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1 **Fungal communities are more sensitive indicators to non-extreme soil moisture**
2 **variations than bacterial communities**

3

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19

20 **Abstract**

21 Many studies have focused on the impact of intense drought and rain events on soil
22 functioning and diversity, but little attention has been paid to the response of microbial
23 communities to non-extreme soil moisture variations. However, small fluctuations of soil
24 water content represent a common situation that ought to be examined before understanding
25 and deciphering the impact of extreme events. Here, we tested the impact of a decrease in
26 average soil water content and small water content fluctuations in non-extreme conditions on
27 microbial community composition and C mineralisation rate of a temperate meadow soil.
28 Two soil microcosm sets were incubated at high and low constant moisture and a third set
29 was subjected to 4 short dry-wet cycles between these two soil moistures. No robust change
30 in bacterial community composition, molecular microbial biomass, and fungal:bacterial ratio
31 were associated with soil water content change. On the contrary, the fungal community
32 composition rapidly alternated between states corresponding to the high and low levels of soil
33 moisture content. In addition, gross C mineralisation was correlated with soil moisture, with a
34 noteworthy absence of a Birch effect (C over-mineralisation) during the wetting. This study
35 suggests that some fungal populations could coexist by occupying different moisture niches,
36 and high fungal community plasticity would classify them as more sensitive indicators of soil
37 moisture than bacteria. Moreover, under non-stressed conditions, the community composition
38 did not affect metabolic performance so a future decrease in average soil moisture content
39 should not result in a supplemental loss in soil carbon stocks by a Birch effect.

40

41 **Keywords**

42 Soil moisture fluctuation; ARISA fingerprinting method; C mineralisation; metabolic
43 activity; pore size; moisture niche.

44

1. Introduction

Microbial community structure could play a role in the ability of communities to realize different functions but also to resist environmental disturbance (Torsvik and Øvreås, 2002). In the context of global change, microbial community structure could be modified and therefore impact ecosystem functioning. Soil moisture is one of the major factors influencing microbial community structure (Brockett et al., 2012) and the shift of microbial community structure is suspected to contribute to important pulses in net mineralisation during the rewetting of dry soils (Borken and Matzner, 2009). But where some authors have suggested that periods of soil water restriction and wetting of dry soil affect microbial community structure through induced osmotic stress and resource competition occasioning selective pressure (Fierer et al., 2003; Castro et al., 2010), others reported no change in microbial community structure (Griffiths et al., 2003).

These contradictory findings in the literature may be due to the specificity of studied ecosystems but also to differences in experimental approaches (Borken and Matzner, 2009). On one hand, the assumed change of the microbial community structure is often made from circumstantial evidence (*e.g.* changes in biomass or activity). On the other hand, the effects of drying and wetting are frequently not distinguished, and “drying soil” and “drought” often merged. Climatic models forecast a decrease of average soil moisture and an intensification of extreme events (IPCC, 2007), but it is still uncertain to what extent the soil system can become unbalanced under these perturbations. While many studies have focused on intense water-stress, little attention has been paid to the response of microbial communities in non-extreme conditions to characterise their stability to natural variability (Meier et al., 2008). However, small fluctuations of soil water content in non-extreme conditions represent a

69 common situation that needs to be explored before understanding and deciphering the impact
70 of extreme events.

71 A limited decrease in soil moisture may be a stressful process for some
72 microorganisms, due to physical constraints that affect bacterial or fungal habitats (Or et al.,
73 2007). At the scale of soil aggregates, the basic units of microbial habitats, drying is
74 heterogeneous and can induce a localized drought that stresses microorganisms; particularly
75 in larger pores (Ruamps et al., 2011). Besides, the decrease of pore connectivity could also
76 modify the microbial community structure by decreasing bacterial mobility and the rate of
77 substrate diffusion (Carson et al., 2010). Fungal communities, on the other hand, are thought
78 to be better adapted to drying than bacteria thanks to hyphal networks which facilitate access
79 to water and nutrients. The question therefore arises as to how fluctuating water content
80 without total drought influences microbial community structure and if the sensitivity of
81 bacterial and fungal community is the same. Beyond the fact that it is still unclear whether a
82 microbial community is associated with a given soil water content, it could be unaltered or
83 alternate between states reflecting the different levels of soil moisture, but also experience
84 another state, reflecting a transient community associated with drying or wetting or both.

85 The objectives of this study were to examine in non-extreme moisture conditions: (i)
86 whether the composition of bacterial and fungal communities is similar at two contrasting
87 water contents; (ii) how the microbial community composition responds to water content
88 fluctuation within a narrow range of soil moisture; and (iii) whether the microbial community
89 composition can contribute to explain soil functioning. We performed a microcosm
90 experiment consisting of two sets of microcosms kept steadily wet at 64% and 33% Water-
91 Holding Capacity (WHC), respectively. A third set was subjected to 4 dry-wet cycles over a
92 one-month period and subsequently kept steadily at 64% WHC for 4 additional months. At
93 several incubation times, the soil bacterial and fungal communities were characterised by

94 molecular tools (crude DNA quantity for molecular microbial biomass, A-RISA
95 fingerprinting method for structure and qPCR for abundance), and C mineralisation rate was
96 measured through CO₂ quantification.

97

98 **2. Materials and methods**

99

100 **2.1. Site description and soil characteristics**

101 Soil was sampled from uncultivated meadow bordering cultural field at Versailles,
102 France (mean annual precipitation: 630 mm; mean annual temperature: 10.5°C); which is an
103 ecosystem with a high ecological importance in urban locations (Manninen et al., 2010).
104 Vegetation (ruderal nitrophilous dominated by *Trifolium repens*) and soil properties of this
105 site are representative of the northern region of France. Fourteen random samples of one
106 kilogram were collected from the topsoil (0-6 cm depth) in August 2010 then combined and
107 homogenized in order to obtain one unique microbial community representative of this
108 ecosystem by lessening spatial heterogeneity. The soil was air-dried, sieved to 4 mm, and
109 vegetation debris, rocks and any fauna visible to the naked-eye were removed before use. The
110 soil is classified as a silty loam (Eutric Cambisol, WRB) with 148 g.kg⁻¹ of clay, 347 g.kg⁻¹ of
111 silt and 496 g.kg⁻¹ of sand. Characterised according to standard methods
112 (http://www.lille.inra.fr/las/methodes_d_analyse/sols), the pH_{H₂O} was 6, and there was 20.6
113 g.kg⁻¹ of organic C, less than 1 g.kg⁻¹ of CaCO₃, 1.6 g.kg⁻¹ of total N hence a C/N of 12.6.
114 Water-holding capacity (WHC) was of 0.41 g of water per g of dry soil.

115 **2.2. Experimental design**

116 Microcosms, consisting of 40 g dry soil equivalent in 126 mL glass bottles, were pre-
117 incubated for 6 weeks at 18.5°C in the dark at desired moisture in order to stabilize the soil
118 microbial communities. Microcosms of high moisture treatment (HM) were maintained at

119 64% WHC ($pF=2.24$), corresponding to the maximum expected microbial activity, and
120 microcosms of low moisture treatment (LM) at 33% WHC ($pF=3.67$) by sealing them with
121 parafilm[®]. Fluctuating moisture treatment (FM) consisted of 4 cycles of air-drying for one
122 week from 64% WHC until about 33% WHC then wetting to 64% WHC by addition of
123 sterile Milli-Q water. As moisture levels were monitored gravimetrically throughout the
124 incubation (Fig. 3a), the quantity of added water balanced the quantity of water lost during
125 the drying. Time 0 is the first day of the first drying period, and the rewetting events for FM
126 were at 7, 14, 21 and 28 days. After these four cycles, microcosms were subsequently sealed
127 and incubated for a further 4 months at 64% WHC. Three replicate microcosms per FM
128 treatment were destructively sampled at the end of drying period (“Fluctuating Moisture” at
129 low moisture, FM-l) and two days after wetting (“Fluctuating Moisture” at high moisture,
130 FM-h) for the four cycles, then at 60, 90 and 145 days at constant moisture, resulting in $n =$
131 33 FM microcosms. Constantly moist microcosms (HM and LM) were sampled in triplicate
132 at 0, 14, 28, 60, 90 and 145 days, resulting in $n = 18$ microcosms per treatment. Soil samples
133 were stored at -20°C until required for microbial analyses.

134 **2.3. DNA extraction: molecular microbial biomass**

135 Total soil DNA was extracted and purified following the GnS-GII procedure, as
136 described in Terrat et al. (2012). The DNA concentration of crude extracts was calculated
137 using a calf thymus standard curve. This soil DNA concentration can be used as a robust
138 indicator of soil microbial biomass (Marstorp et al., 2000) defined by Dequiedt et al. (2011)
139 as the molecular microbial biomass. Crude DNA extracts were purified using PVPP
140 minicolumns (BIORAD, Marne La Coquette, France) and GeneClean Turbo Kit (Q
141 Biogene[®], Illkirch, France) following the manufacturer’s instructions.

142 **2.4. Quantitative PCR: microbial density**

143 The 16S and 18S ribosomal DNA genes copy numbers were determined by real-time
144 PCR using two pairs of universal primers to estimate bacterial (Primer Gold 341F/515R;
145 López-Gutiérrez et al., 2004) and fungal abundances (FR1/FF390; Vainio and Hantula, 2000)
146 following the procedure described in Chemidlin Prévost-Bouré et al. (2011).

147 **2.5. ARISA fingerprinting: genetic structure of microbial community**

148 Automated Ribosomal Intergenic Spacer Analysis (ARISA), a molecular
149 fingerprinting method (Ranjard et al., 2001), has been used to characterise the genetic
150 structure of bacterial (B-ARISA) and fungal (F-ARISA) communities for all samples except
151 the third dry-wet cycle. ARISA was used since it has been demonstrated to be a high-
152 resolution, robust and highly reproducible technique for evaluating microbial communities
153 change through space and time (Ranjard et al., 2001; Jones et al., 2007). The bacterial
154 ribosomal intergenic spacer region (IGS) and the fungal internal transcribe spacer (ITS) were
155 amplified by PCR using primers S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18 and
156 ITS1F/3126T, respectively. Purified PCR products were added to deionized formamide and
157 fragments were resolved on polyacrylamide gels under denaturing conditions as described by
158 Pascault et al. (2010) on a LiCor[®] sequencer (ScienceTec, France).

159 **2.6. C mineralisation rate**

160 C mineralisation was measured as the daily CO₂ gas flux on three replicate
161 microcosms per treatment, the day before the wetting, one hour after the wetting as well as
162 the two consecutive days, then once a week the second month, and finally monthly until the
163 end of incubation. Measurements were carried out using a micro-gas chromatograph as
164 described in Kaisermann et al. (2013).

165 **2.7. Statistical analysis**

166 All statistical analyses were performed using the R software v. 2.13 (R Development
167 Core Team, 2011). The ARISA data were analysed using Non-metric multidimensional

168 scaling (NMDS) implemented in Vegan package (Oksanen et al., 2008) with Bray-Curtis
169 distance measure to generate the dissimilarity matrices. Optimal MDS configurations were
170 determined using 1000 random starts and the lowest stress values were used. The sample
171 discrimination according to treatment, time, and the interaction was tested through a non-
172 parametric manova using the Adonis function (Vegan package; Oksanen et al., 2008) on
173 Bray-Curtis dissimilarity matrices with 999 permutations. This method partitions distance
174 matrices among sources of variations and uses a permutation test with pseudo-F ratios. A first
175 NMDS (Fig. 1a and Fig. 2a) was performed with all samples with the Adonis analysis
176 including 3 treatments (HM, LM, FM) and 7 sampling times. A second NMDS (Fig. 1b and
177 Fig. 2b) was performed for the first month in order to analyse separately the effect of
178 moisture variation in FM treatment with the Adonis analysis including 4 treatments (HM,
179 LM, FM-h, FM-l) and 4 sampling times. Confidence ellipses at 95% were included in
180 ordinations to examine the variability of samples over “time” in the first NMDS, and for
181 “treatment effect” in the second NMDS. Moreover, when the treatment effect was significant,
182 treatments have been compared in a pairwise fashion with independent Adonis tests in order
183 to detail which treatments were different from the others. As the experimental design has
184 nestedness (time effect), we also specified “strata = time” in the Adonis test so that
185 randomization occurs only within each time point.

186 The differences between treatments for molecular microbial biomasses and 18S:16S
187 ratios were analysed with a linear mixed effect model using the *lme* function implemented in
188 the *nmle* package (<http://lme4.r-forge.r-project.org/>) where ‘time’ was included as a random
189 effect. Significant differences ($P < 0.05$) were evaluated by ANOVA.

190 For C mineralisation rates, the dependence of the measurement through time on the
191 same microcosms was integrated with “microcosm ID” as a random effect in the *lme*. When
192 C mineralisation is expressed by $\text{g water.g soil}^{-1}$, the two constantly wet treatments (LM and

193 HM) presented no difference throughout the incubation: the mineralisation rate decreased
194 linearly as a function of time, and the equations describing the relationship in both treatments
195 were not significantly different (treatment effect $P=0.46$, treatment*time effect $P=0.14$;
196 Supplemental data, Fig. S1). Therefore, we used data from LM and HM treatments to
197 determine if the variations of moisture in FM treatment induced a supplemental modification
198 of soil respiration than the one expected due to the only modification of water content. The
199 expected mineralisation rate for LM treatment for a given moisture at a given time was
200 calculated as: $C_{expected} = (-0.0043T + 0.9307) \times Wc$; where $C_{expected}$ is the expected C
201 mineralisation rate in $\mu\text{g C-CO}_2.\text{gsoil}^{-1}.\text{days}^{-1}$, T the time in days and Wc the water content in
202 gwater.gsoil^{-1} . To estimate the effect of drying and wetting on C mineralisation, the observed
203 value of FM was compared to the expected value using lme with treatment and time as fixed
204 effects and the significance estimated by ANOVA.

205

206 **3. Results**

207 **3.1. Genetic structure of bacterial communities**

208 The non-metric multidimensional scale analysis of all B-ARISA profiles showed
209 changes in bacterial community structure over time but without difference between the
210 different treatments (Fig. 1a). The data taken from the first month (Fig. 1b) confirmed the
211 similarity of the bacterial communities between the constant moisture treatments (HM = LM)
212 (Table 1), between high moisture treatments (HM = FM-h) and between low moisture
213 treatments (LM = FM-l). However, the FM treatment after the drying periods (FM-l) was
214 significantly different from the high moisture treatments (HM and FM-h). Therefore,
215 bacterial community composition was identical between the different water contents but a
216 part of the community was different at the end of drying period.

217 **3.2. Genetic structure of fungal communities**

218 The non-metric multidimensional scale analysis of all F-ARISA profiles showed a
219 strong time effect on fungal community structures, especially for days 60 and 145 and also a
220 treatment effect (Fig. 2a). The pairwise comparison showed that fluctuating moisture
221 treatment (FM) was similar to high and low moisture treatments ($P=0.171$ for HM and
222 $P=0.126$ for LM, respectively) when HM and LM fungal communities were significantly
223 different ($P=0.014$).

224 The data taken from the first month (Fig. 2b) revealed significant differences between
225 all treatments expect for the fungal communities of LM and FM-l (Table 1). The statistical
226 difference between HM and FM-h was not confirmed by visual observations of NMDS for
227 times 0 and 14 and actually can only be explained by the high variability of replicates at the
228 time 28 (and corroborated by the strong Time*Treatment effect ($P=0.005$) when the test is
229 done without strata). Therefore, fungal community structure was different between HM and
230 LM, and alternated in the FM treatment between states corresponding to the high and low
231 levels of soil moisture content.

232 **3.3. Microbial molecular biomass, abundance and activity**

233 Molecular microbial biomass (Fig. 3b) and fungi:bacteria ratio measured as 18S:16S
234 ratio (Fig. 3c) were similar between all treatments ($P=0.07$ and $P=0.63$, respectively). The C
235 mineralisation rate of the HM treatment was higher than the LM treatment (Fig. 3d), but was
236 similar when expressed by g water^{-1} (Supplemental data S1). The C mineralisation rate of FM
237 fluctuated between the C mineralisation rates of the HM and LM treatments (“time effect”
238 $P<0.0001$). More precisely, the C mineralisation rates decreased when the soil dried, and
239 became similar to the HM treatment after the wetting. There was no difference between
240 observed values of FM and expected values ($P=0.46$) at all incubation times (“Treatment x
241 time effect” $P=0.14$), showing that moisture variations in FM treatment did not induce a

242 supplemental modification of soil respiration (*i.e.* over-mineralisation) than the one expected
243 due to the modification of water content.

244

245 **4. Discussion**

246 For the two levels of water content for the constant moisture microcosms, the
247 bacterial community compositions were similar whereas the fungal community compositions
248 were distinct. Therefore, only the fungal community presents a specific composition
249 depending on soil moisture. This may reflect the difference of ecological habitat between
250 these two guilds (Chenu et al., 2001). Indeed, fungi could preferentially live in large pores,
251 which are filled at high moisture but empty at low moisture; bacteria in turn would live in
252 smaller pores, better protected against these perturbations. In order to discuss ecological
253 patterns, the overall communities can be examined in terms of populations, or subsets of
254 communities. Therefore, the relative abundance of fungal populations inhabiting the large
255 pores could be decreased in dry conditions while the abundance of other populations could
256 increase at the new air-water interface, which explains the dissimilarity of fungal
257 communities between these two moisture levels. Contrastingly, the lack of difference for the
258 bacterial community is unexpected as different pore or aggregate size classes support distinct
259 bacterial populations (Ruamps et al., 2011; Davinic et al., 2012). This could indicate that
260 bacteria either only inhabit the smallest pores still filled with water at low moisture content,
261 or bacterial populations inhabiting large pores are not impacted when pores are empty at low
262 moisture. The latter could be the result of either (i) the presence of sufficient water on pore
263 walls to ensure favourable living conditions or (ii) that bacterial populations themselves
264 maintain a favourable habitat compliments of their lifestyle strategy, since a lot of soil
265 bacteria are able to live in biofilms, embedded in extracellular polymeric substances (Or et
266 al., 2007).

267 While constant levels of moisture resulted in unchanged bacterial communities,
268 fluctuations resulted in a small modification of the bacterial community structure during the
269 first month. This may result from population shifts within a small portion of the overall
270 community. Indeed, at the aggregate scale, the drainage of pores may be heterogeneous, and
271 since bacteria are dependent on the water in their immediate vicinity, the bacterial
272 community could be only partially in stressful conditions. A finer estimation of how the
273 dynamics of bacterial populations localized in different microenvironments are affected by
274 water fluctuation (i.e. different aggregate size or preferential flow paths; Bundt et al., 2001) is
275 necessary to determine whether a transient community is associated with drying periods.
276 Nevertheless, for the whole of the bacterial community, the community similarity when the
277 water content is half and the lack of large and permanent changes during the water
278 fluctuation, reinforces the hypotheses that either the whole localised bacterial community is
279 adapted to this water disturbance (Griffiths et al., 2003; Meier et al., 2008) or there is no
280 water-stress at this range of soil moisture (Manzoni et al., 2012) at this scale of community
281 integration.

282 The water fluctuation induced a strong change in the fungal community structure that
283 alternated between states reflecting the high and low levels of soil moisture. This transient
284 response suggests that all species could survive and coexist in this order of magnitude of
285 moisture but that different fungal populations within the community would dominate at
286 different moisture levels. Therefore, notions of tolerance range and ecological optimum of
287 Shelford's law are illustrated here for fungal communities (Shelford, 1931). Indeed, the
288 success of an organism depends upon fulfilment of various conditions, the growth being
289 optimal when all the factors are in optimal range. But if one factor, here the water, is
290 deficient or in excess, the limits of survival of an organism are approached (deviation of
291 ecological optimum) and so its growth is decreased. This is also consistent with Lennon et al.

292 (2012) who suggested that some taxa may be able to coexist by partitioning the moisture
293 niche axis. When a moisture shift occurs, a reduction of the activity of dominant fungi
294 adapted to previous moisture content could result in a reduction in competitive ability against
295 other fungal taxa, which will then be able to dominate the community at the new water
296 content (Allison and Treseder, 2008). The rapid observed shift is quite surprising, but could
297 be explained by rapid hyphal turnover (Staddon et al., 2003; de Vries et al., 2009) and the
298 ability of some taxa to grow even in drying periods (Bapiri et al., 2010; Yuste et al., 2011).

299 Therefore, the drought tolerance of fungal communities often claimed in studies could
300 be explained not only by facilitated nutrient access through hyphal networks, but also by a
301 rapid turnover of populations conferring high plasticity to the community. Moreover, the
302 higher variability within the fungal community composition compared to the bacterial
303 community, already highlighted in field condition (Zumsteg et al., 2013), suggests that fungi
304 might be more sensitive indicators of soil moisture than bacteria in non-extreme conditions.
305 Additionally, since such stability of bacterial communities is not always observed after more
306 intense droughts (Fierer et al., 2003; Castro et al., 2010), our study reinforces the concerns
307 about the functioning and resilience of soils undergoing intense droughts because of a
308 permanent impact on bacterial communities.

309 At this range of soil moisture, C mineralisation rates were positively correlated to soil
310 water content. Two outcomes can be drawn from this result. First, it is worth noting the
311 absence of C over-mineralisation after the wetting, a phenomenon known as the ‘Birch
312 effect’, which is commonly observed in many ecosystems (Borken and Matzner, 2009). This
313 suggests that rainfall without prior soil drought did not induce additional C loss, which
314 supports the findings of Fischer (2009), that a minimal water content must be reached before
315 wetting to generate this flush. Secondly, as the molecular microbial biomass and abundance
316 were similar between treatments, the soil respiration variations were probably due to changes

317 in metabolic rates of the overall community. Indeed, it is well documented that when soil
318 water potential decreases, the metabolic activity of some microbial species is decreased
319 (Schimel et al., 2007; Manzoni et al., 2012). However, the activity expressed per gram of
320 water is similar at all the water contents, showing that where there is an activity, the activity
321 rate is preserved. With the assumption that the fungal populations that dominated at low
322 water content have an increased activity (or at least their relative contribution within
323 community activity), metabolic adjustments could occur within the overall community to
324 maintain the same metabolic rate. The lack of a link between composition changes and
325 metabolic activity supports the idea that functional redundancy maintains the metabolic rate
326 in non-extreme moisture conditions. Therefore our study suggests, as already presented by
327 Comte and Del Giorgio (2011) and Baltar et al. (2012) for aquatic ecosystems, that overall
328 metabolic performance of a soil microbial community is determined by environmental drivers
329 and can be achieved through different configurations of community composition.

330 In conclusion, this study illustrates that, taken as a whole and at this range of
331 moisture, (i) the fungal community composition depends on non-extreme moisture conditions
332 in contrast to the bacterial community possibly due to differences in niche preference, (ii) the
333 moisture fluctuation induces a rapid turnover of fungal populations conferring a high
334 community plasticity; therefore fungal community would be a more sensitive indicator of soil
335 moisture than bacteria, (iii) and the community metabolic performance is determined by
336 environmental drivers, the community showing functional redundancy in non-stressed
337 conditions. Finally, our study showed that a microbial community is adapted to cope with
338 non-extreme moisture variation. This reinforces the need to understand why some studies
339 have shown that microbial communities could be lastingly modified by severe drought and
340 evaluate their consequences on ecosystem functioning. For this purpose, it is essential to
341 estimate critical minimum moisture thresholds for microbial community shifts, and thus

342 assess the effects of intensity and duration of drying and wetting events on the stability of
343 microbial communities.

344

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350

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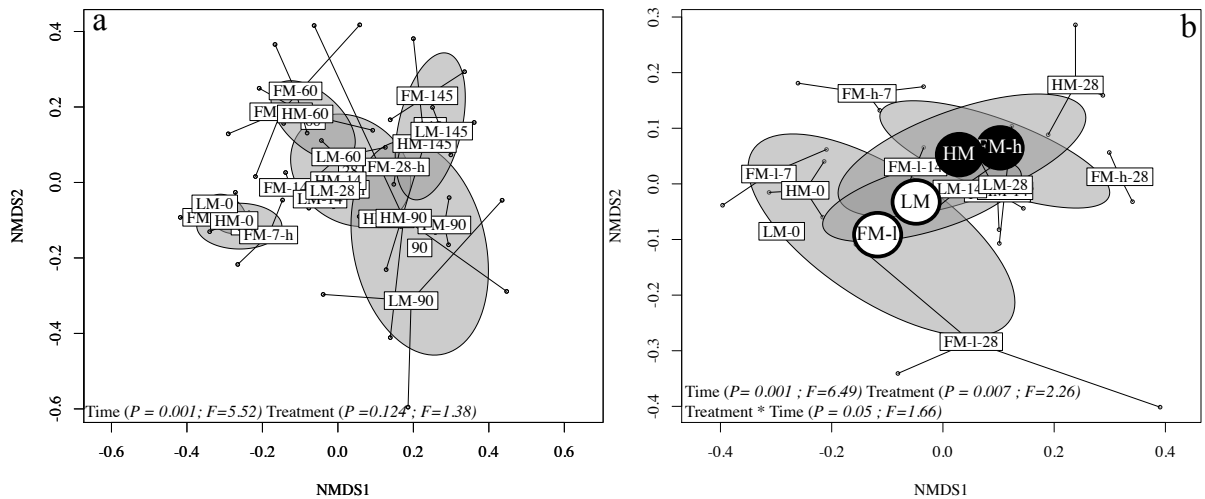
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CAPTIONS

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466 **Table 1.** Summary statistics of pairwise analysis of independent triplicates of
467 bacterial and fungal ARISA profiles for the four treatments High Moisture (HM), Low
468 Moisture (LM) and Fluctuating Moisture at the end of drying periods (FM-l) and two days
469 after the wetting events (FM-h), during the first month of incubation when the moisture
470 fluctuated.

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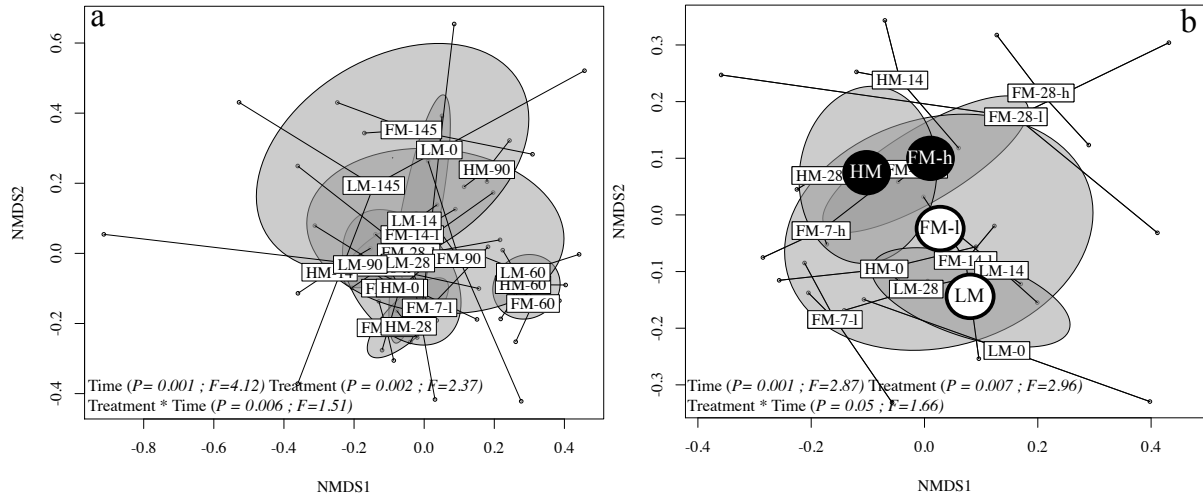


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474 **Fig. 1.** Nonmetric multidimensional scaling analysis generated from independent
475 triplicates of bacterial ARISA profiles for the three treatments High Moisture (HM), Low
476 Moisture (LM) and Fluctuating Moisture (FM) at the end of drying periods (FM-l) and two
477 days after the wetting events (FM-h). Figure (a) shows that the genetic structure of bacterial
478 community from day 0 to day 145 (number indicates the sampling day) is grouped by
479 sampling date. Figure (b) focuses on the first month when the moisture fluctuated, for the
480 first (7), second (14) and fourth (28) dry-wet cycles.

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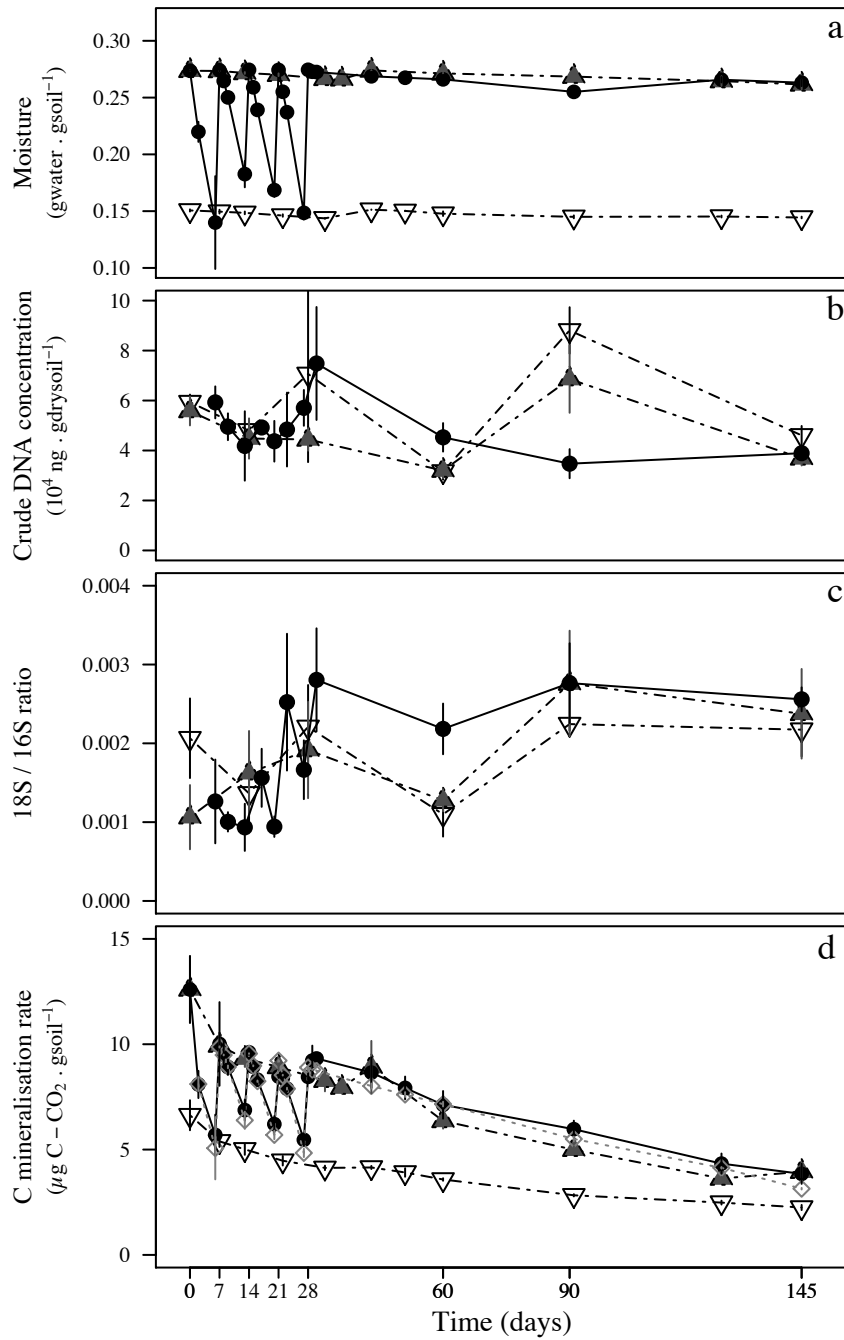
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Fig. 2 Nonmetric multidimensional scaling analysis generated from independent triplicates of fungal ARISA profiles for the three treatments High Moisture (HM), Low Moisture (LM) and Fluctuating Moisture (FM) at the end of drying periods (FM-l) and two days after the wetting events (FM-h). Figure (a) shows the genetic structure of fungal community from day 0 to day 145 (number indicates the sampling day). Figure (b) focuses on the first month when the moisture fluctuated, for the first (7), second (14) and fourth (28) dry-wet cycles.



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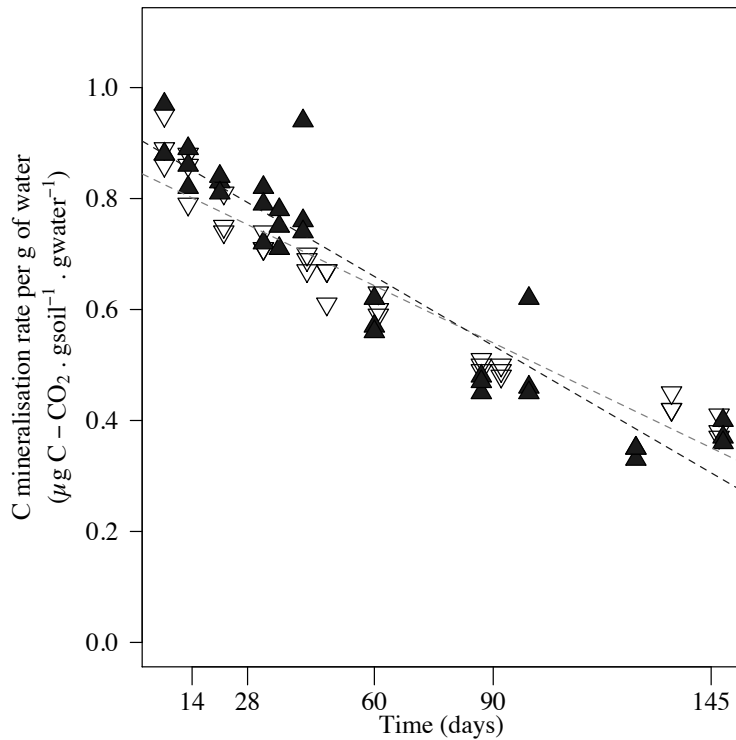
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Fig. 3. Moisture (a), molecular microbial biomass (b), fungal:bacterial ratio (c), and C mineralisation rate (d) for the three treatments High Moisture (HM, black triangles pointing upward), Low Moisture (LM, white triangles pointing downward) and Fluctuating Moisture (FM, black circles). Time 0 is the first day of the first drying period for FM treatment. Grey diamonds represent the C mineralisation calculated with linear model for the FM treatment. Data are mean ± standard deviation, n=3.



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501 **S1.** C mineralisation rate expressed per g of water for Low Moisture (LM, white
 502 triangles pointing downward) and High Moisture (HM, black triangles pointing upward)
 503 treatments. Time 0 is the first day of the first drying period for FM treatment. The dashed
 504 lines are the fitted linear model for each treatment (grey and black for LM and HM,
 505 respectively). This figure shows that the activity per water unit decrease as a function of time
 506 but is similar at the both water content (treatment effect $P=0.46$, treatment*time effect
 507 $P=0.14$).