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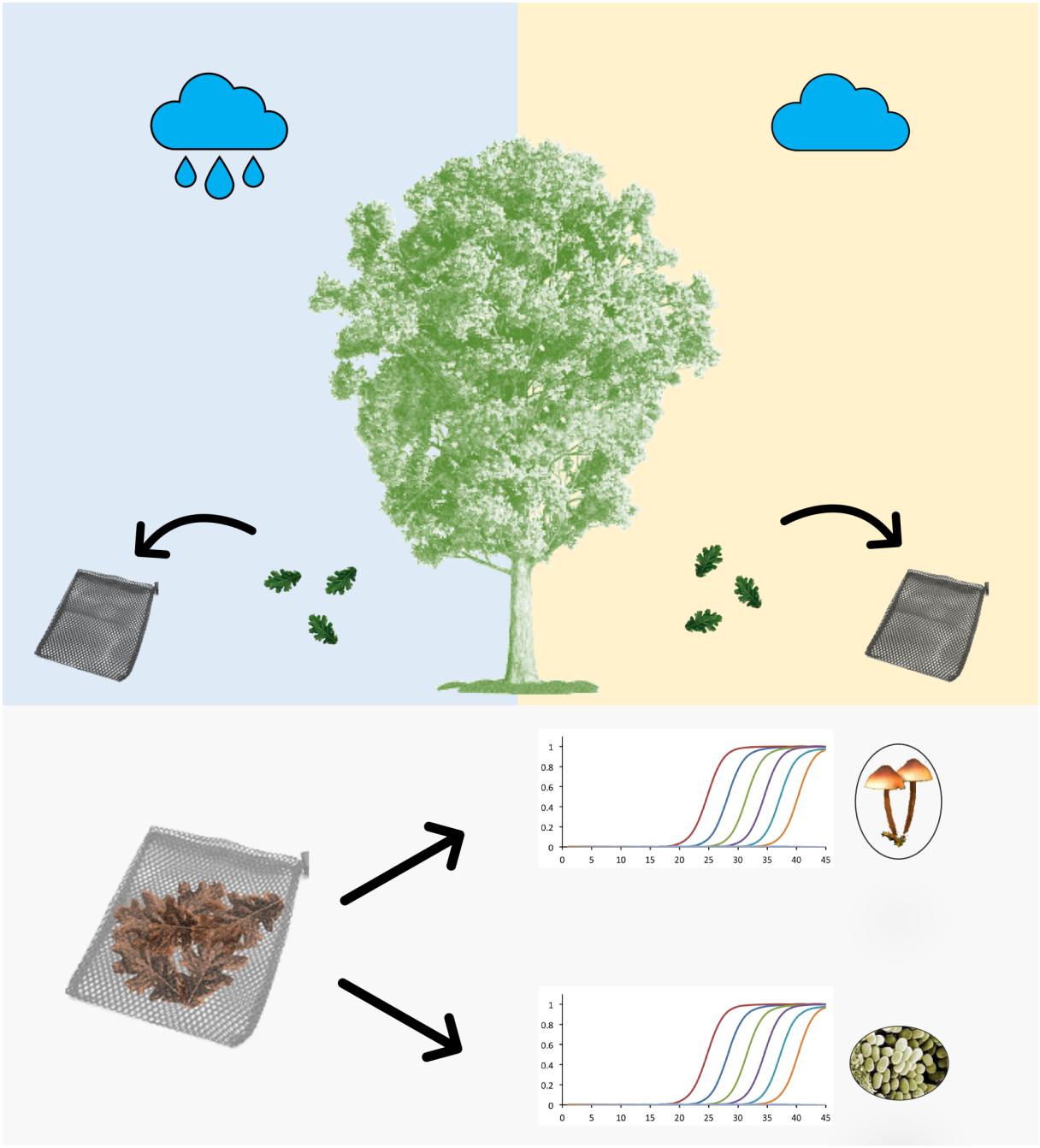
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Litter traits and rainfall reduction alter microbial litter decomposers: the evidence from three Mediterranean forests

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Keywords: *litter decomposition, fungi, actinobacteria, Mediterranean forest, climate change*

Abstract

The objective of the study was to evaluate changes in microbial communities with the predicted arrival of new species to Mediterranean forests under projected intensification of water stress conditions. For that, litter from three Mediterranean forests dominated respectively by *Quercus pubescens* Willd., *Quercus ilex* L. and *Pinus halepensis* Mill. was collected, and placed to their "home" forest but also to the two other forests under natural and amplified drought conditions (i.e. rainfall reduction of 30%). Quantitative PCR showed that overall, actinobacteria and total bacteria were more abundant in *Q. pubescens* and *Q. ilex* than in *P. halepensis* litter. However, the abundance of both groups was dependent on the forest sites: placement of allochthonous litter to *Q. pubescens* and *P. halepensis* forests (i.e. *P. halepensis* and *Q. pubescens*, respectively) increased bacterial and fungal abundances, while no effect was observed in *Q. ilex* forest. *P. halepensis* litter in *Q. pubescens* and *Q. ilex* forests significantly reduced actinobacteria (A/F) and total bacteria (B/F) to fungi ratios. The reduction of rainfall did not influence actinobacteria and bacteria but caused an increase of fungi. As a result, a reduction of A/F ratio is expected with the plant community change towards the dominance of spreading *P. halepensis* under amplified drought conditions.

1. Introduction

Microorganisms are key actors in nutrient cycling and carbon turnover during litter decomposition (Gessner *et al.* 2010; Garcia-Pausas and Paterson 2011). At a global scale, it is estimated that the soil C coming from soil microbial biomass is about 16.7 Pg C, which represents approximately 2.3–2.4% of total organic C found in soil in the top 30 cm (Xu, Thornton and Post 2013). Heterotrophic respiration resulting from microbial litter decomposing activity can account for 10–90% of the CO_2 efflux from soils and substantially affect the atmospheric CO_2 concentration (Manzoni *et al.* 2011). However, annual reduction of rainfall by up to 30% predicted for the Mediterranean basin (Giorgi and Lionello 2008 ; IPCC 2014 ; Hertig and Tramblay 2017) may significantly impact microbial abundance and structure, and thus, also the overall C dynamics (Wardle and Putten 2002).

Studies have already demonstrated shifts in plant community composition in response to the changed climatic conditions (Morales *et al.* 2007; Bertrand *et al.* 2011 ;Guiot and Cramer 2016). In southern France, dominating downy oak (*Quercus pubescens* Willd.) is expected to be replaced by more drought tolerant species: the sclerophylls' evergreen holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Mill.) (Barbero *et al.* 1990 ; Fortunel *et al.* 2009). Additionally, the increase of water stress may induce the synthesis of plant secondary metabolites (e.g. phenolics) known for their phytotoxic activities and anti-appetizing effects, which limit leaf consumption by fauna (Chomel *et al.* 2014). This investment in defence mechanisms over primary metabolic processes may result on the one hand to the decrease of primary productivity (Ogaya and Peñuelas 2007) and thus litter inputs, and on the other hand, in the reduction of litter quality through the increase of recalcitrant organic compounds (i.e. lignin, wax, polyphenolics) (Ormeño *et al.* 2006 ; Hättenschwiler and Jørgensen 2010 ; Sagova-Mareckova *et al.* 2011). Moreover, the reduction of moisture level may induce

physiological stress and limit dispersal of microorganisms, enzymatic activities, and substrate diffusion (Bouskill *et al.* 2013). Thus, the reduction of moisture levels and aboveground litter quality and quantity may impose new community dynamics belowground (Lunghini *et al.* 2013 ; Cleveland *et al.* 2014) and lead to a shift of soil microbial communities to more drought tolerant species, capable of using recalcitrant C sources and resistant to desiccation (Yuste *et al.* 2011 ; Peñuelas *et al.* 2012 ; Allison *et al.* 2013). Actinobacteria are known to thrive in low resource environments (Fierer, Bradford and Jackson 2007) and low soil moisture conditions (Yuste *et al*. 2011; De Vries and Shade 2013; Acosta-Martinez *et al.* 2014) reaching high abundance in arid soils (Okoro *et al.* 2009). They are well protected by a strong, thick interlinked peptidoglycan cell wall, which explains their high resistance to low osmotic potential conditions (Davet 2004). They produce enzymes able to degrade a wide range of biopolymers, whose building blocks including aromatic compounds (McCarthy and Williams 1992) have a vital role in organic matter turnover and humus formations (Anandan, Dharumadurai and Manogaran 2016). They produce a variety of secondary metabolites (Anandan et al. 2016), through which they interact with other microbial groups (Lewin *et al.* 2016). However, little is known about how microbial communities and actinobacteria in particular, would be affected by future reduction of rainfall, both directly by decrease of moisture level and indirectly through the plant species shift. In general, litter types sharing a common history with local microbial communities (autochthonous) have faster decomposition rates than litter types coming from distant (allochthonous) habitats (Gholz *et al.* 2000; Strickland *et al.* 2009). Consequently, changes in biochemical composition of local litter with the arrival of new species may constrain microbial activity and impact negatively litter decomposition (Schimel, Balser and Wallenstein 2007).

In here, we examine how reduction of rainfall and change in plant species may affect belowground microbial community's (actinobacteria, fungi and total bacteria) abundance and structure and consequently also the litter decomposition process. For that, we performed a litter transplanting experiment between three main Mediterranean forests (*Quercus pubescens* Willd., *Quercus ilex* L., and *Pinus halepensis* Mill.) occurring in Southeast of France, under natural and amplified drought conditions for simulating the 30% rainfall reduction. We hypothesised that the arrival of new litter under amplified drought conditions will have a negative impact on microbial abundance and litter decomposition.

2. Materials and Methods

2.1 Sites description

This study was conducted at the three forest sites in the Mediterranean region in southern France: a *Quercus pubescens* forest at Oak Observatory at the "Observatoire de Haute Provence" (O_3HP; Santonja *et al*. 2015), *Quercus ilex* forest at Puéchabon (Limousin *et al.* 2008) and the mixed *Pinus halepensis* forest at Font-Blanche (Table 1). During the study period, the mean annual temperature was 12.1 °C, 13.7°C and 14.0°C and the cumulative annual precipitation was 609 mm, 794 mm and 632 mm, respectively (Table 1, Supplementary Figure 1). There were no significant differences between sites in the mean annual temperature and cumulative annual precipitation during the study period (One-way ANOVA, $P<0.05$). Each site included a control plot (natural drought - ND) and a rain exclusion plot (amplified drought – AD) to mimic the future reduction of rainfall predicted by the climatic model for the Mediterranean Region (about -30% yearly; Table 1 and Supplementary Figure 2).

122 **2.2 Experimental design and sample processing**

123 In 2014, senescent leaves were collected in the control plot of the three forests. For
124 that, litter traps were used during the abscission period that occurred from October to
125 November for the broadleaves (*Q. ilex* and *Q. pubescens*) and from June to September for the
126 needles (*P. halepensis*). Leaves and needles were air dried and stored at room temperature in
127 paper boxes until the beginning of the experiment.

128 Litter decomposition was assessed by using the litter bag method (Swift *et al.* 1979).
129 Four-mm mesh litter bags (20 × 20 cm) containing 10 grams (air-dried) of the senescent
130 material were used to perform the experiment. Litter transplants were made between each site
131 for the three species considered, i.e. a litter bag containing the litter of each species placed on
132 each forest site, under two drought conditions: natural (ND) and amplified (AD) (see
133 Supplementary Figure 3).

134 Thus, the experiment consisted in 18 modalities corresponding to the 3 forests *Q. ilex,*
135 *Q. pubescens* and *P. halepensis*) × 3 plant species (*Q. ilex, Q. pubescens* and *P. halepensis*) ×
136 2 drought conditions (ND and AD). In total, 126 litter bags (18 modalities × 1 sampling date
137 × 7 replicates) were analyzed. Litter bags were placed perpendicularly to the gutters system in
138 Aleppo pine and holm oak forests and under the rain exclusion device in the downy oak
139 forest, by using a transect in blocks (7 columns x 6 lines), equidistantly one from each other
140 (1 m distance between the 7 columns and 0.6 m between the 6 lines). Transects were E-W
141 oriented. They were disposed in the ground floor after the removal of litter layer and fixed to
142 the soil with galvanized nails to prevent movement by animals or wind. Litter layer was then
143 replaced over the litterbags.

144 Litter bags were retrieved after one year (December 2015). Litterbags were placed in
145 zipped plastic bags to prevent the loss of biological material. In the laboratory, the soil and

other debris were removed from the litter bags. Then, they were freeze-dried (Lyovac GT2) for 72 h and the remaining dry mass (%) was calculated. A 1 g of dry mass aliquote was collected, grounded to powder (Retsch® MM400, 30 Hz during 30s) and kept at -20^{o}C for posteriori microbial DNA analysis.

2.3 Initial litter chemical characteristics

At the beginning of the experiment, 5 replicates from each leaf species collected from trees in control plots were grounded to powder (Retsch® MM400, 30 Hz during 30s). Carbon (C) and nitrogen (N) were determined by thermal combustion on a Flash EA 1112 series C/N elemental analyzer (Thermo-Scientific, U.S.A). Phosphorus (P) and cations (Ca, K, Mg and Na) were extracted from 80 mg of ground litter sample with 8 ml of nitric acid and 2 ml of H_2O_2 at 175 °C for 40 min using a microwave digestion system (Ethos One, Milestone SRL, Sorisole, Italy). After the mineralization, each sample was adjusted to 50 ml with demineralized water. Phosphorus concentration was measured at 720 nm using a microplate reader (Victor, Perkin Elmer, Waltham, MA, USA). Cations were determined using an atom absorption spectrometer (AAS, iCE 3000 series, Thermo-Scientific, China). Leaf structural compounds (lignin, cellulose and hemicellulose) and water-soluble compounds (WSC) concentrations were determined according to the van Soest extraction protocol (Van Soest and Wine 1968) using a fiber analyzer (Fibersac 24; Ankom®, Macedon, NJ, USA). Total phenolic concentrations were measured by colorimetry according to the method of Peñuelas *et al.* (1996) using gallic acid as standard. Aqueous extracts were made by dissolving 0.25 g of litter powder in 20 ml of 70% aqueous methanol solution with shaking for 1 h and filtered (0.45 μm). 25 ml of extracts obtained were then mixed with 0.25 ml of Folin-Ciocalteu reagent (Folin and Denis 1915), 0.5 ml of saturated aqueous Na_2CO_3 (to stabilize the

colorimetric reaction) and 4 ml of distilled water. After 60 min, the reaction was completed and the concentration of phenolics was measured at 765 nm using a UV / Vis spectrophotometer (Thermoscientific, U.S.A.).

To determine the water holding capacity (WHC), twenty-one freshly abscised leaves (3 species x 5 replicates) were soaked in distilled water for 24 h, drained and weighted (wet weight). The dry weight was measured after drying the samples for 48 h at 60°C. WHC (in %) was calculated according to the formula: (wet weight/dry weight) × 100 (Santonja *et al.* 2015). Specific leaf area (SLA) was determined by using the Image J software (https://imagej.nih.gov/ij/, MA, USA). SLA was calculated as the ratio between leaf area and leaf dry weight.

2.4 DNA extraction

Environmental DNA from leaf litter samples was extracted with the NucleoSpin® soil kit (Macherey-Nagel, Düren, Germany), following the standard protocol with a slight adjustment. In brief, approximately 250 mg of dry litter was added to the NucleoSpin Bead tubes. Lysis conditions were performed by using buffer SL1 (700 µl, vortex for 5 min, room temperature, 11 000 x g for 2 min), and buffer SL3 (150µl, vortex 5 min, 0-4^{o}C, 14 000 g for 1 min). This procedure was repeated twice using the same sample to optimize the DNA extraction. The filtration of the supernatant was performed in the NucleoSpin Inhibitor Removal Column followed by 11 000 x g for 1 min. DNA binding was done in the Nucleo Spin Soil column after adding 250µl of Buffer SB to the supernatant followed by 11 000 x g for 1 min. A series of successive DNA washing was performed: 500 µl buffer SB (11 000 x g, 3 sec), 550 µl buffer SW1 (11 000 x g, 3 sec), 700 µl SW2 (2 sec vortex, 11 000 x g, 3 sec). At each step, flow-through were discarded. Finally, DNA was eluted in 30 µl of Buffer SE followed by

194 incubation at room temperature for 1 min and 11 000 x g for 30 seconds and kept at -20^{o}C
195 until further analysis. DNA quantification was performed in the ND-1000 spectrophotometer
196 (Nanodrop Technology, Wilmington, DE).
197
198 **2.5 Quantitative real-time PCR analysis (qPCR)**
199 Abundances of total bacteria, actinobacteria and fungi were assessed using a quantitative real-
200 time PCR (qPCR) method. Microbial abundance was quantified as copy number of a target
201 gene. Partial 16S rRNA gene sequences were amplified from the total bacteria using primers
202 COM1 (5' – CAGCAGCCGCGGTAATAC - 3') and 769R (5' –
203 ATCCTGTTTGMTMCCCVCRC - 3', annealing temperature 59^{o}C) (Dorn-In *et al.* 2015),
204 and from actinobacteria using primers S-P-Acti-1154-a-S-19 (5' –
205 GRDACYGCCGGGGTYAACT - 3' annealing temperature 59^{o}C) and S-P-Acti-1339-a-A-18
206 (5' – TCWGCGATTACTAGCGAC - 3') (Pfeiffer *et al.* 2014). Partial 18S rRNA genes from
207 fungi were amplified with primers FF390 (5′-CGATAACGAACGAGACCT-3′) and FR1 (5′-
208 AICCATTCAATCGGTAIT-3′, annealing temperature 50^{o}C) (Vainio and Hantula 2000). All
209 PCR reactions were done 15 µl total volume containing 1× SYBR® Green Gotaq PCR Master
210 Mix (Promega), primers at 600 nM for total bacteria and fungi, and 200 nM for
211 actinobacteria, and 0.2-2 ng environmental DNA. The cycling parameters were: 10 min at
212 95°C followed by 45 cycles at 95°C (15 sec), 59°C (30 sec), 72ºC (30 sec). Melting curves
213 were recorded to ensure qPCR specificity, and gel electrophoresis analyses were performed to
214 confirm the expected size of amplified products. All qPCR reactions were run in triplicate on
215 StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA). To quantify
216 the target gene copy numbers, standard dilution curves of known concentrations of previously

cloned standards of the target genes were determined. Baseline and threshold calculations and data analysis were performed with the StepOne v. 2.2.2 software.

The primer pair recommended by Dorn-In *et al*. (2015) for amplification of bacterial 16S rRNA gene fragment from plant samples, Com1/769R (Fredriksson, Hermansson and Wilén 2013; Rastogi *et al.* 2010) was used for quantitative real-time PCR analysis of the total bacteria. The primer pair does not amplify 16 rRNA genes from chloroplasts, however, consequently also its coverage of the domain Bacteria is incomplete. In silico analysis using TestPrime 1.0 (Klindworth *et al.* 2013) against the Silva database, release 132 (www.arb-silva.de), showed 75% overall coverage of Bacteria with varying representation of the phyla, e.g. 88% Proteobacteria, 81.9% Firmicutes, 93.6% Bacteroidetes, but only 56.2% Actinobacteria, 20.5% Acidobacteria, and 17.5% Verrucomicrobia. Therefore, the results can be used as a proxy of bacterial abundance when comparing between samples, but not to compare with qPCR quantification of individual bacterial phyla or lower taxonomic units using group-specific primers.

2.6 Statistical analysis

All statistical analyses were performed in R v.3.1.3 software (R Development Core Team, 2017). One-way analysis of variance (ANOVA) was used to determine differences between the initial parameters of litter plant species and between forest sites for each litter species and microbial group. Three-way ANOVA was also used to test the effects of forest sites (S), litter species (L) and drought treatment (D) on litter mass loss and microbial abundance (total bacteria (B), actinobacteria (A), fungi (F) and the respective B/F and A/F ratios, followed by

post-hoc Tukey tests. Microbial abundance was log-transformed to fit the assumption of normality and homoscedasticity.

3. Results

3.1 Initial litter composition

Initial litter characteristics varied significantly among species (Table 2, One-way ANOVA, $P < 0.05$). *Q. ilex* showed a significantly higher N (9.62 mg g^{-1}) and P (3.48 mg g^{-1}) contents than the two other species, particularly *P. halepensis* (5.36 mg g^{-1} and 1.56 mg g^{-1}), which resulted in a lowest C/N and C/P ratio for *Q. ilex* and highest ratios for *P. halepensis* (Table 2). Regarding the cations, *Q. pubescens* showed significantly higher content of Ca (32.90 mg g^{-1}) and Mg (2.83 mg g^{-1}), and lower content of K (0.84 mg g^{-1}) and Na (0.04 mg g^{-1}) than the two other species. Concerning the structural compounds of the leaves, *Q. ilex* had higher lignin (337.38 mg g^{-1}) and cellulose content (204.99 mg g^{-1}) than the two other species. Finally, *Q. pubescens* showed higher total phenolics content compared to *Q. ilex*, and the highest specific leaf area (SLA) (133.13 mg g^{-1}) and water holding capacity (WHC) (146.90 mg g^{-1}). *P. halepensis* showed higher water-soluble compounds (WSC) (340.02 mg g^{-1}), and lower WHC (113.89 mg g^{-1}).

3.2 Litter mass loss

After 357 days of decomposition, litter mass loss was statistically different among sites and plant species with interaction effects (Table 3). On average, significantly higher mass loss occurred at *Q. pubescens* forest (36.94%) in comparison with *Q. ilex* forest

(33.88%) and *P. halepensis* forest (33.80%). Regarding the litter, *P. halepensis* (36.81%) and *Q. ilex* (35.07%) lost significantly higher mass than *Q. pubescens* (32.72%). Concerning interactions, *P. halepensis* lost most of its mass at *Q. pubescens* and *P. halepensis* forests compared to the *Q. ilex* forest (Fig. 1a). No significant effect of rain exclusion was found on the litter mass loss (Table 3).

3.3 Microbial abundance and structure

Abundances of actinobacteria, total bacteria and fungi differed significantly according to forest sites, when control and amplified drought conditions were combined (Table 3, Fig. 1) The highest abundances of all microbial groups were at *P. halepensis* forest and lowest at *Q. ilex* forest. However, it differed according to plant litter species producing a site x species interaction effect (Table 3; Fig.1). Actinobacteria and total bacteria had the highest abundance in *Q. ilex* litter and lowest in *P. halepensis* at *Q. ilex* and *P. halepensis* forests, respectively (Fig.1b, c). Roughly, higher abundance of fungi was observed in *P. halepensis* litter at *Q. pubescens* forest, *Q. ilex* litter at *Q. ilex* forest and *Q. pubescens* litter at *P. halepensis* forest (Fig. 1d). Regarding the actinobacteria to fungi (A/F) ratio, it was significantly higher in *Q. pubescens* and *Q. ilex* litter and lower in *P. halepensis* litter at *Q. pubescens* and *Q. ilex* forests (Fig. 1e). Total bacteria to fungi (B/F) ratio was significantly higher in *Q. ilex* litter and lowest in *P. halepensis* litter, at *Q. ilex* and *P. halepensis* forests (Fig. 1f).

Overall, there was a significant increase of fungal abundance and a decrease of A/F ratio under amplified drought (AD) in comparison with natural conditions (Table 3). No main effect of drought conditions was observed for actinobacteria, total bacteria, and bacteria to fungi (B/F) ratio (Table 3). However, an interaction effect was observed between site and drought conditions for total bacteria, fungi and total bacteria to fungi ratio (Table 3; see

287 Supplementary Table 1). Thus, we observed a significant increase of total bacterial (Fig. 2a)
288 and fungal abundance (Fig. 2b) under AD conditions at the *P. halepensis* forest, and a
289 decrease of B/F ratio in *Q. pubescens* forest (Fig. 2c).
290
291 **4. Discussion**
292 In this study, we evaluated how reduction of rainfall and change in plant species may
293 affect belowground microbial community's abundance and structure and consequently the
294 litter decomposition process in three dominant Mediterranean forests of southern France. Our
295 findings are that i) actinobacteria and total bacteria were more affected by changes of plant
296 species than by the decrease of water availability; ii) fungi were positively impacted by the
297 reduction of rainfall and iii) the future arrival of *P. halepensis* litter to *Q. pubescens* and *Q.*
298 *ilex* forest and rainfall reduction could alter the relative proportion of fungi and bacteria in the
299 community hence having implication in microbial decomposers activity.
300
301 **4.1 Effects of litter quality on microbial communities and mass loss**
302 Our results showed the importance of leaf litter initial litter with respect to microbial
303 composition and litter mass loss dynamics. We observed higher abundance of actinobacteria
304 and total bacteria in oak litter than in pine. This is in line with previous studies, which showed
305 a preference of bacteria for high quality litter (i.e. low C/N, C/P, lignin: P and lignin/N ratio)
306 (Hodge, Robinson and Fitter 2000 ; De Boer *et al.* 2005 ; Romaní *et al.* 2006). Both oaks *Q.*
307 *pubescens* and *Q. ilex* are characterized by high N and P content, which are efficiently utilized
308 by bacterial enzymes (Romani et al. 2006) and then are consistently considered key factors in
309 litter decomposition and good predictors of litter mass loss (Moro and Domingo 2000;
310 Garcia-Pausas, Casals and Romanya 2004; Güsewell and Gessner 2009). However, our results

311 only partially corroborate with this by showing that *Q. ilex* and *P. halepensis* litter had higher
312 mass loss than *Q. pubescens*. That is possibly due to higher initial content of total phenolics
313 determined in *Q. pubescens* compared to *Q. ilex*, which are known to inhibit litter microbial
314 communities and N mineralization, during the early stage of decomposition (Souto, Chiapusio
315 and Pellissier 2000 ; Lambers 1993; Ormeño *et al.* 2006 ; Chomel *et al*. 2014). This may
316 explain the lower mass loss of its litter in comparison with *Q. ilex* and *P. halepensis*. The high
317 mass loss of *P. halepensis* litter may be also explained by the leaching of high content of
318 water-soluble compounds (WSC) (Tripathi and Singh 1992 ; Kaushal *et al.* 2012) and high
319 Na content (Gressel *et al.* 1995), which both are known to positive correlated litter decay.
320 Gressel *et al.* (1995) observed that salt accumulation in *P. halepensis* needles enhanced their
321 breakdown compared to *Q. coccifera* leaves in a typical Mediterranean mixed woodland.

322

323 **4.2 Effects of litter-site interaction on litter decomposition processes**

324 The influence of plant species on litter decomposition varied according to the forest sites as
325 shown by strong litter-site interaction. At *Q. pubescens* forest, we observed the highest
326 abundance of fungi associated with *P. halepensis* litter, associated to a reduction of the A/F
327 ratio. This is consistent with the findings by Hodge *et al*. (2000) and Wardle *et al*. (2004) who
328 showed that fungi are favoured by litter with high C/N ratio. In addition, fungi are rather
329 considered specialists and are more equipped enzymatically to assimilate recalcitrant material
330 than more generalist bacteria (Møller, Miller and Kjøller 1999; Romaní *et al.* 2006). The high
331 abundance of total bacteria observed in *P. halepensis* litter may be explained by the
332 synergistic relationship between bacteria and fungi because bacteria may benefit by the
333 presence of fungi, which may provide them resources that bacteria were not able to acquire on
334 their own (Romaní *et al.* 2006). At *Q. ilex* forest, microorganisms were more numerous with

335 *Q. ilex* litter in comparison with the two other species, which suggests the high specificity of
336 decomposers for decomposing the autochthonous litter in this forest (Ayres *et al.* 2009).
337 However, this difference in microbial abundance did not lead to a significant difference in
338 mass loss of *Q. ilex* litter in its own forest compared to the two other forests. This may due to
339 the presence of decomposers able to use different sources of carbon. Decomposers can also
340 develop preferences for some resources they are not used to (St John *et al*., 2011). Taking into
341 account the three litter species, our results showed that *P. halepensis* forest had generally
342 higher microbial abundance (actinobacteria, total bacteria and fungi) than other sites, possibly
343 because this is the only mixed woodland out of the three studied forests and it was
344 demonstrated that an increase of plant diversity promotes litter microbial growth (Chapman
345 and Newman 2010 ; Santonja *et al.* 2017). Indeed, litter dissimilarity may decrease resource
346 competition between functional groups and thus increase the abundance, activity, and hence
347 efficiency of decomposers (Barbe *et al.* 2017).

348

349 **4.3 Effects of rainfall reduction on litter mass loss and microbial community**

350 Contrary to our expectations, amplified drought conditions (AD) had no effect on litter mass
351 loss after one year of decomposition. This may be explained by the extremely dry year during
352 which the study was performed (Saunier *et al.* 2018). Actinobacteria were not directly
353 affected by AD conditions, contrary to previous studies (Bouskill *et al.* 2013 ; Felsmann *et*
354 *al.* 2015). Nevertheless, a reduction in the A/F ratio was observed, probably due to the
355 positive effect of AD conditions on fungal abundance. The increase of fungal abundance
356 under drier conditions was also observed in other Mediterranean forests (Hawkes *et al.* 2011;
357 Sherman, Sternberg and Steinberger 2012 ; Allison *et al.* 2013 ; Alster *et al.* 2013). It is well
358 recognized that fungi are more resistant to drought than bacteria (De Vries and Shade 2013 ;

359 Guhr *et al.* 2015) and might get competitive advantage over bacteria during dry periods (Six
360 2012 ; Yueste *et al.* 2011). Moreover, the expansion of fungal hyphae reported by some
361 studies under drought periods (Allison *et al.* 2013 ; Zeglin *et al.* 2013) may result from the
362 reduction of predatory pressure (i.e. grazers activity or number) under water stressed
363 conditions (Davet 2004 ; Bapiri, Bååth and Rousk 2010 ; Barnard, Osborne and Firestone
364 2013). The observed decrease of the A/F ratio under AD conditions in *Q. pubescens* litter was
365 possibly related to the persistence of phenolics due to the decrease of leaching processes
366 under AD conditions, which delayed microbial colonization by decomposers in that litter
367 (Chomel *et al.* 2014). Contrary to fungi, total bacteria were not affected by changes in drought
368 conditions. Bacterial biomass stability under drought was also observed by Hartmann (2017),
369 and it was explained by independence of bacteria on water film for dispersal
370 (Mohammadipanah and Wink 2016). Nevertheless, those results varied according to forest
371 site and litter type. For example, in the *P. halepensis* forest an increase of total bacteria
372 biomass was observed under AD conditions, which may be explained by i) natural selection
373 of the most drought-tolerant bacterial groups (Barnard, Osborne and Firestone 2013) and/or ii)
374 bacteria resilience to drought resulting from their predominating r-selected life strategy,
375 which enables rapid recovery after a period of disturbance (i.e. drought) (Barnard, Osborne
376 and Firestone 2013; Meisner, Rousk and Bååth 2015).

377

378 **5. Conclusions**

379 Our results showed that microbial litter decomposers respond differently to
380 intensification of water stress conditions in the Mediterranean forests. Actinobacteria and total
381 bacteria were more negatively affected by plant litter changes (*Q. ilex* and *Q. pubescens*
382 higher than *P. halepensis*), than by the direct rainfall reduction. In contrary, fungal

communities seemed to be more tolerant and even favored to water limitation. Also, the potential resilience of bacterial communities was proposed, while fungal response seemed to reflect a higher degree of adaptation to decreased water availability. Finally, the arrival of *P. halepensis* litter to a *Q. pubescens* and *Q. ilex* forest may change litter microbial communities, leading to a decrease of A/F and B/F ratios and promoting fungi and certain groups of bacteria, which are more capable of using complex carbon sources. Therefore, the intensification of drought conditions in Mediterranean forest may introduce a fungi dominated community over bacteria, by either the capability of degrading more recalcitrant litter or/and tolerance to future climate conditions.

6. Funding

This research was financially supported by the Agence Nationale pour la Recherche (ANR) with the project SecPriMe2 (ANR-12-BSV7-0016-01), the BioDivMeX Mistrals program and partially also by the Ministry of Education, Youth and Sports of the Czech Republic, European Regional Development Fund-Project No. CZ.02.1.01/0.0/0.0/16_019/0000845 and grant No. LTC17075 and Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0418.
This work is a contribution to Labex OT-Med (n° ANR-11-LABX-0061) and has received funding from Excellence Initiative of Aix-Marseille University - A*MIDEX, a French "Investissements d'Avenir" programme". We also thank the French Region PACA and Europe for the PhD grant attributed to Susana Pereira.

7. Acknowledgments

We thank all the people that contributed in the set-up of the experiment and in the field campaigns especially to Sylvie Dupouyet, Jean-Philippe Orts and Jean-Marc Ourcival.We thank to Anne Haguenauer from Endoume Marine Station (Marseille, France) for her efficient orientation in the lab and whole team of CRI - Crop Research Institute (Prague, Czech Republic) - for their unconditional help and support. The three experimental sites were annually supported by the research infrastructure AnaEE-France (ANR-11-INBS-0001) and by Allenvi through the SOERE F-ORE-T. We acknowledge the se of the COOPERATE database (Reiter IM, Castagnoli G, Rotereau A 2015 "Cooperate database", https://cooperate.obs-hp.fr/db) run by the CNRS Downloaded from FR3098 ECCOREV and OSU-Pytheas with support from the Labex OT-Med . We also thank the French Region PACA and Europe for the PhD grant attributed to Susana Pereira.

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Fig. 1 – Leaf litter mass loss (a) and associated leaf litter microbial decomposers abundances (actinobacteria (b), bacteria (c), fungi (d)), actinobacteria to fungi (e) and bacteria to fungi (f) ratios, for the three forests and litter species (control and amplified drought conditions were combined). Values are mean ± SE (n=14). Different letters denote significant differences between litter species within forest sites (e.g. both drought conditions combined), with a > b > c. DM = litter dry mass. *Qp* = *Quercus pubescens*, *Qi* = *Quercus ilex*, *Ph* = *Pinus halepensis*.

Fig. 2 – Effects of the two drought conditions (ND= natural drought: AD= amplified drought) on the total bacterial (a), fungal (b) abundances and bacteria to fungi ratio (c). Barplots are mean ± SE. Significant effects are indicated with respective symbols * for P< 0.05 and ** for P < 0.01. *Qp= Quercus pubescens, Qi= Quercus ilex, Ph= Pinus halepensis*.

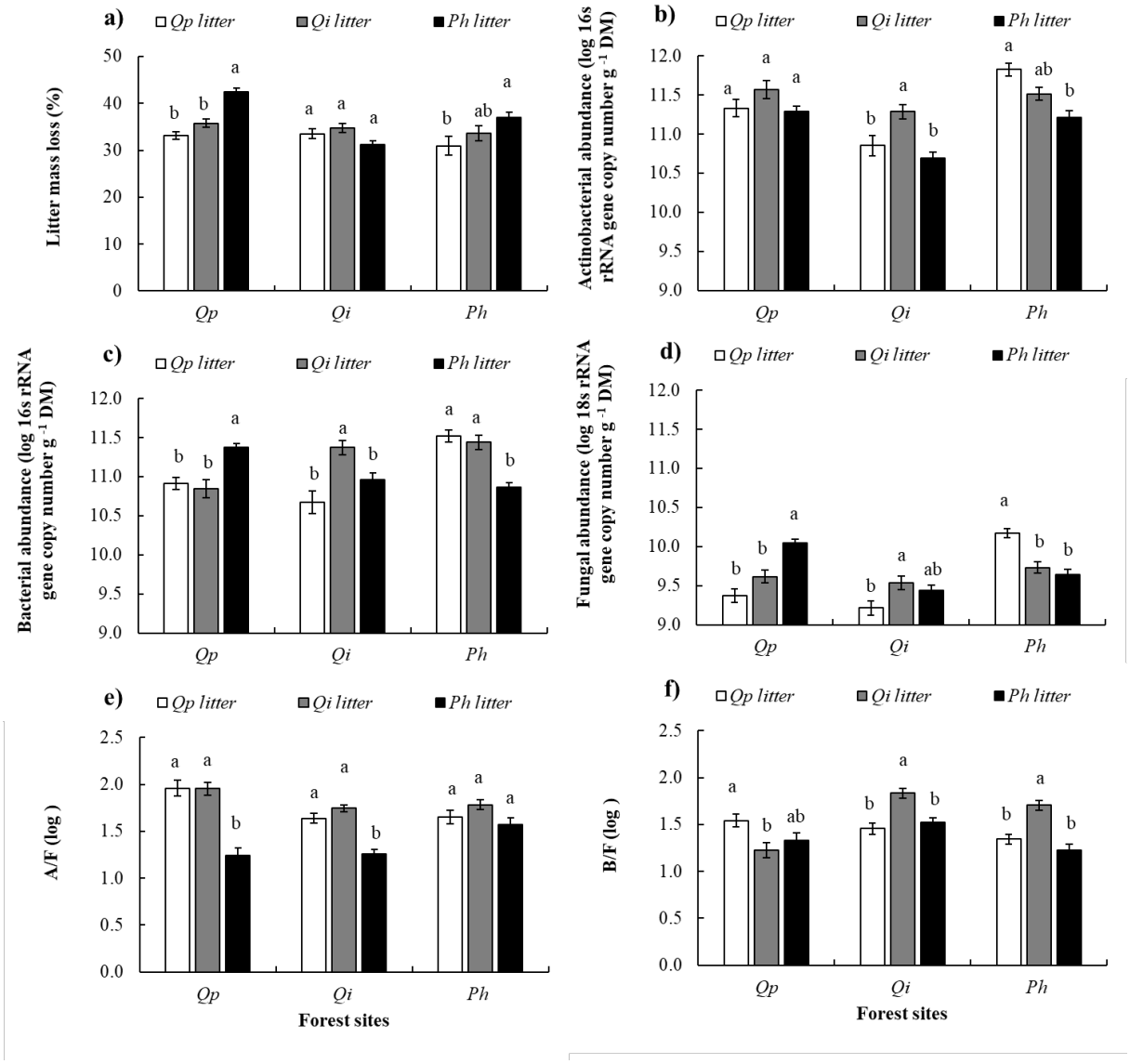
a)
Qp litter
Qi litter
Ph litter
Litter mass loss (%)
b)
Actinobacterial abundance (log 16s rRNA gene copy number g -1 DM)
c)
Bacterial abundance (log 16s rRNA gene copy number g -1 DM)
d)
Fungal abundance (log 18s rRNA gene copy number g -1 DM)
e)
A/F (log)
f)
B/F (log)
Qp
Qi
Ph
Forest sites

Fig.1

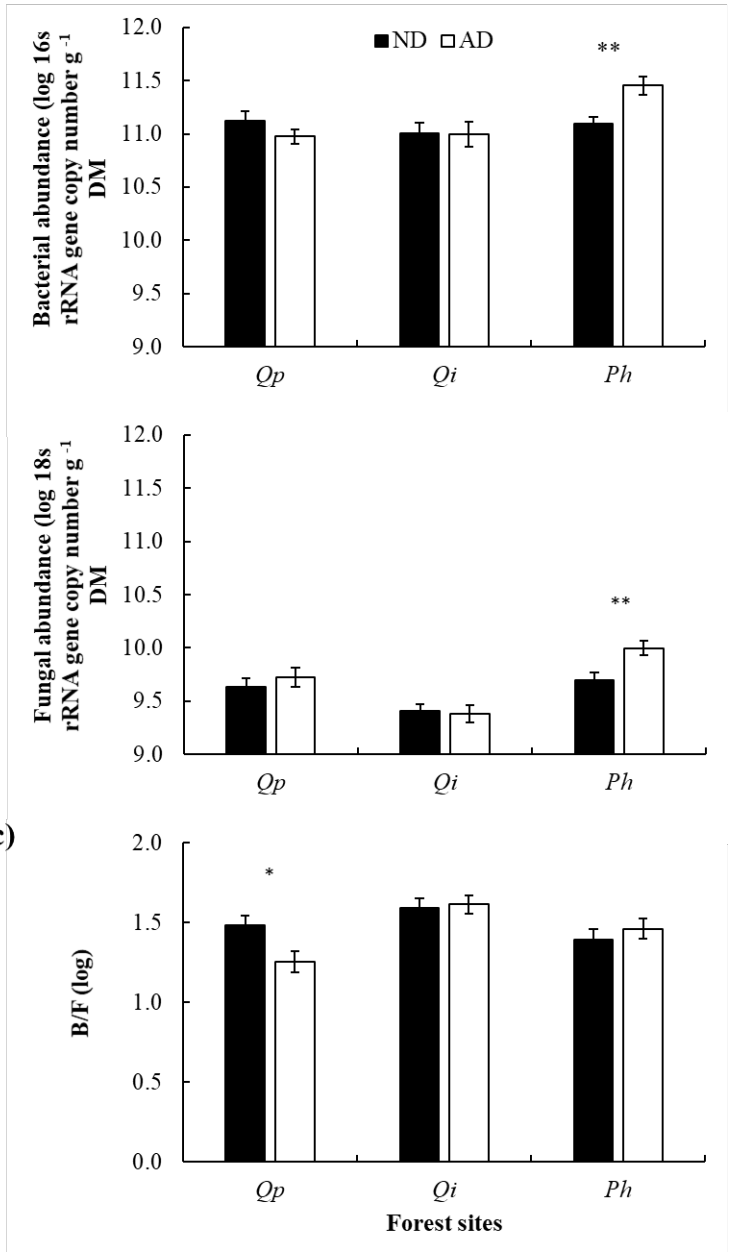
a)
Bacterial abundance (log 16s rRNA gene copy number g-1 DM
12.0
11.5
11.0
10.5
10.0
9.5
9.0
ND AD
**
Qp
Qi
Ph
b)
Fungal abundance (log 18s rRNA gene copy number g-1 DM
12.0
11.5
11.0
10.5
10.0
9.5
9.0
**
Qp
Qi
Ph
c)
B/F (log)
2.0
1.5
1.0
0.5
0.0
*
Qp
Qi
Ph
Forest sites

Fig.2

Table 1 - Main characteristics of the three forest sites selected for this study. MAT= Mean Annual Temperature; MAP= Mean Annual Precipitation (for the study period: December 2014 to December 2015).

Forests	*Quercus pubescens* Willd.	*Quercus ilex* L.	Mixed *Pinus halepensis* Mill.
Sites	Oak Observatory at the Observatoire de Haute Provence	Puéchabon	Font-Blanche
Location	430 56' 115" N, 050 42' 642" E	43° 44' 29"N, 3°35' 45"E	43°14'27" N, 5°40'45" E
Altitude a.s.l. (m)	650	270	425
MAT (°C)	12.25	13.71	14.01
MAP (mm)	609	794	632
Soil type	pierric calcosol	rhodo-chromic luvisol	leptosol
Soil texture	clay	clay loam	clay
Soil pH	6.76	6.6	6.8
Surface rocks cover (%)	23	75	50
Dominant tree species	*Quercus pubescens* Willd.	*Quercus ilex* L.	mixed *Pinus halepensis* Mill. / *Quercus ilex* L.
Other dominant plant species	*Acer monspessulanum* L	*Buxus sempervirens* L.	*Quercus coccifera* L.
	Cotinus coggygria Scop.	*Phyllirea latifolial* L.	*Phyllirea latifolia* L.
		Pistacia terebinthus L.	
		Juniperus oxycedrus L.	
Tree density (stems/ ha)	3503	4500	3368
Forest structure	even-age (70 years)	even-age (74 years)	uneven-age (61 years)
Rain exclusion system dimensions (m^2)	300	140	625
Rain exclusion (%)	33	30	30

Table 2 - Initial leaf litter quality of each litter species: *Quercus pubescens, Quercus ilex* and *Pinus halepensis*. Values are mean ± SE (n=5). One-way ANOVA was performed to test differences between initial litter species. F-Ratio are indicated and P-values with the respective symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. Different letters denote significant differences among species with a>b>c.

	df	*Quercus pubescens*		*Quercus ilex*		*Pinus halepensis*		F-ratio	
Carbon	2	462.6 ± 2.65	c	478.1 ± 1.4	b	516.1 ± 1.6	a	198.80	***
Nitrogen	2	6.4 ± 0.2	b	9.6 ± 0.2	a	5.4 ± 0.1	c	146.30	***
Phosphorus	2	1.9 ± 0.1	b	3.5 ± 0.2	a	1.6 ± 0.1	b	64.6	***
Calcium	2	32.9 ± 0.8	a	25.1 ± 0.6	b	18.4 ± 0.2	c	168.1	***
Potassium	2	0.8 ± 0.01	c	1.8 ± 0.1	a	0.95 ± 0.01	b	370.7	***
Magnesium	2	2.8 ± 0.2	a	1.4 ± 0.01	b	1.5 ± 0.02	c	104.8	***
Sodium	2	0.04 ± 0.00	b	0.11 ± 0.00	a	0.11 ± 0.00	a	149.6	***
Total phenolics	2	40.8 ± 2.6	a	32.9 ± 1.58	b	38.6 ± 1.2	ab	4.7	*
Lignin	2	273.3 ± 9.0	b	337.4 ± 5.5	a	302.6 ± 10.6	b	13.8	***
Cellulose	2	159.1 ± 8.0	b	205 ± 4.2	a	150.8 ± 12.9	b	10.3	**
Hemicellulose	2	274.2 ± 22.5	a	270.0 ± 16.2	ab	206.6 ± 10.4	b	4.7	*
Water-soluble compounds	2	293.4 ± 12.1	b	187.6 ± 10.2	c	340.0 ± 7.1	a	60.9	***
Specific leaf area	2	133.1 ± 0.4	a	51.5 ± 0.9	c	83.5 ± 2.2	b	690.7	***
Water holding capacity	2	146.9 ± 1.0	a	137.2 ± 1.0	b	113.9 ± 1.03	c	285.5	***

Table 3 - ANOVA table of F-Ratio and P-values for the effects of forest sites (S), litter species (L) and drought conditions (D) on the mass loss, microbial biomass (actinobacteria, bacteria and fungi) and actinobacteria/ fungi (A/F) and bacteria/ fungi (B/F) ratios.

		Mass loss		**Actino (A)**		**Bacteria (B)**		**Fungi (F)**		**A/F**		**B/F**	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Sites (S)	2	8.81	**0.0008**	33.92	**<0.0001**	8.86	**0.0003**	30.38	**<0.0001**	6.11	**0.0030**	11.78	**<0.0001**
Litter species (L)	2	8.11	**0.0022**	16.31	**<0.0001**	4.58	**0.0122**	2.41	0.0945	53.55	**<0.0001**	11.34	**<0.0001**
Drought (D)	1	3.70	0.0571	0.09	0.7602	1.91	0.1688	6.20	**0.0143**	12.08	**0.0007**	1.17	0.2828
SxL	4	7.03	**<0.0001**	5.69	**0.0003**	20.56	**<0.0001**	21.01	**<0.0001**	7.67	**<0.0001**	11.49	**<0.0001**
SxD	2	0.43	0.6488	3.00	0.0539	7.25	**0.0011**	3.82	**0.0251**	0.18	0.8328	5.43	**0.0056**
LxD	2	0.14	0.8724	3.83	**0.0246**	1.36	0.2593	1.12	0.3313	3.34	**0.0392**	0.11	0.8986
SxLxD	4	0.10	0.9839	1.11	0.3540	2.72	**0.0335**	1.45	0.2229	2.12	0.0836	1.17	0.3295

Supplementary Table 1 - Summary table of microbial abundance (expressed as 10^{10} gene copy number g^{-1} litter dry mass) for each litter species at each forest site and drought condition (ND = Natural drought; AD = amplified drought). One-way ANOVAs were performed for differences among litter species per drought condition and forest site. F-Ratio are indicated and P-values with the respective symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. Different letters denote significant differences among litter species with a>b. T-tests was performed to test the effect of drought conditions for each species at each forest site. T-test are indicated and significant P-values ($P < 0.05$) are denoted in bold.

Little species				Forest sites				
				Q. pubescens	*Q. ilex*	*P. halepensis*	*F*	*P*
Quercus pubescens	Actinobacteria	ND		49.55 ± 17.89 ab	15.52 ± 4.23 b	74.75 ± 17.67 a	6.05	**
		AD		17.27 ± 5.13 b	7.15 ± 2.94 b	85.62 ± 14.22 a	19.95	***
		t-test		-2.1757	-1.9232	0.93659		
		p-value		ns	ns	ns		
	Bacteria	ND		11.86 ± 3.10 a	10.41 ± 2.76 a	24.58 ± 4.25 a	33.51	ns
		AD		8.12 ± 1.92 b	6.73 ± 3.94 b	53.70 ± 8.17 a	19.54	***
		t-test		-0.65657	-1.3221	2.7689		
		p-value		ns	ns	**0.0176**		
	Fungi	ND		0.30 ± 0.09 b	0.26 ± 0.05 b	1.08 ± 0.17 a	13.91	***
		AD		0.33 ± 0.13 b	0.16 ± 0.05 b	2.26 ± 0.23 a	33.35	***
		t-test		0.089708	-1.559	4.1807		
		p-value		ns	ns	**0.0018**		
	Ratio A/F	ND		171.78 ± 40.21 a	58.32 ± 10.71 b	71.30 ± 18.25 b	5.60	*
		AD		62.77 ± 14.26 a	38.00 ± 5.39 a	37.91 ± 5.47 a	2.34	ns
		t-test		-3.391	-1.6586	-1.5571		
		p-value		**0.0054**	ns	ns		
	Ratio B/F	ND		24.48 ± 4.57 a	48.18 ± 10.46 a	35.92 ± 6.42 a	1.91	ns
		AD		32.56 ± 5.13 a	29.48 ± 7.56 a	23.59 ± 2.65 a	0.67	ns
		t-test		-0.91979	-0.83125	0.16864		
		p-value		ns	ns	ns		
Quercus ilex	Actinobacteria	ND		63.26 ± 18.05 a	30.64 ± 8.66 a	28.44 ± 6.39 a	0.94	ns
		AD		39.9 ± 10.89 ab	19.45 ± 5.14 b	50.03 ± 8.88 a	5.33	*
		t-test		-0.47229	-0.63645	1.8596		
		p-value		ns	ns	ns		
	Bacteria	ND		18.66 ± 12.96 a	35.92 ± 9.87 a	17.00 ± 2.31 a	0.01	ns
		AD		7.27 ± 1.92 b	24.97 ± 5.84 b	55.01 ± 11.58 a	15.89	***
		t-test		-0.56905	-0.44153	3.4846		
		p-value		ns	ns	**0.0061**		
	Fungi	ND		0.47 ± 0.14 a	0.47 ± 0.12 a	0.39 ± 0.06 a	29.61	ns
		AD		0.54 ± 0.09 a	0.41 ± 0.14 a	0.90 ± 0.17 a	4.00	*
		t-test		0.9151	-0.34763	3.2994		
		p-value		ns	ns	**0.0073**		
	Ratio A/F	ND		129.45 ± 22.32 a	63.52 ± 9.21 b	72.76 ± 11.76 b	38.21	*
		AD		70.58 ± 12.05 a	53.67 ± 5.53 a	59.00 ± 6.49 a	0.83	ns
		t-test		-2.2672	-0.78997	-0.45703		
		p-value		**0.0445**	ns	ns		
	Ratio B/F	ND		29.55 ± 10.32 b	77.12 ± 15.33 a	46.96 ± 7.00 ab	7.28	**
		AD		12.96 ± 2.31 b	73.53 ± 14.96 a	64.72 ± 12.05 a	27.71	***
		t-test		-1.9048	-0.19647	1.2028		
		p-value		ns	ns	ns		
Pinus halepensis	Actinobacteria	ND		17.53 ± 5.56 a	5.57 ± 1.79 b	17.98 ± 4.8 a	5.73	*
		AD		27.18 ± 2.79 a	6.58 ± 1.44 b	23.74 ± 7.53 a	14.48	***
		t-test		1.5554	0.43757	0.64576		
		p-value		ns	ns	ns		
	Bacteria	ND		32.19 ± 4.07 a	6.59 ± 1.86 b	5.77 ± 0.89 b	29.41	***
		AD		19.02 ± 1.91 a	17.52 ± 3.94 a	10.85 ± 0.82 a	29.38	ns
		t-test		-2.88	2.7371	2.5945		
		p-value		**0.0185**	**0.0228**	**0.0291**		
	Fungi	ND		1.01 ± 0.1 a	0.23 ±0.05 b	0.41 ±0.07 b	15.48	***
		AD		1.47 ± 0.41 a	0.41 ± 0.07 b	0.59 ±0.08 b	9.75	**
		t-test		0.97788	2.1384	1.7302		
		p-value		ns	ns	ns		
	Ratio A/F	ND		17.56 ± 5.16 b	22.63 ± 1.88 ab	45.56 ± 9.66 a	7.50	**
		AD		24.90 ± 5.37 a	16.79 ± 3.51 a	43.48 ± 13.61 a	30.13	ns
		t-test		0.98631	-1.4713	-0.1249		
		p-value		ns	ns	ns		
	Ratio B/F	ND		32.42 ± 3.27 a	28.16 ± 3.91 ab	19.43 ± 6.12 b	4.24	*
		AD		18.67 ± 5.31 b	44.85 ± 7.37 a	19.67 ± 3.29 b	6.13	**
		t-test		-2.2045	2.0002	0.034746		
		p-value		ns	ns	ns		

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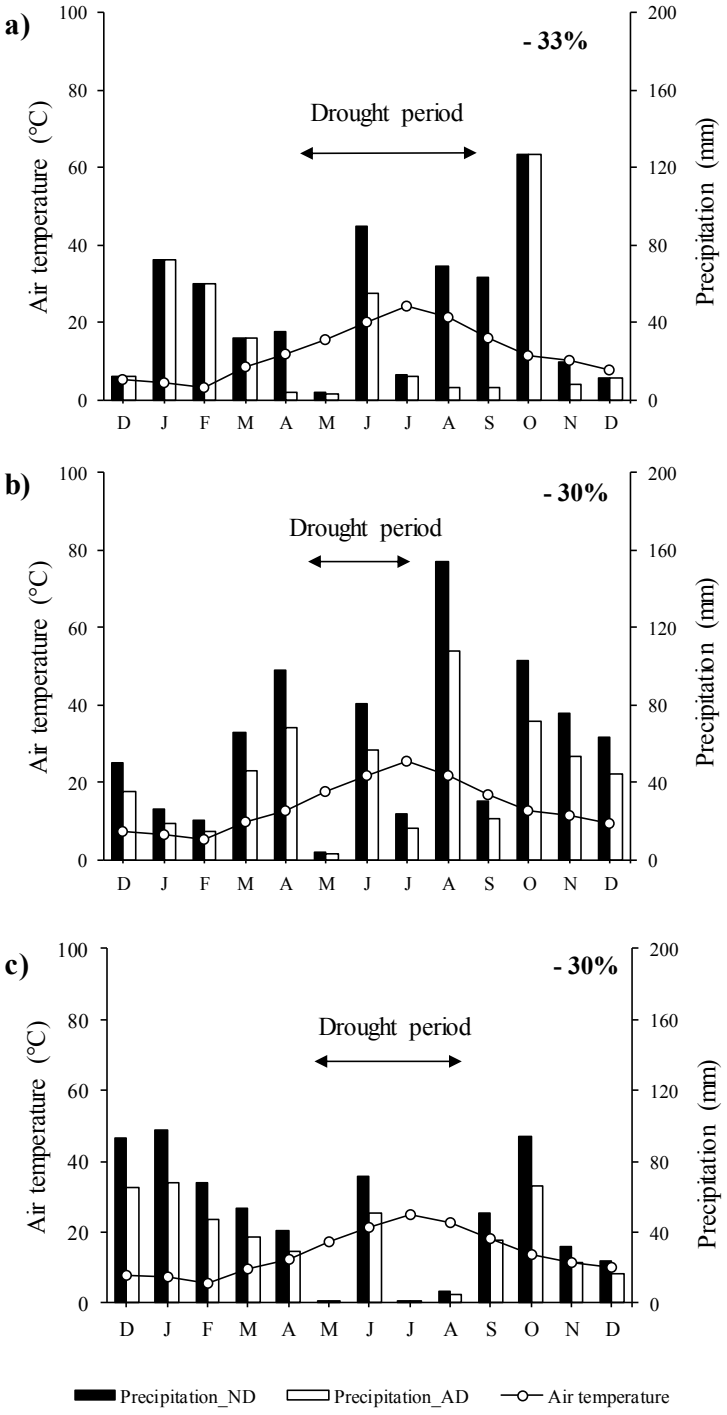

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707 **Supplementary Figure 1** – Ombrothermic diagrams at a) *Quercus pubescens* forest, b)

708 *Quercus ilex* forest and c) *Pinus halepensis* forest study sites between December 2014 to

709 December 2015. Bar represents the mean monthly precipitation (mm) in black (ND= natural

710 drought) and in white (AD = amplified drought). The curve represents the mean monthly

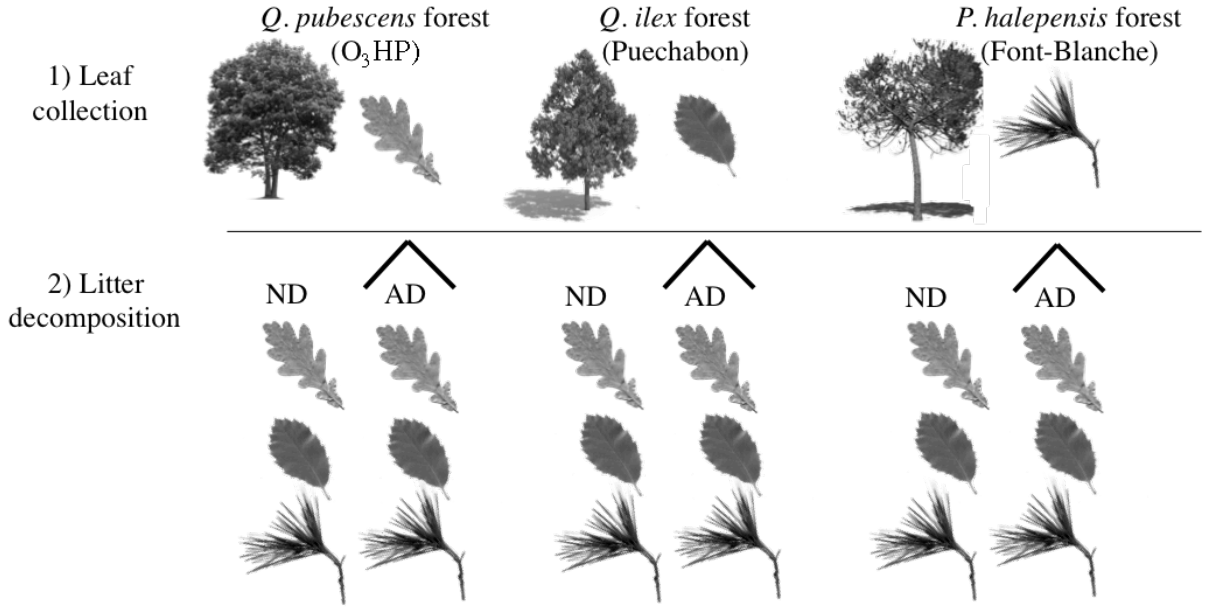
temperature (°C). At each forest site, drought period is indicated by the horizontal arrow and the percentage represents the proportion of excluded rain compared to the natural drought plot.

a)

b)

c)

Supplementary Figure 2 – Rain exclusion devices in the three forest studied (a) *Quercus pubescens* forest at O_3HP, (b) *Quercus ilex* at Puéchabon and (c) *Pinus halepensis* at Font-Blanche.



Supplementary Figure 3 - Litterbags experimental design: 1) leaf litter of the three tree species (*Quercus pubescens, Quercus ilex* and *Pinus halepensis*) coming from natural drought (ND) were redistributed and 2) placed in the three different forests (*Quercus pubescens* O_3HP*, Quercus ilex* Puéchabon and *Pinus halepensis* Font-Blanche forest, respectively), under natural drought (ND) and amplified drought (AD) conditions.

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