECM | UDB017949 | *T. portentosum* | 100 | 1E-120 | 1691 | 410* | 446* | 835 | 1681 | 10* |
| *Inocybe mixtilis* | ECM | JX679372 | *I. mixtilis* | 100 | 2E-97 | 1682 | 515* | 291* | 876 | 922* | 760 |
| *Russula versicolor* | ECM | SH224391 | *R. versicolor* | 99 | 2E-91 | 1656 | 329* | 411* | 916 | 1007 | 649* |
| *Cortinariaceae* sp. | ECM | GQ159878 | *Cortinarius* | 96 | 4E-69 | 1622 | 609 | 642 | 642* | 960 | 662* |

**Table 2.** (a) Assemblage of MOTUs and (b) Beta-diversity of the total fungal community and of representative subgroups: effects of tree genotype (G), season (S), site (Sit) and their interactions assessed by permutation variance analyses and Multivariate Homogeneity of Groups Dispersions, respectively. df = degrees of freedom. F and *p*-value: **p*<0.05; ***p*<0.01;****p*<0.001. ASCO = ascomycetes; BASI = basidiomycetes; ZYGO = zygomycetes; ECM= ectomycorrhizal; SAP = saprotrophic.

| | | TOTAL | | ASCO | | BASI | | ZYGO | | ECM | | SAP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **(a) Assemblage** | df | $R^2$ | F | $R^2$ | F | $R^2$ | F | $R^2$ | F | $R^2$ | F | $R^2$ | F |
| Tree genotype | 2 | 0.04 | **1.8**$^{**}$ | 0.04 | **2.1**$^{**}$ | 0.04 | **1.7**$^{*}$ | 0.03 | **1.8**$^{*}$ | 0.04 | **1.7**$^{*}$ | 0.04 | **1.9**$^{**}$ |
| Season | 1 | 0.05 | **4.5**$^{***}$ | 0.07 | **6.8**$^{***}$ | 0.03 | **2.6**$^{**}$ | 0.19 | **21.6**$^{***}$ | 0.03 | **2.8**$^{**}$ | 0.12 | **11.6**$^{***}$ |
| Site | 2 | 0.25 | **11.8**$^{***}$ | 0.26 | **13.6**$^{***}$ | 0.27 | **12.6**$^{***}$ | 0.18 | **10.2**$^{***}$ | 0.28 | **13.7**$^{***}$ | 0.24 | **13.1**$^{***}$ |
| G x S | 2 | 0.01 | 0.6 | 0.01 | 0.7 | 0.01 | 0.4 | 0.01 | 0.8 | 0.01 | 0.4 | 0.01 | 0.8 |
| G x Sit | 4 | 0.06 | **1.4**$^{**}$ | 0.06 | **1.5**$^{*}$ | 0.07 | **1.5**$^{**}$ | 0.04 | 1.1 | 0.07 | **1.6**$^{**}$ | 0.05 | **1.5**$^{*}$ |
| S x Sit | 2 | 0.03 | 1.2 | 0.03 | 1.4 | 0.02 | 0.9 | 0.05 | **2.8**$^{**}$ | 0.02 | 1.1 | 0.03 | **1.7**$^{*}$ |
| G x S x Sit | 4 | 0.03 | 0.6 | 0.03 | 0.7 | 0.02 | 0.3 | 0.02 | 0.6 | 0.01 | 0.3 | 0.03 | 0.7 |
| **(b) β-diversity** | df | $R^2$ | F | $R^2$ | F | $R^2$ | F | $R^2$ | F | $R^2$ | F | $R^2$ | F |
| Tree genotype | 2 | 0.01 | 0.2 | 0.04 | 1.3 | 0.02 | 0.7 | 0.67 | 0.3 | 0.00 | 0.0 | 0.00 | 0.0 |
| Season | 1 | 0.00 | 0.3 | 0.18 | **1.5**$^{***}$ | 0.04 | 3.0 | 0.37 | **40.7**$^{***}$ | 0.63 | **114**$^{***}$ | 0.32 | **31.3**$^{***}$ |
| Site | 2 | 0.50 | **33.7**$^{***}$ | 0.24 | **10.5**$^{***}$ | 0.42 | **23.8**$^{***}$ | 0.11 | **4.3**$^{*}$ | 0.03 | 1.0 | 0.12 | **4.8**$^{**}$ |
| G x S | 5 | 0.02 | 0.2 | 0.24 | **4.1**$^{**}$ | 0.07 | 0.9 | 0.35 | **6.9**$^{***}$ | 0.60 | **19.1**$^{***}$ | 0.33 | **6.4**$^{***}$ |
| G x Sit | 8 | 0.50 | **7.7**$^{***}$ | 0.29 | **3.1**$^{**}$ | 0.43 | **5.9**$^{***}$ | 0.17 | 1.6 | 0.03 | 0.2 | 0.15 | 1.3 |
| S x Sit | 5 | 0.47 | **11.5**$^{***}$ | 0.35 | **7.0**$^{***}$ | 0.44 | **10.2**$^{***}$ | 0.52 | **14.0**$^{***}$ | 0.55 | **15.3**$^{***}$ | 0.41 | **9.1**$^{***}$ |
| G x S x Sit | 17 | 0.50 | **3.1**$^{***}$ | 0.45 | **2.5**$^{**}$ | 0.46 | **2.6**$^{**}$ | 0.44 | 2.4$^{**}$ | 0.50 | **3.0**$^{***}$ | 0.37 | 1.8 |

**Table 3.** Alpha-diversity of representative fungal families and effects of tree genotype (G) and season (S) and its interaction (G x S) analysed by general linear mixed models with site as random factor. Main test results are shown in the first three columns (F values; *p<0.05; **p<0.01;***p<0.001), followed by post-hoc LSD test analysis (p<0.05) for tree genotype (Atl = Atlantic; Med = Mediterranean; Afr = African) and season; values = means +/- SE; for each factor, different letters denote significant differences (in bold). § = ectomycorrhizal families.

| | Tree genotype | Season | G x S | Tree genotype | | | Season | |
|---|---|---|---|---|---|---|---|---|
| | | | | Atl | Med | Afr | Spring | Autumn |
| *Amanitaceae*§ | **5.2**** | **17.3***** | **3.1*** | **0.8 ± 0.1 b** | **0.6 ± 0.1 ab** | **0.5 ± 0.2 a** | **0.9 ± 0.1 b** | **0.4 ± 0.1 a** |
| *Atheliaceae* | **3.8*** | 2.5 | 0.0 | **12.5 ± 0.8 a** | **14.5 ± 0.9 b** | **15.0 ± 1.0 b** | 14.6 ± 0.7 | 13.3 ± 0.8 |
| *Archaeorhizomycetaceae* | 1.1 | **18.5***** | 0.6 | 3.0 ± 0.3 | 2.8 ± 0.4 | 2.9 ± 0.3 | **2.3 ± 0.2 a** | **3.5 ± 0.2 b** |
| *Bankeraceae*§ | **5.1**** | 0.0 | 0.0 | **0.7 ± 0.4 a** | **1.8 ± 0.5 b** | **1.7 ± 0.4 ab** | 1.5 ± 0.4 | 1.3 ± 0.3 a |
| *Clavulinaceae*§ | 0.3 | 0.2 | 0.2 | 1.0 ± 0.2 | 1.4 ± 0.3 | 0.9 ± 0.2 | 1.1 ± 0.2 | 1.1 ± 0.2 |
| *Cortinariaceae*§ | 1.3 | **4.5*** | 0.3 | 2.7 ± 0.3 | 2.6 ± 0.3 | 3.2 ± 0.4 | **3.2 ± 0.3 b** | **2.5 ± 0.3 a** |
| *Entolomataceae* | **13.3***** | 3.1 | 0.3 | **0.4 ± 0.1 a** | **1.1 ± 0.1 b** | **0.8 ± 0.1 b** | 0.8 ± 0.1 | 0.7 ± 0.1 |
| *Herpotrichiellaceae* | 2.7 | **47.1***** | 0.7 | 14.6 ± 0.9 | 13.7 ± 1.0 | 14.9 ± 0.8 | **12.3 ± 0.6 a** | **16.5 ± 0.7 b** |
| *Hygrophoraceae* | 0.5 | 0.3 | 0.5 | 1.0 ± 0.2 | 0.9 ± 0.2 | 0.9 ± 0.2 | 0.9 ± 0.1 | 1.0 ± 0.2 |
| *Hypocreaceae* | 1.3 | **43.8** | 0.9 | 1.6 ± 0.3 | 1.8 ± 0.3 | 1.9 ± 0.3 | **0.9 ± 0.2 a** | **2.6 ± 0.3 b** |
| *Inocybeaceae*§ | **3.4*** | 2.4 | 0.9 | **9.3 ± 0.7 b** | **8.7 ± 0.6 ab** | **8.1 ± 0.70 a** | 9.3 ± 0.6 | 8.1 ± 0.5 |
| *Mortierellaceae* | 1.3 | **84.9***** | 0.8 | 10.5 ± 0.8 | 10.6 ± 0.7 | 11.7 ± 1.0 | **8.1 ± 0.4 a** | **13.8 ± 0.5 b** |
| *Pezizaceae* | 1.7 | 2.3 | 2.2 | 2.4 ± 0.3 | 2.1 ± 0.4 | 1.9 ± 0.3 | 2.2 ± 0.3 | 2.1 ± 0.3 |
| *Pyronemataceae* | **10.8***** | 0.3 | 0.3 | **1.7 ± 0.2 b** | **1.3 ± 0.2 ab** | **0.9 ± 0.2 a** | 1.4 ± 0.2 | 1.2 ± 0.2 |
| *Rhizopogonaceae*§ | 2.6 | **8.7**** | 0.2 | 2.8 ± 0.2 | 1.9 ± 0.2 | 2.3 ± 0.2 | **2.7 ± 0.2 b** | **2.0 ± 0.2 a** |
| *Russulaceae*§ | 0.9 | 3.0 | 0.8 | 5.9 ± 0.5 | 6.1 ± 0.6 | 6.8 ± 0.7 | 7.0 ± 0.6 | 5.5 ± 0.4 |
| *Sebacinaceae*§ | **7.9***** | 0.1 | 1.0 | **2.8 ± 0.5 a** | **4.8 ± 0.6 b** | **3.3 ± 0.4 ab** | 3.8 ± 0.4 | 3.5 ± 0.4 |
| *Telephoraceae*§ | 0.9 | 0.7 | 0.4 | 11.5 ± 0.7 | 12.1 ± 0.6 | 12.8 ± 0.9 | 12.4 ± 0.6 | 11.9 ± 0.6 |
| *Trichocomataceae* | 0.6 | **7.9**** | 0.5 | 5.5 ± 0.4 | 5.3 ± 0.4 | 5.8 ± 0.4 | **5.0 ± 0.3 a** | **6.0 ± 0.3 b** |
| *Tricholomataceae* | 0.4 | **6.9*** | 1.4 | 2.1 ± 0.3 | 1.8 ± 0.3 | 2.0 ± 0.3 | **2.5 ± 0.3 b** | **1.4 ± 0.2 a** |
| *Tuberaceae*§ | **6.9**** | 1.3 | 0.3 | **1.1 ± 0.1 a** | **1.5 ± 0.1 b** | **1.4 ± 0.1 ab** | 1.4 ± 0.1 | 1.3 ± 0.1 |
| *Umbelopsidaceae* | 2.2 | **28.8***** | 0.1 | 7.6 ± 0.4 | 6.7 ± 0.5 | 7.3 ± 0.5 | **6.2 ± 0.3 a** | **8.2 ± 0.3 b** |

**Table 4.** Generalized linear mixed models testing the response of fungal alpha-diversity to edaphic variables or DBH, and to the factors tree genotype and season. The site was included as random factor within models. Interactions were not significant. Degrees of freedom in all models: numDF =1 and denDF = 62. $R^2$adj, t and *p* values of the α-diversity~edaphic variable models; Significance: . <0.1; * <0.05; ** <0.01; ***<0.001; ns = not significant. RH= relative humidity, EC = electric conductivity, OM = organic matter, N = nitrogen, P = phosphorous, K = potassium, C:N = carbon:nitrogen ratio, DBH = tree diameter at breast height.

| | RH (%) | pH | EC (µS/cm) | OM (%) | N (%) | P (mg/kg) | K (mg/kg) | C:N | DBH (cm) |
|---|---|---|---|---|---|---|---|---|---|
| **Total MOTUs** | | | | | | | | | |
| $R^2_{adj}$ | 0.55 | 0.48 | 0.46 | 0.44 | 0.53 | 0.55 | 0.52 | 0.49 | 0.50 |
| t *p* | 2.4*** | 2.1** | 0.2*** | -1.0 | 1.6** | 3.2 | -0.3* | -3.2*** | -1.5 |
| Tree genotype (*p*) | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Season (*p*) | ns | *** | * | *** | *** | *** | *** | *** | *** |
| **Ascomycetes** | | | | | | | | | |
| $R^2_{adj}$ | 0.73 | 0.75 | 0.73 | 0.74 | 0.74 | 0.75 | 0.73 | 0.76 | 0.74 |
| t *p* | 0.3*** | 3.2*** | -0.2*** | -1.1*** | 0.8 | 1.4** | -1.6*** | -2.3*** | 0.8 |
| Tree genotype (*p*) | ns | * | ns | ns | ns | ns | ns | ns | ns |
| Season (*p*) | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| **Basidiomycetes** | | | | | | | | | |
| $R^2_{adj}$ | 0.25 | 0.12 | 0.11 | 0.20 | 0.24 | 0.14 | 0.14 | 0.11 | 0.30 |
| t *p* | 2.3 | 1.5 | 0.4 | 1.4* | 2.3* | 0.9* | 0.4 | -0.8 | -3.4* |
| Tree genotype (*p*) | * | * | * | ns | ns | * | * | * | ** |
| Season (*p*) | * | ns | ns | ns | ns | ns | ns | ns | ns |
| **Zygomycetes** | | | | | | | | | |
| $R^2_{adj}$ | 0.62 | 0.62 | 0.62 | 0.63 | 0.62 | 0.72 | 0.62 | 0.67 | 0.62 |
| t *p* | 0.5*** | 0.3* | -1.0*** | -1.7* | 0.8** | 4.6*** | -0.1 | -3.0*** | 0.4 |
| Tree genotype (*p*) | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Season (*p*) | ns | * | * | * | * | *** | * | ns | * |
| **Ectomycorrhizal** | | | | | | | | | |
| $R^2_{adj}$ | 0.34 | 0.28 | 0.27 | 0.33 | 0.36 | 0.27 | 0.27 | 0.26 | 0.55 |
| t *p* | 1.7** | 0.1 | 0.4*** | 1.1* | 2.1 | 1.5*** | 2.7 | -1.1 | -4.5* |
| Tree genotype (*p*) | ns | ns | ns | ns | ns | ns | ns | ns | *** |
| Season (*p*) | * | ns | ns | ns | ns | ns | ns | ns | ns |
| **Saprotrophs** | | | | | | | | | |
| $R^2_{adj}$ | 0.67 | 0.71 | 0.67 | 0.66 | 0.67 | 0.67 | 0.67 | 0.66 | 0.66 |
| t *p* | 0.1*** | 2.6*** | -0.2*** | -0.6 | 0.2* | 2.3*** | 0.2** | -1.2*** | 0.2 |
| Tree genotype (*p*) | ns | * | ns | ns | ns | ns | ns | ns | ns |
| Season (*p*) | ns | ns | ns | ns | ns | * | ns | ns | ns |

**Table 5.** Generalized linear mixed models testing the response of functional traits to fungal alpha-diversity and to the factors tree genotype (G) and season (S). Interactions were not significant. The site was included as random factor within models. Degrees of freedom in all models: numDF =1 and denDF = 62. The t (F) and *p* values correspond to the enzyme~α-diversity relationship. Significance of tree genotype (G) and season (S) *p* = . <0.1 *, <0.05, ** <0.01, ***<0.001, ns = not significant.

| | **Total MOTUs** | | | | **Ascomycetes** | | | | **Basidiomycetes** | | | | **Zygomycetes** | | | | **Ectomycorrhizal** | | | | **Saprotrophs** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | α-div | | G | S | α-div | | G | S | α-div | | G | S | α-div | | G | S | α-div | | G | S | α-div | | G | S |
| | $R^2_{adj}$ | $t^p$ | *p* | *p* | $R^2_{adj}$ | $t^p$ | *p* | *p* | $R^2_{adj}$ | $t^p$ | *p* | *p* | $R^2_{adj}$ | $t^p$ | *p* | *p* | $R^2_{adj}$ | $t^p$ | *p* | *p* | $R^2_{adj}$ | $t^p$ | *p* | *p* |
| Glucosidase | 0.35 | -0.5 | ns | * | 0.28 | -1.1$^{**}$ | ns | ns | 0.38 | -2.8$^{*}$ | ns | ns | 0.28 | 0.8 | ns | ns | 0.43 | -2.5$^{**}$ | ns | ns | 0.30 | 0.5 | ns | ns |
| Cellobiohydrolase | 0.27 | -1.1 | ns | * | 0.21 | -0.8$^{*}$ | ns | ns | 0.33 | -2.9$^{**}$ | ns | . | 0.18 | 1.6 | ns | * | 0.37 | -2.9$^{**}$ | ns | . | 0.21 | 1.8$^{*}$ | ns | ns |
| Xylosidase | 0.68 | 2.2$^{***}$ | . | * | 0.68 | 2.0$^{***}$ | . | *** | 0.68 | -0.7$^{**}$ | ns | ** | 0.69 | 1.3$^{***}$ | ns | *** | 0.70 | -0.72$^{***}$ | ns | ** | 0.69 | 2.1$^{***}$ | ns | *** |
| Glucuronidase | 0.38 | 1.9$^{***}$ | ** | ns | 0.38 | 1.3$^{***}$ | * | *** | 0.39 | -0.3$^{**}$ | ** | ns | 0.40 | 0.4$^{***}$ | * | * | 0.39 | -0.2$^{***}$ | ** | ns | 0.40 | 1.0$^{***}$ | ** | * |
| Laccase | 0.57 | 2.9$^{***}$ | ns | . | 0.55 | 0.5$^{***}$ | ns | *** | 0.61 | 0.7 | ns | ns | 0.62 | 2.2$^{***}$ | ns | ns | 0.58 | 0.6$^{***}$ | ns | ns | 0.60 | 0.3$^{***}$ | ns | ** |
| Phosphatase | 0.11 | -0.5 | ns | ns | 0.24 | 0.03 | ns | ns | 0.21 | -1.6 | ns | ns | 0.26 | -1.1 | ns | . | 0.16 | -1.1 | ns | ns | 0.13 | -1.0 | ns | ns |
| Chitinase | 0.01 | -1.5 | ns | ns | 0.05 | -0.2 | ns | ns | 0.09 | -3.0$^{**}$ | ns | ns | 0.02 | -0.8 | ns | ns | 0.06 | -2.2$^{*}$ | ns | ns | 0.01 | 0.2 | ns | ns |
| Leucine | 0.24 | 0.2 | . | * | 0.22 | 0.7 | * | ** | 0.26 | -1.2 | ns | ** | 0.20 | 0.2$^{*}$ | * | . | 0.28 | -0.8 | . | ** | 0.27 | -0.0 | . | ** |

698 **Figure legends**

699 **Fig. 1.** (a) Number of sequences (cursive) and percentages of fungal Molecular Operational
700 Taxonomic Units (MOTUs) by tree genotype (Atlantic, Mediterranean, African), season (spring,
701 autumn), and site (Cabañeros, Riofrío, Espinoso del Rey). Inside squares are MOTUs shared by
702 all (dark grey) or between each two treatments, while MOTUs exclusively found in a treatment
703 are inside circles. (b) Indicator fungal species of different *Pinus pinaster* Ait. genotypes and
704 seasons ($p<0.05$). See Table S2 for additional information of indicator fungal species.
705
706 **Fig. 2.** Alpha-diversity of total fungal community and of representative fungal subgroups
707 associated with (a) different *Pinus pinaster* Ait. genotypes (black = Atlantic; grey =
708 Mediterranean, and white = African), and (b) at different seasons (black = spring, and white =
709 autumn). Boxes represent the interquartile range (IQR) between first and third quartiles and the
710 horizontal line inside is the median. Whiskers denote the lowest and highest values within 1.5 x
711 IQR from the first and third quartiles, respectively. Within each graph, different letters denote
712 significant differences among treatments according to the LSD test ($p<0.05$).
713
714 **Fig. 3.** Path diagrams representing hypothesized causal relationships among the influence of tree
715 productivity, biotic and abiotic predictors and ecosystem functioning. Different colours
716 correspond to different groups of enzymes related with C, N and P cycles. Arrows depict casual
717 relationships: positive effects are indicated by solid lines, and negative effects by dashed lines,
718 with standardized estimated regression weight values (SRW) indicated. Arrow widths are
719 proportional to *p* values. Paths with coefficients non-significant different from 0 ($p>0.08$) are
720 omitted. Fit statistics of the model (NFI, GFI and RMSEA) and sample size (N) are given for all
721 proposed models.
722

**Supplementary Figures**

**Fig. S1.** (a) Genotypes of *Pinus pinaster* Ait. chosen for this study (asterisks) corresponding with Atlantic, Mediterranean, and African origin, and location of sampling sites (circles): CAB = Cabañeros, RIO = Riofrío, ESP = Espinoso del Rey. (b) Diameter at breast height (i.e. proxy of productivity) of the different tree genotypes at the time of the study ($F_{2,26}$ =13.9, P<0.001; tree genotype × site interaction: $F_{4,26}$ =1.65, P>0.1).

**Fig. S2.** Proposed path diagram representing hypothesized causal relationships among the influence of tree productivity, biotic and abiotic predictors and ecosystem functioning. Arrows depict casual relationships.

**Fig. S3.** Assemblage of fungal communities in (a) spring (k=2; stress = 0.16; $R^2$=0.97), and (b) autumn (k=2; stress = 0.12; $R^2$=0.99), by tree genotype (black = Atlantic; grey = Mediterranean; white = African) and site (square = Cabañeros-CAB; circle = Riofrío-RIO; triangle = Espinoso del Rey-ESP), analysed by nonmetric multidimensional scaling (NMDS). Vectors represent the strength/direction of the weight of variables (RH = relative humidity; EC = electric conductivity; K = potassium; P = phosphate; OM = organic matter; N = nitrogen; C:N = carbon/nitrogen ratio; DBH = tree diameter ; Glu = glucosidase; Cell = cellobiohydrolase; Xy = xylosidase; Glucu = glucuronidase; Lac = laccase; Phos = phosphatase; Chi = Chitinase; Leu = leucine ), on the distribution of fungal MOTUs (*p<0.05; **p <0.01; ***p<0.001).

**Fig. S4.** Beta-diversity of the total community and representative fungal subgroups associated with (a) different *Pinus pinaster* Ait. genotypes (black = Atlantic; grey = Mediterranean, and white = African), and (b) at different seasons (black = spring, and white = autumn). Boxes represent the interquartile range (IQR) between first and third quartiles and the horizontal line

748 inside represents the median. Whiskers denote the lowest and highest values within 1.5 x IQR
749 from the first and third quartiles, respectively.
750

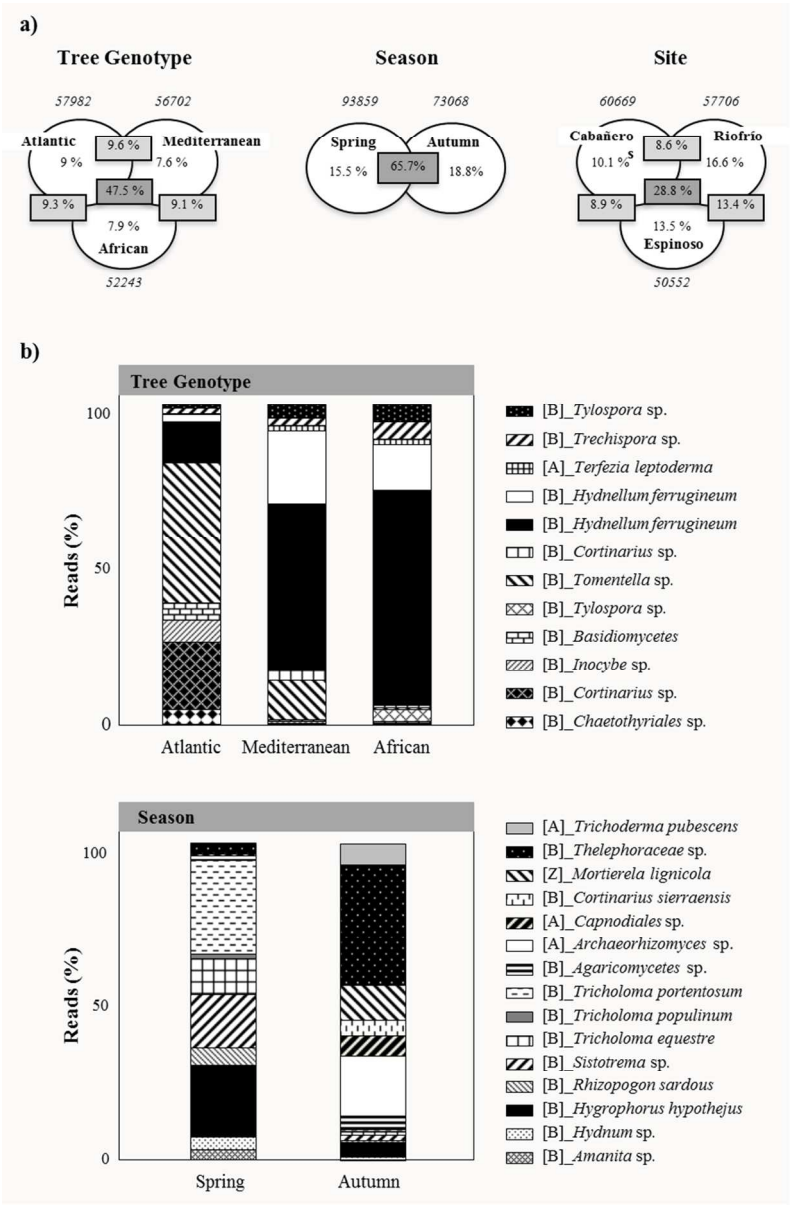


Fig. 1. (a) Number of sequences (cursive) and percentages of fungal Molecular Operational Taxonomic Units (MOTUs) by tree genotype (Atlantic, Mediterranean, African), season (spring, autumn), and site (Cabañeros, Riofrío, Espinoso del Rey). Inside squares are MOTUs shared by all (dark grey) or between each two treatments, while MOTUs exclusively found in a treatment are inside circles. (b) Indicator fungal species of different Pinus pinaster Ait. genotypes and seasons ($p<0.05$). See Table S2 for additional information of indicator fungal species.

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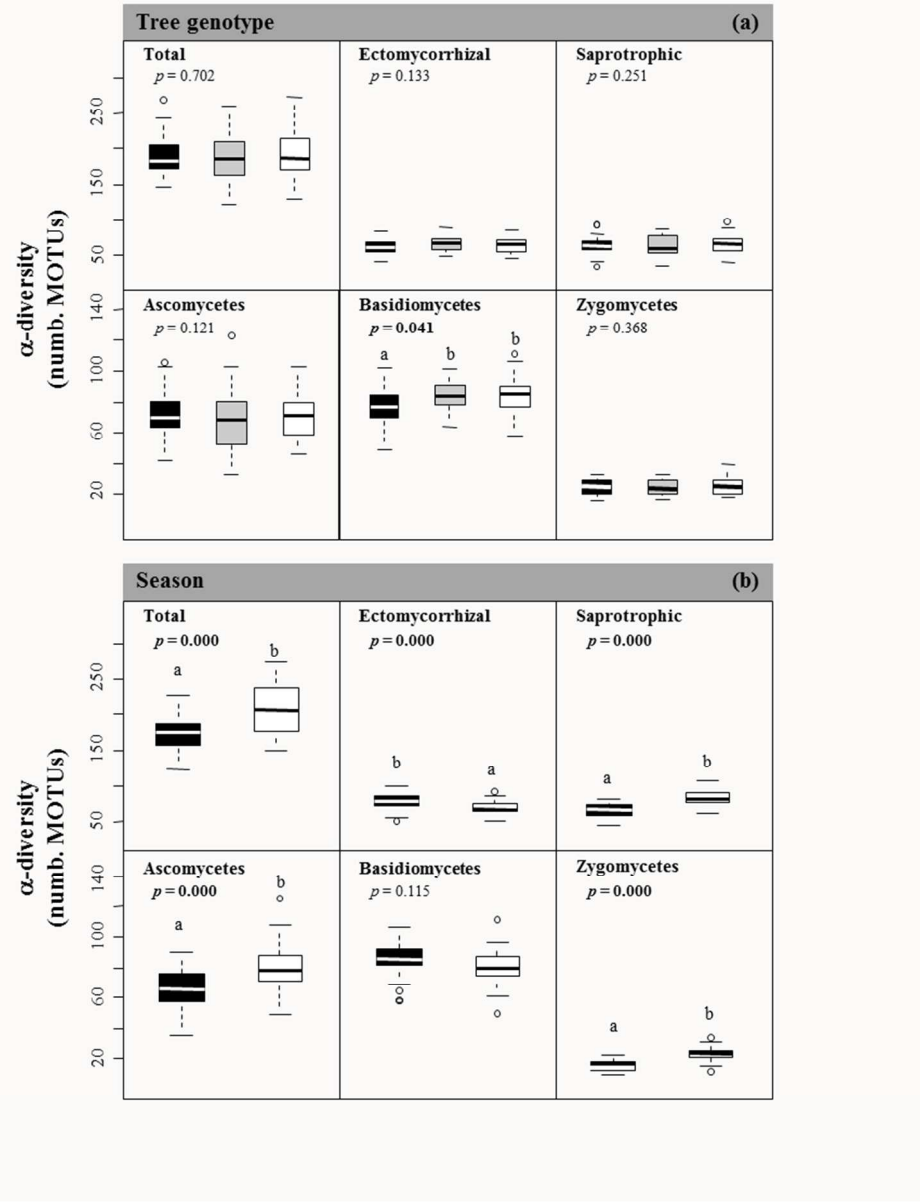


Fig. 2. Alpha-diversity of total fungal community and of representative fungal subgroups associated with (a) different Pinus pinaster Ait. genotypes (black = Atlantic; grey = Mediterranean, and white = African), and (b) at different seasons (black = spring, and white = autumn). Boxes represent the interquartile range (IQR) between first and third quartiles and the horizontal line inside is the median. Whiskers denote the lowest and highest values within 1.5 x IQR from the first and third quartiles, respectively. Within each graph, different letters denote significant differences among treatments according to the LSD test ($p<0.05$).

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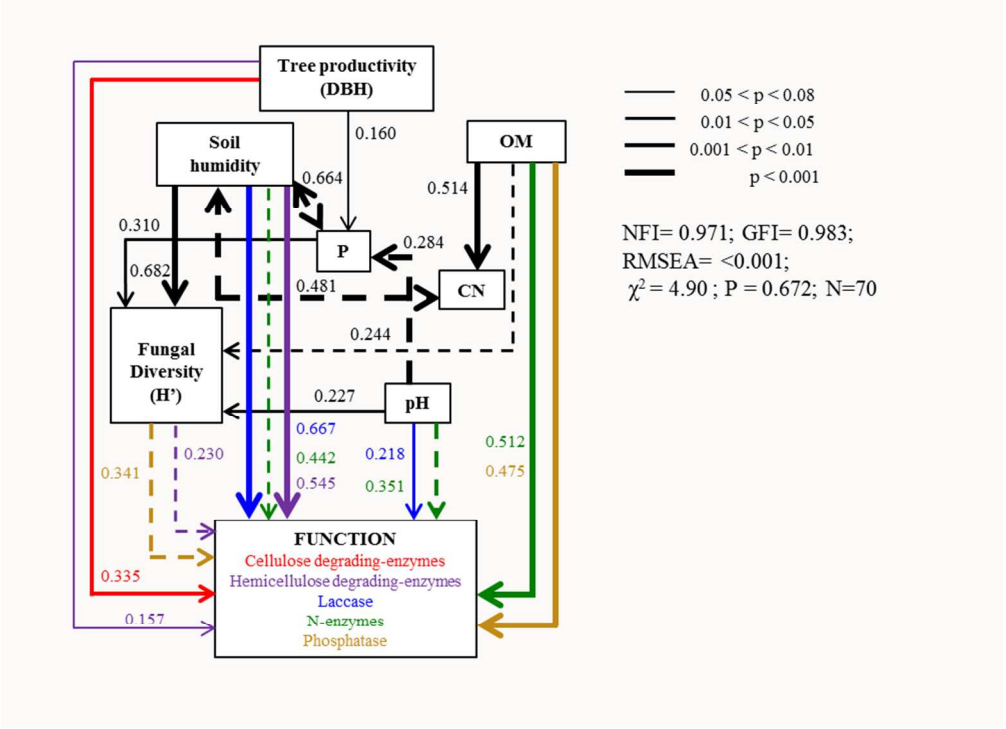


Fig. 3. Path diagrams representing hypothesized causal relationships among the influence of tree productivity, biotic and abiotic predictors and ecosystem functioning. Different colours correspond to different groups of enzymes related with C, N and P cycles. Arrows depict casual relationships: positive effects are indicated by solid lines, and negative effects by dashed lines, with standardized estimated regression weight values (SRW) indicated. Arrow widths are proportional to p values. Paths with coefficients non-significant different from 0 (p>0.08) are omitted. Fit statistics of the model (NFI, GFI and RMSEA) and sample size (N) are given for all proposed models.

169x122mm (150 x 150 DPI)