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► To cite this version:

Adriane Aupic-Samain, Mathieu Santonja, Mathilde Chomel, Susana Pereira, Elodie Quer, et al.. Soil biota response to experimental rainfall reduction depends on the dominant tree species in mature Northern Mediterranean forests. *Soil Biology and Biochemistry*, 2021, 154, pp.108122. 10.1016/j.soilbio.2020.108122 . hal-03101810

HAL Id: hal-03101810

<https://amu.hal.science/hal-03101810>

Submitted on 7 Jan 2021

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Journal Pre-proof

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PII: S0038-0717(20)30418-1

DOI: <https://doi.org/10.1016/j.soilbio.2020.108122>

Reference: SBB 108122

To appear in: *Soil Biology and Biochemistry*

Received Date: 18 March 2020

Revised Date: 16 December 2020

Accepted Date: 21 December 2020

Please cite this article as: Aupic-Samain, A., Santonja, M., Chomel, M., Pereira, S., Quer, E., Lecareux, C., Limousin, J.-M., Ourcival, J.-M., Simioni, G., Gauquelin, T., Fernandez, C., Baldy, V., Soil biota response to experimental rainfall reduction depends on the dominant tree species in mature Northern Mediterranean forests, *Soil Biology and Biochemistry*, <https://doi.org/10.1016/j.soilbio.2020.108122>.

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3

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18

19 **Abstract**

20 Soil organisms play a major role on litter decomposition process and nutrient cycling in forest
21 ecosystems. These organisms are extremely sensitive to environmental conditions such as soil
22 temperature and moisture conditions which control their demographic parameters and activity.
23 The ongoing climate change can therefore directly affect soil biota communities and the
24 processes they drive. Besides, climate change can also indirectly affect soil biota by altering
25 tree functional traits (e.g., N, Ca, Mg, water holding capacity) with cascading effects on the
26 litter quality. The aim of this study was to determine the relative effects of increased drought
27 and litter type on microbial biomass (bacteria and fungi) and mesofauna abundance
28 (Collembola and Acari) in three experimental sites representative of the three main forests
29 encountered in the northern part of the Mediterranean Basin (dominated by either *Quercus*
30 *pubescens*, *Quercus ilex* or *Pinus halepensis*) where rainfall exclusion experiments were
31 taking place. At each site, and in each precipitation treatment (natural and amplified drought
32 plots), we collected and transplanted foliage litters (i.e., species \times drought level). After two
33 years, we reported a litter species effect: *Q. pubescens* litter presented consistently the higher
34 abundance of all soil biota groups compared to *Q. ilex* and *P. halepensis* litters in each forest.
35 Surprisingly, despite that the amplified drought treatment induced a modification of the litter
36 quality, we did not reported an indirect reduced precipitation effect on soil biota parameters.
37 While Oribatid Acari abundance decreased with amplified drought in all three forest types,
38 the direct effects on the other soil biota groups were forest-dependent. In *P. halepensis* forest,
39 amplified drought resulted in higher bacterial and fungal biomasses but lower Collembola
40 abundance. In *Q. ilex* forest both Collembola and predatory Acari abundances decreased with
41 amplified drought. In addition, the positive relationships between Collembola and Oribatida
42 abundances and litter mass loss disappeared under amplified drought conditions in both *Q.*
43 *ilex* and *P. halepensis* forests. These results suggest a key role played by Ca, Mg, specific leaf

44 area (SLA) and water holding capacity (WHC) as drivers of soil biota parameters. Finally, the
45 study highlights that within the same Mediterranean region, climate change could differently
46 alter the soil organisms inhabiting the litter layer and their contributions to the decomposition
47 process depending on the tree species and soil biota group considered.

48

49 **Keywords:** climate change; Mediterranean forest; mesofauna; microorganism; plant-soil
50 (below-ground) interactions; soil biota.

51

52 **1. Introduction**

53 Litter is one of the basal elements of a food web that controls nutrient turnover, carbon
54 sequestration and the overall ecosystem functioning (Wall et al., 2012; Gobat et al., 2013).
55 Among soil biota, mesofauna (mainly Collembola and Acari) drives many biotic interactions
56 which are fundamental for structuring the soil food web and decomposing leaf litter. Firstly,
57 microbi-detrivore organisms (e.g., Collembola and Oribatid Acari) participate directly to the
58 micro-fragmentation of leaf litter, but also control microbial communities through grazing and
59 dispersing spores and mycelium (Berg and Laskowski, 2005; Chahartaghi et al., 2005; Scheu
60 et al., 2005; Anslan et al., 2016). Secondly, predators (e.g., Mesostigmatid and some
61 Prostigmatid Acari) regulate microbi-detrivore organisms by feeding on them (Koehler,
62 1999; Schneider and Maraun, 2009; Thakur et al., 2015) and then indirectly control the leaf
63 litter decomposition.

64 Chemical and physical characteristics of the leaf litter strongly control soil mesofauna
65 demographic parameters and interactions (Hättenschwiler et al., 2005; Chomel et al., 2016;
66 Santonja et al., 2018; Aupic-Samain et al., 2019). Under the specific Mediterranean climatic
67 conditions (summer drought and episodic drying/rewetting cycles; Larcher, 2000; Sardans and
68 Peñuelas, 2013; Gauquelin et al., 2018), trees generally produce sclerophyllous leaves

69 (Coûteaux et al., 1995) characterized by high lignin concentration (Tian et al., 1992; Gallardo
70 and Merino, 1993), low specific leaf area (Wright et al., 2005; Pallardy, 2010) and high
71 diversity and concentration of specialized metabolites (i.e., terpene and phenolic compounds;
72 Macchioni et al., 2003; Fernandez et al., 2009). These particular characteristics of
73 Mediterranean trees could potentially lead to distinct litter quality control over soil mesofauna
74 compared to the other temperate forests for which litter nutrient contents (e.g., C, N and P) are
75 frequently reported as key drivers of soil mesofauna demographic parameters (Jandl et al.,
76 2003; Martinson et al., 2008; Jacob et al., 2009; Maaroufi and De Long, 2020). However, to
77 our knowledge, only few studies investigated this effect of litter quality on soil mesofauna in
78 Mediterranean forests (e.g., Barba et al., 2016; Santonja et al., 2017; Aupic-Samain et al.,
79 2019), necessitating deeper investigation to improve our mechanistic understanding of such
80 relationships.

81 Among terrestrial biomes, Mediterranean ecosystems are recognized as being the most
82 sensitive to climatic change (Sala et al., 2000; Schröter et al., 2005). Regional climate models
83 for the Mediterranean Basin predict a warming of 3.4°C and a decrease of annual
84 precipitations by 30% for the end of the 21st century, which will result in an intensification of
85 summer drought events (Giorgi and Lionello, 2008; IPCC, 2013; Polade et al., 2014).
86 Therefore, by decreasing water availability, climate change in Mediterranean ecosystems may
87 have a direct negative impact on soil microorganisms (e.g., Sardans and Peñuelas, 2010;
88 Talmon et al., 2011) and mesofauna (e.g., Tsiafouli et al., 2005; Santonja et al., 2017). In
89 addition, climate change may indirectly impact microbial and mesofaunal communities by
90 altering litter quality and quantity produced by plants as climatic conditions strongly control
91 plant growth and survival and consequently leaf and litter traits (Wright et al., 2005; Sardans
92 and Peñuelas, 2007; Rodriguez-Ramirez et al., 2017). Indeed, previous studies reported that
93 experimental decrease in water conditions implies lower nutrient content (Chen et al., 2013;

94 Santonja et al., 2019) and higher specialized metabolite content (Hernández et al., 2004;
95 Munné-Bosch and Peñuelas, 2004) with potential negative cascading effects on soil biota
96 (Allison et al., 2013; García-Palacios et al., 2016a; Santonja et al., 2019). In addition, oaks
97 and pines are dominant tree genera that structure both temperate and Mediterranean forests
98 (Ellenberg, 1988; Quézel and Médail, 2003). As oak and pine forests exhibit different
99 microclimatic and soil properties (e.g., pH, soil type, humus forms; Table 1; Gauquelin et al.,
100 2016) as well as chemically and structurally different litters (oak leaves vs. pine needles;
101 Aupic-Samain et al., 2019; Santonja et al., 2015), we could expect that climate change may
102 distinctly affect soil biota in these two forest types. However, our current understanding of
103 soil biota responses to climate change drivers in Mediterranean oak and pine forests is still
104 limited by a lack of studies addressing conjointly the relative contributions of environmental
105 conditions and leaf litter quality and both direct and indirect effects of climate change on
106 these organisms.

107 In this context, we set up a 2-year litter transplant experiment in the three main forests
108 encountered in the northern part of the Mediterranean Basin (*Quercus pubescens*, *Quercus*
109 *ilex* and *Pinus halepensis* dominated forests) in which we manipulated the amounts of
110 precipitation (natural vs. amplified drought), the litter species identity (leaf / needle litters
111 from the three tree species) and the litter type (litters collected from natural or amplified
112 drought plots) in order to determine their relative effects on soil biota, including both
113 microbial (bacteria and fungi) and mesofaunal (Acari and Collembola) communities. We
114 hypothesized that i) microbial biomass and mesofaunal abundance associated with
115 decomposing oak leaves are higher compared to pine needles due to lower amount of
116 refractory compounds (e.g., specialized metabolites); ii) reduced precipitation directly
117 decreases microbial biomass and mesofaunal abundance as water availability is a strong
118 constraining environmental factor; iii) reduced precipitation indirectly decreases microbial

119 biomass and mesofaunal abundance due to a decrease in leaf/ needle litter quality (e.g.,
120 increased specialized metabolite content) and iv) soil biota will be more sensitive to reduced
121 precipitation in oak compared to pine forests where soil biota is already conditioned by more
122 constraining environmental conditions (e.g., litter content).

123

124 **2. Materials and Methods**

125

126 **2.1. Study site**

127 The study was concurrently set up in three Mediterranean experimental sites (Table 1).
128 The first is the Oak Observatory at the “Observatoire de Haute Provence” (O₃HP) located in
129 the Luberon Natural Regional Park (43°45'34.26"N; 5°17'57.84"E), in Provence, SE France
130 (Gauquelin et al., 2011). This oak forest is dominated by deciduous downy oak (*Quercus*
131 *pubescens* Willd.). The second site is located in the Puéchabon State Forest (43°44'30" N;
132 3°35'40" E) in Occitanie, SE France (Misson et al., 2010). This oak forest is dominated by the
133 evergreen holm oak (*Quercus ilex* L.). The third site is located in the departmental forest of
134 Font-Blanche (43°14'25"N; 5°40'40"E) in Provence, SE France (Simioni, 2011). This is a
135 mixed forest, but Aleppo pine (*Pinus halepensis* Mill.) is the most abundant species,
136 contributing around 70% of the basal area. During the two years of field experiment, the mean
137 annual precipitation ranged from 635.6 mm in the *P. halepensis* forest to 1020.9 mm in the *Q.*
138 *ilex* forest, while the mean annual temperature ranged from 12.3°C in the *Q. pubescens* forest
139 to 14.1°C in the *P. halepensis* forest (Supplementary Fig. S2).

140 In order to simulate the intensification of the summer drought period, each site is
141 equipped with a rain exclusion device reducing approximately 30% of annual precipitation
142 (similar to climatic models projection – A2 scenario; Giorgi and Lionello, 2008; IPCC, 2013).
143 In the *Q. pubescens* forest the rain exclusion device consists of a 15 m × 20 m rainout-shelter

144 above the canopy which dynamically excluded precipitations by deploying automated shutters
145 during rainfall events of the vegetation growing season (i.e., from spring to autumn)
146 (Supplementary Fig. S2a). In the *Q. ilex* and *P. halepensis* forests the rain exclusion is
147 performed by using fixed PVC gutters installed below the forest canopy, excluding about 30%
148 at each rainfall event (Supplementary Fig. S2b and c). In each site, we compared control plots
149 (natural drought - ND) and rain exclusion plots (amplified drought – AD) (Table 1 and
150 Supplementary Fig. S1).

151

152 **2.2. Litter collection**

153 Freshly abscised leaves and needles of *Q. pubescens*, *Q. ilex* and *P. halepensis* were
154 collected in ND and AD plots over the litterfall period in 2014. For that, litter traps were used
155 during the abscission period that occurred from June to September for the needles (*P.*
156 *halepensis*) and from October to November for the leaves (*Q. ilex* and *Q. pubescens*).
157 Immediately after collection, the leaves/needles were air dried at room temperature and stored
158 until the beginning of the experiment. Several aliquots of senescent leaves or needles were
159 also frozen at -20 °C, freeze-dried for 72 h and ground prior to chemical analyses.

160

161 **2.3. Experimental design**

162 Plant litter decomposition was studied over 730 days using the litterbags method
163 (Swift et al., 1979). In December 2014, 10 g (in equivalent dry weight) of senescent leaves or
164 needles of either *Q. pubescens*, *Q. ilex* and *P. halepensis* collected from trees either in ND or
165 AD plots were placed in a 4-mm mesh litterbag (20 × 20 cm) designed to allow colonization
166 by microbes and mesofauna. Litter transplants were made between each site for the three
167 species considered, i.e., a litter bag containing the litter of each species placed on each forest
168 site, under the two precipitation conditions, i.e., ND and AD (see Fig. 1).

169 Thus, the experiment consisted in 36 treatment combinations corresponding to 3 forest
170 sites (*Q. pubescens*, *Q. ilex* and *P. halepensis* forests) \times 3 litter species (*Q. pubescens*, *Q. ilex*
171 and *P. halepensis*) \times 2 litter types (litters collected from ND or AD plots) \times 2 precipitation
172 treatments (ND and AD) (Fig. 1). Each modality had 5 replicates for a total of 180 litterbags.
173 Litterbags were placed perpendicular to the gutters system in *Q. ilex* and *P. halepensis*
174 forests and under the rain exclusion device in the *Q. pubescens* forest by using 5 transects
175 (i.e., 5 replicates of the 6 litterbag modalities) equidistant from each other (1 m distance
176 between the 5 transects and 0.6 m between the 6 litterbags). Transects were oriented E-W.
177 They were placed on the ground after the removal of the litter layer and fixed to the soil with
178 galvanized nails to prevent movement by animals or wind. The litter layer was then replaced
179 over the litterbags. In December 2016, i.e., after 730 days of decomposition, all the litterbags
180 were harvested and sealed in plastic bags to prevent the further loss of biological material.

181

182 **2.4. Initial litter characteristics**

183 Initial litter quality of the three litter species (*Q. pubescens*, *Q. ilex* and *P. halepensis*)
184 collected from the two precipitation treatments (ND and AD) was determined from five
185 samples.

186 Carbon (C) and nitrogen (N) concentrations were determined by thermal combustion
187 on a Flash EA 1112 series C/N elemental analyzer (Thermo Scientific®, Waltham, MA,
188 USA). Phosphorus (P) and cations, i.e., calcium (Ca), sodium (Na), potassium (K) and
189 magnesium (Mg), were extracted from 80 mg of grounded litter with 8 ml of HNO₃ and 2 ml
190 of H₂O₂. Then, samples were heated at 175 °C for 40 min using a microwave digestion system
191 (Ethos One, Milestone SRL, Sorisole, Italy). After this mineralization step, every sample was
192 adjusted to 50 ml with demineralized water. P concentration was measured colorimetrically
193 using the molybdenum blue method (Grimshaw et al., 1989). 100 μ l of sample, 100 μ l of

194 NaOH, 50 μ l of mixed reagent (emetic tartrate and ammonium molybdate solution) and 50 μ l
195 of ascorbic acid were mixed directly in a 96 well microplate. After 45 min at 40 °C, the
196 reaction was completed, and P concentration was measured at 720 nm using a microplate
197 reader (Victor, Perkin Elmer, Waltham, MA, USA). Cations concentrations were determined
198 by atomic absorption spectrophotometer. Total phenolic compounds were measured
199 colorimetrically by the adapted method of Peñuelas et al., (1996) using gallic acid as standard
200 (expressed as equivalent acid gallic). 250 mg of litter sample were extracted in 20 ml of a 70
201 % aqueous methanol solution, shaken for 1 h, and then filtered (0.45 μ m filter); 50 μ l of
202 filtered extract were mixed with 200 μ l of saturated aqueous Na₂CO₃ (to stabilize the color
203 reaction), 1650 μ l of distilled water and 100 μ l Folin-Ciocalteu reagent (Folin and Denis,
204 1915). After 30 min, the reaction was completed, and the concentration of phenolics was
205 measured at 765 nm on a UV/Vis spectrophotometer (Thermo Scientific®, Waltham, MA,
206 USA). Lignin, cellulose and hemicellulose as well as water soluble compounds (WSC)
207 concentrations of initial litter materials were determined according to the Van Soest extraction
208 protocol (Van Soest, 1963) using a fiber analyzer (Fibersac 24, Ankom, Macedon, NJ, USA).
209 All concentrations were expressed in mg g⁻¹ of litter dry weigh.

210 To determine the Water Holding Capacity (WHC), intact leaf or needle were soaked in
211 distilled water for 24 h, drained and weighed. The dry weight was determined after drying the
212 samples at 60 °C for 48 h. WHC was calculated as (moist weight / dry weight) \times 100 and
213 expressed in % (Santonja et al., 2015). Specific Leaf Area (SLA) was calculated as the ratio
214 between leaf area (determined by using the Image J software; <https://imagej.nih.gov/ij/>, MA,
215 USA) and dry weight and was expressed in cm² g⁻¹ of dry weigh.

216

217 ***2.5. Mesofauna extraction and identification***

218 Mesofauna was extracted from one litterbag using the Tullgren funnel method for 10
219 days (Berlese, 1905). Collected arthropods were stored in 70 % ethanol, counted by using a
220 binocular microscope and separated between Collembola and Acari, with different suborders
221 for the latter: Oribatid, Mesostigmatid and Prostigmatid Acari (Gisin, 1960; Hopkin, 1997).
222 Collembola and Oribatid Acari were assigned as microbi-detritivores and Mesostigmatid and
223 Prostigmatid Acari as predators (Coleman et al., 2004; Donoso et al., 2013; Crotty et al.,
224 2014; Santonja et al., 2017).

225

226 ***2.6. Litter mass loss estimation***

227 After mesofauna extraction, the litter samples were freeze-dried (Lyovac GT2) for 72
228 h and the remaining dry mass (%) after 730 days of decomposition was calculated.

229

230 ***2.7. PLFA analyses***

231 Since, the phospholipid fatty acids (PLFA) are essential components of all living cells
232 (Tollefson and McKercher, 1983; Zelles, 1999) with a wide structural diversity (Zelles, 1997;
233 Tornberg et al., 2003), we used PLFA as biomarkers of litter microbial communities. The
234 PLFA were extracted from freeze-dried ground litter according to the method from Buyer and
235 Sasser (2012) with modifications. Four ml of Bligh–Dyer extractant containing 4 μ l of 1,2-
236 dinonadecanoyl-sn-glycero-3-phosphocholine (C19:0; Avanti® Polar lipids, Inc.) as internal
237 standard were added to 0.5 g of samples. Lipids separation was performed by solid-phase
238 extraction (SPE) on Phenomenex® (Strata SI-1 with 50 mg of silica, 55 μ m, 70 Å). The
239 resulting fatty acid methyl ester (FAMES) were analysed by gas-chromatography/mass-
240 spectrometry (GC-MS) on an Agilent 7890 system equipped with an MSD5977A Network
241 mass detector, an ALS7693 automatic injector and an HP5-MS apolar column (30 m \times 0.25
242 mm \times 0.25 μ m; J&W Agilent Technologies) and MassHunter software. Qualitative analysis

243 of FAMES resulted by retention time comparison via FAMES mixture (range between C4 to
244 C24). The total PLFA concentration was used as measure of the total microbial biomass,
245 while fungal and bacterial biomasses were estimates through PLFA markers summed
246 (Frostegård and Bååth, 1996). Biomasses were expressed in $\mu\text{g g}^{-1}$ dw of litter. Among the 24
247 identified PLFAs in the samples, 12 microbial specific PLFAs were analysed. The fatty acids
248 i15:0, a15:0, i16:0 and i17:0 were used as biomarkers for Gram-positive bacteria; 16:1 ω 7,
249 18:1 ω 7 and cy19:0 were used as biomarkers of Gram-negative bacteria; 15:0, 17:0 were used
250 as general bacterial markers and 19:1 ω 8 were used as biomarkers of methane oxidizing
251 bacteria (Frostegård et al., 1993). The total bacterial PLFA biomass was calculated by adding
252 Gram-positive, Gram-negative and general bacterial biomarkers while 18:2 ω 6,9 was used as a
253 biomarker of fungi (Bååth and Anderson, 2003; Klamer and Bååth, 2004).

254

255 **2.8. Statistical analysis**

256 Statistical analyses were performed using a combination of univariate and multivariate
257 techniques with R software (version 3.3.1). Statistical significance was evaluated in all cases
258 at $P < 0.05$. Normality and homoscedasticity of the residuals were checked using Shapiro-
259 Wilk and Levene tests, respectively.

260 To analyse the differences of the initial characteristics of the three litter species (*Q.*
261 *pubescens*, *Q. ilex* and *P. halepensis*) and the two litter types (leaves or needles collected from
262 trees in ND or AD plots) we performed a Principle Component Analysis (PCA) followed by
263 pairwise tests with permutational multivariate analyses of variance (PERMANOVA;
264 Anderson, 2005) using the *adonis* function of the *vegan* package (Oksanen et al., 2007, 2013).

265 Four-way analyses of variance (ANOVA), followed by Tukey tests for post-hoc
266 pairwise comparisons, were used to test the effects of forest site, litter species identity,
267 precipitation treatment, litter type and their interactions on the 5 soil biota parameters

268 previously log-transformed: abundances of Collembola, Oribatida, and predator and
269 biomasses of bacteria and fungi. The full models were then simplified to determine the most
270 parsimonious models using the *dredge* function of *MuMIn* package (Barton, 2016), an
271 established model selection procedure with both forward and backward selection algorithms,
272 which ranks all candidate models (all possible combinations of the initial explanatory
273 variables included in the full model) based on the lowest Akaike Information Criterion (AIC).
274 Thus, litter type treatment initially included in the full models, was never retained in the most
275 parsimonious models.

276 Finally, Spearman correlations were performed to link litter mass loss after 24 months
277 of decomposition to the 4 soil biota directly involved in the decomposition process (bacteria,
278 fungi, Collembola and Oribatida).

279

280 **3. Results**

281

282 **3.1. Initial litter characteristics**

283 The PCA (Fig. 2) and PERMANOVA (Pseudo- $F_{\text{litter species}} = 42.5$, $P = 0.001$) revealed a
284 considerable variation of initial litter quality between the three plant species. The first axis of
285 the PCA (43 % of the variance explained) discriminated leaves of *Q. pubescens* from leaves
286 of *Q. ilex* with higher SLA, Mg and lower Na and lignin contents in the former. The second
287 axis of the PCA (34 % of the variance explained) distinguished *P. halepensis* from the two
288 *Quercus* species, with a higher WSC concentration and, on the opposite, lower WHC, Ca and
289 hemicellulose concentrations in the former. In addition, PERMANOVA revealed an effect of
290 litter type (i.e., leaves or needles collected from trees in ND or AD plots; Pseudo- $F_{\text{litter type}} =$
291 3.3 ; $P = 0.033$) indicating a modification of litter quality under amplified drought conditions,
292 as well as an interaction between litter species and litter type (Pseudo- $F_{\text{litter species} \times \text{litter type}} =$

293 2.4; $P = 0.046$) indicating a modification of litter traits according to the tree species
294 considered. AD induced an increase of Mg and Na for both oak species, a decrease of Ca
295 content the three tree species (Supplementary Table S1). SLA and WHC decreased with AD
296 for the two *Quercus* species but did not change for *P. halepensis* (Supplementary Table S1).
297 Finally, AD induced an increase of leaf phenolic content for *Q. ilex* whereas no change was
298 observed for *Q. pubescens* leaves and *P. halepensis* needles (Supplementary Table S1).

299

300 **3.2. Microbial community**

301 Bacterial and fungal biomasses were respectively 16% and 15% higher in *Q.*
302 *pubescens* litter compared to the two other litters (Table 2; Fig. 3a and b) and were not
303 affected by the litter type (i.e., leaves or needles collected from trees in ND or AD plots,
304 Supplementary Fig. S3). The effect of AD on microbial biomass was dependent on the forest
305 considered (significant forest \times precipitation interaction, Table 2). AD treatment had no effect
306 on the fungal or bacterial biomasses in the *Q. pubescens* or *Q. ilex* forests, but increased them
307 by 29 % in the *P. halepensis* forest (Fig. 4a and b).

308

309 **3.3. Soil mesofauna**

310 We collected a total of 27 292 individuals of microarthropods from all the litterbags.
311 Collembola were the most abundant microbi-detritivore arthropods (49 %), compared to
312 Oribatida (39 %), while predatory Acari represented 12 % of the microarthropods community.
313 As reported for microbial biomass, mesofaunal abundance varied according to litter species
314 identity but was not affected by litter type (Table 2, Supplementary Fig. S3). The abundance
315 of all mesofauna groups associated to *Q. pubescens* litter was always two times higher
316 compared to *Q. ilex* and *P. halepensis* litters (Fig. 3c-e).

317 Except for Oribatid Acari, forest type and precipitation treatment interactively affected
318 Collembola and predator abundances (significant forest \times precipitation interaction, Table 2).
319 AD treatment did not affect Collembola abundance in *Q. pubescens* forest but decreased
320 Collembola abundance by 46 % and 48 % in *Q. ilex* and *P. halepensis* forests, respectively.
321 Consequently, the Collembola abundance was similar level across the three Mediterranean
322 forests under AD treatment (Fig. 4c). The AD treatment had a significant effect only in the *Q.*
323 *ilex* forest, with a 50 % decrease of the predator abundance (Fig. 4d). Finally, Oribatid Acari
324 abundance was respectively 87 % and 62 % higher in *Q. ilex* forest compared to *Q. pubescens*
325 and *P. halepensis* forests (Fig. 5a) and was reduced by 33 % under AD conditions in the three
326 forest types (Fig. 5b).

327

328 **3.4. Relationships between soil biota parameters and litter mass loss**

329 While Collembola and Oribatida abundances were positively correlated with litter
330 mass loss under ND condition in both *Q. ilex* and *P. halepensis* forests (Table 3), these
331 relationships disappeared under AD conditions. Collembola abundance was only marginally
332 correlated with litter mass loss under ND treatment in *Q. pubescens* forest (Table 3), and this
333 trend also disappeared under drier conditions. Finally, fungal biomass was positively related to
334 litter mass only in *Q. ilex* forest under AD conditions (Table 3).

335

336 **4. Discussion**

337 Our study highlighted that litter identity strongly controls soil biota in Mediterranean
338 forests after a 24-month field litterbag experiment. In each of the three forest, *Q. pubescens*
339 litter had consistently more abundance of all soil biota groups compared to *Q. ilex* and *P.*
340 *halepensis* litters. Our results suggest a key role played by Ca, Mg, specific leaf area (SLA)
341 and water holding capacity (WHC) as drivers of soil biota parameters. In addition, amplified

342 drought differently affects soil biota with an increase in microbial biomass and a decrease in
343 soil fauna abundance. However, except for Oribatida Acari, negative effect of amplified
344 drought were dependent on the forest considered. Surprisingly, amplified drought did not
345 indirectly affect the soil biota by altering the tree litter quality.

346

347 ***4.1. Effects of litter traits on soil biota***

348 We provided clear evidence that soil biota communities are strongly controlled by
349 litter species identity, but we only partly confirm our first hypothesis of higher abundance of
350 soil biota in oak compared to pine forests. More precisely, the abundance of all soil biota
351 associated with *Q. pubescens* litter was higher compared to *Q. ilex* and *P. halepensis* litters,
352 whatever the forest in which the litter decomposed. *Q. pubescens* leaves exhibited higher
353 specific leaf area (SLA) and water holding capacity (WHC) compared to *Q. ilex* leaves and *P.*
354 *halepensis* needles. Higher litter SLA has been reported to induce an increase in water
355 availability (Castro-Díez et al., 1997; Makkonen et al., 2012, 2013) or in habitat structure for
356 Collembola (Kalinkat et al., 2013; Santonja et al., 2018; Aupic-Samain et al., 2019). WHC is
357 a physical trait corresponding to the litter ability to hold the water, which is necessary for soil
358 biota development and activity (Pflug and Wolters, 2001; Makkonen et al., 2012, 2013;
359 Santonja et al., 2017). In addition, *Q. pubescens* leaves also exhibited higher Ca and Mg
360 concentrations than the two other litters. Ca positively affects fungal growth and activity
361 (Eriksson et al., 1990; Jenkins and Suberkropp, 1995) and is a key constitutive of invertebrate
362 cuticles (Cairns and Yan, 2009). Mg plays an important role in the growth and metabolic
363 functions of microbial cells (Walker, 1994) and is an essential element for invertebrates
364 required for enzymatic reactions, nerve connections or muscle function (National Research
365 Council, 2005). Some previous studies identified these physical (SLA and WHC) and
366 chemical (Ca and Mg) litter traits as important drivers of the litter decomposition process in

367 Mediterranean forests (García-Palacios et al., 2016a), which support our study demonstrating for
368 the first time that these litter traits directly control soil biota in Mediterranean forests and then
369 ecosystem processes. However, we acknowledge that soil biota parameters were analysed
370 only at one sampling time in the present study (after 730 days of decomposition in litterbags),
371 preventing an extrapolation to soil biota dynamics throughout litter decomposition time and
372 necessitating additional experimentations to confirm our findings.

373

374 ***4.2. Direct and indirect effect of precipitation treatment on soil biota***

375 We confirmed our second hypothesis that decreasing precipitation directly affects of
376 soil biota biomass and abundance. Conversely, we did not evidence an indirect effect of
377 amplified drought on soil biota mediated by a shift in litter quality at intraspecific level (Fig.
378 2), in contrast to our third hypothesis. In a climate change context, these findings clearly
379 highlight that reduced water availability prevails the intraspecific shift in litter quality due to
380 plant water stress as a major driver of soil biota communities in our Mediterranean forests.

381 In temperate forest ecosystems, bacteria and fungi are often positively correlated with
382 soil water availability (e.g., Pflug and Wolters, 2001; Taylor and Wolters, 2005; Lensing and
383 Wise, 2007). In Mediterranean ecosystems, in contrast, the regular summer droughts could
384 have selected adapted phenotypes among microbial species (Criquet et al., 2000; Curiel Yuste et
385 al., 2014; Pereira et al., 2019), leading to only weak or an absence of drought effect on soil
386 bacterial and fungal communities (Wilkinson et al., 2002; Sherman et al., 2012; Curiel Yuste
387 et al., 2014). In addition, higher mortality and lower fecundity rates were reported for both
388 Collembola and Acari under drier conditions, due to altered physiological processes
389 (Holmstrup et al., 2001; Houck, 2012; Poinso-Balaguer and Barra, 1991), species behavior
390 (Verhoef and van Selm, 1983) or predator-prey interaction (Santonja et al., 2017).
391 Consistently with these previous studies, microbial biomass was not negatively affected,

392 while soil fauna abundance decreased under amplified drought in the three studied
393 Mediterranean forests.

394 Except for Oribatida Acari, the drought responses of the soil biota varied according to
395 the forest type considered. In *P. halepensis* forest, we observed lower Collembola abundance
396 but higher microbial biomass with the amplified drought treatment. Since Collembola are
397 known to be microbial feeders (Maraun et al., 2003) and soil microbial communities to be
398 drought-tolerant in Mediterranean ecosystems (Sherman et al., 2012; Curiel Yuste et al.,
399 2014), higher microbial biomass under reduced precipitation could be the result of lower
400 Collembola feeding activity on them. Moreover, specialized microorganisms species could
401 also benefit on specific conditions from *P. halepensis* forest (higher specialized metabolites
402 from *P. halepensis* litter) to proliferate at the expense of generalist species. In contrast, in *Q.*
403 *ilex* forest we reported lower Collembola and predatory Acari abundances with amplified
404 drought, but no differences in microbial biomass. Since Acari Mesostigmata and Prostigmata
405 are active predators of Collembola (Koehler, 1999; Schneider and Maraun, 2009) and are
406 more drought-tolerant than Collembola (Santonja et al., 2017), this finding could suggest that
407 amplified drought in *Q. ilex* forest led to a negative cascading effect from Collembola to their
408 predators. However, in *Q. pubescens* forest we observed no effect of reduced precipitation on
409 Collembola, nor on their basale resource (microorganisms) and nor on their predators (Acari).
410 An explanation may lie in the differences among the precipitation reduction set-ups used in
411 three Mediterranean forests: precipitation reduction (~30%) in the *Q. pubescens* forest is
412 concentrated in the summer months, as expected by the climatic models (IPCC, 2013; Polade
413 et al., 2014), while in *Q. ilex* and *P. halepensis* forests, reduced precipitation is effective
414 throughout the year. As sampling was done in winter, soil biota was likely able to recover
415 from the amplified summer drought in *Q. pubescens* forest, given that precipitation patterns,
416 and not only the amounts, could impact the soil biota. A previous assessment of the soil

417 microbial community associated to litter performed in the same *Q. ilex* forest in 2013 reported
418 a significant decrease of their biomass under amplified drought (García-Palacios et al.,
419 2016b), which was not significantly confirmed in December 2016 by the present study. In
420 addition, Santonja et al., (2017) reported a negative effect of amplified drought on both
421 Collembola and Oribatid Acari in the same studied *Q. pubescens* forest between 2012 and
422 2013, while this negative effect was maintained only for Oribatid Acari for the present study.
423 Such discrepancies among studies could be explained by a number of factors, among which
424 are the year to year climatic differences, and the different sampling periods and methods. The
425 experiment of Santonja et al., (2017) in the *Q. pubescens* forest was performed just after the
426 installation of the rain exclusion device in 2011 while the present study was performed 5
427 years later. As Oribatid Acari exhibit lower dispersal ability (Hopkin, 1997), lower fecundity
428 rate (Houck, 2012) or higher habitat specialization (Wehner et al., 2016) than Collembola, we
429 could also speculate why these Oribatid Acari are still negatively affected by amplified
430 drought treatment after several years, while Collembola were able to adapt to the drier
431 conditions. Finally, while increasing Collembola and Oribatida abundances were positively
432 related to litter mass loss in *Q. ilex* and *P. halepensis* forests, amplified drought conditions
433 suppressed these relationships. This last finding highlights that, in addition to the impact on
434 soil biota demographic parameters, drier environmental conditions alter the contributions of
435 soil organisms to the processes they drive in Mediterranean forests.

436

437 **5. Conclusion**

438 The focus of this study was to assess how litter quality and reduced precipitation drive
439 soil biota in three Mediterranean forests. We provide clear evidence that soil biota
440 communities are strongly controlled by litter traits with a common pattern for all taxonomic
441 groups studied. *Q. pubescens* litter exhibited the highest microbial biomasses and mesofaunal

442 abundances in comparison to *Q. ilex* and *P. halepensis* litters whatever the forest in which the
443 litter decomposed, likely due to a better microhabitat (SLA and WHC) as well as nutritive
444 resource (Ca and Mg) conditions. Surprisingly, despite the amplified drought treatment
445 inducing a modification of litter quality, we did not observe an indirect climate change effect
446 on soil biota due to this intraspecific shift in litter quality. However, we observed a direct
447 effect of amplified drought on soil biota and their contributions to the litter decomposition
448 process with different response patterns depending on both the taxonomic group and the
449 Mediterranean forest considered.

450

451 **Author contributions**

452 VB and CF designed the experiment. AAS, SP, CL, MS , CF and VB performed the
453 experiment. AAS and MS analyzed the data and led the writing of the manuscript. All authors
454 contributed critically to the drafts and discussion and gave final approval for publication.

455

456 **Data accessibility**

457 Upon acceptance of this manuscript, the data supporting this article will be available from the
458 Dryad Digital Repository.

459

460 **Acknowledgement**

461 We specially thank to Sylvie Dupouyet, Jean-Philippe Orts and Adoué Adotevi for
462 their technical assistance during the laboratory experiment and Amélie Saunier for his
463 reviewing of the English. Chemical analyses were performed at the Plateforme d'Analyses
464 Chimiques en Ecologie (PACE, LabEx Centre Méditerranéen de l'Environnement et de la
465 Biodiversité, Montpellier, France), as well as at the Mediterranean Institute of Biodiversity
466 and Ecology (IMBE, Marseille, France). We thank Raphaëlle Leclerc, Bruno Buatois and

467 Nicolas Barthes for their assistance during chemical analyses. Funding was provided by the
468 French National Research Agency (ANR) through the project SecPriMe2 (no. ANR-12-
469 BSV7-0016-01) and the program BioDivMeX (BioDiversity of the Mediterranean experiment)
470 of the meta-program MISTRALS (Mediterranean Integrated STudies at Regional And Local
471 Scales). This research is also a contribution to the Labex OT-Med (no ANR-11-LABX-0061)
472 funded by the “Investissements d’Avenir” program of the French National Research Agency
473 through the A*MIDEX project (no ANR-11-IDEX-0001-02). The three experimental sites
474 were annually supported by the research infrastructure AnaEE-France (no ANR-11-INBS-
475 0001) and by Allenvi through the SOERE F-ORE-T. We acknowledge the supporters of the
476 COOPERATE database (Reiter IM, Castagnoli G, Rotureau A, 2015, "COOPERATE
477 database", <https://cooperate.obs-hp.fr/db>): CNRS FR3098 ECCOREV, LABEX OT-Med and
478 OSU-Pythéas. Finally, the authors declare no conflict of interest.

479

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764 **Tables**

765

766 **Table 1.** Main characteristics of the three studied forests. MAT and MAP correspond
 767 respectively to the annual mean values of temperature and precipitation between 2008 and
 768 2019 in natural precipitation (ND) and amplified drought (AD) plots (Supplementary Fig. S2).
 769 These values did not significantly differ between the forest sites (One-way ANOVAs, $F_{\text{site}} =$
 770 0.6 ; $P > 0.05$, $F_{\text{site}} = 1.7$; $P > 0.05$ and $F_{\text{site}} = 1.8$; $P > 0.05$ for MAT, MAP in ND plots and
 771 MAP in AD plots, respectively).

772

Forests	<i>Quercus pubescens</i> Willd.	<i>Quercus ilex</i> L.	Mixed <i>Pinus halepensis</i> Mill.
Sites	Oak Observatory at the Observatoire de Haute Provence (O ₃ HP)	Puéchabon	Font-Blanche
Location	43° 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)	12.6	14.0	13.7
MAP ND (mm)	866.3	955.4	605.0
MAP AD (mm)	639.5	698.9	441.6
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	<i>Quercus pubescens</i> Willd.	<i>Quercus ilex</i> L.	mixed <i>Pinus halepensis</i> Mill. / <i>Quercus ilex</i> L.
Other dominant plant species	<i>Acer monspessulanum</i> L <i>Cotinus coggygria</i> Scop.	<i>Buxus sempervirens</i> L. <i>Phyllirea latifolia</i> L. <i>Pistacia terebinthus</i> L. <i>Juniperus oxycedrus</i> L.	<i>Quercus coccifera</i> L. <i>Phyllirea latifolia</i> L.
Tree density (stems/ ha)	3503	4500	3368
Forest structure	even-age (70 years)	even-age (74 years)	uneven-age (61 years)
Type of rain exclusion system	Dynamic system : moving roof device	Permanent system : PVC gutters	Permanent system : PVC gutters
Rain exclusion system dimensions (m ²)	300	140	625
Rain exclusion device installation	2012	2003	2009

773

774

775 **Table 2.** Effects of the forest type (*Quercus pubescens*, *Quercus ilex* and *Pinus halepensis*
 776 forests), litter species identity, precipitation treatment (natural vs. amplified drought), and
 777 their interactions on microbial (bacterial and fungal biomasses) and mesofaunal (Collembola,
 778 Oribatida, and predator abundances) parameters. d.f. = degrees of freedom. *F*-values and
 779 associated *P*-values (with the respective symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for
 780 $P < 0.001$) are indicated. Litter type treatment was initially included in the full models but it
 781 was never retained in the most parsimonious models.

782

	df	Bacteria		Fungi		Collembola		Oribatida		Predator	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Forest (F)	2	2.9		3.3	*	16.9	***	10.8	***	29.0	***
Species (S)	2	7.6	***	7.5	***	12.0	***	20.2	***	6.9	**
Precipitation (P)	1	0.9		1.5		4.6	*	11.9	***	0.2	
F × S	4	2.5		2.4							
F × P	2	3.4	*	3.4	*	6.1	**	2.1		4.1	*

783

784

785 **Table 3.** Matrix of Spearman correlations between litter mass loss after 24 months of
 786 decomposition and the 4 soil biota directly involved in the decomposition process (bacteria,
 787 fungi, Collembola and Oribatida) according to forest site and precipitation treatment. Values
 788 in this matrix can range from -1.0 to 1.0, with 1.0 indicating perfectly correlated variables and
 789 -1.0 indicating perfectly negative correlations. Significant correlations are indicated with the
 790 respective symbols *** for $P < 0.001$, ** for $P < 0.01$, * for $P < 0.05$ and *ms* for $P < 0.07$. ND
 791 = natural drought and AD = amplified drought.

792

	<i>Q. pubescens</i> forest		<i>Q. ilex</i> forest		<i>P. halepensis</i> forest	
	ND	AD	ND	AD	ND	AD
Bacteria	-0.10	-0.13	-0.14	0.29	-0.17	0.10
Fungi	-0.10	-0.18	-0.14	0.42*	-0.18	0.07
Collembola	0.34 ^{ms}	0.11	0.53**	0.19	0.36*	0.09
Oribatida	0.10	-0.08	0.61***	0.29	0.45**	0.34 ^{ms}

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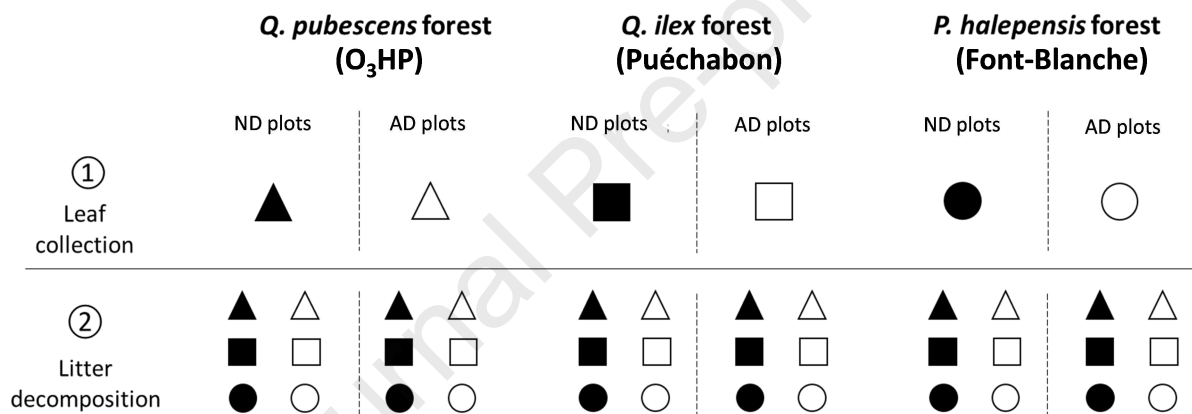
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795 **Figures**

796

797 **Fig. 1.** Schematic design of the field experiment. First, we collected senescent leaves or
 798 needles in three forest sites (*Quercus pubescens* forest, *Quercus ilex* forest or *Pinus*
 799 *halepensis* forests) according to the two precipitation treatments (natural or amplified drought
 800 plots). Second, we performed a 2-year litter decomposition experiment using the two litter
 801 types of the three litter species placed in the two precipitation treatments of the three forests.
 802 ND = natural and AD = amplified drought.

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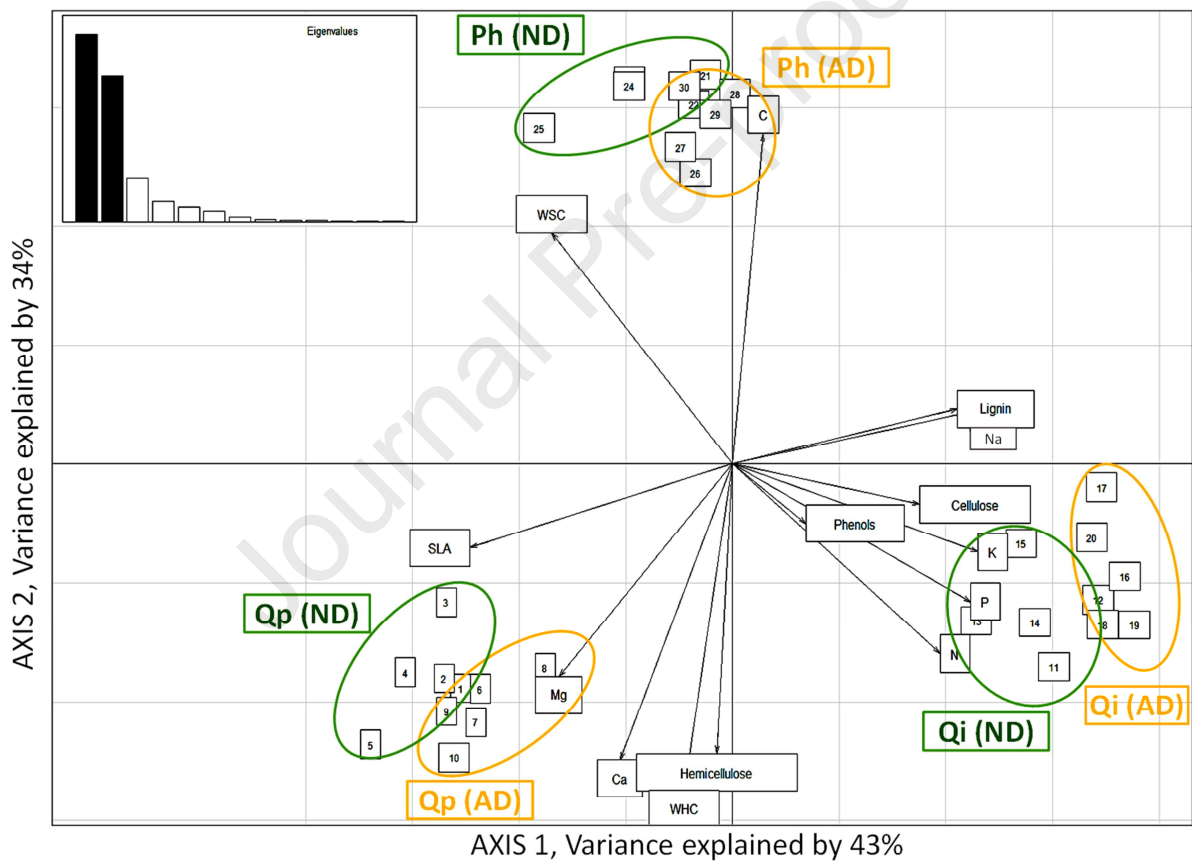


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806 **Fig. 2.** Principal Component Analysis (PCA) based on the 13 initial litter traits (arrows),
 807 arranged by litter species identity (*Quercus pubescens*, *Quercus ilex* and *Pinus halepensis*)
 808 and litter type (leaves / needles collected from trees in ND or AD plots) showed by colored
 809 circles. Qp= *Q. pubescens*, Qi= *Q. ilex*, Ph= *P. halepensis*, ND = natural and AD = amplified
 810 drought, WSC= Water Soluble Compounds, C = Carbon, SLA= Specific Leaf Area, Mg =
 811 Magnesium, Ca = Calcium, WHC = Water Holding Capacity, N = Nitrogen, P =
 812 Phosphorous, and Na = Sodium.

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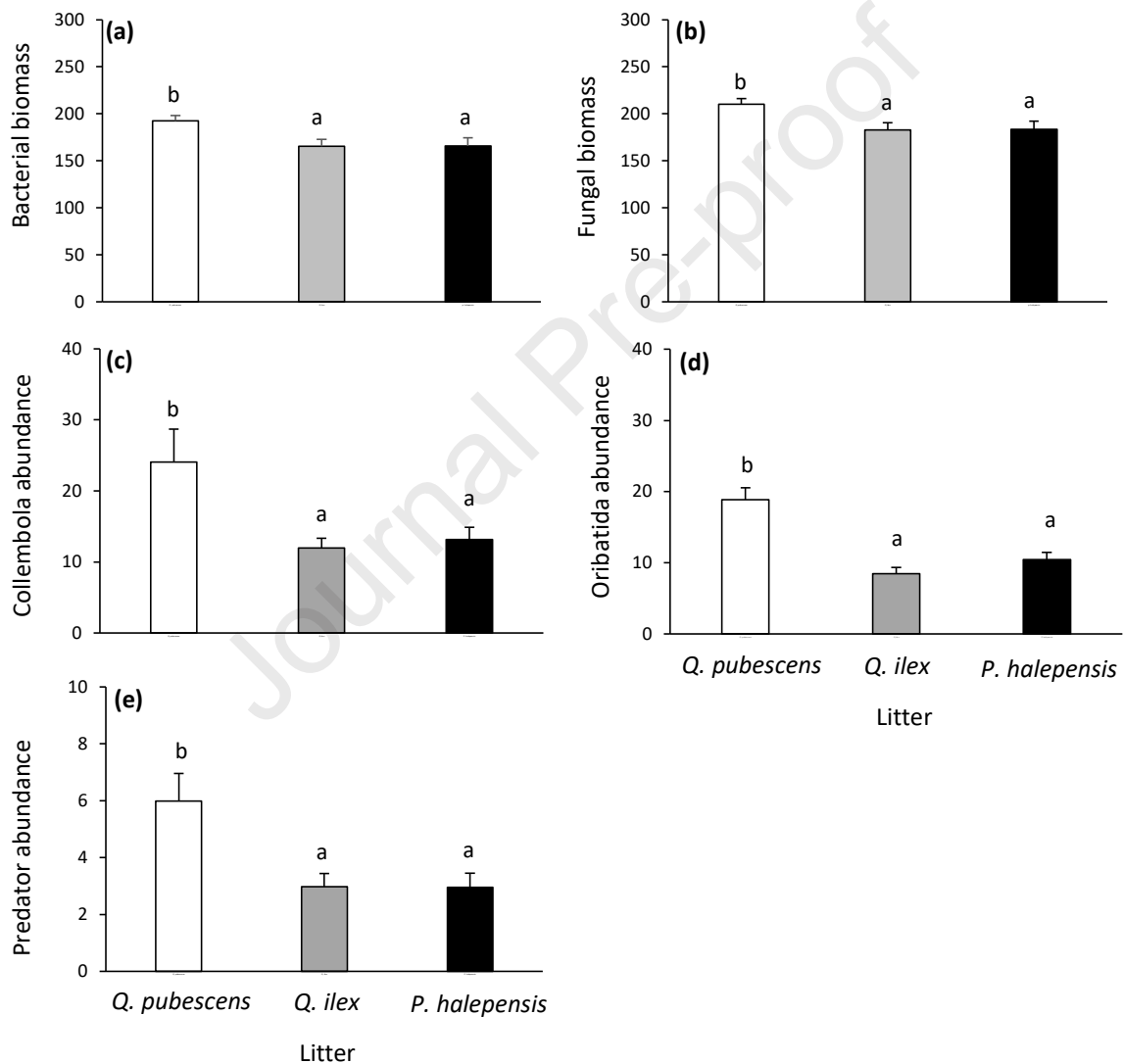


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815

816 **Fig. 3.** Effects of litter species identity on bacterial biomass (a), fungal biomass (b),
817 Collembola abundance (c), Oribatida abundance (d) and predator abundance (e). Values are
818 means \pm SE; $n = 60$. Microbial biomass is expressed as $\mu\text{g. g litter}^{-1}$ and mesofauna
819 abundance as nb of individuals. g litter^{-1} . Different letters denote significant differences
820 between treatments from ANOVA analysis with $a < b$.

821



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824 **Fig. 4.** Bacterial biomass (a), fungal biomass (b), Collembola abundance (c) and predator

825 abundance (d) according to the forest type and the precipitation treatment (Table 2). Values

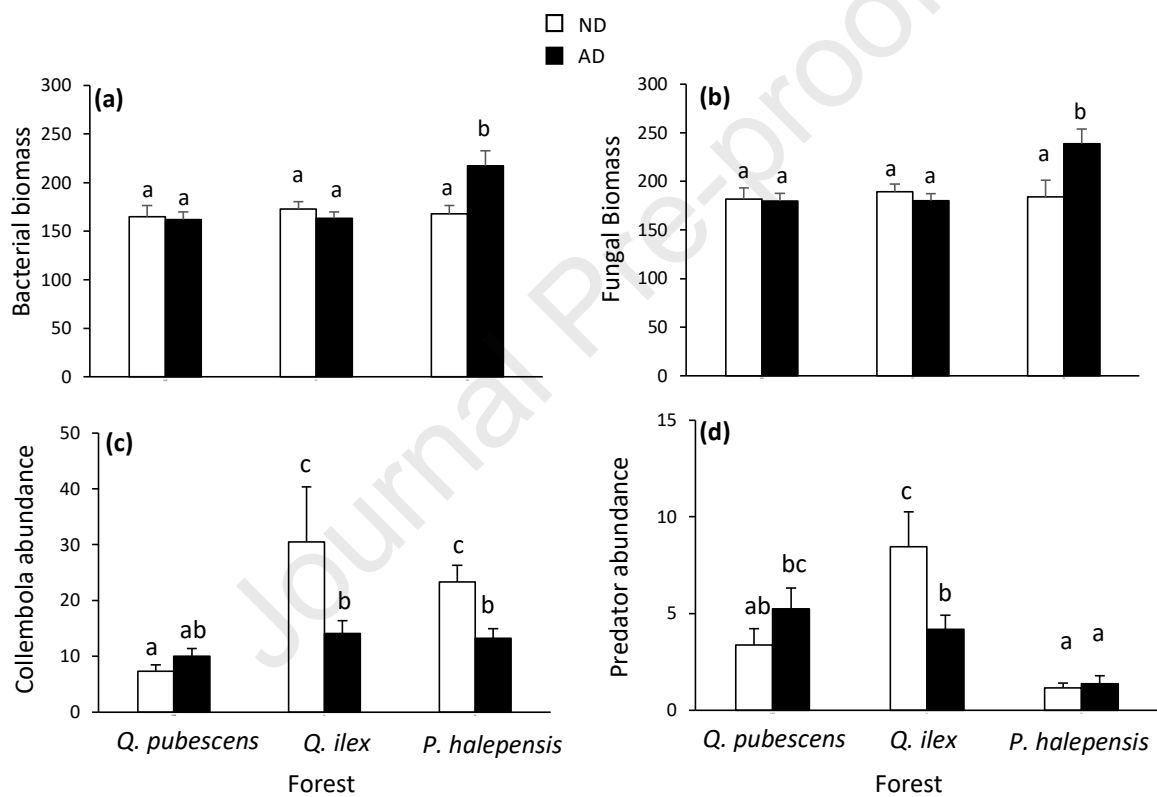
826 are means \pm SE; n= 30. Microbial biomass is expressed as $\mu\text{g. g litter}^{-1}$ and mesofauna

827 abundance as nb of individuals.g litter⁻¹. Different letters denote significant differences

828 between treatments from ANOVA analysis with $a < b < c$. ND = natural and AD = amplified

829 drought.

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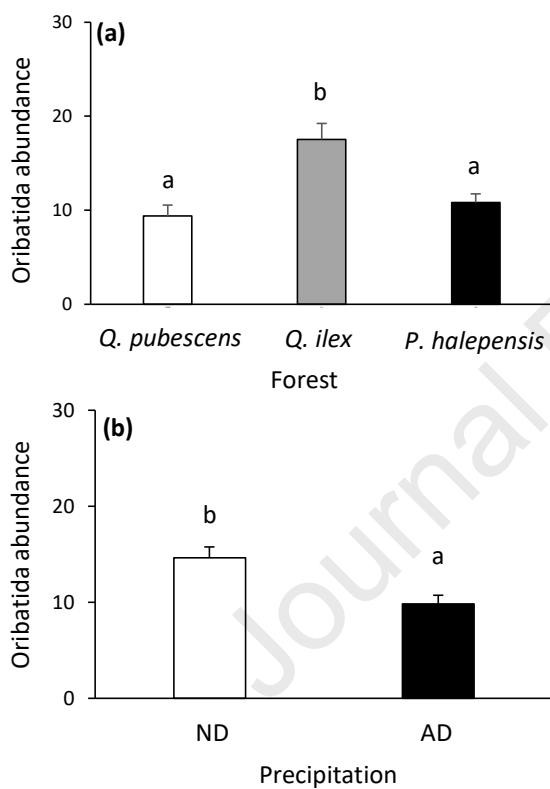


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832

833 **Fig. 5.** Effect of (a) forest site (*Quercus pubescens* forest in white, *Quercus ilex* forest in grey
834 and *Pinus halepensis* forest in black) and (b) precipitation treatment (ND plot in white and
835 AD plot in black) on Oribatida abundance. Values are means \pm SE; $n = 60$ for (a) and $n = 90$
836 for (b). Abundance is expressed as nb of individuals.g litter⁻¹ and μ g. g litter⁻¹, respectively.
837 Different letters denote significant differences between treatments with $a < b$. ND = natural
838 and AD = amplified drought.

839



840

Highlights

- Chemical (Ca, Mg) and physical (WHC, SLA) litter characteristics drive soil biota.
- Drier conditions lead to higher microbial biomass but lower mesofauna abundance.
- Drier conditions alter plant litter quality without cascading effect on soil biota.
- Drier conditions suppress soil fauna-litter decomposition efficiency relationships.

Conflict of interest: The authors declare that they have no conflict of interest.

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