The effect of warming on the vulnerability of subducted organic carbon in arctic soils

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Highlights

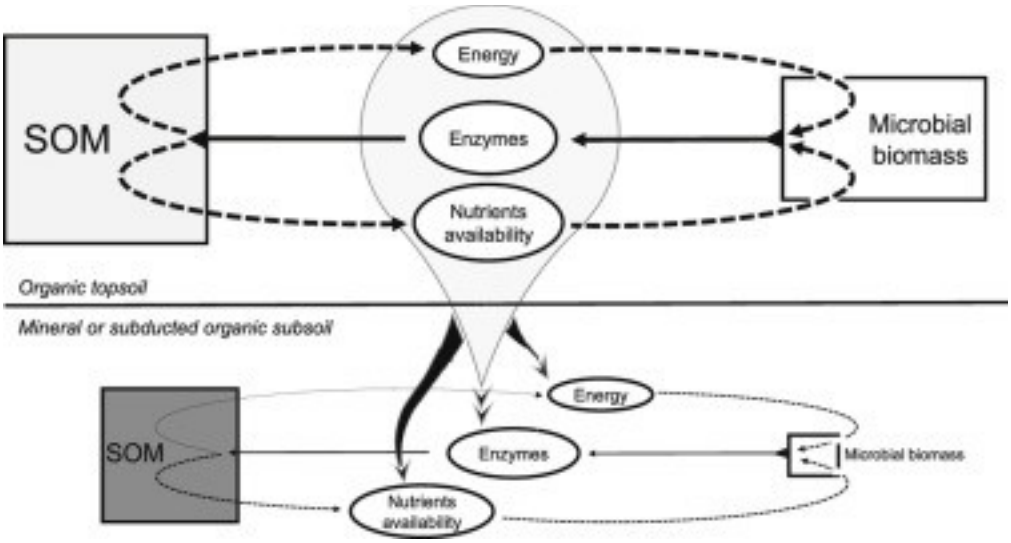
- Temperature sensitivity reflected thermodynamics of aerobic or anaerobic microbial metabolism.
- Subducted organic carbon decomposition was low due to low microbial biomass.
- Microbial biomass preferentially uses allochthonous resources.
- No effect of organic carbon quality on temperature sensitivity of carbon loss was observed.
- Subducted organic carbon is stable.

Abstract

Arctic permafrost soils contain large stocks of organic carbon (OC). Extensive cryogenic processes in these soils cause subduction of a significant part of OC-rich topsoil down into mineral soil through the process of cryoturbation. Currently, one-fourth of total permafrost OC is stored in subducted organic horizons. Predicted climate change is believed to reduce the amount of OC in permafrost soils as rising temperatures will increase decomposition of OC by soil microorganisms. To estimate the sensitivity of OC decomposition to soil temperature and oxygen levels we performed a 4-month incubation experiment in which we manipulated temperature (4–20 °C) and oxygen level of topsoil organic, subducted organic and mineral soil horizons. Carbon loss (C_{LOSS}) was

monitored and its potential biotic and abiotic drivers, including concentrations of available nutrients, microbial activity, biomass and stoichiometry, and extracellular oxidative and hydrolytic enzyme pools, were measured. We found that independently of the incubation temperature, C_{LOSS} from subducted organic and mineral soil horizons was one to two orders of magnitude lower than in the organic topsoil horizon, both under aerobic and anaerobic conditions. This corresponds to the microbial biomass being lower by one to two orders of magnitude. We argue that enzymatic degradation of autochthonous subducted OC does not provide sufficient amounts of carbon and nutrients to sustain greater microbial biomass. The resident microbial biomass relies on allochthonous fluxes of nutrients, enzymes and carbon from the OC-rich topsoil. This results in a “negative priming effect”, which protects autochthonous subducted OC from decomposition at present. The vulnerability of subducted organic carbon in cryoturbated arctic soils under future climate conditions will largely depend on the amount of allochthonous carbon and nutrient fluxes from the topsoil.

Graphical abstract



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Keywords

Subducted organic horizon
Soil carbon loss
Incubation
Temperature

Microbial biomass
Enzymes

1. Introduction

Soils in permafrost areas contain an estimated ~1300 ± 200 Pg of organic carbon (OC), of which ~500 Pg resides in non-permafrost soils or in deeper taliks or is seasonally thawed (i.e. in the “active layer”), while ~800 Pg is perennially frozen (Hugelius et al., 2014). Much of this OC is predicted to be vulnerable to extensive decomposition under warming climate conditions of the northern circumpolar region (Davidson and Janssens, 2006, Zimov et al., 2006, Schuur et al., 2008, Schuur et al., 2009). Several studies in the arctic have already shown increasing carbon loss from upper top and permanently frozen soil horizons under higher temperatures (Oechel et al., 1993, Schuur et al., 2009, Schädel et al., 2014). As well as rising temperatures, recent model scenarios predict an increase of precipitation and the occurrence of more numerous anaerobic sites, which can lead to methane production and release of additional carbon from permafrost-affected soils (Olefeldt et al., 2013). Therefore, both aerobic and anaerobic carbon transformation processes need to be included in predictions of OC vulnerability to decomposition.

Permafrost soils are extensively affected by cryogenic processes (repeated freeze and thaw cycles of the active layer), which result in subduction of carbon rich topsoil organic horizons deeper into the soil profile (Bockheim and Tarnocai, 1998). The amount of OC in subducted organic horizons can make up 90% of total OC in the first meter of soil (Bockheim, 2007), and in total it represents approximately one-fourth of all OC currently stored in permafrost soils (Harden et al., 2012). Recent data indicates lower OC quality and distinctly different microbial community composition and enzyme activities of subducted organic horizons in comparison with topsoil organic horizons (Harden et al., 2012, Gittel et al., 2014, Schnecker et al., 2014, Gentsch et al., 2015a, Gentsch et al., 2015b), which presumably is the cause of the retarded decomposition of subducted OC previously observed (Kaiser et al., 2007, Wild et al., 2014). As a result, the age of organic C in cryoturbated organic pockets could reach several thousand years (Bockheim, 2007, Kaiser et al., 2007, Hugelius et al., 2014, Palmtag et al., 2015). Although the effects of temperature and oxygen level on the rate of OC decomposition are generally well studied and many investigations have documented significant positive effects of both, specific studies on subducted OC are still scarce (Schädel et al., 2014). Without a direct manipulation study, the vulnerability of subducted OC decomposition to warming is currently impossible to predict from these findings. The effect of temperature

on OC decomposition is not uniform across published studies because it is confounded by other factors such as oxygen level, OC quality, nutrients, microbial physiology and enzymatic performance (e.g. Giardina and Ryan, 2000, Brown et al., 2004, Hyvonen et al., 2005, Conant et al., 2008, Allen and Gillooly, 2009, Allison et al., 2010, Davidson et al., 2012, Steinweg et al., 2013). Because of such multifactorial control, no general mechanism of the temperature effect on OC decomposition has become widely accepted (Reichstein et al., 2005, Agren and Wetterstedt, 2007, Allison et al., 2010, Sierra, 2012). According to kinetic theory, the temperature sensitivity of OC decomposition is a function of OC quality (Knorr et al., 2005, Davidson and Janssens, 2006, Conant et al., 2008). The lower the OC quality, the higher is the temperature sensitivity as the decomposition of low quality OC requires more energy. According to metabolic theory, the temperature sensitivity of OC decomposition is determined by the temperature sensitivity of heterotrophic microbial metabolism and thus is independent of OC quality per se (Allen et al., 2005, Yvon-Durocher et al., 2012). Variability in temperature sensitivity of OC decomposition depends entirely on changes in the amount and physiology of microbial biomass, which might be induced by a multitude of different factors (for example, OC quality change). When estimating the effects of temperature and other abiotic or biotic factors on OC decomposition, it is necessary to include not only the effect of OC quality but also effects on microbial activity.

The main objective of the present study was to estimate the temperature sensitivity of OC decomposition in a subducted organic horizon under aerobic and anaerobic conditions and identify key factors determining this sensitivity. We hypothesize that the observed distinctly different composition of microbial communities, low OC quality and inadequate enzymatic activities in the subducted organic horizon pose the barrier for OC utilization by microbial biomass. We expect the increase of OC depolymerization by extracellular enzymes leading to an increase of carbon and nutrient supply to microbial biomass and its increase at higher temperatures. This will result in higher temperature sensitivity of OC decomposition in comparison with regular soil horizons. We further expect slower decomposition of subducted OC and lower temperature sensitivity under anaerobic conditions. To test these hypotheses we set up a 4-month incubation experiment, in which we manipulated the temperature and oxygen level of subducted organic, upper organic and lower mineral horizons. We determined soil carbon loss and the potential biotic and abiotic drivers of OC decomposition, including concentrations of available nutrients, microbial activity and its biomass and stoichiometry, and extracellular oxidative and hydrolytic enzyme pools.

2. Materials and methods

2.1. Soil sampling and preparation

Soil samples for the incubation experiment were collected from a shrubby moss tundra site on the Taymir peninsula, Russia (72°29.57′N, 101°38.62′E). This area is within a continuous permafrost zone. Active layer depth at the sampling site reached 65–90 cm in August 2011. Vegetation was dominated by *Cassiope tetragona*, *Carex arctisibirica* and *Aulacomnium turgidum*. The soil was classified as fine loamy to coarse loamy Typic Aquiturbel according to the US Soil Taxonomy (Soil Survey Staff, 1999) or as Turbic Cryosol according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2007). Bulk samples from three different horizons within the active layer were collected: topsoil organic material from an OA horizon at the surface (further referred to as O horizon), subducted organic material from an Ajj horizon, and mineral subsoil material from the BCg horizon, the latter two from a depth of 50–70 cm. The mineral subsoil material sampled did not include cryoturbated organic material. Living roots were removed from bulk samples after sampling and soil material was kept at 4 °C until processing. Bulk soil material was homogenized before the start of the laboratory incubation and assessed for basic chemical, physical and microbial characteristics (Table 1).

Table 1. Basic physical (*BD – bulk density*, *CEC – cation exchange capacity*, *$\delta^{13}C$ – soil carbon isotopic signature*), chemical (pHH2O – pH in water, pH_{KCl} – exchangeable pH, OC – total soil organic carbon, N_{TOT} – total soil nitrogen, C_{EX} – K_2SO_4 extractable organic carbon, DON – K_2SO_4 extractable organic nitrogen, NH4+ – K_2SO_4 extractable ammonium, NO3− – K_2SO_4 extractable nitrates, P_{EX} – $NaHCO_3$extractable phosphorus) and microbial (*C_{MB}– microbial carbon*, *N_{MB}– microbial nitrogen*, *P_{MB}– microbial phosphorus*) characteristics as well as stoichiometric parameters ($C:N_{TOT}$ – total soil organic carbon to total soil nitrogen ratio, $C:N_{MB}$– microbial carbon to nitrogen ratio, $C:P_{MB}$ – microbial carbon to phosphorus ratio) for organic (*O*), subducted organic (*Ajj*) and mineral (*BCg*) horizons. Numbers listed in the table are means with standard deviations in italic (*n = 3*). The label u.d. indicates a value below the detection limit of the method. The values where no standard deviations are listed were measured without replication.

Horizon	pHH2O	**pH_{KCl}**	**BD**	**CEC**	**OC**	**N_{TOT}**	**$\delta^{13}C$**	**C_{EX}**	**C_{MB}**
			g cm^{-3}	**meq kg^{-1}**	**%**	**%**	**[‰] vs. PDB**	**µmol g^{-1}**	
O	6.2	5.8	1.2	276.8	11.58	0.57	−27.62	28.18	174.07
		0.1		*17.4*	*0.23*	*0.01*	*0.17*	*2.36*	*3.19*
Ajj	6.3	6.3	1.4	150.5	3.97	0.15	−27.56	3.76	10.57
		0.2		*0.8*	*0.07*	*0.00*	*0.17*	*0.76*	*0.50*

Horizon	pHH2O	pH_{KCl}	BD	CEC	OC	N_{TOT}	$\delta^{13}C$	C_{EX}	C_{MB}
			g cm^{-3}	meq kg^{-1}	%	%	[‰] vs. PDB	μmol g^{-1}	
BCg	6.7	6.6	1.8	105.2	0.58	0.03	−26.84	1.22	2.21
		0.3		*4.4*	*0.01*	*0.00*	*0.25*	*0.73*	*0.36*

Horizon	DON	NH4+	NO3−	N_{MB}	P_{EX}	P_{MB}	$C:N_{TOT}$	$C:N_{MB}$	$C:P_{MB}$
	μmol g^{-1}						mol mol^{-1}		
O	7.25	1.99	0.09	9.33	0.21	2.82	20.42	18.66	61.69
	0.09	*0.00*	*0.00*	*0.09*	*0.01*	*0.07*	*0.21*	*0.47*	*2.43*
Ajj	u.d.	0.29	0.16	0.65	0.24	0.15	26.18	16.42	73.97
		0.00	*0.00*	*0.06*	*0.01*	*0.04*	*0.32*	*1.98*	*16.92*
BCg	u.d.	0.29	0.03	0.20	0.06	0.01	18.67	11.72	160.70
		0.00	*0.00*	*0.07*	*0.00*	*0.01*	*0.67*	*4.28*	*49.23*

2.2. Incubation setup

A 19 week-long incubation experiment was performed for each soil horizon (O, Ajj and BCg) at three different temperatures (4, 12 and 20 °C) and three moisture levels (50, 80 and 100% of water holding capacity; WHC) in four replicates. The two lower moisture treatments (50 and 80% WHC) used aerobic conditions, whereas the 100% WHC treatment used anaerobic conditions. Aerobic treatments were regularly flushed with moist air to maintain the oxygen concentration at the atmospheric level and to avoid oxygen limitation. For the anaerobic treatment, the headspaces of the incubation bottles were maintained anoxic by filling them with a He/CO_2 mixture (5% CO_2, 95% He). A CO_2 concentration of 5% was chosen to correspond with CO_2 concentrations commonly detected in anaerobic soils (Nobel and Palta, 1989). As a control, one bottle per temperature and oxygen treatment was incubated without soil. A more detailed description of the incubation setup is given in Supplementary Material and methods.

2.3. Gas analyses

Incubation bottles were kept closed during the whole incubation. CO_2 and CH_4 accumulation and O_2 consumption were measured weekly for the first 3 weeks and then bi-weekly during the rest of the incubation period (11 times in total). After determination of accumulated $CO_2$and CH_4 and consumed O_2, bottles were flushed with ambient air (aerobic bottles) or with 100% He amended with 5% CO_2 (anaerobic bottles). All three gases were measured again approx. 1 h after flushing to acquire

starting concentrations for the calculation of CO_2 and CH_4 production rates and O_2 consumption rate during the next interval.
During the measurements the headspace of incubation vessels was mixed, using a gas-tight membrane pump (KNF Laboport Mini Diaphragm Vacuum Pump, KNF Neuberger, INC., Trenton, USA) in order to remove any stratification of gas layers. The closed loop connecting incubation vessel and membrane pump was equipped with a sampling unit (SwageLok, Solon, USA) from which gas samples (0.2 ml) were taken with 1 ml syringes for immediate gas analysis. CO_2 and CH_4 were analyzed using a gas chromatograph (Agilent 7820A GC, Agilent Technologies, Santa Clara, USA) with a flow rate of 10 ml/min and an oven temperature of 40 °C and equipped with flame ionization and thermal conductivity detectors. Oxygen concentration was measured with an optical method using non-invasive optical oxygen sensors (PSt3, PreSens, Regensburg, Germany).

2.4. Chemical soil parameters

Soil pH was measured in extracts of 1 part soil to 5 parts water. The effective cation exchange capacity (CEC) was determined as the sum of exchangeable base cations (BC_{ex} = sum of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and exchangeable acidity (the sum of Al3+ex and H+ex), each multiplied by the respective number of charges per ion, according to Thomas (1982). The amounts of total soil organic carbon (OC) and of total soil nitrogen (N_{TOT}) were measured using an NC 2100 soil analyzer (Thermo Quest Italia S.p.A., Rodano, MI). For $\delta\ ^{13}C$ determination, an elemental analyzer (Vario micro cube, Elementar Analysen System GmbH, Germany) coupled to an isotope ratio mass spectrometer (IR-MS DELTA plus XL, Finnigan, Germany) was used. Concentrations of available carbon, nitrogen and phosphorus were measured according to Vance et al., 1987, Brookes et al., 1985, Brookes et al., 1982, respectively. Soil samples were free of inorganic carbon (Gentsch et al., 2015a). Details of the analytical procedures used are given in Supplementary Material and methods.

2.5. Microbial biomass and enzyme activities

Microbial carbon, nitrogen and phosphorus concentrations (C_{MB}, N_{MB}, P_{MB}) were estimated by chloroform-fumigation extraction (Brookes et al., 1982, Brookes et al., 1985, Vance et al., 1987). Details are given in Supplementary Material and methods.
Potential extracellular enzyme activities were determined for seven soil enzymes responsible for organic carbon, nitrogen and phosphorus processing. Because the activities of extracellular enzymes were determined under standardized conditions

(unbuffered water extracts at 20 °C) they should be considered as proxies of the enzyme pools. For details on the determination of enzyme activities please refer to Supplementary Material and methods.

The sum of all measured potential enzymatic activities describes the total enzymatic pool in the soil (E_{CNP}). Within this pool there are different classes of enzymes, which we divided into categories according to their product formation with respect to microbial nutrient acquisition. The sum of β-glucosidases and cellobiosidases defines the inherent category of carbon acquisition enzymes (E_C). The sum of leucine and alanine aminopeptidases defines the inherent category of nitrogen acquisition enzymes (E_N), and the sum of phosphatases and phosphodiestarases defines the category of phosphorus acquisition enzymes (E_P). Phenoloxidases are a special case of oxidative enzymes, which can degrade lignin-like compounds and by doing so may serve carbon and nitrogen acquisition (Godbold et al., 2006, Fontaine et al., 2007, Sinsabaugh and Shah, 2012). We treat these enzymes separately as a special case within E_{CNP}.

2.6. Statistical analyses and data evaluation

There was no significant difference between the moisture treatments at 50 and 80% WHC in any of the biochemical or chemical characteristics and gas exchange rates. Therefore, the data from these two moisture treatments was pooled for statistical analyses (further referred to as ‘aerobic treatment’). By doing so, we designed a complete factorial design of the experiment with two treatments differing in oxygen status (aerobic treatment: $n = 8$, and anaerobic treatment: $n = 4$) within each horizon and temperature treatment.

Cumulative carbon loss (C_{LOSS}) from the soil, as a measure of OC decomposability, was calculated as the sum of CO_2 and CH_4 production integrated over the incubation period. A simple exponential function was used to describe C_{LOSS} as a function of temperature:

$$CLOSS=R.ea.T,$$

where T is temperature and R and a are function parameters. Q_{10} as a measure of the temperature sensitivity of C_{LOSS} was calculated from the exponential function as follows:

$$Q10=ea.10,$$

Q_{10} expresses the relative change of C_{LOSS} with a 10 °C increase. To allow direct comparison of temperature sensitivity between soil horizons, we tested the effect of soil horizon on a parameter with nonlinear mixed-effect models, using the program R (R Core Team, 2014) and package nlme (Pinhero et al., 2013). We tested the statistical difference by comparing exponential functions having a parameter fixed for all horizons,

separately estimated for each horizon or randomly varying among horizons. For the comparison we used the Akaike information criterion (AIC).

Absolute differences in C_{LOSS} between horizons, temperature and oxygen status treatments were evaluated by 3-way ANOVA. C_{LOSS} data were log-transformed and normality checked with the Shapiro–Wilk test. To evaluate the differences between the individual treatments we used the post-hoc Tukey HSD test. Since the effect of soil horizon on C_{LOSS} was (in terms of explained variability) much greater than the effects of temperature and oxygen status, the ANOVA was followed by multiple linear regression analysis to find the best predictors of C_{LOSS} across horizons. The best statistical model was chosen from all measured parameters (chemical parameters, microbial parameters and enzyme potential activities), temperature and oxygen status by applying a stepwise algorithm. Because multi-collinearity in soil chemical parameters, microbial parameters or enzyme potential activities often occurs across horizons, ridge regression was used to avoid unstable predictors. Ridge regression was carried out in R (R Core Team, 2014) using package MASS.

The relationship between temperature and oxygen consumption rate was described by a Gaussian model (Tuomi et al., 2008):

$$O2=R.ea.T+b.T2,$$

where O_2 is oxygen consumption rate, T is temperature in degrees Celsius and R, a and b are model parameters. This model allows calculating the temperature at which oxygen consumption rate is maximal (T_{MAX}):

$$TMAX=a-2.b$$

Above T_{MAX}, oxygen consumption rate decreases with temperature. The Gaussian model parameters were estimated using nonlinear mixed-effect models. The effects of chemical and biochemical variables on model parameters were tested. The best model fit was chosen based on the AIC.

For the aerobic treatments the respiration quotient (RQ) was calculated as the molar ratio of CO_2 production to O_2 consumption. An RQ value equal to 1 indicates degradation of simple organic compounds via the citric acid cycle. RQ values below 1 indicate degradation of reduced, more recalcitrant organic compounds, which need more oxygen to be oxidized (Dilly, 2003). RQ was evaluated using 3-way ANOVA with temperature, oxygen status and horizon as factors. Before the analysis, data were root-square transformed (RQ) and normality was checked with the Shapiro–Wilk test. To evaluate the differences between the individual treatments we used the post-hoc Tukey HSD test.

For the statistical evaluation of soil enzymatic classes, microbial biomass, carbon-to-nutrient ratios (C_{MB}, $C:N_{MB}$, $C:P_{MB}$) and soil nutrients (nitrates – $NO3^-$, ammonium ions – $NH4^+$, dissolved organic nitrogen – DON, potassium sulfate extractable carbon – C_{EX}, sodium bicarbonate extractable phosphorus – P_{EX}), generalized linear models with gamma distribution were used. Relationships between enzyme class potential activities and soil nutrients or microbial biomass were tested by linear regression analysis.

3. Results

3.1. Aerobic incubations

3.1.1. Microbial activity and soil carbon loss

Microbial activity, expressing itself as CO_2 production and O_2 consumption rates, was constant over time in all treatments of all horizons throughout the 4-month incubation period, as shown by the constant slopes of cumulative CO_2 production and O_2 consumption (Figs. S2 and S3). The total amount of CO_2 produced during incubation denotes the cumulative carbon loss (C_{LOSS}). C_{LOSS} over the incubation period was consistently higher, at all temperatures, in the O horizon, followed by the Ajj horizon and the BCg horizon ($F = 596.1$, $df = 2$, $p < 0.001$). C_{LOSS} from the O horizon was about ten times higher than from the Ajj horizon, which in turn was ten times higher than from the BCg horizon. The difference between horizons was so large that it accounted for 87% of all explained variability. Normalized to the amount of OC in the respective horizon, C_{LOSS} was still 5 times higher in the O horizon, compared to the BCg and Ajj horizons, which were similar to each other (Fig. 1). Linear regression combined with ridge regression revealed the amounts of carbon and phosphorus in soil microbial biomass as the best and most stable predictors of C_{LOSS} across horizons ($F = 4189.0$, $df = 1$, $p < 0.001$). The control exerted by the microbial biomass over C_{LOSS} is also indicated by the strong correlation between C_{MB} or P_{MB} and C_{LOSS} (Fig. 2). No such correlation, however, was found for N_{MB}.

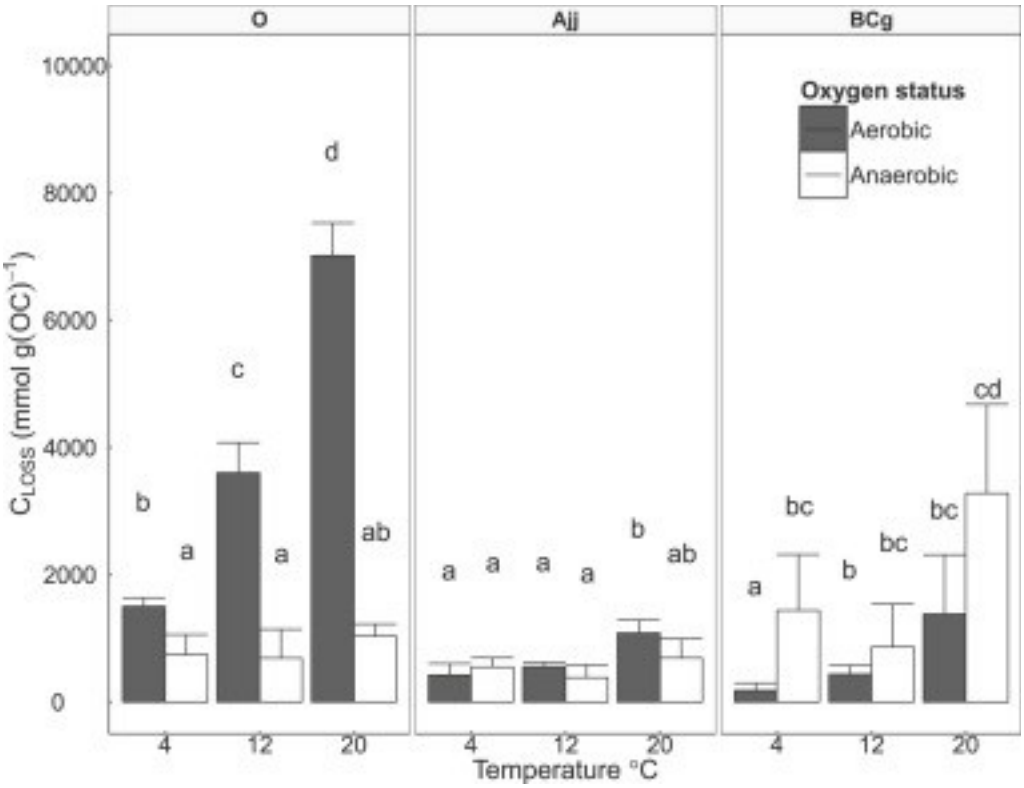

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Fig. 1. Cumulative soil carbon loss (C_{LOSS}) from the organic (O), subducted organic (Ajj) and mineral (BCg) horizon, respectively, in 6 different incubation treatments (combinations of 3 temperatures and 2 oxygen levels). Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard deviations. Results of post-hoc comparisons of means within each horizon are indicated by letters above the bars.

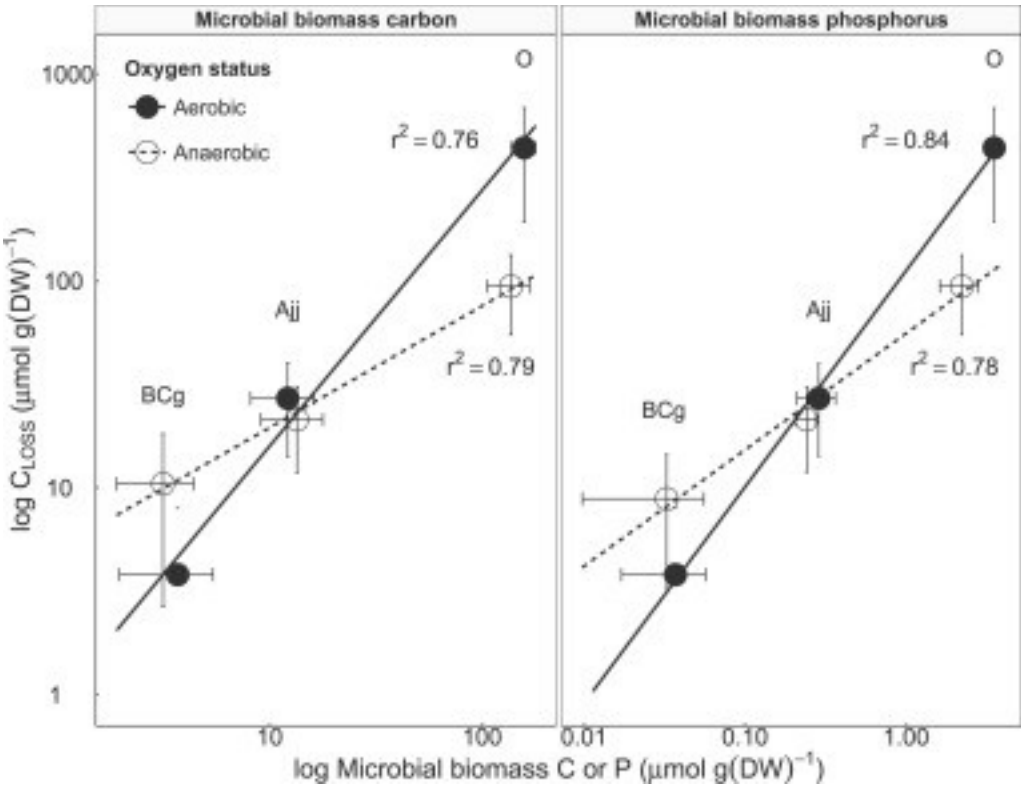

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Fig. 2. Correlations between cumulative soil carbon loss (C_{LOSS}) and concentration of microbial biomass carbon and phosphorus. Open symbols show C_{LOSS} from the organic (O), subducted organic (Ajj) and mineral (BCg) horizon incubated anaerobically, averaged over 3 different temperature treatments (4, 12 and 20 °C), filled symbols show the same for aerobic incubations. Error bars express standard deviation of the mean. Coefficients of determination are given in the plot. Note that both axes use logarithmic scales.

In all horizons, C_{LOSS} increased exponentially with temperature. Temperature sensitivity, expressed as Q_{10}, was statistically indistinguishable between horizons (Fig. S4), with an overall mean of 2.41.

A contrasting temperature sensitivity was, however, found for O_2 consumption. While oxygen consumption increased exponentially with temperature in the O horizon, in the Ajj and BCg horizons it increased only between 4 °C and 12 °C and then decreased again between 12 °C and 20 °C (Fig. 3a). This pattern was consistent during the whole incubation period (Fig. S3). T_{MAX} of O_2 consumption was estimated as 13.1 °C for the Ajj and 12.0 °C for the BCg horizon.

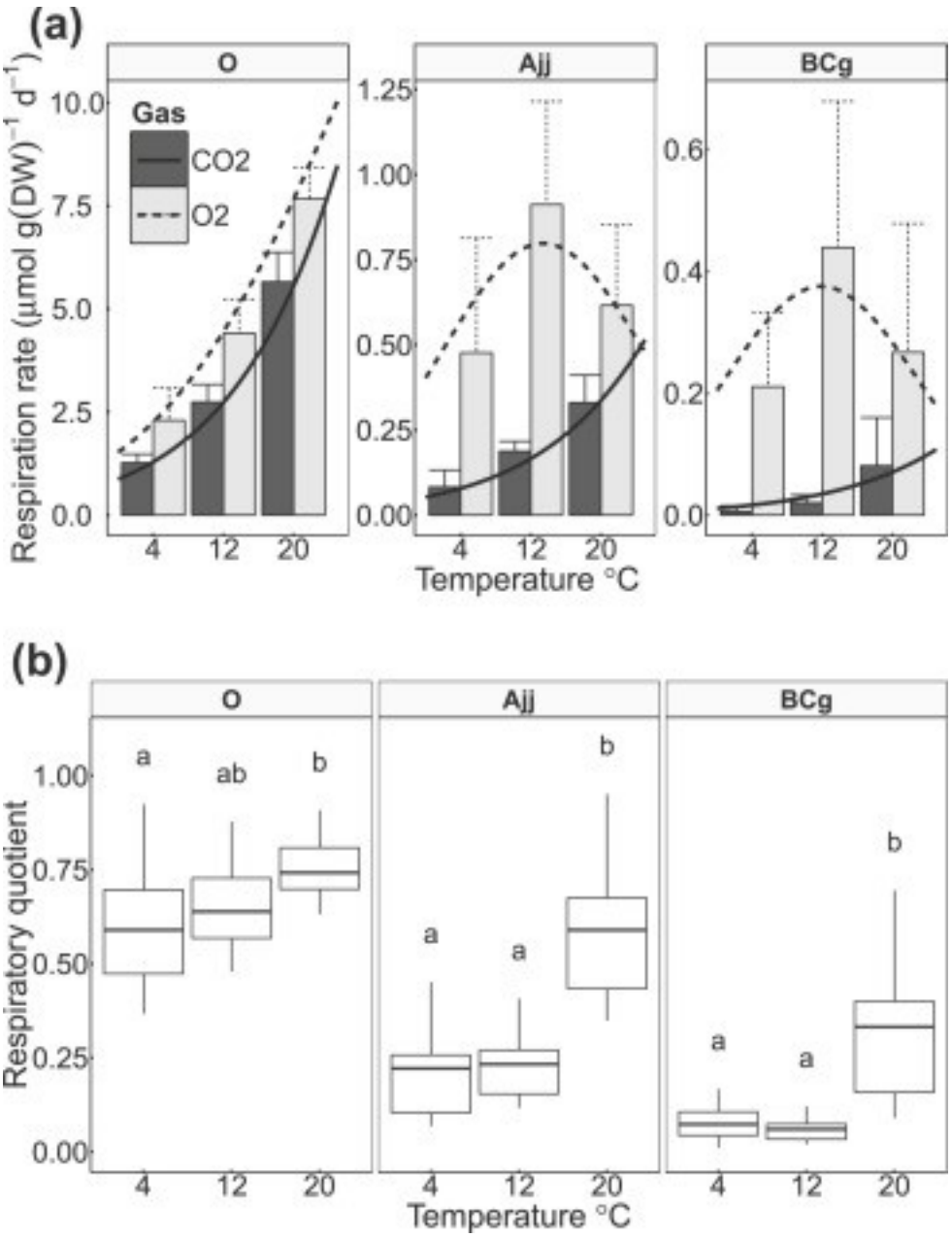

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Fig. 3. (a): Respiration rate in aerobic conditions estimated from CO_2 production (gray bars) or O_2consumption (black bars) in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 3 different temperature treatments. Bar heights represent means and error bars standard deviations. Solid and dashed lines indicate temperature trends according to exponential (CO_2 production) and Gaussian (O_2 production) functions, respectively. (b): Box plots of the ratio of CO_2 production to O_2 consumption rates. The middle line represents median, boxes comprise second and third quartiles, and Whiskers show the lowest datum still within 1.5 IQR of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile.

The respiration quotient (RQ = CO_2/O_2) was significantly higher in the O horizon than in the Ajj and BCg horizons (Fig. 3b). It continuously increased with temperature in the O horizon, but only in a narrow range from 0.59 to 0.74. RQ in the Ajj and BCg horizons significantly increased only between 12 °C and 20 °C, from 0.2 to 0.6 in the Ajj horizon and from 0.1 to 0.3 in the BCg horizon. These large increases were caused by the different temperature responses of CO_2 production and O_2 consumption (Fig. 3a).

3.1.2. Microbial biomass

The microbial biomass (C_{MB}) present at the start of the incubation was highest in the O horizon, with significantly and sequentially lower values in the Ajj and BCg horizons ($F = 454.9$, $df = 2$, $p < 0.001$), reflecting the trend observed for C_{EX} and nutrient contents (Table 1). Normalized to the amount of OC, C_{MB} was 4–5 times higher in the O horizon than in the BCg and Ajj horizons (Fig. 1). During the incubation, C_{MB} decreased at 20 °C in the O horizon ($F = 12.7$, $df = 5$, $p < 0.001$; Fig. 4a) and increased at 12 °C in the Ajj horizon ($F = 3.1$, $df = 5$, $p = 0.02$; Fig. 4a). In the BCg horizon, C_{MB} increased significantly at 12 °C and 20 °C ($F = 6.9$, $df = 2$, $p < 0.001$).

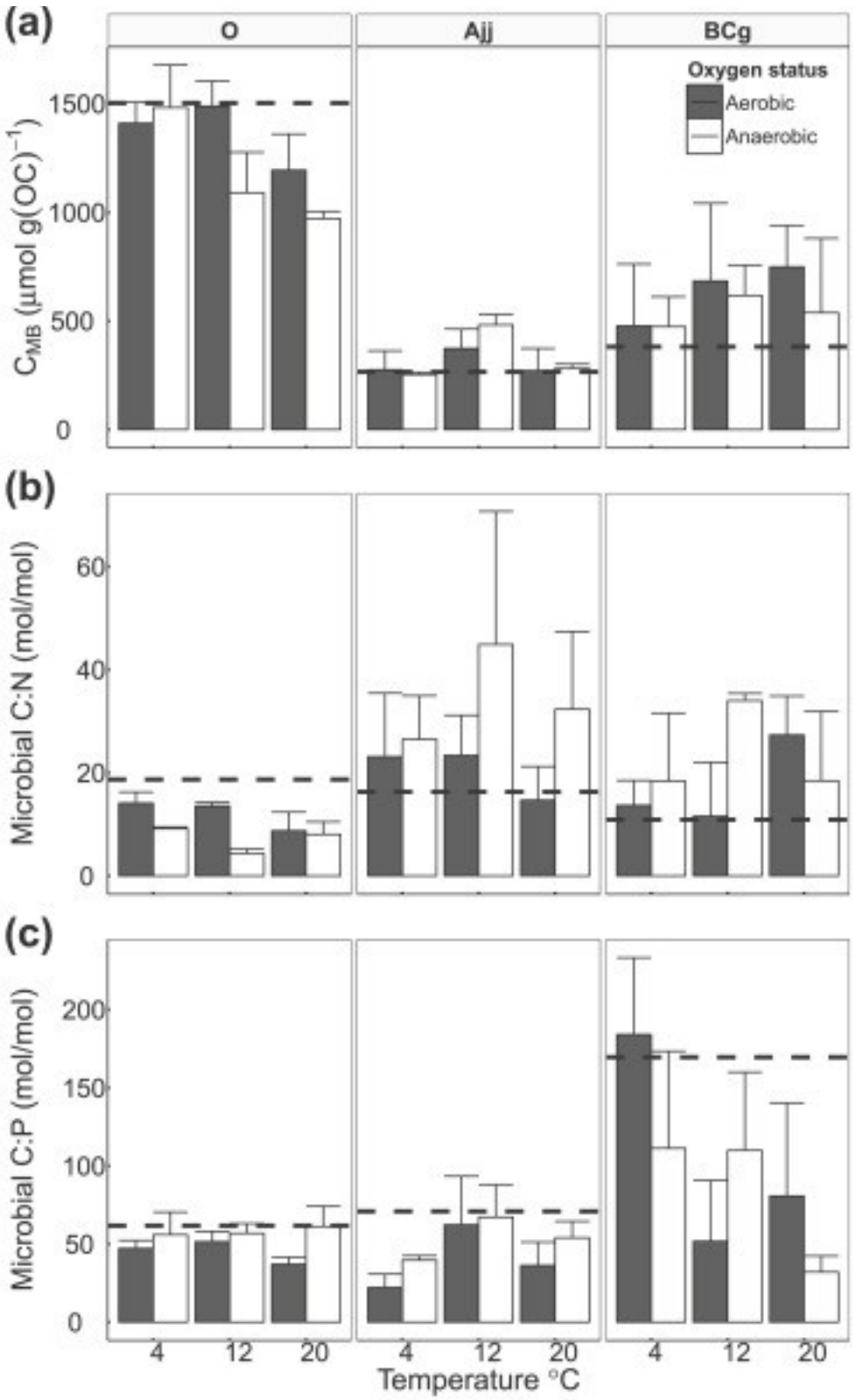

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Fig. 4. Microbial biomass carbon (a), microbial biomass C:N (b) and C:P (c) in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard deviations. Dashed horizontal lines show values at the start of the experiment.

Microbial biomass stoichiometry (Fig. 4b,c; $C:N_{MB}$, $C:P_{MB}$) differed between horizons, with higher initial $C:N_{MB}$ in the O horizon, and sequentially lower values in the Ajj and BCg horizons ($F = 24.4$, $df = 2$, $p < 0.001$) (Table 1). By contrast, initial $C:P_{MB}$ was highest in the BCg horizon, followed by lower values in the Ajj and then the O horizon ($F = 52.2$, $df = 2$, $p < 0.001$) (Table 1). $C:N_{MB}$ and $C:P_{MB}$ changed significantly during the incubation, with $C:P_{MB}$ decreasing in all three horizons, but significantly so only in the O horizon ($F = 176.1$, $df = 1$, $p < 0.001$). $C:N_{MB}$ showed horizon-specific responses: it decreased significantly in the O horizon ($F = 66.6$, $df = 1$, $p < 0.001$), whereas it increased in the Ajj horizon ($F = 7.0$, $df = 1$, $p = 0.011$). In the BCg horizon, $C:N_{MB}$ increased significantly over the incubation period only at 20 °C ($F = 14.3$, $df = 5$, $p < 0.001$) (Fig. 4b).

3.1.3. Soil enzymes

The total enzyme pool (E_{CNP}), per mol of microbial biomass, was highest in the BCg horizon, with sequentially lower values in the Ajj and O horizons, at the beginning of the incubation ($F = 79.6$, $df = 2$, $p < 0.001$) (Fig. S5). During the incubation, E_{CNP} decreased significantly in the Ajj and BCg horizons but not in the O horizon. Regardless of the decrease in Ajj and BCg horizons, E_{CNP} remained lowest in the O horizon ($F = 45.5$, $df = 2$, $p < 0.001$). The initial differences in E_{CNP} between Ajj and BCg horizons decreased during the incubation period, with both horizons yielding similar values at the end of the incubation (Fig. S5). $E_{CNP}$was not significantly affected by temperature in the Ajj and BCg horizons, whereas in the O horizon it increased with temperature ($F = 6.4$, $df = 2$, $p = 0.007$).

Over the incubation period, individual enzymes within E_{CNP} changed in all horizons, and so did the various classes of enzymes grouped with respect to nutrient acquisition (E_C, E_N and E_P; Fig. 5). In all horizons, the E_P and E_N pools decreased ($F = 3021.3$, $df = 5$, $p < 0.001$, and $F = 954.7$, $df = 5$, $p < 0.001$, respectively) compared to their initial values (Fig. 5). The E_C pool increased in the O horizon, but decreased in the Ajj and BCg horizons ($F = 2591.3$, $df = 5$, $p < 0.001$). The increase of the E_C pool in the O horizon reflected the increase of both the hydrolytic enzymes (cellobiosidase and β-glucosidase).

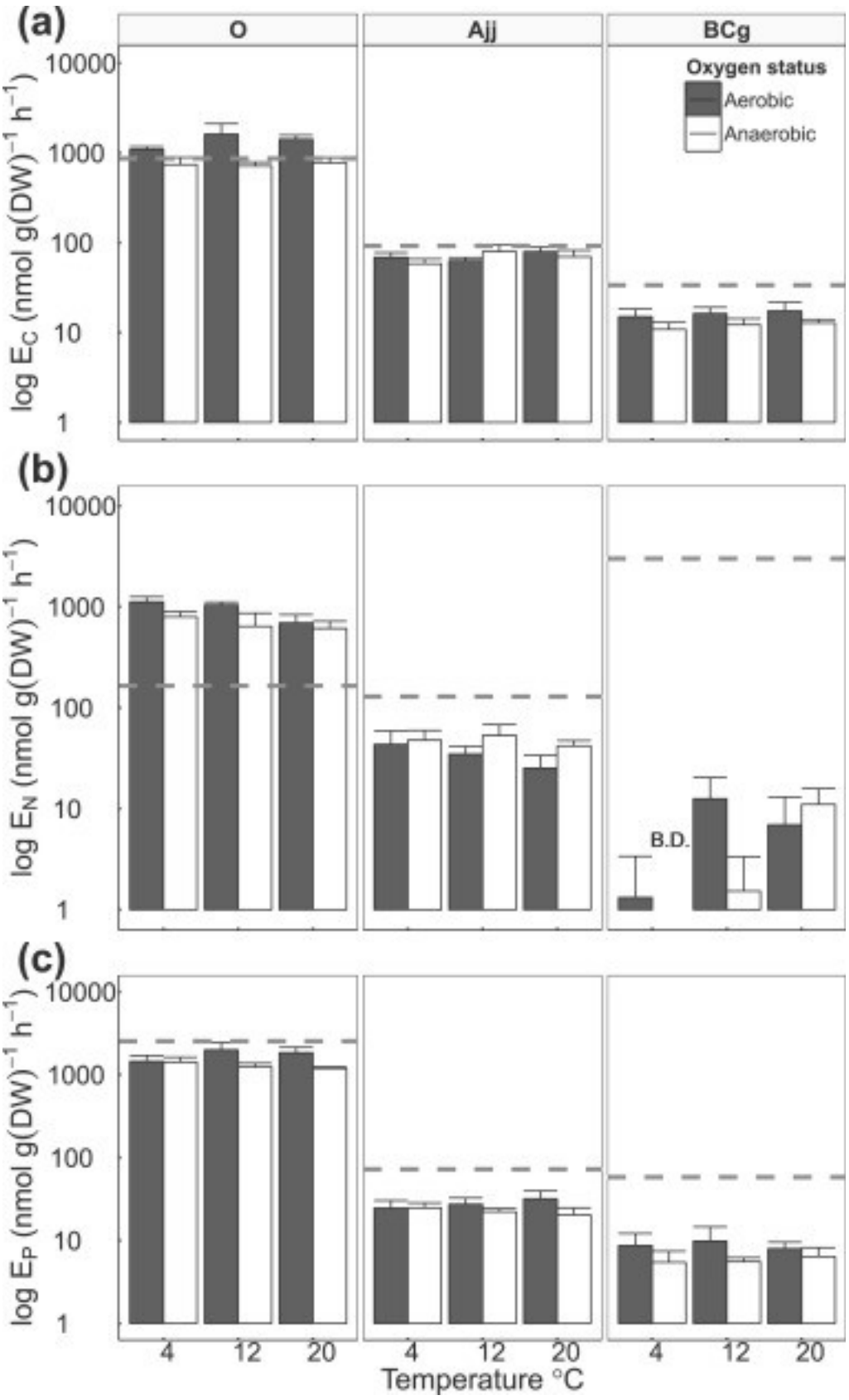
(a)
O
Ajj
BCg
Oxygen status
Aerobic
Anaerobic
log E_C (nmol g(DW)$^{-1}$ h^{-1})
10000
1000
100
10
1
(b)
log E_N (nmol g(DW)$^{-1}$ h^{-1})
B.D.
(c)
log E_P (nmol g(DW)$^{-1}$ h^{-1})
4
12
20
Temperature °C

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Fig. 5. E_C (a), E_N (b) and E_P (c) enzyme classes in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard deviations. Dashed horizontal lines show values at the start of the experiment. All enzyme classes are defined as sums of two different hydrolytic enzymes (E_C = *β-glucosidase + cellobiosidase*; E_N = *alanin-aminopeptidase + leucine-aminopetpidase*; E_P = *phosphoesterase + phosphodiesterase*). Note that y-axes have logarithmic scale.

Like hydrolytic enzymes, phenoloxidases decreased in relation to their initial values in Ajj and BCg horizons (Fig. 6), whereas they increased in the O horizon ($F = 2591.3$, $df = 5$, $p < 0.001$). There was an insignificant increase of phenoloxidases with temperature in the O horizon. In BCg and Ajj horizons, phenoloxidases were highest at 12 °C.

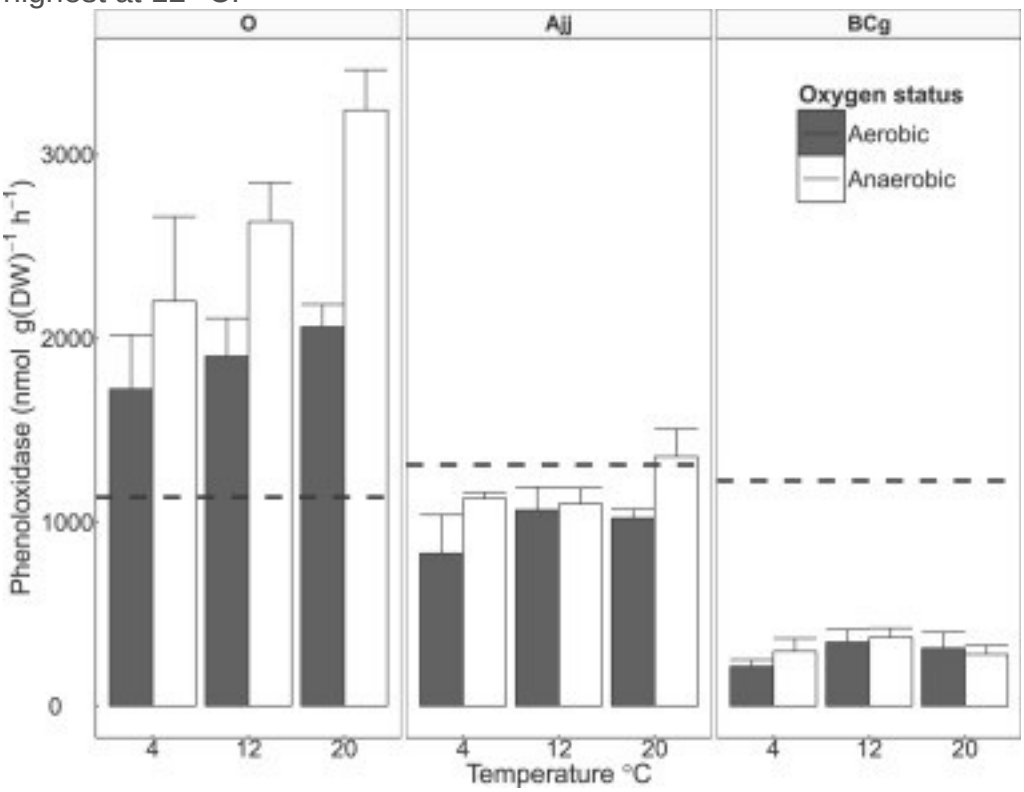

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Fig. 6. Phenoloxidases in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard deviations. Dashed horizontal lines show values at the start of the experiment.

3.2. Anaerobic incubations

3.2.1. Microbial activity and soil carbon loss

As was the case under aerobic conditions, CO_2 production rates under anaerobic conditions were constant throughout the incubation at all temperatures and in all horizons (Fig. S2). The total amount of CO_2 produced by microbial activity during incubation represents the cumulative C_{LOSS} from the Ajj and BCg horizons, where no methane production was detected. In the O horizon, CH_4 production occurred from the 9th week to the end of incubation and thus C_{LOSS} was given by the sum of cumulative CO_2 and CH_4 production (Fig. 1). At the end of the incubation, CH_4 production represented 12, 39 and 42% of C_{LOSS}at 4, 12 and 20 °C, respectively.
As under aerobic conditions, C_{LOSS} was higher at all temperatures in the O horizon, followed by the Ajj and then the BCg horizon ($F = 44.4$, $df = 2$, $p < 0.001$), although the differences were not as pronounced as under aerobic conditions. However, normalized to the amount of OC, C_{LOSS} was highest in the BCg, followed by the O and then Ajj horizon (Fig. 1). C_{LOSS}again strongly correlated with C_{MB} and P_{MB} (Fig. 2), a similar response as in aerobic conditions, although it increased more slowly with both C_{MB} and P_{MB}.
The temperature sensitivity of C_{LOSS} under anaerobic conditions was generally lower than under aerobic conditions (Fig. S4). C_{LOSS} did not increase between 4 °C and 12 °C and increased only slightly between 12 °C and 20 °C (Fig. 1). Temperature sensitivity was again statistically indistinguishable between horizons, regardless of the contribution of CH_4production to C_{LOSS} in the O horizon. The overall mean temperature sensitivity (Q_{10}) for all horizons was 1.38.

3.2.2. Microbial biomass

C_{MB} changes during the anaerobic incubation followed a similar pattern compared with the aerobic treatment (Fig. 4). C_{MB} in the O horizon decreased during incubation at 20 °C but, in contrast to the aerobic treatment, it also decreased at 12 °C ($F = 8.6$, $df = 1$, $p = 0.015$). C_{MB}in the Ajj horizon increased at 12 °C, similarly to the aerobic treatment ($F = 67.3$, $df = 2$, $p < 0.001$). C_{MB} in the BCg horizon increased at all temperatures, but this increase was not significant.
$C:N_{MB}$ (Fig. 4b,c) in the O horizon decreased ($F = 36.5$, $df = 1$, $p < 0.001$) during the incubation, similarly to the aerobic treatment but to a greater degree. $C:P_{MB}$ did not change during the incubation and thus there was no detectable temperature effect. In the Ajj horizon, $C:N_{MB}$ increased at 4 °C, but in contrast to the aerobic treatment, it decreased at higher temperatures. The $C:N_{MB}$ decrease was dependent on incubation temperature ($F = 55.9$, $df = 1$, $p < 0.001$). $C:P_{MB}$ decreased only at 4 and 20 °C,

consistent with the response under aerobic conditions. In the BCg horizon, $C:N_{MB}$ increased at 4 and 12 °C, but decreased at 20 °C ($F = 28.6$, $df = 2$, $p < 0.001$). $C:P_{MB}$ decreased at all temperatures and the decrease was highest at 20 °C ($F = 12.2$, $df = 1$, $p < 0.001$).

3.2.3. Soil enzymes

Total enzyme pools (E_{CNP}), per mol C_{MB}, were nearly identical to those found under aerobic conditions (Fig. S5). The only difference was a steeper increase with temperature in the O horizon. In contrast to the aerobic treatment, all E_C ($F = 2009.2$, $df = 5$, $p < 0.001$), E_N($F = 566.1$, $df = 5$, $p < 0.001$) and E_P ($F = 2788.8$, $df = 5$, $p < 0.001$) pools decreased during the incubation in all horizons (Fig. 5). No temperature effect was found except for an $E_N$increase with temperature in the BCg horizon ($F = 11.5$, $df = 2$, $p = 0.027$).
In general, phenoloxidases showed similar trends to those under aerobic conditions in O and BCg horizons (Fig. 6). In the BCg horizon the trend was identical, but in the O horizon phenoloxidases increased more steeply with temperature and were higher than those found under aerobic conditions at all temperatures. In the Ajj horizon, phenoloxidase activities were higher compared to the aerobic treatment at 4 and 20 °C and were the same at 12 °C.

3.3. Link between microbial biomass, enzymes, C and nutrient availability

Detailed information about C and nutrient availabilities in the different horizons and their changes during incubation is given in Supplementary Results. Across the six temperature/oxygen treatments, changes of microbial biomass, hydrolytic enzymes and the main macronutrients during the incubation were well correlated with each other in the O horizon, but not in the Ajj and BCg horizons (Table 2, Figs. S7 and S8). In the O horizon, E_C, E_N and E_P pools were negatively related to available C (C_{EX}), N (ammonia + nitrates) and P (P_{EX}) concentration, respectively. Furthermore, the E_N pool was positively related to $C:N_{MB}$and both E_C and E_P were negatively related to $C:P_{MB}$. No significant relationship was found in the BCg horizon. The only significant relationship we found in the Ajj horizon was the negative relationship between E_P and $C:P_{MB}$. In contrast to hydrolytic enzymes, phenoloxidases were negatively related to C_{MB} only in the O horizon (Fig. S9). In the Ajj and BCg horizons, we found no relationship between phenoloxidases and any microbial or soil variables.

Table 2. Results of linear regression between different microbial ($C:P_{MB}$ – microbial biomass C:P, $C:N_{MB}$ – microbial biomass C:N) and soil variables (C_{EX} – K_2SO_4 extractable C, N_{MIN} – sum of K_2SO_4 extractable

ammonium and nitrates, P_{EX} – $NaHCO_3$ extractable P) and enzyme categories in respect to C (E_C), N (E_N) and P (E_P) for top organic (O), subducted organic (Ajj) and mineral (BCg) soil horizons incubated under aerobic and anaerobic conditions and 3 different temperatures (4, 12 and 20 °C). Table shows slopes, R^2 and p values of linear regression. Statistically significant regressions are given in bold face.

Horizon	Enzyme category	Microbial variables	Soil variables	R^2	slope	p
O	E_C	$C:P_{MB}$		**0.56**	**−20.8**	**<0.001**
			C_{EX}	**0.39**	**−19.4**	**<0.001**
	E_N	$C:N_{MB}$		**0.54**	**54.5**	**<0.001**
			N_{MIN}	**0.37**	**−32.0**	**<0.001**
	E_P	$C:P_{MB}$		**0.31**	**−18.8**	**<0.001**
			P_{EX}	**0.20**	**−0.8**	**0.004**
Ajj	E_C	$C:P_{MB}$		−0.02	−0.1	0.504
			C_{EX}	−0.03	−0.1	0.962
	E_N	$C:N_{MB}$		0.01	0.3	0.287
			N_{MIN}	−0.02	8.6	0.593
	E_P	$C:P_{MB}$		**0.16**	**−0.2**	**0.012**
			P_{EX}	0.06	−0.1	0.056
BCg	E_C	$C:P_{MB}$		−0.02	0.0	0.458
			C_{EX}	−0.02	−0.3	0.522
	E_N	$C:N_{MB}$		−0.01	0.1	0.389
			N_{MIN}	0.02	30.5	0.227
	E_P	$C:P_{MB}$		−0.04	0.0	0.734
			P_{EX}	0.07	−0.2	0.068

4. Discussion

We have shown that carbon loss from OC subducted through cryogenic soil movements was lower by one order of magnitude than from organic top soil under both aerobic and anaerobic conditions in absolute terms. In relative terms, C_{LOSS} per unit OC was still approx. 5 times lower compared to organic topsoil, which was similar to or lower than in mineral subsoil. We found that the amount of microbial biomass, which is much lower in the subducted organic horizon than in the top organic horizon, is responsible for this difference. We further investigated the factors controlling the amount of microbial biomass in subducted organic horizon. We argue that microbial biomass is not controlled by the carbon and nutrient supply from degradation of subducted OC by extracellular enzymes. While microbial biomass and nutrient availability are related to

extracellular enzyme pools in the organic topsoil, these variables are unrelated in the subducted organic horizon. We suggest that allochthonous material from top organic soil affects microbial biomass in subducted organic and mineral soil horizons. Temperature and oxygen level were identified as secondary controls on C_{LOSS}.

4.1. Temperature sensitivity of soil carbon loss and its link to microbial biomass

C_{LOSS} exponentially increased with temperature, with a mean Q_{10} value of 2.41 across all horizons under aerobic conditions. The 95% confidence interval (95% CI) was 2.36–2.54, so the temperature sensitivity of OC decomposition across horizons was indistinguishable from the value of 2.48, which is predicted by metabolic theory across ecosystems (Brown et al., 2004, Allen et al., 2005, Allen and Gillooly, 2009, Yvon-Durocher et al., 2012). This theory postulates that C_{LOSS} from soil results from two variables: (i) the amount of microbial biomass and (ii) its respiration rate, which is invariantly affected by temperature in all heterotrophic microorganisms using oxygen as electron acceptor. In agreement with the first postulate, absolute C_{LOSS} was well correlated with microbial biomass across all horizons in our experiment. As to the second postulate, the temperature sensitivity of C_{LOSS} was uniform across horizons and we did not observe any major change of microbial biomass within any horizon. Normalized to microbial biomass, the temperature response of microbial specific respiration activity (CO_2 production rate per unit biomass in the last week of the experiment, just before microbial biomass assessment) was, in accord with the metabolic theory, almost identical in all horizons (Fig. S9c).

C_{LOSS} in anaerobic conditions occurred predominantly through CO_2 production while the contribution of methanogenesis was negligible in all horizons, indicating that fermentations and anaerobic respiration were the principal processes driving C_{LOSS}. The temperature sensitivity of C_{LOSS} (Q_{10} = 1.38, *95% CI* = 1.06–1.58) was close to 1.41, the mean value for data obtained from a range of arctic soils (Treat et al., 2015). However, our Q_{10} is still within the range of 0.67–4.10 reported by Treat et al. (2015).

C_{LOSS} showed lower temperature sensitivity than under aerobic conditions, but again it was the same in all soil horizons. In contrast to aerobic conditions, we found a significant decrease of microbial biomass in the O horizon at 12 and 20 °C, which might affect $C_{LOSS}$from the O horizon at higher temperatures. Normalized to microbial biomass, specific respiration activity at the end of the incubation experiment showed different temperature sensitivities for different horizons, being higher in the Ajj and BCg horizons than in the O horizon (Fig. S9c). We suggest that temperature sensitivity of C_{LOSS} under anaerobic conditions reflects the temperature sensitivities of different pathways of

anaerobic metabolism. In anaerobic conditions inorganic and organic electron acceptors are used and metabolic rate as well as specific respiration activity depends on e^- acceptors, which could be expected to differ between horizons. If inorganic e^- acceptors prevail, specific respiration activity is higher than when predominantly organic e^- acceptors are used. It is very likely that organic e^- acceptors prevailed in the O horizon, which lacks inorganic e^- acceptors, and that the role of inorganic e^- acceptors would be greater in Ajj and BCg horizons. Gentsch et al. (2015b) found high concentrations of oxalate-extractable Fe, which goes into solution at the initial stage of dissimilatory Fe (III) reduction in the BCg and Ajj horizons at our study site. In Ajj and BCg horizons, Fe (III) could be an important e^- acceptor, to which microorganisms are able to transfer electrons directly or via humic substances (Lovley et al., 1996), which are especially abundant in the Ajj horizon (Gentsch et al., 2015b).
The effects of temperature and oxygen availability on C_{LOSS} were relatively small compared to the effect of microbial biomass, which explained most of the variability in the data. The proportion of C_{MB} in OC decreased in the order O > BCg > Ajj. The Ajj horizon had the lowest proportion of C_{MB} in OC, and accordingly, the C_{LOSS} per OC observed here was similar or even lower than in the mineral BCg horizon. Similar results were also shown by Wild et al. (2014) and Kaiser et al. (2007) in arctic soils. The low proportion of C_{MB} in OC in the Ajj horizon suggests that the subducted organic horizon contains a large amount of organic carbon that is barely accessible to the microbial community. This could be connected to lower OC quality (Gentsch et al., 2015b) and inefficient enzymatic OC depolymerization (Gittel et al., 2014, Schnecker et al., 2014). Independently of temperature, low quality OC degradation in subducted organic horizons does not provide a sufficient supply of carbon and nutrients to maintain greater microbial biomass. Microbial biomass in the Ajj horizon remained approximately the same at all temperatures (Fig. 4a).

4.2. Broken link between microbial biomass, enzymes, C and nutrient availability

Lower OC quality in subducted organic or mineral soil horizons is the result of a greater degree of OC processing compared with topsoil horizons (Gentsch et al., 2015b). In mineral horizons, most of the OC is associated with clay-sized minerals, and in subducted organic matter as coprecipitates with hydrolyzable Fe and Al as well. Thus, the microbial community has to overcome more constraints to decompose OC in Ajj and BCg horizons than in the O horizon. It explains why C_{MB} relative to OC is lower in Ajj and BCg horizons than in the O horizon (Fig. 4a).

To overcome chemical–physical constraints the microbial community produces extracellular enzymes. First oxidative enzymes cleave aromatic ring structures and break C–C bonds in phenolic and aliphatic compounds, then hydrolytic enzymes can utilize liberated C and N chains, which become available to microbes (Kouno et al., 2002, Sinsabaugh, 2010, Sinsabaugh and Shah, 2012). The reaction of oxidative enzymes with their substrate is considered to be the rate limiting step of low quality OC decomposition (Schimel and Weintraub, 2003, Allison, 2006, Herman et al., 2008). This step requires more energy than the reaction of hydrolytic enzymes with their substrate and is connected with higher activation energy and thus higher temperature sensitivity (Conant et al., 2011). Decomposition of low quality OC is therefore considered to be more temperature sensitive than decomposition of high quality OC (Conant et al., 2008). Based upon that assumption we expected an increase of C and nutrient supply from enzymatic decomposition of low quality OC at higher temperatures (Agren and Wetterstedt, 2007) in the Ajj horizon, followed by microbial biomass increase, which would effectively increase the temperature sensitivity of C_{LOSS}. But microbial biomass remained almost unchanged at all temperatures and we did not observe any difference in temperature sensitivity of C_{LOSS} between horizons, which naturally differ in OC quality. We argue that instead of relying on the energetically demanding production of extracellular enzymes, the microbial community in the Ajj and BCg horizons relies on the flux of allochthonous material from the topsoil organic horizon bypassing chemical–physical constraints (Fig. 7). Nutrient and enzyme pools consist largely of allochthonous nutrients and enzymes in both horizons. By separating the horizons for the experiment we interrupted the flux of enzymes and nutrients from the topsoil horizon. Allochthonous enzymes and nutrients, which were present at the start of the incubation in the Ajj and BCg horizons, were gradually degraded, and autochthonous production was minor, resulting in decreasing nutrient and enzyme pools. This broke the links between microbial biomass and enzymes, C and nutrient availability, respectively (Figs. S6 and S7and Table 2). We see three lines of evidence for that interpretation:

(i)

Enzyme pools in the Ajj and BCg horizons were surprisingly high at the start of the incubation, which is unlikely to be the product of microbial activity in those horizons. There is some uncertainty regarding phenoloxidase assessment in Ajj and BCg horizons. These horizons contain more reactive Fe than the O horizon (Gentsch et al., 2015b). Reactive Fe was shown to be able to oxidize the substrate L-DOPA and cause overestimation of phenoloxidase activity (Hall and Silver, 2013). However, not only phenoloxidases but also all six hydrolytic

enzymes showed high potential activities in Ajj and BCg horizons, especially at the start of the incubation. When enzyme pools were calculated per mol of microbial biomass, they were greater by one order of magnitude than in the O horizon, and 3 to 4 times higher than at the end of the incubation (Fig. S5). Such huge enzymatic pools are unlikely to be composed solely of autochthonous enzymes released by microbes in these horizons. Enzyme production is an energy-demanding process, and due to its negative energy balance, decomposition of low quality OC cannot serve as a sufficient energy source for the required production of enzymes (Fontaine and Barot, 2005, Moorhead and Sinsabaugh, 2006, Fontaine et al., 2007, Allison, 2012). Thus enzyme pools in Ajj and BCg horizons were composed mainly of allochthonous enzymes, which degraded spontaneously during incubation and did not specifically target OC in this horizon.

(ii)

Spontaneity of decrease of both oxidative and hydrolytic enzymes is supported by the fact that enzyme pools decreased without any link to microbial biomass stoichiometry and nutrient availability (Figs. S6 and S7). Hydrolytic enzymes (especially E_N and E_P) decreased even though nutrient concentration decreased ($NH4+$, $NO3-$ and $P\text{-}PO_4$, Table S1). By contrast, the total enzyme pool slightly increased in the O horizon. Enzymes were negatively related to carbon and nutrient availability in this horizon. The greater nutrient availability was, the smaller was the enzyme pool, as microbes were provided with sufficient amounts of nutrients and enzyme production was not needed (Figs. S6 and S7). The enzyme pool was also related to microbial stoichiometry, indicating a direct link between microbes and enzyme activity. This was not seen in Ajj and BCg horizons.

(iii)

Low specificity of enzymes was indicated by a significantly higher ratio of phenoloxidases to hydrolases in the Ajj (7.9) and BCg (12.1) horizons than in the O horizon (0.7), which indicates disruption of the enzyme cascade that is needed for efficient low quality OC degradation and effective supply of C and nutrients to microbial biomass. Unspecific high phenoloxidase activity in the Ajj and BCg horizons led to an increase of DOC and DON and disrupted the ratio between CO_2 production and O_2 consumption (RQ; Fig. 3b). The RQ values below 0.6 reported here (down to 0.1 and 0.2 in the BCg and Ajj horizons, respectively) have not previously been observed in soil incubation studies (Dilly, 2003),

suggesting an additional O_2-consuming process such as oxidative processes mediated by phenoloxidases. The amounts of CO_2 produced and O_2 consumed during microbial metabolism should be proportional. For carbohydrates, which are believed to be the most common carbon source in soils, the RQ is exactly 1. Under natural conditions, the vast majority of studies have shown RQ values ranging from 0.7 to 1.2 in aquatic ecosystems (Berggren et al., 2012), and from 0.6 to 1 in soils (Li et al., 2014). Taking into account the CO_2 production rates in Ajj and BCg horizons and the lowest RQ value found in the literature, we estimated that the potential amount of O_2 used by phenoloxidases could account for up to an average of 75, 70 and 23% of the microbial oxygen demand in the Ajj horizon and 96, 93 and 58% of it in the BCg horizon at 4, 12 and 20 °C, respectively.

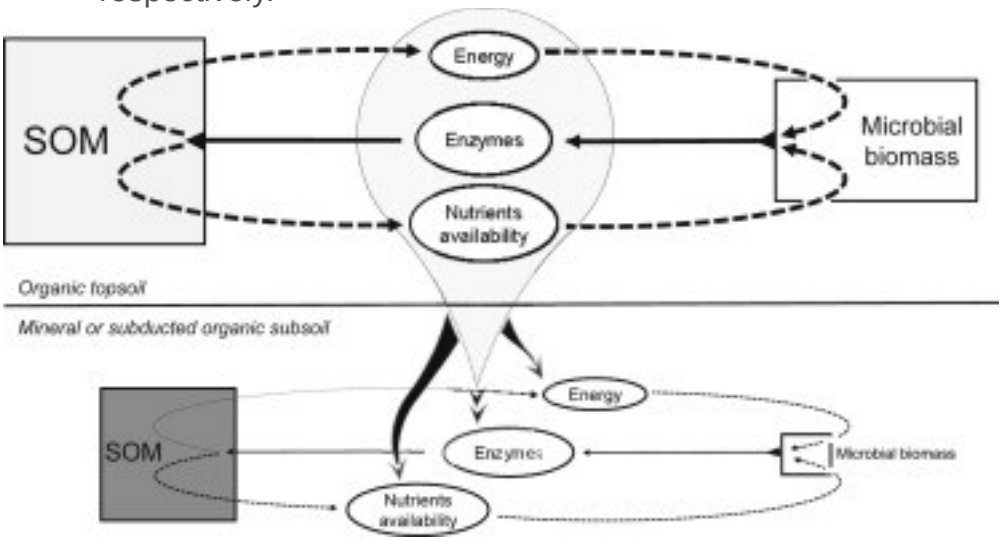

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Fig. 7. Diagram of connections between microbial biomass, soil OC, enzymatic production and nutrient availability within and between soil horizons. The energy availability for enzymatic production within each soil horizon is critical; the low quality soil OC of subsoil horizons provides no energy and thus enzymatic production in subsoil horizons is directly dependent on fresh carbon flow from the topsoil organic horizon. Together with fresh carbon, extracellular enzymes and nutrients are transported down the soil profile, affecting enzymatic pools and nutrient availability of subsoil horizons.

4.3. Subducted organic carbon under climate change

We found that C_{LOSS} from cryoturbated arctic soils is primarily driven by microbial biomass and that the temperature and oxygen level has a secondary effect on the metabolic rate of microorganisms. The subducted organic horizon turned out to contain

little microbial biomass and therefore its C_{LOSS} was low under both aerobic and anaerobic conditions over the whole range of temperatures investigated. The proportion of microbial biomass to OC in the subducted organic horizon was the lowest of all horizons studied. All lines of evidence suggest that this pattern was caused by chemical–physical characteristics of subducted OC (Gentsch et al., 2015a) whose decomposition does not provide the microbial community with a sufficient supply of C and nutrients. In field conditions, the microbial community in subducted organic horizons relies on the allochthonous influx of fresh carbon and nutrients from the topsoil, while decomposition of autochthonous, physically and chemically protected OC lags behind (Fig. 7). It suppresses the effect of autochthonous OC quality on temperature sensitivity of carbon loss and stabilizes subducted OC (Kaiser et al., 2007). This can be perceived as a negative priming effect (Kuzyakov et al., 2000). We believe that this is a common mechanism which “protects” subducted OC from decomposition in cryoturbated arctic soils. Patterns of vertical carbon fluxes in cryoturbated arctic soils were also observed by Gentsch et al. (2015b) and Xu et al. (2009).

Organic carbon supply by the organic topsoil is critical for subducted OC decomposition, as has already been suggested by Wild et al. (2014). If the flux of carbon and nutrients is small, autochthonous subducted OC may be protected from decomposition, and microbes utilize mainly allochthonous C and nutrients from influx (negative priming effect; Kuzyakov et al., 2000). If the flux of C and nutrients is high (e.g. after uplift of subducted OC and root ingrowth), microbial activity is stimulated to a degree that autochthonous subducted OC is decomposed as well (positive priming effect; Kuzyakov et al., 2000, Wild et al., 2014). The positive priming effect, destabilizing subducted OC, may result from input of exudates. Under future climate change the priming may even be enhanced by an expected shift in the composition of the plant community, producing litter of higher quality (Cable et al., 2009, DeMarco et al., 2014). Under this scenario, a substantial loss from subducted OC, which is protected at present, may occur.

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Appendix A. Supplementary data

The following is the supplementary data related to this article:

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$$C_{LOSS} = R \,.\, e^{\,a \,.\, T},$$

where T is temperature and R and a are function parameters. Q_{10} as a measure of temperature sensitivity of C_{LOSS} was calculated from the exponential function as fo

$$Q_{10} = \mathrm{e}^{\,\mathrm{a} \,.\, 10},$$

Q_{10} expresses the relative change of C_{LOSS} with a 10 °C increase. To allow direct comparison of temperature sensitivity between soil horizons, we tested the effect o horizon on a parameter with nonlinear mixed-effect models, using the program R (Team, 2014) and package nlme (Pinhero et al., 2013). We tested the statistical diff comparing exponential functions having a parameter fixed for all horizons, separat estimated for each horizon or randomly varying among horizons. For the compari used the Akaike information criterion (AIC).

Absolute differences in C_{LOSS} between horizons, temperature and oxygen status tr were evaluated by 3-way ANOVA. C_{LOSS} data were log-transformed and normality with the Shapiro–Wilk test. To evaluate the differences between the individual treat used the post-hoc Tukey HSD test. Since the effect of soil horizon on C_{LOSS} was (i explained variability) much greater than the effects of temperature and oxygen stat ANOVA was followed by multiple linear regression analysis to find the best predict C_{LOSS} across horizons. The best statistical model was chosen from all measured parameters (chemical parameters, microbial parameters and enzyme potential acti temperature and oxygen status by applying a stepwise algorithm. Because multi-co in soil chemical parameters, microbial parameters or enzyme potential activities oft across horizons, ridge regression was used to avoid unstable predictors. Ridge reg was carried out in R (R Core Team, 2014) using package MASS.

The relationship between temperature and oxygen consumption rate was describe Gaussian model (Tuomi et al., 2008):

$$O_2 = R \,.\, e^{\,a.\,T \,+\, b \,.\, T^2},$$