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## Seasonality alters drivers of soil enzyme activity in subalpine grassland soil undergoing climate change

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1 **Seasonality alters drivers of soil enzyme activity in**  
2 **subalpine grassland soil undergoing climate change**

3

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31

## 32 **Abstract**

33 In mountain ecosystems with marked seasonality, climate change can affect  
34 various processes in soils, potentially modifying long-term key soil services *via*  
35 change in soil organic carbon (C) storage. Based on a four-year soil  
36 transplantation experiment in Swiss subalpine grasslands, we investigated how  
37 imposed climate warming and reduced precipitation modified the drivers of soil  
38 carbon enzyme potential activities across winter and summer seasons.  
39 Specifically, we used structural equation models (SEMs) to identify biotic  
40 (microbial community structure, abundance and activity) and abiotic (quantity  
41 and quality of organic matter resources) drivers of soil C-enzymes (hydrolase and  
42 oxidase) in two seasons under two different climate scenarios. We found  
43 contrasting impacts of the climate manipulation on the drivers of C-enzymes  
44 between winter and summer. In winter, no direct effect of climate manipulation  
45 (reduced rainfall and warming) on enzyme activity was observed. Yet, climate  
46 indirectly down-regulated enzyme activity through a decrease in the availability

47 of water extractable organic carbon (WEOC) labile resources. During summer,  
48 reduced soil moisture –induced by the climate manipulation– directly reduced  
49 soil microbial biomass, which led to a decrease in C-enzyme activity. In general,  
50 across both seasons, neither microbial community structure, nor organic matter  
51 quality were strong determinants of enzymatic activity. In particular organic  
52 matter recalcitrance (aromaticity) was not found as a general driver of either  
53 hydrolase or oxidase C-enzyme potential activities, though we did observe higher  
54 C-enzyme activities led to an increase of particulate organic matter recalcitrance  
55 in the summer season. Overall, our results highlight the seasonality of climate  
56 change effects on soil organic matter enzymatic decomposition, providing a  
57 comprehensive picture of seasonal potential cause and effect relationships  
58 governing C mineralization in subalpine grasslands.

59

60 Keywords: soil microbial communities; recalcitrance; soil organic matter  
61 fractions; structural equation models; climate manipulation; path analysis

62

63 1- Introduction

64 Soils store vast amounts of carbon (C) as soil organic matter (SOM), which  
65 equals, if not exceeds, the collective C stock in the atmosphere and vegetation  
66 (IPCC 2013). Soil microbial communities play a key role in SOM decomposition  
67 processes, annually releasing ca. 60 GtC as respired CO<sub>2</sub> into the atmosphere  
68 (IPCC 2013, Lal 2008), or roughly double the anthropogenic greenhouse gas  
69 contribution. To decompose SOM, soil microorganisms release soil extracellular  
70 enzymes, which break down SOM through hydrolytic or oxidative processes

71 (Burns et al., 2013; Sinsabaugh, 2010). This enzymatic depolymerisation process  
72 is a crucial step as it has been hypothesized to be the rate-limiting step in SOM  
73 decomposition processes, thus controlling C storage in soil (Bengtson and  
74 Bengtsson, 2007; Conant et al. 2011). In a warmer world, kinetic theory predicts  
75 enzyme activities to increase (Davidson and Janssen 2006). In soil, however,  
76 enzyme activity rates are thought to be primarily determined by the frequency of  
77 substrate-enzyme interactions (Conant et al. 2011). The probability for enzymes  
78 to interact with substrates is controlled by a combination of biological, physical  
79 and chemical drivers (Dungait et al. 2012) which correspond mainly to (i) the  
80 quantity and turnover of the enzyme pool produced by microbial communities, (ii)  
81 the chemistry and availability/protection of OM substrates and (iii) the soil  
82 moisture and temperature conditions that define the physical conditions in which  
83 enzymes operate. However, it is difficult to understand the effects of climate  
84 change on all of these factors combined. Explicit consideration of both direct and  
85 indirect impacts of climate change on soil microorganisms and organic matter  
86 protection are required to understand complex interactions and feedbacks  
87 (Bardgett et al. 2008; Schmidt et al. 2011).

88 Mountain ecosystems cover 12.3% of all terrestrial land area and store  
89 large amounts of soil organic carbon as decomposition processes are limited by  
90 cold temperatures (Körner et al., 2011, Houghton, 2007; Wohlfahrt et al., 2008).  
91 These regions are currently experiencing strong climatic changes with alterations  
92 in temperatures, precipitation and seasonal intensity and duration (Gobiet et al.,  
93 2014). Moreover mountain areas offer an opportunity to test the impact of climate

94 change as elevation gradients represent natural climate change experiments  
95 ideally suited to predicting future climate scenarios (Körner, 2007).

96 Future climate change scenarios for the European Alps predict an increase  
97 in mean annual temperature (MAT), together with a decrease in snow cover in  
98 winter and an increase in the frequency of extreme events such as drought and  
99 heat waves in summer (C2SM. 2011; IPCC. 2013). Such changes have already  
100 been reported to strongly alter the drivers of soil potential enzyme activities  
101 (Henry. 2013). Climate change, particularly warming and drought, is expected to  
102 affect the dynamics of soil microbial communities, organic substrate availability  
103 and therefore enzyme decomposition kinetics (Allison and Vitousek 2005; Conant  
104 et al., 2011; Davidson and Janssens 2006). Although we largely understand the  
105 impact of climate on microbial communities and OM substrate availability, a key  
106 knowledge gap remains to understand how changing ecological conditions affect  
107 interactions between microbial communities and substrate availability in driving  
108 C-degrading enzyme activities. This needs addressing urgently in order to build a  
109 framework to predict the future capacity of soils to act as a C sink (Sinsabaugh,  
110 2010).

111 This study therefore aims to determine the effect of climate change on  
112 multiple interactive drivers of C-enzyme activities in winter and summer seasons  
113 in a subalpine grassland. We sought to perform an integrative analyses on  
114 previously published datasets from an altitudinal transplant experiment (moving  
115 soil turves to a lower altitude) with detailed data on soil microbial activity,  
116 abundance and structure; as well as SOM organic matter resources availability  
117 and chemistry (Puissant et al. 2015, 2017) collected after four years of imposed

118 climate change. Structural equation modelling (SEM) based on path analysis  
119 have been used to evaluate how climate change influenced the interactions  
120 between microbes and SOM protection that driven C-enzyme potential activities. The  
121 climate change manipulation led to a discontinuous and thinner snow cover in  
122 winter and a warmer and drier climate in summer seasons. The effect of the  
123 climate change manipulation on the drivers of C- enzymes potential activities  
124 were evaluated separately in winter and the summer seasons to specifically  
125 examine different seasonal drivers. Our specific objectives were to (i) evaluate  
126 how the climate change manipulation affected C-degrading enzyme potential  
127 activities (hydrolase and oxidase) due to direct effects on microbial communities  
128 as well as effects on SOM resource availability and chemistry; and (ii) to  
129 determine whether the effects were consistent across seasons (winter vs  
130 summer).

131

## 132 2- Materials and methods

### 133 2.1 Study site and experimental manipulations

134 The experiment was located in the Swiss Jura mountain range and consisted of a  
135 high-to-low elevation soil translocation. Our highest site (1350m a.s.l, Combe des  
136 Amburnex, N 46°54', E 6°23') acted as the donor site. Its long-term mean  
137 annual temperature is +4.5 °C and mean annual rainfall is 1750 mm, which  
138 includes over 450 mm of snow. Combe des Amburnex is a species rich grassland  
139 and the soil type is Cambisol (IUSS Working Group WRB, 2007) on Jurassic  
140 limestone with an organic carbon content of 77g.kg<sup>-1</sup> in average (Puissant 2015).

141 We performed a four-year climate manipulation experiment which simulated a  
142 year-round intensive climate change scenario, expected regionally within the 21<sup>st</sup>  
143 century (A2 scenario, Meehl et al. 2007) aiming an average of 4 °C (MAT, +4°C)  
144 temperature increase and 40% decrease in precipitation (MAP, -40%) (Gavazov et  
145 al. 2013). From the donor site (Combe des Amburnex), ten monoliths of  
146 undisturbed soil (30 cm depth) and its vegetation were placed in rectangular PVC  
147 boxes (60 x 80 and 35 cm in height), further referred to as mesocosms. Five  
148 mesocosms were placed back in their home site, i.e. at the same altitude (control,  
149 1350 m a.s.l.), whilst the remaining five mesocosms were brought to a lower-  
150 altitudinal site (570 m a.s.l., Arboretum d'Aubonne, N46°51', E6°37') to  
151 simulate the envisaged climate scenario. All mesocosms were placed in pre-dug  
152 pits.

153 In the winter and summer season of the fourth year of the  
154 transplantation experiment, five intact soil cores (5 cm diameter × 10 cm length),  
155 i.e. one core per replicate mesocosm, were taken, placed in a cool box, and  
156 transported to the lab before analysis.

157

## 158 2.2 Soil microclimate

159 Soil temperature within the topsoil horizon were recorded every minute in each  
160 mesocosm, using Em50 data-loggers (Decagon Devices, Inc., USA) coupled to  
161 ECH2O EC-TM probes inserted at 3 cm depth. The gravimetric soil water content  
162 was measured by drying soil at 105 °C for 48 h according to norm NF ISO 16586  
163 (2003). Winter sampling (February 20th 2013) corresponded to the maximum  
164 snow cover at the control high elevation site, whereas at the low elevation site



165 (570 m a.s.l.), the snow cover had melted completely several times during the  
166 winter, resulting in strong mid-winter soil temperature fluctuations. The daily  
167 average soil temperature at 3 cm depth within the mesocosms was 0.6 and 1.2 °C  
168 and the gravimetric soil moisture content 50 % and 43 % at the high and low  
169 elevation sites, respectively (Puissant et al, 2015). Summer sampling (September  
170 2<sup>nd</sup> 2013) corresponded to a dry period at the end of summer with an average soil  
171 temperature at 3 cm depth of 13.2 and 18.4 °C and gravimetric soil moisture of 33  
172 % and 21 % at the high and low elevation sites, respectively. Overall, our climate  
173 manipulation increased the mean annual soil temperature by 4 °C (November  
174 2012 to October 2013).

175

### 176 2.3 Soil analysis

177 For all chemical soil analyses, samples were dried at 40 °C as indicated in norm  
178 NF ISO 11464 (2006). In order to identify the effect of climate change on the  
179 drivers of potential C-enzymes activities with a structural equation modelling  
180 (SEM) approach, we used published data on the effect of the climate  
181 manipulation on (i) soil microbial activity, abundance and structure (Puissant et  
182 al, 2015) and on (ii) SOM organic matter resources availability and chemistry  
183 (Puissant et al, 2017). Data used to perform SEMs are summarized in Table 1.  
184 Details on each method performed to obtain all the variables used for SEM models  
185 can be found in Supplementary material.

186

### 187 2.4 Structural Equation Modelling (SEM)

188 We organized the dataset into a path-relation network subjected to structural  
189 equation modeling (Fig.1) so as to identify the main seasonal drivers of SOM  
190 enzymatic decomposition in subalpine grasslands that were modified by climate  
191 change (see e.g. Grace et al., 2014).

192 Following current concepts of the SOM enzymatic decomposition processes, we  
193 proposed an *a priori* SEM model of hypothesized relationships within a path  
194 diagram allowing a causal interpretation of SEM outputs (Grace et al. 2012).

195 We chose soil moisture as an exogenous continuous variable in the SEM analyses  
196 in order to reflect within and between treatment natural variability. Soil  
197 moisture can be considered an integrated proxy to climate change as it reflects  
198 ambient air temperature, precipitation and evapotranspiration (Seneviratne et  
199 al., 2006). Indeed, soil gravimetric moisture and soil temperature were strongly  
200 correlated (Pearson  $R^2= 0.94$  and  $p\text{-value}<0.001$ ) within the mesocosm turves.

201 The variance in soil gravimetric moisture was largely explained by our climate  
202 change manipulation ( $R^2=0.53^*$  and  $R^2=0.59^{**}$ ; linear model for winter and  
203 summer season respectively) confirming that this variable integrates the effect of  
204 the climate change manipulation. Moreover, previous investigations of the same  
205 soil transplantation experiment revealed the prevailing soil moisture vs  
206 temperature controls on soil C turnover (Mills et al 2014) and (Gavazov et al  
207 2014). C-enzymes potential activities were split into hydrolase enzymes (mean of  
208  $\beta$ -glucosidases, cellobiohydrolase, xylosidase, lipase) and oxidase enzyme (phenol  
209 oxidase) (Table 1). Oxidases are less stable in the environment than extracellular  
210 hydrolase enzymes and could also respond differently to climate change  
211 (Singsabaugh 2010). Potential drivers of C-enzymes activity were divided into

212 “decomposer variables” (abundance and composition of microbial communities)  
213 and “resource variables” including (i) the abundance of water extractable organic  
214 carbon fraction (WEOC) and of free and intra-aggregate particulate organic  
215 matter (freePOM and occPOM), and (ii) the chemical composition of SOM  
216 fractions estimated by several spectroscopic indices (infrared spectroscopic  
217 indices for POM fractions and an ultraviolet spectroscopic index for the WEOC  
218 fraction, see Fig.1 and Table 1).

219

## 220 2.5 SEM building

221 To understand whether the effects of our climate change manipulation on the  
222 drivers of SOM enzyme decomposition diverged between winter and summer,  
223 SEMs were performed separately for the two seasons. For each season, two  
224 individual SEM path analysis models were built: (i) an ‘abundance SEM’ model  
225 based on the abundance of microbial decomposers and SOM resources; (ii) a  
226 ‘compositional SEM’ model based on the PLFA-derived structure of microbial  
227 decomposers community and the chemistry of SOM resources (Fig. 1). PLFA data  
228 were summarized using the two axis of the principal component analysis  
229 (Puissant et al., 2015; Supplementary material). From the conceptual metamodel  
230 and initial SEMs (Fig.1, Fig.2, Fig.3) we identified the key pathways and C-  
231 enzyme drivers by model simplification using step-wise exclusion of variables  
232 with non-significant regression weights and covariances (Milcu et al., 2013).  
233 Significant SEMs but with weaker model fit are presented in supplementary  
234 material. All SEM analyses were conducted using the *sem* R package (Fox 2006).  
235 Adequate model fit was identified by non-significant chi-square tests ( $P \geq 0.05$ ),

236 low Akaike Information Criterion (AIC), low Root Mean Square Error of  
237 Approximation index ( $RMSEA \leq 0.1$ ), low Standardized Root Mean Square  
238 Residual index ( $SRMR \leq 0.1$ ), and high Comparative Fit Index ( $CFI \geq 0.90$ )  
239 (Grace et al. 2014). Due to non-satisfying fit indices, no compositional SEM was  
240 retained for the winter season.

241

### 242 3- Results

#### 243 3.1 Climate change impact on C-enzymatic drivers in winter season

244 In winter, abundance SEM path analysis showed that decreased soil moisture  
245 content led to a reduction in the amount of water extractable available carbon  
246 (WEOC). The activity of both hydrolase and oxidase enzymes were significantly  
247 affected by the amount of WEOC available (Fig 4.A). The amount of POM  
248 fractions was not a significant driver of C-related enzyme potential activities.  
249 Interestingly soil moisture did not predict directly the amount of microbial  
250 biomass, but higher C-hydrolase activity led to an increase in microbial biomass.

251 Overall, in winter, the abundance SEM (Fig 4.A) showed that lower moisture  
252 content was associated with lower enzyme potential activities and microbial  
253 biomass when the amount of directly available carbon decreased (WEOC). In  
254 winter the climate change manipulation led to a decrease of soil moisture at the  
255 lower elevationsite with -21 % moisture content decreased compare to the control  
256 site (Table 2).

257 The SEM based on compositional data (Fig 4.C) failed to converge, which means  
258 that a stable solution has not been reached. Neither the chemistry of SOM  
259 resources (WEOC and POM fractions), nor the structure of microbial community

260 (PLFAs principal component axis) were sufficient to explain the changes in C-  
261 enzyme potential activities linked to the climate change manipulation.

262

### 263 3.2 Climate change impact on C-enzymatic drivers in summer season

264 In summer we observed a direct effect of climate condition (soil moisture) on the  
265 microbial community. Indeed, the abundance SEM (Fig 4.B) showed that soil  
266 moisture regulated the abundance of soil microbial biomass. Reduced soil  
267 moisture content under climate change conditions (-i.e., at lower elevation, -38%  
268 moisture content, Fig 4. B and Table 2.) led to a decrease in soil microbial  
269 biomass. The strong positive relationship between soil moisture and microbial  
270 biomass was significantly and explained 0.67 of the variance in microbial  
271 biomass (Fig 4.B). Soil microbial biomass was in turn positively controlled by  
272 both hydrolase and phenol oxidase enzymes potential activities. Conversely to  
273 the winter season we did not observe any effect of SOM resource abundance on C-  
274 enzyme activities. Nonetheless, an effect of C-enzyme potential activities was  
275 observed on the abundance of the freePOM fraction. Higher C-hydrolase potential  
276 activities led to a decrease in the quantity of the freePOM fraction (path  
277 coefficient:-0.62\*\*). The summer compositional SEM (Fig 4.D) showed as in the  
278 winter season that SOM resource lability failed to explain C-related enzymes  
279 potential activities. However, higher C-hydrolase potential activities were linked  
280 to higher soil moisture content (Fig 4.D) and were responsible for an increase of  
281 POM aromaticity (path coefficient: 0.67\*\*).

282

283 4- Discussion

284 Climate manipulation (annually reduced precipitation and increased  
285 temperature) significantly reduced soil C-enzyme potential activities and the  
286 drivers of those changes were found to be strongly seasonally dependent. Two  
287 clearly distinct pathways of C-enzyme drivers were found between the winter and  
288 summer seasons. In winter, soil moisture, as affected by climate change  
289 manipulation, impacted C-enzyme potential activities indirectly through  
290 controlling the resource availability (WEOC). In contrast, in summer soil  
291 moisture, as affected by climate change manipulation, directly decreased soil  
292 microbial biomass and then led to reduced C-enzyme potential activities. These  
293 findings shed light on the importance of considering seasonality to better  
294 understand the effect of climate change on C-enzymes potential activities and  
295 thus on soil ecosystem processes.

296 In winter, the climate change manipulation reduced snow cover and led to a  
297 discontinuous snow cover over the winter period with an overall decrease in soil  
298 moisture (Table.2; Puissant et al, 2015). Based on our abundance winter SEM  
299 (Fig 4.A), we showed that the consequences of such changes did not directly  
300 impact the soil microbial biomass but reduced the amount of organic substrate  
301 available, leading to a diminution of C-enzyme potential activities. The reduced  
302 hydrolase C-enzyme potential activities under the climate manipulation were  
303 strongly linked to the reduction of the microbial biomass. Several studies have  
304 reported that soil microbial communities often reached maximal biomass under  
305 snow cover (Schadt et al. 2003; Lipson and Schmidt 2004; Gavazov et al., 2017)  
306 underlying the crucial role of snow cover in regulating soil microbial abundances.  
307 Thermal insulation, soil moisture and organic carbon and nutrient availability

308 have been hypothesized to explain favorable microbial growth conditions under  
309 snow cover (Edwards et al, 2007). However, to our knowledge, no studies evaluate  
310 the direct and indirect pathways which might explain changes in C-enzyme  
311 potential activities and microbial biomass under reduced snow cover. The  
312 statistical approach (SEM) chosen in this study disentangled the direct and  
313 indirect effect of climate change manipulation and shed light on the importance  
314 role of snow cover for preserving substrate availability (WEOC fraction) for  
315 microbial growth. It has been reported that melting of the snowpack coupled with  
316 hydrological activity can lead to important losses of nutrient and substrate from  
317 the soil system (Edwards et al, 2007). Consistent with our study, Gavazov et al  
318 2017 found that snow removal decreased SOM mineralization and microbial  
319 biomass. In winter, in the subalpine grassland studied, water is not limiting for  
320 C-activities and so under these conditions resource availability appeared to limit  
321 SOM enzymatic activity (Brooks et al., 2005; Harrysson Drotz et al., 2009; Öquist  
322 and Laudon, 2008). Such relationships between microbial activity and abundance  
323 and WEOC/DOC content have been reported earlier (Marschner and Kalbitz,  
324 2003; Rees and Parker, 2005), but surprisingly the WEOC degree of aromaticity  
325 normally used as a proxy of WEOC biodegradability (Marschner and Kalbitz  
326 2003) was not found as a driver of soil enzyme activity under the climate change  
327 manipulation. The increase in dissolved organic matter leaching observed  
328 previously in the same experiment (9.9 mg C L<sup>-1</sup> under climate change  
329 manipulation relative to the control site; Gavazov 2013) confirms the potential  
330 losses of directly available substrate in winter due to climate change and leading  
331 to lower C-enzyme potential activities.

332 Contrastingly, in summer WEOC content was not related to C-enzyme potential  
333 activities. Instead, the reduction in soil moisture directly impacted microbial  
334 biomass and led to a strong decrease in both hydrolase and oxidase C-enzyme  
335 potential activities. The strong gravimetric soil moisture decrease due the climate  
336 change manipulation in the summer season (from 34% at the control site to 21%  
337 under the climate change condition; delta -38%, Fig 4.B) might have led to a huge  
338 water stress for the microbial communities with dehydration and diffusion  
339 limiting biological activity (Manzoni et al, 2012). The fact that no organic matter  
340 fractions as proxies of resources were found as a driver of C-enzyme potential  
341 activities confirms the direct effect of water stress on biological activities under  
342 climate change in summer. Moreover, as in winter, a lower aromaticity of soil  
343 organic matter fractions did not promote C-enzyme potential activities. Instead,  
344 we found that freePOM recalcitrance increased with higher C-enzyme potential  
345 activities (path relation 0.69 Fig 4.D) due to the fact that fresh plant material  
346 with less aromaticity chemistry was not yet decomposed under water stress  
347 condition in the summer under climate change (Gavazov et al., 2014). The  
348 accumulation of freePOM due to lower enzyme potential activities (Fig 4.B) adds  
349 further support for fresh plant material accumulation.

350 Interestingly, microbial community composition had no effect on SOM enzymatic  
351 composition, as reported by Schnecker et al (2014). However, the representation  
352 of microbial community structure with PLFA data summarized using the two  
353 axis of the principal component analysis (Puissant et al 2015) may not provide  
354 enough taxonomic resolution to correctly detect changes in microbial taxa which  
355 could influence soil enzyme potential activities under climate change conditions.



356 Additionally, another factor may be that accelerated microbial processes rates  
357 and community shifts are likely to happen after a rain event within hotspots over  
358 short periods of time (Kuzyakov and al, 2015), particularly in summer when the  
359 system is under water stress. In this study, the one-time point sampling does not  
360 allow consideration of such events, possibly obscuring underlying interactions  
361 between microbial community structure and substrate chemistry.

362

### 363 **Conclusion**

364 Overall, our results clearly demonstrate two distinct effects of a climate change  
365 manipulation (reduced precipitation and temperature increase) in winter and  
366 summer seasons in subalpine grassland. Soil moisture change induced by the  
367 climate change manipulation decreased C-enzyme activities by reducing  
368 substrate availability (WEOC) in winter and by decreasing microbial biomass  
369 under water stress condition in summer. Our results provide a comprehensive  
370 picture of potential seasonal cause and effect relationships governing C  
371 mineralization in subalpine grasslands exposed to a natural climate change  
372 scenario. This knowledge will allow better understanding of future changes in  
373 soil processes under climate change in subalpine ecosystems, and permit better  
374 predictions of the likely future impact on soil ecosystem services.

375

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Measurements	Type of SEM	Variable name used in SEMs	Units	Annual average and standard error	Ecological function	Description	Sources methods
<b>Climate conditions</b>							
Soil moisture	Abundance & Compositional	Soil moisture	%	31 ± 16	Climate change manipulation proxy	Gravimetric soil water content	NF ISO 16586 (2003)
<b>Soil enzymes activities</b>							
Cellobiohydrolase; β-glucosidases; xylosidase; lipase	Abundance & Compositional	C-Hydrolase	nmol of product per second per g of dry soil	4.86 ± 0.9	Enzymes activity of C-substrate OM	Fluorogenic methods using 4-MUB Oxidation of ABTS for phenol oxidase	According to Marx et al. (2001) with small modifications Floch et al., 2007)
Phenol oxidase		C-Oxidase		0.65 ± 0.57			
<b>Microbial population characteristics</b>							
Microbial Biomass	Abundance	MB	mg C/gsoil	3.98 ± 1.8	Abundance of decomposer community	Chloroform fumigation extraction	Brookes et al., 1985; Vance et al., 1987
PLFA	Compositional	MCS1 and MCS2	-	-0.8 ± 1.1 and 0.4 ± 5	Proxy for the structure of decomposer community	Two first axis of a PCA on microbial phospholipid fatty acid data (Puissant et al, 2015)	According to Bligh and Dyer (1959) and modified by Börjesson et al. (1998)
<b>SOM resources quantity (physical fractions)</b>							
Water Extractable Organic Carbon (WEOC)	Abundance	WEOC	mg C /g of dry soil	0.12 ± 0.04	Substrate already available for decomposer	Water extraction filtered at 0,45µm	Zsolnay et al (2003) with small modifications
free Particulate Organic Matter (freePOM)		freePOM	g C /kg of dry soil	6.8 ± 5.4	Labile pool of OM	Density fractionation (1,6 g.cm-3)	Leifeld et al. (2005, 2009) and Zimmerman et al. (2009)
Occluded Particulate Organic Matter (occPOM)		occPOM		6.95 ± 2.1	Labile pool of OM but protected by soil macro-aggregates	Density fractionation and macro-aggregates disruption with ultrasonication (22 J.mL <sup>-1</sup> )	
<b>SOM resources quality</b>							

WEOC chemistry	UV280	Relative absorbance	0.08 ± 0.04	WEOC Aromaticity estimating its biodegradability	Ultraviolet (UV) spectroscopy at 280 nm	Kalbitz et al., 2003
POM chemical IR index		Absorbance			Mid-infrared (MIR) spectroscopy	
	Compositional				spectral region corresponding to aromatic C=C bonds	Pengerud et al (2013) and Robroek et al. (2015)
Aromaticity index	POM aromaticity		6.3 x10 <sup>-3</sup> ± 1.4 x10 <sup>-3</sup>	POM Chemistry estimating its biodegradability	1,576–1,618 cm <sup>-1</sup>	

**Table.1: Variables used for performing Structural Equation Models (SEMs). These data are derived from two previous studies on the same experiment focus on either, (i) microbial abundance, structure and activity (Puissant and al, 2015) or, (ii) soil organic carbon pools contribution and chemistry (Puissant et al, 2017). 1→MUB: 4-methylumbelliferone; 2→ABTS: 2.2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt.**

	Summer				Winter			
	p-value	f-value	% difference from control	effect size	p-value	f-value	% difference from control	effect size
<b>Microbial biomass</b>	**	0.6	-54.4	-1.8	ns	0.0	-27.3	-0.6
<b>WEOC</b>	ns	-0.1	0.3	0.0	.	0.3	-34.6	-1.0
<b>C-enzymes hydrolase</b>	***	0.9	-39.3	-6.7	.	0.2	-15.2	-1.3
<b>WEOC aromaticity</b>	ns	0.1	33.9	0.9	ns	0.0	-20.3	-0.6
<b>C-enzyme oxidase</b>	**	0.6	-62.0	-3.4	**	0.8	-59.4	-2.5
<b>freePOM</b>	*	0.5	66.4	2.7	ns	0.3	64.9	1.8
<b>occPOM</b>	ns	-0.1	-3.3	-0.2	ns	0.0	-11.8	-0.6
<b>Soil moisture</b>	**	0.6	-37.7	-3.0	*	0.5	-21.5	-1.9
<b>Soil temperature</b>	***	0.9	39.2	14.3	ns	0.4	122.3	3.7
<b>PLFA MCS1</b>	ns	-0.1	85.3	-0.3	ns	-0.1	7.6	1.2
<b>PLFA MCS2</b>	ns	0.2	-1533.3	1.2	ns	-0.1	-109.6	0.5
<b>POM aromaticity</b>	ns	0.2	-16.3	-1.7	ns	-0.2	-0.5	0.0

**Table.2: Effect of soil transplantation experiment on the main variable used to build SEMs.** The percentage of change from the control site represents for a given variable, the difference between value at the lowest site (570m, Arboretum) corresponding to the climate change

scenario simulated versus value at the control site (1350, Marchairuz) expressed as a percent of the control site value. Effect size value is the difference between value at the lowest site (570m, Arboretum) versus value at the control site (1350, Marchairuz) divided by the standard deviation at the control site. Asterisk symbols indicate significant differences (One-way anova) between winter and summer season at each site (· for  $p < 0.10$ , \* for  $p < 0.05$ , \*\* for  $p < 0.01$ ; \*\*\* for  $p < 0.001$ ).

## Figure captions

**Fig 1. Scheme of the conceptual and hypothetical path-relation network used to perform SEMs.** Green arrows indicate paths involving change in soil organic matter resource quality or quantity. Grey arrows indicate paths involving change of soil microbial community abundance or structure. Double headed arrow indicate that the causal path has been tested in the two direction in two separated different SEM. Abundance SEM and compositional SEM models are the two main kind of SEM performed based on quantity data or quality data. Details of the variables used are given in the Table 1.

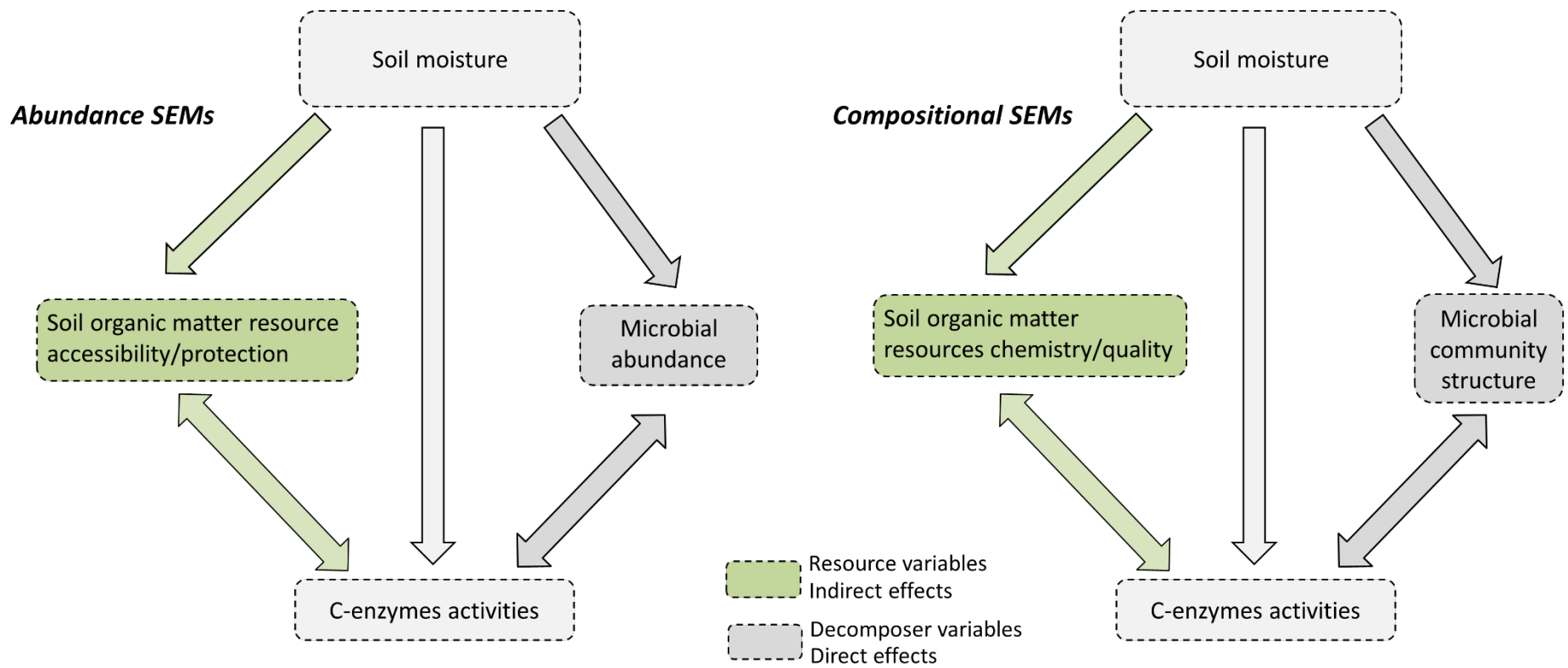
**Fig 2. Abundance initial SEMs showing the different path-relation network used to perform SEMs. Numbers in circle indicate the hypothesis made behind each causal links and presented in the table under SEM figures.** Green arrows indicate paths involving change in soil organic matter resource quantity. Grey arrows indicate paths involving change of soil microbial community abundance.

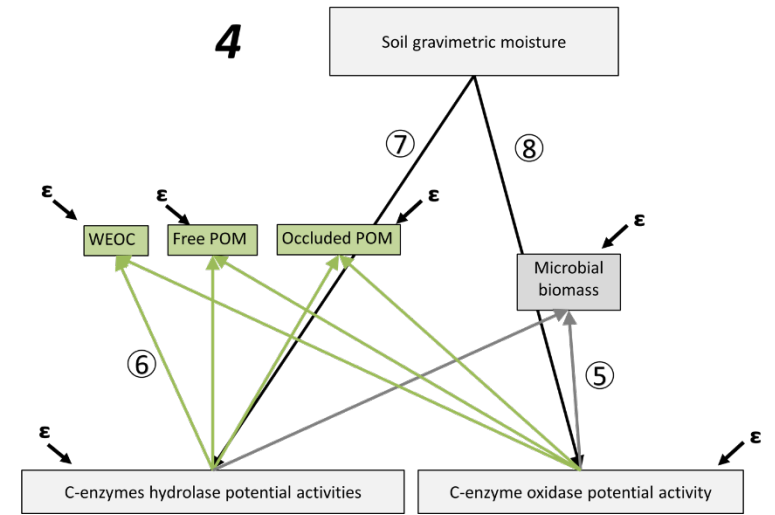
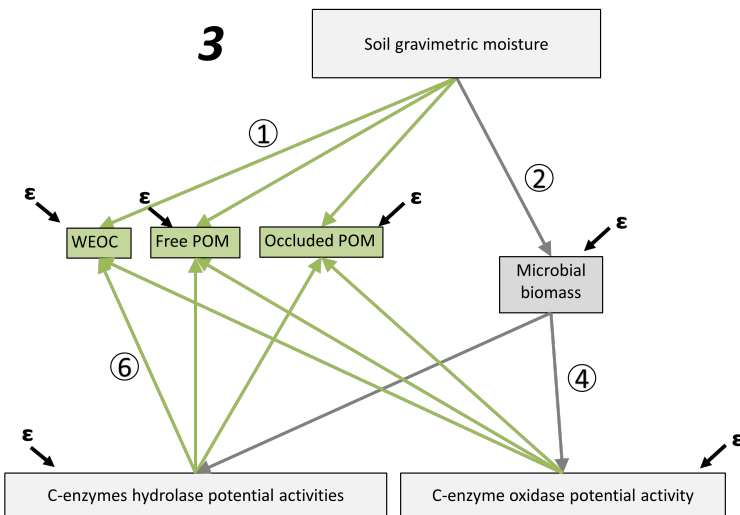
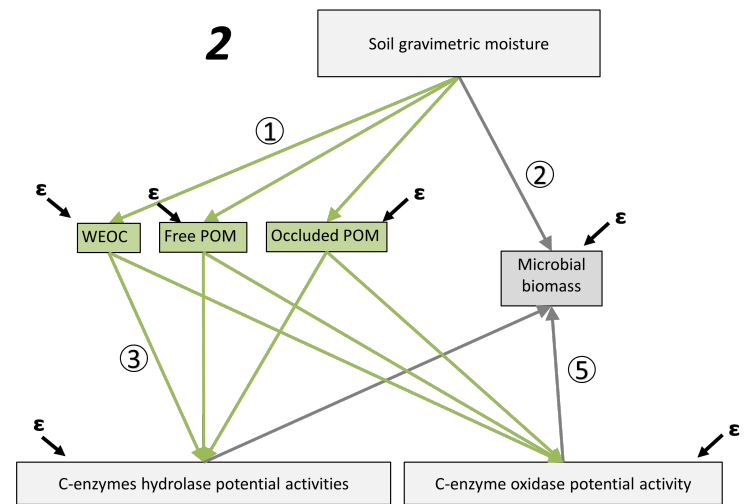
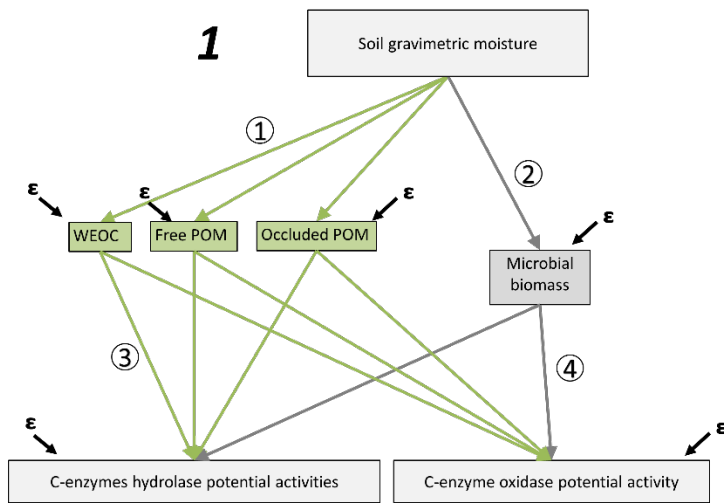
**Fig 3. Compositional initial SEMs showing the different path-relation network used to perform SEMs. Numbers in circle indicate the hypothesis made behind each causal links and presented in the table under SEM figures.** Green arrows indicate paths involving change in soil organic matter resource quality. Grey arrows indicate paths involving change of soil microbial community structure.

**Fig 4. Seasonal SEMs representing the climate effects on the drivers of SOM enzymatic decomposition. A) Winter abundance SEM, B) Summer abundance SEM, C) Winter compositional SEM, D) summer compositional** Values in orange boxes

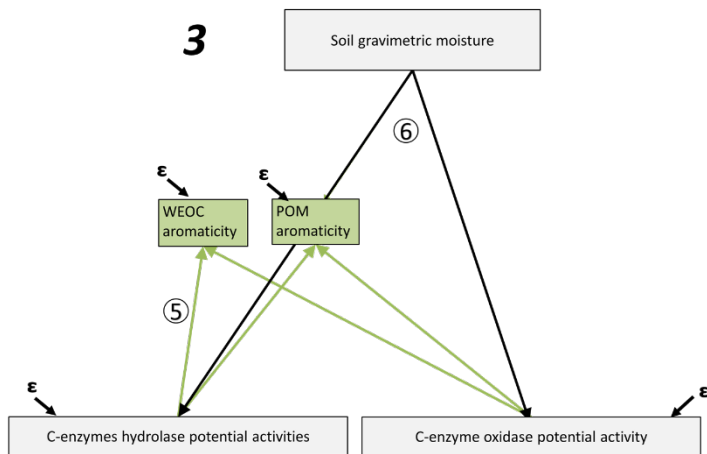
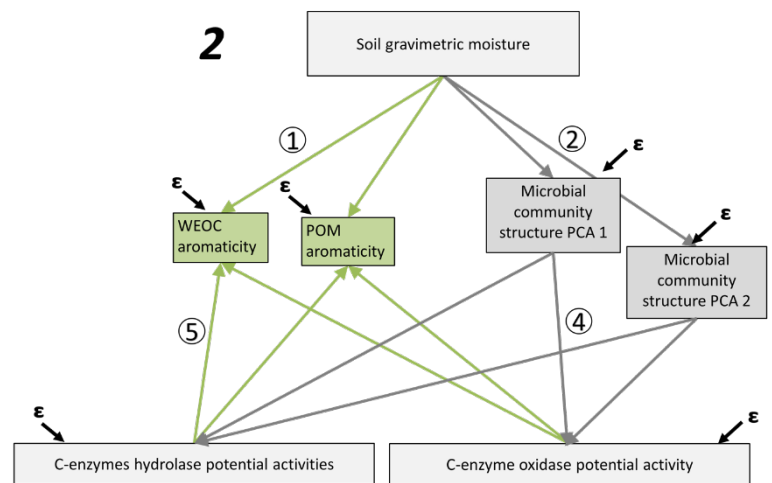
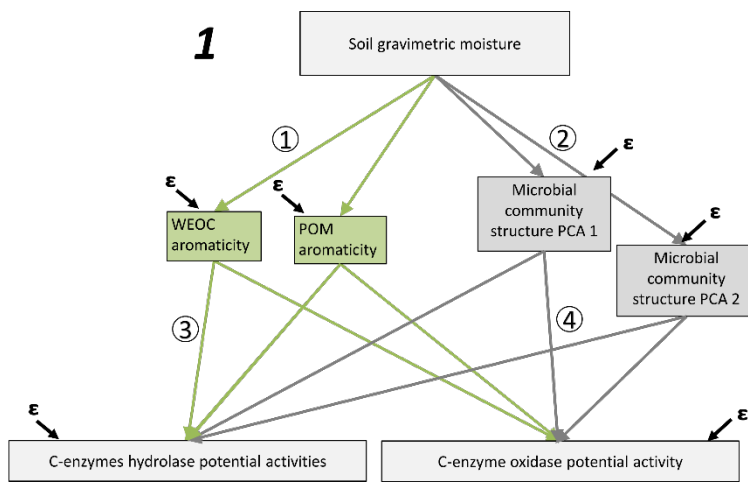
indicate delta change between control site (control, 1350 m a.s.l.) and climate manipulation site (570 m a.s.l.). All delta values are expressed as percentage and are positive or negative indicating respectively a relative increase or decrease compared to the control site. Black boxes and arrows indicate significant factors and paths. The boxes and arrows in grey were not significant and were removed from the models. The numbers beside arrows as the arrow width indicates the strength of the effect.





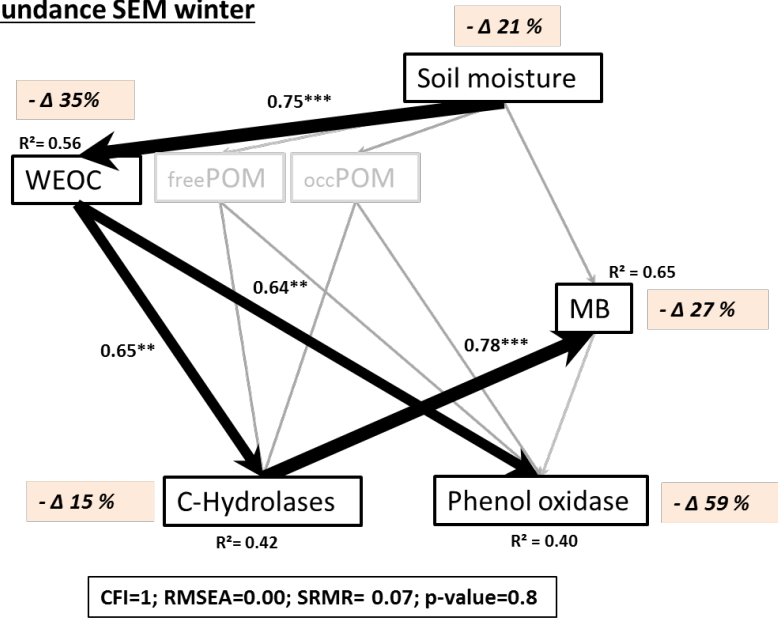


Main path	Pathway	Hypothesized mechanism
<b>Abundance SEMs</b>		
①	Soil moisture → WEOC/freePOM/occPOM	Higher soil moisture increases plant organic matter input and its availability
②	Soil moisture → Microbial biomass	Change in soil moisture affects microbial physiological constraints, and therefore biomass
③	WEOC/freePOM/occPOM → Hydrolase/oxidase activities	Positive effect of SOM resources abundance on enzyme activities
④	Microbial biomass → Hydrolase/oxidase activities	More microbial biomass lead to more enzyme production
⑤	Hydrolase/oxidase activities → Microbial biomass	Higher enzyme activities enable more biomass production
⑥	Hydrolase/oxidase activities → WEOC/freePOM/occPOM	Higher enzyme activities decrease the amount of SOM resource pools
⑦	Soil moisture → Hydrolase/oxidase activities	Soil moisture directly affects enzyme activity

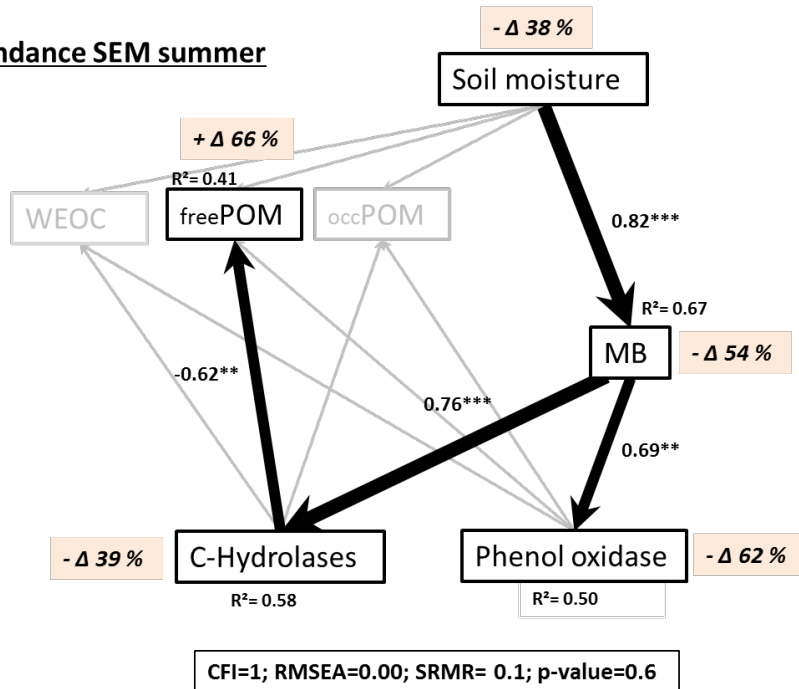


Main path	Pathway	Hypothesized mechanism
<b>Compositional SEMs</b>		
①	Soil moisture → WEOC/POM aromaticity	Higher soil moisture changes plant communities, and therefore organic matter input quality
②	Soil moisture → Microbial community structure	Change in soil moisture affects microbial physiological constraints and therefore microbial community structure
③	WEOC/POM aromaticity → Hydrolase/oxidase activities	Higher resource aromaticity leads to decreased enzyme activities
④	Microbial community structure → Hydrolase/oxidase activities	Change in microbial community leads to change in enzyme production
⑤	Hydrolase/oxidase activities → WEOC/POM aromaticity	Higher enzyme activities leads to increased SOM aromaticity due to preferential degradation of labile resources
⑥	Soil moisture → Hydrolase/oxidase activities	Soil moisture directly affects enzyme activity

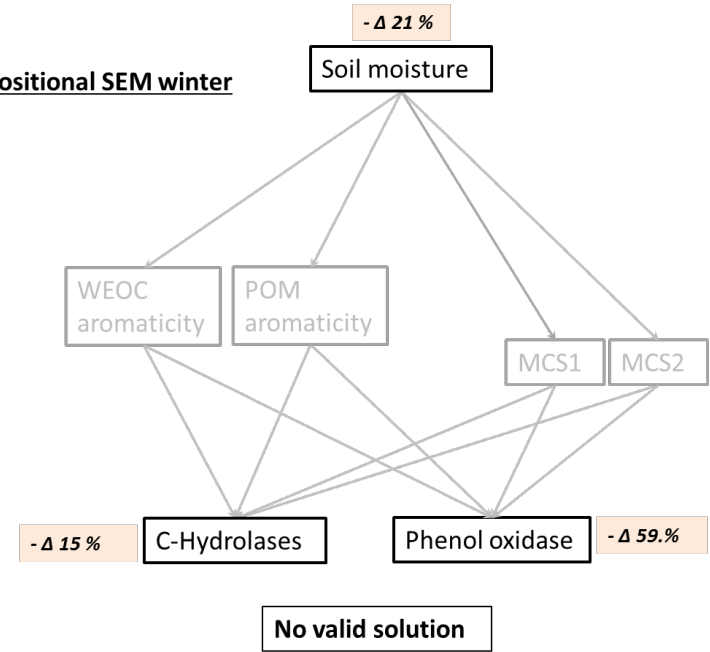
**A.**  
**Abundance SEM winter**



**B.**  
**Abundance SEM summer**



**C.**  
**Compositional SEM winter**



**D.**  
**Compositional SEM summer**

