High fire recurrence reduces ectomycorrhizal fungal diversity of Mediterranean pine forests

1   **High fire recurrence reduces ectomycorrhizal fungal diversity of Mediterranean pine**

2   **forests**

3

4   Pérez-Izquierdo L[1*], Zabal-Aguirre M[1], Verdú M[2], Buée M[3], Rincón A[1]

5

6   [1]Instituto de Ciencias Agrarias, ICA-CSIC. Serrano 115bis, 28006. Madrid, Spain.

7   [2]CIDE-CSIC-UV-GV, Ctra Moncada-Náquera km4.5, 46113, Moncada-Valencia, Spain.

8   [3]INRA, UMR1136 INRA Nancy – Université de Lorraine, Interactions Arbres-

9   Microorganismes Labex ARBRE, 54280 Champenoux, France.

10

11

12   **Corresponding author**: Leticia Pérez-Izquierdo

13   *Current address:

14   Department of Soil and Environment, SLU, Lennart Hjelms väg 9, SE-75007 Uppsala,

15   Sweden.

16   E-mail: leticia.perez@slu.se

17

18   **Running title:** Mycorrhizas in fire-prone Mediterranean ecosystems

19

20

21

22   **Competing interests**

23   The authors declare that they have no competing interests

24

25

26

28   **Abstract**

29   Fire is a major disturbance linked to the evolutionary history and climate of Mediterranean

30   ecosystems, where the vegetation has evolved fire-adaptive traits (e.g., serotiny in pines). In

31   Mediterranean forests, mutualistic feedbacks between trees and ectomycorrhizal (ECM)

32   fungi, essential for ecosystem dynamics, might be drastically threatened by fire. We tested

33   how fire regime alters the structure and function of ECM communities of *Pinus pinaster* and

34   *Pinus halepensis* Mediterranean forests, and analyzed the relative contribution of

35   environmental (climate, soil properties) and tree-mediated (serotiny) factors. For both pines,

36   high fire recurrence significantly reduced local and regional ECM fungal diversity, although

37   certain fungal species were favored by recurrent fire. The high fire recurrence also associated

38   a general decline of ECM root-tip enzymatic activity for *Pinus pinaster*, while it did not

39   imply major functional changes for *Pinus halepensis*. Separated effects of fire regime related-

40   factors such as climate, soil properties or tree phenotype drove these processes. In addition to

41   the main influence of climate, the tree fire-adaptive trait serotiny recovered a great portion of

42   the variation in structure and function of fungal communities associated with the fire regime.

43   Edaphic conditions (especially pH, tightly linked to bedrock type) were an important driver

44   shaping ECM fungal communities, but mainly at the local scale and likely independently of

45   the fire regime. Our results show that fire regime strongly impacts ECM fungal communities,

46   and reveal complex feedbacks among trees, mutualistic fungi and surrounding environment in

47   fire-prone Mediterranean forest ecosystems.

48

49   **Keywords:** ectomycorrhizal communities, enzymatic activity, fire recurrence, Mediterranean

50   pines,   serotiny

Introduction

Mediterranean climate is characterized by a marked seasonality with mild winters and hot dry summers, which gives rise to one of the most fire-prone ecosystems in the world (Pausas, 2004). The evolutionary history of these ecosystems is tightly linked to fire (Keeley, Bond, Bradstock, Pausas, & Rundel, 2011). Recurrent burning has markedly structured plant communities in this area (Verdú & Pausas, 2007), where many species have developed evolutionary mechanisms of resistance and resilience to fire (Pausas, 2015; Tapias, Climent, Pardos, & Gil, 2004). A good example of fire-adaptive trait is serotiny, i.e., the retention of mature seeds in closed cones for more than a year until dispersion and germination is activated by fire, evolved by some representative Mediterranean pine species (Budde et al., 2014; Hernández-Serrano, Verdú, González-Martínez, & Pausas, 2013) and other conifers in fire-dominated systems (Greene et al., 1999). Fire generates phenotypic divergence on serotiny, a heritable trait in Mediterranean pines, ultimately producing local adaptation (Hernández-Serrano et al., 2014). There is evidence that serotiny is related to differences in fire regime, increasing with the frequency of stand-replacing fires (Hernández-Serrano et al., 2013; Radeloff, Mladenoff, Guries, & Boyce, 2004). Changes in the fire regime are closely linked to climate (Pausas, 2004), and predicted climate change scenarios make Mediterranean ecosystems especially vulnerable. Temperature rise and rainfall decrease are expected to increase drought risk and consequently forest wildfires, altering the structure and successional dynamics of Mediterranean forests (Lindner et al., 2010).

Ecosystem development is driven by interactions among climatic conditions, edaphic environment and biotic communities. Given the role of fungi in organic matter turnover and nutrient cycling, they are key players in the plant-soil-microbial feedbacks that determine ecosystem development after disturbances (Clemmensen et al., 2015). Specifically,

75   mutualistic ectomycorrhizal (ECM) fungi, which are tightly linked to the host through

76   specialized symbiotic structures, mediate the uptake and transfer of water and nutrients to

77   trees (Smith & Read, 2008). They channel the photosynthetic carbon (C) fixed by trees into

78   the belowground, influencing soil C storage and nutrient cycling (Clemmensen et al., 2013;

79   Talbot, Allison, & Treseder, 2008). This symbiosis can provide up to the 80% of nutrients to

80   trees and alleviate their hydric stress (Kivlin, Emery, & Rudgers, 2013; van der Heijden,

81   Martin, Selosse, & Sanders, 2015), and therefore can be especially relevant in severe

82   environments e.g., recurrent fire and drought conditions in Mediterranean ecosystems

83   (Egerton-Warburton, Querejeta, & Allen, 2007; Prieto et al., 2016; Querejeta, Egerton-

84   Warburton, & Allen, 2007).

85   Fire affects ECM communities mainly through vegetation damages and altered soil properties

86   (Buscardo et al., 2015; Hart, DeLuca, Newman, MacKenzie, & Boyle, 2005; Rincón &

87   Pueyo, 2010), conditions that may favor fire-tolerant fungi (Glassman, Levine, Dirocco,

88   Battles, & Bruns, 2016; Rincón, Santamaría-Pérez, Ocaña, & Verdú, 2014). Certain fungi can

89   remain for long time in the soil spore banks and be advantaged after fire, as it has been shown

90   in closed-cone pine forest populations with historical highly recurrent and intense fires (Baar,

91   Horton, Kretzer, & Runs, 1999; Bruns et al., 2009; Glassman et al., 2016, 2015). Fire usually

92   decreases root mycorrhizal colonization and fungal richness (Dove & Hart, 2017). More

93   generally, fire drastically disrupts the species composition of the mycorrhizal communities,

94   usually leading to their simplification with the dominance of pioneer fungi (Dove & Hart,

95   2017; Buscardo et al., 2015; de Román & de Miguel, 2005; Rincón et al., 2014; Torres &

96   Honrubia, 1997). Additionally, because intraspecific plant genotypic and phenotypic variation

97   affects the structure of their associated ECM fungi (Gehring & Whitham, 1991; Pérez-

98   Izquierdo et al., 2017, 2019; van der Heijden et al., 2015) it is plausible that genetically fire-

99 driven changes in trees, such as serotiny degree, may co-affect these mutualistic communities

100 for example by determining the C available to exchange in symbiosis. Nevertheless, fires

101 recurrently affect big areas of Mediterranean ecosystems, yet the cumulative effects of

102 recurrent wildfires on ECM fungal communities are less known than those of single wildfires

103 (Buscardo et al., 2015).

104 In our study, we targeted forests of two representative Mediterranean tree species, Maritime

105 pine (*Pinus pinaster* Ait.) and Aleppo pine (*Pinus halepen*sis Mill.), amply distributed in the

106 Iberian Peninsula. While the Maritime pine usually grows in acid soils at 700-1700 m

107 altitude, the Aleppo pine grows in basic substrate and below 800 m (Ruíz, Álvarez-Uria, &

108 Zavala, 2009). *Pinus halepensis* is typically distributed in warm and dry areas, even under

109 extreme drought induced either by climate or soil constituents (e.g., marls, gypsum, rocky

110 slopes) (Ruíz et al., 2009). Both pine species have a life history related to fire adaptation

111 (Tapias et al., 2004) displaying great post-fire colonizing abilities (Barbéro, Loisel, & Quézel,

112 1998) and showing fire-adaptive traits such as serotiny (Pausas, 2015). The natural

113 regeneration and dynamics of these pine forests critically relies on ECM fungal communities,

114 because pine species are obligatory ectomycorrhizal (Nuñez, Horton, & Simberloff, 2009;

115 Smith & Read, 2008). Recurrent fires, tightly linked to climate in these Mediterranean areas,

116 might act as an environmental filter, culling plant and microbial species unable to tolerate

117 conditions at a particular location and thus preventing their establishment or persistence

118 (Kraft et al., 2015).

119 Based on all these premises, we expected that i) due to the habitat filtering imposed by the fire

120 regime, the ECM fungal communities in high fire recurrence sites (HiFi hereafter) would be

121 less diverse and more homogeneous than those in low fire recurrence sites (LoFi hereafter),

122 and that it would imply different functional outcomes. In our study area, the distinct historical

5

123   fire recurrence has induced a sharp serotiny divergence in trees within the HiFi and LoFi

124   populations along time (Hernández-Serrano et al., 2013). Serotiny has been proven to be a

125   heritable trait associated with the genotype of trees (Budde et al., 2014; Castellanos,

126   González-Martínez, & Pausas, 2015; Hernández-Serrano et al., 2013). Because different tree

127   genotypes can influence their associated ECM fungi (Gehring & Whitham, 1991; Pérez-

128   Izquierdo et al., 2017), we further hypothesize that, at the finer scale of individuals, ii) the

129   serotiny degree of trees, i.e., genotypic fire-adaptation, will explain structural and functional

130   divergences of ECM fungal communities.

131   In order to address these predictions, we characterized the structure of root-tip ECM fungal

132   communities of natural *P. pinaster* and *P. halepensis* forests (Hernández-Serrano et al., 2013).

133   For both pine forests, serotinous populations growing under a warm and dry Mediterranean

134   climate subjected to high fire frequency and non-serotinous populations growing under a

135   subhumid climate where fires are rare (Verdú & Pausas, 2007) were surveyed (Figure S1).

136   Additionally, we determined, on excised ECM root-tips, potential fungal enzymatic traits

137   related to C turnover and mobilization of nutrients. These are processes directly implicated in

138   the exchange of resources that support most mycorrhizal symbioses and many essential

139   ecosystem functions (Johnson et al. 2012).

140

141   **Material and Methods**

142   *Study sites and sampling*

143   Surveys were conducted in natural forests of *P. pinaster* (Ppi) and of *P. halepensis* (Pha)

144   located in eastern Spain (Figure S1). We selected nine pine populations located in high fire

145   recurrence sites where crown-fires are historically frequent and most regeneration events are

146   driven by fire (HiFi populations), while the other selected six populations were located in low

147   fire recurrence areas where most regeneration events are independent of fire because fire

148   events are rare (LoFi populations) (Hernández-Serrano et al., 2013; Pausas et al., 2004). In the

149   study area, fire is tightly linked to Mediterranean climatic conditions (i.e., drought) (Pausas,

150   Bradstock, Keith, Keeley, & Network, 2004). Recent fire history information (Pausas &

151   Fernández-Muñoz, 2012; Pausas, 2004) shows that more than 50% of the study area at HiFi

152   conditions burned at least once during the 1978–2001 period, while for LoFi conditions, the

153   proportion was about 15% (Abdel Malak & Pausas, 2006). From a microevolutionary point of

154   view, this distinct fire regime has induced a sharp serotiny divergence within these

155   populations (Hernández-Serrano et al., 2013). More than 500 genes were differentially

156   expressed across the two pine accessions from HiFi and LoFi populations (Pinosio et al.,

157   2014) and three high-differentiation outlier single nucleotide polymorphisms-SNPs were

158   identified between HiFi and LoFi stands, suggesting fire-related selection at the regional scale

159   (Budde et al., 2017). Thus, despite the lack of long-term fire statistics for the specific study

160   sites, there is strong evidence that the fire interval is much shorter in HiFi areas than in LoFi.

161   The serotiny degree of these pine populations growing under distinct fire regime has been

162   accurately characterized in Hernández-Serrano et al., 2013 (Table S1). Briefly, serotiny was

163   estimated considering both the cone age and the proportion of serotinous cones, i.e., the

164   number of closed cones, those remaining closed after maturation, with respect to the total

165   fully ripe (open and closed) cones (see Hernández-Serrano et al., 2013 for details). The

166   characteristics of pine populations related to productivity (diameter at breast height; DBH),

167   fire-adaptation traits (bark thickness and serotiny degree), as well as location and local

168   environmental variables are described in Table S1. All the selected stands were mature pine

169   populations, with DBH between 20.8 and 35.4 cm corresponding to trees of more than 40 yr

170   (Camarero, Olano, & Parras, 2010; Vieira et al., 2015).

171   In May 2013, five trees per population separated by more than 10 m were selected from a

172   total of 15 populations (n = 75). Under each tree, the litter was removed and samples were

173   obtained, approximately 1 m far from the trunk, by excavating 10 x 10 x 20 cm soil holes at

174   the four orientations (north, south, east and west). The four samples per tree were pooled into

175   a combined sample and kept at 4 ºC in plastic bags until processing. Once in the lab, roots

176   were separated from soil, coarse roots discarded (diameter > 2 mm), and remaining roots

177   gently washed with tap water over 2 and 0.5 mm sieves for collecting root tips. All

178   ectomycorrhizal (ECM) root tips per each sample were carefully selected (Rincón et al.,

179   2014), cleaned and sorted per sample under a stereomicroscope for further enzymatic and

180   molecular analyses. Remaining soil was air dried and sieved (2 mm) for analysis.

181   The gravimetric soil moisture of soil samples was determined by drying at 105 ºC for 48 h.

182   Air-dried soils were measured for pH (2 g of soil in 10 ml of H_2O, 1:5, w:v), electrical

183   conductivity (EC) (1:5, w:v in H_2O), organic matter (OM) (Walkley & Black, 1934), total N

184   (Kjeldahl method). Extractable P was determined by the Bray & Kurtz (1945) method, after

185   extraction in an ammonium fluoride and chloride acid solution. Extractable potassium (K)

186   was determined after nitric acid digestion according to Isaac & Kerber (1971). Both P and K

187   extracts were measured by inductively coupled plasma spectrometry (Optima 4300DV,

188   Perkin-Elmer, Waltham, MA, EE.UU.).

189   *Enzymatic tests*

190   The fungal community functioning was evaluated on excised ECM root tips by measuring

191   activities of eight hydrolytic and oxidative exoenzymes secreted by fungi. Seven enzymatic

192   tests were based on fluorogenic substrate release, methylumbelliferone (MU) e.g. β-

193   glucosidase (EC 3.2.1.3 at ExPasy-Enzyme database) and cellobiohydrolase (EC 3.2.1.91)

194   that degrade cellulose, β-xylosidase (EC 3.2.1.37) and β-glucuronidase (EC 3.2.1.31) implied

195   in the degradation of hemicellulose, acid phosphatase (EC 3.1.3.2) involved in the

196   mobilization of phosphorus, N-acetylglucosaminidase or chitinase (EC 3.2.1.14) which

197   hydrolyses chitin, or methylcoumarine (AMC) for L-leucineaminopeptidase (3.4.11.1) related

198   to the mobilization of nitrogen from peptidic substrates. The Laccase (1.10.3.2) activity was

199   determined by a photometric assay based on ABTS substrate (2,2'-Azino-bis (3-ethylbenzo-

200   thiazolin-6-sulfonic acid). This enzyme is related to the degradation of recalcitrant

201   compounds such as lignin. Enzymatic activities were determined following the protocol

202   described by Courty, Pritsch, Schloter, Hartmann, & Garbaye (2005), with modifications. A

203   total of 280 ECM-tips were randomly collected per sample and separated in subsets of 7

204   ECM-tips with 5 replicates per each enzymatic test. Each replicate thus consisted of a tube

205   with 7 pooled ECM-tips that were incubated in buffer during the corresponding time for each

206   enzyme (Courty et al., 2005), after which 100 μl of the respective enzymatic reaction mix was

207   added to 100 μl of stopping buffer in 96-well microplates. Enzymatic activities were

208   measured in a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA),

209   with 355/460 nm excitation/emission wavelengths for the fluorogenic assays and 415 nm for

210   laccase. After reading, the ECM-tips of each replicate were scanned and their area calculated

211   with the software ImageJ 1.49. Enzymatic activities were expressed in pmol min^{-1}mm^{-2}.

212   *Molecular analyses*

213   Per each measured enzyme, the ECM-tips were pooled (7 ECM-tips subsets x 5 replicates =

214   35), added of a pinch of polyvinylpolypyrrolidone (PVPP), and the DNA extracted with the

215   Invisorb®DNA Plant HTS 96 Kit/C kit (Invitek GmbH, Berlin, Germany), making a total of

216   600 DNA extractions (75 tree samples x 8 enzymes) corresponding to 280 root tips per tree.

217   The internal transcribed spacer region ITS-1 of the nuclear ribosomal DNA was amplified

218   with the primer pair ITS1F-ITS2 (Gardes & Bruns, 1993) adapted for Illumina-MiSeq. Each

219   sample was amplified in three independent 20 µl PCR reactions, each containing 2 µl of 10x

220   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

221   Nucleotide Mix, 0.4 µl of 10 mM forward/reverse primers and 0.2 µl of AmpliTaqGold

222   polymerase (5 U ml^{-1}) (Applied Biosystems, Carlsbad, CA, USA). Negative controls without

223   DNA were included in all runs to detect possible contaminations. The PCR conditions were as

224   follow: 3 min 94 ºC, 30 cycles of 1 min 94 ºC, 30 s 53 ºC and 45 s 72 ºC, with a final step of

225   10 min 72 ºC. Independent reactions were combined per sample, and each PCR product was

226   purified (UltraClean PCR clean-up kit of MoBio, Carlsbad, CA, USA), quantified

227   (PicoGreen, Life Technologies, Carlsbad, CA, USA), and finally pooled in an equimolar

228   library containing 75 samples. Sequencing was carried out on an Illumina MiSeq sequencer (2

229   × 300 bp paired-end reads) in an external service (Parque Científico de Madrid, Spain).

230   *Bioinformatic analyses*

231   Sequences were de-multiplexed according to their tags, filtered and trimmed using the

232   *fastq_filter* command and *fastq_truncqual* option of Usearchv.7.0.1001 (Edgar, 2013) for

233   eliminating quality scores ≤ 10. We used FLASH to merge reads with a minimum overlap of

234   110 bp, resulting in 58.3 % of retained sequences (4205677 out of the initial set of 7215915

235   sequences). Then, sequences were dereplicated with the *derep_fulllength* Usearch command.

236   De-replicated sequences were sorted by decreasing abundance, and singletons discarded with

237   the *sortbysize* Usearch command. Finally, 4116377 sequences (57 %) were retained.

238   Operational taxonomic units (OTUs) were generated from abundance-sorted sequences using

239   the *cluster_otus* Usearch command with a 97 % similarity threshold. Extracted ITS sequences

240   were then mapped against the OTU representative sequences using the *usearch_global*

241   Usearch command. Taxonomic assignation of representative sequences for each OTU was

242   done according to the 7.2 UNITE database (Kõljalg et al., 2013). Fungal taxonomic

10

243   assignment served to identify those OTUs closely related to recognized ECM taxa (Nguyen et

244   al., 2016; Tedersoo et al., 2014; Tedersoo & Smith, 2013). The 86 % of the inferred

245   sequences corresponded to ECM fungi and these were used for all subsequent analyses.

246

247   *Statistical analyses*

248   The effect of the fire recurrence (HiFi *vs* LoFi) on the structure (i.e., alpha and beta-diversity)

249   and functioning (i.e., enzymatic activities) of ECM fungal communities (hypothesis 1) was

250   separately evaluated by pine forest. Beta-diversity of total and by phyla ECM fungal

251   communities of the different pine populations was calculated with the functions betadisper

252   and permutest of the vegan package (Anderson, Ellingsen, & McArdle, 2006; Oksanen et al.,

253   2015), and visualized by Principal Component Analyses (PCoA). Beta-diversity was

254   calculated on Bray-Curtis abundance matrix of OTUs previously normalized by variance

255   stabilization according to McMurdie and Holmes (2014) by using the DESeq package

256   (Anders & Huber, 2012).

257   Alpha-diversity was analysed by General Linear Models (GLMs) ($p<0.05$) with the number of

258   fungal OTUs as response variable. In these models, the square root of the total number of

259   sequences obtained per sample was included as a covariate to account for differences in

260   sequencing depth (Tedersoo et al., 2014). Models at lower taxonomic levels were also

261   performed (i.e., phyla and families). In all cases, before modelling, Shapiro and Levene tests

262   were performed to test the normality and homocedasticity of data, and variables were log or

263   square root transformed when needed. Firstly, we modelled the effect of the variable pine

264   forest on ECM fungal alpha-diversity, using pine forest as fixed factor and the site nested

265   within pine forest. Subsequent GLMs analyses were separately performed for each pine

266   species. In these models, the fire regime was introduced as fixed factor and the site (i.e., pine

267   population) nested within fire regime (n = 35 *P. pinaster* trees and n = 40 *P. halepensis* trees).

268   Same GLM syntaxes were used to test the effect of fire recurrence on the different root-tip

269   enzymatic activities. Correlations between enzymatic activities and ECM fungal community

270   assemblages of the pine populations were analyzed by Mantel tests with ade4 R package.

271   To identify representative fungal OTUs of each fire regime per pine species, the Indicator

272   Species Analysis (with OTUs >100 reads to avoid spurious relations) was carried out

273   (*p*<0.05) with the function *multipatt* of the indicspecies R package (Cáceres, Legendre, & He,

274   2013).

275   Climatic and edaphic characteristics are intimately associated with fire regime in

276   Mediterranean ecosystems (Pausas et al., 2004). So, to separate the edaphic and climatic

277   effects from that of the fire regime, we performed CCA on Hellinger-transformed relative

278   fungal abundance data and RDA with the Euclidean distance matrix of enzymatic activities

279   both with forward selection of explanatory variables. We used different analyses due to their

280   different data assumptions, RDA assumes a linear relationship between predictors and data

281   while CCA assumes a unimodal response curve. In these models, the variable fire regime and

282   the edaphic and climatic indexes were included. Previously, to reduce the dimensionality of

283   environmental data, Principal Component Analyses (PCA) were independently run for

284   edaphic variables (pH, OM, EC, N, P, K) and climatic variables (mean annual temperature,

285   mean annual precipitation and altitude), and the first two axes, explaining the maximum

286   amount of variance, of respective PCAs were used as edaphic or climatic indexes (Figure S2).

287   To test our hypothesis 2, effect of tree serotiny degree (i.e., genotypic fire-adaptation) on

288   ECM fungal community, two additional models with the factor serotiny and the edaphic and

289   climatic indexes were included. As before, we performed a CCA model on Hellinger-

290 transformed relative fungal abundance data and a RDA model with the Euclidean distance

291 matrix of enzymatic activities both with forward selection of explanatory variables.

292 Since the distribution of the two pine species considered in our study is closely linked to the

293 bedrock type, i.e., *P. pinaster* preferentially settled on siliceous soils, while *P. halepensis*

294 include both siliceous and calcareous soils (Hernández-Serrano et al., 2013), we made

295 additional analyses for testing possible confounding effects of pine species and bedrock type

296 (both factors included in the variable pine forest). For this, the pine species effect on ECM

297 fungal community structure and enzymatic activities was respectively checked by Canonical

298 Correspondence (CCA) and Redundancy (RDA) analyses, only with data of Sinarcas, the

299 unique location where populations of both pine species grow under a common bedrock

300 environment (Table S1). No significant effects of the pine species identity on the ECM fungal

301 community structure (CCA: n=218; explained variation %=12.4; P=0.204) or on the

302 enzymatic profile (RDA: n=8; explained variation %=44.4; P=0.094) in the location Sinarcas

303 were observed.

304 Ordinations were done with the software CANOCO v.5.0 (Biometris Plant Research

305 International, Wageningen, Netherlands), and the rest of analyses with the R software v.3.5.2

306 (R Core Team, 2014).

308 **Results**

309 *Sequencing yields and taxonomic identification of fungi*

310 A total of 501 ECM fungal OTUs corresponding to 3182548 sequences were identified.

311 Almost half of OTUs were found in both pine species and ~26 % exclusively in one of them

312 (Figure S3a). LoFi and HiFi pine populations shared the 54.8 % of OTUs, while ~21-23 %

313 were only found either in LoFi or HiFi (Figure S3a). Most OTUs belonged to Basidiomycota

314 (89.4 %), 10.2 % to Ascomycota, and 0.4 % to Zygomycota (Figure S3b; Table S2). The

315 taxonomic classification of OTUs allowed identifying 12 fungal orders, 30 families and 47

316 genera, most of them represented across all treatments (Table S2).

317 The majority of the 20 most abundant fungi and of the indicator species found in root tips of

318 *P. pinaster* belonged to *Tomentellaceae*, *Inocybaceae*, *Russulaceae*, and *Rhizopogonaceae*

319 and, in the case of *P. halepensis*, to Pezizales, *Thelephoraceae* and *Sebacinaceae* (Table S3;

320 Figure S4). The indicator species analysis revealed 54 fungal OTUs preferentially associated

321 to *P. pinaster* forests and 37 to *P. halepensis* forests (Table S3). Besides, 31 fungi belonging

322 to 14 different genera were indicators of LoFi populations of *P. pinaster* and 31 fungi

323 belonging to 13 different genera were indicators of LoFi populations of *P. halepensis*. In

324 contrast, 11 indicators from 5 different genera and 6 from 5 different genera were associated

325 with HiFi populations of *P. pinaster* and *P. halepensis* respectively (Table S3).

326

327 *Root-tip ectomycorrhizal fungal diversity*

328 Total fungal α-diversity marginally varied between pine forests (R^2=0.13; F=3.62; P=0.062).

329 At the phylum level, significantly less Basidiomycetes (R^2=0.24; F=6.12; P=0.016) and

330 marginally more Ascomycetes (R^2=0.81; F=3.88; P=0.053) were found in *P. halepensis* forest

331 compared with *P. pinaster*. The root-tips of *P. pinaster* were enriched of *Amanitaceae*,

14

332   *Atheliaceae*, *Cantharellaceae*, *Clavariaceae*, *Cortinariaceae*, *Gloniaceae*, *Rhizopogonaceae*

333   and *Russulaceae* species, while in *P. halepensis* root-tips prevailed species of the families

334   *Pezizaceae*, *Pyronemataceae* and *Sebacinaceae* (Table 1). At regional scale, root-tip ECM

335   fungal β-diversity of both pine forests was similar (R^2=0.01 F=1.34, P=0.265), although

336   Basidiomycetes were more dissimilar in *P. pinaster* than in *P. halepensis* forests (R^2=0.01

337   F=4.44, P=0.033), and the opposite pattern was observed for Ascomycetes (R^2=0.03; F=11.62,

338   P=0.001).

339   The ECM fungal α-diversity was significantly lower in high fire recurrence (HiFi)

340   populations compared with low fire recurrence (LoFi) populations in both pine forests (Figure

341   1a), and this difference was consistent within the two fungal phyla i.e., Ascomycetes and

342   Basidiomycetes (Figure 1b-c). Although the α-diversity of many ECM fungal families

343   decreased in HiFi populations of both pine forest (Table 1), some taxa were more diverse in

344   HiFi, e.g. *Amanitaceae*, *Cantharellaceae* and *Rhizopogonaceae* for *P. pinaster*, or *Pezizaceae*

345   for *P. halepensis* (Table 1). At regional scale, a consistent reduction of ECM fungal β-

346   diversity was observed in HiFi populations of both pine forests (i.e., more homogeneous

347   ECM communities in HiFi than LoFi) (Figure 2a). Likewise, Basidiomycetes were less β-

348   diverse in HiFi than LoFi populations, while no difference was observed for Ascomycetes

349   (Figure 2b-c).

350

351   *Potential enzymatic activity of ECM root-tips*

352   The enzymatic activity of the ECM root tips varied between pine forests (Figure 3). In LoFi

353   populations, the enzymatic activity of ECM root tips was very similar for both pines, except

354   in the case of cellobiohydrolase and phosphatase that were respectively higher and lower in *P.*

355   *halepensis* compared with *P. pinaster* (Figure 3). By contrast, in HiFi populations, the activity

356   of most of the C-degrading enzymes (i.e., cellobiohydrolase, β-xylosidase and β-

357   glucuronidase), and of those mobilizing nitrogen (i.e., chitinase and L-leucineaminopeptidase)

358   was higher for *P. halepensis* than *P. pinaster,* with the exception of laccase that was lower

359   (Figure 3).

360   The ECM fungal species assemblage of *P. pinaster* root-tips significantly correlated with their

361   enzymatic profile (Mantel r=0.14; P=0.003). The HiFi populations of *P. pinaster* had higher

362   root-tip laccase activity and lower hemicellulose degrading activity (i.e., β-xylosidase, β-

363   glucuronidase), and of enzymes mobilizing nitrogen (i.e., chitinase and L-

364   leucineaminopeptidase) and phosphorus (i.e., acid phosphatase) (Figure 3). No significant

365   relation between fungal community structure and enzymatic activity was observed for *P.*

366   *halepensis* (Mantel r=0.03; P=0.287), although laccase activity significantly increased in HiFi

367   populations (Figure 3).

368

369   *Main drivers of the structure and function of ECM fungal communities*

370   Climate, in particular temperature (Climate 1, first axis from PCA in Figure S2), together with

371   fire recurrence, were major factors determining ECM fungal communities of Mediterranean

372   pine forests (Table S4A; Figure 4a). As hypothesized, the tree trait serotiny (selected by fire

373   regime) did affect the structure of root-tip ECM fungal communities (Table S4B; Figure 4a).

374   Higher levels of serotiny significantly correlated with the ECM fungal community structure

375   of HiFi populations in both *P. pinaster* and *P. halepensis* forests (Table S4B; Figure 4a).

376   Contrarily, the edaphic effects were likely related to differences among plots inside LoFi or

377   HiFi populations, i.e. the strength and direction of edaphic vectors along plots displayed in

378   Figure 4a.

379　Regarding ECM functionality (i.e., enzymatic activities), the fire recurrence together with

380　climatic variables explained root-tip enzymatic activity in *P. pinaster* forest (Table S4A;

381　Figure 4b). Moreover, the serotiny degree of *P. pinaster* populations significantly correlated

382　with the ECM root-tip enzymatic profile in HiFi populations, while soil characteristics mainly

383　drove variations in enzymes among sites inside LoFi and HiFi populations (Table S4B; Figure

384　4b). For *P. halepensis*, root-tip enzymatic activity was essentially driven by the surrounding

385　edaphic environment (Table S4; Figure 4b), mainly by high pH and low phosphorus

386　availability (see PC2 in Figure S2a).

387

388　**Discussion**

389　Mutualistic feedbacks between trees and ectomycorrhizal fungi that are essential for

390　ecosystem dynamics can be highly vulnerable to fire in Mediterranean ecosystems. Our

391　results show that fire regime shapes the structure and functioning of root-tip ECM fungal

392　communities in fire-prone Mediterranean forests. Divergent ECM fungal communities

393　associate with *P. pinaster* and *P. halepensis*, which are highly influenced by the edaphic

394　environment and climate. According to our first hypothesis, high fire recurrence reduces ECM

395　fungal diversity and has a homogenizing effect on these communities, which leads to a

396　reduced enzymatic activity for *P. pinaster* (but not for *P. halepensis*). Confirming our second

397　hypothesis, this effect is evidenced at the finer scale of individuals since the serotiny degree

398　of trees, i.e., genotypic fire-adaptation, does significantly affect the structure (for both *P.*

399　*pinaster* and *P. halepensis*) and functioning (in the case of *P. pinaster*) of ECM fungal

400　communities. In this work, relative contribution of main variables related to fire such as

401　climate, soil properties and the tree trait serotiny structuring ECM fungal communities have

402　been disentangled.

17

403

404   *ECM communities in* P. pinaster *and* P. halepensis *forests*

405   The tree species had a marginal effect on ECM fungal communities probably blurred by the

406   strong effect of the local environmental conditions. The degree of tree host specificity for

407   ECM fungi seems to be correlated with the phylogenetic distances between hosts (Tedersoo,

408   Mett, Ishida, & Bahram, 2013). However, it has been shown that the genus *Pinus* associates

409   with specialized ECM fungi, in particular within the genera *Suillus* and *Rhizopogon* (Smith &

410   Read, 2008) and that even the *Pinus* genotype play an essential role in structuring ECM

411   fungal communities (Gehring, Mueller, Haskins, Rubow, & Whitham, 2014; Pérez-Izquierdo

412   et al., 2017, 2019). Given the importance of pH shaping fungal communities (Coince et al.,

413   2014; Glassman, Wang, & Bruns, 2017; Rincón et al., 2015; Tedersoo et al., 2014), these

414   divergent observations could be interpreted as context-dependent, i.e., in our study, soil pH

415   (ranging from 5.1 to 8) was higher, even in Sinarcas where both pine species grew under a

416   similar local environment, than in the mentioned studies (average pH of 5). The strong

417   environmental filter imposed by the bedrock influence, mainly siliceous for *P. pinaster* and

418   calcareous for *P. halepensis* (Ojeda, Pausas, & Verdú, 2010), was reflected in more diverse

419   and more heterogeneous Ascomycetes species together with less diverse and more

420   homogeneous Basidiomycetes in the case of *P. halepensis*, whereas the opposite pattern was

421   observed for *P. pinaster*. We observed a preferential association of *P. halepensis* with fungal

422   species of the genus *Sebacina* and the order Pezizales. So far, no tree host specificity has been

423   observed among the *Sebacina* species (Ray & Craven, 2016; Selosse, Bauer, & Moyersoen,

424   2002). Given the reported positive effects of sebacinous fungi against stresses such as

425   herbivory, salinity or drought (Barazani & Baldwin, 2013; Ray & Craven, 2016; Zarea,

426   Miransari, & Karimi, 2014), they could play a key role in the resistance of *P. halepensis* to

427   limited nutrient availability (i.e., related to high soil pH) and the restrictive conditions

428   imposed by the Mediterranean climate. Similarly, ascomycetous ECM Pezizales, which tend

429   to be favoured in basic soils, have traits such as their melanin production capacity that make

430   them perfect symbionts under stressful conditions including post-fire scenarios (Koide,

431   Fernandez, & Malcolm, 2014; Lamit et al., 2014; Rincón et al., 2014; Rincón & Pueyo, 2010;

432   Tedersoo, Hansen, Perry, & Kjøller, 2006; Torres & Honrubia, 1997). On the other hand,

433   species from the genera *Rhizopogon*, *Craterellus*, *Cenoccocum*, *Russula* and *Lactarius* were

434   enriched under *P. pinaster* (less basic pH). Although ECM fungi tolerate a wide range of pH

435   (Rousk et al., 2010), most of these fungi grow better under acidophilic conditions (Yamanaka,

436   2003).

437

438   *Structural and functional shifts of root-tip ECM fungal communities due to the fire regime*

439   As we firstly hypothesized, for both pine species, the local and regional diversity of root-tip

440   ECM fungi significantly declined in populations where fires are historically frequent, and this

441   implied functional outcomes e.g., laccase activity of ECM root-tips increased in HiFi

442   populations of both pine forests. Many fire studies have ascribed decreases in fungal diversity

443   to the reduction of heat resistant propagules, elimination of rare species from the spore bank,

444   the scarcity of suitable hosts, the direct burning of mycelium and roots, and/or the disturbed

445   soil properties (Glassman et al., 2016; Holden, Gutierrez, & Treseder, 2013; Rincón & Pueyo,

446   2010). These processes do have a great importance on ECM fungal communities in the short

447   term after disturbance, giving the way to other processes such as dispersion through time

448   (Dooley & Treseder, 2011; Kipfer, Moser, Egli, Wohlgemuth, & Ghazoul, 2011; Rincón et

449   al., 2014; Sun et al., 2015), similar to that reported in succession studies where fungal

450   richness increases with forest age (Kyaschenko, Clemmensen, Hagenbo, Karltun, & Lindahl,

451 2017; Twieg, Durall, & Simard, 2007). In our study, it does not seem that ECM fungal

452 diversity has not been yet restored since the last fire (at least more than 40 years ago), because

453 typical late-stage species such as *Amanita* or *Lactarius* (Cairney & Chambers, 2013; Taylor &

454 Bruns, 1999) were indicators in HiFi areas. On the contrary, our results suggest that, the

455 initial ruderal symbionts that facilitate the establishment of seedlings are replaced over time

456 by ECM fungi that respond more efficiently to the new environmental conditions, probably

457 driving plant-soil feedbacks to different directions (Duhamel et al., 2019).

458 The ECM species composition diverged between fire regimes and, although fungal diversity

459 decreased with high fire recurrence, some fungal families were more diverse in HiFi

460 populations, e.g. *Amanitaceae*, *Cantharellaceae* and *Rhizopogonaceae* for *P. pinaster*, or

461 *Pezizaceae* for *P. halepensis*. Different authors have proposed that in ecosystems subjected to

462 frequent fires, the positive response of microorganisms would indicate a selection of the fire-

463 tolerant ones over time (Buscardo et al., 2015; Dooley & Treseder, 2011; Rincón et al., 2014).

464 Fire recurrence in Mediterranean forests is intimately linked to temperature and drought

465 (Pausas, 2004), so as expected, in our work, climate was a strong determinant of fire regime

466 effects on fungal communities. All these results, together with the observed decline in ECM

467 fungal β-diversity, seem to indicate that the habitat filtering was likely the dominant

468 ecological process assembling root-tip ECM fungal communities under high fire recurrence,

469 while without the fire pressure (i.e., low fire recurrence) and milder climate conditions,

470 competition among fungi could prevail, similar to that described across biological groups

471 (Pérez-Valera, Verdú, Navarro-Cano, & Goberna, 2018; Verdú & Pausas, 2007). The

472 diversity of plant and microbial communities is regulated by sequentially operating assembly

473 rules: abiotic filtering is an omnipresent structuring force, and biological interactions e.g.,

474 competition, further fine-tunes the community assemblages (Pérez-Valera et al., 2018; Verdú

475 & Pausas, 2007).

476 Fire regime and climate can filter directly different biotic organisms (i.e., trees and ECM

477 fungi), however our results did provide significant clues about indirect plant-mediated effects

478 of high fire recurrence on ECM fungal communities. We observed that serotiny, previously

479 demonstrated to be a tree heritable trait shaped by recurrent fires (Budde et al., 2014;

480 Castellanos, González-Martínez, & Pausas, 2015; Hernández-Serrano et al., 2014),

481 significantly explained the variation of ECM fungal communities, aside from the accounted

482 climatic effects for both pine species. This could be attributed to climatic constraints in the

483 channel of C from the tree towards the ECM fungi, although, on the other hand, serotiny is a

484 trait that requires resources (water and C) for maintaining a large amount of seeds alive in the

485 canopy with a cost for the plant (Cramer & Midgley, 2009; Harris & Pannell, 2010). Another

486 possible explanation is that the ability of the different tree genotypes/phenotypes to

487 preferentially allocate photosynthates to the more beneficial ECM fungi, or to those with low

488 C demands and/or high abilities to cope with hydric/nutrient stress can be the prevailing

489 mechanism explaining such plant-mediated effects (Bever, Richardson, Lawrence, Holmes, &

490 Watson, 2009; Gehring et al., 2014). This was to some extent supported by our results, at least

491 in *P. pinaster*, for which a direct effect of tree phenotype (serotiny) on enzymatic responses of

492 ECM fungi, but no effect of climatic variables was observed. We have previously shown that

493 particular *P. pinaster* genotypes can impact enzymatic activities through changes in the

494 composition of the associated microbial communities (Pérez-Izquierdo et al., 2017, 2019).

495 Additionally, it cannot be ruled out that, the fire recurrence negatively affects other tree traits

496 such as root development and/or architecture constraining the space for ECM fungal

497 colonization, which would make mechanisms like priority effects particularly important

21

498   (Kennedy, Peay, & Bruns, 2009; Peay, Belisle, & Fukami, 2012). In any case, further

499   experimentation would be needed to shed light on these assumptions.

500   The increased fire recurrence affected ecosystem functions differently in the two pine species

501   by reducing most enzymatic activities in the case of *P. pinaster*, but not for *P. halepensis*.

502   This suggests functional redundancy between ECM fungal communities associated with *P.*

503   *halepensis* in low and high fire recurrence sites, since diversity and assemblage shifts were

504   not translated into functional changes (Jones et al., 2010). These results might indicate a high

505   capacity of *P. halepensis* forests to recover its functionality among recurrent fires in warmer

506   areas probably by shifting plant-microbial feedbacks to readjust to the environment

507   (Clemmensen et al., 2015; Johnstone et al., 2010). The edaphic conditions (mainly pH and P)

508   were main factors explaining differences in enzymatic profiles among populations of both

509   pine species. Among their multiple functions, an outstanding feature of ectomycorrhizal fungi

510   is their ability to mobilize nutrients from the soil (Smith & Read, 2008). Because of their

511   direct access to C from the host, they are able to invest energy (i.e., enzymatic production) to

512   mine for N and/or P from hardly accessible sources (Fernandez, See, & Kennedy, 2020;

513   Lindahl & Tunlid, 2015), as it could be the case for basic soils in our study together with

514   recalcitrant pine litter. Our findings are in line with previous studies indicating that the

515   availability of resources in surrounding soil together with tree host genetics strongly regulate

516   ECM enzymatic production (Aponte, García, Marañón, & Gardes, 2010; Courty et al., 2016;

517   Courty et al., 2011; Schneider et al., 2012). As previously mentioned, the laccase activity

518   increased in high fire recurrence populations of both pines. Laccase is an oxidative enzyme

519   related to several processes like the degradation of recalcitrant C compounds (i.e., lignin), but

520   also to the production of melanin by fungi (Baldrian, 2006; Eisenman et al., 2007). Melanin is

521   a group of complex polymers deposited in the cell wall associated with resistance to stresses

522   such as drought (Koide et al., 2014) and moderately resistant to decomposition (Fernandez,

523   Heckman, Kolka, & Kennedy, 2019). It is also possible that in our study sites, changes in

524   traits like production of melanin by fungi or more litter tannins by trees associated to the fire

525   regime and climatic stress might have affected organic matter turnover and feedback cycles,

526   issues that would deserve further analyses. In fact, in a 2-million-year chronosequence,

527   Albornoz et al. (2016) showed strong variation of ECM fungal communities even within the

528   same hosts, attributable not only to short-term fungal edaphic specialization or different

529   inoculum density and composition, but also likely to a much longer-term ecosystem-level

530   feedbacks among soil, plants and ECM fungi during pedogenesis.

531

532   **Conclusions**

533   In *P. pinaster* and *P. halepensis* Mediterranean forests, the high fire recurrence filters the

534   ECM fungal community composition, even favoring some ECM fungal species, but reduces

535   local and regional ECM diversity. Factors such as climate, which is intimately related to fire,

536   and soil properties in particular pH, affect ECM fungal communities composition and their

537   enzymatic functions. Aside from the climatic filter, local adaptation in pine populations to

538   recurrent fires (i.e., increased serotiny) selects for different ECM fungal communities,

539   accompanying different functional responses. ECM fungal communities associated with

540   serotinous *P. pinaster* trees imply a reduced enzymatic activity, but for *P. halepensis*, a

541   functional redundancy in LoFi and HiFi fire regimes likely exists. Edaphic variables, highly

542   linked to the bedrock type in Mediterranean ecosystems, are main drivers of ECM fungal

543   structure and functioning but generally independent of the fire regime. The bedrock material

544   is also a main factor driving differences in ECM fungal communities associated with *P.*

545   *pinaster* and *P. halepensis*, overriding the possible effect of the tree species. Although fire is a

23

546 main factor shaping Mediterranean ecosystems, changes in fire frequency because of

547 predicted warmer and longer dry periods have the potential to affect the plants and their

548 symbionts altering the structure, functioning and successional dynamics of Mediterranean

549 forests ecosystems. The observed fire regime-related structural and functional shifts in ECM

550 fungal communities might have essential implications for Mediterranean pine forests

551 dynamics, which should be carefully considered to promote the sustainable management of

552 these vulnerable ecosystems and to maintain their resilience under future climatic scenarios.

References

564 Abdel Malak, D., & Pausas, J. G. (2006). Fire regime and post-fire Normalized Difference
565   Vegetation Index changes in the eastern Iberian peninsula (Mediterranean basin).
566   *International Journal of Wildland Fire*, Vol. 15, pp. 407–413. doi: 10.1071/WF0505
567 Albornoz, F. E., Teste, F. P., Lambers, H., Bunce, M., Murray, D. C., White, N. E., &
568   Laliberté, E. (2016). Changes in ectomycorrhizal fungal community composition and
569   declining diversity along a 2-million-year soil chronosequence. *Molecular Ecology*,
570   *25*(19), 4919–4929. doi: 10.1111/mec.13778
571 Anders, S., & Huber, W. (2012). *Differential expression of RNA-Seq data at the gene level–*
572   *the DESeq package*.
573 Anderson, M., Ellingsen, K., & McArdle, B. (2006). Multivariate dispersion as a measure of
574   beta diversity. *Ecology Letters*, *9*(6), 683–693.
575   doi:10.1111/j.14610248.2006.00926.x/full
576 Aponte, C., García, L. V., Marañón, T., & Gardes, M. (2010). Indirect host effect on
577   ectomycorrhizal fungi: Leaf fall and litter quality explain changes in fungal communities
578   on the roots of co-occurring Mediterranean oaks. *Soil Biology and Biochemistry*, *42*(5),
579   788–796. doi: 10.1016/j.soilbio.2010.01.014
580 Baar, J., Horton, T. R., Kretzer, A. M. ., & Runs, T. D. (1999). Mycorrhizal colonization of
581   Pinus muricata from resistant propagules after a stand-replacing wildfire. *New*
582   *Phytologist*, *143*(2), 409–418. Doi: 10.1046/j.1469-8137.1999.00452.x
583 Baldrian, P. (2006). Fungal laccases-occurrence and properties. *FEMS Microbiology Reviews*,
584   *30*, 215–242. doi: 10.1111/j.1574-4976.2005.00010.x
585 Barazani, O., & Baldwin, I. T. (2013). A Mixed Bag: The Plant Growth-Promoting *Sebacina*
586   *vermifera* Impairs Defense Mechanisms Against Herbivores Oz. In *Piriformospora*
587   *indica* (pp. 251–261). doi: 10.1007/978-3-642-33802-1
588 Barbéro, M., Loisel, R., & Quézel, P. (1998). Pines of the Mediterranean basin. In DM
589   Richardson (Ed.), *Ecology and biogeography of Pinus* (pp. 153–170).
590 Bever, J. D., Richardson, S. C., Lawrence, B. M., Holmes, J., & Watson, M. (2009).
591   Preferential allocation to beneficial symbiont with spatial structure maintains
592   mycorrhizal mutualism. *Ecology Letters*, *12*(1), 13–21. doi: 10.1111/j.1461-
593   0248.2008.01254.x
594 Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of
595   phosphorus in soils. *Soil Science*, *59*(1), 39–46.
596 Bruns, T. D., Peay, K. G., Boynton, P. J., Grubisha, L. C., Hynson, N. A., Nguyen, N. H., &
597   Rosenstock, N. P. (2009). Inoculum potential of *Rhizopogon* spores increases with time
598   over the first 4 yr of a 99-yr spore burial experiment. *New Phytologist*, *181*(2), 463–470.
599   doi: 10.1111/j.1469-8137.2008.02652.x
600 Budde, K. B., González-Martínez, S. C., Navascués, M., Burgarella, C., Mosca, E., Lorenzo,
601   Z., … Heuertz, M. (2017). Increased fire frequency promotes stronger spatial genetic
602   structure and natural selection at regional and local scales in *Pinus halepensis* Mill.
603   *Annals of Botany*, *119*(6), 1061–1072. doi: 10.1093/aob/mcw286
604 Budde, K. B., Heuertz, M., Hernández-Serrano, A., Pausas, J. G., Vendramin, G. G., Verdú,
605   M., & González-Martínez, S. C. (2014). In situ genetic association for serotiny, a fire-
606   related trait, in Mediterranean maritime pine (*Pinus pinaster*). *New Phytologist*, *201*(1),
607   230–241. doi: 10.1111/nph.12483
608 Buscardo, E., Rodriguez-Echeverria, S., Freitas, H., De Angelis, P., Pereira, J. S., & Muller,
609   L. A. H. (2015). Contrasting soil fungal communities in Mediterranean pine forests

610   subjected to different wildfire frequencies. *Fungal Diversity*, *70*(1), 85–99. doi:
611   10.1007/s13225-014-0294-5
612 Cáceres, M. D., Legendre, P., & He, F. (2013). Dissimilarity measurements and the size
613   structure of ecological communities. *Methods in Ecology and Evolution*, *4*(12), 1167–
614   1177. doi: 10.1111/2041-210X.12116/full
615 Cairney, J. W., & Chambers, S. M. (2013). *Ectomycorrhizal fungi: key genera in profile*.
616   Springer Science & Business Media.
617 Camarero, J. J., Olano, J. M., & Parras, A. (2010). Plastic bimodal xylogenesis in conifers
618   from continental Mediterranean climates. *New Phytologist*, *185*(2), 471–480. doi:
619   10.1111/j.1469-8137.2009.03073.x
620 Castellanos, M. C., González-Martínez, S. C., & Pausas, J. G. (2015). Field heritability of a
621   plant adaptation to fire in heterogeneous landscapes. *Molecular Ecology*, *24*(22), 5633–
622   5642. doi: 10.1111/mec.13421
623 Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., …
624   Lindahl, B. D. (2013). Roots and Associated Fungi Drive Long-Term Carbon
625   Sequestration in Boreal Forest. *Science*, *339*(6127), 1615–1618. doi:
626   10.1126/science.1231923
627 Clemmensen, K. E., Finlay, R. D., Dahlberg, A., Stenlid, J., Wardle, D. A., & Lindahl, B. D.
628   (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during
629   long-term succession in boreal forests. *New Phytologist*, *205*, 1525–1536. doi:
630   doi:10.1111/nph.13208
631 Coince, A., Cordier, T., Lengellé, J., Defossez, E., Vacher, C., Robin, C., … Marçais, B.
632   (2014). Leaf and root-associated fungal assemblages do not follow similar elevational
633   diversity patterns. *PloS One*, *9*(6), e100668. doi: 10.1371/journal.pone.0100668
634 Courty, P. E., Munoz, F., Selosse, M. A., Duchemin, M., Criquet, S., Ziarelli, F., … Richard,
635   F. (2016). Into the functional ecology of ectomycorrhizal communities: Environmental
636   filtering of enzymatic activities. *Journal of Ecology*, *104*(6), 1585–1598. doi:
637   10.1111/1365-2745.12633
638 Courty, P E, Pritsch, K., Schloter, M., Hartmann, A., & Garbaye, J. (2005). Activity profiling
639   of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New
640   Phytologist*, *167*(1), 309–319. doi: 10.1111/j.1469-8137.2005.01401.x
641 Courty, P. E., Labbé, J., Kohler, A., Marçais, B., Bastien, C., Churin, J., … Le Tacon, F.
642   (2011). Effect of poplar genotypes on mycorrhizal infection and secreted enzyme
643   activities in mycorrhizal and non-mycorrhizal roots. *Journal of Experimental Botany*,
644   *62*(1), 249–260. doi: 10.1093/jxb/erq274
645 Cramer, M. D., & Midgley, J. J. (2009). Maintenance costs of serotiny do not explain weak
646   serotiny. *Austral Ecology*, *34*, 653–662. doi: 10.1111/j.1442-9993.2009.01971.x
647 de Román, M., & de Miguel, A. M. (2005). Post-fire , seasonal and annual dynamics of the
648   ectomycorrhizal community in a *Quercus ilex* L . forest over a 3-year period.
649   *Mycorrhiza*, *15*, 471–482. doi: 10.1007/s00572-005-0353-6
650 Dooley, S. R., & Treseder, K. K. (2011). The effect of fire on microbial biomass: a meta-
651   analysis of field studies. *Biogeochemistry*, *109*(1–3), 49–61. doi: 10.1007/s10533-011-
652   9633-8
653 Dove, N. C., & Hart, S. C. (2017). Fire reduces fungal species richness and in situ
654   mycorrhizal colonization: A meta-analysis. *Fire Ecology*, *13*(2), 37–65. doi:
655   10.4996/fireecology.130237746
656 Duhamel, M., Wan, J., Bogar, L. M., Segnitz, R. M., Dncritts, N. C., & Peay, K. G. (2019).
657   Plant selection initiates alternative successional trajectories in the soil microbial

658   community after disturbance. *Ecological Monographs*, *89*(3), e01367. doi:
659   10.1002/ecm.1367

660   Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon
661   reads. *Nature Methods*, *10*(10), 996–998. doi: 10.1038/nmeth.2604

662   Egerton-Warburton, L. M., Querejeta, J. I., & Allen, M. F. (2007). Common mycorrhizal
663   networks provide a potential pathway for the transfer of hydraulically lifted water
664   between plants. *Journal of Experimental Botany*, *58*(6), 1473–1483. doi:
665   10.1093/jxb/erm009

666   Eisenman, H. C., Mues, M., Weber, S. E., Frases, S., Chaskes, S., Gerfen, G., & Casadevall,
667   A. (2007). *Cryptococcus neoformans* laccase catalyses melanin synthesis from both D -
668   and L -DOPA. *Microbiology*, *153*, 3954–3962. doi: 10.1099/mic.0.2007/011049-0

669   Fernandez, C. W., Heckman, K., Kolka, R., & Kennedy, P. G. (2019). Melanin mitigates the
670   accelerated decay of mycorrhizal necromass with peatland warming. *Ecology Letters*, *22*,
671   498–505. doi: 10.1111/ele.13209

672   Fernandez, C. W., See, C. R., & Kennedy, P. G. (2020). Decelerated carbon cycling by
673   ectomycorrhizal fungi is controlled by substrate quality and community composition.
674   *New Phytologist*, *226*(2), 569–582. doi: 10.1111/nph.16269

675   Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes,
676   application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*, 113–
677   118. doi: 10.1111/j.1365-294X.1993.tb00005.x

678   Gehring, C. A., Mueller, R. C., Haskins, K. E., Rubow, T. K., & Whitham, T. G. (2014).
679   Convergence in mycorrhizal fungal communities due to drought, plant competition,
680   parasitism, and susceptibility to herbivory: Consequences for fungi and host plants.
681   *Frontiers in Microbiology*, *5*(JUN), 1–9. doi: 10.3389/fmicb.2014.00306

682   Gehring, C. A., & Whitham, T. G. (1991). Herbivore-Driven Mycorrhizal Mutualism in
683   Insect-Susceptible Pinyon Pine. *Nature*, *353*(6344), 556–557. doi: 10.1038/353556a0

684   Glassman, S. I., Levine, C. R., Dirocco, A. M., Battles, J. J., & Bruns, T. D. (2016).
685   Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot.
686   *The ISME Journal*, *10*, 1228–1239. doi: 10.1038/ismej.2015.182

687   Glassman, S. I., Peay, K. G., Talbot, J. M., Smith, D. P., Chung, J. A., Taylor, J. W., …
688   Bruns, T. D. (2015). A continental view of pine-associated ectomycorrhizal fungal spore
689   banks : a quiescent functional guild with a strong biogeographic pattern. *New
690   Phytologist*, *205*, 1619–1631. doi: 10.1111/nph.13240

691   Glassman, S. I., Wang, I. J., & Bruns, T. D. (2017). Environmental filtering by pH and soil
692   nutrients drives community assembly in fungi at fine spatial scales. *Molecular Ecology*,
693   *26*(24), 6960–6973. doi: 10.1111/mec.14414

694   Greene, D. F., Zasada, J. C., Sirois, L., Kneeshaw, D., Morin, H., Charron, I., & Simard, M. J.
695   (1999). A review of the regeneration dynamics of North American boreal forest tree
696   species. *Canadian Journal of Forest Research*, *29*(6), 824–839. doi: 10.1139/x98-112

697   Harris, M. S., & Pannell, J. R. (2010). Canopy seed storage is associated with sexual
698   dimorphism in the woody dioecious genus *Leucadendron*. *Journal of Ecology*, *98*, 509–
699   515. doi: 10.1111/j.1365-2745.2009.01623.x

700   Hart, S. C., DeLuca, T. H., Newman, G. S., MacKenzie, M. D., & Boyle, S. I. (2005). Post-
701   fire vegetative dynamics as drivers of microbial community structure and function in
702   forest soils. *Forest Ecology and Management*, *220*(1–3), 166–184. doi:
703   10.1016/j.foreco.2005.08.012

704   Hernández-Serrano, A., Verdú, M., González-Martínez, S. C., & Pausas, J. G. (2013). Fire
705   structures pine serotiny at different scales. *American Journal of Botany*, *100*(12), 2349–

706   2356. doi: 10.3732/ajb.1300182
707   Hernández-Serrano, A., Verdú, M., Santos-Del-Blanco, L., Climent, J., González-Martínez, S.
708   C., & Pausas, J. G. (2014). Heritability and quantitative genetic divergence of serotiny, a
709   fire-persistence plant trait. *Annals of Botany*, *114*(3), 571–577. doi: 10.1093/aob/mcu142
710   Holden, S. R., Gutierrez, A., & Treseder, K. K. (2013). Changes in Soil Fungal Communities,
711   Extracellular Enzyme Activities, and Litter Decomposition Across a Fire
712   Chronosequence in Alaskan Boreal Forests. *Ecosystems*, *16*(1), 34–46. doi:
713   10.1007/s10021-012-9594-3
714   Isaac, R. A., & Kerber, J. D. (1971). Atomic absorption and flame photometry: Techniques
715   and uses in soil, plant and water analysis. In Walsh LM (ed). Soil Science Society
716   America. Madison. WI. (Ed.), *Instrumental Methods for Analysis of Soils and Plant
717   Tissue.*
718   Johnson, D., Martin, F., Cairney, J. W. G., & Anderson, I. C. (2012). The importance of
719   individuals: Intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New
720   Phytologist*, *194*(3), 614–628. doi: 10.1111/j.1469-8137.2012.04087.x
721   Johnstone, J. F., Chapin, F. S., Hollingsworth, T. N., Mack, M. C., Romanovsky, V., &
722   Turetsky, M. (2010). Fire, climate change, and forest resilience in interior AlaskaThis
723   article is one of a selection of papers from The Dynamics of Change in Alaska's Boreal
724   Forests: Resilience and Vulnerability in Response to Climate Warming. *Canadian
725   Journal of Forest Research*, *40*(7), 1302–1312. doi: 10.1139/X10-061
726   Jones, M. D., Twieg, B. D., Ward, V., Barker, J., Durall, D. M., & Simard, S. W. (2010).
727   Functional complementarity of Douglas-fir ectomycorrhizas for extracellular enzyme
728   activity after wildfire or clearcut logging. *Functional Ecology*, *24*(5), 1139–1151. doi:
729   10.1111/j.1365-2435.2010.01699.x
730   Keeley, J. E., Bond, W. J., Bradstock, R. A., Pausas, J. G., & Rundel, P. W. (2011). *Fire in
731   Mediterranean ecosystems: ecology, evolution and management*. Cambridge University
732   Press.
733   Kennedy, P. G., Peay, K. G., & Bruns, T. D. (2009). Root tip competition among
734   ectomycorrhizal fungi: Are priority effects a rule or an exception? *Ecology*, *90*(8), 2098–
735   2107. doi: 10.1890/08-1291.1
736   Kipfer, T., Moser, B., Egli, S., Wohlgemuth, T., & Ghazoul, J. (2011). Ectomycorrhiza
737   succession patterns in Pinus sylvestris forests after stand-replacing fire in the Central
738   Alps. *Oecologia*, *167*(1), 219–228. doi: 10.1007/s00442-011-1981-5
739   Kivlin, S. N., Emery, S. M., & Rudgers, J. A. (2013). Fungal symbionts alter plant responses
740   to global change. *American Journal of Botany*, *100*(7), 1445–1457. doi:
741   10.3732/ajb.1200558
742   Koide, R. T., Fernandez, C., & Malcolm, G. (2014). Determining place and process:
743   Functional traits of ectomycorrhizal fungi that affect both community structure and
744   ecosystem function. *New Phytologist*, *201*(2), 433–439. doi: 10.1111/nph.12538
745   Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., …
746   Larsson, K. H. (2013). Towards a unified paradigm for sequence-based identification of
747   fungi. *Molecular Ecology*, *22*(21), 5271–5277. doi: 10.1111/mec.12481
748   Kraft, N. J. B., Adler, P. B., Godoy, O., James, E. C., Fuller, S., & Levine, J. M. (2015).
749   Community assembly , coexistence and the environmental fi ltering metaphor.
750   *Functional Ecology*, *29*, 592–599. doi: 10.1111/1365-2435.12345
751   Kyaschenko, J., Clemmensen, K. E., Hagenbo, A., Karltun, E., & Lindahl, B. D. (2017). Shift
752   in fungal communities and associated enzyme activities along an age gradient of
753   managed *Pinus sylvestris* stands. *The ISME Journal*, *11*, 863–874. doi:

754   10.1038/ismej.2016.184
755 Lamit, L. J., Lau, M. K., Sthultz, C. M., Wooley, S. C., Whitham, T. G., & Gehring, C. A.
756   (2014). Tree genotype and genetically based growth traits structure twig endophyte
757   communities. *American Journal of Botany*, *101*(3), 467–478. doi: 10.3732/ajb.1400034
758 Lindahl, B. D., & Tunlid, A. (2015). *Ectomycorrhizal fungi – potential organic matter*
759   *decomposers , yet not saprotrophs*. *205*, 1443–1447. doi: 10.1111/nph.13201
760 Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., …
761   Marchetti, M. (2010). Climate change impacts, adaptive capacity, and vulnerability of
762   European forest ecosystems. *Forest Ecology and Management*, *259*(4), 698–709. doi:
763   10.1016/j.foreco.2009.09.023
764 McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbiome data
765   is inadmissible. *PLoS Computational Biology*, *10*(4), e1003531. doi:
766   10.1371/journal.pcbi.1003531
767 Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., … Kennedy, P. G.
768   (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by
769   ecological guild. *Fungal Ecology*, *20*, 241–248. doi: 10.1016/j.funeco.2015.06.006
770 Nuñez, M. A., Horton, T. R., & Simberloff, D. (2009). Lack of belowground mutualisms
771   hinders Pinaceae invasions. *Ecology*, *90*(9), 2352–2359. doi: 10.1890/08-2139.1
772 Ojeda, F., Pausas, J. G., & Verdú, M. (2010). Soil shapes community structure through fire.
773   *Oecologia*, *163*(3), 729–735. doi: 10.1007/s00442-009-1550-3
774 Oksanen, A. J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., Hara, R. B. O., …
775   Wagner, H. (2015). Vegan community ecology package: ordination methods, diversity
776   analysis and other functions for community and vegetation ecologists. Version 2.3-1. In
777   *R package version 2.3-1.*
778 Pausas, J.G. (2015). Evolutionary fire ecology: Lessons learned from pines. *Trends in Plant*
779   *Science*, *20*(5), 318–324. doi: 10.1016/j.tplants.2015.03.001
780 Pausas, J. G., & Fernández-Muñoz, S. (2012). Fire regime changes in the Western
781   Mediterranean Basin: From fuel-limited to drought-driven fire regime. *Climatic Change*,
782   *110*(1–2), 215–226. doi: 10.1007/s10584-011-0060-6
783 Pausas, J. G. (2004). Changes in fire and climate in the eastern Iberian Peninsula
784   (Mediterranean basin). *Climatic Change*, *63*, 337–350.
785 Pausas, J. G, Bradstock, R. A., Keith, D. A., & Keeley, J. E. (2004). Plant functional traits in
786   relation to fire in corwn-fire ecosystems. *Ecology*, *85*(4), 1085–1100. doi: 10.1890/02-
787   4094
788 Peay, K. G., Belisle, M., & Fukami, T. (2012). Phylogenetic relatedness predicts priority
789   effects in nectar yeast communities. *Proceedings. Biological Sciences / The Royal*
790   *Society*, *279*(1729), 749–758. doi: 10.1098/rspb.2011.1230
791 Pérez-Izquierdo, L, Zabal-Aguirre, M., Flores-Rentería, D., González-Martínez, S., Buée, M.,
792   & Rincón, A. (2017). Functional outcomes of fungal community shifts driven by tree
793   genotype and spatial-temporal factors in Mediterranean pine forests. *Environmental*
794   *Microbiology*, 19(4), 1639-1652. doi: 10.1111/1462-2920.13690
795 Pérez-Izquierdo, L., Zabal-Aguirre, M., González-Martínez, S. C., Buée, M., Verdú, M.,
796   Rincón, A., & Goberna, M. (2019). Plant intraspecific variation modulates nutrient
797   cycling through its below-ground rhizospheric microbiome. *Journal of Ecology*, 107(4),
798   1594-1605. doi: 10.1111/1365-2745.13202
799 Pérez-Valera, E., Verdú, M., Navarro-Cano, J. A., & Goberna, M. (2018). Resilience to fire of
800   phylogenetic diversity across biological domains. *Molecular Ecology*, *27*(13), 2896–
801   2908. doi: 10.1111/mec.14729

802   Pinosio, S., González-Martínez, S. C., Bagnoli, F., Cattonaro, F., Grivet, D., Marroni, F., …
803   Vendramin, G. G. (2014). First insights into the transcriptome and development of new
804   genomic tools of a widespread circum-Mediterranean tree species, *Pinus halepensis*
805   Mill. *Molecular Ecology Resources*, *14*(4), 846–856. doi: 10.1111/1755-0998.12232
806   Prieto, I., Roldán, A., Huygens, D., del Mar Alguacil, M., Navarro-Cano, J. A., & Querejeta,
807   J. I. (2016). Species-specific roles of ectomycorrhizal fungi in facilitating interplant
808   transfer of hydraulically redistributed water between *Pinus halepensis* saplings and
809   seedlings. *Plant and Soil*, *406*(1–2), 15–27. doi: 10.1007/s11104-016-2860-y
810   Querejeta, J. I., Egerton-Warburton, L. M., & Allen, M. F. (2007). Hydraulic lift may buffer
811   rhizosphere hyphae against the negative effects of severe soil drying in a California Oak
812   savanna. *Soil Biology and Biochemistry*, *39*(2), 409–417. doi:
813   10.1016/j.soilbio.2006.08.008
814   R Core Team. (2014). R Core Team. *R: A Language and Environment for Statistical*
815   *Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014.*
816   Radeloff, V. C., Mladenoff, D. J., Guries, R. P., & Boyce, M. S. (2004). Spatial patterns of
817   cone serotiny in *Pinus banksiana* in relation to fire disturbance. *Forest Ecology and*
818   *Management*, *189*, 133–141. doi: 10.1016/j.foreco.2003.07.040
819   Ray, P., & Craven, K. D. (2016). Sebacina vermifera: a unique root symbiont with vast
820   agronomic potential. *World Journal of Microbiology and Biotechnology*, *32*(1), 1–10.
821   doi: 10.1007/s11274-015-1970-7
822   Rincón, A., Santamaría-Pérez, B., Ocaña, L., & Verdú, M. (2014). Structure and phylogenetic
823   diversity of post-fire ectomycorrhizal communities of maritime pine. *Mycorrhiza*, *24*,
824   131–141. doi: 10.1007/s00572-013-0520-0
825   Rincón, A., & Pueyo, J. J. (2010). Effect of fire severity and site slope on diversity and
826   structure of the ectomycorrhizal fungal community associated with post-fire regenerated
827   *Pinus pinaster* Ait. seedlings. *Forest Ecology and Management*, *260*(3), 361–369. doi:
828   10.1016/j.foreco.2010.04.028
829   Rincón, A., Santamaría-Pérez, B., Rabasa, S. G., Coince, A., Marçais, B., & Buée, M. (2015).
830   Compartmentalized and contrasted response of ectomycorrhizal and soil fungal
831   communities of Scots pine forests along elevation gradients in France and Spain.
832   *Environmental Microbiology*, *17*, 3009–3024. doi: 10.1111/1462-2920.12894
833   Rousk, J., Baath, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., … Fierer,
834   N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil.
835   *Isme Journal*, *4*, 1340–1351. doi: 10.1038/ismej.2010.58
836   Ruíz, P., Álvarez-Uria, P., & Zavala, M. A. (2009). 9540 Pinares mediterráneos de pinos
837   mesogeanos endémicos. In *VV. AA., Bases ecológicas preliminares para la conservación*
838   *de los tipos de hábitat de interés comunitario en España.* (p. 112). Madrid: Ministerio de
839   Medio Ambiente, y Medio Rural y Marino.
840   Schneider, T., Keiblinger, K. M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G.,
841   Roschitzki, B., … Riedel, K. (2012). Who is who in litter decomposition?
842   Metaproteomics reveals major microbial players and their biogeochemical functions. *The*
843   *ISME Journal*, *6*(9), 1749–1762. doi: 10.1038/ismej.2012.11
844   Selosse, M. A., Bauer, R., & Moyersoen, B. (2002). Basal hymenomycetes belonging to the
845   Sebacinaceae are ectomycorrhizal on temperate deciduous trees. *New Phytologist*,
846   *155*(1), 183–195. doi: 10.1046/j.1469-8137.2002.00442.x
847   Smith, S., & Read, D. (Eds.). (2008). *Mycorrhizal Symbiosis*. Academic Press: London.
848   Sun, H., Santalahti, M., Pumpanen, J., Köster, K., Berninger, F., Raffaello, T., … Heinonsalo,
849   J. (2015). Fungal Community Shifts in Structure and Function across a Boreal Forest

850   Fire Chronosequence. *Applied and Environmental Microbiology*, *81*(22), 7869–7880.
851   doi: 10.1128/AEM.02063-15.Editor
852   Talbot, J. M., Allison, S. D., & Treseder, K. K. (2008). Decomposers in disguise: Mycorrhizal
853   fungi as regulators of soil C dynamics in ecosystems under global change. *Functional*
854   *Ecology*, *22*(6), 955–963. doi: 10.1111/j.1365-2435.2008.01402.x
855   Tapias, R., Climent, J., Pardos, J. a., & Gil, L. (2004). Life histories of Mediterranean pines.
856   *Plant Ecology*, *171*(Richardson 1988), 53–68. doi:
857   10.1023/B:VEGE.0000029383.72609.f0
858   Taylor, D. L., & Bruns, T. D. (1999). Community structure of ectomycorrhizal fungi in a
859   *Pinus muricata* forest: Minimal overlap between the mature forest and resistant
860   propagule communities. *Molecular Ecology*, *8*(11), 1837–1850. doi: 10.1046/j.1365-
861   294X.1999.00773.x
862   Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N. S., Wijesundera, R., …
863   Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213),
864   1256688. doi: 10.1126/science.1256688
865   Tedersoo, L., Hansen, K., Perry, B. A., & Kjøller, R. (2006). Molecular and morphological
866   diversity of pezizalean ectomycorrhiza. *New Phytologist*, *170*(3), 581–596. doi:
867   10.1111/j.1469-8137.2006.01678.x
868   Tedersoo, L., Mett, M., Ishida, T. A., & Bahram, M. (2013). Phylogenetic relationships
869   among host plants explain differences in fungal species richness and community
870   composition in ectomycorrhizal symbiosis. *New Phytologist*, *199*(3), 822–831. doi:
871   10.1111/nph.12328
872   Tedersoo, L., & Smith, M. E. (2013). Lineages of ectomycorrhizal fungi revisited: Foraging
873   strategies and novel lineages revealed by sequences from belowground. *Fungal Biology*
874   *Reviews*, *27*(3–4), 83–99. doi: 10.1016/j.fbr.2013.09.001
875   Torres, P., & Honrubia, M. (1997). *Changes and effects of a natural fire on ectomycorrhizal*
876   *inoculum potential of soil in a Pinus halepensis forest*. *96*, 189–196.
877   Twieg, B. D., Durall, D. M., & Simard, S. W. (2007). Ectomycorrhizal fungal succession in
878   mixed temperate forests. *New Phytologist*, *176*(2), 437–447. doi: 10.1111/j.1469-
879   8137.2007.02173.x
880   van der Heijden, M. G. A., Martin, F. M., Selosse, M. A., & Sanders, I. R. (2015).
881   Mycorrhizal ecology and evolution: The past, the present, and the future. *New*
882   *Phytologist*, *205*(4), 1406–1423. doi: 10.1111/nph.13288
883   Verdú, M., & Pausas, J. G. (2007). Fire drives phylogenetic clustering in Mediterranean Basin
884   woody plant communities. *Journal of Ecology*, *95*(6), 1316–1323. doi: 10.1111/j.1365-
885   2745.2007.01300.x
886   Vieira, J., Campelo, F., Rossi, S., Carvalho, A., Freitas, H., & Nabais, C. (2015). Adjustment
887   capacity of maritime pine cambial activity in drought-prone environments. *PLoS ONE*,
888   *10*(5), 1–15. doi: 10.1371/journal.pone.0126223
889   Walkley, A., & Black, I. (1934). An examination of the Degtjareff method for determining
890   soil organic matter, and a proposed modification of the chromic acid titration method.
891   *Soil Science*, *37*(1), 29–38.
892   Yamanaka, T. (2003). The effect of pH on the growth of saprotrophic and ectomycorrhizal
893   ammonia fungi in vitro. *Mycologia*, *95*(4), 584–589. doi: 10.2307/3761934
894   Zarea, M. J., Miransari, M., & Karimi, N. (2014). *Plant Physiological Mechanisms of Salt*
895   *Tolerance Induced by Mycorrhizal Fungi and* Piriformospora indica. doi: 10.1007/978-
896   1-4939-0721-2

898 **Data Accessibility**

899 Data were deposited in the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) as

900 PRJNA324224.

901

902 **Author contribution**

903 AR and MV designed the experiment; LPI, MZA and AR collected the data; LPI and MB

904 performed the bioinformatics analysis, LPI performed the statistical analyses. LPI and AR

905 wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

906

907

908

909

910

911

912

913

914

915

916

917

918

919

920

921

922 **Table 1.** Alpha-diversity of representative ectomycorrhizal (ECM) fungal families analysed
923 by General Linear Models (GLMs) ($p<0.05$). Main effects of the pine species (Ppi =*Pinus*
924 *pinaster* and Pha =*Pinus halepensis*) (left) and the fire regime (LoFi = low fire recurrence and
925 HiFi = high fire recurrence) (right) are shown. The site was nested within the fix factor fire
926 regime in the GLMs. Data represent means +/- SE. Arrows indicate significantly higher α-
927 diversity, and "=" indicates no variation between pine species according to the GLM (F
928 values; *$p<0.05$; **$p<0.01$; ***$p<0.001$). In the separate analyses on the right, for each pine
929 forest, different letters denote significant differences between fire regimes ($p<0.05$) (in bold).
930 A = Ascomycetes (orange); B = Basidiomycetes (green).

931

932

	Pine Forest			*P. pinaster*		*P. halepensis*	
	Ppi	*Pha*	F (p-value)	LoFi	HiFi	LoFi	HiFi
Amanitaceae[B]	↑		9.2**	**0.1 ± 0.1 a**	**0.9 ± 0.2 b**	0.1 ± 0.1	0.2 ± 0.1
Atheliaceae[B]	↑		6.8*	**9.7 ± 0.6 b**	**6.7 ± 0.6 a**	6.3 ± 0.5	5.9 ± 0.4
Bankeraceae[B]	=	=	2.0	1.8 ± 0.4	1.3 ± 0.3	**1.7 ± 0.5 b**	**0.9 ± 0.2 a**
Cantharellaceae[B]	↑		41.7***	**1.0 ± 0.2 a**	**2.8 ± 0.4 b**	0.7 ± 0.2	0.5 ± 0.2
Clavariaceae[B]	↑		36.9***	0.8 ± 0.2	1.2 ± 0.4	**0.3 ± 0.2 b**	**0.1 ± 0.1 a**
Clavulinaceae[B]	=	=	2.4	**2.9 ± 0.3 b**	**1.7 ± 0.3 a**	**2.7 ± 0.5 b**	**1.3 ± 0.3 a**
Cortinariaceae[B]	↑		16.2***	**2.2 ± 0.7 b**	**0.4 ± 0.2 a**	**0.6 ± 0.4 b**	**0.2 ± 0.1 a**
Gloniaceae[A]	↑		5.7*	**2.5 ± 0.3 b**	**1.2 ± 0.2 a**	**1.7 ± 0.4 b**	**0.9 ± 0.3 a**
Hydnaceae[B]	=	=	0.8	0.3 ± 0.1	0.2 ± 0.1	**0.6 ± 0.2 b**	**0.2 ± 0.1 a**
Inocybeaceae[B]	=	=	1.4	**5.5 ± 0.8 b**	**3.0 ± 0.5 a**	3.1 ± 0.3	2.8 ± 0.3
Pezizaceae[A]		↑	22.8***	0.2 ± 0.1	0.2 ± 0.1	**0.5 ± 0.2 a**	**1.1 ± 0.2 b**
Pyronemataceae[A]		↑	30.7***	0.6 ± 0.2	0.9 ± 0.2	**2.9 ± 0.3 b**	**1.6 ± 0.3 a**
Rhizopogonaceae[B]	↑		29.1***	**0.9 ± 0.2 a**	**1.8 ± 0.2 b**	**0.8 ± 0.2 b**	**0.4 ± 0.1 a**
Russulaceae[B]	↑		14.0***	**7.3 ± 0.6 b**	**5.5 ± 0.7 a**	4.3 ± 0.8	4.3 ± 0.5
Sebacinaceae[B]		↑	33.6***	5.7 ± 1.1	4.5 ± 0.4	8.7 ± 0.8	9.0 ± 0.8
Suillaceae[B]	=	=	0.0	1.6 ± 0.3	1.2 ± 0.2	**1.6 ± 0.2 b**	**1.2 ± 0.2 a**
Telephoraceae[B]	=	=	0.0	11.9 ± 1.0	11.0 ± 1.0	**14.1 ± 1.8 b**	**10.4 ± 0.8 a**
Tuberaceae[A]	=	=	0.0	1.4 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.4 ± 0.2

933

934

935

936

937
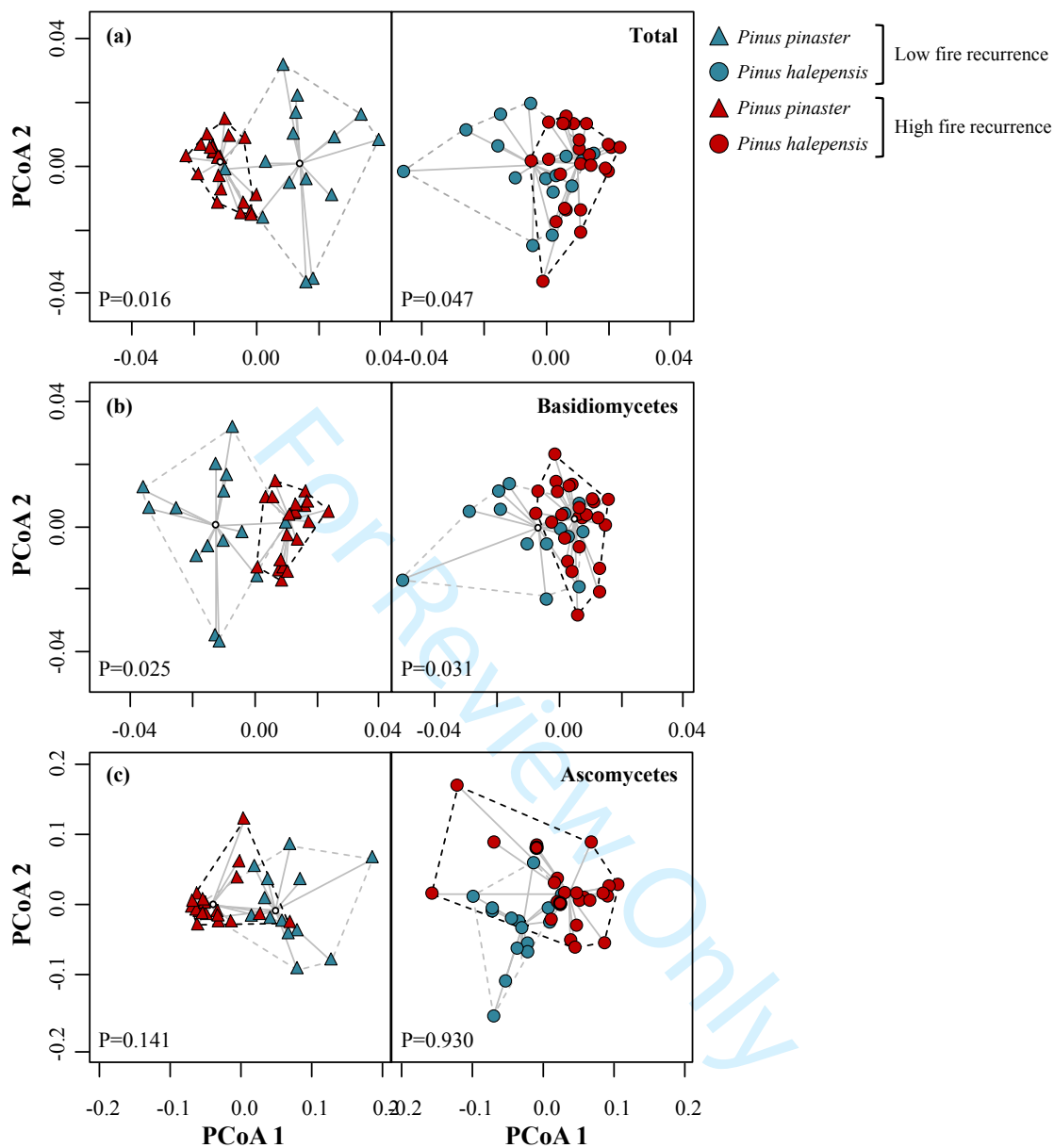
939
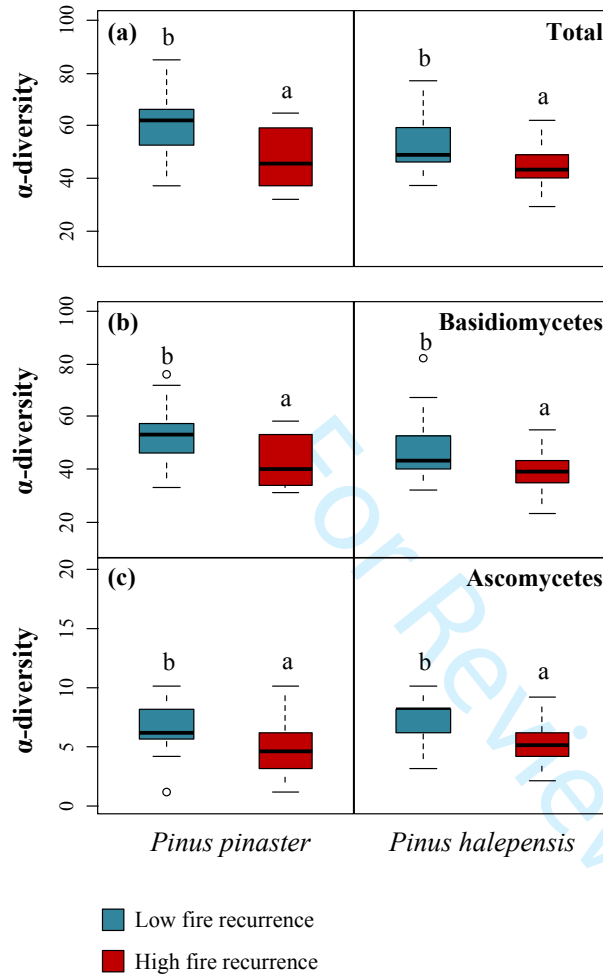
940

Figure 1. Alpha-diversity of (a) total, (b) Basidiomycetes and (c) Ascomycetes ectomycorrhizal fungal communities associated with root-tips of low (blue) or high (red) fire recurrence populations of *Pinus pinaster* and *Pinus halepensis*, analysed by Generalized Linear Models ($p<0.05$). 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 fire regimes.

949

950

951

Figure 2. Beta-diversity of (a) total, (b) Basidiomycetes and (c) Ascomycetes ectomycorrhizal fungal communities associated with root-tips of low (blue) or high (red) fire recurrence populations of *Pinus pinaster* (triangles) and *Pinus halepensis* (circles). The centroids within each group are represented by small white dots. Grey solid lines give the relative position of the point with respect to the respective centroid. Dotted lines reflect the area occupied by all plots of a given treatment, which is inversely proportional to the similarity of their ECM fungal communities.

960
961
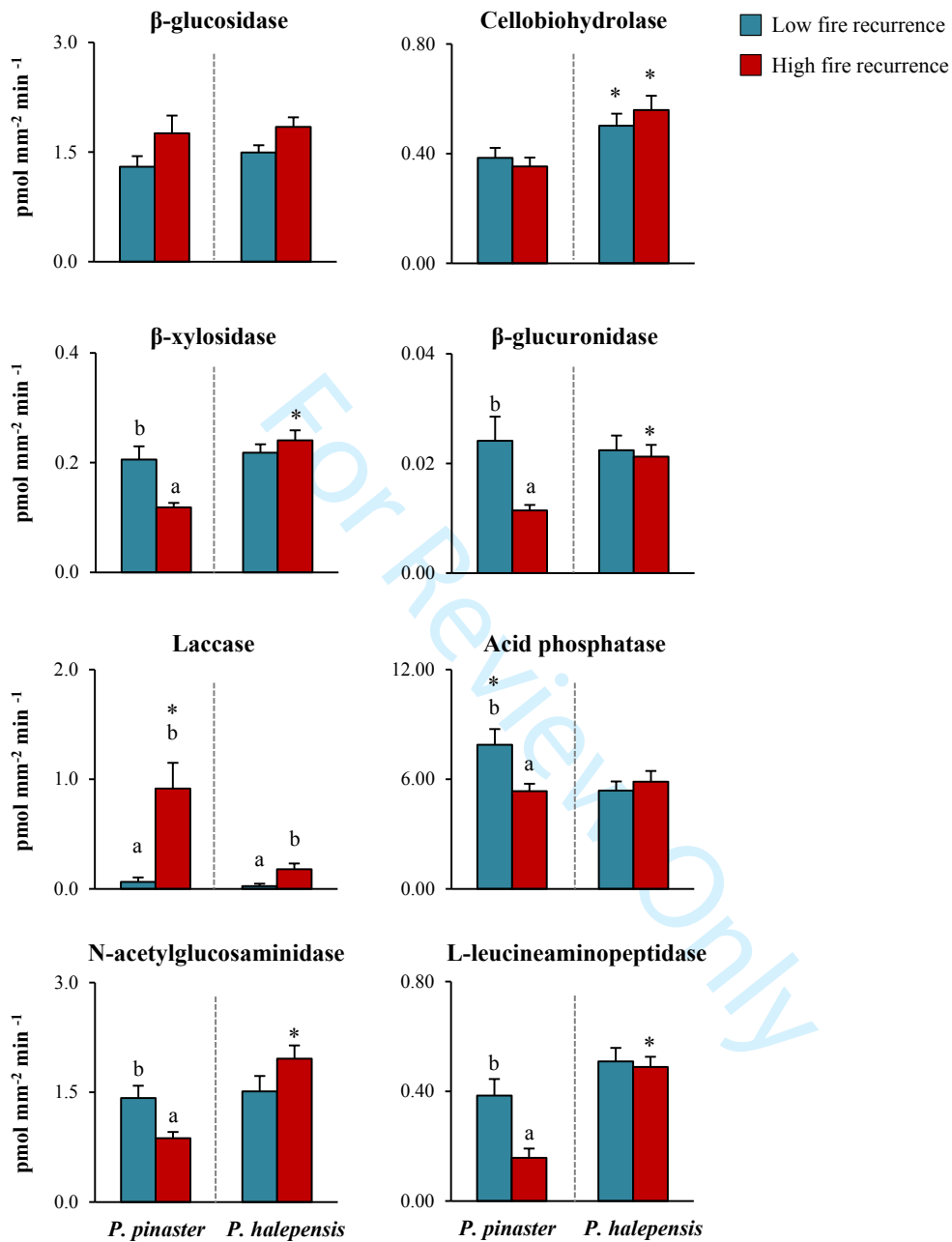
962
963
Figure 3. Enzymatic activity of ectomycorrhizal root tips (means ± SE) of low (LoFi, blue) or
high (HiFi, red) fire recurrence populations of *Pinus pinaster* and *Pinus halepensis*, analysed
by Generalized Linear Models (p<0.05). For each pine species, different letters denote
significant differences between fire recurrence levels, while for each LoFi/HiFi treatment,
asterisks denote significant differences between pine forest levels.
969

37

971

(a) ECM fungal community

(b) Enzymatic activity

▲ *Pinus pinaster*
● *Pinus halepensis* } Low fire recurrence

▲ *Pinus pinaster*
● *Pinus halepensis* } High fire recurrence

972

973
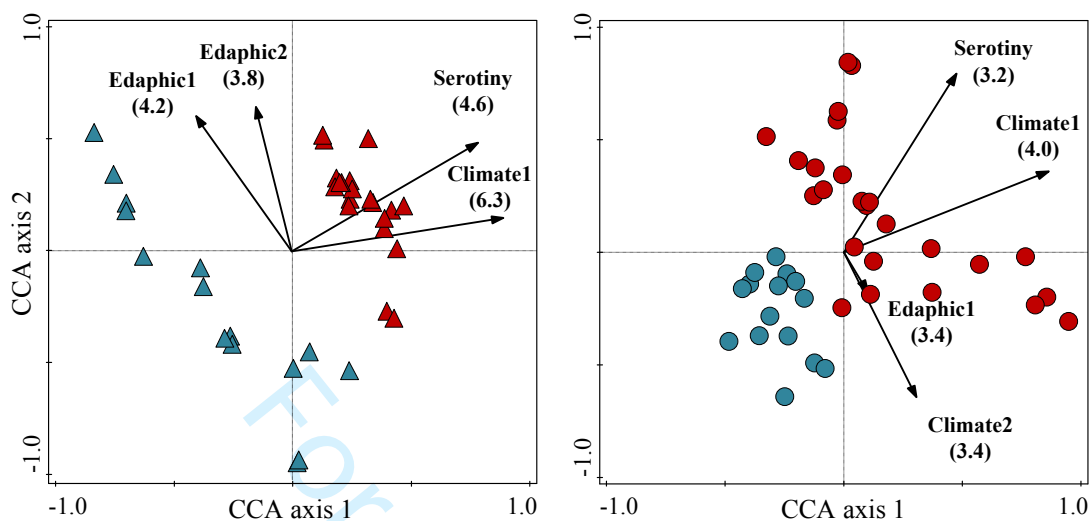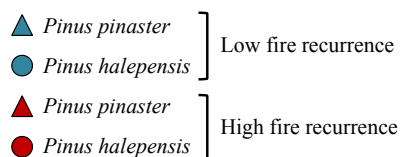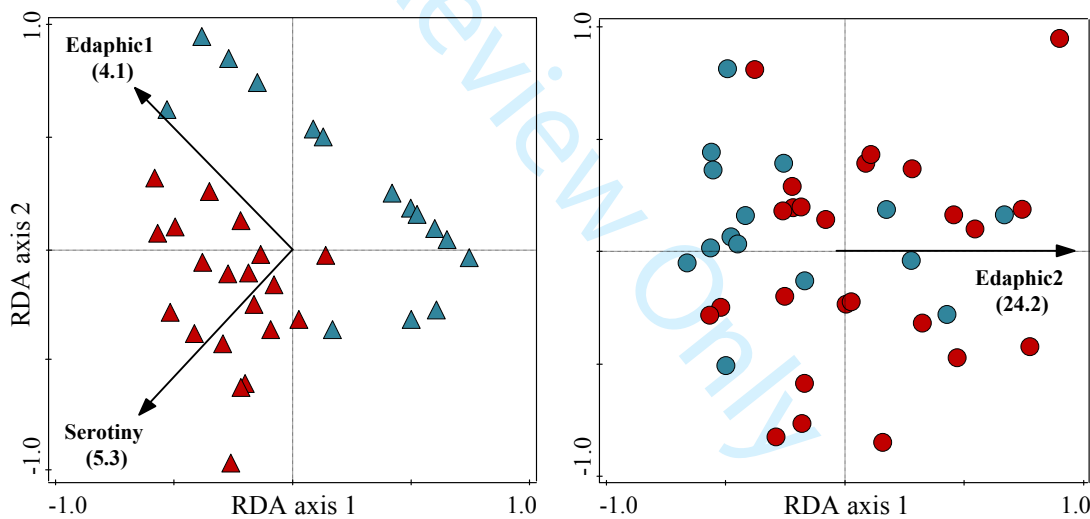
Figure 4. (a) Ectomycorrhizal fungal community structure and (b) enzymatic activities associated with root-tips of *Pinus pinaster* (triangles) and *Pinus halepensis* (circles) forests subjected to low (blue) or high (red) fire recurrence, respectively analyzed by Canonical Correspondence (CCA) and Redundancy (RDA) analyses. In all cases, the weight, direction

978   and separate effects of serotiny, climatic and edaphic conditions is represented by vectors (see
979   model details in Table S4). Serotiny degree is the number of closed cones by total number of
980   cones in the tree. Edaphic and climatic conditions are represented by the first two axes
981   (Edaphic1 and Edaphic2; Climate1 and Climate2) of Principal Coordinates Analyses (PCA)
982   performed considering the pH, electric conductivity and potassium, nitrogen, phosphorus and
983   organic matter content in the first case, and mean temperature, mean precipitation and altitude
984   in the second case (Figure S2). Only significant variables are shown, *p<0.05, **p<0.01,
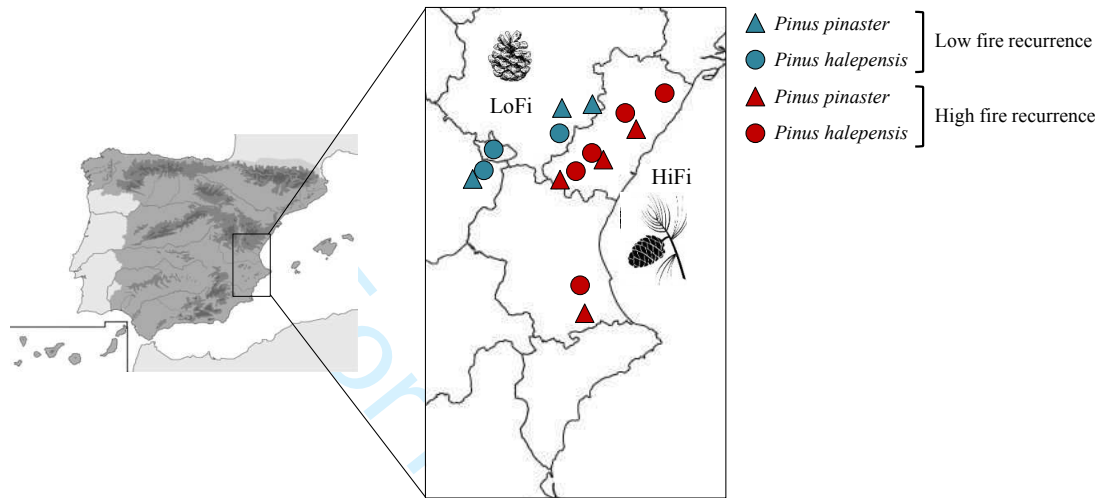985   ***p<0.001.

986   **Supplementary Figures**
987
988   **Figure S1**

989

990

991

992   **Figure S1.** Location of the fifteen study sites with pine populations of *Pinus pinaster* Ait.
993   (triangles) and *Pinus halepensis* Mill. (circles), growing in areas of low (LoFi, blue) and high
994   (HiFi, red) fire recurrence, in eastern Spain. The distinct historical fire regime has induced a
995   sharp serotiny divergence within these pine populations along time. Serotinous populations
996   associate to HiFi while non-serotinous populations associate to LoFi.
997

998

1000   **Figure S2**
1001

(a)

(b)
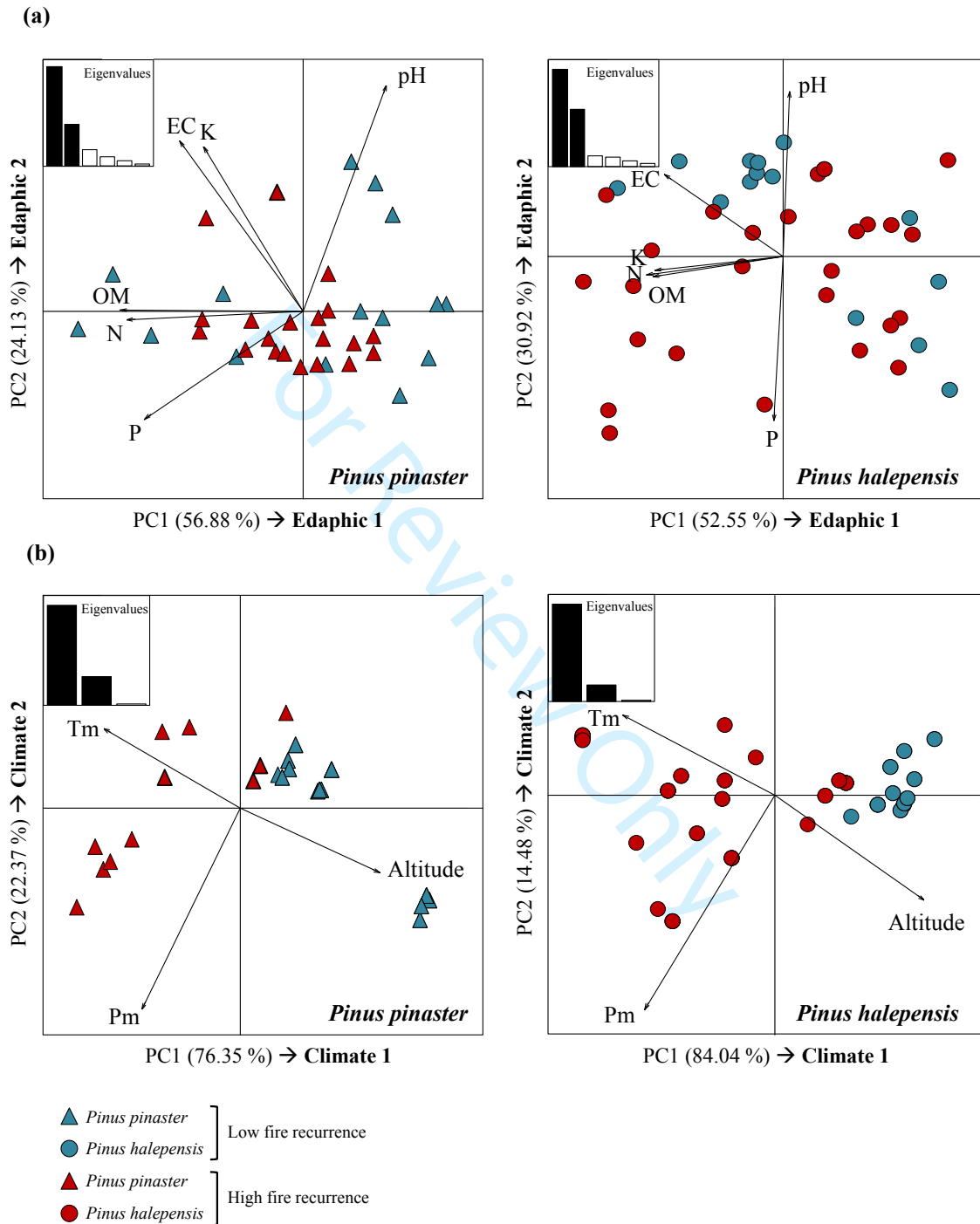
1002

1003   **Figure S2**. Principal component analyses (PCA) performed with (a) the edaphic variables pH,
1004   electric conductivity (EC), potassium (K), nitrogen (N), phosphorus (P) and organic matter
1005   (OM), and (b) the climate-related variables altitude, mean temperature (Tm) and mean

41

1006   precipitation (Pm) of each pine forest. The first two axes of each analysis explaining the
1007   maximum of variance (in parenthesis) were used as edaphic or climatic indexes in models
1008   (i.e., proxies of edaphic and climatic conditions of each pine forest *Pinus pinaster* or *Pinus*
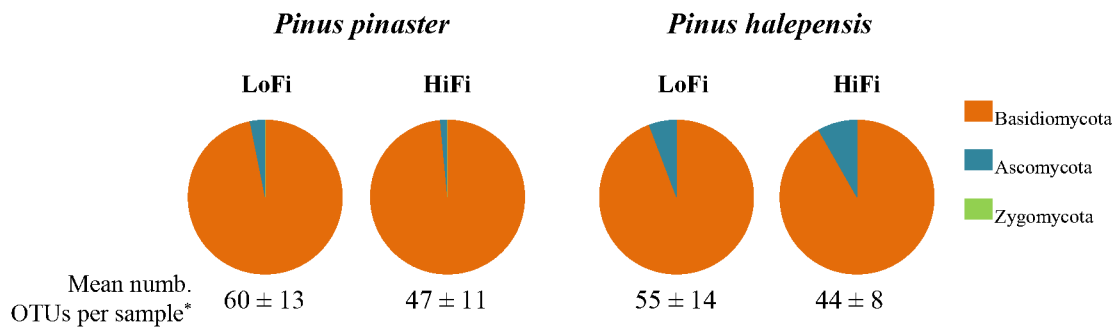1009   *halepensis*).
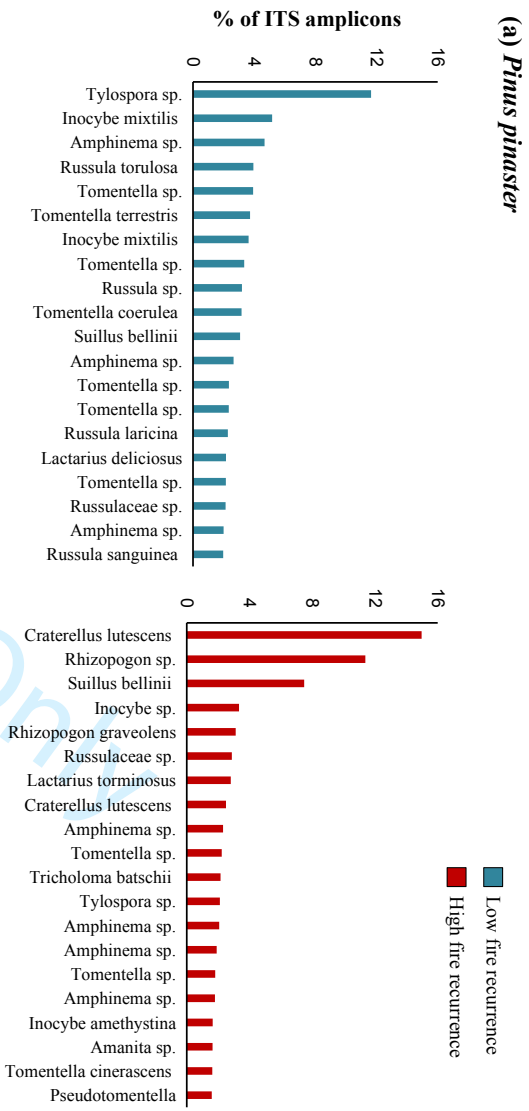
1011   **Figure S3**
1012

a)
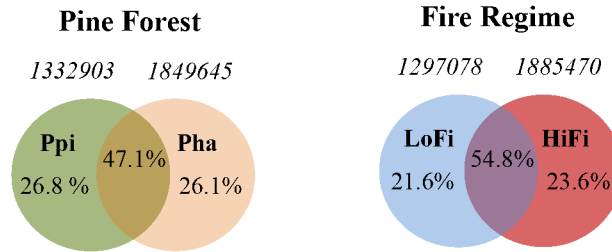
b)

1013

1014

1015   **Figure S3.** (a) Number of sequences (*italics*) and percentage of ectomycorrhizal (ECM)
1016   fungal OTUs by pine forest (Ppi = *Pinus pinaster* and Pha = *Pinus halepensis*) and fire regime
1017   (HiFi and LoFi fire recurrence). (b) Percentages of fungal phyla for each pine species and fire
1018   regime. *Mean ± SD. (See Table S2 for percentages and abundance of other taxonomic ranks
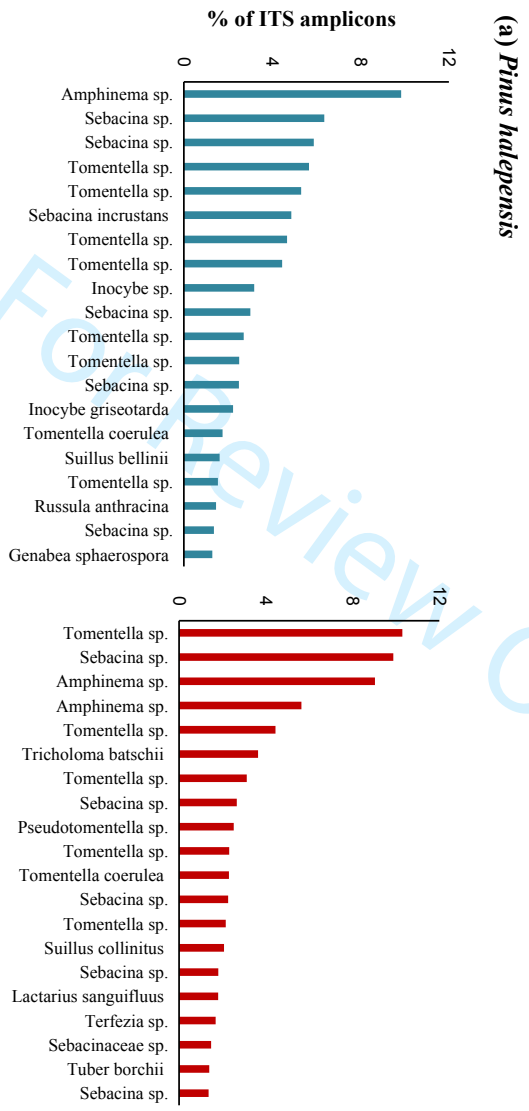1019   across treatments).

1021
1022

Figure S4

(a) *Pinus pinaster*

% of ITS amplicons

Tylospora sp.
Inocybe mixtilis
Amphinema sp.
Russula torulosa
Tomentella sp.
Tomentella terrestris
Inocybe mixtilis
Tomentella sp.
Russula sp.
Tomentella coerulea
Suillus bellinii
Amphinema sp.
Tomentella sp.
Tomentella sp.
Russula laricina
Lactarius deliciosus
Tomentella sp.
Russulaceae sp.
Amphinema sp.
Russula sanguinea

Craterellus lutescens
Rhizopogon sp.
Suillus bellinii
Inocybe sp.
Rhizopogon graveolens
Russulaceae sp.
Lactarius torminosus
Craterellus lutescens
Amphinema sp.
Tomentella sp.
Tricholoma batschii
Tylospora sp.
Amphinema sp.
Amphinema sp.
Tomentella sp.
Amphinema sp.
Inocybe amethystina
Amanita sp.
Tomentella cinerascens
Pseudotomentella

(a) *Pinus halepensis*

% of ITS amplicons

Amphinema sp.
Sebacina sp.
Sebacina sp.
Tomentella sp.
Tomentella sp.
Sebacina incrustans
Tomentella sp.
Tomentella sp.
Inocybe sp.
Sebacina sp.
Tomentella sp.
Tomentella sp.
Sebacina sp.
Inocybe griseotarda
Tomentella coerulea
Suillus bellinii
Tomentella sp.
Russula anthracina
Sebacina sp.
Genabea sphaerospora

Tomentella sp.
Sebacina sp.
Amphinema sp.
Amphinema sp.
Tomentella sp.
Tricholoma batschii
Tomentella sp.
Sebacina sp.
Pseudotomentella sp.
Tomentella sp.
Tomentella coerulea
Sebacina sp.
Tomentella sp.
Suillus collinitus
Sebacina sp.
Lactarius sanguifluus
Terfezia sp.
Sebacinaceae sp.
Tuber borchii
Sebacina sp.

Low fire recurrence
High fire recurrence

1023
1024
1025 **Figure S4.** Abundance rank (%) of the 20 most abundant species of low (blue) or high (red)
1026 fire recurrence populations of (a) *Pinus pinaster* and (b) *Pinus halepensis* forests.
1027
1028
1029
1030
1031

44

1032 **High** ~~Fire regime determines the structure and function of ectomycorrhizal fungal~~

1033 ~~communities of Mediterranean pine forests~~**fire recurrence reduces ectomycorrhizal**

1034 **fungal diversity of Mediterranean pine forests**

1035

1036 Pérez-Izquierdo L[1*], Zabal-Aguirre M[1], Verdú M[2], Buée M[3], Rincón A[1]

1037

1038 [1]Instituto de Ciencias Agrarias, ICA-CSIC. Serrano 115bis, 28006. Madrid, Spain.

1039 [2]CIDE-CSIC-UV-GV, Ctra Moncada-Náquera km4.5, 46113, Moncada-Valencia, Spain.

1040 [3]INRA, UMR1136 INRA Nancy – Université de Lorraine, Interactions Arbres-

1041 Microorganismes Labex ARBRE, 54280 Champenoux, France.

1042

1043

1044 **Corresponding author**: Leticia Pérez-Izquierdo

1045 [*]Current address:

1046 Department of Soil and Environment, SLU, Lennart Hjelms väg 9, SE-75007 Uppsala,

1047 Sweden.

1048 E-mail: leticia.perez@slu.se

1049

1050 **Running title:** ~~ECM fungi~~ Mycorrhizas in fire-prone Mediterranean ecosystems

1051

1052

1053

1054 **Competing interests**

1055 The authors declare that they have no competing interests

1056

1057

1058

1059

1061

1062 **Abstract**

1063 Fire is a major disturbance linked to the evolutionary history and climate of Mediterranean

1064 ecosystems, where the vegetation has evolved fire-adaptive traits (e.g., serotiny in pines). In

1065 Mediterranean forests, mutualistic feedbacks between trees and ectomycorrhizal (ECM)

1066 fungi, essential for ecosystem dynamics, might be drastically threatened by fire. We tested

1067 how fire regime alters the structure and function of ECM communities of *Pinus pinaster* and

1068 *Pinus halepensis* Mediterranean forests, and analyzed the relative contribution of

1069 environmental (climate, soil properties) and tree-mediated (serotiny) factorseffectors. For

1070 both pines, high fire recurrence significantly reduced local and regional ECM fungal diversity,

1071 although certain fungal species were favored by recurrent fire. The high fire recurrence also

1072 associated a general decline of ECM root-tip enzymatic activity for *Pinus pinaster*, while it

1073 did not imply major functional changes for *Pinus halepensis*. Separated effects of fire regime

1074 related-factorseffectors such as climate, soil properties or tree phenotype drove these

1075 processes. In addition to the main influence of climate, the tree fire-adaptive trait serotiny

1076 recovered a great portion of the variation in structure and function of fungal communities

1077 associated with the fire regime. Edaphic conditions (especially pH, tightly linked to bedrock

1078 type) were an important driver shaping ECM fungal communities, but mainly at the local

1079 scale and likely independently of the fire regime. Our results show that fire regime strongly

1080 impacts ECM fungal communities, and reveal complex feedbacks among trees, mutualistic

1081 fungi and surrounding environment in fire-prone Mediterranean forest ecosystems.

1082

1085

Introduction

1086

1087   Mediterranean climate is characterized by a marked seasonality with mild winters and hot dry

1088   summers, which gives rise to one of the most fire-prone ecosystems in the world (Pausas,

1089   2004). The evolutionary history of these ecosystems is tightly linked to fire (Keeley, Bond,

1090   Bradstock, Pausas, & Rundel, 2011). Recurrent burning has markedly structured plant

1091   communities in this area (Verdú & Pausas, 2007), where many species have developed

1092   evolutionary mechanisms of resistance and resilience to fire (Pausas, 2015; Tapias, Climent,

1093   Pardos, & Gil, 2004). A good example of fire-adaptive trait is serotiny, i.e., the retention of

1094   mature seeds in closed cones for more than a year until dispersion and germination is

1095   activated by fire, evolved by some representative Mediterranean pine species (Budde et al.,

1096   2014; Hernández-Serrano, Verdú, González-Martínez, & Pausas, 2013) and other conifers in

1097   fire-dominated systems (Greene et al., 1999). Fire generates phenotypic divergence on

1098   serotiny, an heritable trait in Mediterranean pines, ultimately producing local adaptation

1099   (Hernández-Serrano et al., 2014). There is evidence that serotiny is related to differences in

1100   fire regime, increasing with the frequency of stand-replacing fires (Hernández-Serrano et al.,

1101   2013; Radeloff, Mladenoff, Guries, & Boyce, 2004). Changes in the fire regime are closely

1102   linked to climate (Pausas, 2004), and predicted climate change scenarios make Mediterranean

1103   ecosystems especially vulnerable. Temperature rise and rainfall decrease are expected to

1104   increaserise drought risk and consequently forest wildfires, altering the structure and

1105   successional dynamics of Mediterranean forests (Lindner et al., 2010).

1106   Ecosystem development is driven by interactions among climatic conditions, edaphic

1107   environment and biotic communities. Given the role of fungi in organic matter turnover and

1108   nutrient cycling, they are key players in the plant-soil-microbial feedbacks that determine

1109   ecosystem development after disturbances (Clemmensen et al., 2015). Specifically,

1110   mutualistic ectomycorrhizal (ECM) fungi, which are tightly linked to the host through

1111   specialized symbiotic structures, mediate the uptake and transfer of water and nutrients to

1112   trees (Smith & Read, 2008). They channel the photosynthetic carbon (C) fixed by trees into

1113   the belowground, influencing soil Ccarbon storage and nutrient cycling (Clemmensen et al.,

1114   2013; Talbot, Allison, & Treseder, 2008). This symbiosis can provide up to the 80% of

1115   nutrients to trees and alleviate their hydric stress (Kivlin, Emery, & Rudgers, 2013; van der

1116   Heijden, Martin, Selosse, & Sanders, 2015), and therefore can be especially relevant in severe

1117   environments e.g., recurrent fire and drought conditions in Mediterranean ecosystems

1118   (Egerton-Warburton, Querejeta, & Allen, 2007; Prieto et al., 2016; Querejeta, Egerton-

1119   Warburton, & Allen, 2007).

1120   Fire affects ECM communities are affected by fire mainly through vegetation damages and

1121   altered soil properties (Buscardo et al., 2015; Hart, DeLuca, Newman, MacKenzie, & Boyle,

1122   2005; Rincón & Pueyo, 2010), conditions that may favor fire-tolerantprone fungi (Glassman,

1123   Levine, Dirocco, Battles, & Bruns, 2016; Rincón, Santamaría-Pérez, Ocaña, & Verdú, 2014).

1124   Certain fungi can remain for long time in the soil spore banks and be advantaged after fire, as

1125   it has been shown in closed-cone pine forest populations with historical highly recurrent and

1126   intense fires (Baar, Horton, Kretzer, & Runs, 1999; Bruns et al., 2009; Glassman et al., 2016,

1127   2015). Fire usually decreases root mycorrhizal colonization and fungal richness (Dove &

1128   Hart, 2017). However, exceptions of high colonization rates after fire have been seen for

1129   seedlings of fire-adapted Mediterranean pines. More generally, fire drastically disrupts the

1130   species composition of the mycorrhizal communities, usually leading to their simplification

1131   with the dominance of pioneer fungi (Dove & Hart, 2017; Buscardo et al., 2015; de Román &

1132   de Miguel, 2005; A. Rincón et al., 2014; Torres & Honrubia, 1997) (Torres and Honrubia

1133   1997; de Román and de Miguel 2005; Martin-Pinto et al. 2006; Buscardo et al. 2015; Rincón

1134   and Pueyo 2010; Rincón et al. 2014; Vasquez-Gassibe et al. 2016). Certain fungi can remain

1135   for long time in the soil spores banks and be advantaged after fire, as it has been shown in

1136   closed-cone pine forest populations with historical highly recurrent and intense fires (Baar,

1137   Horton, Kretzer, & Runs, 1999; Bruns et al., 2009; Glassman et al., 2016, 2015).

1138   AdditionallyMoreover, because intraspecific plant genotypic and phenotypic variation affects

1139   the structure of their associated ECM fungi (Gehring & Whitham, 1991; Pérez-Izquierdo et

1140   al., 2017, 2019; van der Heijden et al., 2015) it is and plausibley that , genetically fire-driven

1141   changes in trees, such as serotiny degree, may co-affect these mutualistic communities for

1142   example by determining the Cearbon available to exchange in symbiosis. Nevertheless, fires

1143   recurrently affect big areas of Mediterranean ecosystems, yet the cumulative effects of

1144   recurrent wildfires on ECM fungal communities are less known than those of single wildfires

1145   (Buscardo et al., 2015).

1146   In our study, we targeted forests of two representative Mediterranean tree species, Maritime

1147   pine (*Pinus pinaster* Ait.) and Aleppo pine (*Pinus halepen*sis Mill.), amply distributed in the

1148   Iberian Peninsula. While the Maritime pine usually grows in acid soils at 700-1700 m

1149   altitude, the Aleppo pine grows in basic substrate and below 800 m (Ruíz, Álvarez-Uria, &

1150   Zavala, 2009). *Pinus halepensis* is typically distributed in warm and dry areas, even under

1151   extreme drought induced either by climate or soil constituents (e.g., marls, gypsum, rocky

1152   slopes) (Ruíz et al., 2009). Both pine species have a life history related to fire adaptation

1153   (Tapias et al., 2004) displaying great post-fire colonizing abilities (Barbéro, Loisel, & Quézel,

1154   1998) and showing fire-adaptive traits such as serotiny (Pausas, 2015). The natural

1155   regeneration and dynamics of these pine forests critically relaieys on ECM fungal

1156   communities, because pine species are obligatory ectomycorrhizal (Nuñez, Horton, &

Simberloff, 2009; Smith & Read, 2008). Recurrent fires, tightly linked to climate in these Mediterranean areas, might act as an environmental filter, culling plant and microbial species unable to tolerate conditions at a particular location and thus preventing their establishment or persistence (Kraft et al., 2015).

Based on all these premises, we expected that (i) due to the habitat filtering imposed by the fire regime ~~and subjacent Mediterranean climatic conditions~~, the ECM fungal communities in ~~sites subjected to~~ high fire recurrence sites (HiFi hereafter) would be less diverse and more homogeneous than those in ~~sites exposed to~~ low fire recurrence sites (LoFi hereafter), and ~~that~~this it would imply different functional outcomes. ~~Conversely, increasing diversity might act as an insurance for ecosystem functions during extreme perturbations (e.g. fire and/or drought).~~

In ~~the~~our study area, the distinct historical fire ~~regime~~recurrence~~frequency~~ has induced a sharp serotiny divergence in trees within the HiFi and LoFi populations along time (Hernández-Serrano et al., 2013). Serotiny has been proven to be~~as~~ a heritable trait associated with the genotype of trees (Budde et al., 2014; Castellanos, González-Martínez, & Pausas, 2015; ~~A.~~ Hernández-Serrano et al., 2013). ~~Despite the low specificity of the mycorrhizal symbiosis for pines (Smith & Read, 2008),~~ Because different tree genotypes can influence their associated ECM fungi (Gehring & Whitham, 1991; Pérez-Izquierdo et al., 2017), ~~we~~This led us to further hypothesize that, at the finer scale of individuals, (ii) the ~~trees differing in their~~ serotiny degree of trees, i.e., genotypic fire-adaptation, ~~would~~will explain ~~affect differentially the~~ structural and functional divergences of ~~harbor dissimilar~~ ECM fungal communities. ~~Moreover, given expectable changes in nutrient availability and ECM structural shifts, we assumed (iii) different functional outcomes among root-tip ECM fungi associated~~

1180   with pines growing under distinct fire regime, i.e., either increased or decreased nutrient

1181   mobilization depending on fungal and/or tree adaptation to the new environmental conditions.

1182   In order to address these predictions, we characterized the structure of root-tip ECM fungal

1183   communities of natural *P. pinaster* and *P. halepensis* forests (Hernández-Serrano et al., 2013).

1184   For both pine forests, serotinous populations growing under a warm and dry Mediterranean

1185   climate subjected to high fire frequency and non-serotinous populations growing under a

1186   subhumid climate where fires are rare (Verdú & Pausas, 2007) were surveyed (Figure S1).

1187   Additionally, we determined, on excised ECM root-tips, potential fungal enzymatic traits

1188   related to Ccarbon turnover and mobilization of nutrients. These are processes directly

1189   implicated in the exchange of resources that support most mycorrhizal symbioses and many

1190   essential ecosystem functions (Johnson et al. 2012).

1191

1192   **Material and Methods**

1193   *Study sites and sampling*

1194   Surveys were conducted in natural forests of *P. pinaster* (Ppi) and of *P. halepensis* (Pha)

1195   located in eastern Spain (Figure S1). We selected nNine pine populations are located in high

1196   fire recurrence sites where crown-fires are historically frequent and most regeneration events

1197   are driven by fire (HiFi populations), while the other selected six populations wereare located

1198   in low fire recurrence areas where most regeneration events are independent of fire because

1199   fire events are rare (LoFi populations) (Hernández-Serrano et al., 2013; Pausas et al., 2004).

1200   The serotiny degree of these pine populations growing under distinct fire regime has been

1201   accurately characterized in Hernández-Serrano et al., 2013 (Table S1). Briefly, serotiny was

1202   estimated considering both the cone age and the proportion of serotinous cones the number of

1203   closed cones, those remaining closed after maturation, with respect to the total number (open

1204 ~~and closed).~~ In the study area, fire is tightly linked to Mediterranean climatic conditions (i.e.,

1205 drought) (Pausas, Bradstock, Keith, Keeley, & Network, 2004)~~, and fire history makes it~~

1206 ~~possible a clear differentiation of sites with a much shorter fire return interval than others~~

1207 ~~(Abdel Malak & Pausas, 2006).~~ Recent fire history information (Pausas & Fernández-Muñoz,

1208 2012; Pausas, 2004) shows that more than 50% of the study area at HiFi conditions burned at

1209 least once during the 1978–2001 period, while for LoFi conditions, the proportion was about

1210 15% (Abdel Malak & Pausas, 2006). From a microevolutionary point of view, this distinct

1211 fire regime has induced a sharp serotiny divergence within these populations (Hernández-

1212 Serrano et al., 2013). More than 500 genes were differentially expressed across the two pine

1213 accessions from HiFi and LoFi populations (Pinosio et al., 2014) and three high-

1214 differentiation outlier ~~single nucleotide polymorphisms~~SNPs were identified between HiFi

1215 and LoFi stands, suggesting fire-related selection at the regional scale (Budde et al., 2017).

1216 Thus, despite the lack of long-term fire statistics for the specific study sites, there is strong

1217 evidence that the fire interval is much shorter in HiFi areas than in LoFi. ~~Nine pine~~

1218 ~~populations are located in high fire recurrence sites where crown fires are historically~~

1219 ~~frequent and most regeneration events are driven by fire (HiFi populations), while the other~~

1220 ~~six populations are located in low fire recurrence areas where most regeneration events are~~

1221 ~~independent of fire because fire events are rare (LoFi populations) (Hernández-Serrano et al.,~~

1222 ~~2013; Pausas et al., 2004).~~ The serotiny degree of these pine populations growing under

1223 distinct fire regime has been accurately characterized in Hernández-Serrano et al., 2013

1224 (Table S1). Briefly, serotiny was estimated considering both the cone age and the proportion

1225 of serotinous cones, i.e., the number of closed cones, those remaining closed after maturation,

1226 with respect to the total fully ripe (open and closed) ~~of~~ cones (see Hernández-Serrano et al.,

1227 2013 for details). The characteristics of pine populations related to productivity (diameter at

1228   breast height; DBH), fire-adaptation traits (bark thickness and serotiny degree), as well as

1229   location and local environmental variables are described in Table S1. All the selected stands

1230   were mature pine populations, with DBH between 20.8 and 35.4 cm corresponding to trees of

1231   more than 40 yr (Camarero, Olano, & Parras, 2010; Vieira et al., 2015).

1232   In Mayspring 2013, five trees per population separated by more than 10 m were selected

1233   fromin a total of 15 populations (n = 75). Under each tree, the litter was removed and samples

1234   were obtained., approximately 1 m far from the trunk, by excavating 10 x 10 x 20 cm soil

1235   holes at the four orientations (north, south, east and west). The four samples per tree were

1236   pooled into a combined sample and kept at 4 ºC in plastic bags until processing. Once in the

1237   lab, roots were separated from soil, coarse roots discarded (diameter > 2 mm), and remaining

1238   roots gently washed with tap water over 2 and 0.5 mm sieves for collecting root tips. All

1239   ectomycorrhizal (ECM) root tips per each sample were carefully selected (Rincón et al.,

1240   2014), cleaned and sorted per sample under a stereomicroscope for further enzymatic and

1241   molecular analyses. Remaining soil was air dried and sieved (2 mm) for analysis.

1242   The gravimetric soil moisturerelative humidity (RH) of soil samples was determined by

1243   drying at 1065 ºC for 48 h. Air-dried soils were measured for pH (2 g of soils 1:5, w:v in 10

1244   ml of H_2O, 1:5, w:v), electrical conductivity (EC) (1:5, w:v in H_2O), organic matter (OM)

1245   (Walkley & Black, 1934), total N (Kjeldahl method). Extractable P was determined by the

1246   Bray & Kurtz (1945) method, after extraction in an ammonium fluoride and chloride acid

1247   solution. Extractable potassium (K) was determined after nitric acid digestion according to

1248   Isaac & Kerber (1971). Both P and K extracts were measured by inductively coupled plasma

1249   spectrometry (Optima 4300DV, Perkin-Elmer, Waltham, MA, EE.UU.).

1250   *Enzymatic tests*

1251   The fungal community functioning was evaluated on excised ECM root tips by measuring

1252   activities of eight hydrolytic and oxidative exoenzymes secreted by fungi. Seven enzymatic

1253   tests were based on fluorogenic substrate release, methylumbelliferone (MU) e.g. β-

1254   glucosidase (EC 3.2.1.3 at ExPasy-Enzyme database) and cellobiohydrolase (EC 3.2.1.91)

1255   that degrade cellulose, β-xylosidase (EC 3.2.1.37) and β-glucuronidase (EC 3.2.1.31) implied

1256   in the degradation of hemicellulose, acid phosphatase (EC 3.1.3.2) involved in the

1257   mobilization of phosphorus, N-acetylglucosaminidase or chitinase (EC 3.2.1.14) which

1258   hydrolyses chitin, or methylcoumarine (AMC) for L-leucineaminopeptidase (3.4.11.1) related

1259   to the mobilization of nitrogen from peptidic substrates. The Laccase (1.10.3.2) activity was

1260   determined by a photometric assay based on ABTS substrate (2,2'-Azino-bis (3-ethylbenzo-

1261   thiazolin-6-sulfonic acid). This enzyme is related to the degradation of recalcitrant

1262   compounds such as lignin. Enzymatic activities were determined following the protocol

1263   described by Courty, Pritsch, Schloter, Hartmann, & Garbaye (2005), with modifications. A

1264   total of 280 ECM-tips were randomly collected per sample and separated in subsets of 7

1265   ECM-tips with 5 replicates per each enzymatic test. Each replicate thus consisted of a tube

1266   with 7 pooled ECM-tips that were incubated in buffer during the corresponding time for each

1267   enzyme (Courty et al., 2005), after which 100 μl of the respective enzymatic reaction mix was

1268   added to 100 μl of stopping buffer in 96-well microplates. Enzymatic activities were

1269   measured in a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA),

1270   with 355/460 nm excitation/emission wavelengths for the fluorogenic assays and 415 nm for

1271   laccase. After reading, the ECM-tips of each replicate were scanned and their area calculated

1272   with the software ImageJ 1.49. Enzymatic activities were expressed in pmol $min^{-1}mm^{-2}$.

1273   *Molecular analyses*

1274   Per each measured enzyme, the ECM-tips were pooled (7 ECM-tips subsets x 5 replicates =

1275   35), added of a pinch of polyvinylpolypyrrolidone (PVPP), and the DNA extracted with the

1276   Invisorb®DNA Plant HTS 96 Kit/C kit (Invitek GmbH, Berlin, Germany), making a total of

1277   600 DNA extractions (75 tree samples x 8 enzymes) corresponding to 280 root tips per tree.

1278   The internal transcribed spacer region ITS-1 of the nuclear ribosomal DNA was amplified

1279   with the primer pair ITS1F-ITS2 (Gardes & Bruns, 1993) adapted for Illumina-MiSeq. PCR

1280   amplifications (3 min 94 ºC, 30 cycles of 1 min 94 ºC, 30 s 53 ºC and 45 s 72 ºC, with a final

1281   step of 10 min 72 ºC) were conducted in a Verity Thermal Cycler (Life Technologies), and

1282   eEach sample was amplified in three independent 20 µl PCR reactions, each containing 2 µl

1283   of 10x polymerase buffer, 2.4 µl of 25 mM $MgCl_2$, 1.12 µl of 10 mg ml^{-1} BSA, 0.4 µl of 10

1284   mM Nucleotide Mix, 0.4 µl of 10 mM forward/reverse primers and 0.2 µl of AmpliTaqGold

1285   polymerase (5 U ml^{-1}) (Applied Biosystems, Carlsbad, CA, USA). Negative controls without

1286   DNA were included in all runs to detect possible contaminations. The PCR conditions were as

1287   follow: 3 min 94 ºC, 30 cycles of 1 min 94 ºC, 30 s 53 ºC and 45 s 72 ºC, with a final step of

1288   10 min 72 ºC. Independent reactions were combined per sample, and each PCR product was

1289   purified (UltraClean PCR clean-up kit of MoBio, Carlsbad, CA, USA), quantified

1290   (PicoGreen, Life Technologies, Carlsbad, CA, USA), and finally pooled in an equimolar

1291   library containing 75 samples. Sequencing was carried out on an Illumina MiSeq sequencer (2

1292   × 300 bp paired-end reads) in an external service (Parque Científico de Madrid, Spain).

1293   *Bioinformatic analyses*

1294   Sequences were de-multiplexed according to their tags, filtered and trimmed using the

1295   *fastq_filter* command and *fastq_truncqual* option of Usearchv.7.0.1001 (Edgar, 2013) for

1296   eliminating quality scores ≤ 10. We used FLASH was used to merge reads with a minimum

1297   overlap ofat 97 % of similarity using 110 bp and 160 of minimum and maximum overlap

1298   respectively, resulting inand the 58.3 % of retained sequences was retained (4205677 out of

1299   the initial set of 7215915 sequences). Then, sSequences were dereplicated with the

1300   *derep_fulllength* Usearch command. De-replicated sequences were then sorted by decreasing

1301   abundance, and singletons discarded with the *sortbysize* Usearch command. Finally, 4116377

1302   sequences (57 %) were retained. OMolecular operational taxonomic units (MOTUs) were

1303   generated from abundance-sorted sequences using the *cluster_otus* Usearch command with a

1304   97 % similarity threshold. Extracted ITS sequences were then mapped against the MOTU

1305   representative sequences using the *usearch_global* Usearch command. Taxonomic

1306   assignation of representative sequences for each MOTU was done by using the Basic Local

1307   Alignment Search Tool (BLAST) (Altschul, Gish, Miller, Myers, & Lipman, 1990) according

1308   toagainst the 7.2 UNITE database (Kõljalg et al., 2013). Fungal taxonomic assignment served

1309   to identify those MOTUs closely related to recognized ECM taxa (Nguyen et al., 2016;

1310   Tedersoo et al., 2014; Tedersoo & Smith, 2013). The 86 % of the inferred sequences

1311   corresponded to ECM fungi and these were used for all subsequent analyses.

1312

1313   *Statistical analyses*

1314   To test the hypothesis 1, the effect of the fire recurrencefrequency (HiFi *vs* LoFi) on the

1315   structure (i.e., alpha and beta-diversity) and functioning (i.e., enzymatic activities) of ECM

1316   fungal communities (hypothesis 1) was separately evaluated by pine forest. Beta-diversity of

1317   total and by phyla ECM fungal communities of the different pine populations was calculated

1318   with the functions betadisper and permutest of the vegan package (Anderson, Ellingsen, &

1319   McArdle, 2006; Oksanen et al., 2015), and visualized results of the dissimilarity betadisper

1320   object were plotted by Principal Component Analyses (PCeoA). graphs. Beta-diversity was

1321   calculated on Bray-Curtis abundance matrix of OTUs previously normalized by variance

1322   stabilization according to McMurdie and Holmes (2014) by using the DESeq package

1323   (Anders & Huber, 2012).

1324   Alpha-diversity was analysed by General Linear Models (GLMs) ($p<0.05$) with the number of

1325   fungal OTUs as response variable. In these models, the square root of the total number of

1326   sequences obtained per sample was included as a covariate to account for differences in

1327   sequencing depth (Tedersoo et al., 2014). Models at lower taxonomic levels were also

1328   performed (i.e., phyla and families). In all cases, before modelling, Shapiro and Levene tests

1329   were performed to test the normality and homocedasticity of data, and variables were log or

1330   square root transformed when needed. Firstly, we modelled the effect of the variable pine

1331   forest on ECM fungal alpha-diversity, using pine forest as fixed factor and the site nested

1332   within pine forestand the site as random factor (i.e., pine population). Subsequent GLMs

1333   analyses were separately performed for each pine species. In these models, the fire regime

1334   was introduced as fixed factor and the site (i.e., pine population) nested within fire regime (n

1335   = 35 *P. pinaster* trees and n = 40 *P. halepensis* trees). Same GLM syntaxes were used to test

1336   the effect of fire recurrencefrequency on the different root-tip enzymatic activities.

1337   Correlations between enzymatic activities and ECM fungal community assemblages of the

1338   pine populations were analyzed by Mantel tests with ade4 R package.

1339   To identify representative fungal OTUs of each fire regime per pine species, the Indicator

1340   Species Analysis (with OTUs >100 reads to avoid spurious relations) was carried out

1341   ($p<0.05$) with the function *multipatt* of the indicspecies R package (Cáceres, Legendre, & He,

1342   2013).

1343   Climatic and edaphic characteristics are intimately associated with fire regime in

1344   Mediterranean ecosystems (Pausas et al., 2004). So, to separate the edaphic and climatic

1345   effects from that of the fire regime, we performed CCA on Hellinger-transformed relative

1346   fungal abundance data and RDA with the Euclidean distance matrix of enzymatic activities

1347   both with forward selection of explanatory variables. We used different analyses due to their

1348   different data assumptions, RDA assumes a linear relationship between predictors and data

1349   while CCA assumes a unimodal response curve. In these models, the variable fire regime and

1350   the edaphic and climatic indexes were included. Previously, to reduce the dimensionality of

1351   environmental data, Principal Component Analyses (PCA) were independently run for

1352   edaphic variables (pH, OM, EC, N, P, K) and climatic variables (mean annual temperature,

1353   mean annual precipitation and altitude), and the first two axes, explaining the maximum

1354   amount of variance, of respective PCAs were used as edaphic or climatic indexes (Figure S2).

1355

1356   To test our hypothesis 2, effect of tree serotiny degree (i.e., genotypic fire-adaptation) on

1357   ECM fungal community, two additional models with the factor serotiny and the edaphic and

1358   climatic indexes were included. As before, we performed a CCA model on Hellinger-

1359   transformed relative fungal abundance data and a RDA model with the Euclidean distance

1360   matrix of enzymatic activities both with forward selection of explanatory variables.

1361   Since the distribution of the two pine species considered in our study is closely linked to the

1362   bedrock type, i.e., *P. pinaster* preferentially settled on siliceous soils, while *P. halepensis*

1363   include both siliceous and calcareous soils (A. Hernández-Serrano et al., 2013) (Ojeda,

1364   Pausas, & Verdú, 2010), we made additional analyses for testing possible confounding effects

1365   of pine species and bedrock type (both factors included in the variable pine forest). For this,

1366   the pine species effect on ECM fungal community structure and enzymatic activities was

1367   respectively checked by Canonical Correspondence (CCA) and Redundancy (RDA) analyses,

1368   only with data of Sinarcas, the unique location where populations of both pine species grow

1369   under a common bedrock environment (Table S1). No significant effects of the pine species

1370   identity on the ECM fungal community structure (CCA: n=218; explained variation %=12.4;

1371   P=0.204) or on the enzymatic profile (RDA: n=8; explained variation %=44.4; P=0.094) in

1372   the location Sinarcas were observed.

1373   Ordinations were done with the software CANOCO v.5.0 (Biometris Plant Research

1374   International, Wageningen, Netherlands), and the rest of analyses with the R software v.3.5.2

1375   (R Core Team, 2014).

1376

1378

1379 **Results**

1380 *Sequencing yields and taxonomic identification of fungi*

1381 A total of 501 ECM fungal ~~M~~OTUs corresponding to 3182548 sequences were identified.

1382 Almost half of ~~M~~OTUs were found in both pine species and ~26 % exclusively in one of

1383 them (Figure S3~~2~~a). LoFi and HiFi pine populations shared the 54.8 % of ~~M~~OTUs, while

1384 ~21-23 % were only found either in LoFi or HiFi (Figure S3~~2~~a). Most ~~M~~OTUs belonged to

1385 Basidiomycota (89.4 %), 10.2 % to Ascomycota, and 0.4 % to Zygomycota (Figure S3~~2~~b;

1386 Table S2). The taxonomic classification of OTUs allowed identify~~ing~~ied 12 fungal orders, 30

1387 families and 47 genera, most of them represented across all treatments (Table ~~new~~S2).

1388 The majority of the 20 most abundant fungi and of the indicator species ~~fungi most commonly~~

1389 found in root tips of *P. pinaster* belonged to *Tomentellaceae, Inocybaceae, Russulaceae*, and

1390 *Rhizopogonaceae* and, in the case of *P. halepensis*, to Pezizales, *Thelephoraceae* and

1391 *Sebacinaceae* (Table ~~S2,~~ S3; Figure S4). The indicator species analysis revealed 54 fungal

1392 ~~M~~OTUs preferentially ~~found~~ associated to ~~in~~ *P. pinaster* forests and 37 ~~to~~in *P. halepensis*

1393 forests (Table S3). Besides, 31 fungi belonging to 14 different genera were indicators of LoFi

1394 populations of *P. pinaster* and 31 fungi belonging to 13 different genera were indicators of

1395 LoFi populations of *P. halepensis*. ~~in each respective pine species, while~~In contrast, 11

1396 indicators from 5 different genera ~~for P. pinaster~~ and 6 from 5 different genera ~~for P.~~

1397 ~~halepensis~~ were associated with HiFi populations of *P. pinaster* and *P. halepensis*

1398 respectively ~~indicators~~ (Table S3).

1399

1400 *Root-tip ectomycorrhizal fungal diversity*

1401   Total fungal α-diversity marginally varied between pine forests~~species~~ (R^2=0.13; F=3.62;

1402   P=0.062). At the phylum level, *P. halepensis* ~~harboured~~ significantly less Basidiomycetes

1403   (R^2=0.24; F=6.12; P=0.016) and marginally more Ascomycetes (R^2=0.81; F=3.88; P=0.053)

1404   were found in *P.Pinus* halepensis forest compared with ~~than~~ *P. pinaster* ~~(Figure 1)~~. The root-

1405   tips of *P.inus pinaster* ~~root-tips~~ were enriched of *Amanitaceae, Atheliaceae, Cantharellaceae,*

1406   *Clavariaceae, Cortinariaceae, Gloniaceae, Rhizopogonaceae* and *Russulaceae* species, while

1407   in *P. halepensis* root-tips prevailed species of the families *Pezizaceae, Pyronemataceae* and

1408   *Sebacinaceae* (Table 1). At regional scale, r~~R~~oot-tip ECM fungal β-diversity of both pine

1409   forests~~species~~ was similar (R^2=0.01 F=1.34, P=0.265), although Basidiomycetes were more

1410   dissimilar in *P. pinaster* than in *P. halepensis* forests (R^2=0.01 F=4.44~~51~~, P=0.033~~04~~), and the

1411   opposite pattern was observed for Ascomycetes (R^2=0.03; F=11.62, P=0.001).

1412   The ~~HiFi populations harboured significantly lower~~ ECM fungal α-diversity was significantly

1413   lower in high fire recurrence~~frequency~~ (HiFi) populations compared with~~than~~ low fire

1414   recurrence~~frequency~~ (LoFi) populations in both pine forests~~species~~ (Figure 1a), and this

1415   difference was consistent within the two fungal phyla i.e., Ascomycetes and Basidiomycetes

1416   (Figure 1b-c). ~~Total fungal α-diversity marginally varied between pine species (F=3.62;~~

1417   ~~P=0.062). At the phylum level, *P. halepensis* harboured significantly less Basidiomycetes~~

1418   ~~(F=6.12; P=0.016) and marginally more Ascomycetes (F=3.88; P=0.053) than *P. pinaster*~~

1419   ~~(Figure 1). *Pinus pinaster* root-tips were enriched of *Amanitaceae, Atheliaceae,*~~

1420   ~~*Cantharellaceae, Clavariaceae, Cortinariaceae, Gloniaceae, Rhizopogonaceae* and~~

1421   ~~*Russulaceae* species, while in *P. halepensis* root-tips prevailed species of the families~~

1422   ~~*Pezizaceae, Pyronemataceae* and *Sebacinaceae* (Table 1).~~ Although the α-diversity of many

1423   ECM fungal families decreased in HiFi populations of both pine forest~~species~~ (Table 1), some

1424 taxa were ~~clearly~~ more diverse ~~favoured by fire~~in HiFi, e.g. *Amanitaceae*, *Cantharellaceae*

1425 and *Rhizopogonaceae* for *P. pinaster*, or *Pezizaceae* for *P. halepensis* (Table 1).

1426 At regional scale, a consistent reduction of ECM fungal β-diversity was observed in HiFi

1427 populations of both pine forests~~species~~ (i.e., more homogeneous ECM communities in HiFi

1428 than LoFi) (Figure 2a). Likewise, Basidiomycetes were less β-diverse in HiFi than LoFi

1429 populations, while no difference was observed for Ascomycetes (Figure 2b-c). ~~Root-tip ECM~~

1430 ~~fungal β-diversity of both pine species was similar (F=1.34, P=0.265), although~~

1431 ~~Basidiomycetes were more dissimilar in *P. pinaster* than in *P. halepensis* forests (F=4.51,~~

1432 ~~P=0.004), and the opposite pattern was observed for Ascomycetes (F=11.62, P=0.001).~~

1433

1434 *Potential enzymatic activity of ECM root-tips*

1435 The enzymatic activity of the ECM root tips varied between pine forests (Figure 3). In LoFi

1436 populations, the enzymatic activity of ECM root tips was very similar for both pines, except

1437 in the case of cellobiohydrolase and phosphatase that were respectively higher and lower in *P.*

1438 *halepensis* compared with *P. pinaster* (Figure 3). By contrast, in HiFi populations, the activity

1439 of most of the C-degrading enzymes (i.e., cellobiohydrolase, β-xylosidase and β-

1440 glucuronidase), and of those mobilizing nitrogen (i.e., chitinase and L-leucineaminopeptidase)

1441 was higher for *P. halepensis* than *P. pinaster,* with the exception of laccase that was lower

1442 (Figure 3).

1443 The ECM fungal species assemblage of *P. pinaster* root-tips ~~was~~ significantly

1444 correlated~~associated~~ with their enzymatic profile (Mantel r=0.14; P=0.003) ~~(Figure 3a)~~. The

1445 HiFi populations of *P. pinaster* had higher ~~increased~~ root-tip laccase activity and

1446 lower~~reduced~~ hemicellulose degrading activity (i.e., β-xylosidase, β-glucuronidase), and of

1447 enzymes mobilizing nitrogen (i.e., chitinase and L-leucineaminopeptidase) and phosphorus

1448　(i.e., acid phosphatase) (~~Figure 3a,~~ Figure S3). ~~β -glucosidase and laccase did correlate with~~

1449　~~the ECM fungal families favoured in HiFi, such as *Cantharellaceae* or *Rhizopogonaceae*~~

1450　~~(Table 1; Figure 3a).~~ No significant relation between fungal community structure and

1451　enzymatic activity was observed for *P. halepensis* (Mantel r=0.03; P=0.287) ~~(Figure 3b)~~,

1452　although laccase activity significantly increased~~picked~~ in HiFi populations (~~Figure 3b,~~ Figure

1453　~~S~~3).

1454

1455　*Main drivers of the structure and function of ECM fungal communities*

1456　Climate, in particular temperature (Climate 1, first axis from PCA in Figure S2), together with

1457　fire ~~regime~~recurrence~~frequency~~, ~~and the tree fire-adaptive trait serotiny~~ were~~ere~~ ~~revealed as~~

1458　major ~~fire regime~~ factors determining~~effectors on~~ ECM fungal communities of Mediterranean

1459　pine forests (Table S4A; Figure 4a). ~~As hypothesized, t~~~~The CCA analysis clearly showed that~~

1460　~~s~~~~T~~he tree trait serotiny (selected by fire regime) did ~~also~~ affect~~ted~~ the structure of ~~explained~~

1461　~~differences in~~ root-tip ECM communit~~iesy~~ ~~structure~~ (Table S4~~2~~B; ~~Table 2B;~~ Figure

1462　4a). Higher levels of serotiny significantly correlated with the ECM fungal community

1463　structure~~ies~~ ~~in~~of ~~between LoFi and~~ HiFi populations ~~of~~in ~~apart from the climatic effects,~~

1464　both ~~in~~ *P. pinaster* and *P. halepensis* ~~forests~~ forests (Table S4~~2~~B; Figure 4a). Contrarily, the

1465　edaphic effects were likely related to differences among plots inside LoFi or HiFi populations,

1466　i.e. the ~~(Table 2; Figure 4a)~~strength and direction of edaphic vectors along plots displayed in

1467　Figure 4a.

1468　Regarding ECM functionality (i.e., enzymatic activities), the fire recurrence together with ~~the~~

1469　climatic variables explained~~did not affected~~ root-tip enzymatic activity in *P.~~inus~~ pinaster*

1470　forest ~~any pine forest~~ (Table S4~~2~~A; Figure 4b). Moreover, t~~T~~he serotiny degree of *P. pinaster*

1471　populations significantly correlated with the ECM root-tip enzymatic profile in HiFi

1472 populations, while soil characteristics mainly drove variations in enzymes among sites inside

1473 LoFi and HiFi populations (Table S42B; Figure 4b). For *P. halepensis*, root-tip enzymatic

1474 activity was essentially driven by the surrounding edaphic environment (Table S42; Figure

1475 4b), mainly by high pH and low phosphorus availability (see PC2 in Figure S21a).

1476

1477 **Discussion**

1478 Mutualistic feedbacks between trees and ectomycorrhizal fungi that are essential for

1479 ecosystem dynamics can be highly vulnerable to fire in Mediterranean ecosystems. Our

1480 results show that ~~the~~ fire regime shapes the structure and functioning of root-tip ECM fungal

1481 communities in fire-prone Mediterranean forests. ~~According to our H1D, d~~Divergent ECM

1482 fungal communities associa~~ted~~ted with *P. pinaster* and *P. halepensis*, which ~~–~~are highly

1483 influenced by the ~~bedrock type~~edaphic environment ~~(siliceous vs. calcareous)~~, and ~~by the~~

1484 ~~strong habitat filter imposed by the fire regime and the Mediterranean~~ climate~~these fungal~~

1485 ~~communities differently respond to the fire regime~~. According to our first hypothesis, ~~As we~~

1486 ~~hypothesized (H2h),~~ high fire recurrence reduces ECM fungal diversity and has a

1487 homogenizing effect ~~of ECM fungal~~on these communities, which leads to a reduced

1488 enzymatic activity for ~~one of the pine species~~P. pinaster (but not for *P. halepensis*).

1489 Confirming our second hypothesis, this effect is evidenced at the finer scale of individuals

1490 since the serotiny degree of trees, i.e., genotypic fire-adaptation, does significantly affect the

1491 structure (for both *P. pinaster* and *P. halepensis*) and functioning (in the case of *P. pinaster*)

1492 of ECM fungal communities. In this work, r~~R Our results demonstrate that r~~elative.~~An overall~~

1493 ~~simplification of the ECM community structure is observed under high fire recurrence, which~~

1494 ~~associates a general enzymatic activity decline for P. pinaster but not for P.halepensis.~~

1495 ~~Separated effects~~contribution ~~on ECM fungal communities~~ of main variables related to fire-

related effectors such as climate, soil properties andor the tree trait ee phenotype (serotiny degree) structuring ECM fungal communities have beehavecanenn able to be disentangled in this study.

ECM communities in P. pinaster *and* P. halepensis *forests*

The tree species had a marginal effect on ECM fungal communities probably blurred by the strong effect of the local environmental conditions. .. The contrasted local environment rather than the tree species was the main driver of the differences in ECM fungal assemblages observed between *P. pinaster* and *P. halepensis* forestsThe degree of tree host specificity for ECM fungi seems to be correlated with the phylogenetic distances between hosts (Leho Tedersoo, Mett, Ishida, & Bahram, 2013). However, it has been shown that the genus *Pinus* associates with specialized ECM fungi, in particular within the genera *Suillus* and *Rhizopogon* (Smith & Read, 2008) and Opposite to other mutualistic relationships, the mycorrhizal symbiosis is low specific e.g., for *Pinus* spp. it can reach the genus level (Smith & Read, 2008). However, the tree species identity or even that even the *Pinus* tree genotype has been previously demonstrated to play an essential role in structuring ECM fungal communities (Gehring, Mueller, Haskins, Rubow, & Whitham, 2014; Leticia Pérez-Izquierdo et al., 2017, 2019) (Aponte, García, Marañón, & Gardes, 2010; Barbi et al., 2016; Pérez-Izquierdo et al., 2017). Given the importance of pH shaping fungal communities (Coince et al., 2014; Glassman, Wang, & Bruns, 2017; Ana Rincón et al., 2015; L. Tedersoo et al., 2014) (Coince et al., 2014; Rincón et al., 2015; Rousk et al., 2010; Tedersoo et al., 2014), these divergent observations could be interpreted as context-dependent, i.e., in our study, soil pH (ranging from 5.1 to 8) was two points higher, even in Sinarcas where both pine species grew under a

similar local environment, than in the mentioned studies (average pH of ,5) even in Sinarcas, where both pine species grew under a similar local environment. Thus ,, together with climate limitations, the bedrock influence, mainly siliceous for *P. pinaster*, and calcareous for *P. halepensis*, was aThe strong environmental filter imposed by -the bedrock influence, mainly siliceous for *P. pinaster* and calcareous for *P. halepensis* (Ojeda, Pausas, & Verdú, 2010)(Ojeda, Pausas, & Verdú, 2010), for their associated ECM fungal communities, probably overtaken other possible effect of the tree species. This was also reflected in more diverse and more heterogeneous Ascomycetes species together with less diverse and more homogeneous Basidiomycetes in the case of *P. halepensis*, whereas .,tT and the opposite pattern was observed for *P. pinaster*. In fact, Ascomycetes have been often associated with environmental stressful conditions, by traits such as their melanin production capacity (Koide, Fernandez, & Malcolm, 2014; Treseder & Lennon, 2015). Accordingly, wWe observed a preferential association of *P. halepensis* with fungal species of the genus *Sebacina* and the order Pezizales. So far, noany tree host specificity has been observed among the *Sebacina* species (Ray & Craven, 2016; Selosse, Bauer, & Moyersoen, 2002). Given the reported positive effects of sebacinous fungi against stresses such as herbivorye, salinity or drought (Barazani & Baldwin, 2013; Ray & Craven, 2016; Zarea, Miransari, & Karimi, 2014), they could play a key role in the resistance of *P. halepensis* to limited nutrient availability (i.e., related to high soil pH) and the restrictive conditions imposed by the Mediterranean climate. Similarly, ascomycetous ECM Pezizales, which tend to be favoured in basic soils, have traits that such as their melanin production capacity that make them perfect symbionts under stressful conditions- including post-fire scenariosones (Koide, Fernandez, & Malcolm, 2014; Lamit et al., 2014; A. Rincón et al., 2014; Ana Rincón & Pueyo, 2010; Leho Tedersoo, Hansen, Perry, & Kjøller, 2006; Torres & Honrubia, 1997) (Lamit, Holeski, Flores-Rentería,

1544   Whitham, & Gehring, 2016; Tedersoo, Hansen, Perry, & Kjøller, 2006). On the other hand,

1545   species from the genera *Rhizopogon*, *Craterellus*, *Cenoccocum*, *Russula* and *Lactarius* were

1546   enriched under *P. pinaster* (less basic pH). Although ECM fungi tolerate a wide range of pH

1547   (Rousk et al., 2010), most of these fungi grow better under acidophilic conditions (Yamanaka,

1548   2003).

1549

1550   *Structural and functional shifts of root-tip ECM fungal communities due to the fire regime*

1551   As we firstly hypothesized, for both pine species, the local and regional diversity of root-tip

1552   ECM fungi significantly declined in populations where fires are historically frequent, and this

1553   implied functional outcomes e.g., –laccase activity of ECM root-tips increased in HiFi

1554   populations of both pine forests.

1555   Many fire studies have ascribed decreases in fungal diversity to the reduction of heat resistant

1556   propagules, elimination of rare species from the spore bank, the scarcity of suitable hosts, the

1557   direct burning of mycelium and roots, and/or the disturbed soil properties (Glassman et al.,

1558   2016; Holden, Gutierrez, & Treseder, 2013; Rincón & Pueyo, 2010). These processes do have

1559   a great importance on ECM fungal communities in the short term after relatively post-

1560   disturbance short term, giving the way to other processes such as dispersion through time

1561   (Dooley & Treseder, 2011; Kipfer, Moser, Egli, Wohlgemuth, & Ghazoul, 2011; Rincón et

1562   al., 2014; Sun et al., 2015), similar to that reported in succession studies, where fungal

1563   richness increases with forest age (Kyaschenko, Clemmensen, Hagenbo, Karltun, & Lindahl,

1564   2017; Twieg, Durall, & Simard, 2007). In our study, it does not seems not a plausible

1565   explanation that ECM fungal diversity hasve not been yet restored since the last fire (at least

1566   more than 40 years ago), because typical late-stage species such as *Amanita* or *Lactarius*

1567   (Cairney & Chambers, 2013; Taylor & Bruns, 1999) were indicators in HiFi areas. On the

1568   contrary, our results suggest that, the initial ruderal symbionts that facilitate the establishment

1569   of seedlings are replaced over time by ECM fungi that respond more efficiently to the new

1570   environmental conditions, probably driving plant-soil feedbacks to different directions and

1571   creating a different soil ecosystem (Duhamel et al., 2019).

1572   The ECM species composition diverged between fire regimes and, although fungal diversity

1573   decreased with high fire recurrence, some fungal families were more diverse in HiFi

1574   populationsclearly favoured by fire, e.g. *Amanitaceae*, *Cantharellaceae* and *Rhizopogonaceae*

1575   for *P. pinaster*, or *Pezizaceae* for *P. halepensis*. Different authors have proposed that in

1576   ecosystems subjected to frequent fires, the positive response of microorganisms would

1577   indicate a selection of the fire-tolerant ones over time (Buscardo et al., 2015; Dooley &

1578   Treseder, 2011; Rincón et al., 2014). Fire recurrence in Mediterranean forests is intimately

1579   linked to temperature and drought (Pausas, 2004), so as expected, in our work, climate was a

1580   strong determinant of fire regime effects on fungal communities. Similar to that described for

1581   plant communities (Pausas & Verdú, 2008; Verdú & Pausas, 2007), Aall these results,

1582   together with the observed decline in ECM fungal β-diversity, seem to indicate that the

1583   habitat filtering was likely the dominant ecological process assembling root-tip ECM fungal

1584   communities under high fire recurrence, while without the fire pressure (i.e., low fire

1585   recurrence) and milder climate conditions, competition among fungi could prevail, si-milar to

1586   that described across biological groups (Pérez-Valera, Verdú, Navarro-Cano, & Goberna,

1587   2018; Verdú & Pausas, 2007). The diversity of plant and microbial communities is regulated

1588   by sequentially operating assembly rules: abiotic filtering is an omnipresent structuring force,

1589   and biological interactions e.g., competition, further fine-tunes the community assemblages

1590   (Pérez-Valera et al., 2018; Verdú & Pausas, 2007).

1591

1592   Fire regime and climate can filter directly different ~~Beyond co-variation of~~ biotic organisms

1593   (i.e., trees and ECM fungi) ~~filtered by the fire regime and climate~~, however our results did

1594   provide significant clues about indirect plant-mediated effects of high fire recurrence on ECM

1595   fungal communities. We observed that serotiny, previously demonstrated to be~~as~~ a tree

1596   heritable trait shaped by recurrent fires (Budde et al., 2014; Castellanos, González-Martínez,

1597   & Pausas, 2015; ~~Ana~~ Hernández-Serrano et al., 2014), significantly explained the

1598   variation~~bility~~ of ECM fungal communities, aside from the accounted climatic effects for both

1599   pine species. This could be attributed to climatic constraints in the channel of Carbon~~C~~ from

1600   the tree towards the ECM fungi, although, on the other hand, serotiny is a trait that requires

1601   resources (water and Carbon~~C~~) for maintaining a large amount of seeds alive in the canopy

1602   with a cost for the plant (Cramer & Midgley, 2009; Harris & Pannell, 2010). ~~Thus, we~~

1603   ~~suggest~~Another possible explanation is that the ability of the different tree

1604   genotypes/phenotypes to preferentially allocate photosynthates to the more beneficial ECM

1605   fungi, or to those with low Carbon~~C~~ demands and/or high abilities to cope with hydric/nutrient

1606   stress ~~is~~ can be the prevailing mechanism explaining such plant-mediated effects (Bever,

1607   Richardson, Lawrence, Holmes, & Watson, 2009; Gehring et al., 2014). This was ~~further~~ to

1608   some extent supported by our results, at least in *P. pinaster*, for which a direct effect of tree

1609   phenotype (serotiny) on enzymatic responses of ECM fungi, but no effect of climatic

1610   variables was observed. We have previously shown that particular *P. pinaster* genotypes can

1611   impact enzymatic activities through changes in the composition of the associated microbial

1612   communities (Pérez-Izquierdo et al., 2017, 2019). Additionally, it cannot be ruled out that, ~~as~~

1613   ~~for serotiny,~~ the fire recurrence negatively affects other tree traits ~~,~~ such as root development

1614   and/or architecture constraining ~~, which would consecutively reduce~~ the space for ECM

1615   fungal colonization, which would make mechanisms like priority effects particularly

1616 important ~~making that mechanisms such as priority effects could gain importance~~ (Kennedy,

1617 Peay, & Bruns, 2009; Peay, Belisle, & Fukami, 2012). In any case, further experimentation

1618 would be needed to shed light on these assumptions.

1619

1620 The increased fire recurrence affected ecosystem functions differently in the two pine species

1621 by reducing most enzymatic activities in the case of *P. pinaster*, but not for *P. halepensis*.

1622 This suggests functional redundancy between ~~HiFi and LoFi~~ ECM fungal communities

1623 associated with *P. halepensis* in low and high fire recurrence~~frequency~~ sites, since diversity

1624 and assemblage shifts were not translated into functional changes (Jones et al., 2010). These

1625 results might indicate a high capacity of *P. halepensis* forests to recover its functionality

1626 among recurrent fires in warmer areas probably by shifting plant-microbial feedbacks to

1627 readjust to the environment (Clemmensen et al., 2015; Johnstone et al., 2010). The edaphic

1628 conditions (mainly pH and P) were ~~a~~ main factors~~effector~~ explaining differences in enzymatic

1629 profiles ~~, indistinctively in LoFi and HiFi populations~~ among populations of both pine species.

1630 Among their multiple functions, an outstanding feature of ectomycorrhizal fungi is ~~s,~~

1631 ~~emphasizing again the main role of the bedrock type in nutrient availability and ecosystem~~

1632 ~~functionality in Mediterranean forests. Ectomycorrhizal fungi display multiple functions,~~

1633 ~~among them,~~ their~~y are~~ ability to ~~able to~~ ~~such as~~ mobiliz~~e~~ing nutrients from the soil (Smith &

1634 Read, 2008) ~~. for the host, with particular N mining abilities where litter N is hard to access~~

1635 ~~(Smith & Wang 2019; Fernandez et al., 2020), as could be also the case for pine litter in our~~

1636 ~~study.~~Because of their direct access to C from the host, they are able to invest energy (i.e.,

1637 enzymatic production) to mine for N and/or P from hardly accessible sources (Fernandez,

1638 See, & Kennedy, 2020; Lindahl & Tunlid, 2015)~~which are often limiting nutrients in forests,~~

1639 as it could be the case for ~~pine litter and/or~~ basic soils in our study together with recalcitrant

1640   pine litter~~in our study~~. ~~Our results show that the ECM enzymatic production is mainly~~

1641   ~~regulated by the availability of P which is in turn highly dependent on the bedrock type in~~

1642   ~~Mediterranean ecosystems.~~ ~~Ectomycorrhizal fungi act on soil nutrient and carbon dynamics~~

1643   ~~directly affecting tree productivity, and contributing soil organic matter and priming or~~

1644   ~~inhibiting its turnover in soils (Clemmensen et al., 2013; Fernandez et al., 2020), which can~~

1645   ~~be of prime importance in Mediterranean soil formation.~~ Our findings are in line with

1646   previous studies indicating that the availability of resources in surrounding soil ~~and~~together

1647   with tree host genetics strongly regulates ECM~~microbial~~ enzymatic production ~~in surrounding~~

1648   ~~soil~~~~are among the key factors affecting the enzymatic activity of ECM fungal communities~~

1649   (Aponte, García, Marañón, & Gardes, 2010; ~~P. E.~~ Courty et al., 2016; ~~Pierre Emmanuel~~

1650   Courty et al., 2011; Schneider et al., 2012).

1651   As previously mentioned, the laccase activity ~~of ECM root tips~~ increased in high fire

1652   recurrence populations of both pines~~ forests~~. Laccase is an oxidative enzyme related to several

1653   processes like the degradation of recalcitrant C compounds (i.e., lignin), but also to the

1654   production of melanin by fungi (Baldrian, 2006; Eisenman et al., 2007). Melanin is a group of

1655   complex polymers deposited in the cell wall associated with resistance to stresses such as

1656   drought (Koide et al., 2014) and moderately resistant to decomposition (Fernandez, Heckman,

1657   Kolka, & Kennedy, 2019). It is also possible that in our study sites, changes in traits like

1658   production of melanin by fungi or more litter tannins by trees associated to the fire regime and

1659   climatic stress might have affected organic matter turnover and feedback cycles, issues that

1660   would deserve further analyses. In fact, in a 2-million-year chronosequence, Albornoz et al.

1661   (2016) showed strong variation of ECM fungal communities even within the same hosts,

1662   attributable not only to short-term fungal edaphic specialization or different inoculum density

1663 and composition, but also likely to a much longer-term ecosystem-level feedbacks among soil,

1664 plants and ECM fungi during pedogenesis.

1665

1666 **Conclusions**

1667 In *P. pinaster* and *P. halepensis* Mediterranean forests, the high fire recurrencefrequency

1668 filters the ECM fungal community composition, even favoring some ECM fungal species, but

1669 reduces local and regional ECM diversity. Factors such as climate, which is intimately related

1670 to fire, and soil properties in particular pH, affect ECM fungal communities composition and

1671 their enzymatic functions. Separated effects of fire-regime related effectors such as climate,

1672 soil properties or the tree phenotype are driving these processes in Mediterranean ecosystems.

1673 Aside from the climatic filter, local adaptation in pine populations to recurrent fires (i.e.,

1674 increased serotiny) selects for different ECM fungal communities, accompanying different

1675 functional responses. ECM fungal communities associated with serotinous *P. pinaster* trees

1676 imply a reduced enzymatic activity, but for *P. halepensis*, a functional redundancy in LoFi

1677 and HiFi fire regimes likely exists. Edaphic variables, highly linked to the bedrock type in

1678 Mediterranean ecosystems, are main drivers of ECM fungal structure and functioning but

1679 generally independent of the fire regime. The bedrock material is also a main factor driving

1680 differences in ECM fungal communities associated with *P. pinaster* and *P. halepensis*,

1681 overriding the possible effect of the tree species. Although fire is already a main ecological

1682 factor shaping in the Mediterranean ecosystemsarea, changes in fire frequency because of

1683 predicted warmer and longer dry periods have the potential to affect the plants and their

1684 symbionts altering the structure, functioning and successional dynamics of Mediterranean

1685 forests ecosystems. The observed fire regime-related structural and functional shifts in ECM

1686 fungal communities might have essential implications for Mediterranean pine forests

1687   dynamics, which should be carefully considered to promote the sustainable management of

1688   these vulnerable ecosystems and to maintain their resilience under future climatic scenarios.

1690

Acknowledgements

1701

1702 **References**

1703 Abdel Malak, D., & Pausas, J. G. (2006). Fire regime and post-fire Normalized Difference
1704   Vegetation Index changes in the eastern Iberian peninsula (Mediterranean basin).
1705   *International Journal of Wildland Fire*, Vol. 15, pp. 407–413. doiRetrieved from :
1706   .org/10.1071/WF05052

1707 Albornoz, F. E., Teste, F. P., Lambers, H., Bunce, M., Murray, D. C., White, N. E., &
1708   Laliberté, E. (2016). Changes in ectomycorrhizal fungal community composition and
1709   declining diversity along a 2-million-year soil chronosequence. *Molecular Ecology*,
1710   *25*(19), 4919–4929. doi: 10.1111/mec.13778

1711 Anders, S., & Huber, W. (2012). *Differential expression of RNA-Seq data at the gene level–*
1712   *the DESeq package*.
1713 Retrieved from
1714   http://watson.nci.nih.gov/bioc_mirror/packages/2.11/bioc/vignettes/DESeq/inst/doc/DES
1715   eq.pdf

1716 Anderson, M., Ellingsen, K., & McArdle, B. (2006). Multivariate dispersion as a measure of
1717   beta diversity. *Ecology Letters*, *9*(6), 683–693. Retrieved from
1718   http://onlinelibrary.wiley.com/doi:/10.1111/j.1461-0248.2006.00926.x/full

1719 Aponte, C., García, L. V., Marañón, T., & Gardes, M. (2010). Indirect host effect on
1720   ectomycorrhizal fungi: Leaf fall and litter quality explain changes in fungal communities
1721   on the roots of co-occurring Mediterranean oaks. *Soil Biology and Biochemistry*, *42*(5),
1722   788–796. doi: 10.1016/j.soilbio.2010.01.014

1723 Baar, J., Horton, T. R., Kretzer, A. M. ., & Runs, T. D. (1999). Mycorrhizal colonization of
1724   Pinus muricata from resistant propagules after a stand-replacing wildfire. *New*
1725   *Phytologist*, *143*(2), 409–418. Doi: 10.1046/j.1469-8137.1999.00452.x

1726 Baldrian, P. (2006). Fungal laccases-occurrence and properties. *FEMS Microbiology Reviews*,
1727   *30*, 215–242. doi: 10.1111/j.1574-4976.2005.00010.x

1728 Barazani, O., & Baldwin, I. T. (2013). A Mixed Bag: The Plant Growth-Promoting *Sebacina*
1729   *vermifera* Impairs Defense Mechanisms Against Herbivores Oz. In *Piriformospora*
1730   *indica* (pp. 251–261). doi: 10.1007/978-3-642-33802-1

1731 Barbéro, M., Loisel, R., & Quézel, P. (1998). Pines of the Mediterranean basin. In DM
1732   Richardson (Ed.), *Ecology and biogeography of Pinus* (pp. 153–170).

1733 Bever, J. D., Richardson, S. C., Lawrence, B. M., Holmes, J., & Watson, M. (2009).
1734   Preferential allocation to beneficial symbiont with spatial structure maintains
1735   mycorrhizal mutualism. *Ecology Letters*, *12*(1), 13–21. doi: 10.1111/j.1461-
1736   0248.2008.01254.x

1737 Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of
1738   phosphorus in soils. *Soil Science*, *59*(1), 39–46. Retrieved from
1739   http://journals.lww.com/soilsci/Fulltext/1945/01000/DETERMINATION_OF_TOTAL,_
1740   ORGANIC,_AND_AVAILABLE.6.aspx

1741 Bruns, T. D., Peay, K. G., Boynton, P. J., Grubisha, L. C., Hynson, N. A., Nguyen, N. H., &
1742   Rosenstock, N. P. (2009). Inoculum potential of *Rhizopogon* spores increases with time
1743   over the first 4 yr of a 99-yr spore burial experiment. *New Phytologist*, *181*(2), 463–470.
1744   doi: 10.1111/j.1469-8137.2008.02652.x

1745 Budde, K. B., González-Martínez, S. C., Navascués, M., Burgarella, C., Mosca, E., Lorenzo,
1746   Z., … Heuertz, M. (2017). Increased fire frequency promotes stronger spatial genetic

1747   structure and natural selection at regional and local scales in *Pinus halepensis* Mill.
1748   *Annals of Botany*, *119*(6), 1061–1072. doi: 10.1093/aob/mcw286
1749   Budde, K. B., Heuertz, M., Hernández-Serrano, A., Pausas, J. G., Vendramin, G. G., Verdú,
1750   M., & González-Martínez, S. C. (2014). In situ genetic association for serotiny, a fire-
1751   related trait, in Mediterranean maritime pine (*Pinus pinaster*). *New Phytologist*, *201*(1),
1752   230–241. doi: 10.1111/nph.12483
1753   Buscardo, E., Rodriguez-Echeverria, S., Freitas, H., De Angelis, P., Pereira, J. S., & Muller,
1754   L. A. H. (2015). Contrasting soil fungal communities in Mediterranean pine forests
1755   subjected to different wildfire frequencies. *Fungal Diversity*, *70*(1), 85–99. doi:
1756   10.1007/s13225-014-0294-5
1757   Cáceres, M. D., Legendre, P., & He, F. (2013). Dissimilarity measurements and the size
1758   structure of ecological communities. *Methods in Ecology and Evolution*, *4*(12), 1167–
1759   1177. Retrieved from http://onlinelibrary.wiley.com/doi: /10.1111/2041-210X.12116/full
1760   Cairney, J. W., & Chambers, S. M. (2013). *Ectomycorrhizal fungi: key genera in profile*.
1761   Springer Science & Business Media.
1762   Camarero, J. J., Olano, J. M., & Parras, A. (2010). Plastic bimodal xylogenesis in conifers
1763   from continental Mediterranean climates. *New Phytologist*, *185*(2), 471–480. doi:
1764   10.1111/j.1469-8137.2009.03073.x
1765   Castellanos, M. C., González-Martínez, S. C., & Pausas, J. G. (2015). Field heritability of a
1766   plant adaptation to fire in heterogeneous landscapes. *Molecular Ecology*, *24*(22), 5633–
1767   5642. doi: 10.1111/mec.13421
1768   Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., …
1769   Lindahl, B. D. (2013). Roots and Associated Fungi Drive Long-Term Carbon
1770   Sequestration in Boreal Forest. *Science*, *339*(6127), 1615–1618. doi:
1771   10.1126/science.1231923
1772   Clemmensen, K. arina E., Finlay, R. D., Dahlberg, A., Stenlid, J., Wardle, D. A., & Lindahl,
1773   B. D. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts
1774   during long-term succession in boreal forests. *New Phytologist*, *205*, 1525–1536. doi:
1775   doi:10.1111/nph.13208
1776   Coince, A., Cordier, T., Lengellé, J., Defossez, E., Vacher, C., Robin, C., … Marçais, B.
1777   (2014). Leaf and root-associated fungal assemblages do not follow similar elevational
1778   diversity patterns. *PloS One*, *9*(6), e100668. doi: 10.1371/journal.pone.0100668
1779   Courty, P. -E., Munoz, F., Selosse, M. -A., Duchemin, M., Criquet, S., Ziarelli, F., …
1780   Richard, F. (2016). Into the functional ecology of ectomycorrhizal communities:
1781   Environmental filtering of enzymatic activities. *Journal of Ecology*, *104*(6), 1585–1598.
1782   doi: 10.1111/1365-2745.12633
1783   Courty, P E, Pritsch, K., Schloter, M., Hartmann, A., & Garbaye, J. (2005). Activity profiling
1784   of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New*
1785   *Phytologist*, *167*(1), 309–319. doi: DOI 10.1111/j.1469-8137.2005.01401.x
1786   Courty, P. ierre E. Emmanuel, Labbé, J., Kohler, A., Marçais, B., Bastien, C., Churin, J., … Le
1787   Tacon, F. (2011). Effect of poplar genotypes on mycorrhizal infection and secreted
1788   enzyme activities in mycorrhizal and non-mycorrhizal roots. *Journal of Experimental*
1789   *Botany*, *62*(1), 249–260. doi: 10.1093/jxb/erq274
1790   Cramer, M. D., & Midgley, J. J. (2009). Maintenance costs of serotiny do not explain weak
1791   serotiny. *Austral Ecology*, *34*, 653–662. doi: 10.1111/j.1442-9993.2009.01971.x
1792   de Román, M., & de Miguel, A. M. (2005). Post-fire , seasonal and annual dynamics of the
1793   ectomycorrhizal community in a *Quercus ilex* L . forest over a 3-year period.
1794   *Mycorrhiza*, *15*, 471–482. doi: 10.1007/s00572-005-0353-6

1795 Dooley, S. R., & Treseder, K. K. (2011). The effect of fire on microbial biomass: a meta-
1796   analysis of field studies. *Biogeochemistry*, *109*(1–3), 49–61. doi: 10.1007/s10533-011-
1797   9633-8
1798 Dove, N. C., & Hart, S. C. (2017). Fire reduces fungal species richness and in situ
1799   mycorrhizal colonization: A meta-analysis. *Fire Ecology*, *13*(2), 37–65. doi:
1800   10.4996/fireecology.130237746
1801 Duhamel, M., Wan, J., Bogar, L. M., Segnitz, R. M., Dncritts, N. C., & Peay, K. G. (2019).
1802   Plant selection initiates alternative successional trajectories in the soil microbial
1803   community after disturbance. *Ecological Monographs*, *89*(3), e01367. doi:
1804   10.1002/ecm.1367
1805 Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon
1806   reads. *Nature Methods*, *10*(10), 996–998. doi: 10.1038/nmeth.2604
1807 Egerton-Warburton, L. M., Querejeta, J. I., & Allen, M. F. (2007). Common mycorrhizal
1808   networks provide a potential pathway for the transfer of hydraulically lifted water
1809   between plants. *Journal of Experimental Botany*, *58*(6), 1473–1483. doi:
1810   10.1093/jxb/erm009
1811 Eisenman, H. C., Mues, M., Weber, S. E., Frases, S., Chaskes, S., Gerfen, G., & Casadevall,
1812   A. (2007). *Cryptococcus neoformans* laccase catalyses melanin synthesis from both D -
1813   and L -DOPA. *Microbiology*, *153*, 3954–3962. doi: 10.1099/mic.0.2007/011049-0
1814 Fernandez, C. W., Heckman, K., Kolka, R., & Kennedy, P. G. (2019). Melanin mitigates the
1815   accelerated decay of mycorrhizal necromass with peatland warming. *Ecology Letters*, *22*,
1816   498–505. doi: 10.1111/ele.13209
1817 Fernandez, C. W., See, C. R., & Kennedy, P. G. (2020). Decelerated carbon cycling by
1818   ectomycorrhizal fungi is controlled by substrate quality and community composition.
1819   *New Phytologist*, *226*(2), 569–582. doi: 10.1111/nph.16269
1820 Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes,
1821   application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*, 113–
1822   118. doi: ~~doi.org/~~10.1111/j.1365-294X.1993.tb00005.x
1823 Gehring, C. A., Mueller, R. C., Haskins, K. E., Rubow, T. K., & Whitham, T. G. (2014).
1824   Convergence in mycorrhizal fungal communities due to drought, plant competition,
1825   parasitism, and susceptibility to herbivory: Consequences for fungi and host plants.
1826   *Frontiers in Microbiology*, *5*(JUN), 1–9. doi: 10.3389/fmicb.2014.00306
1827 Gehring, C. A., & Whitham, T. G. (1991). Herbivore-Driven Mycorrhizal Mutualism in
1828   Insect-Susceptible Pinyon Pine. *Nature*, *353*(6344), 556–557. doi: 10.1038/353556a0
1829 Glassman, S. I., Levine, C. R., Dirocco, A. M., Battles, J. J., & Bruns, T. D. (2016).
1830   Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot.
1831   *The ISME Journal*, *10*, 1228–1239. doi: 10.1038/ismej.2015.182
1832 Glassman, S. I., Peay, K. G., Talbot, J. M., Smith, D. P., Chung, J. A., Taylor, J. W., …
1833   Bruns, T. D. (2015). A continental view of pine-associated ectomycorrhizal fungal spore
1834   banks : a quiescent functional guild with a strong biogeographic pattern. *New
1835   Phytologist*, *205*, 1619–1631. doi: 10.1111/nph.13240
1836 Glassman, S. I., Wang, I. J., & Bruns, T. D. (2017). Environmental filtering by pH and soil
1837   nutrients drives community assembly in fungi at fine spatial scales. *Molecular Ecology*,
1838   *26*(24), 6960–6973. doi: 10.1111/mec.14414
1839 Greene, D. F., Zasada, J. C., Sirois, L., Kneeshaw, D., Morin, H., Charron, I., & Simard, M. J.
1840   (1999). A review of the regeneration dynamics of North American boreal forest tree
1841   species. *Canadian Journal of Forest Research*, *29*(6), 824–839. doi: 10.1139/x98-112
1842 Harris, M. S., & Pannell, J. R. (2010). Canopy seed storage is associated with sexual

1843 dimorphism in the woody dioecious genus *Leucadendron*. *Journal of Ecology*, *98*, 509–
1844 515. doi: 10.1111/j.1365-2745.2009.01623.x
1845 Hart, S. C., DeLuca, T. H., Newman, G. S., MacKenzie, M. D., & Boyle, S. I. (2005). Post-
1846 fire vegetative dynamics as drivers of microbial community structure and function in
1847 forest soils. *Forest Ecology and Management*, *220*(1–3), 166–184. doi:
1848 10.1016/j.foreco.2005.08.012
1849 Hernández-Serrano, A., Verdú, M., González-Martínez, S. C., & Pausas, J. G. (2013). Fire
1850 structures pine serotiny at different scales. *American Journal of Botany*, *100*(12), 2349–
1851 2356. doi: 10.3732/ajb.1300182
1852 Hernández-Serrano, A.na, Verdú, M., Santos-Del-Blanco, L., Climent, J., González-Martínez,
1853 S. C., & Pausas, J. G. (2014). Heritability and quantitative genetic divergence of
1854 serotiny, a fire-persistence plant trait. *Annals of Botany*, *114*(3), 571–577. doi:
1855 10.1093/aob/mcu142
1856 Holden, S. R., Gutierrez, A., & Treseder, K. K. (2013). Changes in Soil Fungal Communities,
1857 Extracellular Enzyme Activities, and Litter Decomposition Across a Fire
1858 Chronosequence in Alaskan Boreal Forests. *Ecosystems*, *16*(1), 34–46. doi:
1859 10.1007/s10021-012-9594-3
1860 Isaac, R. A., & Kerber, J. D. (1971). Atomic absorption and flame photometry: Techniques
1861 and uses in soil, plant and water analysis. In Walsh LM (ed). Soil Science Society
1862 America. Madison. WI. (Ed.), *Instrumental Methods for Analysis of Soils and Plant*
1863 *Tissue.*
1864 Johnson, D., Martin, F., Cairney, J. W. G., & Anderson, I. C. (2012). The importance of
1865 individuals: Intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New*
1866 *Phytologist*, *194*(3), 614–628. doi: 10.1111/j.1469-8137.2012.04087.x
1867 Johnstone, J. F., Chapin, F. S., Hollingsworth, T. N., Mack, M. C., Romanovsky, V., &
1868 Turetsky, M. (2010). Fire, climate change, and forest resilience in interior AlaskaThis
1869 article is one of a selection of papers from The Dynamics of Change in Alaska's Boreal
1870 Forests: Resilience and Vulnerability in Response to Climate Warming. *Canadian*
1871 *Journal of Forest Research*, *40*(7), 1302–1312. doi: 10.1139/X10-061
1872 Jones, M. D., Twieg, B. D., Ward, V., Barker, J., Durall, D. M., & Simard, S. W. (2010).
1873 Functional complementarity of Douglas-fir ectomycorrhizas for extracellular enzyme
1874 activity after wildfire or clearcut logging. *Functional Ecology*, *24*(5), 1139–1151. doi:
1875 10.1111/j.1365-2435.2010.01699.x
1876 Keeley, J. E., Bond, W. J., Bradstock, R. A., Pausas, J. G., & Rundel, P. W. (2011). *Fire in*
1877 *Mediterranean ecosystems: ecology, evolution and management*. Cambridge University
1878 Press.
1879 Kennedy, P. G., Peay, K. G., & Bruns, T. D. (2009). Root tip competition among
1880 ectomycorrhizal fungi: Are priority effects a rule or an exception? *Ecology*, *90*(8), 2098–
1881 2107. doi: 10.1890/08-1291.1
1882 Kipfer, T., Moser, B., Egli, S., Wohlgemuth, T., & Ghazoul, J. (2011). Ectomycorrhiza
1883 succession patterns in Pinus sylvestris forests after stand-replacing fire in the Central
1884 Alps. *Oecologia*, *167*(1), 219–228. doi: 10.1007/s00442-011-1981-5
1885 Kivlin, S. N., Emery, S. M., & Rudgers, J. A. (2013). Fungal symbionts alter plant responses
1886 to global change. *American Journal of Botany*, *100*(7), 1445–1457. doi:
1887 10.3732/ajb.1200558
1888 Koide, R. T., Fernandez, C., & Malcolm, G. (2014). Determining place and process:
1889 Functional traits of ectomycorrhizal fungi that affect both community structure and
1890 ecosystem function. *New Phytologist*, *201*(2), 433–439. doi: 10.1111/nph.12538

1891 Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., …
1892   Larsson, K. H. (2013). Towards a unified paradigm for sequence-based identification of
1893   fungi. *Molecular Ecology*, *22*(21), 5271–5277. doi: 10.1111/mec.12481
1894 Kraft, N. J. B., Adler, P. B., Godoy, O., James, E. C., Fuller, S., & Levine, J. M. (2015).
1895   Community assembly , coexistence and the environmental fi ltering metaphor.
1896   *Functional Ecology*, *29*, 592–599. doi: 10.1111/1365-2435.12345
1897 Kyaschenko, J., Clemmensen, K. E., Hagenbo, A., Karltun, E., & Lindahl, B. D. (2017). Shift
1898   in fungal communities and associated enzyme activities along an age gradient of
1899   managed *Pinus sylvestris* stands. *The ISME Journal*, *11*, 863–874. doi:
1900   10.1038/ismej.2016.184
1901 Lamit, L. J., Lau, M. K., Sthultz, C. M., Wooley, S. C., Whitham, T. G., & Gehring, C. A.
1902   (2014). Tree genotype and genetically based growth traits structure twig endophyte
1903   communities. *American Journal of Botany*, *101*(3), 467–478. doi: 10.3732/ajb.1400034
1904 Lindahl, B. D., & Tunlid, A. (2015). *Ectomycorrhizal fungi – potential organic matter*
1905   *decomposers , yet not saprotrophs*. *205*, 1443–1447. doi: 10.1111/nph.13201
1906 Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., …
1907   Marchetti, M. (2010). Climate change impacts, adaptive capacity, and vulnerability of
1908   European forest ecosystems. *Forest Ecology and Management*, *259*(4), 698–709. doi:
1909   10.1016/j.foreco.2009.09.023
1910 McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbiome data
1911   is inadmissible. *PLoS Computational Biology*, *10*(4), e1003531. doi:
1912   10.1371/journal.pcbi.1003531
1913 Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., … Kennedy, P. G.
1914   (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by
1915   ecological guild. *Fungal Ecology*, *20*, 241–248. doi: 10.1016/j.funeco.2015.06.006
1916 Nuñez, M. A., Horton, T. R., & Simberloff, D. (2009). Lack of belowground mutualisms
1917   hinders Pinaceae invasions. *Ecology*, *90*(9), 2352–2359. doi: 10.1890/08-2139.1
1918 Ojeda, F., Pausas, J. G., & Verdú, M. (2010). Soil shapes community structure through fire.
1919   *Oecologia*, *163*(3), 729–735. doi: 10.1007/s00442-009-1550-3
1920 Oksanen, A. J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., Hara, R. B. O., …
1921   Wagner, H. (2015). Vegan community ecology package: ordination methods, diversity
1922   analysis and other functions for community and vegetation ecologists. Version 2.3-1. In
1923   *R package version 2.3-1*.
1924 Pausas, J.G. (2015). Evolutionary fire ecology: Lessons learned from pines. *Trends in Plant*
1925   *Science*, *20*(5), 318–324. doi: 10.1016/j.tplants.2015.03.001
1926 Pausas, J. G., & Fernández-Muñoz, S. (2012). Fire regime changes in the Western
1927   Mediterranean Basin: From fuel-limited to drought-driven fire regime. *Climatic Change*,
1928   *110*(1–2), 215–226. doi: 10.1007/s10584-011-0060-6
1929 Pausas, J. G. (2004). Changes in fire and climate in the eastern Iberian Peninsula
1930   (Mediterranean basin). *Climatic Change*, *63*, 337–350.
1931 Pausas, J. G, Bradstock, R. A., Keith, D. A., & Keeley, J. E. (2004). Plant functional traits
1932   in relation to fire in corwn-fire ecosystems. *Ecology*, *85*(4), 1085–1100. doi: 10.1890/02-
1933   4094
1934 Peay, K. G., Belisle, M., & Fukami, T. (2012). Phylogenetic relatedness predicts priority
1935   effects in nectar yeast communities. *Proceedings. Biological Sciences / The Royal*
1936   *Society*, *279*(1729), 749–758. doi: 10.1098/rspb.2011.1230
1937 Pérez-Izquierdo, L, Zabal-Aguirre, M., Flores-Rentería, D., González-Martínez, S., Buée, M.,
1938   & Rincón, A. (2017). Functional outcomes of fungal community shifts driven by tree

1939 genotype and spatial-temporal factors in Mediterranean pine forests. *Environmental*
1940 *Microbiology*, 19(4), 1639-1652.. doi: 10.1111/1462-2920.13690
1941 ~~Pérez-Izquierdo, Leticia, Zabal-Aguirre, M., Flores-Rentería, D., González-Martínez, S. C.,~~
1942 ~~Buée, M., & Rincón, A. (2017). Functional outcomes of fungal community shifts driven~~
1943 ~~by tree genotype and spatial-temporal factors in Mediterranean pine forests.~~
1944 ~~*Environmental Microbiology*, *19*(4), 1639–1652. doi: 10.1111/1462-2920.13690~~
1945 Pérez-Izquierdo, L.~~eticia~~, Zabal-Aguirre, M., González-Martínez, S. C., Buée, M., Verdú, M.,
1946 Rincón, A., & Goberna, M. (2019). Plant intraspecific variation modulates nutrient
1947 cycling through its below-ground rhizospheric microbiome. *Journal of Ecology*, 107(4),
1948 1594-1605. doi: 10.1111/1365-2745.13202
1949 Pérez-Valera, E., Verdú, M., Navarro-Cano, J. A., & Goberna, M. (2018). Resilience to fire of
1950 phylogenetic diversity across biological domains. *Molecular Ecology*, *27*(13), 2896–
1951 2908. doi: 10.1111/mec.14729
1952 Pinosio, S., González-Martínez, S. C., Bagnoli, F., Cattonaro, F., Grivet, D., Marroni, F., …
1953 Vendramin, G. G. (2014). First insights into the transcriptome and development of new
1954 genomic tools of a widespread circum-Mediterranean tree species, *Pinus halepensis*
1955 Mill. *Molecular Ecology Resources*, *14*(4), 846–856. doi: 10.1111/1755-0998.12232
1956 Prieto, I., Roldán, A., Huygens, D., del Mar Alguacil, M., Navarro-Cano, J. A., & Querejeta,
1957 J. I. (2016). Species-specific roles of ectomycorrhizal fungi in facilitating interplant
1958 transfer of hydraulically redistributed water between *Pinus halepensis* saplings and
1959 seedlings. *Plant and Soil*, *406*(1–2), 15–27. doi: 10.1007/s11104-016-2860-y
1960 Querejeta, J. I., Egerton-Warburton, L. M., & Allen, M. F. (2007). Hydraulic lift may buffer
1961 rhizosphere hyphae against the negative effects of severe soil drying in a California Oak
1962 savanna. *Soil Biology and Biochemistry*, *39*(2), 409–417. doi:
1963 10.1016/j.soilbio.2006.08.008
1964 R Core Team. (2014). R Core Team. *R: A Language and Environment for Statistical*
1965 *Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014*.
1966 Radeloff, V. C., Mladenoff, D. J., Guries, R. P., & Boyce, M. S. (2004). Spatial patterns of
1967 cone serotiny in *Pinus banksiana* in relation to fire disturbance. *Forest Ecology and*
1968 *Management*, *189*, 133–141. doi: 10.1016/j.foreco.2003.07.040
1969 Ray, P., & Craven, K. D. (2016). Sebacina vermifera: a unique root symbiont with vast
1970 agronomic potential. *World Journal of Microbiology and Biotechnology*, *32*(1), 1–10.
1971 doi: 10.1007/s11274-015-1970-7
1972 Rincón, A., Santamaría-Pérez, B., Ocaña, L., & Verdú, M. (2014). Structure and phylogenetic
1973 diversity of post-fire ectomycorrhizal communities of maritime pine. *Mycorrhiza*, *24*,
1974 131–141. doi: 10.1007/s00572-013-0520-0
1975 Rincón, A.~~na~~, & Pueyo, J. J. (2010). Effect of fire severity and site slope on diversity and
1976 structure of the ectomycorrhizal fungal community associated with post-fire regenerated
1977 *Pinus pinaster* Ait. seedlings. *Forest Ecology and Management*, *260*(3), 361–369. doi:
1978 10.1016/j.foreco.2010.04.028
1979 Rincón, A.~~na~~, Santamaría-Pérez, B., Rabasa, S. G., Coince, A., Marçais, B., & Buée, M.
1980 (2015). Compartmentalized and contrasted response of ectomycorrhizal and soil fungal
1981 communities of Scots pine forests along elevation gradients in France and Spain.
1982 *Environmental Microbiology*, *17*, 3009–3024. doi: 10.1111/1462-2920.12894
1983 Rousk, J., Baath, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., … Fierer,
1984 N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil.
1985 *Isme Journal*, *4*, 1340–1351. doi: 10.1038/ismej.2010.58
1986 Ruíz, P., Álvarez-Uria, P., & Zavala, M. A. (2009). 9540 Pinares mediterráneos de pinos

mesogeanos endémicos. In *VV. AA., Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España.* (p. 112). Madrid: Ministerio de Medio Ambiente, y Medio Rural y Marino.

Schneider, T., Keiblinger, K. M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., … Riedel, K. (2012). Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *The ISME Journal*, *6*(9), 1749–1762. doi: 10.1038/ismej.2012.11

Selosse, M. A., Bauer, R., & Moyersoen, B. (2002). Basal hymenomycetes belonging to the Sebacinaceae are ectomycorrhizal on temperate deciduous trees. *New Phytologist*, *155*(1), 183–195. doi: 10.1046/j.1469-8137.2002.00442.x

Smith, S., & Read, D. (Eds.). (2008). *Mycorrhizal Symbiosis*. Academic Press: London.

Sun, H., Santalahti, M., Pumpanen, J., Köster, K., Berninger, F., Raffaello, T., … Heinonsalo, J. (2015). Fungal Community Shifts in Structure and Function across a Boreal Forest Fire Chronosequence. *Applied and Environmental Microbiology*, *81*(22), 7869–7880. doi: 10.1128/AEM.02063-15.Editor

Talbot, J. M., Allison, S. D., & Treseder, K. K. (2008). Decomposers in disguise: Mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Functional Ecology*, *22*(6), 955–963. doi: 10.1111/j.1365-2435.2008.01402.x

Tapias, R., Climent, J., Pardos, J. a., & Gil, L. (2004). Life histories of Mediterranean pines. *Plant Ecology*, *171*(Richardson 1988), 53–68. doi: 10.1023/B:VEGE.0000029383.72609.f0

Taylor, D. L., & Bruns, T. D. (1999). Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: Minimal overlap between the mature forest and resistant propagule communities. *Molecular Ecology*, *8*(11), 1837–1850. doi: 10.1046/j.1365-294X.1999.00773.x

Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N. S., Wijesundera, R., … Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213), 1256688. doi: 10.1126/science.1256688

Tedersoo, L. eho, Hansen, K., Perry, B. A., & Kjøller, R. (2006). Molecular and morphological diversity of pezizalean ectomycorrhiza. *New Phytologist*, *170*(3), 581–596. doi: 10.1111/j.1469-8137.2006.01678.x

Tedersoo, L. Leho, Mett, M., Ishida, T. A., & Bahram, M. (2013). Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. *New Phytologist*, *199*(3), 822–831. doi: 10.1111/nph.12328

Tedersoo, L. eho, & Smith, M. E. (2013). Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews*, *27*(3–4), 83–99. doi: 10.1016/j.fbr.2013.09.001

Torres, P., & Honrubia, M. (1997). *Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a Pinus halepensis forest*. *96*, 189–196.

Twieg, B. D., Durall, D. M., & Simard, S. W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytologist*, *176*(2), 437–447. doi: 10.1111/j.1469-8137.2007.02173.x

van der Heijden, M. G. A., Martin, F. M., Selosse, M. A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, *205*(4), 1406–1423. doi: 10.1111/nph.13288

Verdú, M., & Pausas, J. G. (2007). Fire drives phylogenetic clustering in Mediterranean Basin woody plant communities. *Journal of Ecology*, *95*(6), 1316–1323. doi: 10.1111/j.1365-

2035   2745.2007.01300.x

2036 Vieira, J., Campelo, F., Rossi, S., Carvalho, A., Freitas, H., & Nabais, C. (2015). Adjustment
2037   capacity of maritime pine cambial activity in drought-prone environments. *PLoS ONE*,
2038   *10*(5), 1–15. doi: 10.1371/journal.pone.0126223

2039 Walkley, A., & Black, I. (1934). An examination of the Degtjareff method for determining
2040   soil organic matter, and a proposed modification of the chromic acid titration method.
2041   *Soil Science*, *37*(1), 29–38. Retrieved from
2042   http://journals.lww.com/soilsci/Abstract/1934/01000/AN_EXAMINATION_OF_THE_
2043   DEGTJAREFF_METHOD_FOR.3.aspx

2044 Yamanaka, T. (2003). The effect of pH on the growth of saprotrophic and ectomycorrhizal
2045   ammonia fungi in vitro. *Mycologia*, *95*(4), 584–589. doi: 10.2307/3761934

2046 Zarea, M. J., Miransari, M., & Karimi, N. (2014). *Plant Physiological Mechanisms of Salt*
2047   *Tolerance Induced by Mycorrhizal Fungi and* Piriformospora indica. doi: 10.1007/978-
2048   1-4939-0721-2

2049
2050
2051

2053

Data Accessibility

2055   Data were deposited in the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) as

2056   PRJNA324224.

2057

Author contribution

2059   AR and MV designed the experiment; LPI, MZA and AR collected the data; LPI and MB

2060   performed the bioinformatics analysis, LPI performed the statistical analyses. LPI and AR

2061   wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

2062

2063

2064

2065

2066

2067

2068

2069

2070

2071

2072

2073

2074

2075

2076

2077

2078 **Table 1.** Alpha-diversity of representative ectomycorrhizal (ECM) fungal families analysed
2079 by General Linear Models (GLMs) ($p<0.05$). Main effects of the pine species (Ppi =*Pinus*
2080 *pinaster* and Pha =*Pinus halepensis*) (left) and the fire regime (LoFi = low fire recurrence and
2081 HiFi = high fire recurrence) (right) are shown. The site was nested within the fix factor fire
2082 regime in the ~~Generalized Linear Models (~~GLMs~~)~~. Data represent means +/- SE. Arrows
2083 indicate significantly higher α-diversity, and "=" indicates no variation between pine species
2084 according to the GLM (F values; *$p<0.05$; **$p<0.01$; ***$p<0.001$). In the separate analyses
2085 on the right, for each pine forest, different letters denote significant differences between fire
2086 regimes ($p<0.05$) (in bold). A = Ascomycetes (orange); B = Basidiomycetes (green).

2087

2088

|	Pine Forest			*P. pinaster*		*P. halepensis*	
	Ppi	*Pha*	F (p-value)	LoFi	HiFi	LoFi	HiFi
Amanitaceae[B]	↑		9.2**	**0.1 ± 0.1 a**	**0.9 ± 0.2 b**	0.1 ± 0.1	0.2 ± 0.1
Atheliaceae[B]	↑		6.8*	**9.7 ± 0.6 b**	**6.7 ± 0.6 a**	6.3 ± 0.5	5.9 ± 0.4
Bankeraceae[B]	=	=	2.0	1.8 ± 0.4	1.3 ± 0.3	**1.7 ± 0.5 b**	**0.9 ± 0.2 a**
Cantharellaceae[B]	↑		41.7***	**1.0 ± 0.2 a**	**2.8 ± 0.4 b**	0.7 ± 0.2	0.5 ± 0.2
Clavariaceae[B]	↑		36.9***	0.8 ± 0.2	1.2 ± 0.4	**0.3 ± 0.2 b**	**0.1 ± 0.1 a**
Clavulinaceae[B]	=	=	2.4	**2.9 ± 0.3 b**	**1.7 ± 0.3 a**	**2.7 ± 0.5 b**	**1.3 ± 0.3 a**
Cortinariaceae[B]	↑		16.2***	**2.2 ± 0.7 b**	**0.4 ± 0.2 a**	**0.6 ± 0.4 b**	**0.2 ± 0.1 a**
Gloniaceae[A]	↑		5.7*	**2.5 ± 0.3 b**	**1.2 ± 0.2 a**	**1.7 ± 0.4 b**	**0.9 ± 0.3 a**
Hydnaceae[B]	=	=	0.8	0.3 ± 0.1	0.2 ± 0.1	**0.6 ± 0.2 b**	**0.2 ± 0.1 a**
Inocybeaceae[B]	=	=	1.4	**5.5 ± 0.8 b**	**3.0 ± 0.5 a**	3.1 ± 0.3	2.8 ± 0.3
Pezizaceae[A]		↑	22.8***	0.2 ± 0.1	0.2 ± 0.1	**0.5 ± 0.2 a**	**1.1 ± 0.2 b**
Pyronemataceae[A]		↑	30.7***	0.6 ± 0.2	0.9 ± 0.2	**2.9 ± 0.3 b**	**1.6 ± 0.3 a**
Rhizopogonaceae[B]	↑		29.1***	**0.9 ± 0.2 a**	**1.8 ± 0.2 b**	**0.8 ± 0.2 b**	**0.4 ± 0.1 a**
Russulaceae[B]	↑		14.0***	**7.3 ± 0.6 b**	**5.5 ± 0.7 a**	4.3 ± 0.8	4.3 ± 0.5
Sebacinaceae[B]		↑	33.6***	5.7 ± 1.1	4.5 ± 0.4	8.7 ± 0.8	9.0 ± 0.8
Suillaceae[B]	=	=	0.0	1.6 ± 0.3	1.2 ± 0.2	**1.6 ± 0.2 b**	**1.2 ± 0.2 a**
Telephoraceae[B]	=	=	0.0	11.9 ± 1.0	11.0 ± 1.0	**14.1 ± 1.8 b**	**10.4 ± 0.8 a**
Tuberaceae[A]	=	=	0.0	1.4 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.4 ± 0.2

2089

2090
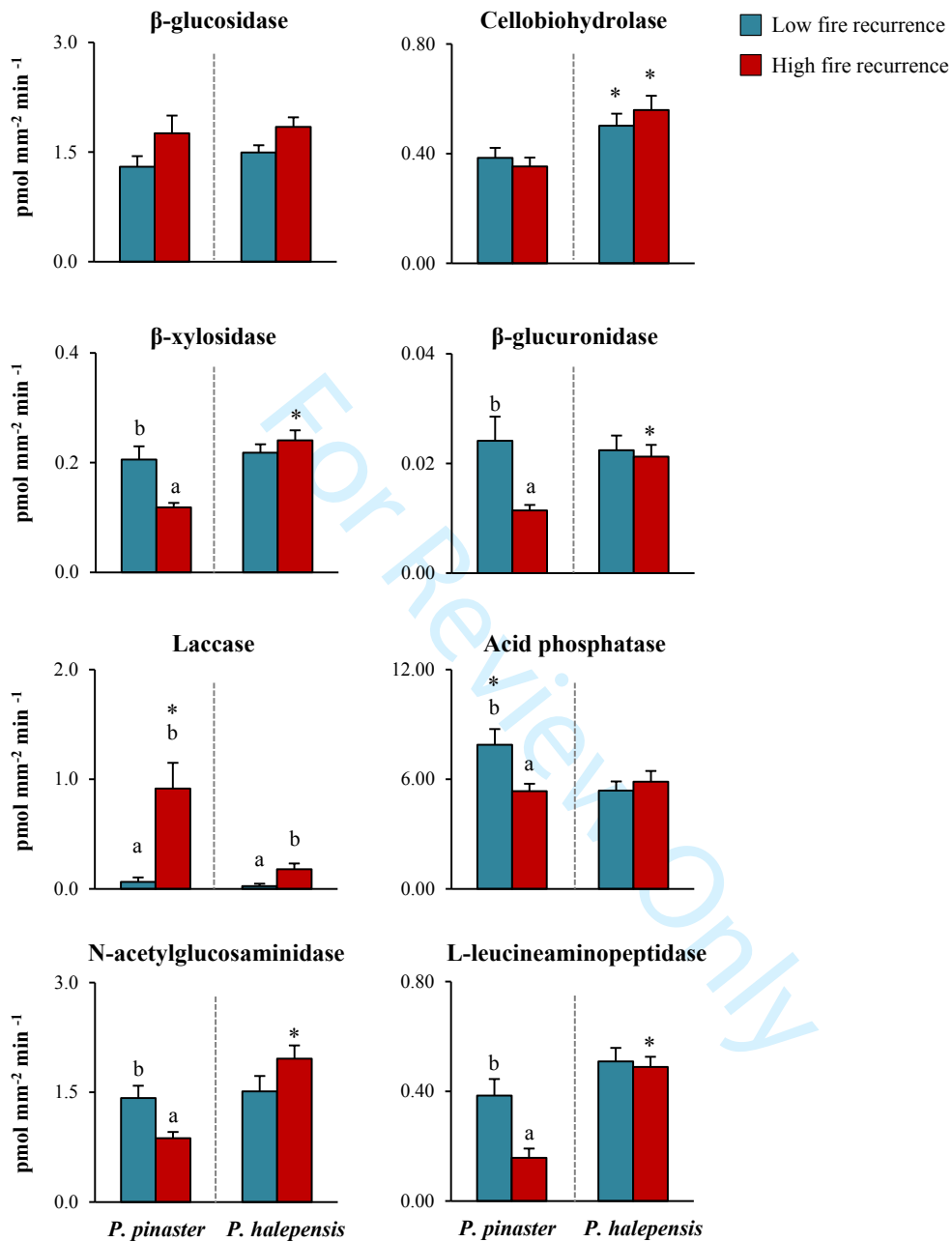
2091

2092

2093

2095
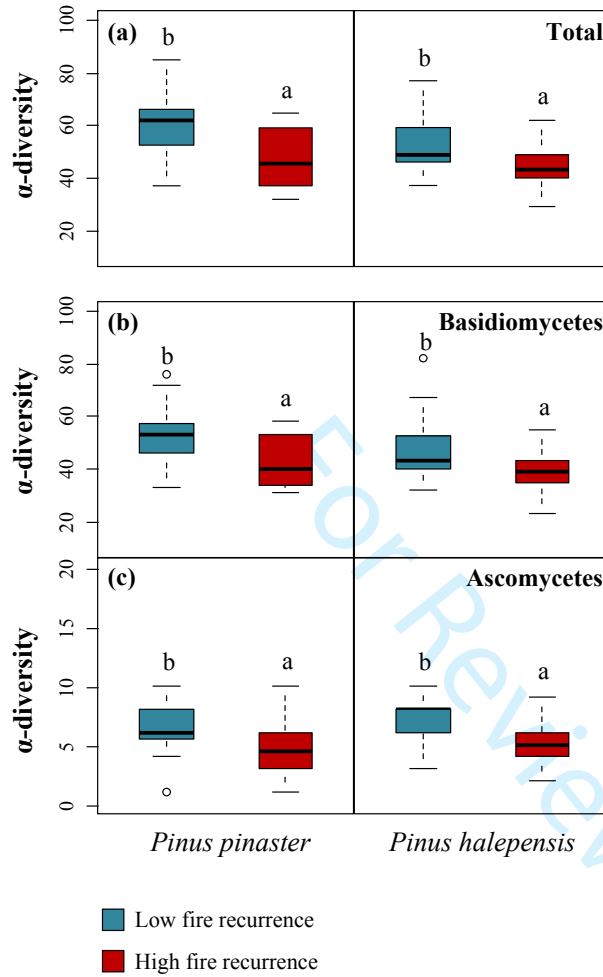
2096

2097

2098   **Figure 1.** Alpha-diversity of (a) total, (b) Basidiomycetes and (c) Ascomycetes
2099   ectomycorrhizal fungal communities associated with root-tips of <u>low (blue) or high (red) fire</u>
2100   <u>recurrence</u>~~LoFi (blue~~light bars) and HiFi (dark bars)~~ populations of *Pinus pinaster* and *Pinus*
2101   *halepensis*, analysed by Generalized Linear Models (p<0.05). Boxes represent the
2102   interquartile range (IQR) between first and third quartiles and the horizontal line inside is the
2103   median. Whiskers denote the lowest and highest values within 1.5 x IQR from the first and
2104   third quartiles, respectively. Within each graph, different letters denote significant differences
2105   among fire regimes.

2107
2108
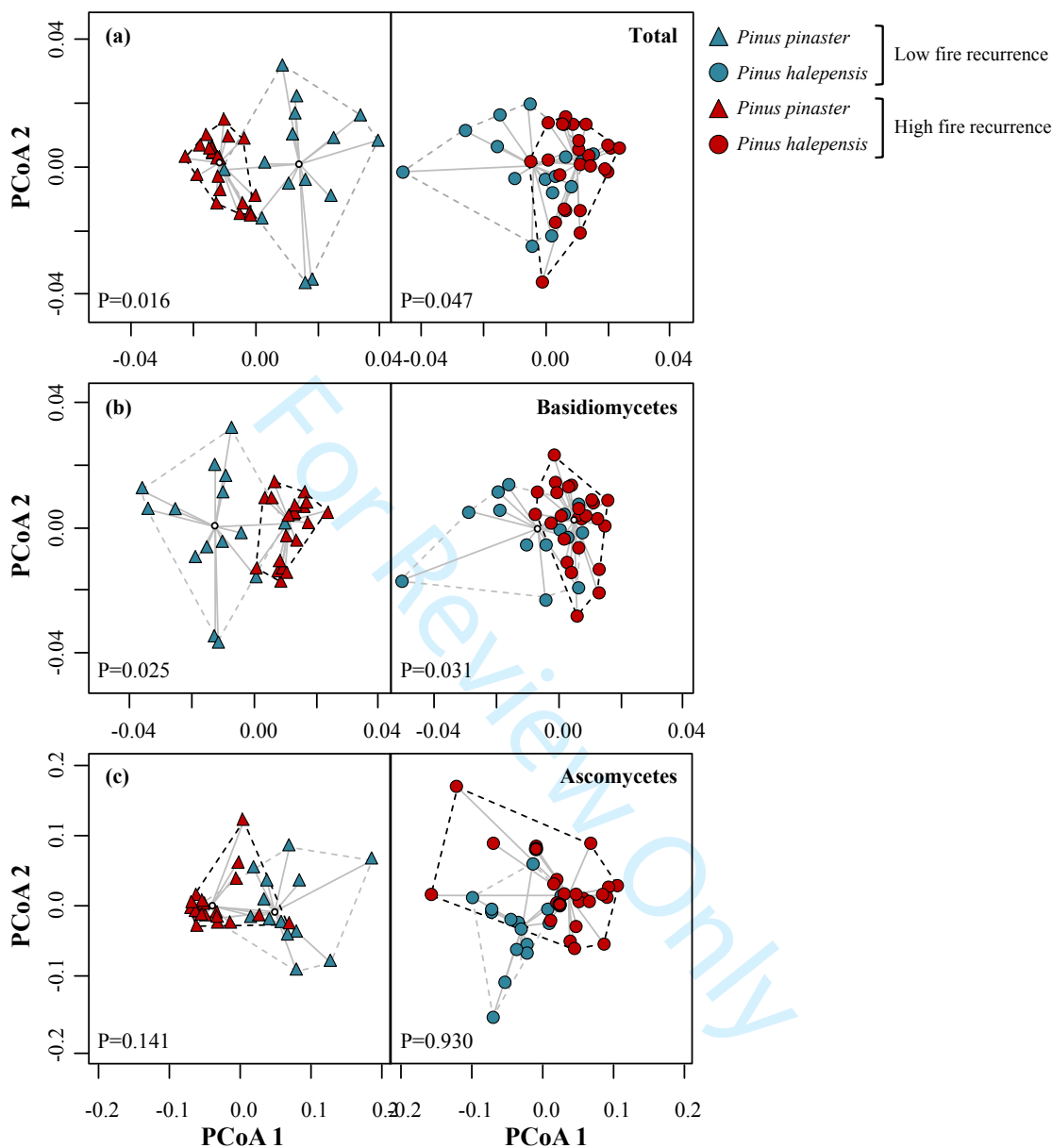
2109
2110

Figure 2. Beta-diversity of (a) total, (b) Basidiomycetes and (c) Ascomycetes ectomycorrhizal fungal communities associated with root-tips of low (blue) or high (red) fire recurrence LoFi (greyblue) and HiFi (blackred) populations of *Pinus pinaster* (triangles) and *Pinus halepensis* (circles). The centroids within each group are represented by small white dots. Grey solid lines give the relative position of the point with respect to the respective centroid. Dotted lines reflect the area occupied by all plots of a given treatment, which is inversely proportional to the similarity of their ECM fungal communities.

Figure 3. Enzymatic activity of ectomycorrhizal root tips (means ± SE) of low (LoFi, blue) or high (HiFi, red) fire recurrence populations of *Pinus pinaster* and *Pinus halepensis*, analysed by Generalized Linear Models (p<0.05). For each pine species, different letters denote significant differences between fire recurrence levels, while for each LoFi/HiFi treatment, asterisks denote significant differences between pine forest levels.
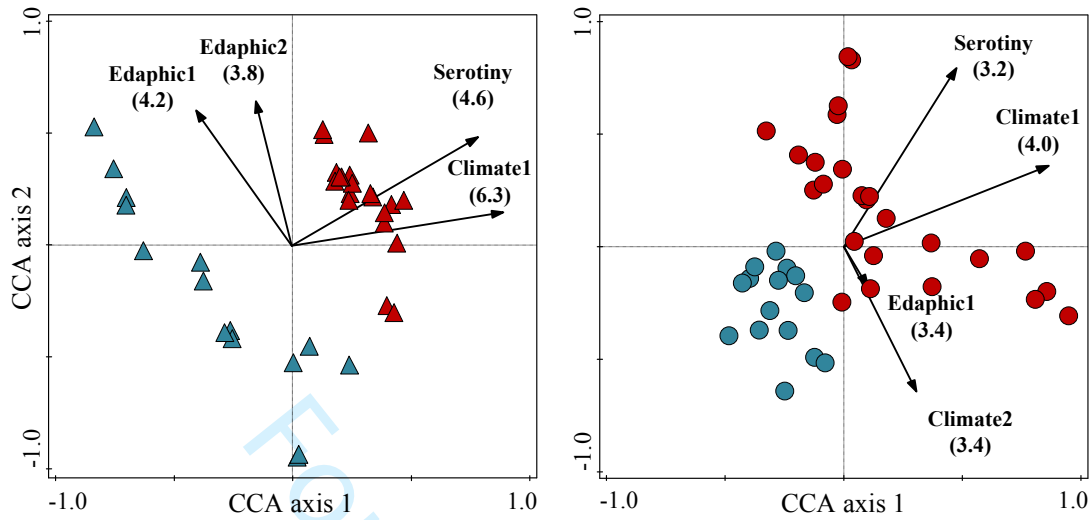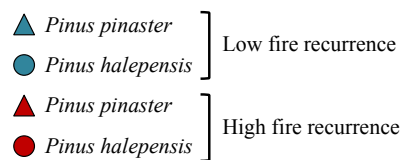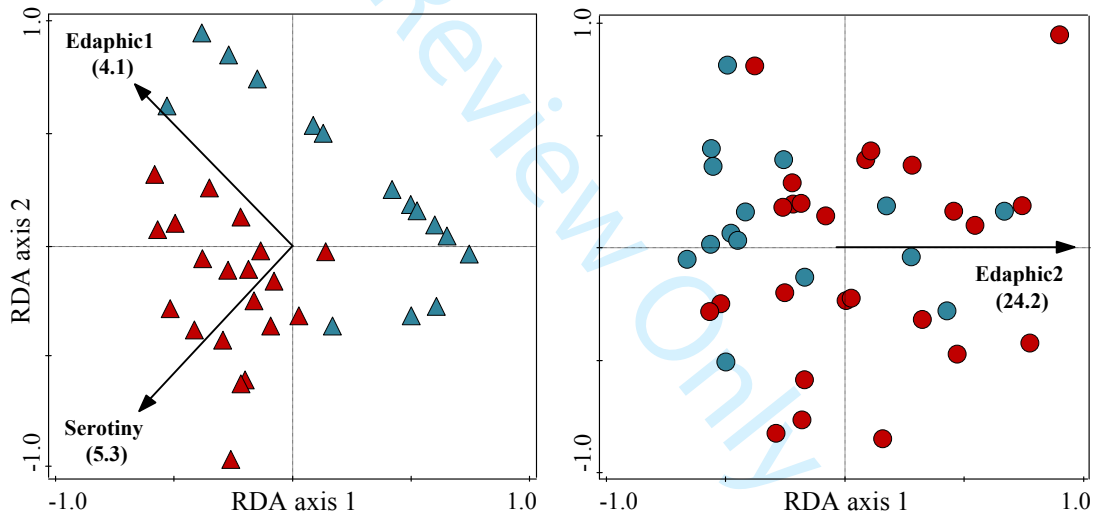
2132

(a) *Pinus pinaster*

Mantel test (r=0.14; P=0.003)

(b) *Pinus halepensis*

Mantel test (r=0.032; P=0.287)
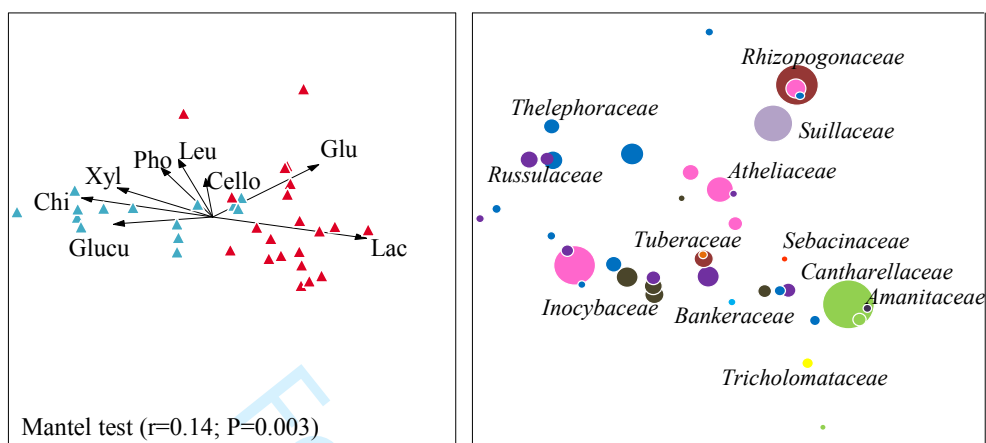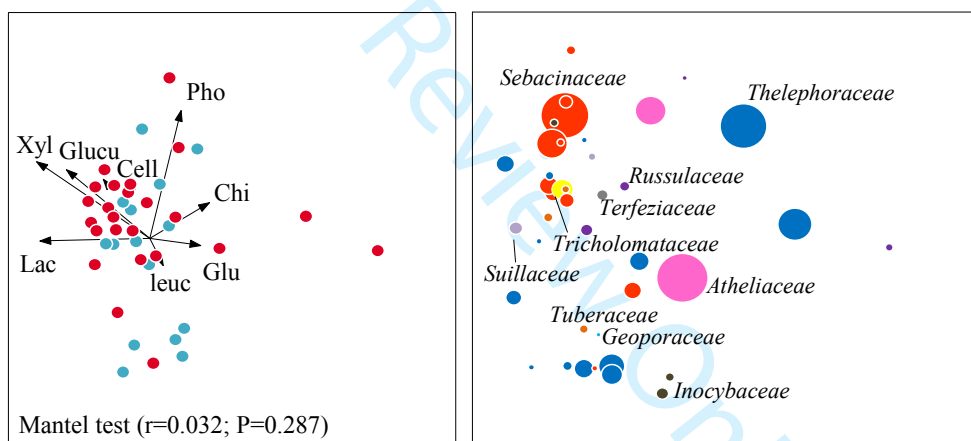
2133

Figure 3. Variation of root-tip ectomycorrhizal (ECM) fungal community composition associated with LoFi (blue) and HiFi (red) populations of (a) *Pinus pinaster* and (b) *Pinus halepensis* by sample plots (left) and fungal families (right) and correlation with enzymatic activities, analyzed by Detrended Correspondence Analysis (DCA). Vectors represent the weight and direction of enzymatic activities plotted in the ordination space. In the fungal species graphs on the right, only the most abundant taxa (proportional to the diameter of the circles) are shown. The correlation between ECM fungal community structure and enzymatic activities dissimilarity matrices is shown through Mantel test.

2142

(a) ECM fungal community

Edaphic1
(4.2)

Edaphic2
(3.8)

Serotiny
(4.6)

Climate1
(6.3)

CCA axis 2

1.0

-1.0

-1.0   CCA axis 1   1.0

Serotiny
(3.2)

Climate1
(4.0)

Edaphic1
(3.4)

Climate2
(3.4)

1.0

-1.0

-1.0   CCA axis 1   1.0

(b) Enzymatic activity

Edaphic1
(4.1)

Serotiny
(5.3)

RDA axis 2

1.0

-1.0

-1.0   RDA axis 1   1.0

Edaphic2
(24.2)

1.0

-1.0

-1.0   RDA axis 1   1.0

△ *Pinus pinaster*
● *Pinus halepensis*] Low fire recurrence

▲ *Pinus pinaster*
● *Pinus halepensis*] High fire recurrence

2143

2144
2145
2146
2147
2148
2149

2150

2151 **Figure 4**. ~~Distribution of~~ (a) ~~E~~ectomycorrhizal ~~(ECM)~~ fungal community structure and (b)
2152 enzymatic activit~~ies~~y ~~composition~~associated with root-tips of ~~LoFi (blue) and HiFi (red)~~
2153 *Pinus pinaster* (triangles) and *Pinus halepensis* (circles) ~~f~~forests subjected to low (blue) or
2154 high (red) fire recurrence, ~~analyzed by Canonical Correspondence Analysis (CCA), and (b)~~
2155 ~~their enzymatic profiles analyzed~~ respectively analyzed by Canonical Correspondence (CCA)
2156 and Redundancy (RDA) ~~a~~Analys~~e~~is. In all cases, ~~(RDA) showing~~ the weight, direction and
2157 separate effects of serotiny, climatic and edaphic conditions ~~in sample plots~~is represented by
2158 vectors (see model details in Table S4). Serotiny degree is the number of closed cones by total
2159 number of cones in the tree. Edaphic and climatic conditions are represented by the first two
2160 axes (Edaphic1 and Edaphic2; Climate1 and Climate2) of Principal Coordinates Analyses
2161 (PCA) performed considering the pH, electric conductivity and potassium, nitrogen,
2162 phosphorus and organic matter content in the first case, and mean temperature, mean
2163 precipitation and altitude in the second case (Figure S2~~1~~). Only significant variables are
2164 shown,   *p<0.05,   **p<0.01,   ***p<0.001.
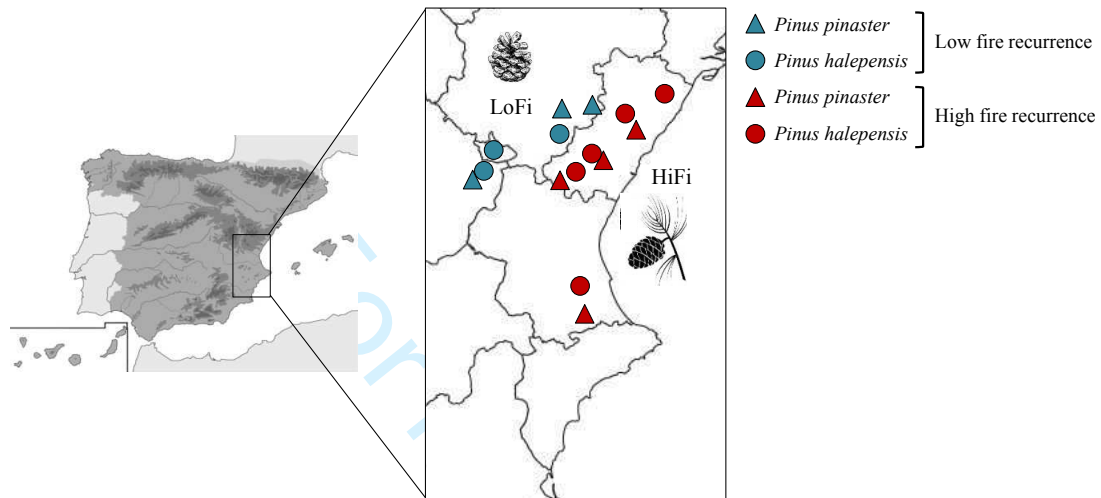
2165
2166 **Supplementary Figures**
2167
2168 <u>**Figure S1**</u>

2169

2170

2171

2172 **Figure S1.** Location of the fifteen study sites with pine populations of *Pinus pinaster* Ait.
2173 (triangles) and *Pinus halepensis* Mill. (circles), growing in areas of low (LoFi, blue) and high
2174 (HiFi, red) fire recurrence, in eastern Spain. The distinct historical fire regime has induced a
2175 sharp serotiny divergence within these pine populations along time. Serotinous populations
2176 associate to HiFi while non-serotinous populations associate to LoFi.
2177

2178

2180
2181 **Figure S2**
2182

(a)

(b)

△ *Pinus pinaster* ⎤
● *Pinus halepensis* ⎦ Low fire recurrence

▲ *Pinus pinaster* ⎤
● *Pinus halepensis* ⎦ High fire recurrence
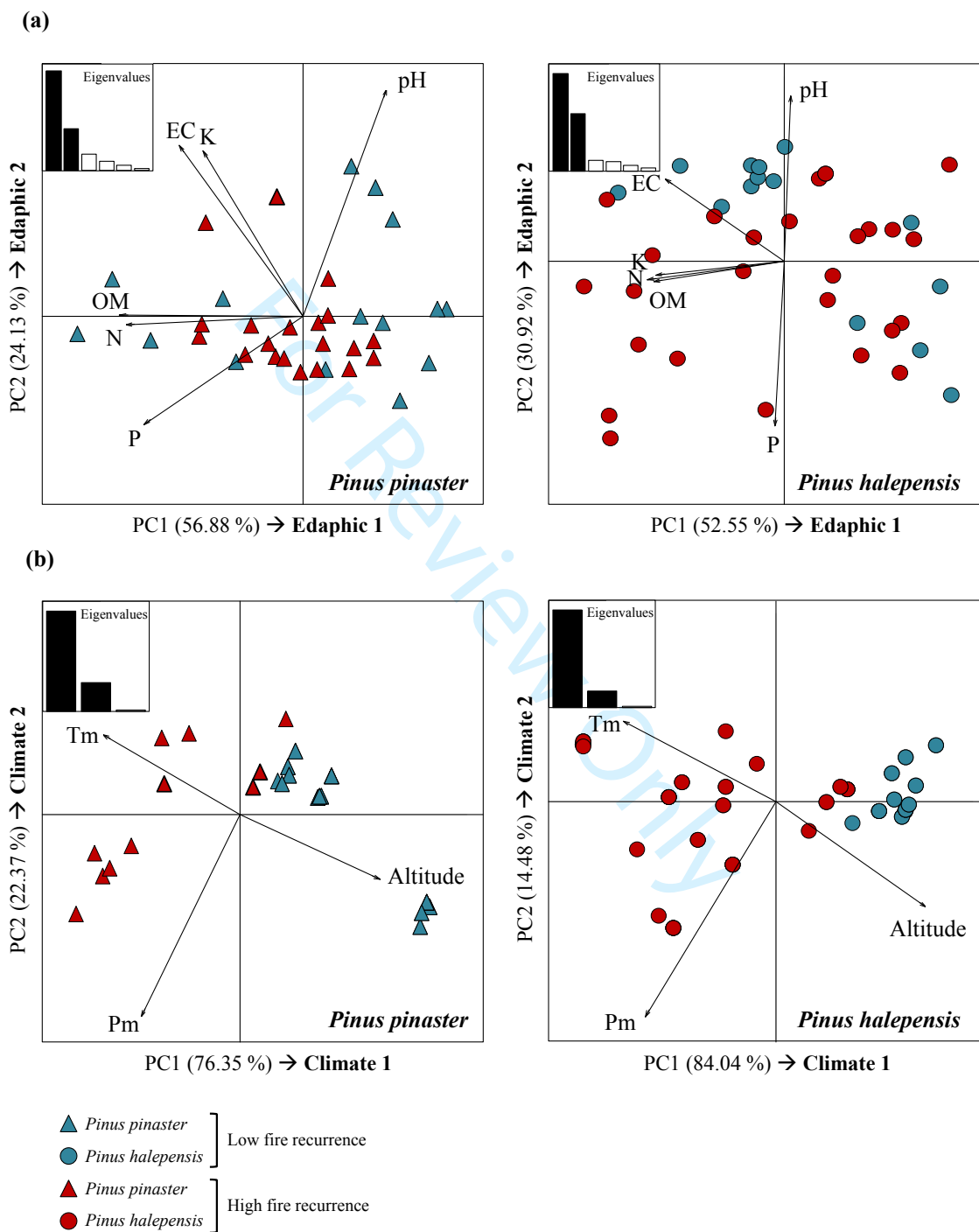
2183
2184

2185

2186

2187

2188   **Figure S21**. Principal component analyses (PCA) performed with (a) the edaphic variables
2189   pH, electric conductivity (EC), potassium (K), nitrogen (N), phosphorus (P) and organic
2190   matter (OM), and (b) the climate-related variables altitude, mean temperature (Tm) and mean
2191   precipitation (Pm) of each pine forest. The first two axes of each analysis explaining the
2192   maximum of variance (in parenthesis) were used as edaphic or climatic indexes in models
2193   (i.e., proxies of edaphic and climatic conditions of each pine forest *Pinus pinaster* or *Pinus*
2194   *halepensis*).

2196
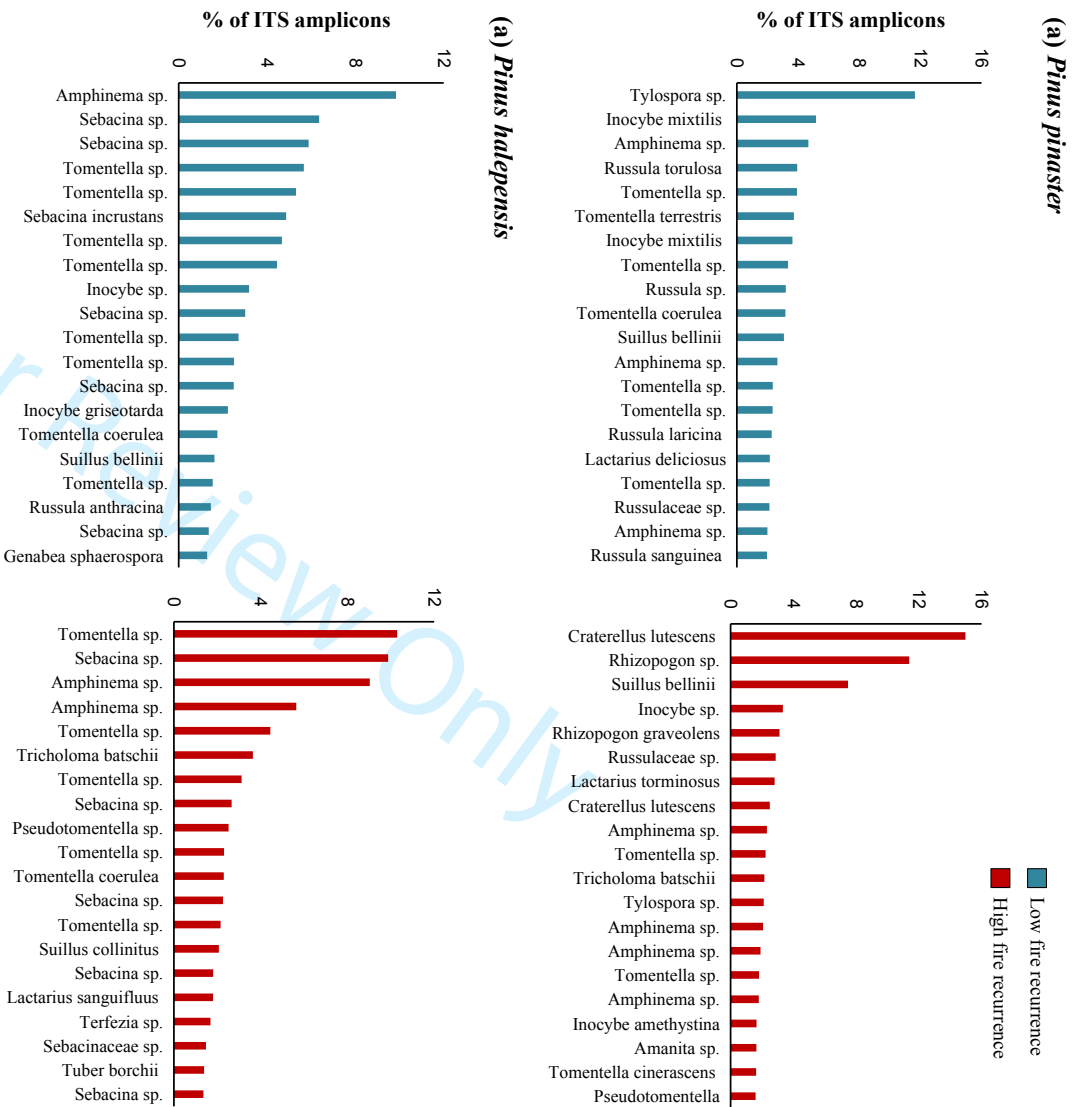2197 **Figure S3**
2198

a)

b)
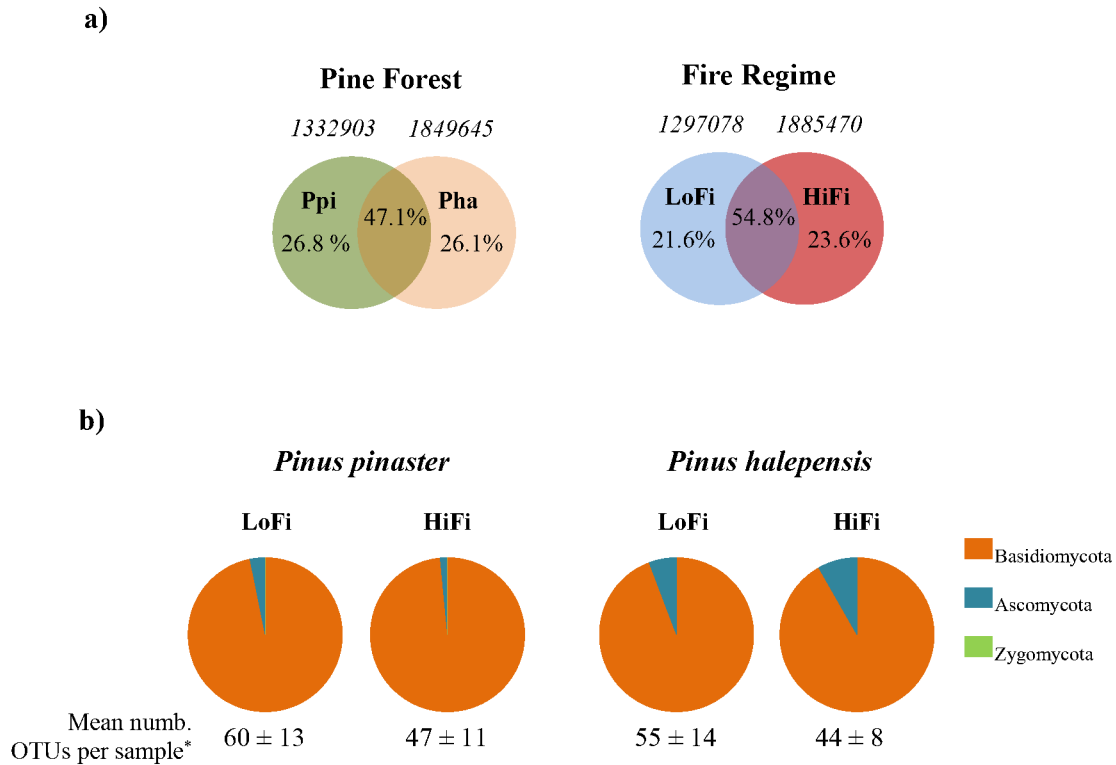
2199
2200

2201 **Figure S32.** (a) Number of sequences (*italics*) and percentage of ectomycorrhizal (ECM)
2202 fungal MOTUs by pine forest (Ppi = *Pinus pinaster* and Pha = *Pinus halepensis*) and fire
2203 regime (HiFi and LoFi fire recurrencefrequency). (b) Percentages of fungal phyla for each
2204 pine species and fire regime. *Mean ± SD. (See Table S2 for percentages and abundance of
2205 other taxonomic ranks across treatments).

2207
2208   **Figure S4**
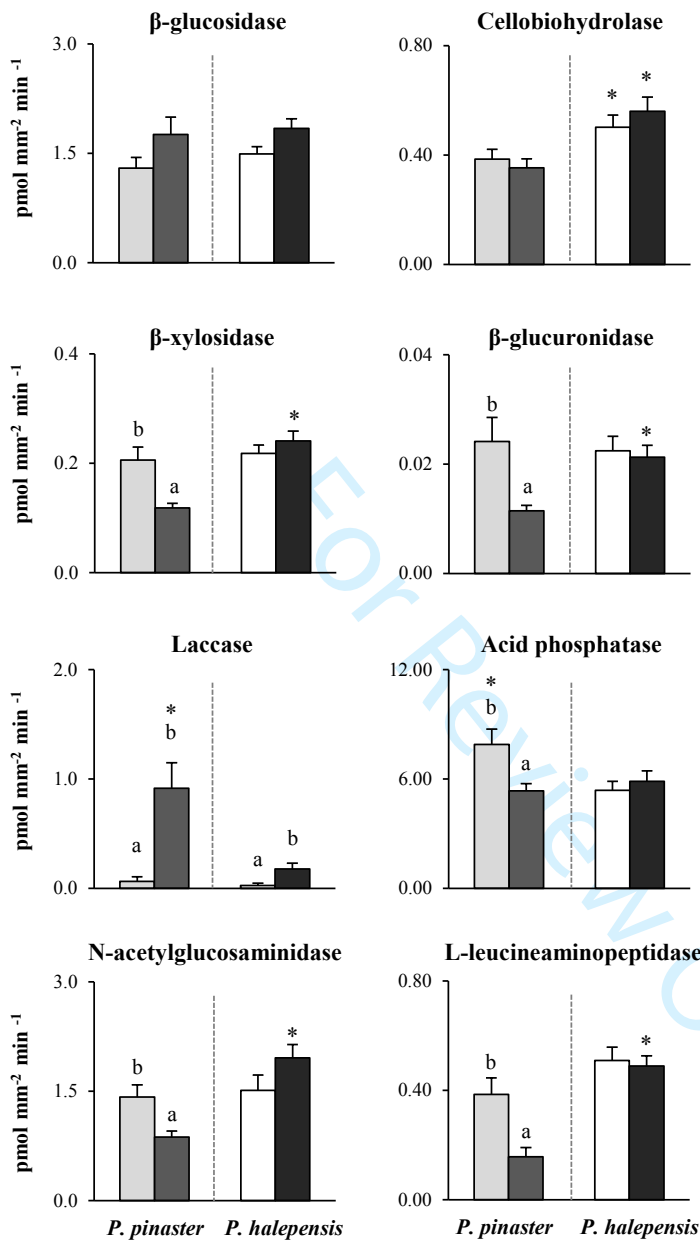2209

2210

2211

2212

2213

2214

2215

2216

2217

Figure S4. Abundance rank (%) of the 20 most abundant species of low (blue) or high (red) fire recurrence populations of (a) *Pinus pinaster* and (b) *Pinus halepensis* forests.

Figure S3. Enzymatic activity of ectomycorrhizal root tips (means ± SE) of LoFi (light bars) and HiFi (dark bars) populations (low and high fire recurrence, respectively) of *Pinus pinaster* (Ppi) and *Pinus halepensis* (Pha), analysed by Generalized Linear Models (p<0.05). For each pine species, different letters denote significant differences between fire recurrence levels, while for each LoFi/HiFi treatment, asterisks denote significant differences between pine forest levels.