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

Jérémy Puissant^{a,b}, Lauric Cécillon^{a,b}, Robert T.E. Mills^{c,d}, Bjorn J.M.
Robroek^{c,d,e}, Konstantin Gavazov^{c,d}, Sébastien De Danieli^{a,b}, Thomas
Spiegelberger^{a,b}, Alexandre Buttler^{c,d,f}, Jean-Jacques Brun^{a,b}

^aIrstea, UR EMGR Ecosystèmes montagnards, 2 rue de la Papeterie-BP 76, F-
38402 Saint Martin d'Hères, France

^bUniv. Grenoble Alpes, F-38402 Grenoble, France

^cEcole Polytechnique Fédérale de Lausanne EPFL, School of Architecture, Civil
and Environmental Engineering ENAC, Laboratory of ecological systems ECOS,
Station 2, 1015 Lausanne, Switzerland

^dSwiss Federal Institute for Forest, Snow and Landscape Research WSL, Site
Lausanne, Station 2, 1015 Lausanne, Switzerland.

^eEcology and Biodiversity, Institute of Environmental Biology, Utrecht
University, Padualaan 8, 3584 CH Utrecht, The Netherlands

^fLaboratoire de Chrono-Environnement, UMR CNRS 6249, UFR des Sciences et
Techniques, 16 route de Gray, Université de Franche-Comté, F-25030 Besançon,
France

* First and corresponding author: Jérémy Puissant

jeremy.puissant@gmail.com

Tel: +33-4-7676-2782;

Abstract

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

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

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

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

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

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

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

1. Introduction

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

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

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

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

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

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

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

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

2. Materials and methods

2.1. Design of the altitudinal soil transplantation experiment

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

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

2.2. Soil microclimate monitoring

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

2.3. Seasonal soil sampling

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

2.4. Basic soil characteristics

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

chemical soil analyses, samples were dried at 40 °C as indicated in norm NF ISO 11464 (2006). Soil pH was measured in H₂O (1:5 vol:vol) according to the norm NF ISO 10390 (2005). Calcareous content was determined following the norm NF ISO 10693 (1995). Organic carbon and total nitrogen concentrations were measured by the Dumas dry combustion after decarbonation (NF ISO 10694, 1995; 13878, 1995, respectively). Decarbonation was achieved by successive additions of a diluted HCl (10%) solution to soil samples.

2.5. 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.

2.6. 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 esters (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).

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

2.7. Soil extracellular enzymes activity (EEA) assays

The potential activity of eight individual extracellular enzymes (see Table 1 for a presentation of all enzymes and their abbreviations) was used to represent the degradation of the main soil biochemical compounds. Seven hydrolytic EEA were measured by fluorogenic methods using 4-MUB (4-methylumbelliferone) and 7-AMC (7-amino-4-methyl coumarin). 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 μ l 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 or 7-AMC) were used. For each substrate, a control including the 4-MUB- or 7-AMC-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 and 342 nm for excitation and 450 and 440 nm for emission for the 4-MUB and the 7-AMC substrate, respectively.

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 $\epsilon_{420} = 36\,000\text{ M}^{-1}\text{cm}^{-1}$ (Ullrich and Nüske, 2004).

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

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

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).

2.8. Statistical analyses

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

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

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

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

3. Results

3.1. Soil microclimate

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

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

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

3.2. Basic soil characteristics

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

3.3. Soil microbial biomass (MB)

We observed significant temporal variation in soil MB, with a higher abundance of soil microorganisms in winter and spring than in summer (Table 2). Interestingly, we also observed a significant transplantation effect on MB, which was stronger than the sampling date effect (Table 3). Soil MB decreased at the two lower experimental sites (570 and 1010 m a.s.l.) compared to the control site

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

3.4. Soil microbial community structure (MCS)

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

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

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

3.5. EEA on a dry soil mass basis

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

Transplanting soils to lower altitudes significantly impacted FDA only in winter, with decreased values at the two lower experimental sites compared to the control site (1350 m a.s.l.; Supplementary Table S1, Fig. 3). For all EEAs, the sampling date effect was stronger than the effect of climate manipulation. Enzymes involved in N (LAP, NAG) and P (PHO) cycle showed clearly higher activity in autumn (Fig. 3). The same pattern is observed for the CB enzyme. Consistently with overall microbial activity (FDA), several EEA's were negatively affected by the climate manipulation, with differences in the magnitude of this negative effect depending on the sampling date (Supplementary Table S1 and Fig. 3). Interestingly, the oxidative POX enzyme was more impacted by the climate manipulation than hydrolytic enzymes, with a significant response at each sampling date (Supplementary Table S1).

3.6. Mass-specific EEA

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

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

3.7. Factors influencing microbial enzymatic activity

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.3. Conclusions

We have shown in our study on mountain subalpine grassland soils of the Swiss Jura that enzyme pool size were regulated by a series of abiotic (soil temperature and moisture) and biotic (soil MB and MCS) factors. Large seasonal variations in enzyme pool size were due to the complex interaction of soil MB, MCS, soil microclimate and also potentially to plant rhizosphere activity. Conversely, the effect of the climate manipulation induced by four years altitudinal soil transplantation on EEA was neither explained by MCS change nor by SOC concentration or quality, but rather by soil microclimate and MB only. Surprisingly, the medium term loss in SOC content induced by the four years climate manipulation could not be linked to any microbial data assessed in this study. This underlines the difficulties to relate microbial information with the actual dynamics of soil carbon concentration. More research is needed on the

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

Acknowledgements

This work has been funded by Irstea, by the CCES (Competence Center Environment and Sustainability of the ETH Domain, Switzerland) as part of the Mountland project, and supported by a grant from Labex OSUG@2020 (Investissements d'avenir – ANR10 LABX56) and by a grant from the French Ministry of Higher Education and Research (Ph.D. thesis of JP, EDISCE Doctoral School). BJMR was supported through the Netherlands Organization for Scientific Research (NWO; Research Innovation Scheme 863.10.014). The Laboratoire d'Ecologie Alpine (LECA) and T. Goïtré (Irstea) are thanked for providing the equipment and help for soil enzymes measurements. Two anonymous reviewers are thanked for their constructive comments which strongly improved this paper.

References

- Adam, G., Duncan, H., 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology and Biochemistry* 33, 943–951.
- Allison, S.D., 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecology Letters* 8, 626–635. doi:10.1111/j.1461-0248.2005.00756.x
- Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* 14, 2898–2909. doi:10.1111/j.1365-2486.2008.01716.x

- 642 Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to
643 warming dependent on microbial physiology. *Nature Geoscience* 3, 336–340.
644 doi:10.1038/ngeo846
- 645 Alster, C.J., German, D.P., Lu, Y., Allison, S.D., 2013. Microbial enzymatic
646 responses to drought and to nitrogen addition in a southern California grassland.
647 *Soil Biology and Biochemistry* 64, 68–79. doi:10.1016/j.soilbio.2013.03.034
- 648 Bahn, M., Rodeghiero, M., Anderson-Dunn, M., Dore, S., Gimeno, C., Drösler, M.,
649 Williams, M., Ammann, C., Berninger, F., Flechard, C., Jones, S., Balzarolo, M.,
650 Kumar, S., Newesely, C., Priwitzer, T., Raschi, A., Siegwolf, R., Susiluoto, S.,
651 Tenhunen, J., Wohlfahrt, G., Cernusca, A., 2008. Soil Respiration in European
652 Grasslands in Relation to Climate and Assimilate Supply. *Ecosystems* 11, 1352–
653 1367. doi:10.1007/s10021-008-9198-0
- 654 Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T., Valášková,
655 V., 2013. Responses of the extracellular enzyme activities in hardwood forest to
656 soil temperature and seasonality and the potential effects of climate change. *Soil*
657 *Biology and Biochemistry* 56, 60–68. doi:10.1016/j.soilbio.2012.01.020
- 658 Bardgett, R.D., Bowman, W.D., Kaufmann, R., Schmidt, S.K., 2005. A temporal
659 approach to linking aboveground and belowground ecology. *Trends in Ecology &*
660 *Evolution* 20, 634–41. doi:10.1016/j.tree.2005.08.005
- 661 Bardgett, R.D., Lovell, R.D., Hobbs, P.J., Jarvis, S.C., 1999. Seasonal changes in
662 soil microbial communities along a fertility gradient of temperate grasslands. *Soil*
663 *Biology and Biochemistry* 31, 1021–1030.

- 664 Bell, T.H., Klironomos, J.N., Henry, H.A.L., 2010. Seasonal Responses of
665 Extracellular Enzyme Activity and Microbial Biomass to Warming and Nitrogen
666 Addition. *Soil Science Society of America Journal* 74, 820.
667 doi:10.2136/sssaj2009.0036
- 668 Bengtson, P., Bengtsson, G., 2007. Rapid turnover of DOC in temperate forests
669 accounts for increased CO₂ production at elevated temperatures. *Ecology letters*
670 10, 783–90. doi:10.1111/j.1461-0248.2007.01072.x
- 671 Björk, R.G., Björkman, M.P., Andersson, M.X., Klemedtsson, L., 2008. Temporal
672 variation in soil microbial communities in Alpine tundra. *Soil Biology and*
673 *Biochemistry* 40, 266–268. doi:10.1016/j.soilbio.2007.07.017
- 674 Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and
675 purification. *Canadian Journal of Biochemistry and Physiology* 37, 911–917.
- 676 Bond-Lamberty, B., Thomson, A., 2010. A global database of soil respiration data.
677 *Biogeosciences* 7, 1915–1926. doi:10.5194/bg-7-1915-2010
- 678 Börjesson, G., Sundh, I., Tunlid, A., Svensson, B.H., 1998. Methane oxidation in
679 landfill cover soils, as revealed by potential oxidation measurements and
680 phospholipid fatty acid analyses. *Soil Biology and Biochemistry* 30, 1423–1433.
- 681 Bradford, M.A., 2013. Thermal adaptation of decomposer communities in
682 warming soils. *Frontiers in Microbiology* 4, 333. doi:10.3389/fmicb.2013.00333
- 683 Brookes, P.C., Kemmitt, S.J., Addiscott, T.M., Bird, N., 2009. Reply to Kuzyakov
684 et al.’s comments on our paper: “Kemmitt, S. Lanyon, C. V., Waite, I.S., Wen, Q.,
685 O’Donnell, A.G., Brookes, P.C., 2008. Mineralization of native soil organic matter

686 is not regulated by the size, activity or composition of the soil microbial biomass –
 687 a new perspective 40, 61-73”. Soil Biology and Biochemistry 41, 440–443.
 688 doi:10.1016/j.soilbio.2008.09.002

689 Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform
 690 fumigation and the release of soil nitrogen: A rapid direct extraction method to
 691 measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17,
 692 837–842.

693 Budge, K., Leifeld, J., Egli, M., Fuhrer, J., 2011. Soil microbial communities in
 694 (sub)alpine grasslands indicate a moderate shift towards new environmental
 695 conditions 11 years after soil translocation. Soil Biology and Biochemistry 43,
 696 1148–1154. doi:10.1016/j.soilbio.2011.02.001

697 Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E.,
 698 Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a
 699 changing environment: Current knowledge and future directions. Soil Biology
 700 and Biochemistry 58, 216–234. doi:10.1016/j.soilbio.2012.11.009

701 C2SM, 2011. Swiss Climate Change Scenarios CH2011. doi:10.3929/ethz-a-
 702 006720559

703 Cécillon, L., Cassagne, N., Czarnes, S., Gros, R., Brun, J.-J., 2008. Variable
 704 selection in near infrared spectra for the biological characterization of soil and
 705 earthworm casts. Soil Biology and Biochemistry 40, 1975–1979.
 706 doi:10.1016/j.soilbio.2008.03.016

- 707 Chowdhury, T.R., Dick, R.P., 2012. Standardizing methylation method during
708 phospholipid fatty acid analysis to profile soil microbial communities. *Journal of*
709 *Microbiological Methods* 88, 285–291.
- 710 Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson,
711 P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R.,
712 Kirschbaum, M.U.F., Lavalley, J.M., Leifeld, J., Parton, W.J., Megan Steinweg,
713 J., Wallenstein, M.D., Martin Wetterstedt, J.Å., Bradford, M.A., 2011.
714 Temperature and soil organic matter decomposition rates - synthesis of current
715 knowledge and a way forward. *Global Change Biology* 17, 3392–3404.
716 doi:10.1111/j.1365-2486.2011.02496.x
- 717 Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon
718 decomposition and feedbacks to climate change. *Nature* 440, 165–73.
719 doi:10.1038/nature04514
- 720 Djukic, I., Zehetner, F., Watzinger, A., Horacek, M., Gerzabek, M.H., 2013. In
721 situ carbon turnover dynamics and the role of soil microorganisms therein: a
722 climate warming study in an Alpine ecosystem. *FEMS Microbiology Ecology* 83,
723 112–24. doi:10.1111/j.1574-6941.2012.01449.x
- 724 Dray, S., Dufour, A.B., 2007. The ade4 Package: Implementing the Duality
725 Diagram for Ecologists. *Journal of Statistical Software* 22, 1 – 20.
- 726 Erman, D.O.P.G., Hacon, S.T.S.C., 2011. Substrate concentration and enzyme
727 allocation can affect rates of microbial decomposition. *Ecology* 92, 1471–1480.

- 728 Floch, C., Alarcon-Gutiérrez, E., Criquet, S., 2007. ABTS assay of phenol oxidase
729 activity in soil. *Journal of Microbiological Methods* 71, 319–24.
730 doi:10.1016/j.mimet.2007.09.020
- 731 Frei, C., Schöll, R., Fukutome, S., Schmidli, J., Vidale, P.L., 2006. Future change
732 of precipitation extremes in Europe: Intercomparison of scenarios from regional
733 climate models. *Journal of Geophysical Research* 111, D06105.
734 doi:10.1029/2005JD005965
- 735 Frey, S.D., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional
736 capacity, and community structure after 12 years of soil warming. *Soil Biology
737 and Biochemistry* 40, 2904–2907. doi:10.1016/j.soilbio.2008.07.020
- 738 Gavazov, K., 2013. Subalpine Pasture-Woodlands in a Changing Climate: the
739 Role of Land Use Type in Ecosystem Resistance to Simulated Climate Change.
740 Thesis EPFL 5989. Lausanne, Switzerland.
- 741 Gavazov, K., Peringer, A., Buttler, A., Gillet, F., Spiegelberger, T., 2013.
742 Dynamics of Forage Production in Pasture-woodlands of the Swiss Jura
743 Mountains under Projected Climate Change Scenarios. *Ecology and Society* 18,
744 art38. doi:10.5751/ES-04974-180138
- 745 Gavazov, K., Spiegelberger, T., Buttler, A., 2014. Transplantation of subalpine
746 wood-pasture turfs along a natural climatic gradient reveals lower resistance of
747 unwooded pastures to climate change compared to wooded ones. *Oecologia* 174,
748 1425–35. doi:10.1007/s00442-013-2839-9

- 749 German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L.,
750 Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for
751 ecosystem studies. *Soil Biology and Biochemistry* 43, 1387–1397.
752 doi:10.1016/j.soilbio.2011.03.017
- 753 Green, V.S., Stott, D.E., Diack, M., 2006. Assay for fluorescein diacetate
754 hydrolytic activity: Optimization for soil samples. *Soil Biology and Biochemistry*
755 38, 693–701. doi:10.1016/j.soilbio.2005.06.020
- 756 Hassan, W., Chen, W., Cai, P., Huang, Q., 2013. Oxidative Enzymes, the
757 Ultimate Regulator: Implications for Factors Affecting Their Efficiency. *Journal*
758 *of Environmental Quality* 42, 1779–1790.
- 759 Henry, H.A.L., 2013. Reprint of “Soil extracellular enzyme dynamics in a
760 changing climate”. *Soil Biology and Biochemistry* 56, 53–59.
761 doi:10.1016/j.soilbio.2012.10.022
- 762 Hu, S., Chapin, F.S., Firestone, M.K., Field, C.B., Chiariello, N.R., 2001. Nitrogen
763 limitation of microbial decomposition in a grassland under elevated CO₂. *Nature*
764 409, 188–91. doi:10.1038/35051576
- 765 IUSS Working Group WRB. 2007. World Reference Base for Soil Resources 2006,
766 first update 2007. World Soil Resources Reports No. 103. FAO, Rome.
- 767 Jing, X., Wang, Y., Chung, H., Mi, Z., Wang, S., Zeng, H., He, J.-S., 2013. No
768 temperature acclimation of soil extracellular enzymes to experimental warming
769 in an alpine grassland ecosystem on the Tibetan Plateau. *Biogeochemistry* 117,
770 39–54. doi:10.1007/s10533-013-9844-2

- 771 Jingguo, W., Bakken, L.R., 1997. Competition for nitrogen during mineralization
772 of plant residues in soil: Microbial response to C and N availability. *Soil Biology
773 and Biochemistry* 29, 163–170. doi:10.1016/S0038-0717(96)00292-1
- 774 Kemmitt, S.J., Lanyon, C.V., Waite, I.S., Wen, Q., Addiscott, T.M., Bird, N.R.A.,
775 O'Donnell, A.G., Brookes, P.C., 2008. Mineralization of native soil organic matter
776 is not regulated by the size, activity or composition of the soil microbial
777 biomass—a new perspective. *Soil Biology and Biochemistry* 40, 61–73.
778 doi:10.1016/j.soilbio.2007.06.021
- 779 Lawrence, C.R., Neff, J.C., Schimel, J.P., 2009. Does adding microbial
780 mechanisms of decomposition improve soil organic matter models? A comparison
781 of four models using data from a pulsed rewetting experiment. *Soil Biology and
782 Biochemistry* 41, 1923–1934. doi:10.1016/j.soilbio.2009.06.016
- 783 Legendre, P., Gallagher, E., 2001. Ecologically meaningful transformations for
784 ordination of species data. *Oecologia* 129, 271–280. doi:10.1007/s004420100716
- 785 Lipson, D.A., Schmidt, S.K., 2004. Seasonal Changes in an Alpine Soil Bacterial
786 Community in the Colorado Rocky Mountains Seasonal Changes in an Alpine
787 Soil Bacterial Community in the Colorado Rocky Mountains. *Applied and
788 Environmental Microbiology* 70, 2867–2879. doi:10.1128/AEM.70.5.2867
- 789 Löffler, U.C.M., Cypionka, H., Löffler, J., 2008. Soil microbial activity along an
790 arctic-alpine altitudinal gradient from a seasonal perspective. *European Journal
791 of Soil Science* 59, 842–854. doi:10.1111/j.1365-2389.2008.01054.x

- 792 Marx, M., Wood, M., Jarvis, S., 2001. A microplate fluorimetric assay for the
793 study of enzyme diversity in soils. *Soil Biology and Biochemistry* 33, 1633–1640.
- 794 McGuire, K.L., Treseder, K.K., 2010. Microbial communities and their relevance
795 for ecosystem models: Decomposition as a case study. *Soil Biology and*
796 *Biochemistry* 42, 529–535. doi:10.1016/j.soilbio.2009.11.016
- 797 Meehl, G.A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye, J.M.
798 Gregory, A. Kitoh, R. Knutti, J.M. Murphy, A. Noda, S.C.B. Raper, I.G.
799 Watterson, A.J. Weaver and Z.-C. Zhao, 2007: Global Climate Projections. In:
800 Climate Change 2007: The Physical Science Basis. Contribution of Working
801 Group I to the Fourth Assessment Report of the Intergovernmental Panel on
802 Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B.
803 Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press,
804 Cambridge, United Kingdom and New York, NY, USA
- 805 Mills, R.T.E., Gavazov, K.S., Spiegelberger, T., Johnson, D., Buttler, A., 2014.
806 Diminished soil functions occur under simulated climate change in a sup-alpine
807 pasture, but heterotrophic temperature sensitivity indicates microbial resilience.
808 *Science of the Total Environment* 473-474, 465–72.
809 doi:10.1016/j.scitotenv.2013.12.071
- 810 NF ISO 10693, Soil quality., 1995. Determination of carbonate content.
811 Volumetric method. AFNOR.
- 812 NF ISO 10690, Soil quality., 2005. Determination of pH. AFNOR

- 813 NF ISO 10694, Soil quality., 1995. Determination of organic and total carbon
814 after dry combustion (elementary analysis). AFNOR.
- 815 NF ISO 11464, Soil quality., 2006. Pretreatment of samples for physico-chemical
816 analysis. AFNOR.
- 817 NF ISO 13878, Soil quality., 1995. Determination of total nitrogen content by dry
818 combustion ("elemental analysis"). AFNOR.
- 819 NF ISO 16586, Soil quality., 2003. Determination of soil water content as a
820 volume fraction on the basis of known dry bulk density - Gravimetric method.
821 AFNOR.
- 822 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L.,
823 Solymos, P., Stevens, M.H.H., Wagner, H., 2013. vegan: Community Ecology
824 Package. R package version 2.0-10. <http://CRAN.R-project.org/package=vegan>
- 825 Park, J.-H., Meusburger, K., Jang, I., Kang, H., Alewell, C., 2014. Erosion-
826 induced changes in soil biogeochemical and microbiological properties in Swiss
827 Alpine grasslands. *Soil Biology and Biochemistry* 69, 382–392.
828 doi:10.1016/j.soilbio.2013.11.021
- 829 Pascault, N., Cécillon, L., Mathieu, O., Hénault, C., Sarr, A., Lévêque, J., Farcy,
830 P., Ranjard, L., Maron, P.-A., 2010. In situ dynamics of microbial communities
831 during decomposition of wheat, rape, and alfalfa residues. *Microbial Ecology* 60,
832 816–828. doi:10.1007/s00248-010-9705-7

- 833 Paterson, E., 2009. Comments on the regulatory gate hypothesis and implications
834 for C-cycling in soil. *Soil Biology and Biochemistry* 41, 1352–1354.
835 doi:10.1016/j.soilbio.2009.02.012
- 836 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core, T.R., 2014. nlme: Linear and
837 Nonlinear Mixed Effects Models. R package version 3.1-117, [http://CRAN.R-](http://CRAN.R-project.org/package=nlme)
838 [project.org/package=nlme](http://CRAN.R-project.org/package=nlme).
- 839 Rinnan, R., Michelsen, A., Bååth, E., Jonasson, S., 2007. Fifteen years of climate
840 change manipulations alter soil microbial communities in a subarctic heath
841 ecosystem. *Global Change Biology* 13, 28–39. doi:10.1111/j.1365-
842 2486.2006.01263.x
- 843 Robroek, B.J.M., Heijboer, A., Jassey, V.E.J., Hefting, M.M., Rouwenhorst, T.G.,
844 Buttler, A., Bragazza, L., 2012. Snow cover manipulation effects on microbial
845 community structure and soil chemistry in a mountain bog. *Plant and Soil* 369,
846 151–164. doi:10.1007/s11104-012-1547-2
- 847 Saccone, P., Morin, S., Baptist, F., Bonneville, J.-M., Colace, M.-P., Domine, F.,
848 Faure, M., Geremia, R., Lochet, J., Poly, F., Lavorel, S., Clément, J.-C., 2012. The
849 effects of snowpack properties and plant strategies on litter decomposition during
850 winter in subalpine meadows. *Plant and Soil* 363, 215–229. doi:10.1007/s11104-
851 012-1307-3
- 852 Saenger, A., Cécillon, L., Sebag, D., Brun, J.-J., 2013. Soil organic carbon
853 quantity, chemistry and thermal stability in a mountainous landscape: A Rock–
854 Eval pyrolysis survey. *Organic Geochemistry* 54, 101–114.
855 doi:10.1016/j.orggeochem.2012.10.008

- 856 Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., Zechmeister-
857 Boltenstern, S., 2011. Experimental warming effects on the microbial community
858 of a temperate mountain forest soil. *Soil Biology and Biochemistry* 43, 1417–
859 1425. doi:10.1016/j.soilbio.2011.03.005
- 860 Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics
861 of soil. *Soil Biology and Biochemistry* 42, 391–404.
862 doi:10.1016/j.soilbio.2009.10.014
- 863 Sjögersten, S., Alewell, C., Cécillon, L., Hagedorn, F., Jandl, R., Leifeld, J.,
864 Martinsen, V., Schindlbacher, A., Sebastià, M.-T., van Miegroet, H., 2011.
865 Mountain soils in a changing climate – vulnerability of C stocks and ecosystem
866 feedbacks, in: *Soil Carbon in Sensitive European Ecosystems: From Science to*
867 *Land Management*. Wiley-Blackwell. pp. 118–148.
- 868 Stauffer, M., Leyval, C., Brun, J.-J., Leportier, P., Berthelin, J., 2014. Effect of
869 willow short rotation coppice on soil properties after three years of growth as
870 compared to forest, grassland and arable land uses. *Plant and Soil* 377, 423–438.
871 doi:10.1007/s11104-013-1986-4
- 872 Steger, K., Jarvis, Å., Smårs, S., Sundh, I., 2003. Comparison of signature lipid
873 methods to determine microbial community structure in compost. *Journal of*
874 *Microbiological Methods* 55, 371–382.
- 875 Steinweg, J.M., Dukes, J.S., Paul, E.A., Wallenstein, M.D., 2013. Microbial
876 responses to multi-factor climate change: effects on soil enzymes. *Frontiers in*
877 *Microbiology* 4, 146. doi:10.3389/fmicb.2013.00146

- 878 Stevenson, B.A., Hunter, D.W.F., Rhodes, P.L., 2014. Temporal and seasonal
879 change in microbial community structure of an undisturbed, disturbed, and
880 carbon-amended pasture soil. *Soil Biology and Biochemistry*.
881 doi:10.1016/j.soilbio.2014.04.010
- 882 Sundh, I., Nilsson, M., Borga, P., 1997. Variation in microbial community
883 structure in two boreal peatlands as determined by analysis of phospholipid
884 Fatty Acid profiles. *Applied and Environmental Microbiology* 63, 1476–1482.
- 885 Tan, B., Wu, F., Yang, W., He, X., 2014. Snow removal alters soil microbial
886 biomass and enzyme activity in a Tibetan alpine forest. *Applied Soil Ecology* 76,
887 34–41. doi:10.1016/j.apsoil.2013.11.015
- 888 Ullrich, R., Nüske, J., 2004. Novel haloperoxidase from the agaric basidiomycete
889 *Agrocybe aegerita* oxidizes aryl alcohols and aldehydes. *Applied and*
890 *Environmental Microbiology* 70, 4575–4581. doi:10.1128/AEM.70.8.4575–
891 4581.2004
- 892 Vanhala, P., Karhu, K., Tuomi, M., Björklöf, K., Fritze, H., Hyvärinen, H., Liski,
893 J., 2011. Transplantation of organic surface horizons of boreal soils into warmer
894 regions alters microbiology but not the temperature sensitivity of decomposition.
895 *Global Change Biology* 17, 538–550. doi:10.1111/j.1365-2486.2009.02154.x
- 896 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for
897 measuring soil microbial biomass C. *Soil Biology and Biochemistry*. 19, 703-707
898 doi:10.1016/0038-0717(87)90052-6

- 899 Waldrop, M.P., Firestone, M.K., 2006. Seasonal dynamics of microbial community
900 composition and function in oak canopy and open grassland soils. *Microbial*
901 *Ecology* 52, 470–9. doi:10.1007/s00248-006-9100-6
- 902 Wallenstein, M.D., McMahon, S.K., Schimel, J.P., 2009. Seasonal variation in
903 enzyme activities and temperature sensitivities in Arctic tundra soils. *Global*
904 *Change Biology* 15, 1631–1639. doi:10.1111/j.1365-2486.2008.01819.x
- 905 Wang, G., Post, W.M., Mayes, M.A., 2013. Development of microbial-enzyme-
906 mediated decomposition model parameters through steady-state and dynamic
907 analyses. *Ecological Applications* 23, 255–72.
- 908 Weedon, J.T., Aerts, R., Kowalchuk, G.A., van Bodegom, P.M., 2011. Enzymology
909 under global change: organic nitrogen turnover in alpine and sub-Arctic soils.
910 *Biochemical Society Transactions* 39, 309–14. doi:10.1042/BST0390309
- 911 Yao, H., Bowman, D., Shi, W., 2011. Seasonal variations of soil microbial biomass
912 and activity in warm- and cool-season turfgrass systems. *Soil Biology and*
913 *Biochemistry* 43, 1536–1543. doi:10.1016/j.soilbio.2011.03.031
- 914 Zeglin, L.H., Bottomley, P.J., Jumpponen, a, Rice, C.W., Arango, M., Lindsley, a,
915 McGowan, a, Mfombep, P., Myrold, D.D., 2013. Altered precipitation regime
916 affects the function and composition of soil microbial communities on multiple
917 time scales. *Ecology* 94, 2334–45.
- 918 Zhang, W., Parker, K.M., Luo, Y., Wan, S., Wallace, L.L., Hu, S., 2005a. Soil
919 microbial responses to experimental warming and clipping in a tallgrass prairie.
920 *Global Change Biology* 11, 266–277. doi:10.1111/j.1365-2486.2005.00902.x

921 **Tables**

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

922

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

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

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

928

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

932

933

934

935

936

937

938

939

Figure captions

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

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

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

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

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

965

966

967

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

author-produced version of the article published in: Soil biology and biochemistry, vol 80, p.296-305														
	sampling date		altitude		sampling date		autumn		winter		spring		summer	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Soil Characteristics														
Soil moisture	161.0	***	11.0	**	1.1	0.4	2.7	0.1	3.6	0.1	4.5	*	23.2	***
Soil field moisture (3 cm)	120.0	***	6.3	*	2.0	0.1	3.4	0.1	1.2	0.3	9.5	**	11.1	**
Soil temperature	5033.0	***	100.0	***	69.0	***	138.0	***	1.2	0.4	91.6	***	101.1	***
pH [H2O]	1.7	0.2	4.6	*	3.8	**	7.2	**	0.6	0.6	1.5	0.3	0.8	0.5
Organic carbon	0.0	1.0	7.6	**	0.6	0.8	1.5	0.3	4.3	*	7.0	*	4.3	*
Organic nitrogen	4.1	*	6.6	*	5.7	**	4.9	*	4.2	*	6.7	*	6.2	*
C/N ratio	10.3	***	1.8	0.2	7.0	***	10.1	**	0.8	0.5	0.1	0.9	2.1	0.2
Microbial biomass (MB)	9.6	***	14.4	***	0.9	0.5	-	-	2.1	0.2	9.9	**	10.6	**
F/B ratio	12.3	**	0.3	0.6	0.0	0.9	-	-	0.3	0.6	-	-	0.2	0.7
EEA														
LAP	51.0	***	4.6	*	4.2	**	2.5	0.1	7.7	**	5.2	*	5.4	*
CB	192.0	***	2.1	0.2	2.2	0.1	8.0	*	0.4	0.7	1.0	0.4	4.3	*
NAG	149.0	***	7.4	**	0.9	0.5	1.7	0.2	5.7	*	2.6	0.1	4.3	*
FDA	278.0	***	1.5	0.3	2.9	*	0.2	0.8	8.3	**	2.3	0.1	3.2	0.1
BG	82.0	***	8.7	**	0.5	0.8	4.5	*	1.2	0.3	3.8	0.1	1.7	0.2
BX	90.0	***	10.6	**	3.1	*	0.1	0.9	0.9	0.4	11.7	**	8.6	**
LIP	90.0	***	1.9	0.2	1.2	0.3	5.3	*	1.4	0.3	0.7	0.5	20.9	***
PHO	169.0	***	9.6	**	1.4	0.3	2.2	0.1	1.7	0.2	1.5	0.3	9.1	**
POX	53.0	***	14.0	***	2.7	*	15.7	***	11.5	**	6.6	*	4.8	*
Mass-specific EEA														
LAP	11.8	***	0.2	0.8	0.1	1.0	-	-	0.1	0.9	0.2	0.9	0.1	0.9
CB	21.3	***	3.7	*	0.8	0.5	-	-	1.8	0.2	1.4	0.3	4.6	*
NAG	4.5	*	0.0	1.0	1.2	0.3	-	-	0.8	0.5	0.1	0.9	0.9	0.5
FDA	35.7	***	6.3	*	0.8	0.6	-	-	0.6	0.6	8.5	**	4.0	*
BG	17.9	***	1.4	0.3	0.4	0.8	-	-	0.5	0.6	1.2	0.3	1.1	0.4
BX	35.2	***	3.2	0.1	1.3	0.3	-	-	0.7	0.5	3.0	0.1	3.4	0.1
LIP	21.3	***	3.0	0.1	1.9	0.1	-	-	0.6	0.6	10.0	**	4.6	*
PHO	3.4	*	8.4	**	0.4	0.8	-	-	1.7	0.2	2.8	0.1	5.1	*
POX	24.8	***	1.3	0.3	1.7	0.2	-	-	1.0	0.4	1.4	0.3	2.2	0.2

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

EEA on a dry soil mass basis (nKatal/g dry soil)