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1 **Seasonal influence of climate manipulation on microbial**  
2 **community structure and function in mountain soils**

3

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25 **Abstract**

26

27 Microbial communities drive soil organic matter (SOM) decomposition through  
28 the production of a variety of extracellular enzymes. Climate change impact on  
29 soil microbial communities and soil enzymatic activities can therefore strongly  
30 affect SOM turnover, and thereby determine the fate of ecosystems and their role  
31 as carbon sinks or sources.

32 To simulate projected impacts of climate change on Swiss Jura subalpine  
33 grassland soils, an altitudinal soil transplantation experiment was set up in  
34 October 2009. On the fourth year of this experiment, we measured microbial  
35 biomass (MB), microbial community structure (MCS), and soil extracellular  
36 enzymatic activities (EEA) of nine hydrolytic and oxidative extracellular enzymes  
37 in the transplanted soils on a seasonal basis.

38 We found a strong sampling date effect and a smaller but significant effect  
39 of the climate manipulation (soil transplantation) on EEA. Overall EEA was  
40 higher in winter and spring but enzymes linked to N and P cycles showed higher  
41 potential activities in autumn, suggesting that other factors than soil  
42 microclimate controlled their pool size, such as substrate availability. The climate  
43 warming manipulation decreased EEA in most cases, with oxidative enzymes  
44 more concerned than hydrolytic enzymes. In contrast to EEA, soil MB was more  
45 affected by the climate manipulation than by the seasons. Transplanting soils to  
46 lower altitudes caused a significant decrease in soil MB, but did not affect soil  
47 MCS. Conversely, a clear shift in soil MCS was observed between winter and  
48 summer. Mass-specific soil EEA (EEA normalized by MB) showed a systematic

49 seasonal trend, with a higher ratio in winter than in summer, suggesting that the  
50 seasonal shift in MCS is accompanied by a change in their activities.

51 Surprisingly, we observed a significant decrease in soil organic carbon (SOC)  
52 concentration after four years of soil transplantation, as compared to the control  
53 site, which could not be linked to any microbial data.

54 We conclude that medium term (four years) warming and decreased  
55 precipitation strongly affected MB and EEA but not MCS in subalpine grassland  
56 soils, and that those shifts cannot be readily linked to the dynamics of soil carbon  
57 concentration under climate change.

58

59 **Keywords: Climate change; PLFA; soil enzymes; soil organic carbon;**  
60 **mountain grasslands; soil transplantation experiment**

## 61 1. Introduction

62

63 The persistence of the large amount of organic carbon stored in mountain  
64 grassland soils strongly depends on the expected modification of the rate of  
65 microbial decomposition of soil organic matter (SOM) under a changing climate  
66 (Saenger et al., 2013; Sjögersten et al., 2011). Climate control on the rate of SOM  
67 decomposition can be evaluated by considering soils as a “black box”, and  
68 monitoring soil C outputs such as soil respiration under various climatic  
69 conditions (Bahn et al., 2008; Bond-Lamberty and Thomson, 2010). However, the  
70 emergence of a new generation of models of soil organic C (SOC) dynamics,  
71 integrating some principles of microbial ecology (biomass, community structure  
72 and metabolic activities of microorganisms), aims for a better understanding of  
73 the climate dependency of microbial mechanisms involved in SOM decomposition  
74 (Allison et al., 2010; Lawrence et al., 2009; McGuire and Treseder, 2010; Wang et  
75 al., 2013).

76 Soil microbial communities affect SOM decomposition by a variety of  
77 extracellular enzymes (EEs), exudated to the soil matrix; each EE being specific  
78 to a certain chemical bond. Soil EEs depolymerize SOM through several types of  
79 chemical reactions, such as hydrolysis (hydrolytic EEs) or oxidation (oxidative  
80 EEs), the latter having received relatively little attention in soil research  
81 (Sinsabaugh, 2010). Even though the regulation of SOM turnover by soil  
82 microorganisms has recently been questioned (Bradford, 2013; Kemmitt et al.,  
83 2008), SOM enzymatic depolymerisation has been hypothesised to be the rate-  
84 limiting step in SOM decomposition (Bengtson a Bengtsson, 2007; Conant et al.,

85 2011). This key soil process is tightly linked to soil microclimate (temperature  
86 and moisture), and also to EE pool size, which is mediated by the complex  
87 strategies of soil microbial communities (Allison and Treseder, 2008; Burns et al.,  
88 2013; Steinweg et al., 2013).

89 Only few studies have assessed the effect of *in situ* climate change (using *e.g.*  
90 overhead infrared heaters, open top chambers, soil altitudinal transplantation)  
91 on potential soil extracellular enzyme activities (EEA), and most of them focused  
92 on the growing season only (Burns et al., 2013). Climate change could lead to  
93 contradictory effects on microbial decomposition with decreased enzyme  
94 production (synthesis and secretion), increased enzyme-catalysed reactions, and  
95 modified enzyme stability in soils (Burns et al., 2013). Altered precipitation  
96 generally has a greater impact on soil EEA than temperature change, which is  
97 often not significant (Henry, 2013). Seasonal climate effects are generally known  
98 to be stronger on soil EEA than experimental climate manipulations (see Weedon  
99 et al., 2011 for a review), suggesting other drivers like plant nutrient demand  
100 and/or substrate quality and availability for soil EEA (German et al., 2011). On  
101 the other hand, climate warming affects also seasonal development, typically in  
102 mountain regions with a shortening of the snow period, which affects soil  
103 processes (Robroek et al., 2012). However, seasonal changes in soil EEA are not  
104 unidirectional, and depend on the studied enzyme and ecosystem type (Baldrian  
105 et al., 2013; Löffler et al., 2008; Steinweg et al., 2013). As such, it appears  
106 difficult to predict modifications in the rate of SOM enzymatic depolymerisation  
107 under projected climate change (Burns et al., 2013; Davidson and Janssens,  
108 2006). Moreover, there is a lack of knowledge on the effect of climate change on

109 the relationships between soil microbial biomass (MB), microbial community  
110 structure (MCS) and EEA, which impedes building a predictive framework  
111 (Sinsabaugh, 2010). Apart from generally observed seasonal shift in MCS and  
112 MB (Bardgett et al., 1999; Lipson and Schmidt, 2004; Pascault et al., 2010;  
113 Waldrop and Firestone, 2006), climate manipulations (mostly long term  
114 warming, i.e. >10 years) also impact on MCS (Budge et al., 2011; Rinnan et al.,  
115 2007). The link between MB and soil EEA is not clear, with a majority of studies  
116 showing no correlation as evidenced by climate induced modifications of mass-  
117 specific EEA (i.e. EEA per mg MB C; Schindlbacher et al., 2011; Steinweg et al.,  
118 2013; Waldrop and Firestone, 2006), while some enzyme-based models explicitly  
119 hypothesize that EEA is tightly linked to MB (e.g. Wang et al., 2013).

120 In this study, we used an altitudinal transplantation experiment of  
121 grassland turfs in mesocosm boxes to investigate the climate impact (both the  
122 seasonal influence and the climate-manipulation effect on the fourth year of  
123 experimentation) on microbial decomposition in subalpine grasslands of the  
124 Swiss Jura. The transplantation experiment simulated two realistic climate  
125 change scenarios, with increased air temperatures ranging between 2 °C and 4 °C  
126 and decreased precipitation ranging between 20% and 40%. These changes reflect  
127 current predictions of climate change for the 21<sup>th</sup> century in temperate mountain  
128 regions (Frei et al., 2006; Meehl et al., 2007; C2SM, 2011). Previous results from  
129 the same experimental plots have already shown (i) a significant decrease in soil  
130 respiration (Gavazov, 2013; Mills et al., 2014), and (ii) a significant increase in  
131 soil dissolved organic carbon (DOC) concentration in soil solution collected by  
132 zero tension lysimeters (Gavazov, 2013) under both climate change scenarios, and

133 (iii) a significant decrease in aboveground biomass production under the most  
134 intensive climate change scenario (+4 °C and 40% decrease in precipitation;  
135 Gavazov et al., 2014).

136 Here, we investigated three key elements related to microbial  
137 decomposition: soil microbial abundance/biomass, soil microbial C, N and P  
138 enzymatic activities and soil microbial community structure across seasons. Our  
139 main objectives were to assess (i) the seasonal dynamics of various enzyme pool  
140 sizes in mountain grassland soils, and their relationships with microbial  
141 abundance and community structure; (ii) the medium term (four years) impact of  
142 two climate change scenarios on soil microbial abundance, community structure  
143 and activities across seasons, and their links with SOC concentration in  
144 mountain grassland soils.

145

## 146 **2. Materials and methods**

147

### 148 **2.1. Design of the altitudinal soil transplantation experiment**

149

150 The experimental design of this study was based on a mesocosm turf  
151 transplantation carried out in 2009 (Gavazov et al., 2013, 2014), and focused on  
152 the response of subalpine grasslands in the Swiss Jura mountains to two  
153 intensities of climate change. This manipulation successfully simulated year-  
154 round moderate and intensive climate change scenarios expected regionally in  
155 the 21<sup>st</sup> century (A1B and A2 scenarios, respectively; Frei et al., 2006; Meehl et  
156 al., 2007). Briefly, 15 mesocosms, made of rectangular PVC boxes (60 x 80 and 35



157 cm in height), containing monoliths of undisturbed soil (30 cm depth), and their  
158 intact herbaceous vegetation typical of open grasslands (consisting mainly of  
159 graminoids with few forbs; Gavazov et al., 2014) were taken from a subalpine  
160 pasture located at 1350 m a.s.l. (Combe des Amburnex, N46°54', E6°23'). The soil  
161 type is Cambisol (IUSS Working Group WRB, 2007), and the parent material is  
162 Jurassic limestone. The 15 mesocosms were then transplanted (5 per site) in  
163 common gardens at the site of origin (control: 1350 m a.s.l.) and two lower-  
164 altitudinal sites: 1010 m a.s.l. (Saint-George, N46°52', E6°26') and 570 m a.s.l.  
165 (Arboretum d'Aubonne, N46°51', E6°37'). Climate conditions in the Swiss Jura  
166 are typical for an oceanic mountain climate, with significant amount of  
167 precipitation and large annual temperature variation. At the highest site  
168 (control; 1350m a.s.l.), the mean annual temperature is +4.5 °C and the mean  
169 annual rainfall is 1750 mm, including over 450 mm of snow. The soil  
170 transplantation simulates a climate warming with an average 2 °C and 4 °C  
171 temperature increase and 20% and 40% decrease in precipitation at the  
172 intermediate site and the lowest site, respectively (see Gavazov et al. 2013 for a  
173 more detailed description of climate data).

174

## 175 2.2. Soil microclimate monitoring

176

177 Soil temperature and volumetric water content of topsoil horizon were recorded  
178 every minute in each mesocosm, using Em50 data-loggers (Decagon Devices, Inc.,  
179 USA) and ECH2O EC-TM probes inserted at 3 cm depth. Soil temperature and  
180 moisture data used here (Nov. 2012 – Oct. 2013) are daily averages.

181

### 182 2.3. Seasonal soil sampling

183

184 Four sampling campaigns (corresponding to each season) were performed over  
185 the fourth year of the soil transplantation experiment. Sampling dates were  
186 chosen according to the main climatic drivers at the control site (1350 m a.s.l.):  
187 just before snow cover (autumn, November 23<sup>rd</sup> 2012), at maximum snow cover  
188 (winter, February 20<sup>th</sup> 2013), just after snow-melt (spring, May 27<sup>th</sup> 2013) and at  
189 the end of summer (September 2<sup>nd</sup> 2013). For MB, EEA and basic soil  
190 characteristics, five intact soil cores (5 cm diameter × 10 cm length) per site (one  
191 core per mesocosm) were taken at each sampling date, immediately placed in a  
192 cool box and transported to the lab where the cores were stored at field moisture  
193 content and 4 °C before analysis. Samples were analysed within one week after  
194 sampling for EEA and MB (see below). For PLFA analysis, 20 g of soil from 0 to  
195 10 cm were sampled at two sampling date (winter and summer) in each  
196 mesocosm, only at the highest and lowest altitudes. These soil samples were  
197 sieved to 2 mm directly in the field and stored immediately at -79 °C into carbon  
198 ice before analysis.

199

### 200 2.4. Basic soil characteristics

201

202 Basic soil properties of the intact soil cores (0-10 cm depth) were analysed using  
203 standardized methods. The gravimetric soil water content was measured by  
204 drying soil at 105 °C for 48 h according to norm NF ISO 16586 (2003). For all

205 chemical soil analyses, samples were dried at 40 °C as indicated in norm NF ISO  
206 11464 (2006). Soil pH was measured in H<sub>2</sub>O (1:5 vol:vol) according to the norm  
207 NF ISO 10390 (2005). Calcareous content was determined following the norm NF  
208 ISO 10693 (1995). Organic carbon and total nitrogen concentrations were  
209 measured by the Dumas dry combustion after decarbonation (NF ISO 10694,  
210 1995; 13878, 1995, respectively). Decarbonation was achieved by successive  
211 additions of a diluted HCl (10%) solution to soil samples.

212

### 213 2.5. Soil microbial biomass (MB)

214

215 Soil MB was assessed as microbial C, using the chloroform fumigation extraction  
216 method (Brookes et al., 1985; Vance et al., 1987) on subsamples of 10 g of sieved  
217 (2 mm) soils incubated in the dark, overnight. An extraction coefficient of 0.45  
218 was used for calculating microbial C. Soil MB measurements are available only  
219 for the winter, spring and summer sampling times.

220

### 221 2.6. Soil microbial community structure (MCS)

222

223 Soil MCS was assessed by analysing the microbial phospholipid fatty acid (PLFA)  
224 composition. PLFAs were extracted according to Bligh and Dyer (1959), and  
225 modified by Börjesson et al. (1998). Total lipids were extracted overnight from 4 g  
226 freeze-dried soil in a solvent phase of 3.0 ml 50mM phosphate buffer (pH = 7.0),  
227 3.8 ml chloroform (CHCl<sub>3</sub>), 7.6 ml methanol (MeOH), and 4 ml Bligh and Dyer  
228 (1959) reagent (CHCl<sub>3</sub>: MeOH: P-buffer; 1: 2: 0.8 (v/v/v)). Total lipids were  
229 separated into neutral lipids, glycolipids, and phospholipids by dissolving the

230 total lipid fraction using chloroform, acetone and methanol solutions, which were  
231 respectively added over Discovery<sup>®</sup> DSC-Si SPE Tubes (Sigma-Aldrich). PLFA  
232 19:0 (Larodan Malmö, Sweden) was added as internal standard to the  
233 phospholipid fraction. PLFAs were trans-esterified to fatty acid methyl esters  
234 (FAMES) using 1 ml 0.2 M methanolic-KOH (Chowdhury and Dick, 2012; Sundh  
235 et al., 1997). PLFAs were analysed on a gas chromatograph according to Steger et  
236 al. (2003).

237         The ratio of fungal to bacterial biomass (F/B) in soil was calculated from  
238 PLFA data, following the method used and described by Bardgett et al, (1999).

239

## 240 2.7. Soil extracellular enzymes activity (EEA) assays

241

242 The potential activity of eight individual extracellular enzymes (see Table 1 for a  
243 presentation of all enzymes and their abbreviations) was used to represent the  
244 degradation of the main soil biochemical compounds. Seven hydrolytic EEA were  
245 measured by fluorogenic methods using 4-MUB (4-methylumbelliferone) and 7-  
246 AMC (7-amino-4-methyl coumarin). Enzyme assays were processed in acetate  
247 buffer solution (pH = 5) which was chosen to be close to soil field pH, and for  
248 stabilizing the fluorescence intensity which is dependent on pH fluctuation  
249 (German et al., 2011). Enzyme assays were performed according to Marx et al.  
250 (2001) with small modifications. Briefly, 2.5 g of moist soil sieved at 2 mm was  
251 mixed with 40 ml of acetate buffer in 50ml sterile tubes. These tubes were placed  
252 for twenty minutes into a shaker at 250 rpm to obtain a homogenous soil  
253 solution. Then, 30  $\mu$ l of soil solution was added to a 96-well microplate with 30  $\mu$ l

254 of fluorometric substrate (300 mM, saturated concentration) and completed to  
255 250  $\mu$ l with acetate buffer solution. Enzymatic reactions were incubated in the  
256 dark for 5 hours at 28 °C, with one fluorometric measure per hour. For each  
257 sample, three methodological replicates (sample + buffer + substrate) and a  
258 quenched standard (sample + buffer + 4-MUB or 7-AMC) were used. For each  
259 substrate, a control including the 4-MUB- or 7-AMC-linked substrate and the  
260 buffer solution alone were used to check the evolution of fluorescence without  
261 enzyme degradation over the duration of assay. The fluorescence intensity was  
262 measured using a Varioskan flash spectrophotometer set to 330 and 342 nm for  
263 excitation and 450 and 440 nm for emission for the 4-MUB and the 7-AMC  
264 substrate, respectively.

265 The potential activity of phenol oxidase (POX), an oxidative EE, was  
266 measured by absorbance. The protocol described by (Floch et al., 2007) was used  
267 with small modifications. Oxidation of ABTS (2,2'-Azino-bis (3-  
268 ethylbenzothiazoline-6-sulfonic acid) diammonium salt) was determined by using  
269 the same soil solution prepared for fluorogenic enzyme assays. POX reactions  
270 were processed for 10 minutes at 37 °C in 2 ml centrifuge tubes containing 0.4 ml  
271 of soil solution, 1 ml of acetate buffer (pH = 5) and 0.1 ml of ABTS (50 mM).  
272 Blanks were measured with 0.4 ml of soil solution and 1.1 ml of acetate buffer.  
273 Additionally, a control of substrate absorbance was performed with 0.1 ml of  
274 ABTS (50 mM) and 1.4 ml of acetate buffer. Absorbance was measured at 420 nm  
275 and the extinction value was  $\epsilon_{420} = 36\ 000\ \text{M}^{-1}\text{cm}^{-1}$  (Ullrich and Nüske, 2004).

276 Finally, hydrolysis of fluorescein diacetate (FDA) was measured to  
277 estimate the overall microbial activity in soil (Green et al., 2006). FDA was

278 assayed with the same protocol as described above for hydrolytic EEA, with little  
279 adaptations. For FDA, the reaction was processed in sodium phosphate buffer  
280 (pH = 7.6) as recommended by Green et al. (2006), and the fluorescence intensity  
281 was measured at 490 nm for excitation and 523 nm for emission.

282 All enzymes activities were calculated in nanokatal (nmol of product per  
283 second) and normalized by (i) g of dry soil (EEA on a dry soil mass basis), (ii) mg  
284 of microbial biomass (mass-specific EEA, reflecting microbial strategy of enzymes  
285 production).

286

## 287 2.8. Statistical analyses

288

289 The effect of climate manipulation (altitudinal effect) on MB, F/B, EEA and basic  
290 soil characteristics was assessed at each sampling date using a one-way ANOVA  
291 followed by Tukey's HSD post hoc tests. Assumptions of normality and  
292 homoscedasticity of the residuals were verified visually using diagnostic plots  
293 and a Shapiro-Wilk test. Log or square root transformations were used when  
294 necessary to respect these assumptions.

295 The effects of sampling date and climate manipulation (altitudinal effect)  
296 and their interactions on MB, F/B, EEA and basic soil characteristics were  
297 assessed by repeated measures ANOVA. Fixed factors were sampling date and  
298 site, while mesocosm was added as a random factor. It is important to note that  
299 in this study the seasonal dynamics is evaluated with one sampling date per  
300 season, with spatial replicates instead of temporal replicates.

301 To identify MCS pattern, a principal component analysis (PCA) based on  
302 Hellinger-transformed PLFA data was performed (Legendre and Gallagher,  
303 2001). We used a permutational multivariate ANOVA (ADONIS) based on  
304 Euclidean distance to test the effect of sampling date and altitude on MCS  
305 pattern. For each sample, PLFA data were normalized by total PLFA abundance  
306 to obtain relative abundances. Sums of squares and resulting F-tests from  
307 permutations of the raw data were calculated to test for the significance of the  
308 transplantation site and the sampling date on PLFAs data. Two indices PC 1 and  
309 PC 2 corresponding to axis 1 and 2 of the PCA were extracted so as to summarize  
310 MCS data in subsequent statistical analyses. Finally, the effects of the soil  
311 microbial compartment (MB and MCS, summarized as PC 1 and PC 2 indices)  
312 and substrate availability (SOC content and C/N) on EEA (dry soil mass basis)  
313 were tested using redundancy analysis (RDA). RDA analysis was applied to the C  
314 (=BG + BX + CB), N (=NAG +NAP), P, LIP, POX enzyme pool size, using  
315 microbial parameters (MB and MCS), and soil substrate characteristics (SOC  
316 content and C/N) as explanatory variables. RDA was based on data collected in  
317 winter and summer at the control site (1350 m a.s.l.) and the lowest experimental  
318 site (570 m. a.s.l.; the most intensive climate change scenario). The significance of  
319 explanatory variables was tested by a permutation test (9999 permutations).

320 All statistical analyses were performed in R 2.14.0 (R Development Core  
321 Team 2011), using the R packages vegan (Oksanen et al., 2013), ade4 (Dray and  
322 Dufour, 2007) and NLME (Pinheiro et al., 2014).

323

### 324 **3. Results**

325

## 326 3.1. Soil microclimate

327

328 Soil temperatures during the period of investigation (Fig. 1) showed a long stable  
329 soil temperature period at 0°C in winter under snow cover and a daily maximum  
330 temperature of ca. 20 °C in summer at the control site (1350 m a.s.l.). The  
331 transplantation sites at lower elevation, in contrast, exhibited longer and warmer  
332 plant growing seasons. At the lowest experimental site (570 m a.s.l.), the snow  
333 cover melted completely several times during the winter, resulting in strong mid-  
334 winter soil temperature fluctuations. For each experimental site, soil moisture  
335 (volumetric water content) at 3 cm depth remained rather constant across the  
336 year except in summer when soil moisture decreased strongly (Fig. 1).

337 The effect of soil transplantation (climate manipulation) on soil  
338 microclimate was more proportional to altitude for soil temperature than for soil  
339 moisture (Fig. 1). From November 2012 to October 2013, averaged annual soil  
340 temperature and moisture at 3 cm depth were respectively 6.6°C and 53% at the  
341 control site (1350 m a.s.l.), 9.2°C and 56% at the intermediate experimental site  
342 (1010 m a.s.l.), and 11.2°C and 36% at the lowest experimental site (570 m a.s.l.).  
343 These values were consistent with microclimate data measured from the start of  
344 the experiment (Gavazov, 2013). The three experimental sites thus showed a  
345 clear gradient of soil temperature, with decreasing temperature related to the  
346 elevation of the experimental site. Soil moisture slightly decreased with altitude  
347 except at the intermediate site (1010 m a.s.l) where soil moisture was either  
348 similar to the highest site or to the lowest site, depending on the measured soil  
349 depth (0-3 cm or 0-10 cm, Table 2). Globally, on the four sampling dates, the soil



350 microclimate was consistent with soil temperature and moisture patterns  
351 recorded during the four corresponding seasons (Fig. 1).

352

### 353 3.2. Basic soil characteristics

354

355 Soil pH neither differed among sampling dates, nor among experimental sites  
356 (mean pH = 5.5), but in autumn when soil pH was higher at the control (1350 m  
357 a.s.l.) site (Table 2). Calcareous content was about 2 g kg<sup>-1</sup> and did not differ  
358 among experimental sites or among sampling dates (Table 2). Soil organic C  
359 concentration did not change along the year. In contrast, SOC concentration was  
360 affected by the soil transplantation. SOC concentration was decreased  
361 significantly at the intermediate experimental site (in winter, spring and  
362 summer) and at the lowest site (summer) compared to the control site after four  
363 years of soil transplantation (Table 2). Soil total N concentration showed a  
364 similar pattern (Table 2), except in autumn, where total N concentration was  
365 lower at the control and intermediate sites as compared to the lowest  
366 experimental site.

367

### 368 3.3. Soil microbial biomass (MB)

369

370 We observed significant temporal variation in soil MB, with a higher abundance  
371 of soil microorganisms in winter and spring than in summer (Table 2).

372 Interestingly, we also observed a significant transplantation effect on MB, which  
373 was stronger than the sampling date effect (Table 3). Soil MB decreased at the  
374 two lower experimental sites (570 and 1010 m a.s.l.) compared to the control site

375 (1350 m a.s.l.). These differences in soil MB due to climate manipulation were  
376 significant in spring and summer but not in winter even though the trend was  
377 similar (Tables 2 and 3).

378

### 379 3.4. Soil microbial community structure (MCS)

380

381 The PCA based on PLFA data explained 76.9% of total MCS variance on the first  
382 two axes (66.4% and 10.5%, Fig.2). PCA ordination diagram identified two  
383 distinct non-overlapping groups of soil microbial communities corresponding to  
384 the winter and the summer samplings (Fig.2). Conversely, soil transplantation  
385 had no effect on MCS, as indicated by the close location of the centroids  
386 corresponding to the control (1350 m a.s.l.) and the lowest (570 m a.s.l.)  
387 experimental sites on the ordination map (Fig. 2). This was also confirmed by the  
388 variance partitioning analysis (ADONIS), which pointed out significant effects of  
389 sampling date ( $p=0.002$ ,  $R^2=0.30$ ), but neither an effect of climate manipulation  
390 ( $p=0.33$ ) nor of the interaction between both factors ( $p=0.87$ ) on MCS.

391 Examination of the PCA loadings showed that PLFA cy19:0 contributed mainly  
392 to the first PCA axis, while PLFAs 20:5 $\omega$ 3, 18:1 $\omega$ 9 and i-16:0 contributed mainly  
393 to the second PCA axis. Soil microclimate variables (temperature and moisture)  
394 were correlated with the first PCA axis ( $r = 0.7$  and  $0.6$ , respectively,  $p < 0.05$ ).

395 We observed a significant temporal variation in F/B between winter and  
396 summer, with a higher abundance of fungi microorganisms in winter than in  
397 summer (Table 2 and Supplementary Table 1). Conversely, soil transplantation  
398 had no effect on F/B.

### 399 3.5. EEA on a dry soil mass basis

400

401 On a dry soil mass basis, overall microbial activity potential (FDA) was three  
402 times higher in winter and spring ( $1.2 \text{ nkatal.g}^{-1}$  of soil) than in summer and  
403 autumn ( $0.4 \text{ nkatal.g}^{-1}$  of soil), irrespective of site (Fig. 3).

404 Transplanting soils to lower altitudes significantly impacted FDA only in  
405 winter, with decreased values at the two lower experimental sites compared to  
406 the control site (1350 m a.s.l.; Supplementary Table S1, Fig. 3). For all EEAs, the  
407 sampling date effect was stronger than the effect of climate manipulation.

408 Enzymes involved in N (LAP, NAG) and P (PHO) cycle showed clearly higher  
409 activity in autumn (Fig. 3). The same pattern is observed for the CB enzyme.

410 Consistently with overall microbial activity (FDA), several EEA's were negatively  
411 affected by the climate manipulation, with differences in the magnitude of this  
412 negative effect depending on the sampling date (Supplementary Table S1 and  
413 Fig. 3). Interestingly, the oxidative POX enzyme was more impacted by the  
414 climate manipulation than hydrolytic enzymes, with a significant response at  
415 each sampling date (Supplementary Table S1).

416

### 417 3.6. Mass-specific EEA

418

419 Mass-specific EEA (potential EEA per mg MB C) were significantly different  
420 between sampling dates, with a systematic seasonal trend (Supplementary Table  
421 S1). Indeed, we found lower mass-specific EEA for all hydrolytic or oxidative  
422 enzymes, and for overall microbial activity in summer (Supplementary Fig. S1).

423           Climate manipulation impacted mass-specific EEA of some hydrolases in  
424 spring and summer. Conversely, no effect of soil transplantation on mass-specific  
425 EEA was observed in winter for none of the investigated enzymes. Contrary to  
426 EEA on a dry soil mass basis, mass-specific EEA associated to oxidative reaction  
427 (POX) and the N cycle (NAG+LAP) did not differ between sites for all sampling  
428 dates (Supplementary Table S1 and Fig. S1).

429

### 430 3.7. Factors influencing microbial enzymatic activity

431

432 Differences between winter and summer sampling dates were mostly observed on  
433 axis 1 of RDA, while differences between experimental sites were represented on  
434 axis 1 and also on axis 2 for winter (Fig. 4). The soil EEAs were significantly  
435 explained by soil MCS (F-value = 7.2,  $p = 0.008$  for PC 1 index; PC 2 index:  $p =$   
436 0.08) and also by soil MB (F-value = 6.29,  $p = 0.01$ ). Conversely, substrate  
437 concentration and quality (SOC content and C/N) were not related to soil enzyme  
438 pool size (F-value = 0.8,  $p = 0.41$  and F-value = 0.4,  $p = 0.61$ , respectively).

439

## 440 4. Discussion

441

442 4.1. Seasonal dynamics of enzymatic activities and their relationships with  
443 microbial biomass and structure

444

445           We found a strong effect of seasonal change on microbial enzymatic  
446 activity as assessed by standard laboratory tests of potential hydrolytic and  
447 oxidative EEA. Such EEA measurements under controlled temperature

448 conditions (27°C) provide a reliable assessment of enzyme pool size (Henry,  
449 2013). The fact that the pool size of most soil enzymes changed seasonally is  
450 consistent with most studies on intra-annual variation of microbial enzymatic  
451 activities (Baldrian et al., 2013; Löffler et al., 2008; Wallenstein et al., 2009; Yao  
452 et al., 2011). According to other works, the season corresponding to the highest  
453 soil enzyme pool size is strongly ecosystem dependent. Indeed, several studies  
454 found higher potential EEAs in the warmer seasons (Baldrian et al., 2013; Jing et  
455 al., 2013; Löffler et al., 2008), whereas others found higher EEA in colder periods  
456 (Alster et al., 2013; Bell et al., 2010). In our study, the observed seasonal pattern  
457 in FDA showed (i) an increase in the enzyme pool size during the winter and  
458 spring, when, respectively, snow cover reduces soil micro-climate  
459 variability/amplitude and when plants draw nutrients and release root exudates;  
460 (ii) a decrease in the enzyme pool size in summer under dryer conditions and in  
461 autumn. FDA was used as an indicator of overall microbial activity, as this  
462 substrate is hydrolysed by many non-specific enzymes such as esterases,  
463 proteases and lipases (Adam and Duncan, 2001). This indicator remains,  
464 however, seldom used in soil enzyme studies which generally measure specific  
465 hydrolytic enzymes (but see e.g. Cécillon et al., 2008; Stauffer et al., 2014). In  
466 mountain ecosystems, EEA peaks typically occur at the snowmelt period (Weedon  
467 et al., 2011). Bell and colleagues (2010) suggested that in winter, microbes could  
468 increase enzyme production at low temperature so as to compensate lower  
469 enzyme reaction kinetics. Moreover, during the growing season, competition  
470 between plants and microorganisms for resources is often observed (Hu et al.,  
471 2001; Jingguo and Bakken, 1997; Yao et al., 2011; Zhang et al., 2005a), which

472 could lead to increased enzyme production by soil microorganisms. These  
473 explanations seem to be confirmed in our study by the high mass-specific EEAs  
474 in winter and spring, as compared to summer, for most soil enzymes. This  
475 systematic seasonal trend for mass-specific EEA underlines a clear decoupling  
476 between enzyme pool size and the abundance of soil microbes (MB) in these  
477 mountain grassland soils. Such decoupling could be linked to the strong seasonal  
478 shift in soil MCS and the fungal to bacterial ratio, with higher fungal activity in  
479 winter. Indeed, in winter fungi could access decomposing plant residues, while in  
480 summer bacteria are more competitive due to the availability of easily degradable  
481 root exudates (Bardgett et al., 2005). This emphasizes the need to explicitly  
482 distinguish enzyme pool size from microbial biomass in enzyme-based models of  
483 SOC dynamics (Steinweg et al., 2013; Wang et al., 2013), and shows that soil MB  
484 may not be a relevant proxy for soil enzyme pool size. The decrease in enzyme  
485 pool size in drier summer conditions is consistent with a conceptual model (Henry  
486 et al., 2013), which shows hypothetical variations in EEA along a soil moisture  
487 gradient depending on soil drainage status. The decrease in EEA during summer  
488 is associated with a strong decrease in MB that might be related to a hydrological  
489 stress (i.e. summer drought). In autumn, soils showed a water content similar to  
490 that of winter and spring. Nevertheless, the FDA potential activity remained  
491 surprisingly very low in this season. Whilst this might have been linked to freeze  
492 thaw cycles, which are frequent in mountain grassland soils in autumn (with no  
493 snow cover), and can deeply affect soil microbial decomposition (e.g. Saccone et  
494 al., 2012), soil temperature measurements did not show any soil frost at 3 cm  
495 depth during this time (Fig. 1). Substrate availability and quality are known to

496 be key factors in controlling EE production (Allison., 2005, Erman and Hacon.,  
497 2011), but in this study, the RDA did not reveal any link between EEA (on a dry  
498 soil mass basis) and SOC content or C/N. However, some more specific  
499 information on the actual microbial substrate (dissolved organic matter) and its  
500 chemistry could potentially explain the contrasted enzyme pool size for C, N and  
501 P cycles observed in autumn (Fig. 3; see e.g., Park et al., 2014). Indeed, our  
502 results showed contrasting responses of the various enzyme groups to seasonal  
503 changes, with a higher activity in autumn for CB, N-acquiring, and P-acquiring  
504 enzymes, while all other EEAs had their highest intensities in winter and spring.  
505 Each season is associated with a specific soil microclimate, but also a specific  
506 plant activity and secretion of root exudates and/or soil microbial substrate  
507 quality. Their combination results in contrasted MB, MCS and EEA across  
508 seasons, as previously shown (see e.g, Pascault et al., 2010; Stevenson et al.,  
509 2014; Tan et al., 2014). Overall, our results on the seasonal dynamics of these  
510 three microbial compartments are consistent with the literature, as they also  
511 showed contrasting soil MB and MCS across seasons, which significantly  
512 explained the seasonal changes of EEAs, as revealed by the RDA (Fig. 4).

513

514 4.2. Impact of climate manipulation on microbial biomass and structure and  
515 enzymatic activities across seasons and their link with soil organic carbon  
516 dynamics

517

518 With a significant decrease under both climate change scenarios at all seasons,  
519 soil MB appeared to be the most sensitive microbial parameter to the medium

520 term experimental climate manipulation in our study. Indeed, a decrease in  
521 topsoil MB under experimental warming is often observed in climate change  
522 experiments (Rinnan et al., 2007; Vanhala et al., 2011, but see Tan et al., 2014 ;  
523 Zhang et al., 2005b).

524         Conversely, we observed no modifications of soil MCS under both climate  
525 change scenarios (at the 570 and 1010 m a.s.l. experimental sites) compared to  
526 the control site (1350 m a.s.l.; Fig. 2), with no interactions between climate  
527 manipulation and seasons. This result indicates a high resistance of microbial  
528 communities to the four years experimental climate manipulation, the only  
529 changes in MCS of soil transplants being induced by temporal variations within  
530 each transplantation site. A previous investigation of the same soil  
531 transplantation experiment has revealed a strong effect of the most intensive  
532 climate change scenario on aboveground plant biomass and diversity (Gavazov et  
533 al., 2014). Thus, our results combined with Gavazov et al. (2014), hint more so  
534 towards a dominant effect of seasonality (including both seasonal variations in  
535 climate and plant activity) on MCS than an effect of plant biomass and diversity.  
536 Nonetheless, despite the fact that PLFA is a relevant method to detect changes in  
537 soil MCS (Björk et al., 2008; Waldrop and Firestone, 2006; Zeglin et al., 2013), its  
538 relatively low resolution in profiling MCS could hide finer MCS changes due to  
539 the climate manipulation. However, according to the literature, soil microbial  
540 community structure is mostly impacted by experimental climate warming  
541 and/or decreased precipitation in longer climate manipulation experiments (>10  
542 years; Frey et al., 2008; Rinnan et al., 2007, but see Djukic et al., 2013).



543           Contrary to MCS, transplanting soils to lower altitudes had a moderate  
544 but significant effect on potential soil EEAs. Overall, enzyme pool size decreased  
545 under both climate change scenarios compared to the control site, with a  
546 diversity of responses depending on the enzyme and the season considered. These  
547 results on EEAs are consistent with soil respiration measurements performed in  
548 2011 on the same soil transplantation experiment (Gavazov, 2013; Mills et al.,  
549 2014). Interestingly, the oxidative POX enzyme was more heavily affected by the  
550 climate manipulation than the hydrolytic enzymes. This highlights the need for  
551 improving our knowledge on the specific response to climate change of oxidative  
552 enzyme reactions, which are seldom assessed in soil enzyme studies (Burns et al.,  
553 2013; Hassan et al., 2013; Sinsabaugh, 2010). Our results are thus contradictory  
554 with many studies simulating climate warming, which did not show any climate  
555 manipulation impact on soil enzyme pool size (Allison and Treseder, 2008; Henry,  
556 2013; Jing et al., 2013; Steinweg et al., 2013). However, to our knowledge, our  
557 study is the first investigation on the response of such a large panel of soil  
558 hydrolytic and oxidative enzyme pool sizes to a climate manipulation generated  
559 by a soil transplantation experiment.

560           Most mass-specific EEAs including FDA were significantly increased by  
561 climate manipulation, similarly to results from Steinweg et al. (2013), with a  
562 medium warming (+2°C). This suggests an adaptation of microbial communities,  
563 which increase their enzyme production under stressful conditions. However,  
564 Steinweg et al. (2013) showed a decline in mass-specific EEAs under intensive  
565 warming (ca. +3°C), which contradicts our results. In our study, we found a  
566 significant effect of climate manipulation on EEA (on both a dry soil mass basis

567 and a mass-specific basis), which could be explained by an adaptation of  
568 microorganisms but also to other factors controlling soil enzyme pool size. For  
569 instance, the stability of soil EE can change under climate change: warming and  
570 decrease in precipitation could lead to decreased enzyme stability in soil (Burns  
571 et al., 2013). However, our enzyme assays were not suitable to investigate  
572 enzyme stability in soils.

573

574 In this study, we found a significant decrease in SOC content in the topsoil  
575 (0-10 cm) at the intermediate (in winter, spring and summer) and at the lowest  
576 experimental site (summer) compared to the control site, whereas no differences  
577 in SOC concentration were found in the topsoil layer (0-4 cm) two years before  
578 (Mills et al., 2014). This could indicate that (i) the dynamics of SOC concentration  
579 is different between the 0-4 cm and the 0-10 cm soil layers, possibly due to roots  
580 exudates dynamics, or that (ii) the effect of climate manipulation on SOC  
581 concentration became significant only on the fourth year of the experiment.  
582 Indeed, the decrease in SOC concentration in the 0-10 cm layer reached the  
583 significance threshold during the fourth year (respectively in winter 2012 and in  
584 summer 2013 at the intermediate and the lowest experimental sites).

585 Interestingly, the observed trends for EEAs were not consistent with the  
586 trend of SOC concentration. This highlights that the actual SOC dynamics  
587 cannot be easily predicted from the assessment of a simple proxy of SOC outputs  
588 such as potential soil enzyme activity tests. Other variables may explain the  
589 trend in SOC concentration, such as a decrease in C inputs or an increase in C  
590 leaching from the mesocosms. Indeed, Gavazov et al. (2014) and Gavazov (2013)

591 respectively reported a decreased aboveground biomass production at the lowest  
592 site but similar yields at the intermediate site compared to the control site, and  
593 increased DOC concentration in soil solution collected by zero tension lysimeters.  
594 Overall, the discordance between our microbial parameters and the trend in SOC  
595 concentration would add to a controversial debate in soil biogeochemistry  
596 (Bradford, 2013; Brookes et al., 2009; Paterson, 2009), with emerging conceptual  
597 models on SOC turnover, which discusses the ability of microbial community  
598 characteristics such as MCS or EEA to be directly linked to SOC dynamics  
599 (Kemmitt et al., 2008).

600

#### 601 4.3. Conclusions

602

603 We have shown in our study on mountain subalpine grassland soils of the Swiss  
604 Jura that enzyme pool size were regulated by a series of abiotic (soil temperature  
605 and moisture) and biotic (soil MB and MCS) factors. Large seasonal variations in  
606 enzyme pool size were due to the complex interaction of soil MB, MCS, soil  
607 microclimate and also potentially to plant rhizosphere activity. Conversely, the  
608 effect of the climate manipulation induced by four years altitudinal soil  
609 transplantation on EEA was neither explained by MCS change nor by SOC  
610 concentration or quality, but rather by soil microclimate and MB only.

611 Surprisingly, the medium term loss in SOC content induced by the four years  
612 climate manipulation could not be linked to any microbial data assessed in this  
613 study. This underlines the difficulties to relate microbial information with the  
614 actual dynamics of soil carbon concentration. More research is needed on the

615 regulation of SOM enzymatic depolymerisation by microbial communities  
616 according to climate and to substrate quantity, quality and availability, which  
617 may improve enzyme-based models of SOC dynamics.

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630

631

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921 **Tables**

<b>Abbreviation</b>	<b>EC number</b>	<b>Enzyme</b>	<b>Substrate</b>	<b>Function</b>
PHO	3.1.3.1	alkaline phosphatase	4-MUB-phosphate	Phosphorus mineralization
BG	3.2.1.21	$\beta$ -glucosidases	4-MUB- $\beta$ -D-glucopyranoside	Releases glucose from cellulose
CB	3.2.1.91	cellobiohydrolase	4-MUB- $\beta$ -D-cellobioside	Releases disaccharides from cellulose
NAG	3.2.1.30	N-acetyl-glucosaminidase	4-MUB-N-acetyl- $\beta$ -D-glucosaminide	Degrades chitin
BX	3.2.1.37	xylosidase	4-MUB- $\beta$ -D-xylopyranoside	Degrades hemicellulose
LIP	3.1.1.3	lipase	4-MUB-heptanoate	Degrades lipids
FDA	3.1.1	fluorescein diacetate hydrolysis	Fluorescein diacetate	Overall microbial activity potential
POX	1.10.3.2	phenol oxidase	ABTS	Oxidation of aromatic compound (lignin)
LAP	3.4.11.1	leucine-amino-peptidase	L-Leucine-7-AMC	Degrades protein into amino acids

922

923 **Table 1: Soil extracellular enzymes assayed for potential activity with**  
924 **abbreviation and enzyme commission classification (EC), substrate and**  
925 **enzymes functions.** 7-AMC = 7-amido-4-methylcoumarin hydrochloride, 4-MUB:  
926 4-methylumbelliferyl. ABTS = 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic  
927 acid) diammonium salt.

	Sampling dates	autumn (November 23 <sup>rd</sup> 2012)			winter (February 20 <sup>th</sup> 2013)			spring (May 27 <sup>th</sup> 2013)			summer (September 2 <sup>nd</sup> 2013)		
	Units												
Altitude	m a.s.l.	570	1010	1350	570	1010	1350	570	1010	1350	570	1010	1350
Soil moisture (0-10 cm; laboratory measurements)	%	42 ± 2	43 ± 2	48 ± 1	43 ± 2	44 ± 2	50 ± 2	44 ± 1 $a$	45 ± 2 $ab$	51 ± 2 $b$	21 ± 1 $a$	26 ± 2 $a$	33 ± 1 $b$
Soil moisture (field measurements at 3 cm)	%	38 ± 2	55 ± 4	54 ± 11	47 ± 8	60 ± 4	57 ± 9	45 ± 5 $a$	70 ± 1 $b$	60 ± 8 $ab$	14 ± 2 $a$	22 ± 1 $b$	23 ± 1 $b$
Soil temperature (field measurements at 3 cm)	°C	7.7 ± 0.3 $a$	6.1 ± 0.1 $b$	2.4 ± 0.2 $c$	1.2 ± 0.4	1.2 ± 0.2	0.6 ± 0.2	14.1 ± 0.4 $a$	10.7 ± 0.2 $b$	7.2 ± 0.4 $c$	18.4 ± 0.4 $a$	16.1 ± 0.1 $b$	13.2 ± 0.2 $c$
pH [in H <sub>2</sub> O]	-	5.2 ± 0.1 $a$	5.3 ± 0.3 $a$	6.5 ± 0.3 $b$	5.7 ± 0.2	5.9 ± 0.2	5.7 ± 0	5.3 ± 0.1	5.6 ± 0.2	5.5 ± 0.1	5.4 ± 0.2	5.7 ± 0.2	5.6 ± 0.1
Calcareous content	g.kg <sup>-1</sup>	2.6 ± 0.2	2.4 ± 0.2	7.8 ± 4.1	2.6 ± 0.2	2.2 ± 0.2	2 ± 0.5	2.2 ± 0.6	1.4 ± 0.6	1.2 ± 0.5	3.6 ± 1.1	2.4 ± 0.2	1.6 ± 0.4
Organic carbon	g.kg <sup>-1</sup>	71.1 ± 5.2	61 ± 6.9	74.6 ± 6	66.5 ± 4.3 $ab$	60.7 ± 4.6 $b$	77.5 ± 2.1 $a$	68.7 ± 2.9 $ab$	56.5 ± 6.9 $b$	82.1 ± 3.8 $a$	69.8 ± 6.7 $a$	57.2 ± 5.3 $a$	81.1 ± 4.1 $b$
Total nitrogen	g.kg <sup>-1</sup>	6.7 ± 0.3 $a$	5 ± 0.5 $b$	5.2 ± 0.1 $b$	6.1 ± 0.4 $ab$	5.7 ± 0.5 $a$	7.4 ± 0.2 $b$	6.3 ± 0.2 $ab$	5.2 ± 0.6 $a$	7.5 ± 0.4 $b$	5.8 ± 0.4 $a$	5.2 ± 0.5 $a$	7.5 ± 0.4 $b$
C/N	-	10.6 ± 0.3 $a$	12.2 ± 0.6 $ab$	14.3 ± 0.8 $b$	10.8 ± 0.2	10.7 ± 0.2	10.5 ± 0.1	10.9 ± 0.3	10.8 ± 0.3	11 ± 0.2	12 ± 0.8	10.9 ± 0.2	10.8 ± 0.1

<b>Microbial biomass</b>	<b>mg C.g<sup>-1</sup>soil</b>	-	-	-	3.6 ± 0.5	4.7 ± 0.4	6.2 ± 1.4	4.3 ± 0.5 <b>a</b>	4.2 ± 0.5 <b>a</b>	6.9 ± 0.4 <b>b</b>	2.3 ± 0.4 <b>a</b>	2.6 ± 0.2 <b>a</b>	5.1 ± 0.7 <b>b</b>
<b>F/B ratio</b>	-	-	-	-	0.09 ± 0.02	-	0.08 ± 0.01	-	-	-	0.05 ± 0.01	-	0.05 ± 0.01

928

929 **Table 2: Basic soil characteristics, soil microbial biomass and F/B ratio for each sampling date and experimental**  
 930 **site.** Values represent the mean (n=5) with the associated standard error (SE). Post hoc HSD tests are only represented with  
 931 different bold letters to indicate significant differences (p<0.05).

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940 **Figure captions**

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942 **Fig.1. Daily mean soil temperature and moisture of soil mesocosms at**  
943 **each site throughout the year 2012-2013.** Control site (1350 m a.s.l.) in black  
944 line; intermediate site (1010 m a.s.l.) in dashed line; lowest site (570 m a.s.l.) in  
945 grey line. The arrows indicate the four dates of soil sampling.

946

947 **Fig.2. Principal component analysis (PCA) ordination diagram based on**  
948 **soil microbial community structure data (PLFAs).** Centroids of season and  
949 site are written framed. Triangle = 1350 m a.s.l. site, circle = 570 m a.s.l. site;  
950 filled in = winter, empty = summer.

951

952 **Fig.3. Soil extracellular enzymatic activities (dry soil mass basis) across**  
953 **seasons for each site.** Error bars represent the standard errors of the mean  
954 values (n = 5). Circle= control site (1350 m a.s.l.); triangle = intermediate site  
955 (1010 m a.s.l.); dark square = lowest site (570 m a.s.l.). Aut, Win, Spr, Sum  
956 respectively stand for autumn, winter, spring and summer. See Table 1 for  
957 abbreviations.

958

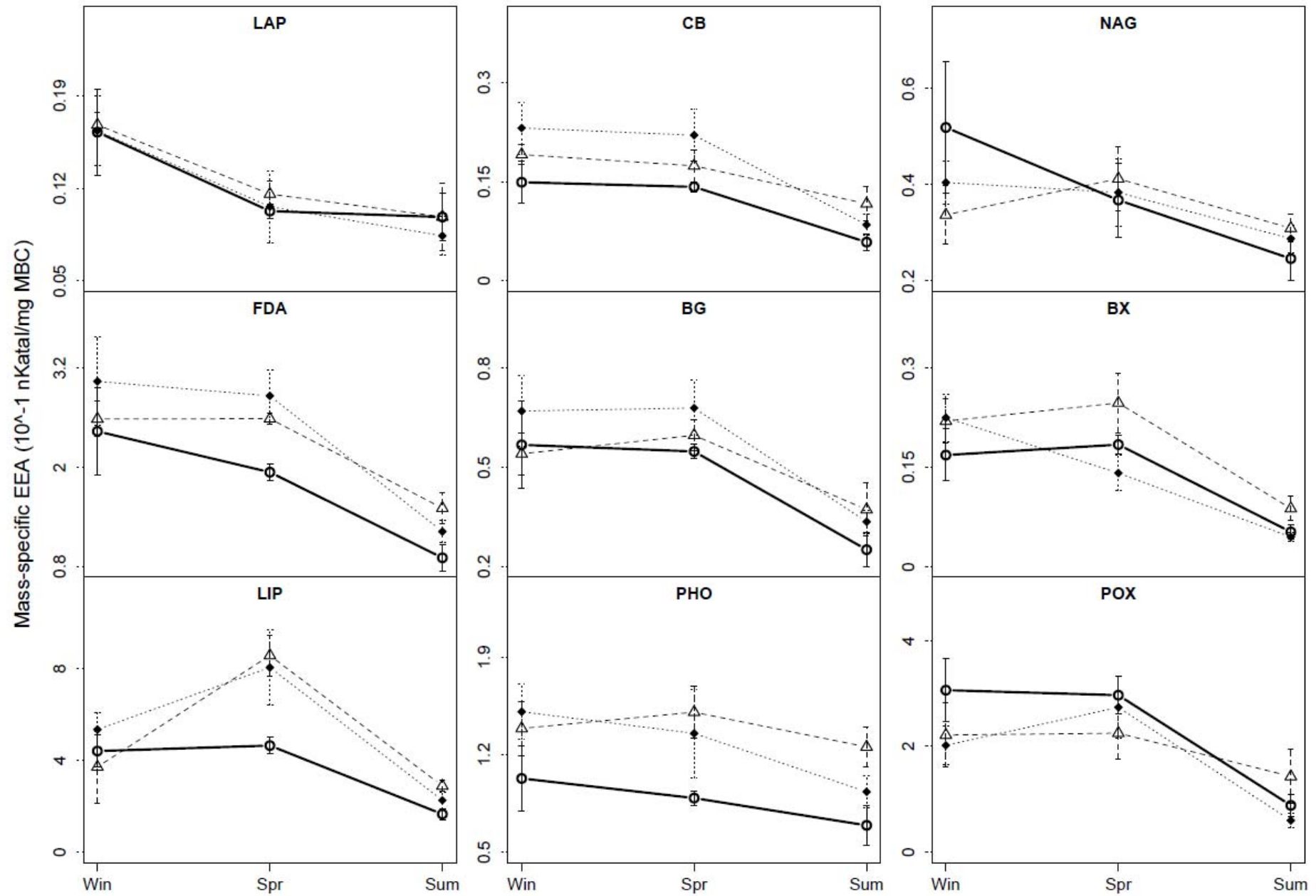
959 **Fig.4. Redundancy analysis (RDA) of EEA (dry soil mass basis) using**  
960 **microbial biomass (MB), microbial community structure (PC 1; PC 2),**  
961 **SOC and C/N as constraining variables.** Triangle = control site (1350 m  
962 a.s.l.), circle = lowest site (570 m a.s.l.); filled in = winter, empty = summer. Grey

963 arrows and black arrows represent non-significant and significant explanatory  
964 variables, respectively ( $p < 0.05$ ).

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**Fig.S1. Mass-specific soil extracellular enzymes activities across seasons for each site.** Error bars represent the standard errors of the mean values (n = 5). Circle= control site (1350 m a.s.l.); triangle = intermediate site (1010 m a.s.l.); dark square = lowest site (570 m a.s.l.). Win, Spr, Sum respectively stand for winter, spring and summer. See Table 1 for abbreviations.

	sampling date		altitude		sampling date		autumn		winter		spring		summer	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
	<b>Soil Characteristics</b>													
Soil moisture	161.0	***	11.0	**	1.1	0.4	2.7	0.1	3.6	0.1	4.5	*	23.2	***
Soil field moisture (3 cm)	120.0	***	6.3	*	2.0	0.1	3.4	0.1	1.2	0.3	9.5	**	11.1	**
Soil temperature	5033.0	***	100.0	***	69.0	***	138.0	***	1.2	0.4	91.6	***	101.1	***
pH [H2O]	1.7	0.2	4.6	*	3.8	**	7.2	**	0.6	0.6	1.5	0.3	0.8	0.5
Organic carbon	0.0	1.0	7.6	**	0.6	0.8	1.5	0.3	4.3	*	7.0	*	4.3	*
Organic nitrogen	4.1	*	6.6	*	5.7	**	4.9	*	4.2	*	6.7	*	6.2	*
C/N ratio	10.3	***	1.8	0.2	7.0	***	10.1	**	0.8	0.5	0.1	0.9	2.1	0.2
Microbial biomass (MB)	9.6	***	14.4	***	0.9	0.5	-	-	2.1	0.2	9.9	**	10.6	**
F/B ratio	12.3	**	0.3	0.6	0.0	0.9	-	-	0.3	0.6	-	-	0.2	0.7
<b>EEA</b>														
LAP	51.0	***	4.6	*	4.2	**	2.5	0.1	7.7	**	5.2	*	5.4	*
CB	192.0	***	2.1	0.2	2.2	0.1	8.0	*	0.4	0.7	1.0	0.4	4.3	*
NAG	149.0	***	7.4	**	0.9	0.5	1.7	0.2	5.7	*	2.6	0.1	4.3	*
FDA	278.0	***	1.5	0.3	2.9	*	0.2	0.8	8.3	**	2.3	0.1	3.2	0.1
BG	82.0	***	8.7	**	0.5	0.8	4.5	*	1.2	0.3	3.8	0.1	1.7	0.2
BX	90.0	***	10.6	**	3.1	*	0.1	0.9	0.9	0.4	11.7	**	8.6	**
LIP	90.0	***	1.9	0.2	1.2	0.3	5.3	*	1.4	0.3	0.7	0.5	20.9	***
PHO	169.0	***	9.6	**	1.4	0.3	2.2	0.1	1.7	0.2	1.5	0.3	9.1	**
POX	53.0	***	14.0	***	2.7	*	15.7	***	11.5	**	6.6	*	4.8	*
<b>Mass-specific EEA</b>														
LAP	11.8	***	0.2	0.8	0.1	1.0	-	-	0.1	0.9	0.2	0.9	0.1	0.9
CB	21.3	***	3.7	*	0.8	0.5	-	-	1.8	0.2	1.4	0.3	4.6	*
NAG	4.5	*	0.0	1.0	1.2	0.3	-	-	0.8	0.5	0.1	0.9	0.9	0.5
FDA	35.7	***	6.3	*	0.8	0.6	-	-	0.6	0.6	8.5	**	4.0	*
BG	17.9	***	1.4	0.3	0.4	0.8	-	-	0.5	0.6	1.2	0.3	1.1	0.4
BX	35.2	***	3.2	0.1	1.3	0.3	-	-	0.7	0.5	3.0	0.1	3.4	0.1
LIP	21.3	***	3.0	0.1	1.9	0.1	-	-	0.6	0.6	10.0	**	4.6	*
PHO	3.4	*	8.4	**	0.4	0.8	-	-	1.7	0.2	2.8	0.1	5.1	*
POX	24.8	***	1.3	0.3	1.7	0.2	-	-	1.0	0.4	1.4	0.3	2.2	0.2

**Supplementary Table 1: Left hand side: effects of sampling date, climate manipulation and interactions of both factors on basic soil characteristics, F/B, MB and EEAs (overall repeated measures ANOVA tests); right hand side: within season effect of climate manipulation on basic soil characteristics, MB and EEAs (one-way ANOVA tests). Significance codes for ANOVA's are (\*\*\*)  $p < 0.001$ ; (\*\*)  $p < 0.01$ ; (\*)  $p < 0.05$ .**

