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

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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

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

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

1- Introduction

Soils store vast amounts of carbon (C) as soil organic matter (SOM), which equals, if not exceeds, the collective C stock in the atmosphere and vegetation (IPCC 2013). Soil microbial communities play a key role in SOM decomposition processes, annually releasing ca. 60 GtC as respired CO_2 into the atmosphere (IPCC 2013, Lal 2008), or roughly double the anthropogenic greenhouse gas contribution. To decompose SOM, soil microorganisms release soil extracellular 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

change as elevation gradients represent natural climate change experiments 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

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 $77g.kg^{-1}$ 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 21st

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 monotliths 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 mesocosm 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 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 2nd 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 perfomed 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-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   ß-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 ($P \geq 0.05$),

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

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-1 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.

Contrastingly, in summer WEOC content was not related to C-enzyme potential activities. Instead, the reduction in soil moisture directly impacted microbial biomass and led to a strong decrease in both hydrolase and oxidase C-enzyme potential activities. The strong gravimetric soil moisture decrease due the climate change manipulation in the summer season (from 34% at the control site to 21% under the climate change condition; delta -38%, Fig 4.B) might have led to a huge 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.

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.

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References

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry 37, 937–944. doi:10.1016/j.soilbio.2004.09.014

Bardgett, R.D., Freeman, C., Ostle, N.J., 2008. Microbial contributions to climate change through carbon cycle feedbacks. The ISME journal 2, 805–814. doi:10.1038/ismej.2008.58

Bengtson, P., Bengtsson, G., 2007. Rapid turnover of DOC in temperate forests accounts for increased CO_2 production at elevated temperatures. Ecol. Lett. 10, 783–90. doi:10.1111/j.1461-0248.2007.01072.x

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology 37, 911–917.

Blois, J.L., Williams, J.W., Fitzpatrick, M.C., Jackson, S.T., Ferrier, S., 2013. Space can substitute for time in predicting climate-change effects on biodiversity. Proceedings of the National Academy of Sciences of the United States of America 110, 9374–9.

Börjesson, G., Sundh, I., Tunlid, A., Svensson, B.H., 1998. Methane oxidation in landfill cover soils, as revealed by potential oxidation measurements and phospholipid fatty acid analyses. Soil Biology and Biochemistry 30, 1423–1433.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837–842.

Brooks, P.D., McKnight, D., Elder, K., 2005. Carbon limitation of soil respiration under winter snowpacks: Potential feedbacks between growing season and winter carbon fluxes. Global Change Biology 11, 231–238. doi:10.1111/j.1365-2486.2004.00877.x

418   Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E.,

419   Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a

420   changing environment: Current knowledge and future directions. Soil

421   Biology and Biochemistry 58, 216–234. doi:10.1016/j.soilbio.2012.11.009

422   C2SM, 2011. Swiss Climate Change Scenarios CH2011. doi:10.3929/ethz-a-

423   006720559

424   Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E. a., Eliasson,

425   P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R.,

426   Kirschbaum, M.U.F., Lavallee, J.M., Leifeld, J., Parton, W.J., Megan

427   Steinweg, J., Wallenstein, M.D., Martin Wetterstedt, J.Å., Bradford, M. a.,

428   2011a. Temperature and soil organic matter decomposition rates - synthesis

429   of current knowledge and a way forward. Global Change Biology 17, 3392–

430   3404. doi:10.1111/j.1365-2486.2011.02496.x

431   Davidson, E. a, Janssens, I. a, 2006. Temperature sensitivity of soil carbon

432   decomposition and feedbacks to climate change. Nature 440, 165–73.

433   doi:10.1038/nature04514

434   Dungait, J.A.J., Hopkins, D.W., Gregory, A.S. et Whitmore, A.P., 2012. Soil

435   organic matter turnover is governed by accessibility not recalcitrance. Global

436   Change Biology 18, 1781–1796. doi:10.1111/j.1365-2486.2012.02665.x

437   Edwards, A.C., Scalenghe, R. et Freppaz, M., 2007. Changes in the seasonal snow

438   cover of alpine regions and its effect on soil processes: A review. Quaternary

439   International 162-163, 172–181. doi:10.1016/j.quaint.2006.10.027

440   Floch, C., Alarcon-Gutiérrez, E., Criquet, S., 2007. ABTS assay of phenol oxidase

441   activity in soil. Journal of microbiological methods 71, 319–24.

442   doi:10.1016/j.mimet.2007.09.020

443   Gavazov, K.S., 2013. Subalpine pasture-woodlands in a changing climate: The

444   role of land use type in ecosystem resistance to simulated climate change.

445   Thesis EPFL 5989. Lausanne, Swizerland. doi:10.5075/epfl-thesis-5989

446   Gavazov, K., Peringer, A., Buttler, A., Gillet, F., Spiegelberger, T., 2013.

447   Dynamics of Forage Production in Pasture-woodlands of the Swiss Jura

448   Mountains under Projected Climate Change Scenarios. Ecol. Soc. 18, art38.

449   doi:10.5751/ES-04974-180138

450   Gavazov, K., Mills, R., Spiegelberger, T., Lenglet, J., Buttler, A., 2014. Biotic and

451   Abiotic 664 Constraints on the Decomposition of Fagus sylvatica Leaf Litter

452   Along an Altitudinal 665 Gradient in Contrasting Land-Use Types.

453   Ecosystems 17, 1326–1337. 666 doi:10.1007/s10021-014-9798-9

454   Gavazov, K.; Ingrisch, J.; Hasibeder, R.; Mills, R. T. E.; Buttler, A.; Gleixner, G.;

455   Pumpanen, J.; and Bahn, M., 2017. Winter ecology of a subalpine grassland:

456   Effects of snow removal on soil respiration, microbial structure and function.

457   Science of The Total Environment. doi:10.1016/j.scitotenv.2017.03.010

458   Gobiet, A., Kotlarski, S., Beniston, M., Heinrich, G., Rajczak, J., Stoffel, M., 2014.

459   21st century climate change in the European Alps-A review. Science of the

460   Total Environment 493, 1138–1151. doi:10.1016/j.scitotenv.2013.07.050

461   Grace, J. B., D. R. Schoolmaster Jr., G. R. Guntenspergen, A. M. Little, B. R.

462   Mitchell, K. M. Miller, and E. W. Schweiger. 2012. Guidelines for a graph-

463   theoretic implementation of structural equation modeling. Ecosphere 3(8):73.

464   http://dx.doi.org/10.1890/ES12-00048.1

465   Grace, J.B., Adler, P.B., Stanley Harpole, W., Borer, E.T., Seabloom, E.W., 2014.

466   Causal networks clarify productivity-richness interrelations, bivariate plots

467   do not. Functional Ecology. 28: 787–798. doi:10.1111/1365-2435.12269

468   Green, V.S., Stott, D.E., Diack, M., 2006. Assay for fluorescein diacetate

469   hydrolytic activity: Optimization for soil samples. Soil Biology and

470   Biochemistry 38, 693–701. doi:10.1016/j.soilbio.2005.06.020

471   Harrysson Drotz, S., Tilston, E.L., Sparrman, T., Schleucher, J., Nilsson, M.,

472   Öquist, M.G., 2009. Contributions of matric and osmotic potentials to the

473   unfrozen water content of frozen soils. Geoderma 148, 392–398.

474   doi:10.1016/j.geoderma.2008.11.007

475 Henry, H.A.L., 2013. Reprint of "Soil extracellular enzyme dynamics in a
476   changing climate." Soil Biology and Biochemistry 56, 53–59.
477   doi:10.1016/j.soilbio.2012.10.022

478

479 Houghton, R.A., 2007. Balancing the Global Carbon Budget. Annual Review of
480   Earth and Planetary Sciences 35, 313–347.
481   doi:10.1146/annurev.earth.35.031306.140057

482 IPCC, 2013. Intergovernmental Panel on Climate Change. Climate Change 2013
483   : The Physical Science Basis. IPCC Working Group I Contribution to AR5

484 Jassey, V. E. J., Lamentowicz, M., Bragazza, L., Hofsommer, M. L., Mills, R. T.
485   E., Buttler, A., et al. (2016). Loss of testate amoeba functional diversity with
486   increasing frost intensity across a continental gradient reduces microbial
487   activity in peatlands. European Journal of Protistology, 1–13.
488   http://doi.org/10.1016/j.ejop.2016.04.007

489 Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E., 2003. Biodegradation of
490   soil-derived dissolved organic matter as related to its properties. Geoderma
491   113, 273–291. doi:10.1016/S0016-7061(02)00365-8

492 Körner, C., 2007. The use of "altitude" in ecological research. Trends in Ecology &
493   Evolution 22, 569–74. doi:10.1016/j.tree.2007.09.006

494 Körner, C., Paulsen, J., Spehn, E.M., 2011. A definition of mountains and their
495   bioclimatic belts for global comparisons of biodiversity data. Alpine Botany
496   121, 73–78. doi:10.1007/s00035-011-0094-4

497 Kuzyakov, Y. et Blagodatskaya, E., 2015. Microbial hotspots and hot moments in
498   soil: Concept & review. Soil Biology and Biochemistry 83, 184–199.
499   doi:10.1016/j.soilbio.2015.01.025

500 Lal, R. 2008. Sequestration of atmospheric CO_2 in global carbon pools.

501 Energy & Environmental Science, 86-100. doi:10.1039/B809492F

502   Leifeld, J., Fuhrer, J., 2009. Long-term management effects on soil organic

503   matter in two cold, high-elevation grasslands: clues from fractionation and

504   radiocarbon dating. European Journal of Soil Science 60, 230–239.

505   doi:10.1111/j.1365-2389.2008.01111.x

506   Leifeld, J., Kögel-Knabner, I., 2005. Soil organic matter fractions as early

507   indicators for carbon stock changes under different land-use? Geoderma 124,

508   143–155. doi:10.1016/j.geoderma.2004.04.009

509   Leifeld, J., Zimmermann, M., Fuhrer, J., Conen, F., 2009. Storage and turnover of

510   carbon in grassland soils along an elevation gradient in the Swiss Alps.

511   Global Change Biology 15, 668–679. doi:10.1111/j.1365-2486.2008.01782.x

512   Lorenz, K., Lal, R., Preston, C.M., Nierop, K.G.J., 2007. Strengthening the soil

513   organic carbon pool by increasing contributions from recalcitrant aliphatic

514   bio(macro)molecules. Geoderma 142, 1–10.

515   doi:10.1016/j.geoderma.2007.07.013

516   Manzoni, S. et Porporato, A. Soil carbon and nitrogen mineralization : Theory

517   and models across scales. Soil Biology and Biochemistry 41, 1355-1379

518   Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability

519   of dissolved organic matter in soils. Geoderma 113, 211–235.

520   doi:10.1016/S0016-7061(02)00362-2

521   Marx, M., Wood, M., Jarvis, S., 2001. A microplate fluorimetric assay for the

522   study of enzyme diversity in soils. Soil Biology and Biochemistry 33, 1633–

523   1640.

524   Milcu, A., Allan, E., Roscher, C., Jenkins, T., Meyer, S.T., Flynn, D., Bessler, H.,

525   Buscot, F., Engels, C., Gubsch, M., Kö Nig, S., Lipowsky, A., Loranger, J.,

526   Renker, C., Scherber, C., Schmid, B., Thébault, E., Wubet, T., Weisser, W.W.,

527   Scheu, S., Eisenhauer, N., 2013. Functionally and phylogenetically diverse

528   plant communities key to soil biota. Ecology 94, 1878–1885.

529   Mills, R.T.E., Gavazov, K.S., Spiegelberger, T., Johnson, D., Buttler, A., 2014.

530   Diminished s773 soil functions occur under simulated climate change in a

531   sup-alpine pasture, but 774 heterotrophic temperature sensitivity indicates

532   microbial resilience. The Science of the 775 total environment 473-474, 465–

533   72. doi:10.1016/j.scitotenv.2013.12.071

534   Öquist, M.G., Laudon, H., 2008. Winter soil frost conditions in boreal forests

535   control growing season soil CO2 concentration and its atmospheric exchange.

536   Global Change Biology 14, 2839–2847. doi:10.1111/j.1365-2486.2008.01669.x

537   Pengerud, A., Cécillon, L., Johnsen, L.K., Rasse, D.P., Strand, L.T., 2013.

538   Permafrost Distribution Drives Soil Organic Matter Stability in a Subarctic

539   Palsa Peatland. Ecosystems 16, 934–947. doi:10.1007/s10021-013-9652-5

540   Puissant, J., Cécillon, L., Mills, R.T.E., Robroek, B.J.M., Gavazov, K., De Danieli,

541   S., Spiegelberger, T., Buttler, A., Brun, J.-J., 2015. Seasonal influence of

542   climate manipulation on microbial community structure and function in

543   mountain soils. Soil Biology and Biochemistry 80, 296–305.

544   doi:10.1016/j.soilbio.2014.10.013

545   Puissant, J., Mills, R.T.E., Robroek, B.J.M., Gavazov, K., Perrette Y., De Danieli,

546   S., Spiegelberger, T., Buttler, A., Brun, J.-J., Cécillon, L., 2017. Climate

547   change effects on the stability and chemistry of soil organic carbon pools in a

548   subalpine grassland. Biogeochemistry 132: 123. doi:10.1007/s10533-016-

549   0291-8

550   Puissant, J., Effets des changements climatiques sur la dynamique de

551   décomposition microbienne du carbone organique du sol en prairie subalpine

552   calcaire. Biodiversité et Ecologie. Université Grenoble Alpes, 2015. Français.

553   <NNT : 2015GREAS008>. <tel-01259628>

554   Rees, R.M., Parker, J., 2005. Filtration increases the correlation between water

555   extractable organic carbon and soil microbial activity. Soil Biology &

556   Biochemistry 37, 2240–2248. doi:10.1016/j.soilbio.2005.03.024

557   Robroek, B. J. M., Jassey, V. E. J., Kox, M. A. R., Berendsen, R. L., Mills, R. T. E.,

558   Cécillon, L., Puissant, J., Meima-Franke, M., Bakker, P. A. H. M., Bodelier,

559   P. L. E. (2015), Peatland vascular plant functional types affect methane

560   dynamics by altering microbial community structure. Journal of Ecology,
561   103: 925–934. doi: 10.1111/1365-2745.12413

562 Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of
563   previously unknown fungal lineages in tundra soils. Science 301:1359–1361

564 Schnecker J, Wild B, Hofhansl F, et al. Effects of Soil Organic Matter Properties
565   and Microbial Community Composition on Enzyme Activities in
566   Cryoturbated Arctic Soils. Öpik M, ed. PLoS ONE. 2014;9(4):e94076.
567   doi:10.1371/journal.pone.0094076.

568 Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G.,
569   Janssens, I. a., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. a.
570   C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence
571   of soil organic matter as an ecosystem property. Nature 478, 49–56.
572   doi:10.1038/nature10386

573 Seneviratne, S.I., Lüthi, D., Litschi, M., Schär, C., 2006. Land-atmosphere
574   coupling and climate change in Europe. Nature 443, 205–209.
575   doi:10.1038/nature05095

576 Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics
577   of soil. Soil Biology and Biochemistry 42, 391–404.
578   doi:10.1016/j.soilbio.2009.10.014

579 Sjögersten, S., Alewell, C., Cécillon, L., Hagedorn, F., Jandl, R., Leifeld, J.,
580   Martinsen, V., Schindlbacher, A., Sebastian, M.T., Van Miegroet, H., 2011.
581   Mountain Soils in a Changing Climate - Vulnerability of Carbon Stocks and
582   Ecosystem Feedbacks, in: Soil Carbon in Sensitive European Ecosystems:
583   From Science to Land Management. John Wiley and Sons, pp. 118–148.
584   doi:10.1002/9781119970255.ch6

585 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for
586   measuring soil microbial biomass C. Soil Biology and Biochemistry.
587   doi:10.1016/0038-0717(87)90052-6

588 Von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger,

589   G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in

590   temperate soils: mechanisms and their relevance under different soil

591   conditions - a review. European Journal of Soil Science 57, 426–445.

592   doi:10.1111/j.1365-2389.2006.00809.x

593 Wohlfahrt, G., Anderson-Dunn, M., Bahn, M., Balzarolo, M., Berninger, F.,

594   Campbell, C., Carrara, A., Cescatti, A., Christensen, T., Dore, S., Eugster,

595   W., Friborg, T., Furger, M., Gianelle, D., Gimeno, C., Hargreaves, K., Hari,

596   P., Haslwanter, A., Johansson, T., Marcolla, B., Milford, C., Nagy, Z.,

597   Nemitz, E., Rogiers, N., Sanz, M.J., Siegwolf, R.T.W., Susiluoto, S., Sutton,

598   M., Tuba, Z., Ugolini, F., Valentini, R., Zorer, R., Cernusca, A., 2008. Biotic,

599   abiotic, and management controls on the net ecosystem CO_2 exchange of

600   European mountain grassland ecosystems. Ecosystems 11, 1338–1351.

601   doi:10.1007/s10021-008-9196-2

602 Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y., Ccahuana, J.., 2009. Climate

603   dependence of heterotrophic soil respiration from a soil-translocation

604   experiment along a 3000 m tropical forest altitudinal gradient. European

605   Journal of Soil Science 60, 1–12. doi:10.1007/s10533-010-9562-y

606 Zsolnay, Á., 2003. Dissolved organic matter: Artefacts, definitions, and functions.

607   Geoderma 113, 187–209. doi:10.1016/S0016-7061(02)00361-0

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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 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			
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		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	g C /kg of dry soil	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		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	Compositional	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.

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
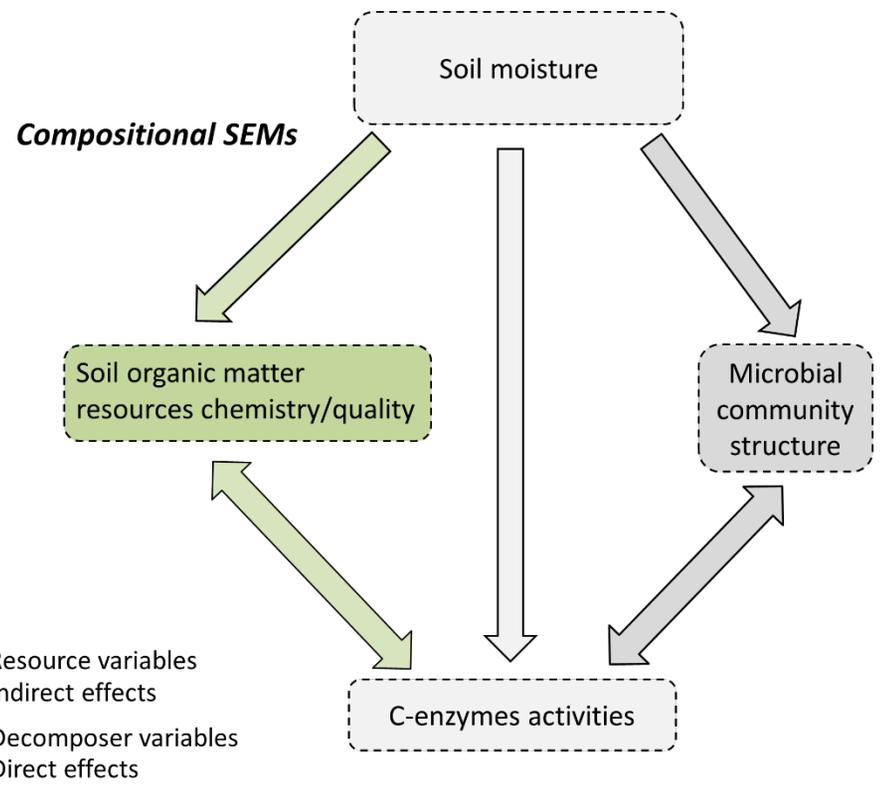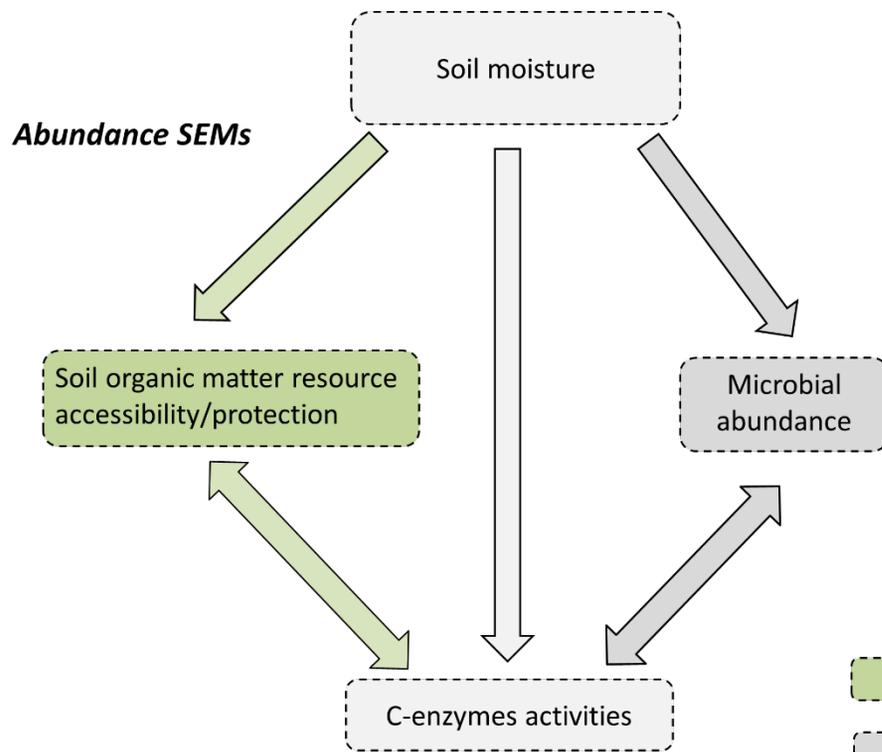
Resource variables
Indirect effects

Decomposer variables
Direct effects
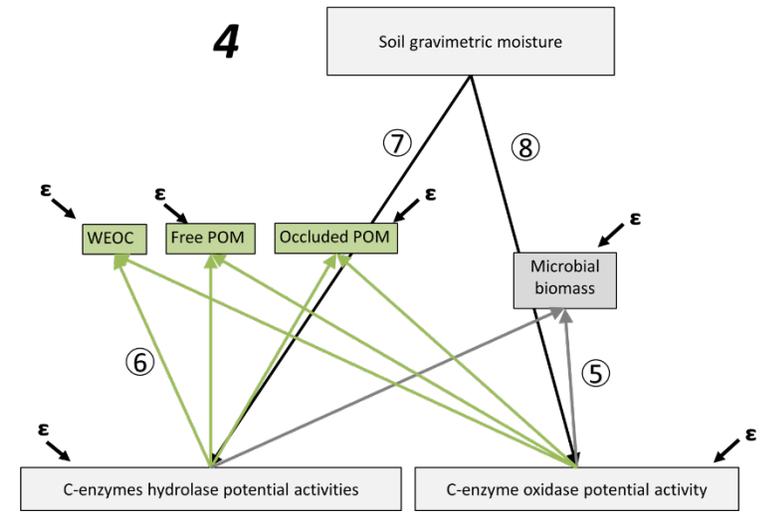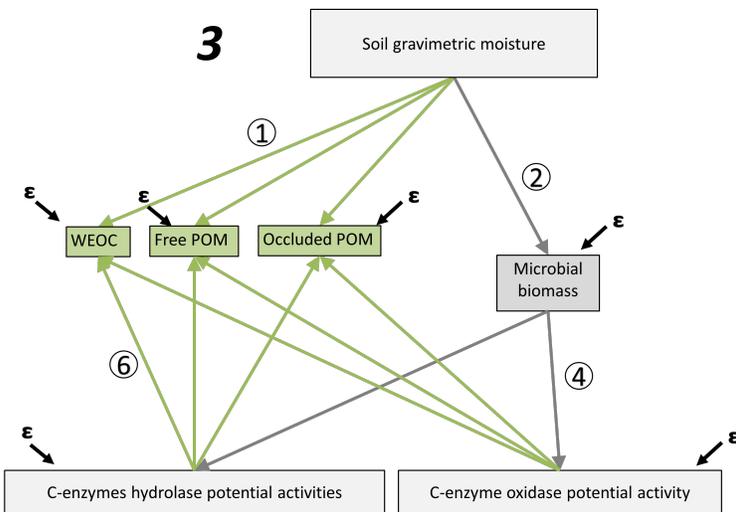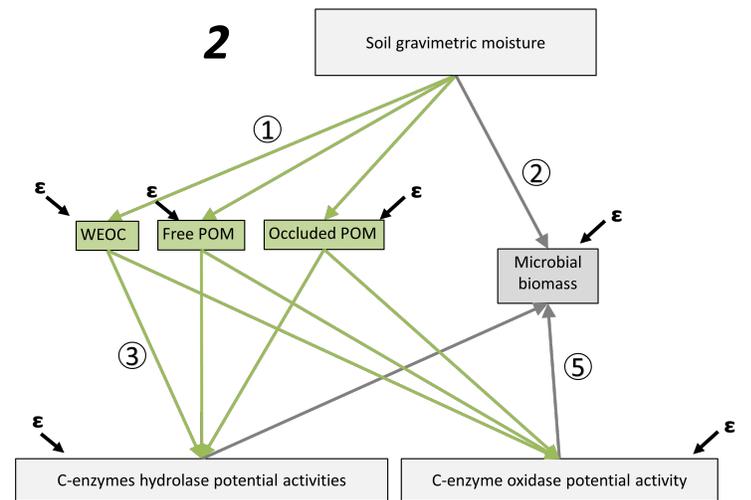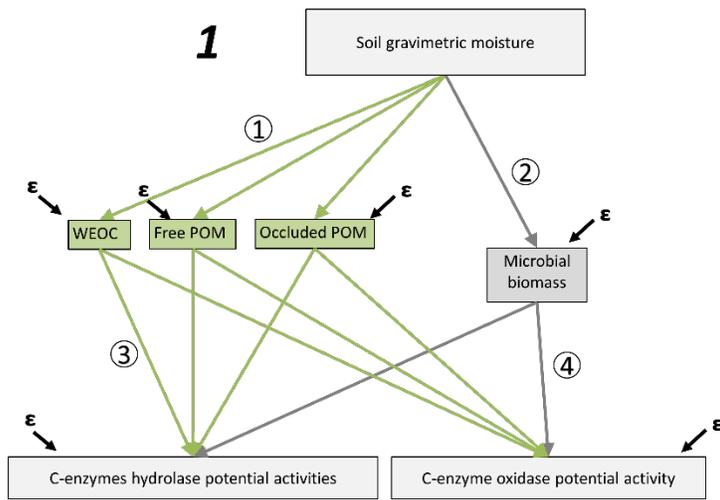
Main path	Pathway	Hypothesized mechanism
Abundance SEMs		
❶	Soil moisture→ WEOC/freePOM/occPOM	Higher soil moisture increases plant organic matter input and its availability
❷	Soil moisture→ Microbial biomass	Change in soil moisture affects microbial physiological constraints, and therefore biomass
❸	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
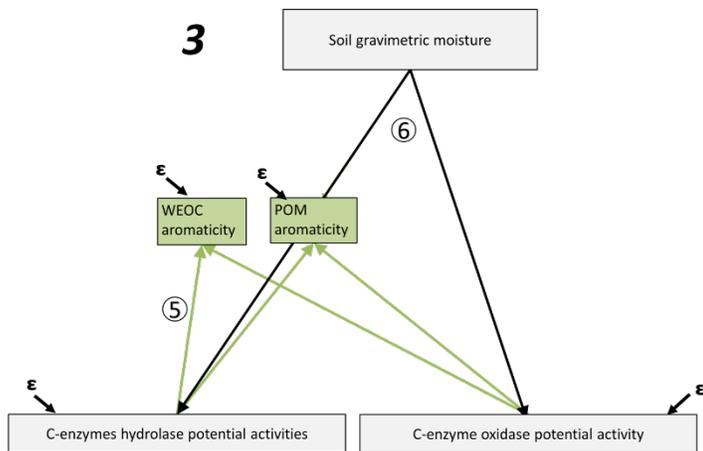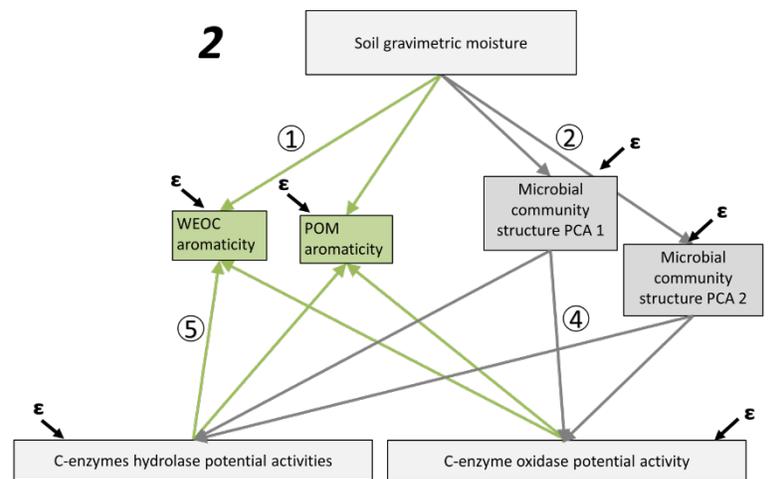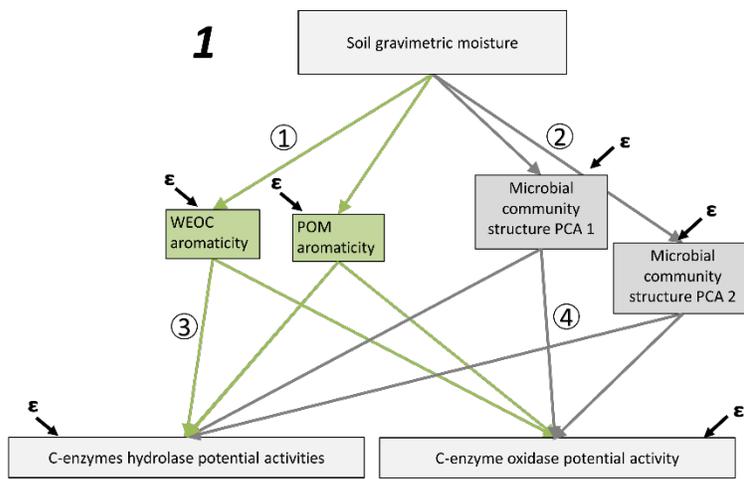
| Main path	Pathway	Hypothesized mechanism
Compositional SEMs		
❶	Soil moisture→ WEOC/POM aromaticity	Higher soil moisture changes plant communities, and therefore organic matter input quality
❷	Soil moisture→ Microbial community structure	Change in soil moisture affects microbial physiological constraints 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%
R²= 0.56
WEOC

freePOM   occPOM

0.75***

R² = 0.65
MB   - Δ 27 %

0.64**

0.65**

0.78***

- Δ 15 %
C-Hydrolases
R²= 0.42

Phenol oxidase
R² = 0.40   - Δ 59 %

CFI=1; RMSEA=0.00; SRMR= 0.07; p-value=0.8
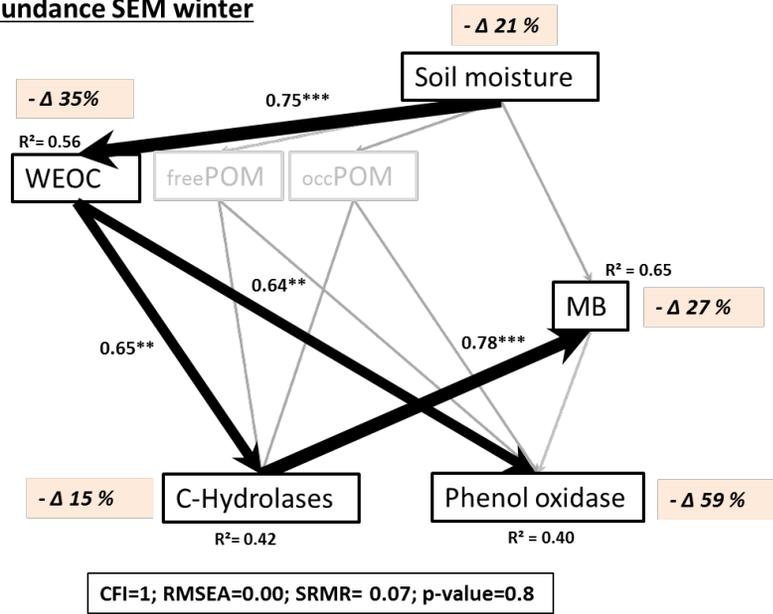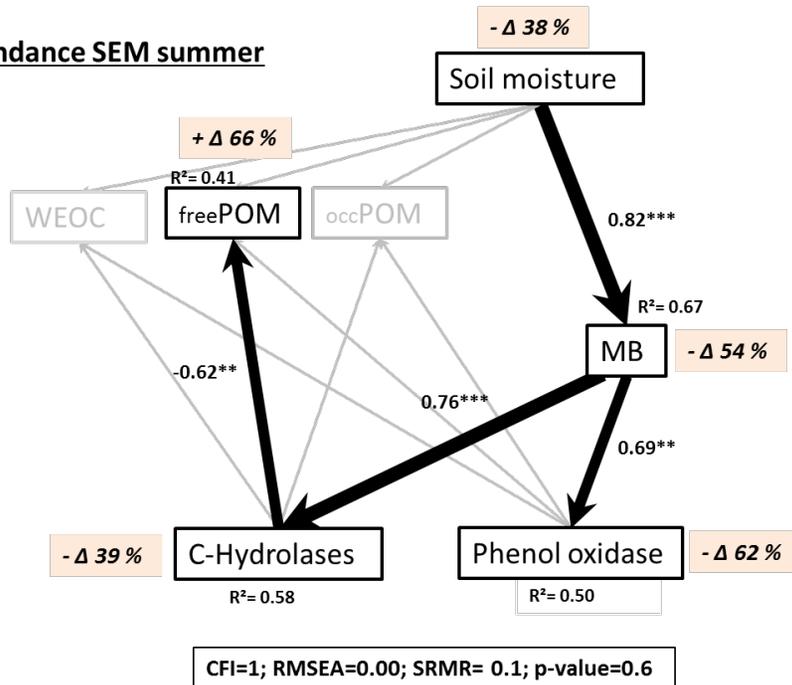
C.

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
freePOM

WEOC   occPOM

0.82***

R²= 0.67
MB   - Δ 54 %

-0.62**

0.76***

0.69**

- Δ 39 %
C-Hydrolases
R²= 0.58

Phenol oxidase
R²= 0.50   - Δ 62 %

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
freePOM Aromaticity

WEOC Aromaticity   occPOM Aromaticity

0.63 **

0.76***

0.67**

- Δ 39 %
C-Hydrolases
R²= 0.40

Phenol oxidase
R²= 0.57   - Δ 61 %

CFI=1; RMSEA=0.00; SRMR= 0.1; p-value=0.49