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# Temporal changes of bacterial communities in the *Tuber melanosporum* ectomycorrhizosphere during ascocarp development

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1 **Temporal changes of bacterial communities in the *Tuber melanosporum* ectomycorrhizosphere**  
2 **during ascocarp development**

3  
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34 **Abstract**

35

36 Ectomycorrhizae form a multitrophic ecosystem made of the association between tree roots, mycelium  
37 of the ectomycorrhizal fungus, and a complex microbiome. Despite the expected importance of these  
38 microorganisms for the physiology of the tree host and the functioning of the ectomycorrhizal symbiosis,  
39 few studies have carried out in depth analyses of ectomycorrhiza associated bacterial community  
40 composition and their temporal dynamics. Our objective was to investigate the composition and the  
41 dynamic from summer to winter time of bacterial communities associated with ectomycorrhizae (EcM)  
42 from *Tuber melanosporum* in a hazelnut tree *Corylus avellana* plantation. We used 16S rRNA based  
43 pyrosequencing to compare the bacterial community structure and the richness in *T. melanosporum*'s  
44 EcMs with those of bulk soil. The EcMs of *T. melanosporum* harboured distinct bacterial communities  
45 from those of the bulk soil, with an enrichment in Alpha- and Gamma-Proteobacteria. In contrast to the  
46 bacterial communities colonising truffle ascocarps that deeply varies in composition and richness during  
47 the maturation of the fruiting body and to those from the bulk soil, *T. melanosporum* EcM-associated  
48 bacterial community composition stayed rather stable from September to January. Our results fit with  
49 the recent demonstration performed on the same experimental site at the same period that a continuous  
50 supply of carbohydrates and nitrogen occurs from EcMs to the fruiting bodies during the maturation of  
51 the ascocarps, that could potentially create a stable niche in the ectomycorrhizosphere while the  
52 phenology of the tree changes.

53

54

55 **Keywords:** *Tuber melanosporum*, bacterial communities, temporal evolution, soil-ectomycorrhizae  
56 interface, ectomycorrhizosphere, 16S rRNA based pyrosequencing.

57

58 **Introduction**

59

60 Among soil microorganisms, ectomycorrhizal fungi (EcM) that form symbiotic associations with plants  
61 are important actors in terrestrial ecosystems. Owing to their ability to forage large volumes of soil  
62 where they access nutrients such as phosphorous, nitrogen (N) and inorganic compounds, to degrade  
63 organic matter, and to weather minerals, EcM fungi improve the nutrient uptake capacity of their  
64 associated trees (Smith and Read 2008; Courty et al. 2010). In exchange, trees provide the symbiotic  
65 fungi with carbohydrates that are transferred to the fungus in the ectomycorrhizae, a mixed organ  
66 formed by tree roots and fungal mycelium. This particular interface, called the ectomycorrhizosphere,  
67 connects tree roots to the surrounding soil and forms an unique environment both in terms of their  
68 physicochemistry and microbiology (Rambelli 1973; Linderman 1988). The mycorrhizosphere is  
69 comparable to the rhizosphere by its intense bacterial colonisation, but differs from the rhizosphere by  
70 the nature of the main chemicals released in soil. Especially, mycorrhizal roots are characterised by  
71 elevated concentrations of fungal derivatives such as trehalose, mannitol or N-acetyl glucosamine,  
72 which are less represented in the rhizosphere (Martin et al. 1984; Martin et al. 1985). The bacterial  
73 communities of the mycorrhizosphere interact physically with mycorrhizal fungi through the symbiotic  
74 mantle or the hyphal network, and also chemically by metabolising mycorrhizal root exudates (Frey-Klett  
75 et al. 2005; Uroz et al. 2007). Through these physicochemical characteristics, mycorrhizal fungi enrich  
76 their vicinity with specific bacterial communities capable of improving fungal and plant growth through  
77 the access to inorganic nutrients or the production of hormones and/or vitamins (Uroz et al. 2007;  
78 Deveau et al. 2010; Uroz et al. 2013).

79 Although the enrichment of specific bacterial communities in the mycorrhizosphere has been  
80 demonstrated in multiple studies, these analyses were focused only on few fungal species and mostly  
81 based on culture-dependent and fingerprinting approaches giving a partial view on the real community  
82 composition and structure (Uroz et al. 2007; Izumi et al. 2007; Burke et al. 2008; Izumi and Finlay 2011).  
83 The factors that impact bacterial selection, and the parameters that drive their community composition  
84 and diversity in mycorrhizosphere are yet to be understood. While there are indications that the  
85 ectomycorrhizal fungi themselves might impact the associated bacterial community composition, some  
86 studies suggest that fungi alone are not the only drivers in EcM bacterial community structuration (Izumi  
87 et al. 2007; Burke et al. 2008; Izumi and Finlay 2011; Uroz et al. 2012). Furthermore, seasonal  
88 variations of the associated bacterial communities (diversity, structure, and composition) are expected  
89 to occur because the transfer of photosynthates from the tree to the mycorrhizal partner has a direct  
90 impact on the fungal metabolism and is known to vary along seasons (Epron et al. 2011; Churchland  
91 and Grayston 2014). Although seasonal changes have been evidenced in the rhizosphere of various

92 plants (Rasche et al. 2011; Collignon et al. 2011; López-Mondéjar et al. 2015), the relative stability or  
93 dynamics of the EcM associated bacterial communities are not known.

94 Determining such temporal evolution of natural mycorrhizosphere bacterial communities remains a  
95 technical challenge as it requires to be able to collect samples at different time from the same  
96 mycorrhizal species. In general, tree roots are colonised by complex and dynamic EcM fungal  
97 communities that evolve along time, and thus reducing the probability to sample the same EcM species  
98 through time. To reduce this problem we took advantage of the controlled 'mycorrhization' process that  
99 reduces the variability owing to the high level of mycorrhization by a single species. We used *Corylus*  
100 *avellana* trees that were highly mycorrhized with *Tuber melanosporum* and planted in the experimental  
101 truffle orchard of Rollainville (North of France). This made possible to collect EcMs of the same species  
102 at different dates. *Tuber melanosporum* Vittad. is an Ascomycete ectomycorrhizal fungus well known for  
103 its production of hypogeous fruiting bodies (ascocarps) called Périgord black truffles. Life cycle of *Tuber*  
104 species differs from many other EcM fungi in the duration taken to develop their fruiting bodies (Le  
105 Tacon et al. 2015a). Ascocarps take several weeks to few months to grow, compared to merely few  
106 days for other well-known epigeous EcM fungi such as *Boletus* sp. or *Cantharellus* sp. The *T.*  
107 *melanosporum* ascocarp is thought to be functionally linked to its host tree through EcMs from its birth  
108 in late spring to its maturation during the following winter (Le Tacon et al. 2013; Le Tacon et al. 2015a;  
109 Le Tacon et al. 2015b). From the latter studies, we inferred that *T. melanosporum* EcMs serve as a  
110 nutritional pipeline during the entire duration of the ascocarp development (Le Tacon et al. 2013; Le  
111 Tacon et al. 2015b).

112 We demonstrated previously that *T. melanosporum* ascocarps are heavily colonised by complex  
113 bacterial communities and the community compositions evolved along the maturation of the ascocarps  
114 (Antony-Babu et al. 2014). We also demonstrated that the ectomycorrhizosphere of *T. melanosporum*  
115 was highly colonised by complex bacterial communities that differed significantly in terms of structure,  
116 diversity and composition from those of the ascocarps (Antony-Babu et al. 2014); the study was made  
117 at only one sampling date (November 2010) on the Rollainville experimental truffle orchard. EcMs being  
118 connected to the ascocarp for the entire period of growth and maturation, we can wonder whether the  
119 ascocarpic and the EcM microbial communities are connected and whether they vary similarly along the  
120 ascocarp development in autumn and winter. Thus, our objective was to assess whether temporal  
121 changes occurred in the composition, richness and structure of the EcM bacterial communities along  
122 the development and maturation stages of truffle ascocarps. A subsidiary objective was to confirm that  
123 *T. melanosporum* EcMs select in its vicinity specific bacterial communities compared to the surrounding  
124 bulk soil and the ascocarps. Our strategy was to collect spatially distant soil samples and  
125 ectomycorrhizal roots under the same tree from September 2010 to January 2011, as well as ascocarps

126 (Antony-Babu *et al.* 2014). We analysed bacterial community structure, composition and diversity using  
127 16S rRNA gene amplicon pyrosequencing on EcM, ectomycorrhizosphere and bulk soil samples.

128

## 129 **Materials & methods**

### 130 **Sampling site**

131 All samples were collected under a single hazel tree (*Corylus avellana*) labelled “A11” in the truffle  
132 orchard of Rollainville (48° 21' N, 5° 44' E; Lorraine region, France) during the truffle maturation process  
133 from September 2010 to January 2011. Characteristics of the truffle orchard and soil properties are  
134 described in Antony-Babu *et al.* (2014).

135

### 136 **Sampling method and storage**

137 Three types of samples were collected: *T. melanosporum* ectomycorrhizal root tips (EcM), the soil at  
138 the interface with the EcM (EcM-I - soil adhering to the ectomycorrhizal root tip) and the surrounding  
139 bulk soil (BS). At each sampling date (28<sup>th</sup> of September 2010, 17<sup>th</sup> of November 2010, 22<sup>nd</sup> of  
140 December 2010 and 27<sup>th</sup> of January 2011), samples were collected from three or four independent  
141 locations around the tree labelled “A11” and spaced about 2 meters apart from the trunk (Table 1). The  
142 samples were stored on ice during transport to the laboratory. At the laboratory, ectomycorrhizal root  
143 tips were collected under a microscope in a laminar flow cabinet. Ectomycorrhizae were characterised  
144 both by morphotyping and using specific molecular probes as described by Paolocci (Paolocci 1999).  
145 One to 5 grams of non adhering bulk soils surrounding ectomycorrhizae (BS) were collected. Soil  
146 adhering ectomycorrhizae (ECM-I; 100 to 300 mg) were obtained using needles and forceps. All  
147 samples were stored in -80°C directly without addition of any preservative additives. A total of 48  
148 samples was obtained (Table 1).

149

### 150 **DNA extraction**

151 Due to the different type of samples considered in the study (mycorrhizal tips and soil), two different  
152 extraction methods were necessary to isolate high quality genomic DNA from the samples compatibles  
153 with 16S rRNA gene amplicon pyrosequencing (Antony-Babu *et al.* 2013). Soil samples were processed  
154 using Fast-DNA Spin kit (Q-Biogene, Heidelberg, Germany) and ectomycorrhizal samples were isolated  
155 using DNeasy Plant Mini Kit (Qiagen SA, Courtaboeuf, France), according to manufacturer’s guidelines.

156

### 157 **Barcoded pyrosequencing, DNA sequence processing and taxonomic analyses**

158 The extracted DNA samples covering different habitats (BS, ECM and ECM-I) through time were used  
159 as templates to generate 16S rRNA gene amplicon libraries. The primers 787r (xxxxx-

160 ATTAGATACCYTG TAGTCC) (Nadkarni et al. 2002) and 1073F (xxxxx-  
161 ACGAGCTGACGACARCCATG) (On et al. 1998) were used to generate PCR fragments of ca 250 bp.  
162 where "xxxxx" represents the sample identification barcode sequences. PCR amplifications were carried  
163 out with the following conditions: 94°C for 10 min, 30 cycles of 30 seconds at 94°C (denaturation),  
164 annealing temperature starting at 48°C for 45 seconds followed by 72°C for 90 seconds (extension).  
165 The reactions concluded with final extension for 10 minutes at 72°C. PCR amplicons were purified  
166 using Qiagen PCR purification kit or Qiagen gel purification kit for samples showing multiple bands.  
167 Quality of the purified PCR amplifications were checked on 1% agarose gels using the Low DNA mass  
168 ladder (Invitrogen). PCR product quantifications were performed using both gel electrophoresis and  
169 nanodrop spectrometry. The PCR barcoded amplicons were pooled into respective libraries in  
170 equimolar concentrations. Amplicon pyrosequencing was performed on the GS-FLX 454 Titanium  
171 platform of Beckman Coulter Genomics (Danvers, MA, USA) as half a run.

172 The sequences obtained were analysed using MOTHUR (Schloss et al. 2009). After demultiplexing  
173 and quality control (quality score=30). The sequences were then processed using MOTHUR suite to  
174 determine operational taxonomic units (OTUs, 97 % sequence similarity), rarefaction analysis, richness  
175 estimates Chao1 and Shannon-Weaver index. Rarefactions curves were generated using the R  
176 package ([www.r-project.org](http://www.r-project.org)). After quality trimming and denoising, sets of 16S rRNA sequences were  
177 adjusted randomly at 1,245 (corresponding to the smallest sample). Phylogenetic affiliation was  
178 performed (80% sequence similarity) with the MG-RAST platform (Metagenome Rapid Annotation using  
179 Subsystem Technology, Meyer et al. 2008) and the RDP database (Ribosomal data project, Cole et al.  
180 2009).

181

## 182 **Statistical analyses**

183 The different relative values (%) used in this study were transformed by arcsin sqrt for statistical  
184 analyses. The habitat and sampling times effects on the structure of the bacterial communities were  
185 determined by analysis of variance (ANOVA) at a threshold level of  $P=0.05$  associated with a Fisher test  
186 and analyses of similarity (ANOSIM) based on Bray-Curtis distances were performed 16S rRNA  
187 pyrosequence data using the anosim function of R Vegan package (Oksanen et al. 2015). Analysis of  
188 variance and multivariate analyses were performed with XLStat 2011 (Addinsoft, Paris, France).

189

190 **Nucleotide sequence accession numbers.** The 454 sequences generated have been deposited on  
191 the Sequence Read Archive of the NCBI under the the SAMN04103598 to SAMN04103628 and  
192 SUB291410 accession numbers.

193

## 194 **Results**

195

### 196 **Pyrosequencing results**

197 A total of 400,965 raw pyrotag reads were obtained across the bulk soil, soil-ectomycorrhiza interface  
198 and ectomycorrhiza mantle samples. After quality filtering and chimera removal 180,206 sequences  
199 remained to be analysed, showing a number of sequences per sample varying between 1,245 and  
200 8,920. To be able to compare the 48 samples, the same level of surveying effort (n=1,245 sequences  
201 per sample) was applied on each sample of the dataset. A total of 33,207 OTUs were identified after  
202 bioinformatics analyses, but a detailed analysis revealed that only 422 OTUs were represented by 10  
203 sequences or more across the samples. Regardless of the sampling date, slightly less numbers of  
204 OTUs were detected in ECM compared to the two other compartments (926 +/- 33 vs 1100 +/- 19  
205 observed OTUs ; ANOVA p<0.001). In contrast, the number of OTUs did not vary significantly between  
206 BS and ECM-I notwithstanding the sampling dates considered. Non-parametric analyses also confirmed  
207 this trend. A lower richness was predicted in the ectomycorrhizosphere compared to the bulk soil and  
208 the soil-ectomycorrhiza interface by the Shannon and InvSimpson indexes (P<0.001 ANOVA, Table 2).

209

### 210 **Comparison of bacterial communities associated to Bs, EcM-I and EcM**

211 The overall analysis revealed that the most abundant phyla that are found across different  
212 compartments were Proteobacteria (15 to 17%), Actinobacteria (17 to 20%), Bacteroidetes (8 to 10%),  
213 Firmicutes (1 to 5%), Verrucomicrobia (1 to 5 %), Acidobacteria (0.2 to 4%), Planctomycetes (0.4 to 2%),  
214 Chloroflexi (0.3 to 1%) and Chlamydiae (0.2 to 1%) as shown by the taxonomic assignment (Figure 1).  
215 Among the Proteobacteria, Alpha-proteobacteria were the most represented (4.1 to 9.4 %) followed by  
216 Delta- (2.0 to 7.1 %) Beta- (2.3 to 5.6 %), Gamma- (1.0 to 5.9 %), and Epsilon-proteobacteria (0 to  
217 0.1 %).

218 Nevertheless, the above distribution was not uniform among the compartments as highlighted by  
219 multivariate and ANOSIM analyses performed on the relative distribution of these phyla. In all the time  
220 points that were considered, the EcM compartment was distinct from those of BS and EcM-I according  
221 to the first axis of the PCoA that explained 50 % of the variance in the data at the class level (Figure 2,  
222 ANOSIM p<0.05). Such similar pattern was observed also at the genus level. Regardless of the  
223 taxonomic hierarchy used, the BS and ECM-I compartments were more closely related to each other  
224 than to the EcM compartment. When the taxonomic resolution of the analyses were increased it was  
225 observed that sequences from Alpha- and Gamma-proteobacteria and especially the sequences  
226 assigned to the *Bradyrhizobium* genus, and the Caulobacterales and Rhodospirillales orders were more  
227 prevalent in the EcM compartment than the other two (Table 3). In contrast, sequences related to Delta-



228 proteobacteria, Acidobacteria (*Acidobacterium* and *Candidatus Koribacter*) Flavobacteria  
229 (*Flavobacterium*), Firmicutes (*Bacillus*, *Finegoldia*) Verrucomicrobia (*Pedosphaera*, *Verrucomicrobium*),  
230 Planctomycetia (*Pirellula*) were less in the EcM compartment. Actinobacterial genera such as  
231 *Mycobacterium*, *Nocardioides*, *Rubrobacter* were less frequent in the EcM compartment while  
232 representatives of the genus *Thermoleophilum* were enriched in the EcMs. Furthermore, reads that  
233 were unassigned to any known taxa were more common in EcM than in the other two compartments,  
234 representing 20 to 40 % and 4 to 15% of the reads, respectively.

235

### 236 **Temporal evolution of bacterial communities associated to BS, EcM-I and EcM**

237 Richness of the bacterial communities in the three compartments considered did not vary along the  
238 sampling season regardless of the compartment considered (Table 2). However, the constituents of the  
239 bacterial assemblages differed from September 2010 to January 2011 and varied according to the  
240 compartment considered. According to PCoA, the bacterial community dynamics in the BS  
241 compartment was more affected by seasonal changes than those associated with the EcM  
242 compartment. Indeed, BS and EcM-I samples were separated by sampling time according to the second  
243 axis of the PCoA (18 % of the variance), but not the EcM one's (Figure 2). Three phyla showed  
244 statistically significant differences to temporal variations in BS and EcM-I compartments: Acidobacteria,  
245 Bacteroidetes and beta-Proteobacteria. Each of these three taxa exhibited different abundance patterns  
246 along the sampling time. Since BS and EcM-I samples showed similar patterns, only BS samples are  
247 given in figure 3a to simplify the reading. Beta-proteobacteria were highly represented in September in  
248 comparison to the other sampling dates. In contrast, Acidobacteria (*Candidatus Koribacter*) followed an  
249 opposite trend as their levels increased in November, December and January compared to September.  
250 Bacteroidetes (*Microscilla*) communities increased in November. Actinobacteria did not show  
251 significant variation at the phylum level but different significant patterns of variation were observed  
252 depending on the genus considered. While reads pertaining to the genus *Rubrobacter* were more  
253 abundant in December and January than in November, genus *Terrimonas* and *Nocardioides* were more  
254 abundant in November and December, respectively (data not shown).

255 Although a temporal differentiation in EcM communities was not explicit in the PCoA analyses, some  
256 specific genera were consistently more enriched at some periods (ANOVA,  $p < 0.05$ ). For example  
257 Actinobacterial genera *Thermoleophilum* and *Pseudonocardia* were more frequently represented in EcM  
258 than in BS and ECM-I in September and November, and in January, respectively. In contrast,  
259 Bacteroidetes representatives of the genus *Microscilla* and Actinobacteria from the genus *Nocardioides*  
260 were not detected in the EcMs collected in November and in December, respectively. Alpha-

261 proteobacteria showed a negative trend where it decreased from September to January, albeit this trend  
262 was not statistically significant (Figure 3b).

263 As some of the ascocarpic samples of Antony-Babu *et al.* (2014) were collected at the same dates  
264 (November, December and January) and using the same molecular and bioinformatic procedure, we  
265 performed comparison of the EcM and ascocarp compartments across time. Both multivariate and  
266 ANOVA analyses revealed that the EcM bacterial communities were separated from those of the  
267 ascocarp compartments (gleba and peridium) (Figure S1). Whatever the sampling date considered,  
268 Actinobacteria, Chloroflexi, Planctomycetes, Delta-proteobacteria and Verrucomicrobia appeared  
269 significantly enriched in the EcM compartment compared to the gleba ( $p < 0.05$ ; Figure 4). Significant  
270 variations were also observed between EcM and gleba compartments for other taxa such as Firmicutes,  
271 Alpha-, Beta- and Gamma-proteobacteria at specific time points (Table S1).

272

## 273 Discussion

274

275 The recent advancements of high throughput sequencing methods has led to the “re”discovery that  
276 almost all eukaryotic organisms host a complex microbiome. It is interesting to note that in most cases  
277 the composition and the activity of these microbiomes strongly impact the physiology, health and  
278 resilience to perturbations of their hosts (Bulgarelli *et al.* 2012; Goh *et al.* 2013; Shafquat *et al.* 2014;  
279 Rådecker *et al.* 2015). For example, it is now evident that the human gut and skin microbiomes  
280 influence human immunity, physiology and even behaviours (Schommer and Gallo 2013; Yano *et al.*  
281 2015). Similar studies on plant models such as *Arabidopsis thaliana* have also highlighted the strong  
282 impact of the plant microbiome on host functioning (Lebeis 2014; Panke-Buisse *et al.* 2015). In  
283 comparison to these examples, studies deciphering the composition and potential role of tree  
284 microbiomes are scarce. Although EcMs are known for decades to host specific bacterial communities  
285 that physically and metabolically interact with the symbiotic fungus and the host plant (Linderman 1988),  
286 only three studies have thoroughly analysed the microbiomes of EcMs using high throughput  
287 sequencing: the first one performed on EcMs between *Quercus petraea* and *Xerocomus pruinatus* and  
288 *Scleroderma citrinum* (Uroz *et al.* 2012), the second on EcMs of the herbaceous plant *Bistorta vivipara* –  
289 one of a few herbs that form EcMs (Vik *et al.* 2013) - and the third on EcMs between *Tuber aestivum*  
290 and *Carpinus betulus* (Gryndler *et al.* 2013). Through the study presented here we provide description  
291 of a fourth system, i.e the bacterial communities of EcMs between *T. melanosporum* and *C. avellana*.

292

293 Firstly, this study confirms the selection of specific bacterial communities in the ectomycorrhizosphere  
294 of *T. melanosporum*, as previously known for other EcM fungi (Mogge *et al.* 2000; Assigbetse *et al.*

295 2005; Frey-Klett et al. 2005; Uroz et al. 2007; Izumi et al. 2008; Calvaruso et al. 2010). Those  
296 communities differed from their respective bulk soil all through the sampling period. Bacterial  
297 communities of *T. melanosporum* ectomycorrhizosphere were characterised by the exclusion of taxa  
298 such as Acidobacteria, Flavobacteria, Firmicutes and Verrucomicrobia while other taxa among which  
299 Alpha-Proteobacteria and Actinobacteria of the genus *Thermoleophilum* were enriched in the EcMs. Vik  
300 et al. (2013) also observed a significant exclusion of members of Planctomycetes, Firmicutes and  
301 Acidobacteria from the ectomycorrhizosphere of *B. vivipara*. However, Uroz et al. (2012) observed that  
302 Acidobacteria were dominant in EcMs of *X. pruinatus* and *S. citrinum* in temperate and acidic soils.  
303 Similar to the study presented here, members of the *Bradyrhizobium* genus were also highly  
304 represented in EcMs of *X. pruinatus* and *S. citrinum*. Thereby, these data support the hypothesis,  
305 previously proposed based on less exhaustive methods, that EcMs bacterial communities are quite  
306 diverse in their composition and that several factors mediate their structuration: the host tree (Calvaruso  
307 et al. 2010), the fungal symbiont (Izumi and Finlay, 2011), the soil characteristics and the composition of  
308 the soil bacterial communities. However some common trends among bacterial communities from  
309 different EcM species and host trees also exist, suggesting a possible core bacterial community (Burke  
310 et al. 2008; Uroz et al. 2012). Sampling of a large number of EcMs species is required to test whether  
311 EcMs of different species and tree host share a core bacterial community. Such a study would also aid  
312 in the identification of factors that drive the composition of the accompanying specific bacterial  
313 community.

314

315 Our results revealed that the overall composition of *T. melanosporum* EcM bacterial communities was  
316 very close to the one obtained in *T. aestivum* EcMs of hornbeam using similar 454 pyrosequencing tools  
317 (Gryndler et al. 2013). In both cases, a dominance of Actinobacteria and Proteobacteria among which  
318 Alpha-, Beta- and Delta-Proteobacteria, were retrieved. Interestingly, Actinomycetes were isolated from  
319 *T. borchii* EcMs (Sbrana et al. 2002). Within the Actinomycetes phylum, members of the  
320 Pseudonocardinae sub-order were abundant in both *T. aestivum* and *T. melanosporum* EcMs, and were  
321 part of the ten most represented genera in January samples. Members of the Pseudonocardinae order  
322 are often found colonising cave walls, thanks to their ability to degrade recalcitrant humic substances  
323 and lignocellulose (Stomeo et al. 2008; Porca et al. 2012). They are also found to be associated with  
324 leaf cutting ants that culture fungal gardens (Barke et al. 2010; Schoenian et al. 2011). Although their  
325 direct fungal helper functions are unknown, they are envisaged to protect the fungi from other  
326 competing fungal contaminants (Schoenian et al. 2011). Unfortunately, no cultivable bacterial strains of  
327 this order have been isolated from truffles so far, thus making difficult any conjecture on their potential  
328 functional interaction with *Tuber* sp. EcMs.

329

330 A striking contrast between *T. aestivum* and *T. melanosporum* EcM bacterial communities are their  
331 difference regarding *Bradyrhizobium*. This genus was found to be generally excluded from EcMs of *T.*  
332 *aestivum* while they are particularly enriched in *T. melanosporum* EcMs, as well as in all the truffle  
333 species ascocarps analysed so far (Barbieri et al. 2005; Barbieri et al. 2010; Antony-Babu et al. 2014).  
334 *Bradyrhizobium* strains were also retrieved at high frequency from other EcM fungi such as  
335 *Cenococcum geophilum* (Kataoka et al. 2008). Many of these bacteria, although not all, have the ability  
336 to fix N, leading some to hypothesize that these bacteria contribute towards the fungal nutritional  
337 requirements for N (Kataoka et al. 2008; Barbieri et al. 2010; Antony-Babu et al. 2014). However, a  
338 demonstration of N-fixed transfer from these bacteria to the fungus is missing so far. Functional tests on  
339 cultivable bacterial isolates and genomic mining may help to better understand what specifically links  
340 this bacterial genus to truffles.

341

342 Development of *T. melanosporum* ascocarps occur while the physiology of the tree host changes: it  
343 starts at the end of summer/beginning of autumn while trees are still photosynthetically active and it  
344 ends during the dormant period of trees (November to March). Changes in the tree phenology are  
345 coupled to seasonal modifications of edaphic parameters; soil temperature decreases and soil moisture  
346 increases at fall (Brockett et al. 2012). These environmental parameters have been shown to strongly  
347 impact soil bacterial community composition (Cleveland et al. 2006; Tabuchi et al. 2008; Stres et al.  
348 2008; Rasche et al. 2011; López-Mondéjar et al. 2015). In addition, the fall of leaves in October-  
349 November contributes to litter renewal, thereby providing new organic carbon (C) and N sources to the  
350 soil microorganisms. Few studies have documented the response of deciduous forest soil bacterial  
351 communities to temporal or seasonal variations (Rasche et al. 2011; López-Mondéjar et al. 2015). In  
352 line with these two studies, we observed a modification of the soil bacterial community composition  
353 from summer to winter. In our study, this change cannot be attributed to the input of fresh nutrients  
354 coming from litter decomposition. As part of the experimental set-up, the falling leaves were collected  
355 with a net, preventing a leaf litter development. The falling leaves were removed to enable a parallel <sup>13</sup>C  
356 isotopic labelling of the same hazelnut tree considered in our study so as to assess the heterotrophic  
357 behaviour of the ascocarp for C (Le Tacon et al. 2013). Alternatively, an increase of the soil moisture  
358 content may have influenced the abundances of Acidobacteria and Beta-proteobacteria in the soil: the  
359 Acidobacteria abundance increased from September to January meanwhile Beta-Proteobacteria  
360 abundance decreased. Acidobacteria can be particularly favoured by high soil moisture content, in  
361 contrast to Beta-Proteobacteria (Rasche et al. 2011). This inverted pattern may also be associated to

362 changes in the available C concentration in soil since Beta-Proteobacteria are copiotrophic  
363 microorganisms while Acidobacteria are oligogotrophic (Fierer et al. 2007).  
364 The composition of EcM-associated bacterial communities was less affected by temporal variations  
365 comparatively to the soil and ascocarp compartments, suggesting that a parameter(s) stabilised the  
366 ECM communities along time. Such stability may be related to the nutrient exchanges occurring between  
367 the tree and its symbiont. We made a  $^{13}\text{CO}_2$  isotopic labelling of this 'A11' hazelnut tree shoots at the  
368 same period. Results indicated that C remobilized from storage compartements of the host tree was  
369 continuously transferred from the EcMs to the ascocarps along the growing period of the ascocarp (Le  
370 Tacon et al. 2013). This continuous C supply to EcMs during the transition from the photosynthetically  
371 active season to the dormant season may create a stable environment in the EcMs. Part of the  
372 transferred C may be available to bacterial communities all along ascocarp development and maturation,  
373 thus explaining the low variations observed in the bacterial communitiy composition. It also suggests  
374 that the fungus could be the main driver of the composition of *T. melanosporum* EcM-associated  
375 bacterial communities, in contrast to other few EcM fungi studied so far (Izumi and Finlay 2011; Uroz et  
376 al. 2012). Whether this pattern is due to the peculiar long term development of the ascocarps of *T.*  
377 *melanosporum* that would act as continous sink for C from summer to winter or whether it is more  
378 widespread among EcMs fungi remains to be determined. Nevertheless, the relative stability of the *T.*  
379 *melanosporum* EcMs bacterial communities composition strongly contrasts with the high dynamic of the  
380 bacterial community composition observed in the ascocarps during their maturation.

381

## 382 **Conclusion**

383 This study provides for the first time an exhaustive analysis of the diversity, structure and composition  
384 of the bacterial communities associated to EcMs of *T. melanosporum* during the development of its  
385 ascocarps. Our data, coupled to the previous ones of Mello *et al.* (2013) and Antony-Babu *et al.* (2014)  
386 obtained at other stages of the fungus life cycle, indicate that *T. melanosporum* influences bacterial  
387 communities in its vicinity. Based on these studies, we suggest that *T. melanosporum* creates at least  
388 four different habitats: the Brulé i.e the soil area almost completely devoid of vegetation around trees  
389 colonised by *T. melanosporum* (Mello et al. 2013), the gleba, the peridium (Antony-Babu et al. 2014)  
390 and the EcMs (this study). Each of these habitats harbour distinct bacterial communities that are most  
391 probably selected from the bulk soil reservoir and that vary along the seasons and depending of the  
392 habitat considered. While the bacterial community composition of the ascocarps strongly varies with the  
393 stage of development and maturation of the ascocarp, the composition of the bacterial communities  
394 colonizing the EcMs remains rather stable along time. We hypothesize that this stability is associated to  
395 the continuous supply of nutrients coming from tree host and transferred to the ascocarps through the

396 EcMs. Thus a rather stable niche would be created in the ectomycorrhizosphere of *T. melanosporum*  
397 despite the modification of the tree host physiology and phenology at fall time. The functional properties  
398 of the bacteria that colonize these different niches, and the role they play in the life cycle of the fungus  
399 remain to be explored. A combination of functional analyses on cultivable strains and  
400 metatranscriptomic analyses may help answer these questions.

401

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406

#### 407 **References**

- 408 Antony-Babu S, Deveau A, Van Nostrand JD, et al. (2014) Black truffle-associated bacterial  
409 communities during the development and maturation of *Tuber melanosporum* ascocarps and  
410 putative functional roles. *Environ Microbiol* 16:2831–47. doi: 10.1111/1462-2920.12294
- 411 Antony-Babu S, Murat C, Deveau A, et al. (2013) An improved method compatible with metagenomic  
412 analyses to extract genomic DNA from soils in *Tuber melanosporum* orchards. *J Appl Microbiol*  
413 115:163–170.
- 414 Assigbetse K, Gueye M, Thioulouse J, Duponnois R (2005) Soil bacterial diversity responses to root  
415 colonization by an ectomycorrhizal fungus are not root-growth-dependent. *Microb Ecol* 50:350–  
416 359. doi: 10.1007/s00248-004-0229-x
- 417 Barbieri E, Bertini L, Rossi I, et al. (2005) New evidence for bacterial diversity in the ascoma of the  
418 ectomycorrhizal fungus *Tuber borchii* Vittad. *FEMS Microbiol Lett* 247:23–35.
- 419 Barbieri E, Ceccaroli P, Saltarelli R, et al. (2010) New evidence for nitrogen fixation within the Italian  
420 white truffle *Tuber magnatum*. *Fungal Biol* 114:936–942.
- 421 Barke J, Seipke RF, Gruschow S, et al. (2010) A mixed community of actinomycetes produce multiple  
422 antibiotics for the fungus farming ant *Acromyrmex octospinosus*. *BMC Biol* 8:109. doi:  
423 10.1186/1741-7007-8-109
- 424 Brockett BFT, Prescott CE, Grayston SJ (2012) Soil moisture is the major factor influencing microbial  
425 community structure and enzyme activities across seven biogeoclimatic zones in western Canada.  
426 *Soil Biol Biochem* 44:9–20. doi: 10.1016/j.soilbio.2011.09.003
- 427 Bulgarelli D, Rott M, Schlaeppi K, et al. (2012) Revealing structure and assembly cues for *Arabidopsis*  
428 root-inhabiting bacterial microbiota. *Nature* 488:91–95.

- 429 Burke DJ, Dunham SM, Kretzer AM (2008) Molecular analysis of bacterial communities associated with  
430 the roots of Douglas fir (*Pseudotsuga menziesii*) colonized by different ectomycorrhizal fungi.  
431 FEMS Microbiol Ecol 65:299–309.
- 432 Calvaruso C, Turpault MP, Leclerc E, et al. (2010) Influence of Forest Trees on the Distribution of  
433 Mineral Weathering-Associated Bacterial Communities of the *Scleroderma citrinum*  
434 Mycorrhizosphere. Appl Environ Microbiol 76:4780–4787. doi: 10.1128/AEM.03040-09
- 435 Churchland C, Grayston SJ (2014) Specificity of plant-microbe interactions in the tree mycorrhizosphere  
436 biome and consequences for soil C cycling. Front Microbiol 5:1–20. doi:  
437 10.3389/fmicb.2014.00261
- 438 Cleveland CC, Nemergut DR, Schmidt SK, Townsend AR (2006) Increases in soil respiration following  
439 labile carbon additions linked to rapid shifts in soil microbial community composition.  
440 Biogeochemistry 82:229–240. doi: 10.1007/s10533-006-9065-z
- 441 Cole JR, Wang Q, Cardenas E, et al. (2009) The Ribosomal Database Project: improved alignments  
442 and new tools for rRNA analysis. Nucleic Acids Res 37:D141–145.
- 443 Collignon C, Uroz S, Turpault M-P, Frey-Klett P (2011) Seasons differently impact the structure of  
444 mineral weathering bacterial communities in beech and spruce stands. Soil Biol Biochem  
445 43:2012–2022. doi: 10.1016/j.soilbio.2011.05.008
- 446 Courty P-E, Buée M, Diedhiou AG, et al. (2010) The role of ectomycorrhizal communities in forest  
447 ecosystem processes: new perspectives and emerging concepts. Soil Biol Biochem 42:679–698.
- 448 Deveau a, Brulé C, Palin B, et al. (2010) Role of fungal trehalose and bacterial thiamine in the improved  
449 survival and growth of the ectomycorrhizal fungus *Laccaria bicolor* S238N and the helper  
450 bacterium *Pseudomonas fluorescens* BBc6R8. Environ Microbiol Rep 2:560–8. doi:  
451 10.1111/j.1758-2229.2010.00145.x
- 452 Epron D, Ngao J, Dannoura M, et al. (2011) Seasonal variations of belowground carbon transfer  
453 assessed by in situ <sup>13</sup>C CO<sub>2</sub> pulse labelling of trees. Biogeosciences 8:1153–1168. doi:  
454 10.5194/bg-8-1153-2011
- 455 Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology  
456 88:1354–64.
- 457 Frey-Klett P, Chavatte M, Clausse ML, et al. (2005) Ectomycorrhizal symbiosis affects functional  
458 diversity of rhizosphere fluorescent pseudomonads. New Phytol 165:317–328.
- 459 Goh C-H, Veliz Vallejos DF, Nicotra AB, Mathesius U (2013) The impact of beneficial plant-associated  
460 microbes on plant phenotypic plasticity. J Chem Ecol 39:826–39. doi: 10.1007/s10886-013-0326-8
- 461 Gryndler M, Soukupová L, Hršelová H, et al. (2013) A quest for indigenous truffle helper prokaryotes.  
462 Environ Microbiol Rep 5:346–352. doi: 10.1111/1758-2229.12014
- 463 Izumi H, Cairney JWG, Killham K, et al. (2008) Bacteria associated with ectomycorrhizas of slash pine  
464 (*Pinus elliotii*) in south-eastern Queensland, Australia. FEMS Microbiol Lett 282:196–204. doi:  
465 10.1111/j.1574-6968.2008.01122.x

- 466 Izumi H, Finlay RD (2011) Ectomycorrhizal roots select distinctive bacterial and ascomycete  
467 communities in Swedish subarctic forests. *Environ Microbiol* 13:819–30. doi: 10.1111/j.1462-  
468 2920.2010.02393.x
- 469 Izumi H, Moore E, Killham K, et al. (2007) Characterisation of endobacterial communities in  
470 ectomycorrhizas by DNA- and RNA-based molecular methods. *Soil Biol Biochem* 39:891–899. doi:  
471 10.1016/j.soilbio.2006.10.011
- 472 Kataoka R, Taniguchi T, Ooshima H, Futai K (2008) Comparison of the bacterial communities  
473 established on the mycorrhizae formed on *Pinus thunbergii* root tips by eight species of fungi.  
474 *Plant Soil* 304:267–275. doi: 10.1007/s11104-008-9548-x
- 475 Lebeis SL (2014) The potential for give and take in plant-microbiome relationships. *Front Plant Sci*  
476 5:287. doi: 10.3389/fpls.2014.00287
- 477 Le Tacon F, Rubini A, Murat C, et al. (2015a) Certainties and uncertainties about the life cycle of the  
478 Périgord black truffle (*Tuber melanosporum* Vittad.). *Ann For Sci*. doi: 10.1007/s13595-015-0461-1
- 479 Le Tacon F, Zeller B, Plain C, et al. (2013) Carbon transfer from the host to *Tuber melanosporum*  
480 mycorrhizas and ascocarps followed using a <sup>13</sup>C pulse-labeling technique. *PLoS One* 8:e64626.  
481 doi: 10.1371/journal.pone.0064626
- 482 Le Tacon F, Zeller B, Plain C, et al. (2015b) Study of nitrogen and carbon transfer from soil organic  
483 matter to *Tuber melanosporum* mycorrhizas and ascocarps using <sup>15</sup>N and <sup>13</sup>C soil labelling and  
484 whole-genome oligoarrays. *Plant Soil*. doi: 10.1007/s11104-015-2557-7Linderman R (1988)  
485 Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect.  
486 *Phytopathology* 78:366–371.
- 487 López-Mondéjar R, Voříšková J, Větrovský T, Baldrian P (2015) The bacterial community inhabiting  
488 temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. *Soil Biol*  
489 *Biochem* 87:43–50. doi: 10.1016/j.soilbio.2015.04.008
- 490 Martin F, Canet D, Marchal J (1985) <sup>13</sup>C nuclear magnetic resonance study of mannitol cycle and  
491 trehalose synthesis during glucose utilization by the ectomycorrhizal *Cenococcum graniforme*.  
492 *Plant Physiol* 77:499–502.
- 493 Martin F, Canet D, Marchal J, Brondeau J (1984) In Vivo Natural-Abundance <sup>13</sup>C Nuclear Magnetic  
494 Resonance Studies of Living Ectomycorrhizal Fungi. *Plant Physiol* 75:151–153.
- 495 Mello A, Ding GC, Piceno YM, et al. (2013) Truffle Brulés Have an Impact on the Diversity of Soil  
496 Bacterial Communities. *PLoS One*. doi: 10.1371/journal.pone.0061945
- 497 Meyer F, Paarmann D, D'Souza M, et al. (2008) The metagenomics RAST server - a public resource for  
498 the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9:386.  
499 doi: 10.1186/1471-2105-9-386
- 500 Mogge B, Loferer C, Agerer R, et al. (2000) Bacterial community structure and colonization patterns of  
501 *Fagus sylvatica* L. ectomycorrhizospheres as determined by fluorescence in situ hybridization and  
502 confocal laser scanning microscopy. *Mycorrhiza* 9:271–278. doi: 10.1007/PL00009991



- 503 Nadkarni MA, Martin FE, Jacques NA, Hunter N (2002) Determination of bacterial load by real-time  
504 PCR using a broad-range (universal) probe and primers set. *Microbiology* 148:257–66. doi:  
505 10.1099/00221287-148-1-257
- 506 Oksanen J, Blanchet F, Kindt R, et al. (2015) *Vegan: Community Ecology Package*.
- 507 On SLW, Atabay HI, Corry JEL, et al. (1998) Emended description of *Campylobacter sputorum* and  
508 revision of its infrasubspecific (biovar) divisions, including *C. sputorum* biovar *paraureolyticus*, a  
509 urease-producing variant from cattle and humans. *Int J Syst Bacteriol* 48:195–206. doi:  
510 10.1099/00207713-48-1-195
- 511 Panke-Buisse K, Poole AC, Goodrich JK, et al. (2015) Selection on soil microbiomes reveals  
512 reproducible impacts on plant function. *ISME J* 9:980–9. doi: 10.1038/ismej.2014.196
- 513 Paolocci F (1999) Rapid molecular approach for a reliable identification of *Tuber* spp. ectomycorrhizae.  
514 *FEMS Microbiol Ecol* 28:23–30. doi: 10.1016/S0168-6496(98)00088-9
- 515 Porca E, Jurado V, Žgur-Bertok D, et al. (2012) Comparative analysis of yellow microbial communities  
516 growing on the walls of geographically distinct caves indicates a common core of microorganisms  
517 involved in their formation. *FEMS Microbiol Ecol* 81:255–266. doi: 10.1111/j.1574-  
518 6941.2012.01383.x
- 519 Rådecker N, Pogoreutz C, Voolstra CR, et al. (2015) Nitrogen cycling in corals: the key to  
520 understanding holobiont functioning? *Trends Microbiol* 23:490–7. doi: 10.1016/j.tim.2015.03.008
- 521 Rambelli A (1973) The rhizosphere of mycorrhizae. In: Marks G, Kozlowski T (eds) *Ectomycorrhizae*  
522 their Ecol. *Physiol.* Academic Press, New York, pp 299–343
- 523 Rasche F, Knapp D, Kaiser C, et al. (2011) Seasonality and resource availability control bacterial and  
524 archaeal communities in soils of a temperate beech forest. *ISME J* 5:389–402. doi:  
525 10.1038/ismej.2010.138
- 526 Sbrana C, Agnolucci M, Bedini S, et al. (2002) Diversity of culturable bacterial populations associated to  
527 *Tuber borchii* ectomycorrhizas and their activity on *T. borchii* mycelial growth. *FEMS Microbiol Lett*  
528 211:195–201. doi: 10.1016/S0378-1097(02)00712-7
- 529 Schloss PD, Westcott SL, Ryabin T, et al. (2009) Introducing mothur: open-source, platform-  
530 independent, community-supported software for describing and comparing microbial communities.  
531 *Appl Environ Microbiol* 75:7537–41. doi: 10.1128/AEM.01541-09
- 532 Schoenian I, Spittler M, Ghaste M, et al. (2011) Chemical basis of the synergism and antagonism in  
533 microbial communities in the nests of leaf-cutting ants. *Proc Natl Acad Sci U S A* 108:1955–1960.  
534 doi: 10.1073/pnas.1008441108
- 535 Schommer NN, Gallo RL (2013) Structure and function of the human skin microbiome. *Trends Microbiol*  
536 21:660–8. doi: 10.1016/j.tim.2013.10.001
- 537 Shafquat A, Joice R, Simmons SL, Huttenhower C (2014) Functional and phylogenetic assembly of  
538 microbial communities in the human microbiome. *Trends Microbiol* 22:261–6. doi:  
539 10.1016/j.tim.2014.01.011

- 540 Smith S, Read D (2008) *Mycorrhizal Symbiosis*, 3rd ed. Academic Press, London
- 541 Stomeo F, Portillo MC, Gonzalez JM, et al. (2008) Pseudonocardia in white colonizations in two caves  
542 with Paleolithic paintings. *Int Biodeterior Biodegrad* 62:483–486. doi: 10.1016/j.ibiod.2007.12.011
- 543 Stres B, Danevcic T, Pal L, et al. (2008) Influence of temperature and soil water content on bacterial,  
544 archaeal and denitrifying microbial communities in drained fen grassland soil microcosms. *FEMS*  
545 *Microbiol Ecol* 66:110–22. doi: 10.1111/j.1574-6941.2008.00555.x
- 546 Tabuchi H, Kato K, Nioh I (2008) Season and soil management affect soil microbial communities  
547 estimated using phospholipid fatty acid analysis in a continuous cabbage ( *Brassica oleracea* var.  
548 *capitata* ) cropping system. *Soil Sci Plant Nutr* 54:369–378. doi: 10.1111/j.1747-  
549 0765.2008.00242.x
- 550 Uroz S, Calvaruso C, Turpault MP, et al. (2007) Effect of the mycorrhizosphere on the genotypic and  
551 metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil.  
552 *Appl Environ Microbiol* 73:3019–3027. doi: 10.1128/AEM.00121-07
- 553 Uroz S, Courty PE, Pierrat JC, et al. (2013) Functional profiling and distribution of the forest soil  
554 bacterial communities along the soil mycorrhizosphere continuum. *Microb Ecol* 66:404–15. doi:  
555 10.1007/s00248-013-0199-y
- 556 Uroz S, Oger P, Morin E, Frey-Klett P (2012) Distinct ectomycorrhizospheres share similar bacterial  
557 communities as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Appl Environ*  
558 *Microbiol* 78:3020–4. doi: 10.1128/AEM.06742-11
- 559 Vik U, Logares R, Blaaid R, et al. (2013) Different bacterial communities in ectomycorrhizae and  
560 surrounding soil. *Sci Rep* 3:3471. doi: 10.1038/srep03471
- 561 Yano JM, Yu K, Donaldson GP, et al. (2015) Indigenous bacteria from the gut microbiota regulate host  
562 serotonin biosynthesis. *Cell* 161:264–276. doi: 10.1016/j.cell.2015.02.047
- 563

564 **Tables**

565 **Table 1.** Types and number of samples analysed in this study. Samples of *Tuber melanosporum*  
 566 ectomycorrhizal root tips (EcM), of soil at the interface with the EcM (EcM-I - soil adhering to the  
 567 ectomycorrhizal root tip) and of the surrounding bulk soil (BS) have been collected.  
 568

	September	November	December	January
<b>BS</b>	3	4	4	4
<b>EcMs - I</b>	3	3	4	4
<b>EcMs</b>	3	4	3	3

569 **Table 2.** Alpha-diversity indices expressed as average values of three to 4 samples +/-SE  
 570  
 571

Time	Origin	Phylotypes	Shannon	Inv Simpson
<b>Sept</b>	<b>BS</b>	1097 ± 9	6.92 ± 0.01	2828 ± 325
	<b>EcM-I</b>	1110 ± 11	6.95 ± 0.02	3523 ± 326
	<b>EcM</b>	892 ± 26	6.44 ± 0.07	352 ± 70
<b>Nov</b>	<b>BS</b>	1104 ± 8	6.94 ± 0.01	3637 ± 244
	<b>EcM-I</b>	1092 ± 6	6.92 ± 0.01	3092 ± 212
	<b>EcM</b>	921 ± 39	6.46 ± 0.19	535 ± 165
<b>Dec</b>	<b>BS</b>	1150 ± 15	7.00 ± 0.02	6037 ± 1004
	<b>EcM-I</b>	1111 ± 10	6.95 ± 0.02	3417 ± 376
	<b>EcM</b>	972 ± 47	6.60 ± 0.16	698 ± 330
<b>Jan</b>	<b>BS</b>	1121 ± 17	6.97 ± 0.02	4423 ± 473
	<b>EcM-I</b>	1093 ± 15	6.93 ± 0.02	3292 ± 296
	<b>EcM</b>	919 ± 70	6.46 ± 0.19	553 ± 331

572 **Table 3.** Comparison of compartments at the class, order and genus levels. Class, order and genera  
 573 appear in a decreasing order of abundance. The 15 more abundant genera representing at least more  
 574 than 1% in a compartment are figured in the last column. Bold letters are used to highlight statistically  
 575 significant differences according to a one factor ANOVA followed by Tukey post test (p<0.01). Class,  
 576 order or genus for which a significant higher number of sequences was found in the EcM compartement  
 577 compared to the other compartment are underlined.  
 578  
 579  
 580

Class	Order	Genus
Actinobacteria	Actinomycetales	<i>Terrimonas</i>
BS=EcM-I<EcM	BS=EcM-I=EcM	BS=EcM-I=EcM
Bacteroidetes	Sphingobacteriales	<i>Streptomyces</i>
BS=EcM-I>EcM	BS=EcM-I=EcM	BS=EcM-I=EcM
<b>Alpha-proteobacteria</b>	Cytophagales	<b><i>Mycobacterium</i></b>
BS=EcM-I<EcM	BS=EcM-I>EcM	BS<EcM-I>EcM
<b>Delta-proteobacteria</b>	<b>Rhizobiales</b>	<b><i>Bradyrhizobium</i></b>
BS>EcM-I>EcM	BS=EcM-I<EcM	BS=EcM-I<EcM
<b>Verrucomicrobia</b>	<b>Verrucomicrobiales</b>	<b><i>Pedospaera</i></b>
BS>EcM-I>EcM	BS=EcM-I>EcM	BS=EcM-I>EcM
Beta-proteobacteria	Clostridiales	<b><i>Microscilla</i></b>
BS=EcM-I=EcM	BS=EcM-I>EcM	BS=EcM-I>EcM
<b>Firmicutes</b>	Burkholderiales BS=EcM-	<b><i>Flavobacterium</i></b>
BS=EcM-I>EcM	I=EcM	BS=EcM-I>EcM

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<p><b>Acidobacteria</b> BS=EcM-I&gt;EcM</p> <p><b>Planctomycetes</b> BS=EcM-I&gt;EcM</p> <p>Gamma-proteobacteria BS=EcM-I&lt;EcM</p>	<p><b>Planctomycetales</b> BS=EcM-I&gt;EcM</p> <p><b>Flavobacteriales</b> BS=EcM-I&gt;EcM</p> <p>Coriobacteriales BS=EcM-I=EcM</p> <p><b>Bacillales</b> BS=EcM-I&gt;EcM</p> <p><u><b>Thermoleophilales</b></u> BS=EcM-I&lt;EcM</p> <p><b>Rubrobacterales</b> BS=EcM-I&gt;EcM</p> <p><b>Acidobacteriales</b> BS=EcM-I&gt;EcM</p>	<p><u><b>Thermoleophilum</b></u> BS=EcM-I&lt;EcM</p> <p><i>Cytophaga</i> BS=EcM-I=EcM</p> <p><b>Candidatus Koribacter</b> BS=EcM-I&gt;EcM</p> <p><b>Gordonibacter</b> BS&lt;EcM-I&gt;EcM</p> <p><b>Rubrobacter</b> BS=EcM-I&gt;EcM</p> <p><b>Bacillus</b> BS=EcM-I&gt;EcM</p> <p><b>Pirellula</b> BS=EcM-I&gt;EcM</p> <p><b>Acidobacterium</b> BS=EcM-I&gt;EcM</p>
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582

583 **Figure legends.**

584

585 **Figure 1.** Relative abundance of main bacterial classes and Proteobacteria sub classes in bulk soil  
586 (black), EcM-soil interface (grey) and EcM samples (white). Data are expressed as the mean values +/-  
587 SE. Value with a same letter are not statistically different according to a one-way (sample origin)  
588 ANOVA ( $p > 0.05$ ) followed by a Fisher post-hoc test.

589

590 **Figure 2.** Multivariate analysis of the bacterial communities associated to *T. melanosporum* EcM, EcM-I  
591 and BS along maturation time. Principal Coordinate axes 1 and 2 explain most of the variance in the  
592 data cumulatively (F1 = 49.9 % F2 = 18.4 %). Circles, squares and triangles represent bulk soil, EcM-  
593 soil interface and EcM samples, respectively. White, light grey, dark grey and black fillings represent  
594 September, November, December 2010 and January 2011 sampling times.

595

596 **Figure 3.** Relative abundance of main bacterial classes and Proteobacteria sub classes in bulk soil (A)  
597 and EcM samples (B). Data for EcM-I samples are not represented to simplify the figure as they show  
598 identical pattern to the one's of the BS. September, November, December and January samples are  
599 represented by black, dark grey, light grey, white colors, respectively. Data are expressed as the mean  
600 values +/- SE. Value with a same letter are not statistically different according to a one-way (sampling  
601 time) ANOVA ( $p > 0.05$ ) followed by a Fisher post-hoc test.

602

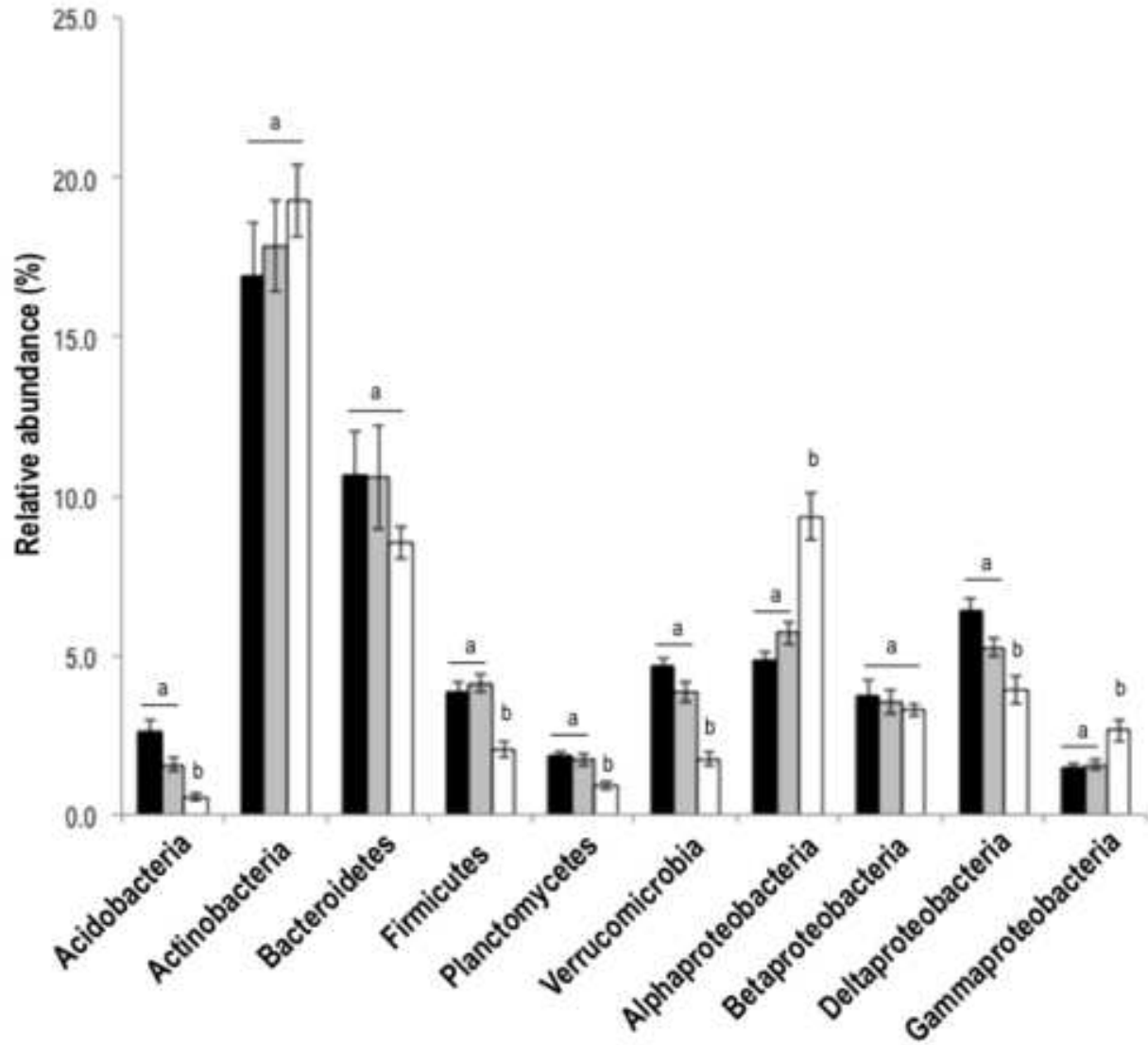
603 **Figure 4.** Relative abundance of main bacterial classes and Proteobacteria sub classes in bulk soil,  
604 EcM, peridium and gleba in November, December and January.

605

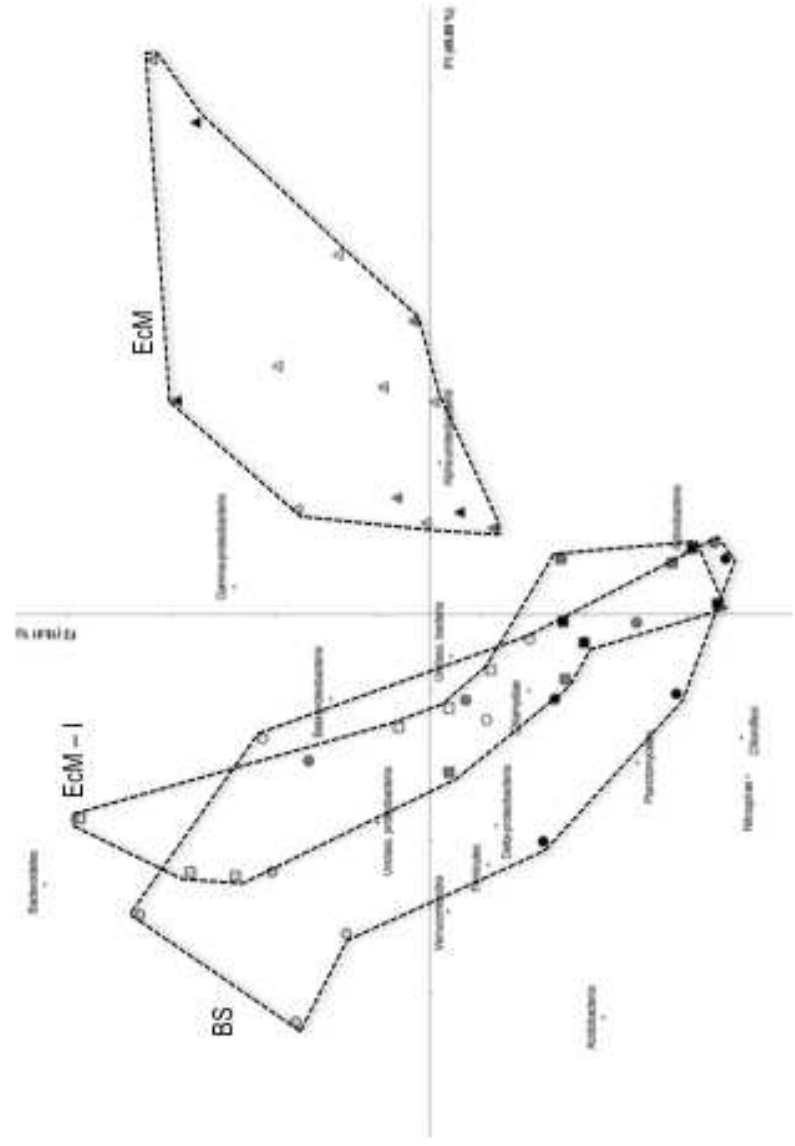
606 **Supplemental Figure 1.** Multivariate analyses of the bacterial communities associated to *T.*  
607 *melanosporum* EcM, BS and ascocarps (peridium and gleba) in November (A), December (B) and  
608 January (C). MBS: bulk soil, MYCO: EcM, AIN: gleba, AOUT: peridium.

609

Figure

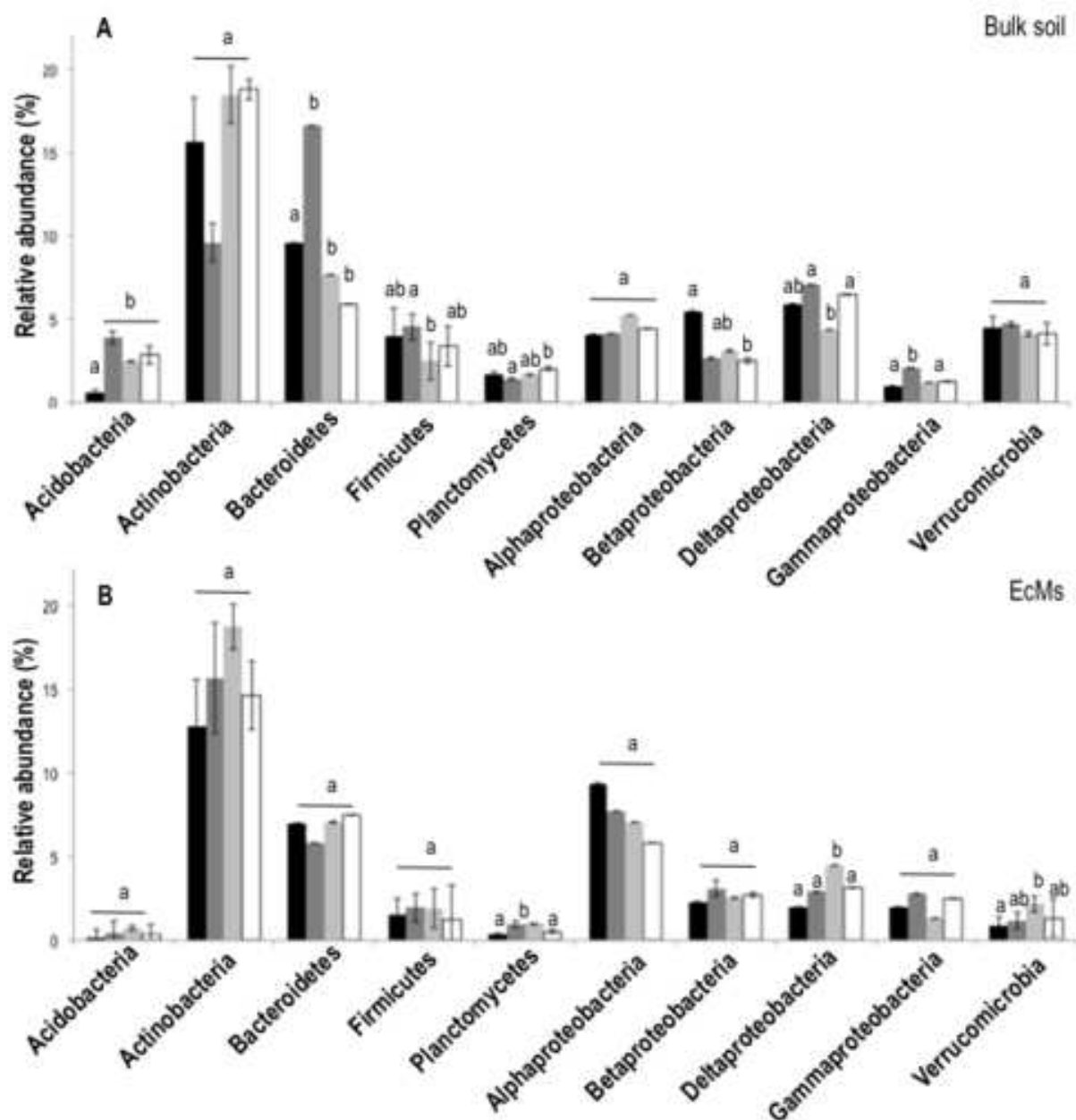


**Fig 1** Relative abundance of main bacterial classes and Proteobacteria sub classes in bulk soil (black), EcM-soil interface (grey) and EcM samples (white). Data are expressed as the mean values +/- SE. Value with a same letter are not statistically different according to a one-way (sample origin) ANOVA ( $p > 0.01$ ) followed by a Fisher post-hoc test



**Fig 2** Multivariate analysis of the bacterial communities associated to *T. melanosporum* EcM, EcM-I and BS along maturation time. Principal Coordinate axes 1 and 2 explain most of the variance in the data cumulatively (F1 = 49.9 % F2 = 18.4 %). Circles, squares and triangles represent bulk soil, EcM-soil interface and EcM samples, respectively. White, light grey, dark grey and black fillings represent September, November, December 2010 and January 2011 sampling times

Figure



**Fig 3** Relative abundance of main bacterial classes and Proteobacteria sub classes in bulk soil (A) and EcM samples (B). Data for EcM-I samples are not represented to simplify the figure as they show identical pattern to the one's of the BS. September, November, December and January samples are represented by black, dark grey, light grey, white colors, respectively. Data are expressed as the mean values +/- SE. Value with a same letter are not statistically different according to a one-way (sampling time) ANOVA ( $p > 0.05$ ) followed by a Fisher post-hoc test



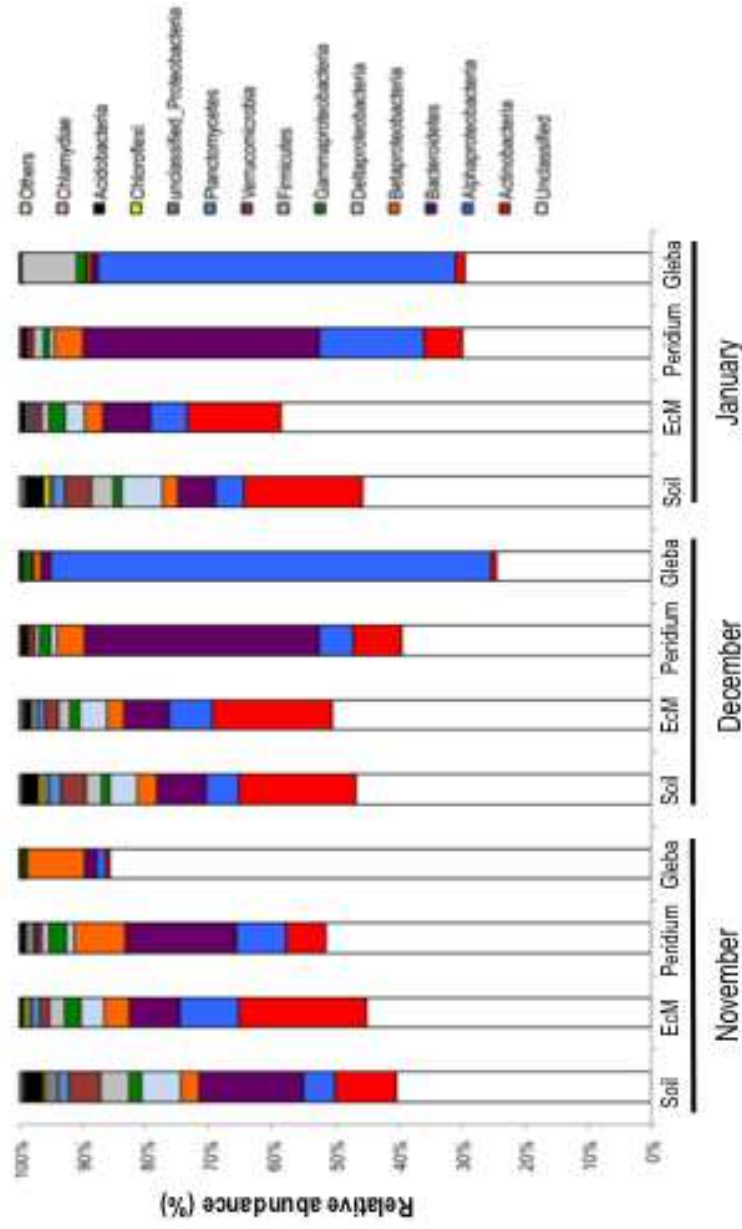
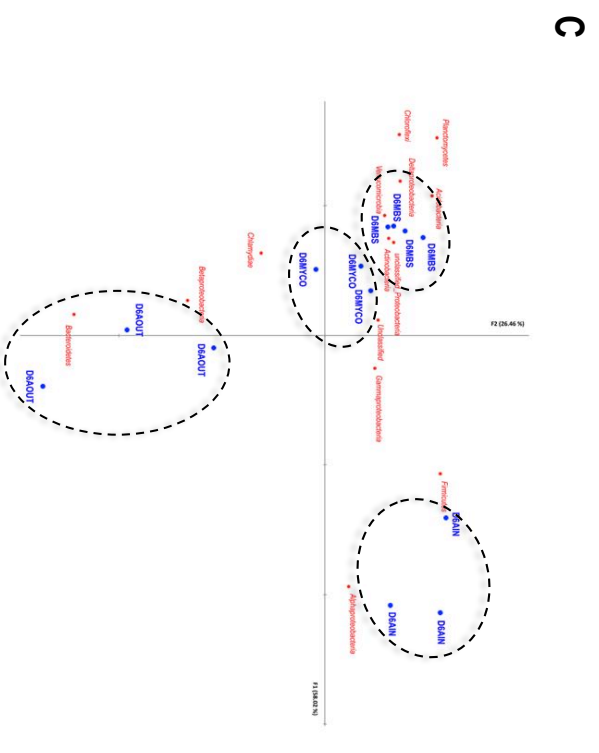
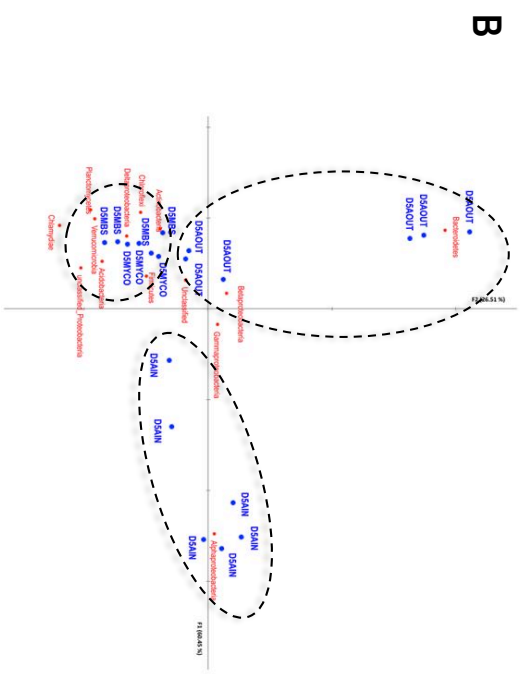
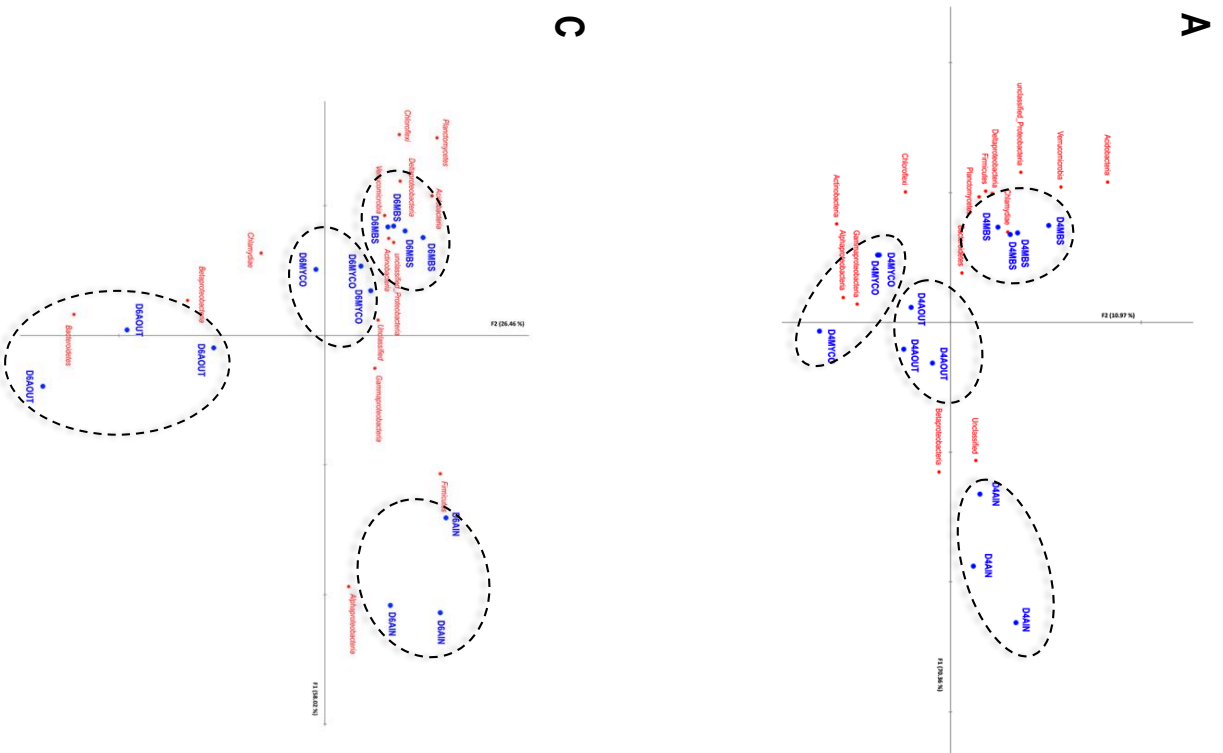


Fig 4 Relative abundance of main bacterial classes and Proteobacteria sub classes in bulk soil, EcM, peridium and gleba in November, December and January



**Supplemental Fig 1** Multivariate analyses of the bacterial communities associated to *T. melanosporum* ECM, BS and ascocarps (peridium and gleba) in November (A), December (B) and January (C). MBS: bulk soil, MYCO: ECM, AIN: gleba, AOUI: peridium

**Table S1: Comparison of EcM and ascocarps compartments (gleba and peridium).** Relative abundance of the main phyla detected in both EcM and ascocarp compartments were used to performed ANOVA analyses. Taxa presenting a significant enrichment whatever the date are presented in column 'all dates' and those presenting significant variations at one or several dates are presented in the column 'specific date(s)'. The symbol '>' means significant enrichment. D4, D5 and D6 mean respectively November, December and January. For ascocarp, data coming from Antony-Babu et al. (2014) have been used.

<b>Taxa</b>	<b>All dates</b>	<b>Specific date(s)</b>
Actinobacteria	EcM > Gleba and peridium	
Chloroflexi	EcM > Gleba	
Planctomycetes	EcM > Gleba	
Delta-Proteobacteria	EcM > Gleba and peridium	
Verrucomicrobia	EcM > Gleba	
Firmicutes		D6 Gleba > D6 EcM ; D4 and D5 EcM > D4 and D5 Gleba
Alpha-Proteobacteria		D5 and D6 Gleba > D5 and D6 EcM
Beta-Proteobacteria		D4 Gleba > D4 EcM D6 EcM > D6 Gleba
Gamma-Proteobacteria		D4 EcM > D4 Gleba