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

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Abstract

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

47 labile resources. During summer, reduced soil moisture –induced by the climate
48 manipulation– directly reduced soil microbial biomass, which led to a decrease in
49 C-enzyme activity. In general, across both seasons, neither microbial community
50 structure, nor organic matter quality were strong determinants of enzymatic
51 activity. In particular organic matter recalcitrance (aromaticity) was not found as
52 a general driver of either hydrolase or oxidase C-enzyme potential activities,
53 though we did observe higher C-enzyme activities led to an increase of particulate
54 organic matter recalcitrance in the summer season. Overall, our results highlight
55 the seasonality of climate change effects on soil organic matter enzymatic
56 decomposition, providing a comprehensive picture of seasonal potential cause and
57 effect relationships governing C mineralization in subalpine grasslands.
58
59 Keywords: soil microbial communities; recalcitrance; soil organic matter fractions;
60 structural equation models; climate manipulation; path analysis
61
62 1- Introduction
63 Soils store vast amounts of carbon (C) as soil organic matter (SOM), which
64 equals, if not exceeds, the collective C stock in the atmosphere and vegetation
65 (IPCC 2013). Soil microbial communities play a key role in SOM decomposition
66 processes, annually releasing ca. 60 GtC as respired CO_2 into the atmosphere
67 (IPCC 2013, Lal 2008), or roughly double the anthropogenic greenhouse gas
68 contribution. To decompose SOM, soil microorganisms release soil extracellular
69 enzymes, which break down SOM through hydrolytic or oxidative processes (Burns
70 et al., 2013; Sinsabaugh, 2010). This enzymatic depolymerisation process is a

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

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

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

This study therefore aims to determine the effect of climate change on multiple interactive drivers of C-enzyme activities in winter and summer seasons in a subalpine grassland. We sought to perform an integrative analyses on previously published datasets from an altitudinal transplant experiment (moving soil turves to a lower altitude) with detailed data on soil microbial activity, abundance and structure; as well as SOM organic matter resources availability and chemistry (Puissant et al. 2015, 2017) collected after four years of imposed climate change. Structural equation modelling (SEM) based on path analysis have been used to evaluate how climate change influenced the interactions between

119 microbes and SOM protection that driven C-enzyme potential activities. The climate
120 change manipulation led to a discontinuous and thinner snow cover in winter and
121 a warmer and drier climate in summer seasons. The effect of the climate change
122 manipulation on the drivers of C- enzymes potential activities were evaluated
123 separately in winter and the summer seasons to specifically examine different
124 seasonal drivers. Our specific objectives were to (i) evaluate how the climate
125 change manipulation affected C-degrading enzyme potential activities (hydrolase
126 and oxidase) due to direct effects on microbial communities as well as effects on
127 SOM resource availability and chemistry; and (ii) to determine whether the effects
128 were consistent across seasons (winter vs summer).
129
130 2- Materials and methods
131 2.1 Study site and experimental manipulations
132 The experiment was located in the Swiss Jura mountain range and consisted of a
133 high-to-low elevation soil translocation. Our highest site (1350m a.s.l, Combe des
134 Amburnex, N 46°54′, E 6°23′) acted as the donor site. Its long-term mean annual
135 temperature is +4.5 °C and mean annual rainfall is 1750 mm, which includes over
136 450 mm of snow. Combe des Amburnex is a species rich grassland and the soil type
137 is Cambisol (IUSS Working Group WRB, 2007) on Jurassic limestone with an
138 organic carbon content of 77g.kg^{-1} in average (Puissant 2015).
139 We performed a four-year climate manipulation experiment which simulated a
140 year-round intensive climate change scenario, expected regionally within the 21st
141 century (A2 scenario, Meehl et al. 2007) aiming an average of 4 °C (MAT, +4°C)
142 temperature increase and 40% decrease in precipitation (MAP, -40%) (Gavazov et

143 al. 2013). From the donor site (Combe des Amburnex), ten monotliths of
144 undisturbed soil (30 cm depth) and its vegetation were placed in rectangular PVC
145 boxes (60 x 80 and 35 cm in height), further referred to as mesocosms. Five
146 mesocosm were placed back in their home site, i.e. at the same altitude (control,
147 1350 m a.s.l.), whilst the remaining five mesocosms were brought to a lower-
148 altitudinal site (570 m a.s.l., Arboretum d'Aubonne, N46°51′, E6°37′) to simulate
149 the envisaged climate scenario. All mesocosms were placed in pre-dug pits.
150 In the winter and summer summer season of the fourth year of the transplantation
151 experiment, five intact soil cores (5 cm diameter × 10 cm length), i.e. one core per
152 replicate mesocosm, were taken, placed in a cool box, and transported to the lab
153 before analysis.
154
155 2.2 Soil microclimate
156 Soil temperature within the topsoil horizon were recorded every minute in each
157 mesocosm, using Em50 data-loggers (Decagon Devices, Inc., USA) coupled to
158 ECH2O EC-TM probes inserted at 3 cm depth. The gravimetric soil water content
159 was measured by drying soil at 105 °C for 48 h according to norm NF ISO 16586
160 (2003). Winter sampling (February 20th 2013) corresponded to the maximum snow
161 cover at the control high elevation site, whereas at the low elevation site (570 m
162 a.s.l.), the snow cover had melted completely several times during the winter,
163 resulting in strong mid-winter soil temperature fluctuations. The daily average
164 soil temperature at 3 cm depth within the mesocosms was 0.6 and 1.2 °C and the
165 gravimetric soil moisture content 50 % and 43 % at the high and low elevation
166 sites, respectively (Puissant et al, 2015). Summer sampling (September 2nd 2013)

167 corresponded to a dry period at the end of summer with an average soil
168 temperature at 3 cm depth of 13.2 and 18.4 °C and gravimetric soil moisture of 33
169 % and 21 % at the high and low elevation sites, respectively. Overall, our climate
170 manipulation increased the mean annual soil temperature by 4 °C (November 2012
171 to October 2013).
172
173 2.3 Soil analysis
174 For all chemical soil analyses, samples were dried at 40 °C as indicated in norm
175 NF ISO 11464 (2006). In order to identify the effect of climate change on the drivers
176 of potential C-enzymes activities with a structural equation modelling (SEM)
177 approach, we used published data on the effect of the climate manipulation on (i)
178 soil microbial activity, abundance and structure (Puissant et al, 2015) and on (ii)
179 SOM organic matter resources availability and chemistry (Puissant et al, 2017).
180 Data used to perform SEMs are summarized in Table 1. Details on each method
181 perfomed to obtain all the variables used for SEM models can be found in
182 Supplementary material.
183
184 2.4 Structural Equation Modelling (SEM)
185 We organized the dataset into a path-relation network subjected to structural
186 equation modeling (Fig.1) so as to identify the main seasonal drivers of SOM
187 enzymatic decomposition in subalpine grasslands that were modified by climate
188 change (see e.g. Grace et al., 2014).

189 Following current concepts of the SOM enzymatic decomposition processes, we
190 proposed an *a priori* SEM model of hypothesized relationships within a path
191 diagram allowing a causal interpretation of SEM outputs (Grace et al. 2012).
192 We chose soil moisture as an exogenous continuous variable in the SEM analyses
193 in order to reflect within and between treatment natural variability. Soil moisture
194 can be considered an integrated proxy to climate change as it reflects ambient air
195 temperature, precipitation and evapotranspiration (Seneviratne et al., 2006).
196 Indeed, soil gravimetric moisture and soil temperature were strongly correlated
197 (Pearson R^2= 0.94 and p-value<0.001) within the mesocosm turves. The variance
198 in soil gravimetric moisture was largely explained by our climate change
199 manipulation (R^2=0.53* and R^2=0.59**; linear model for winter and summer
200 season respectively) confirming that this variable integrates the effect of the
201 climate change manipulation. Moreover, previous investigations of the same soil
202 transplantation experiment revealed the prevailing soil moisture vs temperature
203 controls on soil C turnover (Mills et al 2014) and (Gavazov et al 2014). C-enzymes
204 potential activities were split into hydrolase enzymes (mean of ß-glucosidases,
205 cellobiohydrolase, xylosidase, lipase) and oxidase enzyme (phenol oxidase) (Table
206 1). Oxidases are less stable in the environment than extracellular hydrolase
207 enzymes and could also respond differently to climate change (Singsabaugh 2010).
208 Potential drivers of C-enzymes activity were divided into “decomposer variables”
209 (abundance and composition of microbial communities) and “resource variables”
210 including (i) the abundance of water extractable organic carbon fraction (WEOC)
211 and of free and intra-aggregate particulate organic matter (freePOM and occPOM),
212 and (ii) the chemical composition of SOM fractions estimated by several

spectroscopic indices (infrared spectroscopic indices for POM fractions and an ultraviolet spectroscopic index for the WEOC fraction, see Fig.1 and Table 1).

2.5 SEM building

To understand whether the effects of our climate change manipulation on the drivers of SOM enzyme decomposition diverged between winter and summer, SEMs were performed separately for the two seasons. For each season, two individual SEM path analysis models were built: (i) an 'abundance SEM' model based on the abundance of microbial decomposers and SOM resources; (ii) a 'compositional SEM' model based on the PLFA-derived structure of microbial decomposers community and the chemistry of SOM resources (Fig. 1). PLFA data were summarized using the two axis of the principal component analysis (Puissant et al., 2015; Supplementary material). From the conceptual metamodel and initial SEMs (Fig.1, Fig.2, Fig.3) we identified the key pathways and C-enzyme drivers by model simplification using step-wise exclusion of variables with non-significant regression weights and covariances (Milcu et al., 2013). Significant SEMs but with weaker model fit are presented in supplementary material. All SEM analyses were conducted using the *sem* R package (Fox 2006). Adequate model fit was identified by non–significant chi-square tests ($P \geq 0.05$), low Akaike Information Criterion (AIC), low Root Mean Square Error of Approximation index ($RMSEA \leq 0.1$), low Standardized Root Mean Square Residual index ($SRMR \leq 0.1$), and high Comparative Fit Index ($CFI \geq 0.90$) (Grace et al. 2014). Due to non-satisfying fit indices, no compositional SEM was retained for the winter season.

3- Results

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

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

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

The SEM based on compositional data (Fig 4.C) failed to converge, which means that a stable solution has not been reached. Neither the chemistry of SOM resources (WEOC and POM fractions), nor the structure of microbial community (PLFAs principal component axis) were sufficient to explain the changes in C-enzyme potential activities linked to the climate change manipulation.

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

In summer we observed a direct effect of climate condition (soil moisture) on the microbial community. Indeed, the abundance SEM (Fig 4.B) showed that soil

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

4- Discussion

Climate manipulation (annually reduced precipitation and increased temperature) significantly reduced soil C-enzyme potential activities and the drivers of those changes were found to be strongly seasonally dependent. Two clearly distinct pathways of C-enzyme drivers were found between the winter and summer seasons. In winter, soil moisture, as affected by climate change manipulation, impacted C-enzyme potential activities indirectly through

285 controlling the resource availability (WEOC). In contrast, in summer soil moisture,
286 as affected by climate change manipulation, directly decreased soil microbial
287 biomass and then led to reduced C-enzyme potential activities. These findings shed
288 light on the importance of considering seasonality to better understand the effect
289 of climate change on C-enzymes potential activities and thus on soil ecosystem
290 processes.

291 In winter, the climate change manipulation reduced snow cover and led to a
292 discontinuous snow cover over the winter period with an overall decrease in soil
293 moisture (Table.2; Puissant et al, 2015). Based on our abundance winter SEM (Fig
294 4.A), we showed that the consequences of such changes did not directly impact the
295 soil microbial biomass but reduced the amount of organic substrate available,
296 leading to a diminution of C-enzyme potential activities. The reduced hydrolase C-
297 enzyme potential activities under the climate manipulation were strongly linked
298 to the reduction of the microbial biomass. Several studies have reported that soil
299 microbial communities often reached maximal biomass under snow cover (Schadt
300 et al. 2003; Lipson and Schmidt 2004; Gavazov et al., 2017) underlying the crucial
301 role of snow cover in regulating soil microbial abundances. Thermal insulation, soil
302 moisture and organic carbon and nutrient availability have been hypothesized to
303 explain favorable microbial growth conditions under snow cover (Edwards et al,
304 2007). However, to our knowledge, no studies evaluate the direct and indirect
305 pathways which might explain changes in C-enzyme potential activities and
306 microbial biomass under reduced snow cover. The statistical approach (SEM)
307 chosen in this study disentangled the direct and indirect effect of climate change
308 manipulation and shed light on the importance role of snow cover for preserving

309 substrate availability (WEOC fraction) for microbial growth. It has been reported
310 that melting of the snowpack coupled with hydrological activity can lead to
311 important losses of nutrient and substrate from the soil system (Edwards et al,
312 2007). Consistent with our study, Gavazov et al 2017 found that snow removal
313 decreased SOM mineralization and microbial biomass. In winter, in the subalpine
314 grassland studied, water is not limiting for C-activities and so under these
315 conditions resource availability appeared to limit SOM enzymatic activity (Brooks
316 et al., 2005; Harrysson Drotz et al., 2009; Öquist and Laudon, 2008). Such
317 relationships between microbial activity and abundance and WEOC/DOC content
318 have been reported earlier (Marschner and Kalbitz, 2003; Rees and Parker, 2005),
319 but surprisingly the WEOC degree of aromaticity normally used as a proxy of
320 WEOC biodegradability (Marschner and Kalbitz 2003) was not found as a driver
321 of soil enzyme activity under the climate change manipulation. The increase in
322 dissolved organic matter leaching observed previously in the same experiment (9.9
323 mg C L-1 under climate change manipulation relative to the control site; Gavazov
324 2013) confirms the potential losses of directly available substrate in winter due to
325 climate change and leading to lower C-enzyme potential activities.
326 Contrastingly, in summer WEOC content was not related to C-enzyme potential
327 activities. Instead, the reduction in soil moisture directly impacted microbial
328 biomass and led to a strong decrease in both hydrolase and oxidase C-enzyme
329 potential activities. The strong gravimetric soil moisture decrease due the climate
330 change manipulation in the summer season (from 34% at the control site to 21%
331 under the climate change condition; delta -38%, Fig 4.B) might have led to a huge
332 water stress for the microbial communities with dehydration and diffusion limiting

biological activity (Manzoni et al, 2012). The fact that no organic matter fractions as proxies of resources were found as a driver of C-enzyme potential activities confirms the direct effect of water stress on biological activities under climate change in summer. Moreover, as in winter, a lower aromaticity of soil organic matter fractions did not promote C-enzyme potential activities. Instead, we found that freePOM recalcitrance increased with higher C-enzyme potential activities (path relation 0.69 Fig 4.D) due to the fact that fresh plant material with less aromaticity chemistry was not yet decomposed under water stress condition in the summer under climate change (Gavazov et al., 2014). The accumulation of freePOM due to lower enzyme potential activities (Fig 4.B) adds further support for fresh plant material accumulation.

Interestingly, microbial community composition had no effect on SOM enzymatic composition, as reported by Schnecker et al (2014). However, the representation of microbial community structure with PLFA data summarized using the two axis of the principal component analysis (Puissant et al 2015) may not provide enough taxonomic resolution to correctly detect changes in microbial taxa which could influence soil enzyme potential activities under climate change conditions. Additionally, another factor may be that accelerated microbial processes rates and community shifts are likely to happen after a rain event within hotspots over short periods of time (Kuzyakov and al, 2015), particularly in summer when the system is under water stress. In this study, the one-time point sampling does not allow consideration of such events, possibly obscuring underlying interactions between microbial community structure and substrate chemistry.

Conclusion

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

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Measurements	Type of SEM	Variable name used in SEMs	Units	Annual average and standard error	Ecological function	Description	Sources methods
Climate conditions							
Soil moisture	Abundance & Compositional	Soil moisture	%	31 ± 16	Climate change manipulation proxy	Gravimetric soil water content	NF ISO 16586 (2003)
Soil enzymes activities							
Cellobiohydrolase; ß-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	According to Marx et al. (2001) with small modifications
Phenol oxidase		C-Oxidase		0.65 ± 0.57		Oxidation of ABTS for phenol oxidase	Floch et al., 2007)
Microbial population characteristics							
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)
SOM resources quantity (physical fractions)							
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 ultra-sonication (22 J.mL−1)	
SOM resources quality							

WEOC chemistry	Compositional	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	
Aromaticity index		POM aromaticity		$6.3 \times 10^{-3} \pm 1.4 \times 10^{-3}$	POM Chemistry estimating its biodegradability	spectral region corresponding to aromatic C=C bonds 1,576–1,618 cm^{-1}	Pengerud et al (2013) and Robroek et al. (2015)

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**
Microbial biomass	**	0.6	-54.4	-1.8	ns	0.0	-27.3	-0.6
WEOC	ns	-0.1	0.3	0.0	.	0.3	-34.6	-1.0
C-enzymes hydrolase	***	0.9	-39.3	-6.7	.	0.2	-15.2	-1.3
WEOC aromaticity	ns	0.1	33.9	0.9	ns	0.0	-20.3	-0.6
C-enzyme oxidase	**	0.6	-62.0	-3.4	**	0.8	-59.4	-2.5
freePOM	*	0.5	66.4	2.7	ns	0.3	64.9	1.8
occPOM	ns	-0.1	-3.3	-0.2	ns	0.0	-11.8	-0.6
Soil moisture	**	0.6	-37.7	-3.0	*	0.5	-21.5	-1.9
Soil temperature	***	0.9	39.2	14.3	ns	0.4	122.3	3.7
PLFA MCS1	ns	-0.1	85.3	-0.3	ns	-0.1	7.6	1.2
PLFA MCS2	ns	0.2	-1533.3	1.2	ns	-0.1	-109.6	0.5
POM aromaticity	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.

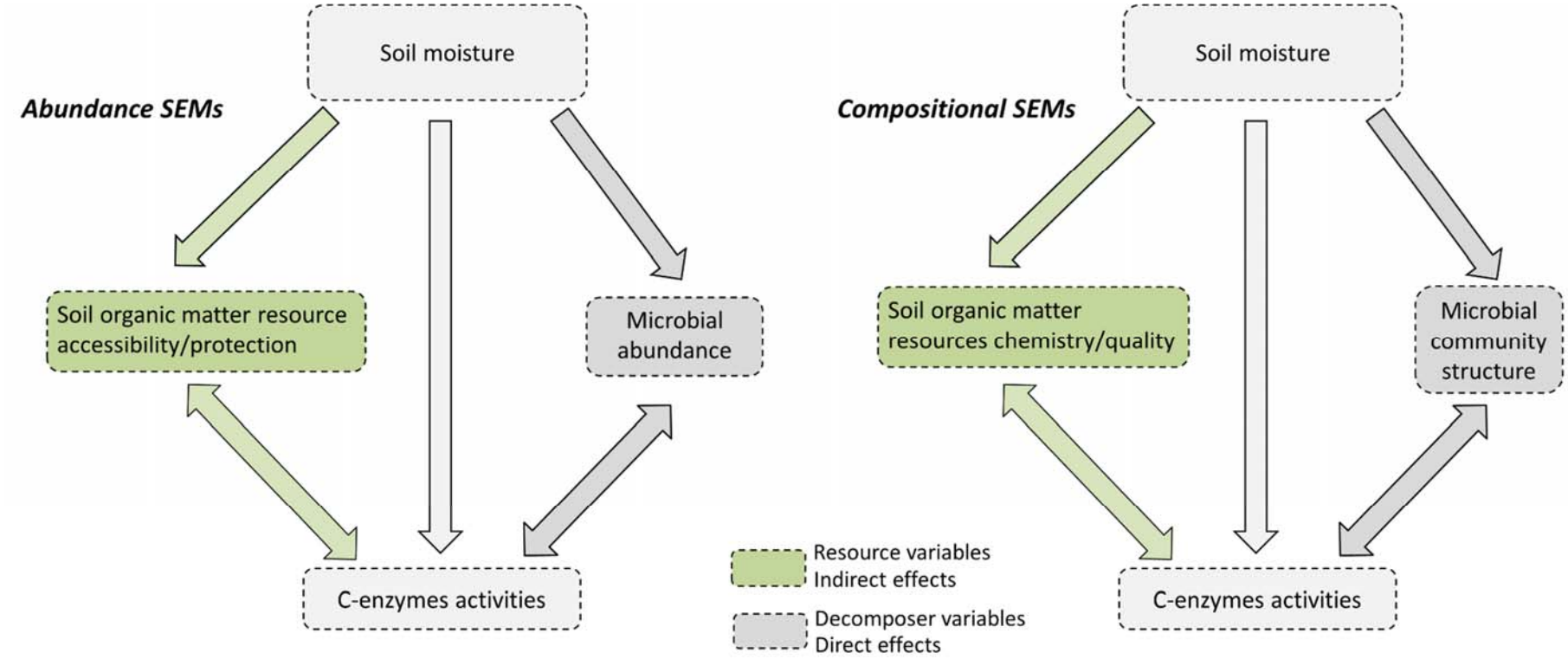
Fig 3. Compositional initial SEMs showing the different path-relation network used to perform SEMs. Numbers in circle indicate the hypothesis made behind each causal links and presented in the table under SEM figures. Green arrows indicate paths involving change in soil organic matter resource quality.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

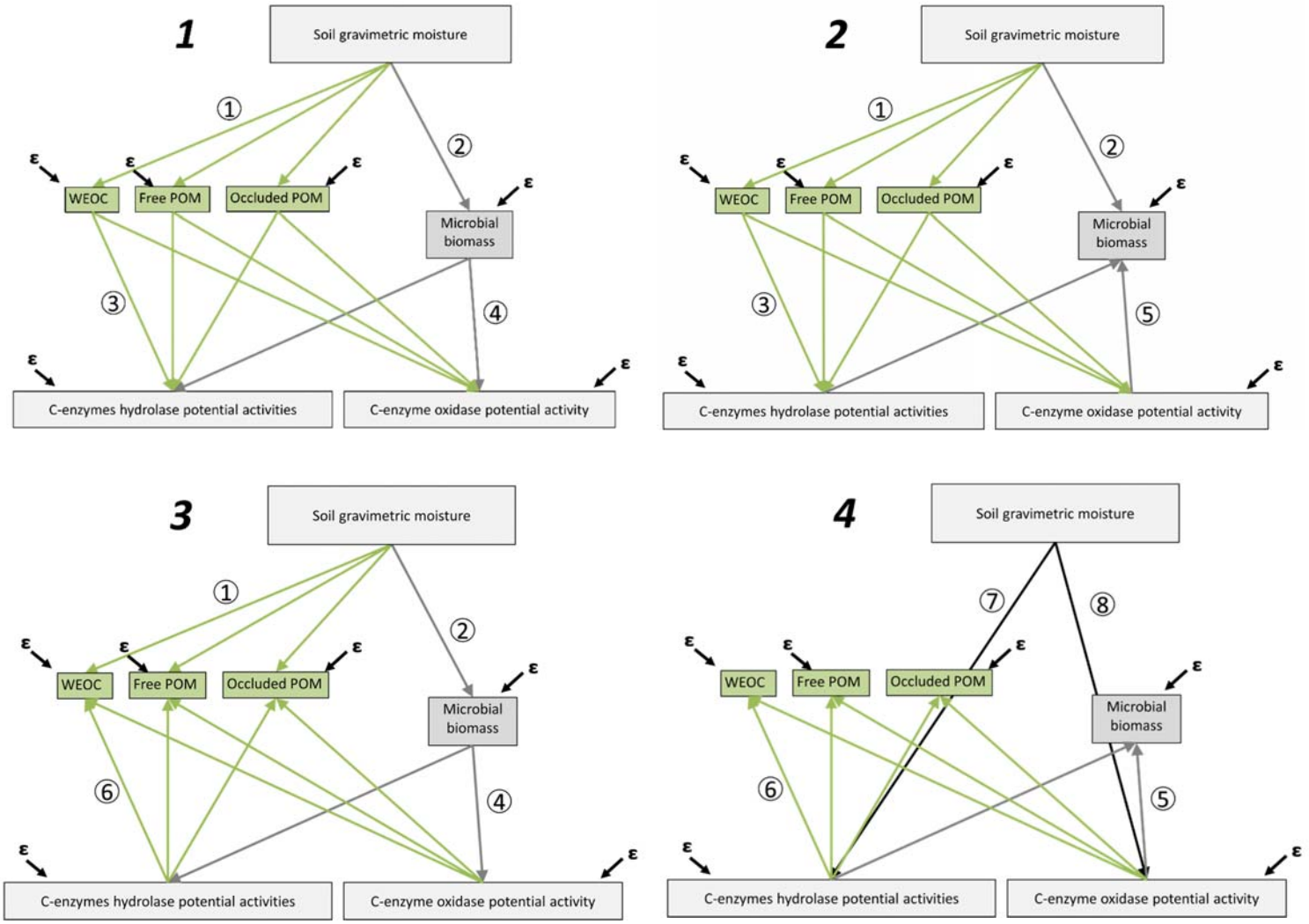
Fig 4. Seasonal SEMs representing the climate effects on the drivers of SOM enzymatic decomposition. A) Winter abundance SEM, B) Summer abundance SEM, C) Winter compositional SEM, D) summer compositionalValues in orange boxes

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.

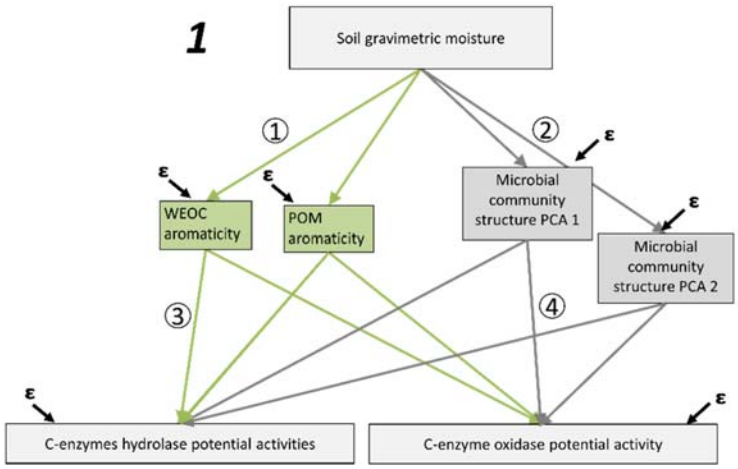
Highlights

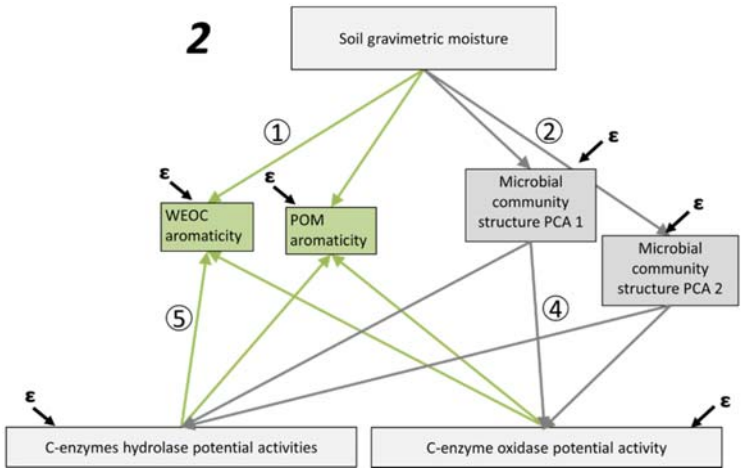
- Contrasting impacts of the climate manipulation on the drivers of carbon enzymes between winter and summer
- In winter, the reduced availability of water extractable organic carbon downregulated enzyme activity
- In summer, reduced soil microbial biomass led to a decrease of C-enzyme activity

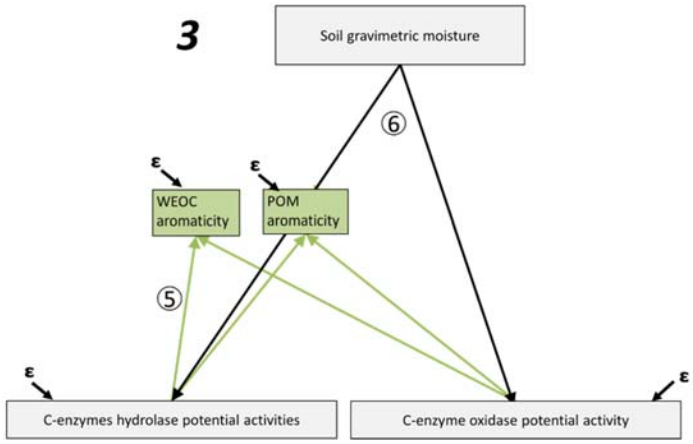

Abundance SEMs
Soil moisture
Soil organic matter resource accessibility/protection
Microbial abundance
C-enzymes activities
Compositional SEMs
Soil moisture
Soil organic matter resources chemistry/quality
Microbial community structure
C-enzymes activities
Resource variables
Indirect effects
Decomposer variables
Direct effects



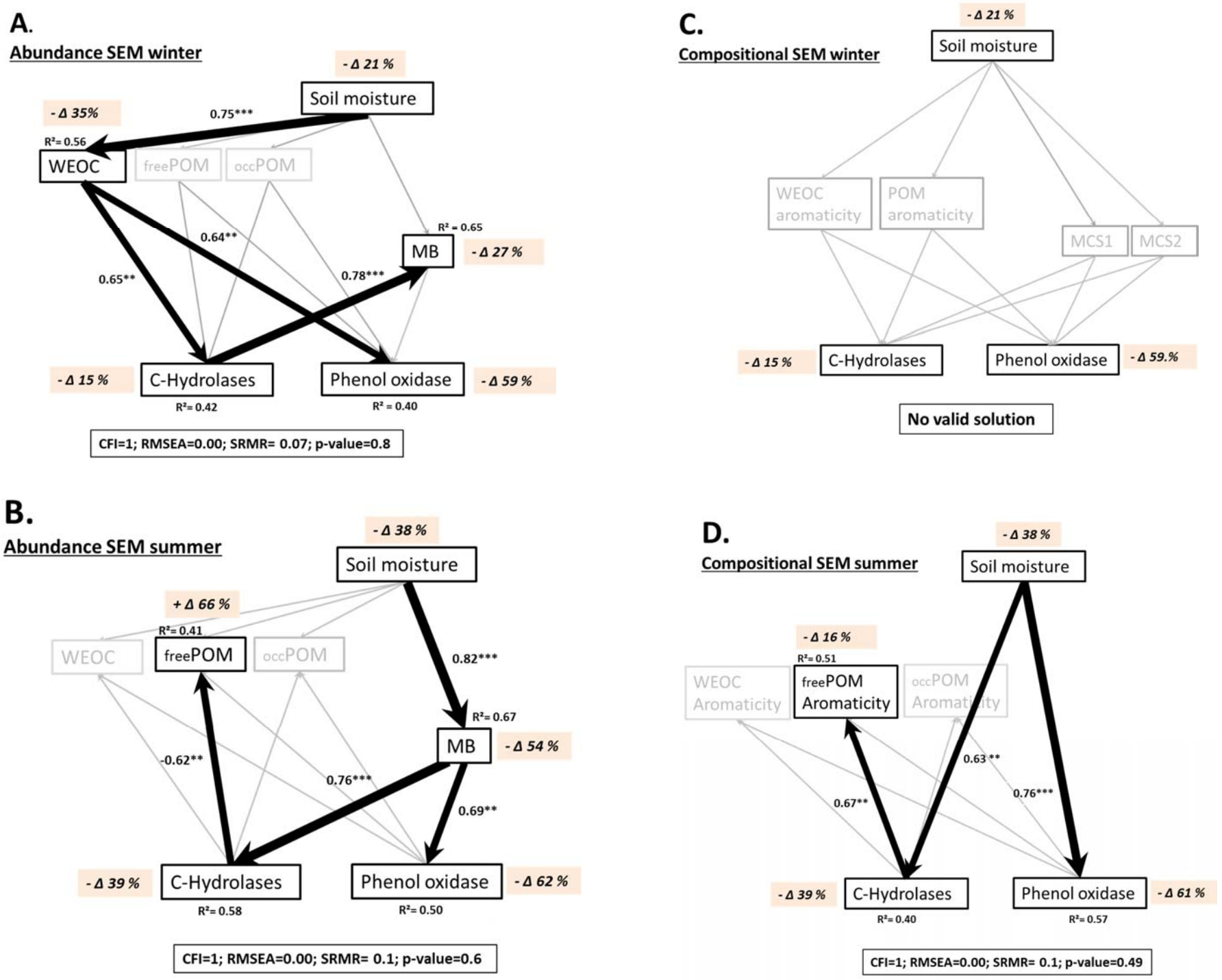
Main path	Pathway	Hypothesized mechanism
	Abundance SEMs	
❶	Soil moisture→ WEOC/freePOM/occPOM	Higher soil moisture increases plant organic matter input and its availability
❷	Soil moisture→ Microbial biomass	Change in soil moisture affects microbial physiological constraints, and therefore biomass
❸	WEOC/freePOM/occPOM→ Hydrolase/oxidase activities	Positive effect of SOM resources abundance on enzyme activities
❹	Microbial biomass→ Hydrolase/oxidase activities	More microbial biomass lead to more enzyme production
❺	Hydrolase/oxidase activities→ Microbial biomass	Higher enzyme activities enable more biomass production
❻	Hydrolase/oxidase activities→ WEOC/freePOM/occPOM	Higher enzyme activities decrease the amount of SOM resource pools
❼	Soil moisture→ Hydrolase/oxidase activities	Soil moisture directly affects enzyme activity







Main path	Pathway	Hypothesized mechanism
	Compositional SEMs	
❶	Soil moisture→ WEOC/POM aromaticity	Higher soil moisture changes plant communities, and therefore organic matter input quality
❷	Soil moisture→ Microbial community structure	Change in soil moisture affects microbial physiological constraints and therefore microbial community structure
❸	WEOC/POM aromaticity→ Hydrolase/oxidase activities	Higher resource aromaticity leads to decreased enzyme activities
❹	Microbial community structure→ Hydrolase/oxidase activities	Change in microbial community leads to change in enzyme production
❺	Hydrolase/oxidase activities→ WEOC/POM aromaticity	Higher enzyme activities leads to increased SOM aromaticity due to preferential degradation of labile resources
❻	Soil moisture→ Hydrolase/oxidase activities	Soil moisture directly affects enzyme activity


A.
Abundance SEM winter
- Δ 21 %
Soil moisture
- Δ 35%
0.75***
R²= 0.56
WEOC
freePOM
occPOM
R² = 0.65
0.64**
MB
- Δ 27 %
0.65**
0.78***
- Δ 15 %
C-Hydrolases
Phenol oxidase
- Δ 59 %
R²= 0.42
R² = 0.40
CFI=1; RMSEA=0.00; SRMR= 0.07; p-value=0.8
C.
Compositional SEM winter
- Δ 21 %
Soil moisture
WEOC aromaticity
POM aromaticity
MCS1
MCS2
- Δ 15 %
C-Hydrolases
Phenol oxidase
- Δ 59.%
No valid solution
B.
Abundance SEM summer
- Δ 38 %
Soil moisture
+ Δ 66 %
R²= 0.41
WEOC
freePOM
occPOM
0.82***
R²= 0.67
MB
- Δ 54 %
-0.62**
0.76***
0.69**
- Δ 39 %
C-Hydrolases
Phenol oxidase
- Δ 62 %
R²= 0.58
R²= 0.50
CFI=1; RMSEA=0.00; SRMR= 0.1; p-value=0.6
D.
Compositional SEM summer
- Δ 38 %
Soil moisture
- Δ 16 %
R²= 0.51
WEOC Aromaticity
freePOM Aromaticity
occPOM Aromaticity
0.63 **
0.67**
0.76***
- Δ 39 %
C-Hydrolases
Phenol oxidase
- Δ 61 %
R²= 0.40
R²= 0.57
CFI=1; RMSEA=0.00; SRMR= 0.1; p-value=0.49

I. Microbial data used to perform SEMs (from Puissant et al, 2015)

I-1.Soil microbial biomass (MB)

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

I-2.Soil microbial community structure (MCS)

Soil MCS was assessed by analysing the microbial phospholipid fatty acid (PLFA) composition. PLFAs were extracted according to Bligh and Dyer (1959), and modified by Börjesson et al. (1998). Total lipids were extracted overnight from 4 g freeze-dried soil in a solvent phase of 3.0 ml 50mM phosphate buffer (pH = 7.0), 3.8 ml chloroform (CHCl3), 7.6 ml methanol (MeOH), and 4 ml Bligh and Dyer (1959) reagent (CHCl3: MeOH: P-buffer; 1: 2: 0.8 (v/v/v)). Total lipids were separated into neutral lipids, glycolipids, and phospholipids by dissolving the total lipid fraction using chloroform, acetone and methanol solutions, which were respectively added over Discovery® DSC-Si SPE Tubes (Sigma-Aldrich). PLFA 19:0 (Larodan Malmö, Sweden) was added as internal standard to the phospholipid fraction. PLFAs were trans-esterified to fatty acid methyl esthers (FAMEs) using 1 ml 0.2 M methanolic-KOH (Chowdhury and Dick, 2012; Sundh et al., 1997). PLFAs were analysed on a gas chromatograph according to Steger et al. (2003). To identify MCS pattern, a principal component analysis (PCA) based on Hellinger-transformed PLFA data was performed (Legendre and Gallagher, 2001). For each sample, PLFA data were normalized by total PLFA abundance to obtain relative abundances. Two indices PC 1 and PC 2 corresponding to axis 1 and 2 of the PCA were extracted so as to summarize MCS data in subsequent statistical analyses.

I-3.Soil extracellular enzymes activity (EEA) assays

Hydrolytic EEA (Cellobiohydrolase, 4-MUB-ß-D-cellobioside; ß-glucosidases, 4-MUB-ß-D-glucopyranoside; xylosidase, 4-MUB-ß-D-xylopyranoside; lipase, 4-MUB-heptanoate) were measured by fluorogenic methods using 4-MUB (4-methylumbelliferone) Enzyme assays were processed in acetate buffer solution (pH = 5) which was chosen to be close to soil field pH, and for stabilizing the fluorescence intensity which is dependent on pH fluctuation (German et al., 2011). Enzyme assays were performed according to Marx et al. (2001) with small modifications. Briefly, 2.5 g of moist soil sieved at 2 mm was mixed with 40 ml of acetate buffer in 50ml sterile tubes. These tubes were placed for twenty minutes into a shaker at 250 rpm to obtain a homogenous soil solution. Then, 30 µl of soil solution was added to a 96-well microplate with 30 µl of fluorometric substrate (300 mM, saturated concentration) and completed to 250 µl with acetate buffer solution. Enzymatic reactions were incubated in the dark for 5 hours at 28 °C, with one fluorometric measure per hour. For each sample, three methodological replicates (sample + buffer + substrate) and a quenched standard (sample + buffer + 4-MUB) were used. For each substrate, a control including the 4-MUB-linked substrate and the buffer solution alone were used to check the evolution of fluorescence without enzyme degradation over the duration of assay. The fluorescence intensity was measured using a Varioskan flash spectrophotometer set to 330 for excitation and 450 for emission for the 4-MUB

The potential activity of phenol oxidase (POX), an oxidative EE, was measured by absorbance. The protocol described by (Floch et al., 2007) was used with small modifications. Oxidation of ABTS (2.2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) was determined by using the same soil solution prepared for fluorogenic enzyme assays. POX reactions were processed for 10 minutes at 37 °C in 2 ml centrifuge tubes containing 0.4 ml of soil solution, 1 ml of acetate buffer (pH = 5) and 0.1 ml of ABTS (50 mM). Blanks were measured with 0.4 ml of soil solution and 1.1 ml of acetate buffer. Additionally, a control of substrate absorbance was performed with 0.1 ml of ABTS (50 mM) and

1.4 ml of acetate buffer. Absorbance was measured at 420 nm and the extinction value was ε420 = 36 000 M-1cm-1 (Ullrich and Nüske, 2004).

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

II. Soil organic matter resources data used to perform SEMs (from Puissant et al, 2017)

II-1. Water-extractable organic C fraction

To obtain the WEOC fraction, 40 mL of deionized water was added to 10 g of moist sieved (2 mm) soil, and shaken for 20 minutes at 250 rpm. Samples were then centrifuged at 10,000 g for 10 minutes, after which the solution was filtered through 0.45 mm Millipore filter and immediately stored at -20 °C until analysis. Soil WEOC content was measured using a total organic carbon analyzer (Shimadzu Inc., Kyoto, Japan). The analyzer was calibrated for total dissolved C (TDC) and dissolved inorganic C (DIC) using a calibration solution of potassium hydrogen phthalate ($C_8H_5KO_4$) and a solution containing a mixture of sodium hydrogen carbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3) for TDC and DIC respectively. WEOC was calculated as the difference between TDC and DIC and expressed in mg C.g-1 soil.

II-2. Soil organic matter density fractionation

Three SOM fractions (freePOM, OccPOM) were separated by density fractionation of oven dried (40 °C) and sieved (< 2 mm) soil samples following Leifeld et al. (2005, 2009). Briefly, 15 g of soil were placed into a 50 mL centrifuge tube. A sodium polytungstate solution (density = 1.6 g cm-3) was added up to the 50 mL line and the tube was gently inverted several times. After 2 hours, floating materials (<1.6 g cm-3) corresponding to the freePOM fraction, were collected and washed thoroughly with deionized water through 0.45 µm nitrocellulose membrane filters.

This first step was repeated four times to obtain all remaining freePOM. Then the remaining pellet was re-suspended in sodium polytungstate and treated with ultra-sonication (22 J mL−1 in an ice bath using a Branson 250 calibrated according to Schmidt et al (1999) so as to breakdown all soil macro-aggregates (Leifeld and Kögel-Knabner, 2005). After sonication, samples were centrifuged at 10,000 g for 10 minutes and floating materials (occPOM fraction) were collected and washed thoroughly with deionized water through 0.45 µm nitrocellulose membrane filters. This step was repeated four times to collect all occPOM released by the sonication treatment. We used 0.45 µm nitrocellulose membrane filters so as to characterize the SOC fraction until the WEOC size definition. All washed fractions were oven dried at 40 °C and weighed. Organic C and total N concentrations of the freePOM, occPOM and Organic C and total N concentrations of SOM fractions (expressed as g C or N kg-1 SOM fraction) were then expressed as percent of the SOC and total N contents of bulk soil samples (i.e. SOC and total N distribution in SOM fractions).

II-3. Chemistry of the soil organic matter fractions

II-3.1. Chemistry of the WEOC fraction

The chemistry of the WEOC fraction was qualitatively assessed using ultraviolet (UV) spectroscopy. The absorbance of the WEOC fraction at 280 nm was used as an indicator of its aromaticity (Kalbitz et al., 2003).

II-3.2. Chemistry of the POM fractions

The chemistry of the POM fractions (freePOM and occPOM) was assessed using mid-infrared (MIR) spectroscopy. Prior to these analyses, POM fractions were ball-milled (< 0.25 mm using a Retsch ZM 200) and further dried overnight at 40 °C to limit interferences with water, without altering OM chemistry. Crushed samples were analyzed using a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific Inc., Madison, WI, USA). Spectral acquisition was performed by diamond

attenuated total reflectance (MIR-ATR) spectroscopy over the spectral range 4,000–650 cm-1, with spectral resolution of 4 cm-1 and 16 scans per replicate (2 replicates per sample). All MIR-ATR spectra were corrected for atmospheric interferences (H2O and CO2). Spectral data were further processed and analyzed using the hyperSpec (Beleites and Sergo, 2011), signal (signal developers, 2013) and ptw (Bloemberg et al 2010) packages in the R environment, software version 2.14.0 (R Development Core Team 2011). Spectral regions corresponding to 1,576–1,618 cm-1 was chosen for C=C bonds aromatic index according to Pengerud et al (2013) and Robroek et al. (2015).

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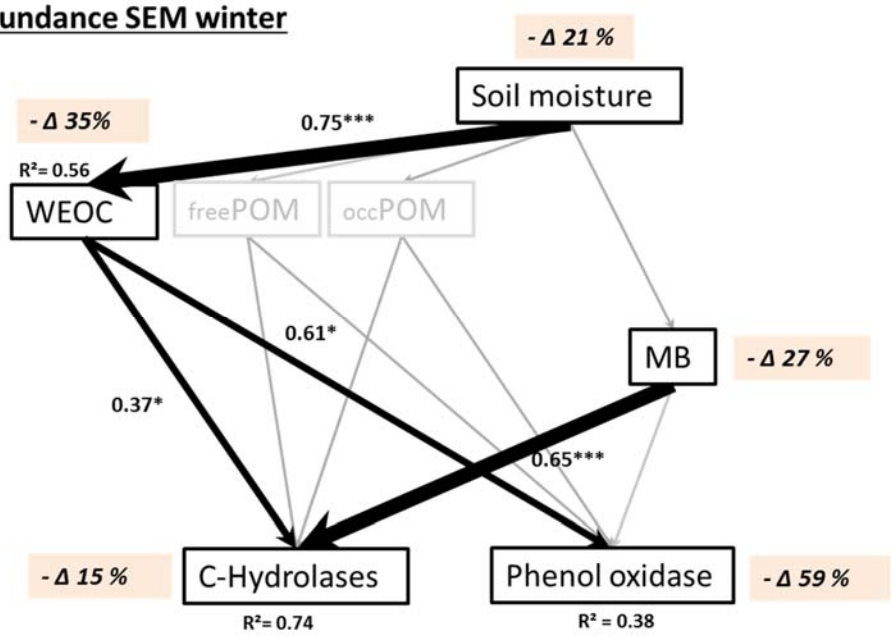
A. From initial SEM 1

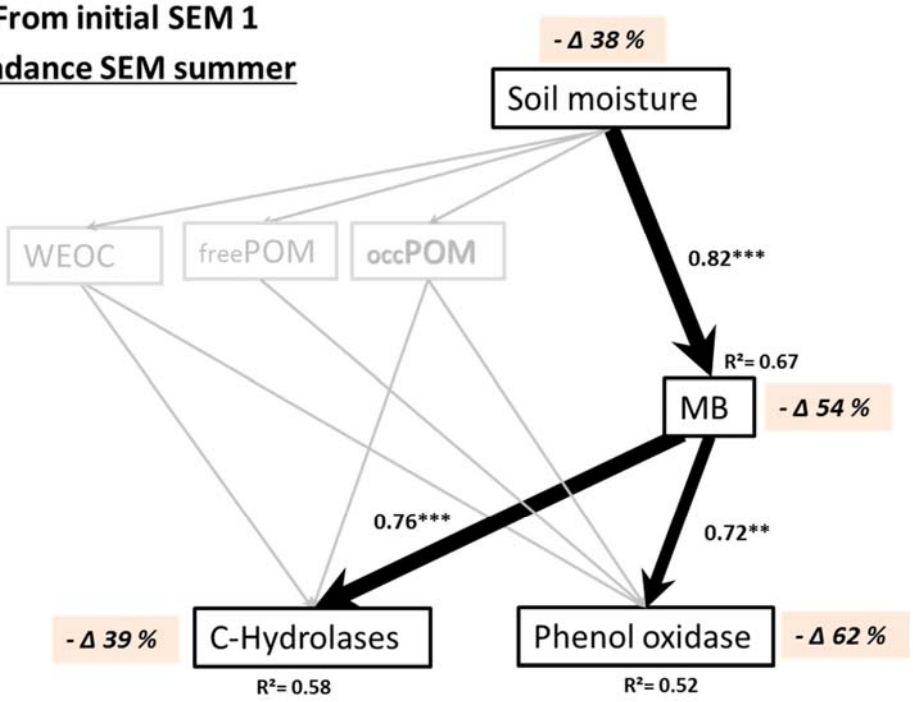
Abundance SEM winter



CFI=1; RMSEA=0.00; SRMR= not valid (0.159); p-value=0.42

B. From initial SEM 1

Abundance SEM summer



CFI=1; RMSEA=0; SRMR= 0.05; p-value=0.5