

1 **Mycorrhizae support oaks growing in a phylogenetically distant**
2 **neighbourhood**

3

4 **Benjamin Yguel^{1,8}; Pierre-Emmanuel Courty²; Hervé Jactel³; Xu Pan⁴, Olaf Butenschoen⁵,**
5 **Phil J. Murray⁶, Andreas Prinzing^{1,7}**

6 ¹University of Rennes 1/Centre National de la Recherche Scientifique, research unit UMR 6553,
7 « Ecosystems, Biodiversity, Evolution », Campus Beaulieu, Bâtiment 14 A, 263 Av. du Général
8 Leclerc, 35042, Rennes cedex, France;

9 ²Zurich-Basel Plant Science Center, Department of Environmental Sciences, Botany, University
10 of Basel, Hebelstrasse 1, 4056, Basel, Switzerland

11 ³ UMR BIOGECO – INRA. Laboratory of Forest Entomology & Biodiversity. 69, route
12 d'Arcachon, 33612 CESTAS cedex, France.

13 ⁴State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese
14 Academy of Sciences, Beijing 100093, China.

15 ⁵J. F. Blumenbach Institute of Zoology and Anthropology, Georg August University Göttingen,
16 Berliner Str. 28, 37073 Göttingen, Germany

17 ⁶Sustainable Soils and Grassland Systems Department, Rothamsted Research, North Wyke,
18 Okehampton, Devon, EX20 2SB, UK

19 ⁷ Alterra Centre for Ecosystem Studies, Wageningen University and Research Centre, PO Box
20 47, 6700AA Wageningen, The Netherlands

21 ⁸German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher
22 Platz 5e, 04103 Leipzig, Germany

23

24 Corresponding author : Benjamin Yguel:

25 Mail: benyguel@hotmail.fr, Tel: +33 6 13 74 55 19; Fax: +33 2 23 23 50 26

- 26 Type of article: Research article
- 27 Number of words: Abstract(209), Main text (5325)
- 28 Number of references: 50
- 29 Number of figures and tables: 4 figures and 1 table; supporting information (10 figures).

30 **Abstract**

31 Host-plants may rarely leave their ancestral niche and in which case they tend to be surrounded
32 by phylogenetically distant neighbors. Phylogenetically isolated host-plants might share few
33 mutualists with their neighbours and might suffer from a decrease in mutualist support. In
34 addition host plants leaving their ancestral niche might face a deterioration of their abiotic and
35 biotic environment and might hence need to invest more into mutualist partners. We tested
36 whether phylogenetic isolation of hosts from neighbours decreases or increases abundance and
37 activity of their mutualists and whether mutualist activity may help to compensate deterioration
38 of the environment. We study oak-hosts and their ectomycorrhizal fungi mutualists established in
39 the litter layer formed by the phylogenetically closely or distantly related neighbourhood. We
40 find that oaks surrounded by phylogenetically distant neighbors show increased abundance and
41 enzymatic activity of ectomycorrhizal fungi in the litter. Moreover, oaks surrounded by
42 phylogenetically distant neighbors also show delayed budburst but ectomycorrhizal fungi
43 activity partly compensates this negative effect of phylogenetic isolation. This suggests
44 decreased nutrient availability in a phylogenetically distant litter partly compensated by
45 increased litter-degradation by ectomycorrhizal fungi activity. Most observed effects of
46 phylogenetic isolation cannot be explained by a change in baseline soil fertility (as reflected by
47 nutritional status of fresh oak litter, or soil microbial biomass and activity) nor by simple
48 reduction of percentages of oak neighbours, nor by the presence of gymnosperms. Our results
49 show that colonizing new niche represented by the presence of distantly related neighbours may
50 delay plant phenology but may be supported by mycorrhizal mutualists. Studies on other host-
51 plant species are required to generalize our findings.

52

53 **Key words:** community phylogeny, mycorrhiza, forest trees, enzymatic activity, breaking with
54 niche conservatism, mutualism strength, budburst phenology

56 **1. Introduction**

57 Mutualists provide essential services to numerous species. For instance, more than 80% of
58 terrestrial plant species interact with mutualistic mycorrhizal fungi (Parnischke, 2008) that play a
59 vital role for the plants. Mycorrhizal fungi take up nutrients from the soil via their extraradical
60 mycelium and translocate these nutrients to the plant partner, receiving carbohydrates in return
61 (Smith and Read, 2008). However, mutualistic relationships are exposed to threats. In particular
62 spatial isolation between individual hosts of the same plant species may lead to a loss of
63 mutualist species and a decrease in their ecological functions, as already observed with
64 pollinators (Ghazoul and Shaanker, 2004), predators of plant enemies (Magrath et al., 2011) and
65 mycorrhizal fungi (Dickie and Reich, 2005; Peay et al., 2010; Frank et al., 2009).

66 Mutualists may be host-plant-specific and may conserve hosts throughout evolutionary history
67 (e.g. Powell et al., 2009; Shefferson et al., 2010 but see van der Heijden and Horton 2009 and
68 below). Thus phylogenetically close host-species may be expected to share mutualists and their
69 services (Peay et al. 2010 and above references). It has been shown that host plant species
70 surrounded by distantly related plant neighbors (i.e. phylogenetically isolated hosts) can have
71 lower herbivore load than host plant species surrounded by closely related species (Goßner et al.,
72 2009; Yguel et al., 2011). Similar conclusions may apply to mutualists: phylogenetic isolation of
73 host plants from neighboring species could lead to a decrease in abundance of host mutualists
74 and associated services. However, some mutualists are not or only partly host-specific (Molina et
75 al., 1992; Fontaine et al., 2009; Morris et al., 2009), and phylogenetically distant host species
76 may hence harbor the same generalist mutualist species (Selosse et al., 2006). In such case, host
77 plants surrounded by phylogenetically distant plant species may not experience any loss in
78 mutualist species.

79 Individual host plants that are surrounded by phylogenetically distant species might also face a
80 deterioration of their living conditions. Phylogenetically distantly related species may have
81 different functional traits resulting in different levels of litter decomposition (Pan et al. in press).
82 Therefore colonizing an environment composed of distantly related species may correspond to
83 different and often deteriorated abiotic living conditions like nutrient availability in the
84 litter. Moreover, as a general tendency, phylogenetically distant species often have different
85 environmental preferences (Wiens et al. 2010 for multiple examples and references), and thus, an
86 environment dominated by phylogenetically distant neighbours is likely suboptimal for a given
87 focal species, independent of the impact of these neighbours on the environment. As an extreme
88 example, fish have conserved an aquatic niche. Thus, a distantly related species like an ape for
89 instance surrounded by fish will be in extreme abiotic living conditions. In addition, within the
90 environment colonized, and possibly created by phylogenetically distant species, these species
91 are likely the superior competitors. Mutualistic interactions may then be particularly important to
92 compensate for such deteriorated and suboptimal living conditions (Hacker and Gaines, 1997;
93 Dickie and Reich, 2005). In that case, host plants surrounded by phylogenetically distant species
94 would need to invest more into mutualistic interactions. As a consequence, the abundance and
95 activity of mutualists are expected to increase with increasing phylogenetic isolation of a host
96 plant.

97 Three contradictory hypotheses are hence plausible: for a given host plant species, interactions
98 with mutualists (i) decrease, (ii) remain unchanged or (iii) increase with increasing phylogenetic
99 distance of host plants to their plant neighbors. In this study, we tested these hypotheses with
100 plant-mycorrhizae interactions, focusing on deciduous oak trees, their ectomycorrhizal fungi
101 (EMf) mutualists and their enzymatic activity related to budburst, a vital function. Deciduous
102 oaks (*Quercus petraea/rubra*) are particularly suitable to test such hypotheses: these oaks occur
103 in a wide range forest compositions and neighbourhoods, and are important for forestry.

104 In spring, oaks and other deciduous trees need to quickly produce their leaves within a narrow
105 time window between winter frosts and the annual peak of solar irradiation in order to maximize
106 annual carbon uptake (e.g. Morecroft et al., 2003). Oak budburst starts in a period without
107 support from photosynthetic apparatus resulting in an unbalanced plant carbon-budget during this
108 period. Budburst could be delayed by deteriorated soil or microclimates as they may occur in
109 phylogenetically distant neighborhoods (Courty et al., 2007). But budburst may be supported by
110 mycorrhiza: Breda et al. (2013) have shown that carbon is derived from litter during spring
111 reactivation and is channelled from the soil to oak roots via EMf, supporting the hypothesis that
112 oaks are partially mixotrophic plants (Courty et al., 2007). EMf may do so by producing
113 extracellular and cell wall-bound hydrolytic and oxidative enzymes to degrade C- and N-
114 compounds contained in soil organic matter (Courty et al., 2010; Rineau et al., 2012; Tedersoo et
115 al., 2012). In fact, in deciduous oaks, spring reactivation modifies the activity of EMf resulting in
116 greater mobilization of carbon and nitrogen from soil organic matter (Courty et al., 2007).
117 Without the contribution of EMf to nutrient uptake, budburst would be significantly delayed
118 (Dickie et al., 2007; Breda et al., 2013). Such and other functions of EMF can now be portrayed
119 by measures of enzymatic activities of excised ECM root tips. Such a functional approach may be
120 more powerful than looking at taxonomic identity of ECMf, given that enzymatic functions can
121 vary within ECMf taxa or remain constant between ECMf taxa (Courty et al., 2005, Buée et al.,
122 2007). This functional approach permits testing whether phylogenetic distant neighborhoods
123 change ECMf support and whether this support may compensate for a possible delay in budburst.
124 We studied the effect of phylogenetic isolation of oak trees (*Quercus robur* L. and *Quercus*
125 *petraea* Mattuschka Liebl) from their tree neighbours on budburst phenology and on the
126 abundance and activity of associated EMf in root tips within the litter layer formed by this
127 neighborhood. We focused on enzymes involved in the mobilization of carbon, but also nitrogen
128 during budburst. In particular, we addressed the following questions:

129 - Does the phylogenetic isolation of oak trees change the abundance and activity of litter EMf?

130 - Does phylogenetic isolation delay budburst phenology?

131 - Can enzymatic activity of litter EMf compensate for the negative effect of phylogenetic

132 isolation on budburst phenology?

133 We also explored multiple environmental variables by which the neighborhood of a tree or the

134 corresponding baseline conditions may operate on a focal tree. We finally explored whether the

135 effect of phylogenetic isolation can be explained by the percentage of oaks in the neighborhood

136 or the presence of gymnosperms.

137

138

139 **2. Materials & methods**

140 **2.1. Site description and experimental design**

141 Given that this study is on adult trees, experimentation is virtually impossible, so we followed a
142 correlative approach, profiting from a natural experiment of variation in phylogenetic canopy
143 composition around a focal species across a forest canopy. Such an approach cannot entirely
144 control for sampling effects, such as a high abundance of oaks being related to a low
145 phylogenetic distance, but this can be accounted for in later analyses (see below) Specifically,
146 our study was conducted in the Forest of Rennes (surface area: 2000 ha), Brittany (France;
147 Supporting information S1). A total of 17 different tree species were in contact with the focal
148 oaks canopy. Twenty two c. 60-year-old oaks were sampled, with age estimated from tree
149 circumference at breast height [total mean equal to 62.1 cm (SD = 16.7)] and from information
150 from forest managers. Oak trees were chosen by pair, with one surrounded mainly by other oaks
151 and beech trees, and the other surrounded mainly by pine and holly trees, plus some other
152 angiosperm trees. Because oaks in pine stands are generally in the lower part of the canopy, we
153 restricted ourselves to such low-canopy trees everywhere. Oak trees within a pair were close to
154 each other (30–150 m apart), and belonged to the same species, *Quercus petraea* or *Q. robur*
155 (note that these oak species are closely related and tend to hybridize). Pairs were spread across
156 the entire forest. Such an approach of pairing or blocking has been recommended to partial out
157 spatially varying environmental factors such as soil composition (Legendre et al., 2004).

158

159 **2.2. Phylogenetic isolation of host trees within the surrounding canopy**

160 For each focal oak, we quantified its mean phylogenetic distance to all neighboring trees with
161 which its crown was in contact. Phylogenetic distances were extracted from published
162 phylogenies (Table S1) following procedures applied previously (Vialatte et al., 2009; Yguel et

163 al., 2011) and using phylogenetic classification (APG, 2009). In order to quantify phylogenetic
164 distance, we used the younger of the crown ages of the two lineages involved, i.e. of the two
165 ages of earliest diversification within the two lineages. For instance, we ranked the comparison
166 between oak and pine species as a comparison between two classes, Gymnosperms and
167 Angiosperms, between which the younger is approximately 140 million years old (the crown age
168 of angiosperms), and the phylogenetic distance is hence 140 million years. Thus, the younger of
169 the two crown ages represents biologically the time when the oak lineage and the other lineage
170 started to be physically and physiologically distinct from a point of view of mycorrhizal fungi or
171 of other species tightly interacting with the tree. Moreover, this age also avoids giving overly
172 weight to gymnosperms, in contrast to stem-age distance which would in many cases simply be a
173 descriptor of % of gymnosperms in the neighborhood. Overall, mean phylogenetic isolation
174 ranged from 10 to 125.66 million years, and varied continuously between these extremes.

175

176 **2.3. Abundance and activities of EMf**

177 For each focal oak tree, we took four soil samples at a distance of 0.5-1.5m from the trunk in the
178 four cardinal directions to take into account possible within-tree variation in EMf colonization or
179 activity (note that neighbouring trees were at a distance mostly superior to 2 m, often much
180 more). One sample corresponded to a soil core of 4 cm in diameter by 10 cm depth (125 cm³).
181 This depth exceeds the litter layer, but we found that 100% of the root tips were restricted to the
182 litter layer, notably the litter corresponding to the previous falls. Sampling was repeated twice,
183 before and after budburst, the 21st of March and the 21st of April 2011, respectively. Litter
184 thickness was measured for each sample. Oak roots from soil cores were soaked in tap water for
185 15 min before being gently washed. Shape and colour were used to separate oak roots from those
186 of other tree species. Moreover, genetic analyses on two root tips per sample confirmed this
187 determination (See below, verification of root tree species)

188 Oak root tips were observed in water with a stereomicroscope (x40) and the total number of root
189 tips with EMf was recorded in each sample. Abundance of root tips with EMf per tree was
190 calculated as mean of abundances in the four soil samples. Then, ten root tips with EMf were
191 analyzed for activity using the high-throughput microplate assays described by Courty et al.
192 (2005) and recently optimized by Pritsch et al. (2011). The enzymatic activities were measured
193 successively on each root tip with EMf: decomposition of cellulose and hemi-cellulose by β -
194 glucosidase (EC 3.2.1.3) and β -glucuronidase (EC 3.2.1.31), and decomposition of phenolic
195 substrates by laccase (EC 1.10.3.2) activities (Courty et al., 2007; Breda et al., 2013). As a
196 control we also considered β -glucosidase, which expression is independent to oak budburst
197 (Courty et al., 2007; Breda et al., 2013). Enzyme activities were expressed per unit of time and
198 per EMf root tips projected area as described in Pritsch et al. (2011). The mean activity of the 10
199 sampled root tips with EMf was then calculated per tree.

200 As explained in the Introduction we chose a functional approach to characterize EMf based on
201 enzymatic activity, rather than a taxonomic approach identity given that enzymatic functions can
202 vary within taxa or be constant between (Courty et al., 2005; Buée et al., 2007). Thus, EMf
203 species were not identified.

204

205 **2.4. Verification of species identity of roots**

206 Two root-tips in each soil sample were snap frozen and kept individually at -20°C in Eppendorf
207 tubes. DNA was extracted using the REDExtract-N-Amp Plant PCR Kit (XNAP, Sigma,
208 FRANCE). The oak ITS was amplified according to the procedure detailed in Courty *et al.*
209 (2008) and with oak specific primers (Oak-ITSF, 5'-CGAAACCTGCACAGCAGAACGACCC-
210 3'; Oak-ITSR, 5'-CGCGGGATTCGTGCAATTCACACC -3'). PCRs were run on a 0.8%
211 agarose gel (Bioprobe, QBiogene) in 1% Tris-Acetate-EDTA buffer and stained with Midori

212 Green (LabGene, Switzerland). The expected size of the band (320 bp) was verified with a 1-kb
213 ladder (Gibco BRL, France), confirming the species identity of the root. All 352 root tips tested
214 were identified as oak trees (*Quercus robur* or *petraea*). We note that the correct species identity
215 does not guarantee that the roots belong to the adjacent oak, but the probability that they do is
216 very high.

217

218 **2.5. Measurement of soil humidity, temperature and pH**

219 Soil humidity, temperature and pH were measured within a few cm from the root samples.
220 Humidity and temperature were measured with a wet sensor (WET-2 - WET Sensor, AT delta-T
221 devices) in March and April 2011 while pH (pH-H₂O) was measured only once between March
222 and April. Two statistical outliers of abiotic conditions data were identified graphically and
223 excluded from the analyses presented in the results section. But including or not these outliers
224 did not change the effect of phylogenetic isolation on activity of EMf (Tables S2). The mean, the
225 standard deviation and the range of the soil temperatures are respectively: 11.09, 1.79 and 10.20
226 in March and 16.47, 2.84 and 16.60 in April. The mean, the standard deviation and the range of
227 the soil humidity are respectively: 33.07, 11.47 and 60.80 in March, and 22.97, 12.29 and 57.80
228 in April. The mean, the standard deviation and the range of the soil pH are respectively: 4.03,
229 0.24 and 1.14.

230

231 **2.6. Measurement of air temperature and humidity**

232 Temperature and humidity were measured from the 25th March to the 30th April 2011. We used a
233 sensor placed in the middle of the crown and in a mesh bag under a branch, protected from
234 precipitation as well as sun (DS1923 Hygrochron Temperature/Humidity Logger iButton, 8KB
235 Data-Log Memory), with hourly records, averaged per day and summed per month. Four sensors

236 did not work at all and three were lost, thus 7 trees were excluded from all analysis with
237 temperature and humidity of air (n=15 for all measurements).The mean, the standard deviation
238 and the range of the air temperatures are respectively: 85.61, 1.76 and 6.61 in March and 412.55,
239 9.19 and 31.09 in April. The mean, the standard deviation and the range of the air humidity are
240 respectively: 83.53, 21.23 and 94.60 in March and 72.75, 10.70 and 48.72 in April.

241

242 **2.7. Inferring variation in soil fertility for trees and microbes**

243 We used an integrative approach to infer variation of the nutritional soil status in which the oaks
244 and the mycorrhizal fungi were growing. A site dominated by phylogenetically distant
245 neighbours may be characterized by abiotic baseline conditions that are suboptimal, regardless
246 of the impact of the neighbourhood on the litter. First, we used the chemical composition of oak
247 leaves; A low C/N ratio of tree leaves indicates a high nutritional status of the leaves and hence
248 of the soil in which the tree is growing. We studied C,N and C/N ratios both in the fresh leaves
249 in spring and in the fresh litter of the oaks. Both types of samples led to the same results and we
250 only present the latter as it better integrates across the entire year. Fresh litter of the oak trees
251 was sampled during autumn 2010 by gently shaking branches, i.e. the sampled leaves were in
252 turn of abscission and leaves had not touched the ground and its decomposers at the ground.
253 Carbon and nitrogen concentrations and carbon isotope ratios of litters were measured by dry
254 combustion on a NA 1500 elemental analyser (Carlo Erba, Rodana, Italy).

255 Second, a high microbial biomass of the soil may be related to high soil nutritional status, and
256 high microbial activity or biomass of the soil is directly related to high carbon mineralization
257 (Doran and Parkin, 1994). Soils were sampled in August 2011 and February 2012 under each
258 tree. Microbial biomass of the soil was analyzed using the substrate-induced respiration (SIR)
259 method (Anderson and Domsch, 1978). The microbial respiratory response was measured in an

260 electrolytic O₂ microcompensation apparatus at hourly intervals for 24 h at 22°C (Scheu, 1992).
261 Microbial activity (basal respiration; mg O₂ h⁻¹g⁻¹ dw) was determined without substrate. The
262 mean, the standard deviation and the range are respectively 1.17, 0.45 and 2.22 for nitrogen
263 concentration, 49.50, 3.11 and 15.35 for carbon concentration, 45.53, 10.69 and 4.40 for the ratio
264 C/N, 23.50, 15.16, 59.61 for microbial activity in February, 2453.82, 1782.61, 7479.78 for
265 microbial biomass in February and 15.01, 5.10, 21.24 for microbial activity in August and
266 1781.76, 655.39, 2487 for microbial biomass in August.

267

268 **2.8. Budburst phenology**

269 Budburst phenology was monitored from the 15th March to the end of budburst in 2011, by
270 scoring the phenological state of 10 random apical buds from the upper layer of the crown of
271 each sampled oak, every 3.5 days. Phenological state corresponds to a three-rank scale described
272 in Wesolowski and Rowinski (2008). Then, we calculated the budburst phenology as the number
273 of days required to reach the score indicating full budburst for all buds. The sampling procedure
274 is detailed in Yguel et al. (2011). The effect of tree pair and the effect of the focal oak species on
275 budburst phenology were non-significant (respectively d.f. = 11; F=1.10; *p*=0.43 and d.f. = 20;
276 F=2.66; *p*=0.11). Hence, these variables were not included in further analyses.

277

278 **2.9. Statistical analysis**

279 We used simple regression analyses to test the effect of phylogenetic isolation of the host, the
280 effect of the abiotic soil conditions (i.e. soil temperature, soil humidity, soil pH, litter thickness
281 and more generally all factors related to tree pair) and the effect of focal oak species (i.e. *Q.*
282 *petraea* or *Q. robur*) on abundance and activity of EMf. In this and all further analyses we
283 confirmed that residuals approached normality and homoscedasticity We also performed

284 multiple linear regressions in order to explore the effect of phylogenetic isolation on abundance
285 and enzymatic activity of EMf while accounting for the effect of other co-variables. Only co-
286 variables that scored at least marginally significant in simple regression analyses were included
287 in the latter multiple regression analyses. In multiple regression analyses, standardized regression
288 coefficients are presented instead of unstandardized regression coefficient, because the former
289 allow a better comparison between the effects of independent variables (Schiezeth, 2010).As
290 month had an effect on most of the variables, data from March and April were analyzed
291 separately.

292 We tested the effect of phylogenetic isolation, EMf abundance and enzymatic activity on
293 budburst using simple regression analyses. We tested the interaction effects between
294 phylogenetic isolation and activity of EMf enzymes on budburst to investigate the hypothesis of
295 compensation of a negative effect of phylogenetic isolation by increased EMf activity. Contrary
296 to the other hypotheses this hypothesis is one tailed and so were the tests of the interaction terms
297 (Tab. 1). It was not possible to incorporate in the same model all possible explanatory variables,
298 i.e. phylogenetic isolation, all enzymatic activities, abiotic factors and their respective
299 interactions due to strong multicollinearity and limited sample size. We hence made an effort to
300 reduce the number of covarying independent variables in the model explaining budburst
301 phenology. We discarded the abiotic factors as in simple regression analyses they had a lower
302 explanatory power than either phylogenetic isolation or enzymatic activities. Due to strong
303 correlations among activities of different enzymes, we calculated a separate model for each
304 enzyme separately. We only retained enzymes for which the activity was significantly correlated
305 with budburst phenology in simple regression analyses. Even in these models multicollinearity
306 among independent variables was still strong. In order to decrease the remaining collinearity, we
307 transformed data by centering the enzymatic activities, phylogenetic isolation and the interaction

308 term by their own means. Using these transformed data increased the tolerance values (Table S3)
309 and hence decreased collinearity but did not change the significance of the interactions term.

310 We used the method described in Aiken & West (1991) to illustrate graphically the interaction
311 effect between two quantitative predictors. This method uses the unstandardized coefficients of
312 the regression model including the interaction, i.e. $Y = a + b_1X + b_2Z + b_3X*Z$, with a the
313 intercept, b_1 the regression coefficient of the phylogenetic isolation, b_2 , the regression coefficient
314 of the enzymatic activity, b_3 the regression coefficient of the interaction term, Y the budburst
315 phenology, Z the enzymatic activity and X the phylogenetic isolation. The equation was then
316 rearranged in order to obtain a linear equation to be used for graphical illustration: $Y = (b_1 + b_3Z)$
317 $X + (a + b_2Z)$. Three values of Z were used to represent medium, low and high enzymatic
318 activity: the mean of observed enzymatic activity, plus and minus the standard deviation
319 respectively. Phylogenetic isolation is given as X axis, covering the observed values from 10 to
320 125.5 million years. We predicted Y (budburst) for each of 50 levels of phylogenetic isolation
321 separated by an increment of 2.31 million years (i.e. $(125.5-10)/50=2.31$).

322 All statistical analyses were performed using Statistica Version 9.0 (Statsoft, Maisons-Alfort,
323 France).

324

325

326 **3. Results**

327 **3.1. Abundance of EMf increased with phylogenetic isolation**

328 Abundance of EMf (recorded in the litter layer only) increased with increasing phylogenetic
329 isolation of host trees in simple regression analyses. This relationship was significant in April and
330 marginally significant in March (Fig. 1; Table S4a). Phylogenetic isolation, however, explained
331 only 16 and 18% of the variation of the EMf abundance. Focal oak species (*Q. robur* vs. *Q.*

332 *petraea*) had also a significant effect on EMf abundance in March but not in April (Table
333 S4). There was no significant effect of nutritional status of fresh oak litter, or of soil microbial
334 biomass or activity on ectomycorrhizal abundance (Table S4). In the multiple regression analyses
335 including environmental condition as covariables, phylogenetic isolation had a significant effect
336 on EMf abundance in April in all models. In March, the effect of phylogenetic isolation on EMf
337 abundance was marginally significant with focal oak species as co-variable and non-significant
338 with focal oak species and soil humidity as co-variables (Table S4b, S4c). Phylogenetic isolation
339 was always among the two most important explanatory variables of EMf abundance (see
340 standardized regression coefficients in Table S4b, S4c).

341

342 **3.2. Enzymatic activities of EMf increased with phylogenetic isolation**

343 In simple regression analyses, the laccase activity in March and April, and the β -glucuronidase
344 activity in April only significantly increased with the phylogenetic isolation of host trees (Fig. 2a,
345 2b, tables S5a, S5d). Phylogenetic isolation had no significant effect on β -glucosidase
346 activity, which is unrelated to budburst (Figure 2c and Table S5g). None of the enzymatic
347 activities were significantly related to nutritional status of fresh oak litter, microbial biomass or
348 activity with one exception: strangely soil microbial activity in February has a significant effect
349 on β -glucuronidase activity in March (See table S5a, c, d, g). After removing two outliers, soil
350 humidity had a significant effect on β -glucuronidase activity in April and laccase activity in
351 March and April (Tables S2a). Soil pH had also a significant effect on β -glucuronidase in April
352 and on laccase activity in March (Table S2b). Air temperature had a significant effect on β -
353 glucuronidase activity in April, and marginally significant on laccase activity in March
354 (Tables S5a, S5d). Humidity of air had a marginally significant effect on β -glucuronidase and β -
355 glucosidase activity in March and a significant effect on laccase in April (Tables S5a, S5d, S5g).

356 However, in multiple regression analyses accounting for abiotic variables and phylogenetic
357 isolation of the host tree as independent variables, phylogenetic isolation was the only predictor
358 which had a significant effect on β -glucuronidase activity in April, and on laccase activity in
359 March and April (Tables S5b, S5c, S5e, S5f). Removing the two outliers did not change the
360 results; in multiple regression analyses, phylogenetic isolation was still the only predictive
361 variable which had a significant effect on β -glucuronidase activity in April and laccase activity
362 in March and April (Tables S2c, S2d, S2e, S2f). The effect of phylogenetic isolation on β -
363 glucosidase activity remained non-significant in March and in April (Tables S5h, S5i).
364 Phylogenetic isolation was always the variable with the strongest effect on laccase and
365 glucuronidase activities (see standardized regression coefficients in tables S5b, S5c, S5e, S5f).

366

367 **3.3. Budburst phenology was delayed with phylogenetic isolation**

368 In simple regression analysis, budburst phenology was significantly delayed with increasing
369 phylogenetic isolation of host trees (Fig. 3, Table S6). The low r^2 may be explained by the lack of
370 experimental control and unknown environmental variation and the fact that budburst was a
371 semi-quantitative variable, with three categories, recorded every 3.5 days, reducing inevitably
372 the explicable variance. Budburst was also significantly delayed with increasing laccase activity in
373 March and April, and also with increasing β -glucuronidase activity in April (Table S6).
374 Inversely, budburst phenology was accelerated marginally significantly with increasing air
375 temperature in March and April (Table S6). We note that in a prior study in 2010 we observed
376 the same effect of phylogenetic isolation but not of air temperature on budburst (Yguel et al.,
377 2014 in press). Thus the effect of phylogenetic isolation on budburst, though of limited R^2 , is
378 consistent across year and could not be due to year to year fluctuation (See Yguel et al., 2014 in

379 press). Besides, there was no significant effect of nutritional status of fresh oak litter, soil
380 microbial biomass or activity on budburst phenology (Table S6).

381

382 **3.4. Enzymatic activity of EMf partly compensated for the delay of budburst in** 383 **phylogenetically isolated trees**

384 We performed multiple regression analyses including an interaction term between phylogenetic
385 isolation of host trees and the enzymatic activities of EMf that were significant in simple
386 regression analyses (i.e. laccase activity in March and April; β -glucuronidase in April). The
387 interaction between laccase activity in April and phylogenetic isolation had a significant effect on
388 budburst phenology (Table 1). Figure 4 illustrates the direction of the interaction. Budburst was
389 far less delayed in phylogenetically isolated oaks when they showed high laccase activity. No
390 other tested interactions were significant.

391

392 **3.5. Most effects significant phylogenetic isolation cannot be fully explained by a lower** 393 **percentages of oaks in the neighbourhood of the focal tree.**

394 We explored whether the significant effects ($p \leq 0.05$) of phylogenetic isolation described above
395 could be explained simply by a reduction in the percentage of oaks in phylogenetically isolated
396 neighbourhoods. For this purpose we included percentage oaks as a covariable in the
397 corresponding analyses (plus percentage of oaks x laccase activity in the analysis of possible
398 compensatory effects). We found that in two cases phylogenetic isolation became non-significant
399 and less important than percentage of oaks after inclusion of percentage of oaks: on laccase
400 activity in March and tree budburst (Tables S7 c, e). For these two processes phylogenetically
401 isolation hence operates primarily via the absence of very closely related individuals (i.e. other
402 *Quercus*). In one case phylogenetic isolation became non-significant but more important than

403 percentage oaks: EMf abundances in April (Table S7. a). In three cases the effect of phylogenetic
404 isolation remained significant: on β -glucuronidase activity in April and on laccase activity in
405 April, and, most interestingly, the interaction term laccase activity x phylogenetic isolation on
406 budburst (Tables S7. b, d, f, g). Overall, the effect of phylogenetic isolation on abundance and
407 enzymatic activity in April represent more than just a dilution of oaks. While budburst delay is
408 more due to a dilution of oaks, the compensatory effect of enzymatic activity on budburst is
409 triggered by phylogenetic isolation.

410

411

412

413 **4. Discussion**

414 In our study, EMf abundance increased significantly (although only moderately) with the
415 phylogenetic isolation of a host tree. The effect of phylogenetic isolation was much stronger on
416 enzymatic activity involved in C and N mobilization during budburst while an enzyme not
417 related to budburst was not affected. These EMf were entirely in the litter layer, formed by the
418 oak and its respective neighbours. Phylogenetic isolation also delayed budburst but this effect
419 was due to the dilution of oaks. Interestingly, the increase in EMf enzymatic activity appeared to
420 partly compensate for the negative effect of phylogenetic isolation on budburst phenology. Most
421 of these effects of phylogenetic isolation could not be entirely explained by an effect of
422 percentage of oak neighbors, notably the compensatory effect of laccase activity on delayed
423 budburst in phylogenetically distant neighborhoods.

424

425 **4.1. Why should EMf abundance and enzymatic activities increase with phylogenetic**
426 **isolation ?**

427 Phylogenetic isolation might have affected EMf via changes in abiotic or biotic conditions,
428 which indeed varied strongly among trees (Methods). Abiotic conditions might in part reflect the
429 baseline environments dominated by phylogenetically distant species rather than the impact of
430 the neighbours themselves. First, we indeed found that with increasing phylogenetic
431 isolation, soil humidity, air temperature and pH decreased (Table S8). These abiotic factors may
432 indeed affect abundance and activity of EMf (Bago, 1998; Courty et al., 2008). Nevertheless,
433 these effects of abiotic factors were probably negligible compared to biotic factors since effects
434 of abiotic factors were less significant than those of phylogenetic isolation and not significant
435 anymore when accounting for phylogenetic isolation. Second, a dominance of phylogenetically
436 distant neighbours might, theoretically, reflect a low soil fertility for our focal oaks and low soil
437 fertility in turn might trigger an increase in EMf abundance and activity. However we found
438 neither a change of soil-fertility indicators (i.e. nutritional status of oak leaves, soil microbial
439 biomass/activity) with phylogenetic isolation nor a clear effect of these variables on mycorrhizal
440 abundance or activity (see Table S8). Thus, a difference in fertility is unlikely in order to explain
441 the increasing activity and abundance of EMf in phylogenetically isolated trees.

442 In our study, biotic factors appear hence to be the most important factors affecting abundance
443 and enzymatic activities of EMf. Increasing phylogenetic isolation of host trees corresponds to a
444 change in neighboring tree species composition. This change affects litter composition which
445 could in turn modify the composition and activity of EMf communities (Conn and Dighton,
446 2000; Tedersoo et al., 2003; Buee et al., 2007). Increasing phylogenetic isolation from
447 neighboring species may correspond to an increasing difference in chemical composition
448 between oak litter and that of tree neighbors. This is for instance the case with gymnosperm
449 neighbours compared to angiosperm neighbours, both occurring in our study system. Litter from
450 angiosperm species is known to be more easily decomposed than that of gymnosperm species
451 (Weedon et al., 2009; Osono, 2011). In particular, lignin (Osono, 2011) and hemicellulose

452 (Weedon et al., 2009) decompose more slowly in gymnosperms than in angiosperms. Also,
453 lignin concentration is often higher in gymnosperms compared to angiosperms (Weedon et al.,
454 2009) whereas the opposite is true for phosphorus or nitrogen (Weedon et al., 2009). We
455 therefore suggest that both the quality and the decomposition rate of the litter decreased with
456 increasing phylogenetic isolation of oak trees. This was also supported in our study by the fact
457 that litter thickness also increased with increasing phylogenetic isolation of host trees ($p \leq 0.03$,
458 results not shown). Phylogenetic isolation might hence decrease the mobility of carbon in
459 cellulose, hemicellulose and lignin, which should necessitate increasing enzymatic activity
460 related to cellulose, hemicellulose and lignin degradation (Colpaert and van Tichelen, 1996;
461 Conn and Dighton, 2000). This is actually what we observed: enzymatic activity strongly
462 increased with phylogenetic isolation. We note that the relationship between phylogenetic
463 isolation and enzymatic activity was not solely due to an increase in abundance of gymnosperm
464 neighbours. In fact, studying only the oaks exclusively surrounded by angiosperm neighbours
465 gave the same results in April (Table S9).

466 Overall the effect of phylogenetic isolation on enzymatic activity appears to be more related to
467 functional differences reflected by phylogenetic distance in general, including among
468 Angiosperms. It is not only the difference between gymnosperms and angiosperms that
469 counts. Nevertheless, we acknowledge that our results might be specific to our focal species,
470 oaks, which may require more and invest more into support by EMf than other tree species.
471 Studies on other host-plant species are therefore required to verify the generality of our findings.
472 Moreover, future research will need to identify the precise functional traits conveying the effect
473 of a phylogenetically distant neighbourhood, including little known physiological and root traits
474 that may influence interactions of trees with mycorrhizal fungi. Finally, further soil parameters
475 may help to definitively conclude about the role of abiotic baseline conditions on which distantly

476 related neighbours dominate vs. the deterioration in conditions (e.g. litter composition) caused by
477 these neighborhood.

478 Besides litter decomposability there might be biotic factor that could explain the positive
479 relationship between phylogenetic isolation and EMf abundance. Natural enemies of EMf, i.e.
480 fungivores, might have been less abundant around phylogenetically isolated host trees. Actually,
481 fungivores of EMf associated with neighbouring phylogenetically distant tree to might not accept
482 EMf associated with oak or, if they do, might suffer reduced fitness and population growth
483 (Bertheau et al., 2010). On the other hand, fungivores specialized on oak EMf might not
484 penetrate phylogenetically distant neighborhoods (see also Prinzing, 2003). Such reduced
485 abundance of consumers and associated “consumption” in phylogenetically isolated trees may be
486 equivalent to the reduced insect herbivore abundance and herbivory (Vialatte et al., 2009; Yguel
487 et al., 2011).

488 Finally, the increased EMf and activity on roots of phylogenetically isolated trees might simply
489 reflect an increased microbial activity of these litters fertility. However, delayed budburst in a
490 more active litter is little plausible. Moreover, we found no relationship between phylogenetic
491 isolation and microbial biomass or respirational activity in the litter (unsigned $t < 1.34$, $p > 0.19$,
492 results not shown), or between either of the latter and EMf abundance or enzymatic activity
493 (unsigned $t < 1.57$, $p > 0.13$, except for a negative relationship microbial activity vs. EMf
494 glucuronidase activity (April at $t = -2.2$, $p = 0.041$, results not shown). This, tentatively,
495 suggests that denser and more active mycorrhiza colonizers of roots are more than a random
496 sample from the ambient microbial litter community.

497

498

499 **4.2. Why budburst phenology was delayed with phylogenetic isolation, and how this might**
500 **be compensated by higher enzymatic activity of EMf?**

501 Carbon sources are required to achieve budburst but carbon reserves in oak are largely depleted
502 before budburst (Courty et al., 2007). Hence, oaks need additional source of carbon to achieve
503 budburst and associated EMf could partly mobilize this missing carbon (Courty et al., 2007;
504 Bréda et al., 2013). As previously mentioned, phylogenetic isolation of trees was probably
505 associated with a change in litter composition that resulted in a lower decomposition rate of the
506 litter, which in turn delayed budburst. In addition, phylogenetic isolation may be associated with
507 a deterioration of microclimatic conditions during budburst such as stronger shading under
508 gymnosperm crowns. Again this would render budburst more difficult, limit the photosynthetic
509 activity of budding leaves and increase the need for soil-derived carbon. Air temperatures are
510 indeed related to phylogenetic isolation of crowns in April (Table S8) and we found that
511 budburst can be related to air temperature (Table S6). However, as we state in the results, the
512 effect of air temperatures was not consistent across years. Regardless of the exact mechanism by
513 which phylogenetic isolation affects budburst, increasing enzymatic activities of EMf might
514 partly compensate for the delaying effect of phylogenetic isolation on budburst time. This is
515 what we found for laccase: increased laccase activity decreased the negative effect of
516 phylogenetic isolation on budburst. Such partial compensation of the effect of phylogenetic
517 isolation on the physiological performance of trees during budburst may dampen any farther-
518 reaching effects of phylogenetic isolation on tree growth (and, consistently, Yguel et al., unpubl.,
519 find such effects). However, the observed delay of 3-4 days in budburst between phylogenetically
520 isolated and non-isolated oaks could nevertheless have other major consequences by altering
521 competitive balance between plant species and thereby geographic distribution of tree species
522 (Vitasse et al., 2013).

523

524 **4.3. Why budburst was still delayed?**

525 Budburst was still delayed in phylogenetically isolated trees. Several reasons might explain this
526 result. On the one hand, EMf might be unable to entirely compensate for the deterioration of
527 abiotic and biotic conditions triggered by phylogenetic isolation. On the other hand, trees
528 generally have to reward EMf for their services (for arbuscular mycorrhizal plants: see Kiers and
529 Van der Heijden, 2006; Kiers et al., 2011). Hence, trees might have to “pay back” more for the
530 high activity of their EMf under high phylogenetic isolation than for the low activity of EMf
531 under low phylogenetic isolation. High costs for sustaining interactions with mutualists may
532 limit tree performance and delay budburst.

533

534 **4.4. What are the possible evolutionary implications and future directions?**

535 At least for mixotrophic or heterotrophic plants, our study suggest that phylogenetic isolation of
536 host plants may enhance the recruitment and activity of mutualists in response to the
537 deterioration of environmental conditions. Phylogenetic isolation from neighbours can be
538 interpreted in terms of niche evolution. Many lineages show phylogenetic conservatism in
539 species niches (Wiens et al., 2010 or “signal” sensu Losos 2008), including the flora considered
540 in our study (Prinzing et al., 2001). In case of such phylogenetic conservatism we expect that the
541 ancestral niche is dominated by closely related species and a non-ancestral one is dominated by
542 distantly related species. A plant colonizing such a non-ancestral environment would hence find
543 itself phylogenetically isolated from its neighbours. If leaving the ancestral niche exposes an
544 individual to a deterioration of its environmental conditions, we might hypothesize that a
545 stronger support from mutualists is then required for plants to colonize a new niche (Brundett et
546 al., 2002). Such support from mutualists may be a case of niche construction i.e. a process that
547 improves the response to a niche attribute affecting the fitness of individuals (Kylafis and

548 Loreau, 2011). In our study, niche construction was likely achieved by increasing investment
549 into interactions with EMf that decompose litter and hence facilitate nutrient uptake. Inversely,
550 EMf may benefit from host plants breaking with niche conservatism due to increased investment
551 of energy by host-plants into the support by mutualists. This is consistent with the idea that
552 mutualistic interactions between fungi and plants have evolved particularly during colonization
553 of new niches (Prinzing, 1999; Brundrett et al., 2002).

554

555 **5. Conclusion**

556 Here, we study a situation in which oak individuals converge with individuals of distantly related
557 species onto the same local environment. Success of such oaks would, theoretically, contribute to
558 an evolutionary expansion of niches of oaks towards niches of distantly related species. Our
559 study shows that EMf contribute to this success. The evidence remains correlative and
560 experiments manipulating mycorrhiza across the full life span of trees are needed to identify the
561 outcome of mutualist support for tree fitness. Moreover, future studies have to investigate
562 whether more intense interactions between EMf species and trees in the new niche involve the
563 same EMf species as in the ancestral niche, or whether new associations between trees and EMf
564 species are being formed. In other words to answer the following question: Do ancestral partners
565 help their hosts to colonize a new niche - or does the new niche provides colonists with the
566 partners they need to succeed in their colonization?

567

568 **Acknowledgements**

569 We thank Frederic Jean for his help in sampling in 2010 and 2011, Amaury Brault for the
570 phenologymeasurement in 2010, and the Office National des Forêts for logistic support. We also
571 thank Alain Roques for his useful comments on our study. This work is supported by an

572 ACOMB grant from the Région Bretagne, an ATIP grant from CNRS, and a doctoral grant from
573 Région Bretagne and CNRS. Pierre-Emmanuel Courty gratefully acknowledges financial support
574 from the Swiss National Science Foundation (grant N° PZ00P3_136651). Benjamin Yguel
575 acknowledges additional funding by sDiv, the Synthesis Centre for Biodiversity Sciences - a unit
576 of the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig (DFG
577 FZT 118).

578

579

580 **References**

- 581 Anderson, J. P. E., and K. H. Domsch. 1978. Physiological method for quantitative measurement
582 of microbial biomass in soils. *Soil Biology & Biochemistry* 10:215-221.
- 583 Angiosperm Phylogeny Group. 2009. "An update of the Angiosperm Phylogeny Group 43
584 classification for the orders and families of flowering plants: APG III", *Botanical Journal of*
585 *Linnean Society* 161, 105-121.
- 586 Aiken, L.S., West, S.G., 1991. *Multiple regression: Testing and interpreting interactions*.
587 Newbury Park, London, Sage.
- 588 Bago, B., Azcon-Aguilar, C., Piche, Y., 1998. Architecture and developmental dynamics of the
589 external mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown under
590 monoxenic conditions. *Mycologia*90, 52-62.
- 591 Bertheau, C., Brockerhoff, E.G., Roux-Morabito, G., Lieutier, F., Jactel, H., 2010. Novel insect-
592 tree associations resulting from accidental and intentional biological 'invasions': a meta-analysis
593 of effects on insect fitness. *Ecology Letters*13, 506-515.
- 594 Breda, N., Maillard, P., Montpied, P., Brechet, C., Garbaye, J., Courty, P.E., 2013. Isotopic
595 evidence in adult oak trees of a mixotrophic lifestyle during spring reactivation. *Soil Biology and*
596 *Biochemistry*58, 136-139.
- 597 Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. *New*
598 *Phytologist*154, 275-304.
- 599 Buee, M., Courty, P.E., Mignot, D., Garbaye, J., 2007. Soil niche effect on species diversity and
600 catabolic activities in an ectomycorrhizal fungal community. *Soil Biology and Biochemistry*39,
601 1947-1955.
- 602 Colpaert, J.V., Van Tichelen, K.K., 1996. Decomposition, nitrogen and phosphorus mineralization
603 from beech leaf litter colonized by ectomycorrhizal or litter-decomposing basidiomycetes. *New*
604 *Phytologist*134, 123-132.

605 Conn, C., Dighton, J., 2000. Litter quality influences on decomposition, ectomycorrhizal
606 community structure and mycorrhizal root surface acid phosphatase activity. *Soil Biology and*
607 *Biochemistry*32, 489-496.

608 Courty, P.E., Pritsch, K., Schloter, M., Hartmann, A., Garbaye, J., 2005. Activity profiling of
609 ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New*
610 *Phytologist*167, 309-319.

611 Courty, P.E., Breda, N., Garbaye, J., 2007. Relation between oak tree phenology and the
612 secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and
613 during bud break. *Soil Biology and Biochemistry*39, 1655-1663.

614 Courty, P.E., Franc, A., Pierrat, J.C., Garbaye, J., 2008. Temporal changes in the ectomycorrhizal
615 community in two soil horizons of a temperate oak forest. *Applied and Environmental*
616 *Microbiology*74, 5792-5801.

617 Courty, P.E., Buee, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P.,
618 Uroz, S., Garbaye, J., 2010. The role of ectomycorrhizal communities in forest ecosystem
619 processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry*42, 679-698.

620 Dickie, I.A., and Reich, P.B., 2005. Ectomycorrhizal fungal communities at forest edges. *Journal*
621 *of Ecology* 93, 244-255.

622 Dickie, I.A., Montgomery, R.A., Reich, P.B., Schnitzer, S.A., 2007. Physiological and
623 phenological responses of oak seedlings to oak forest soil in the absence of trees. *Tree*
624 *Physiology*27, 133-140.

625 Doran J.W., Parkin T.B., 1994. Defining and assessing soil quality. In: Doran JW, Coleman DC,
626 Bezdicsek DF, Stewart BA (eds) Defining soil quality for sustainable environment. Special
627 Pub.35. Soil Science Society of America, Inc., Madison

628 Fontaine, C., Thebault, E., Dajoz, I., 2009. Are insect pollinators more generalist than insect
629 herbivores? *Proceedings of the Royal Society B-Biological Sciences*276, 3027-3033.

630 Frank, J.L., Anglin, S., Carrington, E.M., Taylor, D.S., Viratos, B., Southworth, D., 2009.
631 Rodent dispersal of fungal spores promotes seedling establishment away from mycorrhizal
632 networks on *Quercus garryana*. *Botany-Botanique*87, 821-829.

633 Ghazoul, J., Shaanker, R.U., 2004. Sex in space: Pollination among spatially isolated plants.
634 *Biotropica*36, 128-130.

635 Hacker, S.D., Gaines, S.D., 1997. Some implications of direct positive interactions for
636 community species diversity. *Ecology*78, 1990-2003.

637 Kiers, E.T., van der Heijden, M.G.A., 2006. Mutualistic stability in the arbuscular mycorrhizal
638 symbiosis: Exploring hypotheses of evolutionary cooperation. *Ecology*87, 1627-1636.

639 Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., Fellbaum,
640 C.R., Kowalchuk, G.A., Hart, M.M., Bago, A., Palmer, T.M., West, S.A., Vandenkoornhuise,
641 P., Jansa, J., Bucking, H., 2011. Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal
642 Symbiosis. *Science*333, 880-882.

643 Kylafis, G., Loreau, M., 2011. Niche construction in the light of niche theory. *Ecology Letters*14,
644 82-90.

645 Legendre, P., Dale, M.R.T., Fortin, M.J., Casgrain, P., Gurevitch, J., 2004. Effects of spatial
646 structures on the results of field experiments. *Ecology*85, 3202-3214.

647 Losos JB. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship
648 between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*11,
649 995-1003.

650 Magrath, A., Guitian, J., Larrinaga, A.R., 2011. Land-use and edge effects unbalance seed
651 dispersal and predation interactions under habitat fragmentation. *Ecological Research*26, 851-
652 861.

653 Molina, R., Massicotte, H.B., Trappe, J.M., 1992. Ecological role of specificity phenomena in
654 ectomycorrhizal plant-communities - potentials for interplant linkages and guild development.
655 *Mycorrhizas in ecosystems*. pp. 106-112. C a B International, Wallingford.

656 Morecroft, M.D., Stokes, V.J., Morison, J.I.L., 2003. Seasonal changes in the photosynthetic
657 capacity of canopy oak *Quercus robur* leaves: the impact of slow development on annual carbon
658 uptake. *International Journal of Biometeorology*47, 221-226.

659 Morris, M.H., Perez-Perez, M.A., Smith, M.E., Bledsoe, C.S., 2009. Influence of host species on
660 ectomycorrhizal communities associated with two co-occurring oaks *Quercus* spp. in a tropical
661 cloud forest. *FEMS Microbiology Ecology*69, 274-287.

662 Osono, T., 2011. Diversity and functioning of fungi associated with leaf litter decomposition in
663 Asian forests of different climatic regions. *Fungal Ecology*4, 375-385.

664 Pan, X., Cornelissen, J.H.C., Zhao, W.-W., Liu, G.-F., Hu, Y.-K., Prinzing, A., Dong, M.,
665 Cornwell, W.K. 2014 Experimental evidence that the Ornstein-Uhlenbeck model best describes
666 the evolution of leaf litter decomposability. In press in *Ecology and Evolution*

667 Parniske, M., 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature*
668 *Reviews Microbiology*6, 763-775.

669 Peay, K.G., Kennedy, P.G., Davies, S.J., Tan, S., Bruns, T.D., 2010. Potential link between plant
670 and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of
671 tropical ectomycorrhizal fungi across a plant and soil ecotone. *New Phytologist*185, 529-542.

672 Powell, J.R., Parrent, J.L., Hart, M.M., Klironomos, J.N., Rillig, M.C., Maherali, H., 2009.
673 Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular
674 mycorrhizal fungi. *Proceedings of the Royal Society B-Biological Sciences*276, 4237-4245.

675 Prinzing, A.J., 1999. Wind-acclimated thallus morphogenesis in a lichen *Evernia prunastri*,
676 *Parmeliaceae* probably favored by grazing disturbances. *American Journal of Botany*86, 173-
677 183.

678 Prinzing A., Durka W., Klotz S, Brandl R. 2001. The niche of higher plants: Evidence for
679 phylogenetic conservatism. Proceedings of the Royal Society of London Series B-Biological
680 Sciences268: 2383-2389.

681 Prinzing, A., 2003. Are generalists pressed for time? An interspecific test of the Time-Limited
682 Disperser Model. Ecology84, 1744-1755.

683 Rineau, F., Roth, D., Shah, F., Smits, M., Johansson, T., Canback, B., Olsen, P.B., Persson, P.,
684 Grell, M.N., Lindquist, E., Grigoriev, I.V., Lange, L., Tunlid, A., 2012. The ectomycorrhizal
685 fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot
686 mechanism involving Fenton chemistry. Environmental Microbiology14, 1477-1487.

687 Scheu, S. 1992. Automated measurement of the respiratory response of soil microcompartments
688 - Active microbial biomass in earthworm feces. Soil Biology & Biochemistry 24:1113-1118.

689 Schielzeth, H., 2010. Simple means to improve the interpretability of regression coefficients.
690 Methods in Ecology and Evolution1, 103-113.

691 Selosse, M.A., Richard, F., He, X.H., Simard, S.W., 2006.Mycorrhizal networks: des liaisons
692 dangereuses? Trends in Ecology & Evolution21, 621-628.

693 Shefferson, R.P., Cowden, C.C., McCormick, M.K., Yukawa, T., Ogura-Tsujita, Y., Hashimoto,
694 T., 2010. Evolution of host breadth in broad interactions: mycorrhizal specificity in East Asian
695 and North American rattlesnake plantains *Goodyera* spp. and their fungal hosts. Molecular
696 Ecology19, 3008-3017.

697 Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, Ed 3. Academic Press, Cambridge, UK

698 Tedersoo, L., May, T.W., Smith, M.E., 2003. Ectomycorrhizal lifestyle in fungi: global diversity,
699 distribution, and evolution of phylogenetic lineages. Mycorrhiza20, 217-263.

700 Tedersoo, L., Naadel, T., Bahram, M., Pritsch, K., Buegger, F., Leal, M., Koljalg, U., Poldmaa,
701 K., 2012. Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to
702 phylogeny and exploration types in an afrotrropical rain forest. New Phytologist195, 832-843.

703 Van der Heijden, M.G.A., Horton T.R., 2009. Socialism in soil? The importance of mycorrhizal
704 fungal networks for facilitation in natural ecosystems. *Journal of Ecology*97, 1139-1150.

705 Vialatte, A., Bailey, R.I., Vasseur, C., Matocq, A., Gossner, M.M., Everhart, D., Vitrac, X.,
706 Belhadj, A., Ernoult, A., Prinzing, A., 2010. Phylogenetic isolation of host trees affects assembly
707 of local Heteroptera communities. *Proceedings of the Royal Society B-Biological Sciences*277,
708 2227-2236.

709 Vitasse, Y., Francois C., Delpierre N., Dufrene E., Kremer A., Chuine I., and Delzon S. 2011.
710 Assessing the effects of climate change on the phenology of European temperate trees.
711 *Agricultural and Forest Meteorology* 151:969-980.

712 Wesolowski, T., Rowinski, P. 2008. Late leaf development in pedunculate oak *Quercus robur*:
713 An antiherbivore defence? *Scandinavian Journal of Forest Research*23, 386-394.

714 Wiens J.J., Ackerly D.D., Allen A.P., Anacker B.L., Buckley L.B., Cornell H.V., Damschen E.I.,
715 Davies T.J., Grytnes J.A., Harrison S.P., Hawkins B.A., Holt R.D., McCain C.M., Stephens P.R.,
716 2010.Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology*
717 *Letters*13: 1310-1324.

718 Yguel, B., Bailey, R., Tosh, N.D., Vialatte, A., Vasseur, C., Vitrac, X., Jean, F., Prinzing, A.,
719 2011. Phytophagy on phylogenetically isolated trees: why hosts should escape their relatives.
720 *Ecology Letters*14, 1117-1124.

721 Yguel B., Bailey R. I., Villemant C., Brault A., Jactel H., Prinzing A. 2014. Insect herbivores
722 should follow plants escaping their relatives. In press in *Oecologia*

723

724

725 **SUPPORTING INFORMATION**

726

727 **Appendix S1.** Details on the Rennes Forest and the species composition of trees surrounding the
728 focal oaks studied.

729 **Table S1.** Phylogenetic distance between oak and neighboring species

730 **Table S2.** Relationship between soil humidity or pH and enzymatic activity excluding two
731 outliers.

732 **Table S3.** Effect of phylogenetic isolation, enzymatic activities and the interaction term on the
733 budburst phenology analysed without any transformation of the data.

734 **Table S4.** Effect of phylogenetic isolation, abiotic conditions and focal oak species on EMf
735 abundance in simple and multiple regression analyses

736 **Table S5.** Effect of phylogenetic isolation, abiotic conditions and focal oak species on enzymatic
737 activity of EMf in simple and multiple regression analyses

738 **Table S6.** Effect of phylogenetic isolation, abiotic conditions abundance and enzymatic activity
739 of EMf on budburst phenology in simple regression analyses

740 **Table S7.** Variables significantly related to phylogenetic isolation: the effect of accounting for
741 percentage of oaks as co-variable.

742 **Table S8.** Effect of phylogenetic isolation on abiotic conditions

743 **Table S9.** Effect of phylogenetic isolation on enzymatic activity of EMf in March and April, in
744 simple regression analyses, considering only trees without gymnosperm neighbors.

745

746 **TABLES AND FIGURES**

747

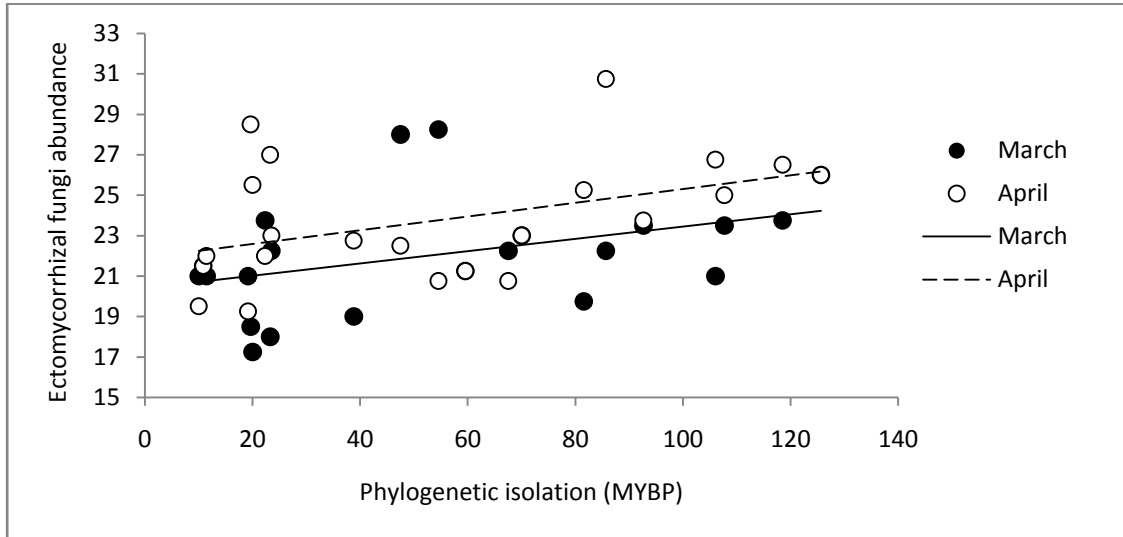
748 **Table 1.** Multiple regression analyses explaining budburst phenology by the effect of
 749 phylogenetic isolation and enzymatic activity of EMf, and the interaction term between both.
 750 Enzymes considered show significant relationship to budburst in simple regression analysis.
 751 Phylogenetic isolation is expressed in million of year before present (MYBP) and enzymatic
 752 activity is expressed per unit of time and per EMf root tips projected area ($\mu\text{M mm}^{-2} \text{min}^{-1}$). Data
 753 are centered by their means (see Appendix S10 for analysis of uncentered data). Tolerances
 754 characterize the mutual independence among independent variables (i.e. 1 if an independent
 755 variable is entirely uncorrelated to the other independent covariables). P-values are for one tailed
 756 hypotheses.

		<i>Effect on budburst phenology</i>				
		Df	T	P	Standardized regression coefficient	Tolerance
Model 1	Phylogenetic isolation[V1]		0.08	0.46	0.07	0.04
P=0.06	EMf β -glucuronidase (April)[V2]	18	0.55	0.29	0.46	0.05
$r^2=0.31$	V1* V2		-0.69	0.24	-0.19	0.45
Model 2	Phylogenetic isolation[V1]		0.44	0.33	0.13	0.39
P=0.03	EMf Laccase (March) [V2]	18	1.41	0.08	0.44	0.35
$R^2=0.37$	V1*V2		-0.72	0.24	-0.15	0.77
Model 3	Phylogenetic isolation[V1]		1.67	0.05	0.98	0.10
P=0.04	EMf Laccase (April) [V2]	18	-0.79	0.26	-0.45	0.10
$R^2=0.35$	V1*V2		-1.85	0.04	-0.50	0.48

757

758

759 **Figure 1.** Relationship between phylogenetic isolation of host trees and abundance of
760 ectomycorrhizal fungi in March and April per soil core. The statistics for these relationships are
761 respectively: d.f. = 20, $r^2 = 0.16$, $t = 1.99$, $P = 0.06$ (March); d.f. = 20, $r^2 = 0.18$, $t = 2.16$, $P =$
762 0.04 (April).



763

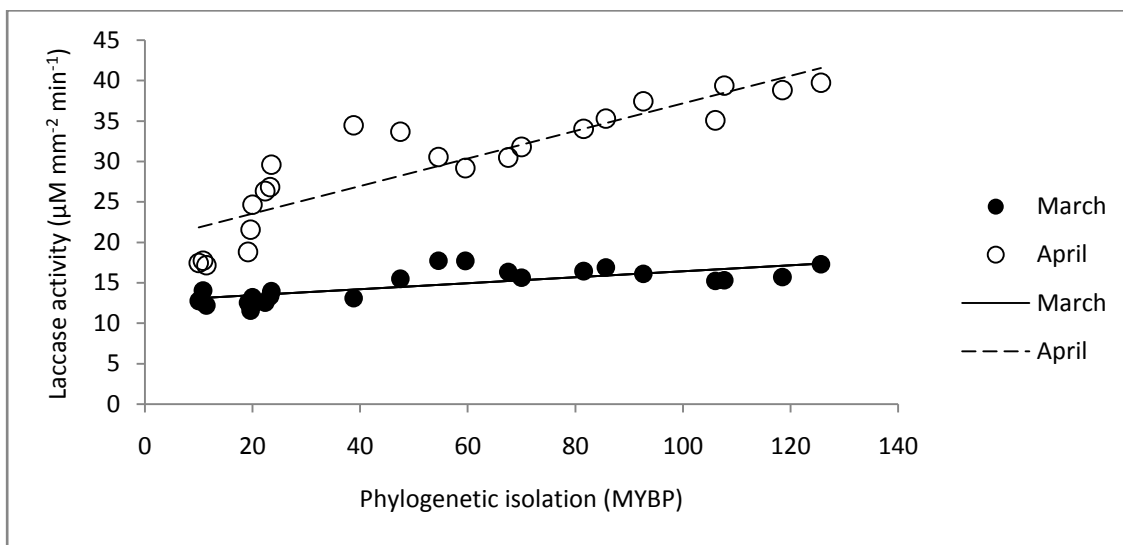
764

765

766

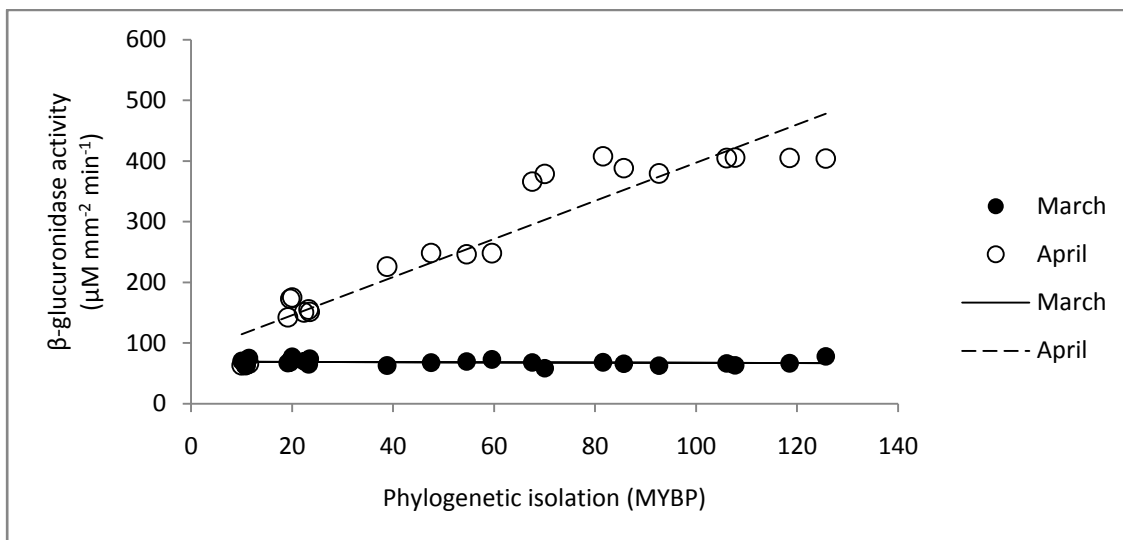
767 **Figure 2.** Relationship between phylogenetic isolation of host tree and EMf laccase activity in
 768 March and April (a), EMf β -glucuronidase activity in March and April (b), and EMf β -
 769 glucosidase activity in March and April (c). The statistics for these relationships are respectively:
 770 (a) March d.f. = 20, $r^2 = 0.54$, $t = 4.92$, $P = 8 \times 10^{-5}$; April d.f. = 20, $r^2 = 0.78$, $t = 8.61$, $P = 3 \times 10^{-8}$;
 771 (b) March d.f. = 20, $r^2 = 0.02$, $t = -0.73$, $P = 0.47$; April d.f. = 20, $r^2 = 0.89$, $t = 12.96$, $P = 3 \times 10^{-11}$;
 772 11 ; (c) March d.f. = 20, $r^2 = 0.04$, $t = -0.98$, $P = 0.33$; April d.f. = 20, $r^2 = 0.09$, $t = -1.43$, $P = 0.16$.

773 (a)



774

775 (b)

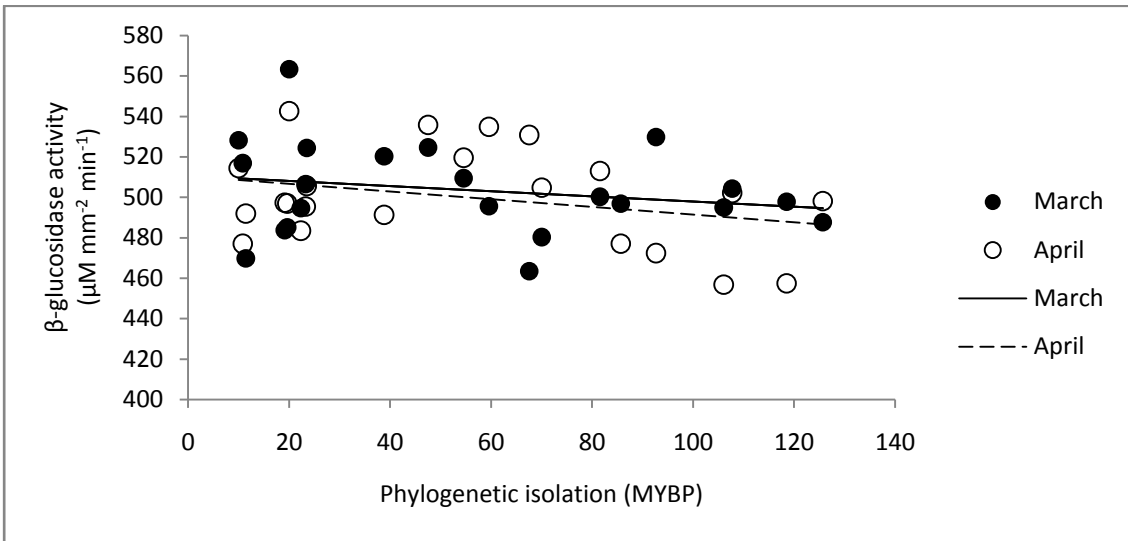


776

777

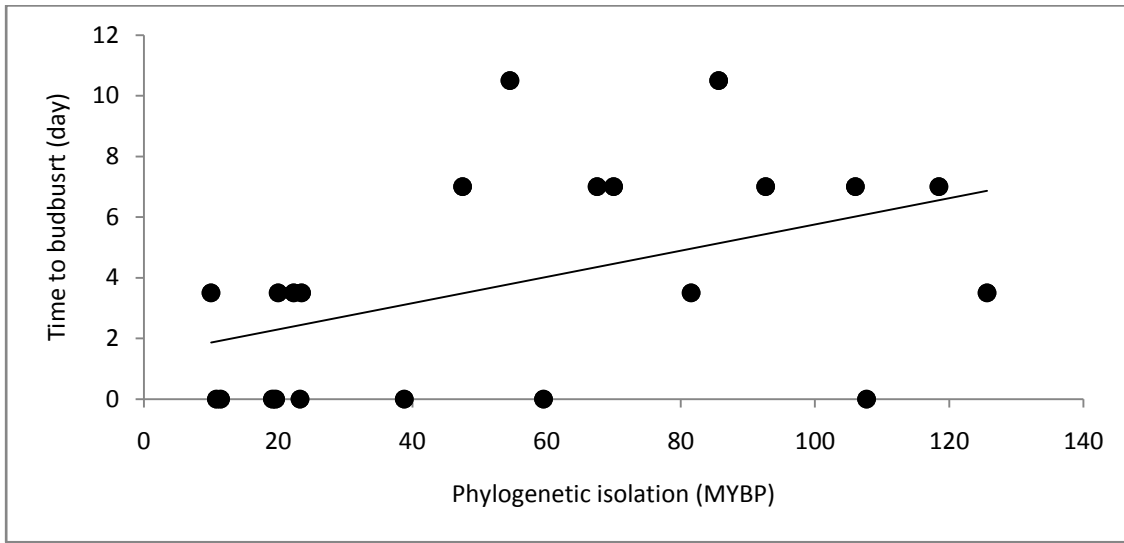
778

779 (c)



780
781
782
783
784

785 **Figure 3.** Relationship between phylogenetic isolation and budburst phenology in oak trees. The
786 statistics of the relationship are : $df=20$; $t=2.34$; $p=0.02$; $r^2=0.21$.



787

788

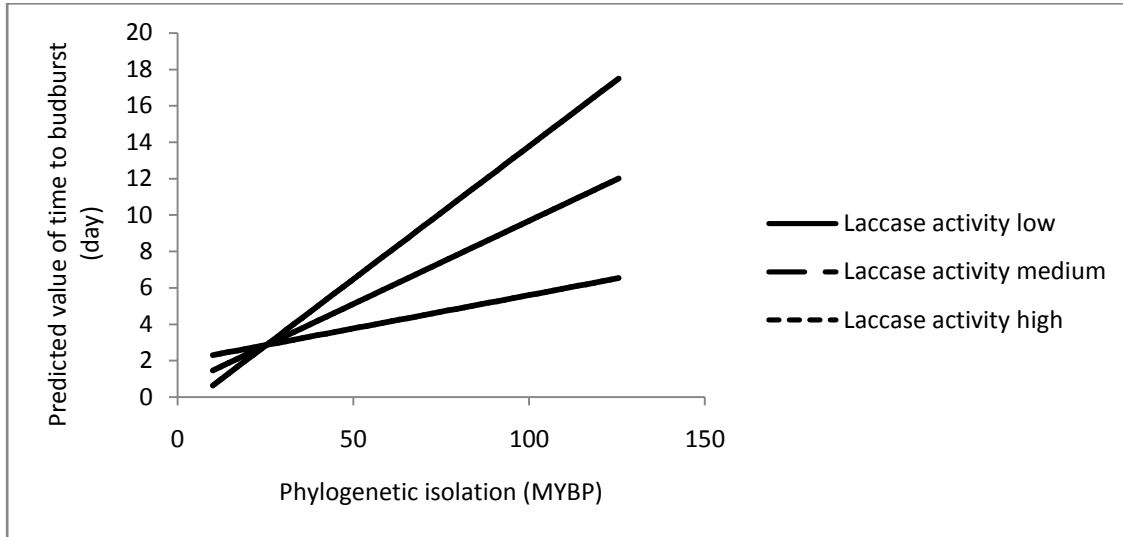
789

790

791

792

793 **Figure 4.**Representation of the statistical interaction effect between phylogenetic isolation and
794 laccase activity of ectomycorrhizal fungi in April on budburst phenology based on the non-
795 standardized regression coefficients of the variables in the interactive model (See Methods).
796 Phylogenetic isolation strongly delayed budburst in oak trees with low laccase activity See table
797 1 for tests statistics.



798
799
800
801
802