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### ► To cite this version:

Eve Hellequin, Françoise Binet, Olivier Klarzynski, Sara Hallin, Jaanis Juhanson, et al.. Shaping of soil microbial communities by plants does not translate into specific legacy effects on organic carbon mineralization. *Soil Biology and Biochemistry*, 2021, 163, pp.108449. 10.1016/j.soilbio.2021.108449 . hal-03430174

HAL Id: hal-03430174

<https://univ-rennes.hal.science/hal-03430174v1>

Submitted on 16 Nov 2021

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# 1 Shaping of soil microbial communities by plants does not translate into 2 specific legacy effects on organic carbon mineralization

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## 14 Abstract

15 Plants shape soil microbial communities through their root architecture, their rhizodeposits  
16 and return of dead plant material to the soil. These interactions can have a strong influence  
17 on the soil organic carbon dynamics. However, it is unclear whether the plant species effects  
18 on the soil microbial community could influence the organic carbon mineralization through  
19 plant legacy effects. Therefore, we examined how and to what extent a short-term plant  
20 growing phase affected the total and active soil microorganisms and through a possible plant  
21 legacy, also the mineralization of soil organic carbon, a central ecosystem function. Using a  
22 controlled pot experiment, we first showed that the two phylogenetically distinct plants,  
23 *Arabidopsis thaliana* and *Triticum aestivum*, differently shaped the soil microbial  
24 communities when recruiting from the same soil community. Although both plants recruited  
25 plant-growth promoting bacteria in the vicinity of their roots, *A. thaliana* had a stronger  
26 effect than *T. aestivum* and also recruited saprophytic fungi, while inhibiting fungal  
27 pathogens. Due to plant legacy effects on the soil microbial communities, different microbial  
28 successions occurred in the two previously planted soils when subjected to plant litter. By  
29 contrast, plant legacy effects on soil basal respiration were not plant-specific, with basal  
30 respiration increasing similarly in both cases and moreover did not translate to changes in  
31 litter carbon mineralization in the short-term of 49 days. Our results suggest that the soil  
32 nutrient dynamics rather than changes in soil microbial community composition drive the  
33 organic carbon mineralization of added litter. The present study brings new insights in how

34 the relationships between plants, microorganisms and soil nutrient dynamics affect litter  
35 carbon cycling.

36 **Key-words:** plant legacy, litter mineralization, soil bacteria, soil fungi, *Arabidopsis thaliana*,  
37 *Triticum aestivum*, microbial succession

## 38 **Introduction**

39 Soil harbors a large amount of phylogenetically and functionally diverse  
40 microorganisms (Torsvik and Øvreås, 2002) that interact with plants and contribute to  
41 above-below ground exchanges. The “plant-soil feedbacks” concept is defined as plant-  
42 mediated changes in soil microbiota and soil nutrient availability that influence the fitness of  
43 the same or other plants in return (Perkins et al., 2011a; Van der Putten et al., 2013). Plants  
44 shape the composition and diversity of soil microbiota through rhizodeposits and roots  
45 architecture, litter C:N ratio and litter carbon quality (Heal et al., 1997; Grayston et al., 1998;  
46 Zak et al., 2003; Gu et al., 2012). By providing new habitats and resources in the vicinity of  
47 their roots, plants induce the colonization of microorganisms, therefore the rhizosphere is  
48 known to be a microbial hotspot (Lynch and Whipps, 1990 ; Lundberg et al., 2012; Reviewed  
49 in Muller et al., 2016). The microbial diversity declines sequentially from bulk soil to  
50 rhizosphere, suggesting increasingly stronger competition among microorganisms (Muller et  
51 al., 2016) or a selection of specific microorganisms induced by the plant. Thus, plant roots  
52 shape the microbial community directly or indirectly by enriching and/or activating specific  
53 microorganisms, but also by depleting and/or inhibiting other ones (Berg and Smalla, 2009).

54 The modifications induced by plants on soil microbiota may persist after their  
55 disappearance (i.e. legacy effects) (Van der Putten et al., 2016), for example, via their  
56 rhizodeposits or litter. Such legacy effects of plants have been shown to influence soil carbon  
57 storage (Lange et al., 2015) and soil respiration (de Oliveira et al., 2020), and could therefore  
58 affect the mineralization of organic carbon and nutrients from litter. The capacity of the soil  
59 to mineralize organic matter is attributed to the production of specific enzymes that allow  
60 specialized microorganisms to use carbon and nutrients of organic matter with low  
61 nutritional value, known as “metabolic ability” (Keiser et al., 2011). Depending on the  
62 functional capacity of the decomposer communities, soils have different metabolic abilities  
63 (Keiser et al., 2014, Veen et al., 2018). Thus, for the same litter, differences in its

64 decomposition rate can occur depending on the microbial community present and its active  
65 fraction. While active soil microorganisms have been shown to only contribute 2% of the  
66 total microbial biomass in soils, they are linked to soil metabolic ability (Blagodatskaya and  
67 Kuzyakov, 2013) and may be more relevant than the total microbial community to assess  
68 plant legacies on soil function. Hence, by shaping the microbial communities, plants may  
69 indirectly also influence the soil organic matter decomposition and, in the long-term,  
70 potentially accelerate its own litter decomposition by selecting an adapted decomposing  
71 microbial community (Ayres et al., 2009a; Veen et al., 2015a).

72 In the present study, we asked whether plant species shape the soil microbial  
73 community and if such plant-specific effects could influence, the organic carbon  
74 mineralization through plant legacy effects. For this purpose, we conducted an experiment  
75 with two phases, with the first phase denoted the “plant-soil experiment”, in which we  
76 aimed at examining how a short-term (two months) planted agricultural soil affects the total  
77 and the active microbial communities and to what extent it depends on plant species. In the  
78 second phase referred to the “litter-carbon mineralization experiment”, we determined if  
79 such changes induce plant legacy effects that affect the litter-carbon mineralization. Two  
80 phylogenetically distinct plants, a common plant model (*Arabidopsis thaliana*) and a crop  
81 (*Triticum aestivum*) were grown in the plant-soil experiment and followed by the litter-  
82 carbon mineralization experiment using the soils from phase one to which litter of *T.*  
83 *aestivum* was added. In this experiment, differences in the daily and cumulative  
84 mineralization rates of organic carbon from litter and subsequent nutrient release were  
85 determined. To identify early plant legacy effects on soil microbiota, both DNA (total  
86 microbial community) and RNA (active microbial community) were used to quantify  
87 microbial abundances (quantitative PCR) and identify the changes in microbial diversity using  
88 a 16S rRNA gene and ITS amplicon sequencing approach.

89 We hypothesized that i) specific microbial communities would develop according to  
90 plant species, through specific recruitment from the soil and ii) specific plant-induced  
91 changes in microbial communities, soil nutrient and carbon contents would then translate  
92 into plant-specific legacy effects on the mineralization of added litter to soil, despite the  
93 generalist feature of this function.

## 94 2. Materials and methods

### 95 2.1. Plant-soil experiment (phase 1)

96 The soil was an agricultural topsoil (0-20 cm depth) from the agronomic experimental site of  
97 the Brittany regional chamber of agriculture at Kerbernez (Plomelin, France; 47°56'39.3"N /  
98 4°07'47.9"O), collected in April 2018 in the field border where grass was growing. It was air-  
99 dried at ambient room temperature for 48 h and sieved at 4 mm prior to storage in the dark  
100 at 4 °C. The soil was used for the plant-soil experiment, performed in May 2018. Soil  
101 physical, chemical and microbial properties were determined following sieving at 2 mm and  
102 according to Carter and Gregorich (2008) and are presented in Table 1. The soil was planted  
103 with either *A. thaliana* or *T. aestivum* or not planted (bare soil). The *A. thaliana* seeds from  
104 the Ecotype Columbia-O and the *T. aestivum* L. cv. "Baldus" seeds were kindly provided by  
105 IsoLife BV (Wageninigen, the Netherlands). The seeds were surface-sterilized, directly sown  
106 on 10 kg equivalent dry soil in sterile planters (length: 50 cm, width: 17 cm, height 15 cm) at  
107 a density of  $2.6 \times 10^{-6}$  and  $1.22 \times 10^{-5}$  seeds per  $m^2$  for *T. aestivum* and *A. Thaliana*,  
108 respectively. The planters were placed in an environmental growth chamber (Percival  
109 scientific, Perry, Iowa, USA) with the following conditions: 16 hours light (day), 8 hours dark  
110 (night) and light intensity of  $160 \mu\text{mol m}^{-2} \text{s}^{-1}$ , air temperature of 20 °C and relative humidity  
111 of 80%. Soils were watered with sterile water periodically to maintain WHC around 60% by  
112 weighting the planters. After 63 days, which corresponded to the flowering stage of *A.*  
113 *thaliana*, the bare soil from the not planted planter and the soil in the vicinity of the roots of  
114 *A. thaliana* and *T. aestivum* were harvested. Four replicates of the original soil (blank) and  
115 four soil replicates (30 g of dry weight equivalent for each replicate) from the same bare and  
116 planted planters (Bare soil, *A. thaliana* and *T. aestivum*) were sampled and stored at -20 °C  
117 and -80 °C for further chemical and microbial DNA- and RNA-based analyses, respectively  
118 (n=16). The remaining soil was used for setting-up the second experimental phase.

### 119 2.2 Litter-carbon mineralization experiment (phase 2)

120 In the second experimental phase, we used soil microcosms to study the plant legacy  
121 effects on C mineralization based on a 3 x 2 factorial design (three soils from phase 1 without  
122 and with *T. aestivum* litter addition). For each of the six treatments, four replicates were  
123 independently designed for each of the five incubation times (0, 3, 7, 21 and 49 days) after

124 litter addition to soil, allowing destructive soil sampling. The design corresponded to a total  
125 of 120 soil microcosms. Each soil (e.g. bare soil (control), and previously planted soils with *A.*  
126 *thaliana* or *T. aestivum*) was sieved at 2 mm according to the AFNOR standard (AFNOR XP U  
127 44–163) and soil aliquots of 30 g weight equivalent dry soil were mixed with or without 184  
128 mg of *T. aestivum* sterile chopped straw, corresponding to an input of 40 mg of organic  
129 carbon and 0.3 mg of organic nitrogen. The straw was previously chopped into fine pieces of  
130 1 mm. To avoid soil contamination by external microorganisms, the straw was sterilized by  
131 Ionisos (Dagneux, France) with gamma irradiation at 25 KGy. The sterilization efficiency was  
132 checked by none amplification of the 16S rRNA gene with a polymerase chain reaction (PCR).  
133 Each soil microcosm was placed in a hermitically closed 1 L glass jar that was incubated in  
134 the dark at 28 °C. At each incubation time, except the initial time point, the CO<sub>2</sub> produced  
135 and accumulated in the headspace was measured by micro gas chromatography (Agilent,  
136 Santa Clara, California, USA) and expressed as mg of C-CO<sub>2</sub> g<sup>-1</sup> dry soil. Before each  
137 measurement, the air of the headspace was homogenized with a 50 ml syringe. After each  
138 measurement, the air of the headspace was entirely renewed, to ensure that the headspace  
139 did not become anoxic, and the soil moisture was maintained at 60% WHC by weighting and  
140 adding sterilized water if needed. At day 0 and after each CO<sub>2</sub> measurement (days 3, 7, 21  
141 and 49) a time series of microcosms was destroyed and the soil was stored at -20 °C and -80  
142 °C for further chemical and microbial RNA-based analyses, respectively.

### 143 **2.3 Chemical analyses**

144 The soil chemical analyses are detailed in Hellequin et al. (2020). Briefly, the total  
145 organic carbon and nitrogen were determined with an elemental analyzer (Elementar, Vario  
146 PYRO Cube, Lyon, France). The dissolved organic carbon and the dissolved inorganic nitrogen  
147 (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) were measured by an automated photometric analyzer following K<sub>2</sub>SO<sub>4</sub>  
148 extraction. The Olsen phosphorus concentrations were determined according to Olsen et al.  
149 (1954). The microbial carbon and nitrogen biomass (MBC and MBN, respectively) were  
150 determined according to Vance et al. (1987).

### 151 **2.4 Microbial quantification and metabarcoding**

152 The DNA and RNA extractions were performed following the protocol described by  
153 Griffiths et al. (2000) with some modifications according to Nicolaisen et al., (2008) and  
154 Monard et al. (2013). Further details on DNA extraction and subsequent quantitative PCR  
155 (qPCR) are available in supplementary materials (section 1). Half of the extract was stored at-  
156 20 °C before further DNA-based analyses (original soil and 0 day samples). The other half  
157 was subjected to a DNase treatment for further RNA-based analyses (original soil and  
158 samples from day 0, 3, 7, 21 and 49). RNA (200 ng) were used for reverse transcription (RT).  
159 Half of the cDNA products was stored at -20 °C to prepare 16S rRNA and ITS1 amplicons for  
160 the quantification of the total bacteria and fungi by qPCR and the other half was used for 2 X  
161 300 Illumina sequencing. The libraries were constructed using a two-step PCR approach "16S  
162 metagenomics sequencing library preparation" protocol provided by Illumina.

## 163 **2.5 Data analyses**

164 The microbial sequences are available on the sequence read archive (SRA) database  
165 (Bioproject PRJNA540147). The microbial sequence analyses were performed according to  
166 Hellequin et al. (2020) with the following changes: i) only the bacterial and fungal OTUs  
167 present in at least 4 out of 128 samples and representing at least 0.0005% of all sequences  
168 were retained, which corresponded to OTUs with a minimum of 12 and 29 sequences for  
169 bacteria and fungi, respectively. In the final data set, a total of 1,253,087 bacterial sequences  
170 corresponding to 8,911 OTUs and 3,113,807 fungal sequences corresponding to 1,351 OTUs  
171 were obtained.

172 All the statistical analyses were performed using R (v3.4.3, Core Team, 2017). The  
173 number of sequences per sample was randomly subsampled to the lowest number, with  
174 bacterial and fungal DNA and cDNA amplicons being normalized separately using the *rarefy*  
175 function from the *vegan* package version 2.5-4 (Oksanen et al., 2019); 6 399 and 15 589 for  
176 bacterial and fungal DNA, and 7 000 and 6 000 for bacterial and fungal cDNA. Twenty-one  
177 out of the 256 samples were below these thresholds and were removed before further  
178 analyses. The rarefaction curves at each sampling date and before and after normalization  
179 were performed using the *rarecurve* function from the *vegan* package (Figures S1 and S2).  
180 For each sample, the abundance of each OTU was calculated based on its relative abundance  
181 from the sequencing analysis and to the bacterial 16S rRNA and fungal ITS quantifications

182 performed simultaneously. The richness (number of OTUs) (Figure S11) and Shannon  
183 diversity index were calculated to estimate the alpha diversity using the *phyloseq* package  
184 (McMurdie and Holmes, 2013).

185 To test for statistical differences between plant-soil treatments, we used analysis of variance  
186 (ANOVA) and Tukey's post-hoc test on the following endpoints: emissions, bacterial and  
187 fungal Shannon diversities, microbial carbon and nitrogen biomasses, soil nutrient (N and P)  
188 contents, and total and active bacterial and fungal abundances. To test for significant  
189 difference in the composition of the total and active bacterial and fungal communities at the  
190 end of the plant growth phase, powered partial least squares discriminant analysis (PPLS-DA)  
191 were generated using DNA-based OTU or RNA-based OTU tables using the *RVAideMemoire*  
192 package version 0.9-72 (Hervé, 2019) and the *p/s* package version 2.7-0 (Mevik et al., 2018).  
193 ANOVAs were carried out on each OTU to identify significant differences in their  
194 abundances. The significant DNA-based or RNA-based OTUs were determined as enriched or  
195 activated *versus* depleted or inhibited by the plants, respectively. Over the incubation period  
196 in the second experiment, PPLS-DA were generated with the RNA-based OTUs and at each  
197 sampling date to compare the active bacterial and fungal community compositions between  
198 soils derived from bare soil, *A. thaliana* or *T. aestivum* and amended or not with litter.  
199 ANOVAs were carried out on each OTU to identify significant differences in their abundances  
200 according to the PPLS-DA results. In parallel, we used co-inertia analyses to identify the  
201 relationships between the OTUs that contributed significantly to the differences in  
202 community composition between soils and the soil dissolved organic carbon and nutrient  
203 contents. Only the sampling times (i.e. day 7 for bacteria and days 0 and 49 for fungi) at  
204 which both the PPLS-DA and the co-inertia resulted in significant differences were kept to  
205 discuss the results. To identify microbial successions during the incubation, additional PPLS-  
206 DA on the RNA-based OTUs were generated to compare the composition of active bacterial  
207 and fungal communities over time.

208

### 209 **3. Results**

#### 210 **3.1 Planting effects on soil carbon and nutrients (N, P) contents**



211 Without plants, the total content of dissolved mineral nitrogen increased significantly  
212 after 63 days due to a higher amount of  $\text{NO}_3^-$ , whereas the content of  $\text{NH}_4^+$  remained the  
213 same (Figure 1). In soils with plants, dissolved soil organic carbon content increased  
214 significantly, whereas the dissolved  $\text{NO}_3^-$  significantly decreased compared with the bare soil.  
215 Both plants tended to increase the microbial carbon (MBC) (significant for *A. thaliana*) and  
216 nitrogen biomass (MBN). The soil P content was significantly depleted by *A. thaliana*, but not  
217 by *T. aestivum* (Figure 1). Furthermore, *A. thaliana* increased the ratio of carbon from soil  
218 microbial biomass to total soil organic carbon compared to the original soil.

### 219 3.2. Planting effects on total and active soil microorganisms

220 Both bacteria and fungi were affected after 63 days of incubation without plants  
221 present. A decrease in diversity of the total and active community of both bacteria and fungi  
222 was observed as well as a decrease in the abundances of the active bacterial and fungal  
223 communities and the RNA:DNA ratio (Figure 2). The active bacteria and fungi were even less  
224 abundant in soils with plants. With *T. aestivum*, the original levels of total and active  
225 bacterial diversity were maintained, whereas they increased in soil with *A. thaliana*. For the  
226 total fungal diversity, the original level was maintained with both plant species, but the  
227 diversity of the active fungal community decreased even though *A. thaliana* showed higher  
228 diversity levels than bare soil. Interestingly, the copy numbers of the 16S rRNA was 3-fold  
229 higher than its gene (Figure 2).

230 All soils were dominated by the same main bacterial phyla, i.e. *Alphaproteobacteria*,  
231 *Acidobacteria*, *Actinobacteria* and *Planctomycetes*, but *A. thaliana* enriched for *Zygomycota*,  
232 *Ascomycota* and some unknown fungi (Figure 3). The growth of *A. thaliana* and *T. aestivum*  
233 induced significant changes in the structure of the total bacterial and fungal communities  
234 and the active fungal community in the vicinity of their roots (Figure 4 A, E & G,  $P = 0.004$ ,  
235 89.81 % of variance explained;  $P = 0.028$ , 76.36 %;  $P = 0.003$ , 98.17 %, respectively), while  
236 the structure of the active bacterial community was similar between planted and bare soils  
237 (Figure 4 C,  $P = 0.215$ , 88.09 % of variance explained).

238 We further identified the OTUs driving the observed differences, either because they  
239 were enriched or depleted (from DNA) and activated or inhibited (from cDNA) by the plants  
240 (Figure 4 B, E, D, H). While the plants induced depletion or inhibition of a high number of  
241 bacterial OTUs, they mainly induced enrichment or activation of specific fungal OTUs (Figure

242 4). *Bacillus* (OTUs 1 and 7) and *Methyloligellaceae* (OTU 17) were the most depleted bacteria  
243 by the two plants while the *Xanthobacteraceae* (OTUs 5, 4 and 9) was specifically depleted  
244 by *A. thaliana* (Figure 4B). Among the most abundant bacterial OTUs inhibited by the two  
245 plants, some were depleted by both plants (OTUs 1, 4 and 7), and the bacterial OTUs  
246 specifically inhibited by *A. thaliana* were all affiliated to *Bradyrhizobium* (OTUs 5, 3 and 2)  
247 (4D). For fungi, the only OTU inhibited by the two plants was affiliated to *Sordariomycetes*  
248 (OTU 1) (4H).

249 On top of these general trends, we found a stronger enrichment by *A. thaliana* than *T.*  
250 *aestivum* that was more pronounced for fungi than bacteria (Figure 4 B & E). A total of 46  
251 and 70 DNA-based bacterial and fungal OTUs, respectively, were enriched with *A. thaliana*,  
252 against 32 and 14 with *T. aestivum*. *Proteobacteria* (delta, beta and alpha) were mainly  
253 enriched by *A. thaliana* while *T. aestivum* mainly enriched *Actinobacteria*, *Acidobacteria*,  
254 *Bacteroidetes* and *Planctomycetes*, (data not shown). For fungi, the enriched OTUs by *A.*  
255 *thaliana* were highly abundant and mainly affiliated to *Mortierella* (OTUs 5, 11 and 13) (4F).  
256 We detected a stronger activation of bacteria with *T. aestivum* than with *A. thaliana*, which  
257 instead activated more fungi when compared with the total microbial communities (4 D &  
258 G). The bacterial and fungal OTUs that were activated were also different from those that  
259 were enriched (data not shown).

### 260 3.3. Plant legacies on basal respiration and carbon-litter mineralization

261 The modifications observed in the bacterial and fungal communities after 63 days of plant-  
262 growth were plant specific. In the following litter-carbon mineralization experiment these  
263 changes were associated with functional changes in the soil basal respiration rate. In soil  
264 without litter addition, the basal respiration rate in soils that previously had plants,  
265 regardless of plant species, was always higher than in the bare soil (Figure 5). The daily  
266 emissions were peaking at day 7, with *T. aestivum* having the strongest effect, before  
267 decreasing until day 49 independent of the previous treatment in phase 1.

268 Following soil C-enrichment (litter addition), the soil respiration rate increased in all soils as  
269 expected (Figure 5). By contrast to soil without litter addition, the respiration rate and  
270 cumulative C-respired were higher in the bare soil than in soils that previously had plants, at  
271 days 7 and 21. At day 49, these observations reverted, the respiration rate being higher in  
272 the soils that had plants than in the bare soil, with no differences between plants (Figure 5).

273

274 **3.4. Plant legacies on active microbial successions**

275 The temporal dynamic of abundances and composition of active bacterial and fungal  
276 communities varied according to the soils being either C-enriched or not by litter (Figure 3).  
277 In the previously bare soil without litter, the abundances of active bacteria and fungi  
278 gradually decreased over time. In previously planted soils without litter addition we  
279 observed an increase in the abundance of active fungi and bacteria at day 3. For fungi, this  
280 was due to an activation of *Ascomycota* (Figure S8). At day 49, the bacterial and fungal  
281 abundances were similar to those at the beginning of the second phase experiment.

282 In soils with litter addition, bacterial and fungal abundances gradually increased until day 21,  
283 but then reverted to their initial abundance at day 49. The DNA-based OTUs significantly  
284 enriched by the growth of *A. thaliana* or *T. aestivum* (Figure 4 B & F) were compared to their  
285 corresponding RNA-based OTUs at all time points of the litter-carbon mineralization  
286 experiment. Among the bacterial OTUs enriched by *A. thaliana* or *T. aestivum*, some were  
287 activated at the beginning of the incubation (days 3 and 7), but did not remain activated  
288 until day 49 (Figures S4 & S5). The fungal OTUs enriched by *A. thaliana* were not more  
289 activated along the litter-carbon mineralization experiment (Figure S6), but for those  
290 enriched by *T. aestivum*, we observed higher abundances at day 21 (Figure S7).

291

292 The soil C-enrichment with litter did not conceal the plant legacy effects detected without  
293 litter addition. In *A. thaliana* soil, similar microbial dynamics as without litter were observed,  
294 however, with litter, the changes in abundances were less pronounced. The increase in  
295 microbial abundances observed at day 3 was due to an activation of *Alphaproteobacteria*  
296 (*Rhizobiales*) and *Ascomycota* (*Sordariomycetes*) (Figure S9). Further, we observed an  
297 activation of *Deltaproteobacteria* (*Myxococcales*) and *Zygomycota* at day 49. In the *T.*  
298 *aestivum* soil, the community dynamics were different with litter than without, with high  
299 bacterial and fungal abundances at day 21 and lower abundances at day 49 compared to day  
300 0. The decreased fungal abundance at day 49 was mainly due to a decrease in active  
301 *Ascomycota* (*Sordariomycetes*) (Figure S9).

302 When the structure of the active bacterial or fungal communities was different  
303 between soils, we used co-inertia analyses to identify if there was any relationships between  
304 the OTUs that contributed significantly to the differences between soils and the measured  
305 soil variables. The  $\text{NO}_3^-$  soil content was the sole variable explaining the differences between  
306 soils. Such significant relationships between planted soil history and soil parameters were  
307 identified for bacteria at day 7 with litter and for fungi at day 0 and 49 with and without  
308 litter, respectively (Figure S10). For bacteria, OTUs mainly affiliated to *Alphaproteobacteria*  
309 (*Caulobacteraceae* and *Sphingomonadaceae*), *Bacteroidetes* (*Cytophagaceae* and  
310 *Microscillaceae*) and *Gammaproteobacteria* (*Burkholderiaceae* and *Cellvibrio*) were more  
311 abundant in the bare soil and positively correlated with the  $\text{NO}_3^-$  soil content (Figure S10).  
312 For fungi, the OTUs that were more abundant in the bare soil and positively correlated with  
313 the  $\text{NO}_3^-$  content were affiliated to *Ascomycota* (*Sordariomycetes* and *Dothideomycetes*).  
314 Lastly, at day 49, the OTUs that were more abundant in the *A. thaliana* soil compared to the  
315 bare soil, were negatively correlated with  $\text{NO}_3^-$  soil content and affiliated to *Zygomycota*  
316 (*Mortierella*) and *Basidiomycota* (*Solicoccozyma* and *Tremellales*).

317

## 318 4. Discussion

### 319 4.1. Plant-specific effects on the total and active soil microorganisms and soil nutrients

320 As hypothesized, the growth of both *A. thaliana* and *T. aestivum* induced the  
321 selection of specific soil bacteria and fungi in the vicinity of their roots. We showed that the  
322 effect of *A. thaliana* was stronger, especially for fungal communities. Root architecture as  
323 well as rhizodeposits are highly plant species-specific and could thus differentially act on  
324 microbial communities depending on plant species, leading to specific recruitment of root-  
325 and rhizosphere-associated microorganisms (Berg and Smalla, 2009). In the present study  
326 the presence of plants, irrespective of plant species, resulted in an increase in the dissolved  
327 soil organic carbon content and carbon in microbial biomass, likely reflecting the inflow of  
328 carbon to soil microbes through plant carbon deposition. At the same time, a depletion in  
329 mineral nitrogen was observed, which could be due to plant N uptake that occurred  
330 concomitantly with plant carbon deposition during plant growth. Tkacz et al. (2015) showed  
331 that changes in the soil microbiota following plant growth appeared quickly after only one  
332 plant generation and were plant-species dependant. However, in our study we showed a

333 stronger effect of *A. thaliana* than *T. aestivum* on abundances and community composition  
334 of total bacteria and fungi, while Tkacz et al. (2015) showed the opposite, probably due to its  
335 extensive root system (Tkacz et al., 2015). In our experiment the plants grew longer than in  
336 the study of Tkacz et al. (2015), allowing *A. thaliana* to reach its flowering state. This could  
337 explain the differences as it has been shown that the plant effects on soil microbiota depend  
338 on the growth stage of the plant, with the strongest effect at the flowering stage, whereas  
339 the effect is weaker during earlier growth stages (Cheng and Kuzyakov, 2005). The growth  
340 stage might thus have overtaken the effect of root density in shaping rhizospheric  
341 microbiota leading to different results than those of Tkacz et al. (2015). Another explanation  
342 could be the differences in the properties of the soils used. While we used a sandy-loam  
343 agricultural soil, Tkacz et al. (2015) used artificial soils consisting of compost or sand. They  
344 observed that plant selection on the rhizosphere microbiota depended strongly on soil  
345 composition probably due to contrasted organic nutrient contents. Nevertheless, both  
346 studies results point toward a specific plant effect on soil microbial communities that  
347 depends on the properties of the original soil.

348 Differences between total and active microbial community have already been demonstrated,  
349 with active microorganisms being a subset of the total community (Romanowicz et al.,  
350 2016). However, for bacteria, it is well known that the copy number of the 16S rRNA gene  
351 varies between taxa (Větrovský and Baldrian, 2013) and Blazewicz et al., (2013) pointed out  
352 that the relationship between rRNA concentration and bacterial growth rate can differ  
353 greatly among taxa. These differences could explain why we observed higher 16S rRNA copy  
354 numbers than its gene. Globally, we showed that the enriched or activated OTUs differed  
355 between plant species, indicating a plant-specific effect on both total or active bacteria and  
356 fungi. However, most of the depleted and inhibited OTUs were common to both plants and  
357 the negative interaction therefore seemed less plant-specific. The depletion or inhibition of  
358 certain OTUs was more pronounced for bacteria than for fungi. Surprisingly, both plants  
359 depleted or inhibited *Bacillus sp.* and *Bradyrhizobium sp.*, of which some are known to be  
360 plant growth-promoting bacteria (reviewed in Govindasamy et al., 2010). However, both  
361 plants also recruited plant growth-promoting bacteria, which can provide direct benefits to  
362 plant growth and health by influencing the nutrient status or affecting plant-pathogen  
363 interaction. *T. aestivum* selected more total and active bacteria, while *A. thaliana* recruited

364 preferentially total and active fungi and inhibited fungal pathogens like *Alternaria* sp. Some  
365 of these species, e.g. *Mycrosphaerella tassiana* and *Pyrenophora teres* (similar to OTUs 9, 7  
366 and 2, respectively) are known to cause leaf diseases, such as net blotch (Thomma, 2003;  
367 El-said et al., 2014; Moya et al., 2018). Further, *A. thaliana* enriched *Mortierella*, *Tremellales*  
368 and *Sordariomycetes*, in agreement with both Tkacz et al., (2015) and Fabianska et al.,  
369 (2019). *Mortierella* and *Tremellales* are known to be root associated saprotrophic fungi  
370 involved in litter decomposition (Keim et al., 2014; Kuramae et al., 2013). Regarding the  
371 main bacterial phyla enriched by *A. thaliana*, the *Proteobacteria* have already been detected  
372 as recruited by this plant (Micallef et al., 2009; Bulgarelli et al., 2012; Lundberg et al., 2012)  
373 and members of this phylum possess several plant growth-promoting mechanisms (see  
374 references in Naz et al., 2014) and/or are associated with diseases suppression (Mendes et  
375 al., 2011). *T. aestivum* enriched other bacteria, such as *Planctomycetes*, *Acidobacteria*,  
376 *Bacteroidetes* and *Actinobacteria*, which is in agreement with those identified by Mahoney  
377 et al. (2017). *Actinobacteria* and *Bacteroidetes* are known to be plant-growth-promoting  
378 bacteria through the synthesis of different phytohormones, siderophores and enzymes and  
379 also their ability for solubilization of mineral nutrients such as phosphorus, potassium, and  
380 zinc (reviewed in Yadav et al., 2018). The soil phosphorus contents we measured in soil  
381 planted with *T. aestivum* are in accordance with higher microbial ability for providing  
382 phosphorus from soil matrix due to enrichment of plant growth-promoting bacteria by *T.*  
383 *aestivum*. A renewal of the soil phosphorus pool through solubilization could explain the  
384 differences in the soil content of phosphorus between the two plants, with the phosphorus  
385 pool decreasing in *A. thaliana* but not in *T. aestivum*. Also root architecture and mycorrhizal  
386 colonization can influence the efficiency of plants phosphorus uptake (Richardson et al.,  
387 2009; Zuo et al., 2014).

#### 388 **4.2. No plant legacy effects on the mineralization of soil organic carbon despite higher** 389 **plant-induced basal respiration**

390 Based on the observed plant species-specific effects on the microbial community  
391 composition in the plant-soil experiment and the difference in the quantity or quality of the  
392 carbon (from rhizodeposits or litter) between plant species (Johnson et al., 2007; Gul et al.,  
393 2012), we expected different basal respiration rates during the mineralization experiment.  
394 However, there were no plant-species effects, but rather a plant effect. The quantity of

395 organic carbon and the respiration rates were enhanced in both soils that previously had  
396 plants compared to the bare soil when no litter was added. The previously planted soils also  
397 had higher diversity of active and total bacteria as well as total fungi compared to the  
398 previously bare soil. These results suggest functional redundancy within the communities,  
399 which could be expected for carbon respiration, which is a generalist function of central  
400 importance for soil ecosystem resilience (Bender et al., 2016). Moreover, we cannot exclude  
401 that the plant legacy might have been reduced by the soil disturbance and the  
402 homogenization that occurred during the soil sieving for conducting the carbon litter  
403 mineralization experiment.

404 We did not observe any plant-specific effect following the addition of *T. aestivum*  
405 litter. The seeds of *T. aestivum* used for the plant-soil experiment and the litter used for the  
406 mineralization experiment came from the same variety and batch. Hence, in the context of  
407 plant-soil feedbacks and legacy effect, we could have expected some litter specificity (Veen  
408 et al., 2015a). Due to previous plant uptake, the mineral nutrient availability in the soil was  
409 lower and could thus have limited the degradation of high C:N litter, as previously shown  
410 (Partey et al., 2014). This nutrient limitation would explain the lower mineralization rates in  
411 previously planted soil compared to the bare soil when litter was added. In agreement, we  
412 observed a pulse of emission at day 7 in the bare soil with litter and simultaneously, we  
413 identified that diazotrophic bacteria, such as *Caulobacteraceae* (*Alphaproteobacteria*),  
414 *Burkholderia* and *Cellvibrio* (*Gammaproteobacteria*) (Suarez et al., 2014; Pagé et al., 2019),  
415 were activated and positively correlated to the soil  $\text{NO}_3^-$  content. Their activity would  
416 thereby support the nitrogen demand needed for degradation of the high C:N ratio litter  
417 from *T. aestivum*. Moreover, the limitation of the litter carbon mineralization that we  
418 observed and which was linked to the inorganic nitrogen status, is in agreement with the  
419 regulatory gate theory proposed by Brookes et al. (2008). This theory suggests that the rate  
420 of the soil organic matter mineralization is directly governed by abiotic processes, such as  
421 nutrients dynamics in the soil matrix, rather than by soil microbial communities themselves  
422 (composition, abundance or activity).

423 A microbial legacy due to specific plant species was observed until the end of the  
424 mineralization experiment for both plants, although microbial successions with different  
425 responses to the addition of litter according to soils were demonstrated. Among the OTUs

426 enriched by *A. thaliana*, some bacterial OTUs were also activated in the beginning of the  
427 incubation period of the litter experiment but, globally, these OTUs did not remain activated  
428 until the end of the experiment. We previously highlighted that both plant species recruited  
429 total plant growth-promoting bacteria during their growth. Due to these intimate  
430 interactions, the disappearance of the plants could lead to the inactivation of these specific  
431 bacteria during the mineralization experiment. The peak in abundance of active bacteria  
432 observed at day 3 was potentially due to the activation of copiotrophic microorganisms  
433 (*Alphaproteobacteria* and *Ascomycota*), while at the end of the incubation, the oligotrophic  
434 *Deltaproteobacteria* were stimulated (Bastian et al., 2009; Verastegui et al. 2014; Bastida et  
435 al., 2016). Microbial abundances shared similar temporal patterns in the *T. aestivum* and *A.*  
436 *thaliana* soils without litter, but not when the soil was C-enriched with litter. The peak of  
437 active fungal abundances at day 21 was mainly due to unknown fungi and their decrease at  
438 day 49 was mainly due to the copiotrophic *Ascomycota*. At this timepoint, the higher  
439 abundances of active microorganisms in the soil that previously had plants were  
440 concomitant with higher daily emissions.

441 To conclude, the effect of plants on the soil microbiome was plant-species specific.  
442 While both plants recruited plant growth-promoting bacteria in the vicinity of their roots, *A.*  
443 *thaliana* had a stronger effect on fungi than *T. aestivum*. The species specific responses were  
444 also different between active and total bacterial and fungal communities. However, this was  
445 not reflected in the observed plant legacy effect on the carbon mineralization from litter as  
446 higher basal respiration rates were detected in planted soils irrespective of plant species.  
447 Our results suggest that the soil nutrient dynamics induced by the presence of plants rather  
448 than changes in soil microbial communities drive the organic carbon mineralization of added  
449 litter. Nevertheless, soils with a planting history either maintained or displayed a higher  
450 abundance of active soil microorganisms, which was concomitant with higher daily  
451 respiration rates. In addition, the present study further highlights the importance to consider  
452 both total (DNA-based) and active (RNA-based) microbial communities to be able to  
453 differentiate how plants control enrichment/depletion and activation/inhibition of specific  
454 microorganisms.

455

456 **Acknowledgments**



457 This research was supported by the "Association Nationale de la Recherche et de la  
458 Technologies » (ANRT) through the convention N° 2016/0091 to F. Binet and O. Klarzynski  
459 and by the University of Rennes 1 through its international mobility programm N° 12/  
460 2018 to E. Hellequin. This research was also partly funded by the 2015-2016 BiodivERsA call  
461 by The Swedish Research Council Formas (contract 2016-0194). The authors thank Mikael  
462 Fauchaux for sampling the soil in the field before the experiment and Nathalie Le Bris and  
463 Marion Chorin from the EcoChim platform for the soil nutrient measurements  
464 (<https://ecobio.univ-rennes1.fr/plateforme-ecochimie-ecochim-presentation.php>).

### 465 **Competing Interests**

466 The authors declare no competing interests.

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755

## 756 Figure legends

757 **Figure 1: Carbon, nitrogen and Olsen phosphorus content in the original soil and at the end**  
758 **of the plant-growing experiment (*A. thaliana*, *T. aestivum* and bare soil).** The different  
759 letters indicate significant differences between treatments according to ANOVA and Tukey's



760 test ( $n=4$ ,  $p<0.05$ ). The red dot in each box indicates the mean value. MBC: microbial  
761 biomass carbon, MBN: microbial biomass nitrogen.

762

763 **Figure 2: Microbial community abundances and diversity in the original soil and after**  
764 **terminating the plant-soil experiment (*A. thaliana*, *T. aestivum* and bare soil).** The  
765 abundance and diversity (Shannon diversity index) of the total bacteria and fungi were  
766 obtained from DNA extracts and the abundance and diversity of active bacteria and fungi  
767 from RNA extracts. The different letters indicate significant differences between treatments  
768 according to ANOVA and Tukey's tests ( $n=4$ ,  $p<0.05$ ).

769 **Figure 3: Legacy effects of previously cultivated *A. thaliana* and *T. aestivum* in soil without**  
770 **or with litter addition on composition of the active bacterial and fungal communities.** The  
771 composition of the total bacterial and fungal communities present just after the plant-  
772 growth phase (63 days) is represented on the left part of the figure.

773 **Figure 4: Structure and composition of the total and active bacterial and fungal**  
774 **communities in unplanted (bare soil) or planted soil with *A. thaliana* or *T. aestivum* after**  
775 **63 days of plant-growth.** A, C, E, G – Powered partial least squares discriminant analysis  
776 (PPLS-DA) describing the total and active bacterial and fungal community structures at the  
777 OTU level after plant-growth. B, D, F, H - Ternary plots describing the distribution of the  
778 individual bacterial and fungal DNA-based or RNA-based OTUs between the soil treatments  
779 after plant-growth (grey circles). Color code: Enriched (DNA based) or activated (RNA based)  
780 OTUs by *A. thaliana* (dark green circles) or by *T. aestivum* (blue circles), depleted (DNA  
781 based) or inhibited (RNA based) OTUs by *A. thaliana* (light green) or by *T. aestivum* (light  
782 blue), enriched or activated OTUs by both plants (orange) and depleted or inhibited by both  
783 plants (yellow). The numbers below *A. thaliana* or *T. aestivum* corresponded to the OTUs  
784 that were only present in these soils. The inserted boxes zoom on the most abundant OTUs.

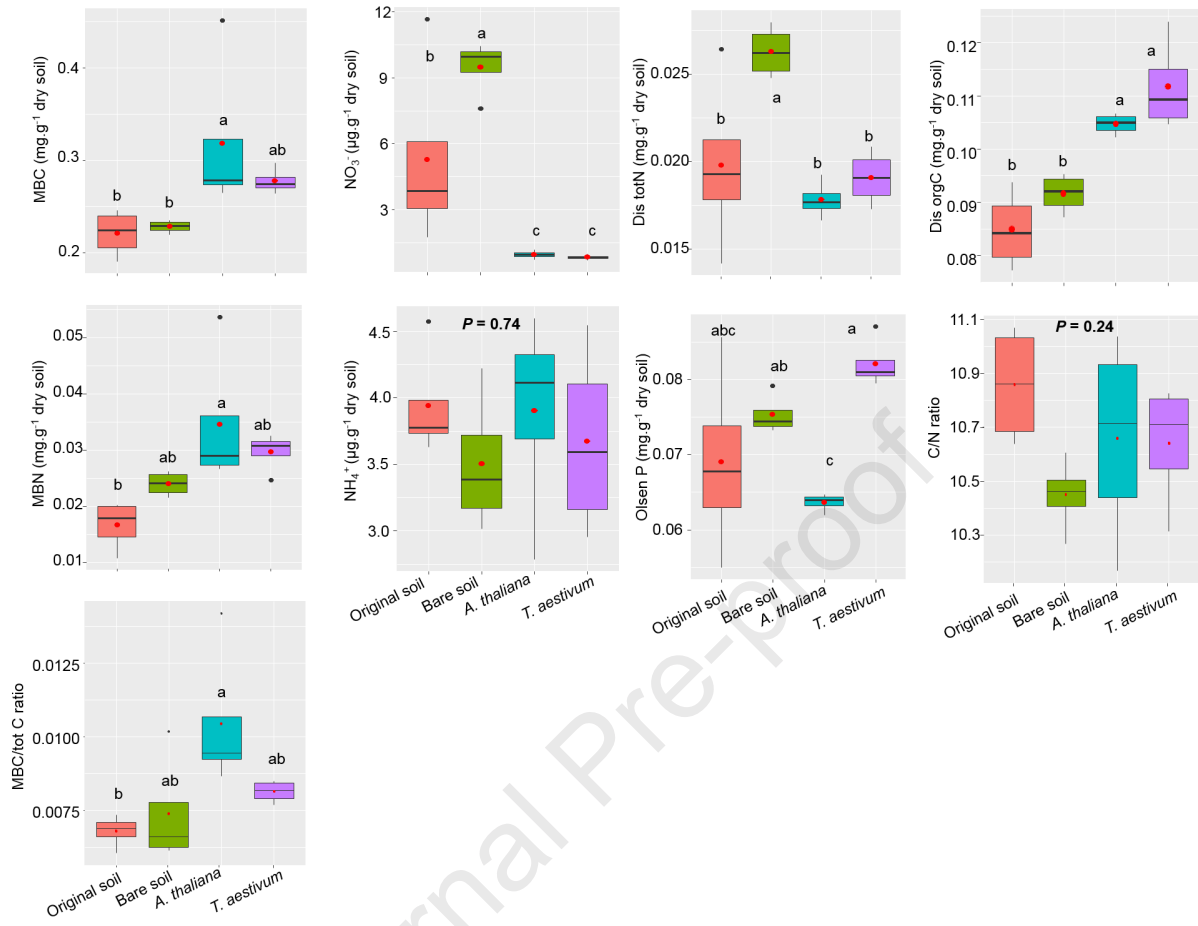
785 **Figure 5: Daily and cumulative C-CO<sub>2</sub> emissions at different sampling times in different**  
786 **soils without or with litter addition.** Different letters indicate significant differences at each  
787 day between treatments according to ANOVA and Tukey's test ( $n=4$ ,  $p<0.05$ ). Bare soil: not  
788 planted soil; *A. thaliana*: soil previously planted with *A. thaliana*; *T. aestivum*: soil previously  
789 planted with *T. aestivum*; Bare soil-litter: not planted soil with plant litter addition;  
790 *A.thaliana*-litter: soil previously planted with *A. thaliana* and with plant litter addition; *T.*  
791 *aestivum*: soil previously planted with *T. aestivum* and with plant litter addition

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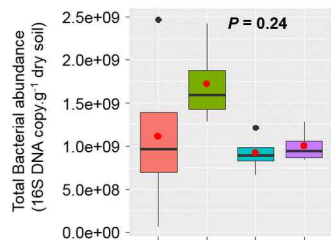
**Table 1: Physical, chemical and microbiological properties of the soil used for plant-soil experiment (phase 1).**  
(Means and standard errors, n=4).

Physical and chemical	Data (mean, n=4)	Microbiological properties	Data (mean, n=4)
% Clay	12.9	MBC ( $\mu\text{g g dry soil}^{-1}$ )	221 $\pm$ 13
% silt	40.2	MBN ( $\mu\text{g g dry soil}^{-1}$ )	17 $\pm$ 2
% sand	46.8	Total fungi TS (copy g dry soil <sup>-1</sup> )	2.29E+07 $\pm$ 1.15E+07
% organic matter	4.9	Total bacteria 16S (copy g dry soil <sup>-1</sup> )	1.12E+09 $\pm$ 5E+08
Tot orgC ( $\text{mg g dry soil}^{-1}$ )	33 $\pm$ 1	DNA ( $\text{ng g dry soil}^{-1}$ )	21323 $\pm$ 8299
tot N ( $\text{g kg dry soil}^{-1}$ )	3 $\pm$ 0.12	Active fungi (ITS (copy g dry soil <sup>-1</sup> ))	9.80E+07 $\pm$ 7.81E+06
Diss tot N ( $\mu\text{g g dry soil}^{-1}$ )	20 $\pm$ 23	Active bacteria 16S (copy g dry soil <sup>-1</sup> )	1.66E+12 $\pm$ 3.84E+10
Diss NO <sub>3</sub> <sup>-</sup> ( $\mu\text{g g dry soil}^{-1}$ )	5 $\pm$ 2	RNA ( $\text{ng g dry soil}^{-1}$ )	13371 $\pm$ 1153
Diss NH <sub>4</sub> <sup>+</sup> ( $\mu\text{g g dry soil}^{-1}$ )	4 $\pm$ 0.2		
Diss orgC ( $\mu\text{g g dry soil}^{-1}$ )	85 $\pm$ 4		
Olsen PO <sub>4</sub> ( $\text{mg g dry soil}^{-1}$ )	69 $\pm$ 6		
P ( $\text{mg kg dry soil}^{-1}$ )	212		
Ca ( $\text{mg kg dry soil}^{-1}$ )	1159		
Mg ( $\text{mg kg dry soil}^{-1}$ )	182		
K ( $\text{mg kg dry soil}^{-1}$ )	392		
pH <sub>water</sub>	5.5		
CEC	10.8		

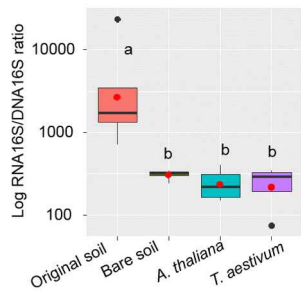
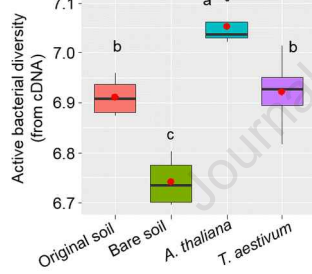
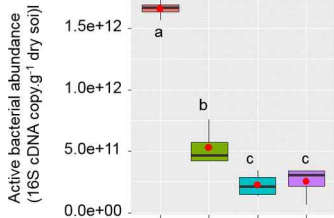
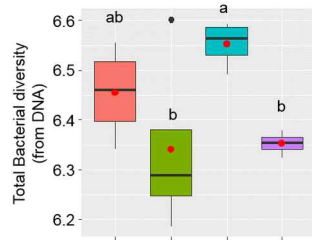
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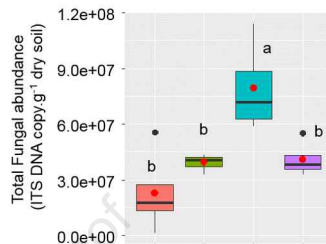
## Abundances



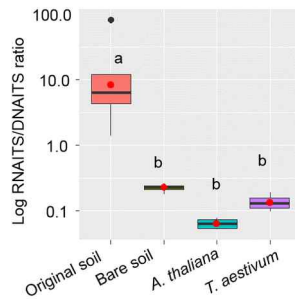
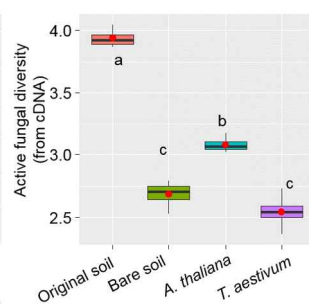
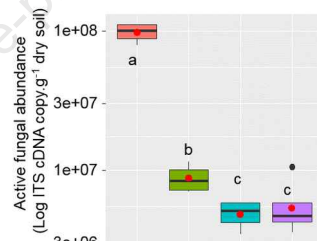
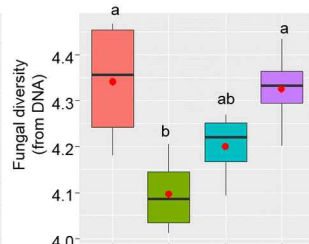
## Diversity



## Abundances

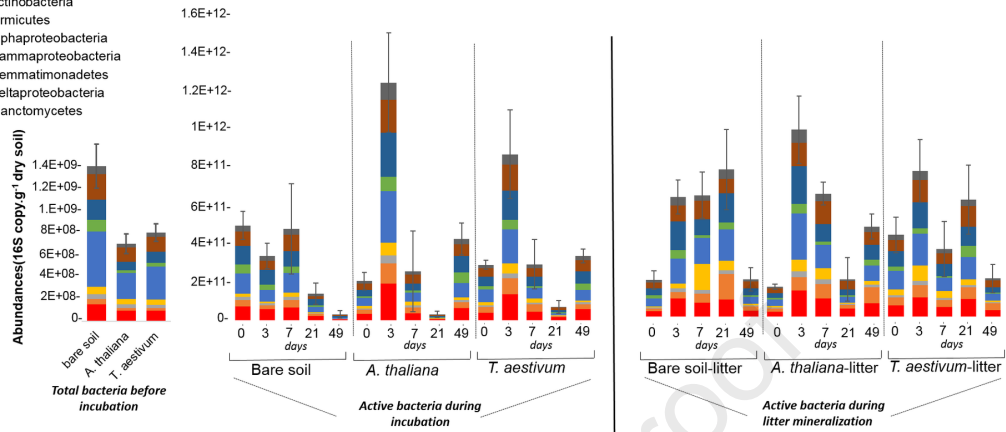


## Diversity



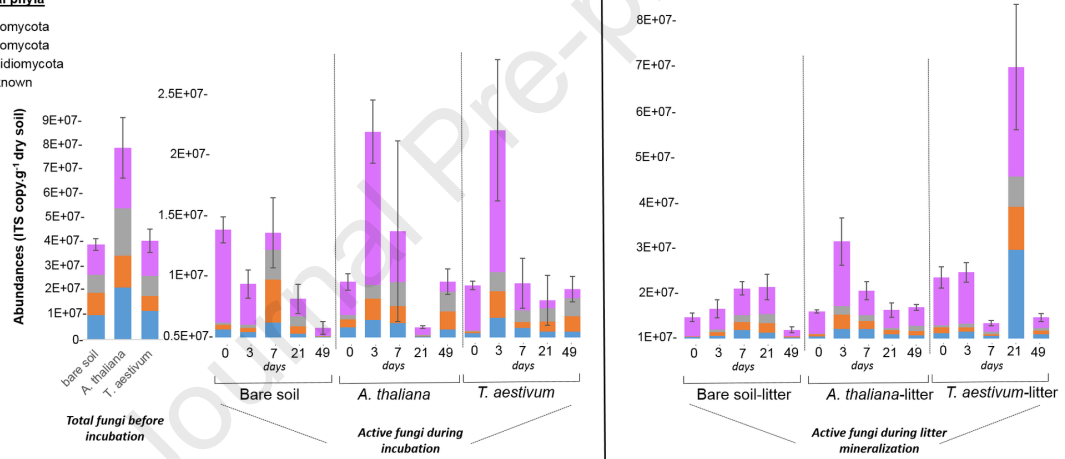
**Bacterial phyla**

- Chloroflexi
- Acidobacteria
- Actinobacteria
- Firmicutes
- Alphaproteobacteria
- Gammaproteobacteria
- Gemmatimonadetes
- Deltaproteobacteria
- Planctomycetes

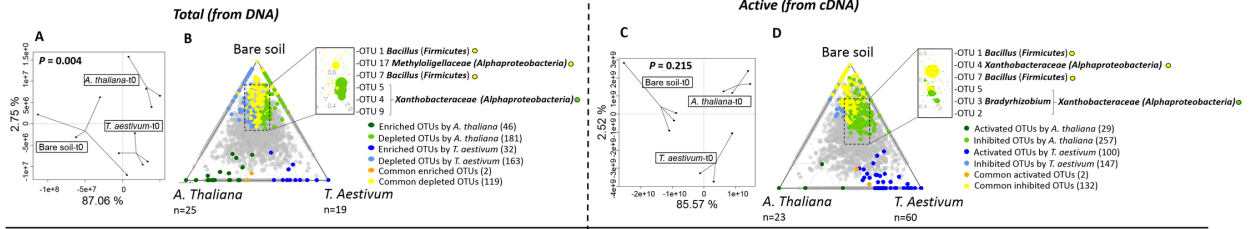


**Fungal phyla**

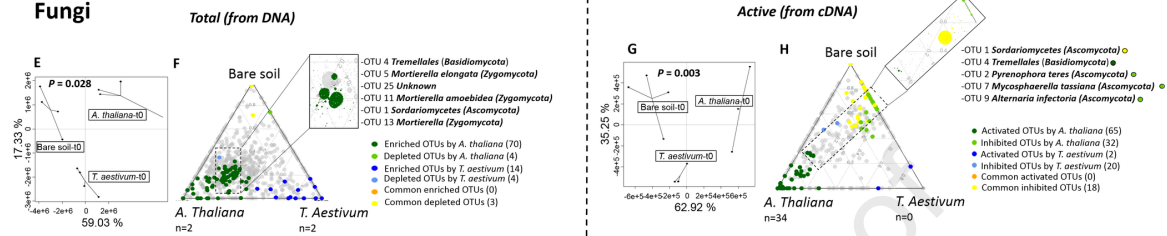
- Ascomycota
- Zygomycota
- Basidiomycota
- Unknown



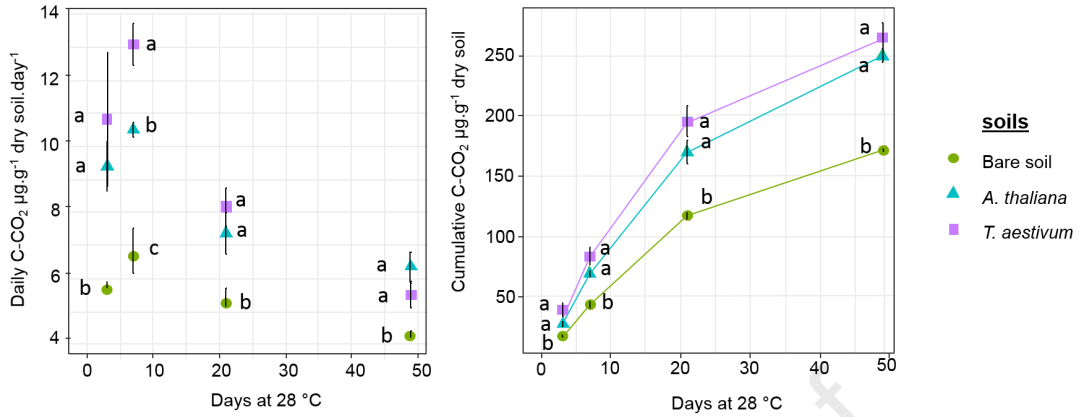
**Bacteria**



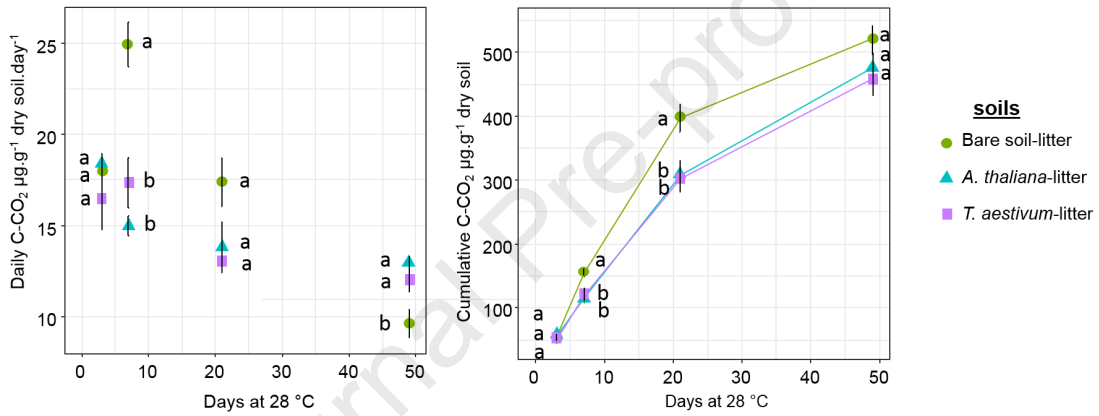
**Fungi**



## Without litter



## With litter





- *A. thaliana* and *T. aestivum* differently shaped the soil microbial communities
- *A. thaliana* had a stronger effect, especially for fungal communities
- PGPB were recruited in the vicinity of the roots of both *A. thaliana* and *T. aestivum*
- Plant legacy effects traduced in changes in soil organic carbon and nutrient dynamics.
- Plant legacy effects linked to nutrient dynamics rather than to microbial community

Journal Pre-proof

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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