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Vulnerability of organic matter in permafrost-affected soils and potential greenhouse gas emissions

Ph.D. Thesis

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Annotation

Degradability of soil organic carbon and potential greenhouse gas emissions were studied in permafrost-affected soils. Special attention was paid to cryoturbated horizons in upland tundra and cryogenic bare ground features exposing deep peat layers on a surface of permafrost peatland. The presented studies aimed to identify driving factors influencing degradability of organic carbon in arctic soils and the effects of temperature, oxygen availability and addition of plant-derived organic compounds were targeted by several laboratory incubation experiments. Role of nitrogen availability in C cycling and GHGs production was further investigated in combination of field measurements and laboratory experiments.

Declaration [in Czech]

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List of papers and author´s contributions

The thesis is based on the following papers:

I. Čapek, P., **Diáková, K.**, Dickopp, J.-E., Bárta, J., Wild, B., Schnecker, J., Alves, R.J.E., Aiglsdorfer, S., Guggenberger, G., Gentsch, N., Hugelius, G., Lashchinsky, N., Gittel, A., Schleper, C., Mikutta, R., Palmtag, J., Shibistova, O., Urich, T., Richter, A., Šantrůčková, H., 2015. The effect of warming on the vulnerability of subducted organic carbon in arctic soils. Soil Biology and Biochemistry **90**, 19-29. (IF = 4.86)

Kateřina Diáková took part in designing and running the incubation experiment, in soil and gas sample analyses, and in data evaluation and participated on the manuscript revision.

II. **Diáková, K.**, Čapek, P., Kohoutová, I., Mpamah, P. A., Bárta, J., Biasi, C., Martikainen, P., Šantrůčková H., 2016. Heterogeneity of carbon loss and its temperature sensitivity in East-European subarctic tundra soils. FEMS Microbiology Ecology **92**, fiw140. (IF = 3.72)

Kateřina Diáková took part in designing and running the incubation experiment, in soil and gas sample analyses, participated in data evaluation and wrote the manuscript.

III. Wild B., Gentsch, N., Čapek, P., **Diáková, K.**, Alves, R. J. E., Bárta, J., Gittel, A., Hugelius, G., Knoltsch, A., Kuhry, P., Lashchinskiy, N., Mikutta, R., Palmtag, J., Schleper, C., Schnecker, J., Shibistova, O., Takriti, M., Torsvik, V.L., Urich, T., Watzka, M., Šantrůčková, H., Guggenberger, G., Richter, A., 2016. Plant-derived compounds stimulate the decomposition of organic matter in arctic permafrost soils. Scientific Reports **6**, 25607. (IF = 4.26)

Kateřina Diáková took part in designing and running the incubation experiment, in soil and gas sample analyses, and participated on the manuscript revision.

IV. **Diáková, K.**, Biasi, C., Čapek, P., Martikainen, P., Marushchak, M. E., Patova, E. N., Šantrůčková H., 2016. Variation in N_2 fixation in subarctic tundra in relation to landscape position and nitrogen pools and fluxes. Arctic Antarctic and Alpine Research **48**, 111-125. (IF = 1.78)

Kateřina Diáková designed and performed the field measurements and laboratory experiments, analysed majority of gas and soil samples, evaluated data and wrote the manuscript.

List of abbreviations

C	carbon
CH_4	methane
C_{LOSS}	loss of carbon from soil or eosystem in form of CO_2 and CH_4
CO_2	carbon dioxide
C:N	ratio of C and N in soil organic matter
GHG	greenhouse gas
GWP	global warming potential
N	nitrogen
N_2	molecular atmospheric nitrogen
NH_4^+	ammonium
NO_3^-	nitrates
N_2O	nitrous oxide
OC	organic carbon
O_2	oxygen
Q_{10}	relative increase of reaction rate with 10°C temperature rise
SOM	soil organic matter

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1 GENERAL INTRODUCTION

1.1 STOCKS OF ORGANIC MATTER IN ARCTIC SOILS

The northern circumpolar permafrost region spans over 17.8 x 10^6 km^2 which represents about 16% of the total global soil area (Tarnocai *et al.* 2009). The primary production rates of tundra ecosystem are low due to low mean annual temperature and short vegetation season. Still, large stocks of soil organic matter (SOM) accumulated (sequestration) because decomposition (cleavage of organic compounds into smaller organic or inorganic molecules) and mineralization (degradation of organic compounds to simple inorganic molecules as end products) rates are lower than primary production rates. Therefore, Arctic has been a sink of SOM over thousands of years which represents a carbon (C) pool exceeding the amount of C present in the atmosphere. According to the latest estimates, total soil organic carbon (OC) stored in this region is about 1307 Pg C (Hugelius *et al.* 2014). About 800 Pg of arctic OC is perennially frozen in permafrost layer (Hugelius *et al.* 2014) thus virtually inactive and preserved from decomposition. The rest is located in active layer and takes part on the active C cycling. Climate warming can increase the rates of decomposition and thus disturb the balance between sequestration and mineralization. When C mineralization prevails over the C sequestration, C is lost from the ecosystem in gaseous forms, CO_2 and CH_4, to atmosphere (C_{LOSS}). In respect to the size of the OC pool in arctic soils, this has a high potential to impact global greenhouse gas (GHG) concentrations in the atmosphere and enforce positive feedback of the ecosystem to climate warming.

Besides the high OC stocks, approximately 67 Pg nitrogen (N) is contained in arctic soils (Harden *et al.* 2012). Nitrogen abundance in soils and C:N ratio strongly affect rate of SOM decomposition, (chapter 1.2.3) and therefore it is crucial to study the dynamics of C mineralization in context of N availability. Although low N availability is one of the factors limiting plant productivity (Shaver & Chapin 1980, 1986, Chapin & Shaver 1989, Jonasson *et al.* 1999) and decomposition (Lavoie *et al*. 2011, Sistla *et al*. 2012) in tundra ecosystems, hotspots of faster N cycling in N-rich soils have been reported from the arctic region (Repo *et al.* 2009, Elberling *et al*. 2010, Marushchak *et al.* 2011, Abbott & Jones 2015). There, fast N mineralization exceeds N demand, abundant ammonium (NH_4^+) can be turned into nitrates (NO_3^-)

and accelerated nitrification and denitrification can both lead to emissions of N_2O (Baggs & Philippot 2010), the strong N-derived GHG.

Arctic landscape is diverse mosaic of various habitats with different contents, distribution, quality and age of SOM. Commonly, the highest content of SOM in the soil profile is located in the thin uppermost organic layer (organic topsoil, Fig. 1 a) and contains high portion of easily degradable compounds of the most recent origin (Fig. 1 b). The amount and quality of OC is lower in deeper mineral layers (mineral subsoil). Obviously, comparably little of SOM has been incorporated from topsoil into the mineral subsoil during history (Fig. 1 a) by means of soil mixing, root ingrowth or by leaching of dissolved organic compounds and the SOM has been degraded by microbial decomposition and depleted of easily degradable compounds over the time (Fig. 1 b). The increase of OC age and degree of decomposition with depth in the soil profile is linked to increasing portion of recalcitrant organic compounds. Advanced SOM decomposition is further coupled with decrease in bulk C:N ratio towards deeper soil layers (Kuhry & Vitt 1996, Kaiser *et al.* 2007, Hugelius *et al.* 2014, Schädel *et al.* 2014, Palmtag *et al.* 2016, Fig. 1) because C is lost in gaseous forms from the soil during SOM mineralization while majority of mineralized N is retained in the internal nutrient cycle and incorporated into stable organic material (Malmer & Holm 1984, Melillo *et al.* 1989, Chapin *et al*. 2002).

The C content and quality patterns in soil profile are altered in some of the permafrost soils affected either by I) cryoturbation, the frost-driven vertical mixing of soil layers or II) accumulation of deep organic deposits in permafrost peatlands. These soils represent the habitats with the highest OC concentration (kg C m^{-2}) due to the abundant OC in deep layers (Tarnocai *et al.* 2009, Hugelius *et al.* 2014).

I) Cryoturbated soils cover 31% of northern circumpolar permafrost region and store about 454 Pg C (Hugelius *et al.* 2014). Cryoturbation is a cryogenic process in active layer of moist mineral permafrost soils with poor drainage driven by seasonal freeze-thaw cycle (Bockheim & Tarnocai 1998, Bockheim 2007, Michaelson *et al*. 1996) when organic matter from topsoil is translocated downwards into mineral subsoil (Fig. 2). Intrusions of cryoturbated material with documented OC age between 1240 – 2500 ybp (Kaiser *et al.* 2005, Gentsch *et al.* 2018) are rich in OC as compared to the surrounding mineral subsoil and can account for about one third of the total C stocks in these soils (Kaiser *et al.* 2007, Gentsch *et al.* 2015a, Palmtag *et al.* 2015, Palmtag *et al.* 2016). Chemical composition (e.g. OC content, $\delta^{13}C$, C:N ratio) and degree of decomposition of SOM in cryoturbated horizons are similar to the mineral topsoil (Gentsch *et al.* 2015a, Fig. 1) but the age is up to three-times higher (Kaiser *et al.* 2007) which implies that the decomposition of cryoturbated

SOM have been considerably delayed in respect to its age (Gentsch *et al.* 2015a). The cryoturbated SOM is likely stabilized to major extend by abiotic conditions in subsoil (Kaiser *et al.* 2007) and by organo-mineral associations which can decrease accessibility of organic molecules to microbes (Gentsch *et al.* 2015a, Fouché *et al.* 2014). It was further suggested that microbial community activity and composition might hold additional control over the C mineralization from cryoturbated organic

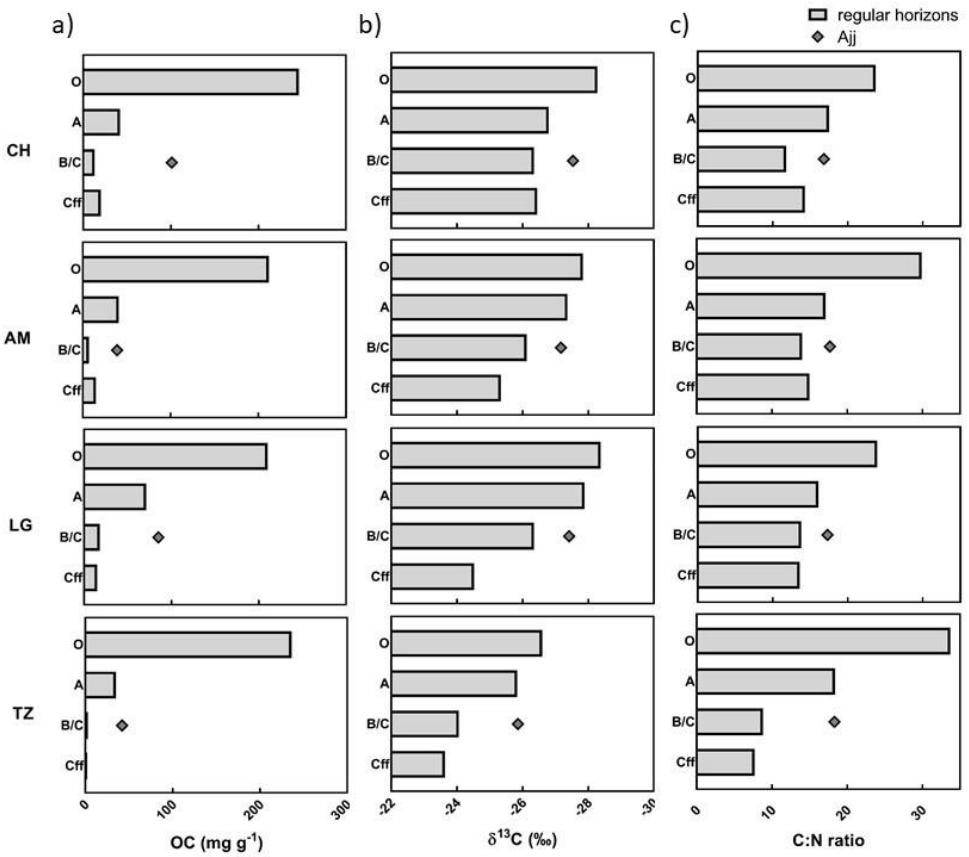

Figure 1: *Vertical patterns of OC content (a), $\delta^{13}C$ values representing a measure of OM transformation - the lower the $\delta^{13}C$, the higher the transformation state of SOM (b) and C:N ratios in separate horizons of soil profiles from cryoturbated tundra (c). Regular soil horizons marked as dark grey columns: O – organic topsoil, A – mineral topsoil, B and C – mineral subsoil, Cff – permafrost subsoil. Cryotubated organic horizons Ojj and Ajj depicted as diamond point. Soils from localities used in this thesis are presented: CH – Cherskiy (East Siberia), AM – Ari-Mas and LG – Logata (Central Siberia), TZ – Tazovskiy (West Siberia); values presented as mean ± standard deviation (data collected from Gentsch et al. 2015a).*

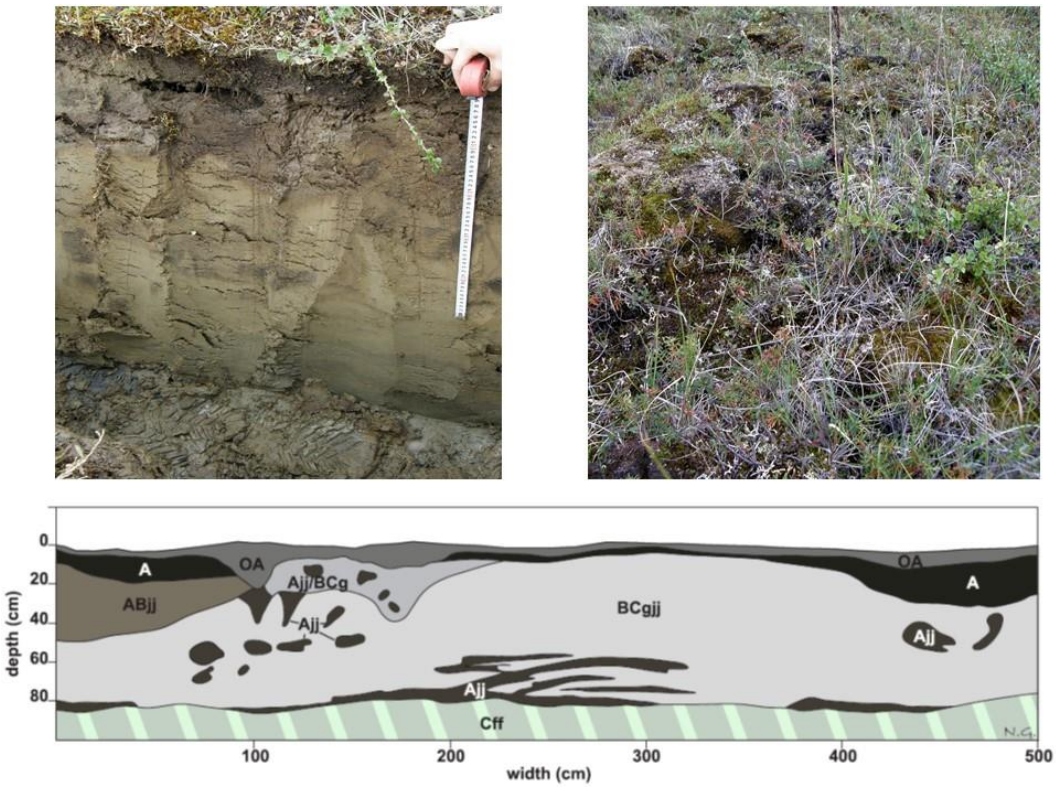

Figure 2*: Cryoturbated soil profile in Ari-Mas, Taymir peninsula, Russia (upper left photo), vegetation cover at the sampling site (upper right photo) and scheme of cryoturbated soil profile based on real profile photo from Ari-Mas sampling site: OA – organic topsoil, A – mineral topsoil, Ajj – cryoturbated horizons, BCg – mineral subsoil, Cff - permafrost (both photos taken by Petr Čapek, soil profile scheme adopted from Gentsch et al. 2015a)*.

horizons (Kaiser *et al.* 2007, Gittel *et al.* 2014a, Gittel *et al.* 2014b, Schnecker *et al.* 2014) because of their low adaptability to the substrate and environmental conditions in subsoil (Schnecker *et al.* 2014, Wild *et al.* 2014, Gentsch *et al.* 2015a). However, the constraining factors of SOM decomposition in cryoturbated organic horizons are still not well understood.

II) Permafrost peatlands take up a small area of arctic region (about 8%) but they represent a significant storage of about 300 Pg C (Hugelius *et al.* 2014) in up to 4 m deep organic deposits (Kaverin *et al.* 2016, Routh *et al.* 2014). They gradually accumulated over the Holocene period in water-saturated conditions, first as a fen where the vegetation is in contact with naturally nutrient-rich groundwater, later as a raised bog fed by nutrient-poor rain water, with occasional permafrost aggradation

during the development (Routh *et al.* 2014, Ronkainen *et al.* 2015, Treat *et al.* 2015). Permafrost can be fully evolved as continuous frozen layer in northern peat plateaus or in a form of scattered permafrost cores creating isolated palsas within northern mires. Changes related to the freeze-thaw processes in peatlands are connected either i) with permafrost thaw and surface subsidence or ii) with localized deep-ice accretion in peat profile and further surface elevation. Permafrost degradation (i) leads to a collapse of thawing peat profiles forming thermokarst, a landscape with relatively recently formed surface depressions or lakes and full-profile exposures at their walls (Fig. 3 c). Ice-lense formation (ii) provokes creation of mounds followed by subsequent loss of surface layers and brings deep peat layers to the surface (Payette *et al.* 2004, Kuhry 2008, Marushchak *et al.* 2011), which leads to origin of circular bare ground features on subarctic peat plateaus, so called peat circles (Kaverin *et al.* 2016; Fig. 3 a, Fig. 4, Fig. 5). Similar process forms also bare palsas (Fig. 3 b). Deep peat is exposed to aerobic ambient conditions on top of the peat circles and palsas, as well as on the thermokarst walls (Fig. 3). Further in this study, bare peat circles are studied as an example of deep peat layers on the surface. The age of surfaced organic soil in peat circles was dated back to 3500 – 5900 ybp (Biasi *et al.* 2014, Ronkainen *et al.* 2015) which corresponds to layers 1 - 2 m deep in undisturbed peat profile (Routh *et al.* 2014). Deep layers originated thousands of years ago in the early stage of peatland development as fen peat, which is naturally richer in nutrients as compared to the topsoil peat of the peat plateau with bog vegetation. An advanced decomposition stage of deep peat layers likely contributes to a lower C:N ratio and leads to a higher portion of complex organic compounds as compared to the topsoil of the vegetated peat plateau (Pengerud *et al.* 2017 , Routh *et al.* 2014). Bare peat circles exhibit C and N dynamics contrasting with the topsoil peat. They reflect low OC quality and low C:N ratio of the peat from deeper layers and inorganic N species (NO_3^- and NH_4^+) are abundant here. Rates of N transformations are high, especially denitrification and N_2O emissions were detected in high rates in the bare peat in field and in laboratory experiments (Repo *et al.* 2009, Marushchak *et al.* 2011, Palmer *et al.* 2012, Voigt *et al.* 2016). Interestingly, the denitrification as microbially facilitated process which requires relatively easily available organic substrate is fast here (Repo *et al.* 2009, Marushchak *et al.* 2011, Gil *et al.* 2017) even though the old peat is characterized by high portion of recalcitrant compounds. On the bare surface without shading plants, photodegradation, the organic matter breakdown by solar irradiance, could hypothetically represent a source of simple organic molecules (Mayer *et al.* 2006, Rutledge *et al.* 2010, King *et al.* 2012) but so far, little is known about key factors

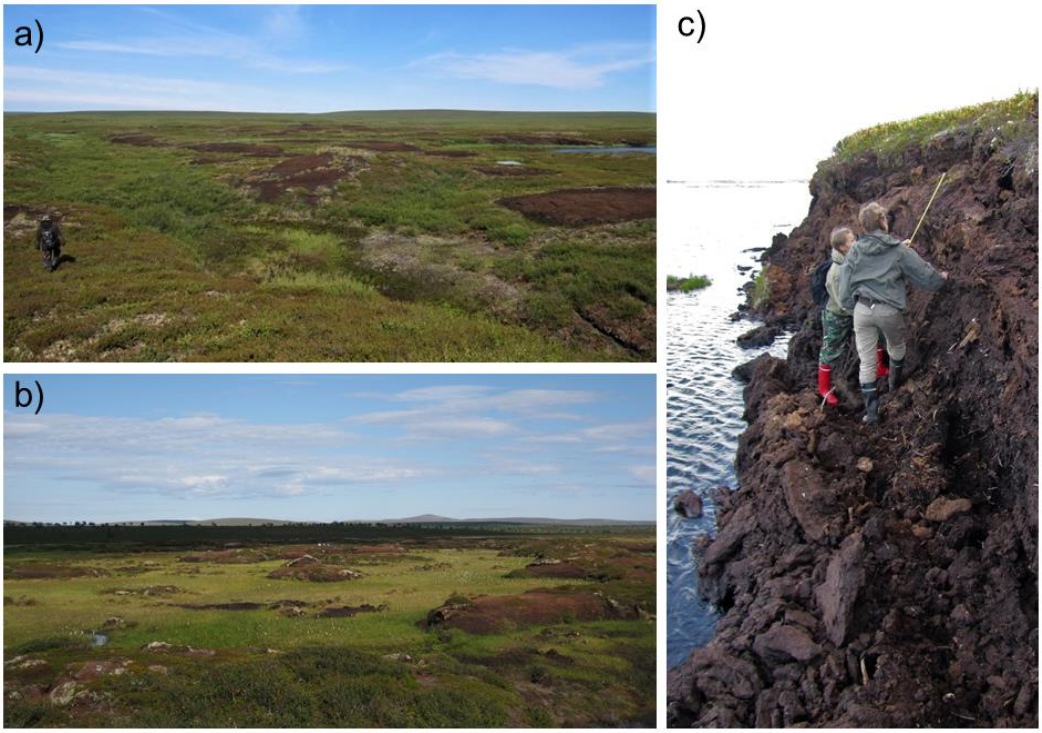

Figure 3*: Forms of exposed bare old peat material in arctic landscape; peat circles at peat plateau at the study site Seida, Komi Republic, Russia (a, photo by Kateřina Diáková), bare palsa surfaces in palsa mire in western Utsjoki, Finland (b, photo by Maija Marushchak) and thermokarst lake wall at the study site Seida, Komi Republic, Russia (c, photo by Richard Lamprecht)*

affecting peat decomposition and N dynamics. It is assumed that exposition of deep peat to ambient conditions at the surface can escalate microbial activity and degradation of SOM previously preserved in deep layers by environmental conditions unfavorable for microbial activity (Kaverin *et al.* 2016). Thus, naturally exposed bare peat could significantly contribute to C_{LOSS} from permafrost peatlands in nearby future (Schmidt *et al.* 2011, Biasi *et al.* 2014).

While OC and N stocks and their dynamics in topsoils have currently received high attention due to their faster OC and N cycling (Jonasson *et al.* 1999, Schmidt *et al.* 2002, Weintraub & Schimel 2003, Shaver *et al.* 2006, Lavoie *et al.* 2011, Biasi *et al.* 2014), little is known about vulnerability of the abundant stocks of SOM in deeper layers (Schmidt *et al.* 2011) and the fate of SOM after cryogenic transposition upwards and downwards along the soil profile (Kaiser *et al.* 2005, Kaiser *et al.* 2007).

The main concerns of current science are which factors control the decomposition of arctic OC stocks, how are these factors going to alter in the future (Davidson & Janssens 2006) and how vulnerable is the SOM to climate-induced environmental changes (Schädel *et al.* 2014, Schuur *et al.* 2015).

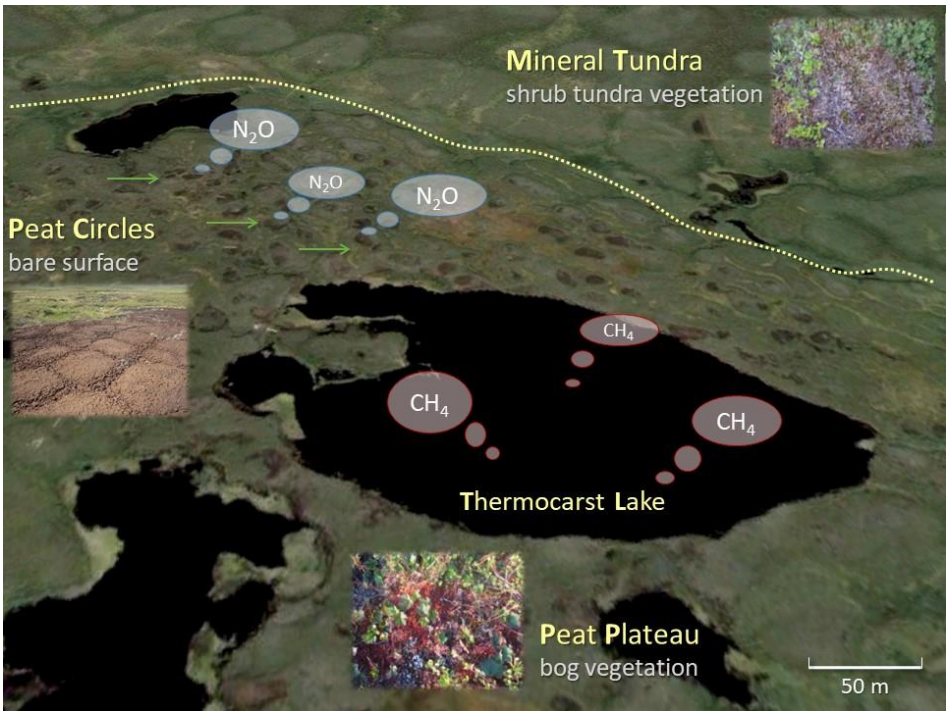

Figure 4*: Satellite image of Seida study site adopted from Map data ©2016 Google, DigitalGlobe, with surface photos and vegetation specification for each of the studied habitats; peat plateau, peat circles (marked with green arrows) and mineral tundra. Significant emissions of strong GHGs (methane CH_4 and nitrous oxide N_2O) are marked in the landscape scheme.*

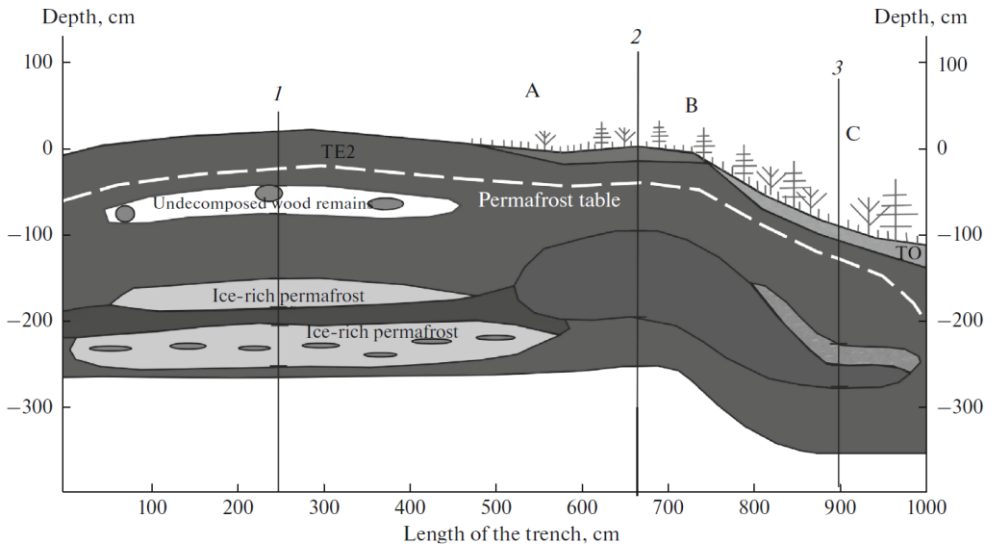

Figure 5*: Cross-section through peat circle formation in subarctic peat plateau. Deep ice aggradation causes uplift of above-laying peat layers creating a mound. Surface vegetation and peat layers are lost by wind erosion exposing old peat layers to ambient conditions. Vegetation cover zones: A – bare surface with marginal cover of mosses and lichens, B – dwarf shrubs, C – shrubs. Dashed white line depicts the permafrost table, light grey areas represent ice-rich permafrost. White area below 3 m depth was not sampled but the peat deposits reach the depth of 4.5 m (adopted from Kaverin et al. 2016).*

1.2 MICROBIAL DECOMPOSITION OF SOIL ORGANIC MATTER IN ARCTIC SOILS

Low temperatures and abundant water-saturated conditions in the arctic soils have suppressed the decomposition of organic material (Davidson & Janssens 2006, Ping *et al.* 2015) and contributed to accumulation of high stocks of SOM. These conditions are considered as the primary controls (Hobbie *et al.* 2002) but SOM decomposition is further influenced by multiple chemical and biological factors, e.g. nutrient availability, OC quality (Schädel *et al.* 2014) or microbial community composition and interactions with plants (Hartley *et al.* 2012, Walker *et al.* 2016, Walz *et al.* 2017). Cryoturbated organic horizons and peat material now on the surface of peat circles developed at deeper soil layers under abiotic conditions comparably less favorable for microbial activity than in the topsoils, such as lower

summer season temperature, higher soil moisture, and longer season when the ground is frozen. Slow microbial degradation over long period of time lead to SOM less decomposed than would correspond to its age but still more processed by microbes than SOM in topsoils (Gentsch *et al.* 2015a, Hugelius *et al.* 2012). Similarly, OC degradability in peat circles is lower than in the vegetated peat topsoil (Biasi *et al.* 2014). Because OC of recent origin in topsoils have different qualities and is inhabited by different biota than OC stored in cryoturbated organic horizons (Kaiser *et al.* 2007, Gittel *et al.* 2014a, Gittel *et al.* 2014b, Schnecker *et al.* 2014, Gittel *et al.* 2014a, Wild *et al.* 2014, Gentsch *et al.* 2015a) and peat circles (Palmer *et al.* 2012, Routh *et al.* 2014, Hugelius *et al.* 2012), distinct decomposition controls can be presumed.

1.2.1 TEMPERATURE SENSITIVITY

Temperature sensitivity of C mineralization is usually described on basis of two different theories. Intrinsic temperature response of C mineralization is uniform according to the metabolic theory which postulates that microbial aerobic respiration is invariantly dependent on temperature and the temperature response can be enhanced by growth of microbial biomass under higher temperature (Brown *et al.* 2004, Allen *et al.* 2005, Allen & Gillooly 2009, Yvon-Durocher *et al.* 2012). However, evident temperature sensitivity of C mineralization was found highly variable among arctic soils (Schädel *et al.* 2016, Moni *et al.* 2015) which can be explained by further modulation by several factors, such as OC quality and substrate availability, or activity of extracellular enzymes (Fierer *et al.* 2006, Davidson & Janssens 2006, Allison 2006, Conant *et al.* 2011). According to the kinetic theory, temperature sensitivity of aerobic mineralization is positively correlated to substrate complexity; the more complex substrate, the higher temperature sensitivity (Mikan *et al.* 2002, Knorr *et al.* 2005, Davidson & Janssens 2006, Conant *et al.* 2008, Wetterstedt *et al.* 2010) but results of laboratory studies bring equivocal, often contrasting evidences (Biasi *et al.* 2005, Fierer *et al.* 2006, Gershenson *et al.* 2009). All the influential factors can interact with each other and some are temperature dependent themselves, such as enzyme activity or substrate availability when strength of bonds between SOM and mineral particles depends on temperature (Davidson & Janssens 2006, Gillabel *et al.* 2010, Gentsch *et al.* 2018). Čapek (2016) aimed to test the two above-mentioned theories (metabolic and kinetic theory) using experimental data from cryoturbated upland tundra soils. He suggested that temperature response of aerobic C mineralization among soil

horizons of cryoturbated soil profile in mineral tundra was invariant at the steady-state microbial biomass which supported the temperature sensitivity predicted by metabolic theory. Temperature response of microbial biomass and C mineralization in extensive arctic peat deposits has not been thoroughly studied yet (Schmidt *et al.* 2011) and needs better understanding.

1.2.2 OXYGEN AVAILABILITY

High water content or complete water saturation abundant in arctic soils restrain oxygen (O_2) diffusion and set up anaerobic conditions in the soil profile. Decomposition under limited access of O_2 is far less effective and yields less energy for microbial decomposers than aerobic metabolic pathways. Under anaerobic conditions, microorganisms use alternative electron acceptors instead of O_2, such as nitrates (denitrification), organic molecules (fermentation), multivalent ions of iron (Fe^{3+} reduction) and mangan (Mn^{4+} reduction), sulphates (sulphate reduction) and acetate or CO_2 (methanogenesis). These metabolic pathways have in this order decreasing yield of energy and thus are less effective in SOM mineralization. Carbon mineralization is not always completed in anaerobic conditions and degradation intermediates cumulate in soil. As a result, anaerobic conditions diminish total C_{LOSS} from arctic soils by factor of 3.4 in average (Schädel *et al.* 2016) and generate not only CO_2 but also CH_4 as a product of C mineralization. The CO_2:CH_4 ratio depends on C content (organic < mineral), soil layer (active layer < permafrost), vegetation cover (herbaceous < mosses, lichens; Treat *et al.* 2015b) and temperature (lower CO_2:CH_4 at higher temperature; Schädel *et al.* 2016, Marushchak *et al.* 2016). Anaerobic conditions also tend to diminish temperature sensitivity of SOM mineralization as compared to aerobic conditions (Schädel *et al.* 2016). Thus, it can be presumed that production of CO_2 and CH_4 in anaerobic soils will increase less with the rising temperature then in aerobic soils.

1.2.3 ORGANIC CARBON QUALITY

Organic C quality is often perceived as continuum between labile and recalcitrant (Kleber & Johnson 2010; von Lützow *et al.* 2007; Simpson & Simpson 2012) or fast-cycling and passive C pools (Schädel *et al.* 2014), which is linked to the chemical composition of OC and turnover time, respectively. The general understanding is that SOM with higher abundance of complex organic macro-molecules, such as phenolic compounds, lignin or lipids, is less degradable with longer turnover time than SOM with higher portion of simple organic molecules

(Davidson & Janssens 2006, Knorr *et al.* 2005). While chemical composition of OC had been shown to influence directly C mineralization in arctic soils (Schädel *et al.* 2014), recent studies reporting fast turnover times of "recalcitrant" groups of organic compounds (Qualls 2005) challenged the traditional point of view and it has been suggested that the OC quality plays less important role in SOM decomposition control as compared to environmental and biological factors (Schmidt *et al.* 2011). Availability of OC can be impeded by heterogenic spatial distribution of organic substrate within soil matrix and possible physical disconnection of immobile microbial cells from the OC source. Also presence of organo-mineral complexes makes organic compounds in mineral soils less available to microbes because more energy is needed for dissociation of SOM complexes with clay minerals or co-precipitated forms with Fe^{3+} and Al^{3+} oxides.

The SOM decomposition as a sequence of consecutive catabolic reactions is mediated by extracellular enzymes produced by microbial community and released to the soil. Complex organic compounds are first cleaved into smaller subunits by non-specific oxidative enzymes (peroxidases, phenoloxidases) produced by primary decomposers and then gradually broken down by hydrolytic enzymes into simple organic monomers (e.g. simple sugars, simple organic acids or amino acids) or functional groups (e.g. NH_4^+, PO_4^{3-}) which can be taken up by microbial cell as a source of OC and energy, or nutrients. Ability to produce different sorts of extracellular enzymes varies among microbes, for instance ability of synthesis of oxidative enzymes is rare among bacteria and has been attributed mainly to fungi. (Sinsabaugh *et al.* 2002). The degradability of complex OC therefore depends largely on abundance and activity of adequade enzymes. The diversity and magnitude of the enzyme pool is linked with microbial community composition. In addition, the enzyme activity depends on their life-time in the soil (on a rate of enzyme degradation or physical protection in mineral associations) as well as on current environmental conditions, e.g. on temperature (chapter 1.2.1) or O_2 availability (Schnecker *et al.* 2015, Tveit *et al.* 2012). For instance, anaerobic conditions in soil inhibit activity of oxidases which use O_2 for the enzymatic reaction (Ekschmitt *et al.* 2008) and thus the complex OC can remain unavailable as a substrate at low access of O_2.

1.2.4 NITROGEN AVAILABILITY

Besides the complexitiy of chemical structure of organic molecules, nutrient availability and stoichiometry of SOM, i.e. ratio of C and nutrients in the substrate,

play an important role in microbial growth, activity and decomposition (Schimel & Bennett 2004, Sistla *et al.* 2012). The primary production and SOM decomposition in arctic ecosystem are presumed to be N-limited (Giblin *et al.* 1991, Shaver & Chapin 1986, Atkin 1996, Schimel *et al.* 2004) and, indeed, Schädel *et al.* (2014) found a strong correlation between the C:N ratio of SOM and C minerazation across collected studies on arctic soils. Substrate with high C:N ratio is likely to provide insufficient source of N while being rich in OC, and vice versa for substrate with low C:N ratio. Nitrogen abundance in most of the arctic topsoils is low (Sistla *et al.* 2012, Wild *et al.* 2014). Plants and soil microbes compete for N resources and immobilize N in their biomass which leads to low rates of net N mineralization and low availability of mineral N species in the topsoil (Schmidt *et al.* 2002, Schimel *et al.* 2004, Kaiser *et al.* 2007, Paré & Bedard-Haughn 2012, Wild *et al.* 2014). The C:N ratio usually decreases with depth in the soil profile as described earlier in the text (see chapter 1.1). This C:N gradient infers that N limitation in the topsoils may flip into deficiency of available OC for microbial decomposers in the subsoils (Fierer *et al.* 2003, Wild *et al.* 2014) which means that not only N but also OC can limit the microbial mineralization of SOM in arctic soils. However, assuming that N in complex organic matter is bound in hardly decomposable organic compounds in more processed SOM of subsoils, microbes need to release extracellular enzymes to mine for N from the complex substrate.

During cryoturbation, poorly decomposed SOM from topsoil with relatively high C:N is subducted into subsoil where the microbial activity is limited (see chapter 1.1, paragraph I) and thus the SOM remains relatively unprocessed over time (Schirrmeister *et al.* 2011). Although the subducted SOM can be also mixed with surrounding mineral subsoil with higher N content, which might partly mitigate N deficiency, high enzymatic activity is still needed to quarry N as well as OC from SOM. Low N availability in combination with low enzyme activity may represent a bottleneck in decomposition of cryoturbated organic matter. Frequent vertical cryogenic moves along the cryoturbated soil profile can bring subducted SOM to the surface again, which would induce higher soil temperature, higher O_2 availability and input of root exudates. More favorable conditions could enhance microbial activity and support enzyme production. Therefore advanced comprehension of factors determining decomposition of the abundant subducted SOM is essential for assessment of its vulnerability.

Inputs of N into arctic soils are scarce; new N is implemented to the soil either by decomposition of N-poor plant material or by N_2 fixation. Nitrogen fixation, the only biological pathway of converting the atmospheric N into bioavailable N

species, is energy demanding process. Nitrogen fixation rates are generally low in the Arctic as compared to other ecosystems (Grimm & Petrone 1997) because the process is constrained by low temperature, limited energy inputs, low availability of other nutrients, e.g. phosphorus or low pH (Chapin & Bledsoe 1992). Still, N fixed by cyanobacteria (Liengen & Olsen 1997, Liengen 1999, Stewart *et al.* 2011, Sorensen & Michelsen 2011, Zielke *et al.* 2002) or heterotrophic soil bacteria (Nosko *et al.* 1994, Vile *et al.* 2014, Larmola *et al.* 2014, Knorr *et al.* 2015) can represent a crucial input of N into northern ecosystems (Hobara *et al.* 2006, Solheim *et al.* 2006, Vile *et al.* 2014) but its importance and contribution to N budget in different habitats of arctic tundra and the main driving factors are not yet well understood. The new N coming to the soil via N_2 fixation enters the cycle of N transformations; N assimilation, SOM depolymerization and N mineralization. Enhanced N abundance can contribute to higher rates of nitrification and denitrification and N_2O can be released as an intermediate of each of the two processes under certain conditions, namely high N availability and aerobic conditions for nitrification while anaeobic for denitrification (Baggs & Philippot 2010).

The N availability for microbial community in cryoturbated organic horizons is retained low because income of new N in subsoils is limited due to scarce input of plant material and deficiency of high energy substrate for N_2 fixation. Significant emissions of N_2O under these conditions are unlikely and indeed, virtually no N_2O fluxes have been reported from cryoturbated mineral tundra soils (Chen *et al.* 2014, Paré & Bedard-Haughn 2012, Marushchak *et al.* 2011). On the other hand, bare peat in peat circles is characterized with an excessive availability of mineral N which is linked to high N_2O emissions (Repo *et al.* 2009, Marushchak *et al.* 2011, Palmer *et al.* 2012). Several processes might play along here towards release of reactive N. Photodegradation of SOM with low C:N might represent not only a source of simplier organic molecules (see chapter 1.1, paragraph II) but also origin of additional NH_4^+ (Mayer *et al.* 2012, King *et al.* 2012), Otherwise high enzymatic activity is needed to support relevant rates of microbial N mineralization in the complex SOM. Further, N_2 fixation might constitute an additional N source. In fact, higher surface temperatures (Kaverin *et al.* 2016, Voigt *et al.* 2016) with abundant access of light, observation of dark crusts and detected low rates of photosynthesis at the bare surface (Voigt *et al.* 2016) lead to anticipation of occurence of autotrophic N_2-fixing microorganisms. In addition, all mineral N remains available to microbes due to absence of plants and lack of competition. Until present, however, high abundance of mineral N species in peat circles has not been completely clarified yet.

1.2.5 PRIMING EFFECT [1]

Positive change in input of fresh plant material such as root exudates or plant litter into soil can alter C mineralization not only by a simple addition of new substrate and its mere decomposition but also it can stimulate or reduce decomposition of older SOM (Kuzyakov *et al.* 2000). This so called priming effect is likely to play a role especially where microbial community is limited by nutrients (Fontaine *et al.* 2004). Fresh organic material contains nutrients and easily degradable compounds rich in OC and energy. They become available during the substrate decomposition and can partially release nutritional limitations of current microbial community. However, OC and nutrients released from the new substrate have high C:N ratio and the flux of nutrients to the soil under natural condition is relatively low. This supply is usually too low and nutritionally unbalanced for microbes to build new biomass but microorganisms can invest earned energy and nutrients into production of extracellular enzymes to break down more complex polymeric organic structures from SOM which would be otherwise out of reach for them without the substrate surplus. This way, the fresh substrate addition results in extra C mineralization derived from the older SOM (Fig. 6 a) and is referred to as positive priming (Kuzyakov *et al.* 2000). Under lower supply of fresh organic material, however, the substrate addition can reduce the decomposition of older SOM instead of stimulation which is known as negative priming (Kuzyakov *et al.* 2000). The added substrate would only fill in the needs of energy-limited microbes to maintain basic cell structures and basal metabolism but the microbes would not invest into building enzymes. They would further preferably use the simple, easily available substrate and refrain from complex SOM utilization. Thus, total C mineralization from the soil would increase to a lesser extend than could be expected from the direct addition of new-substrate mineralization because decomposition of SOM is diminished (Fig. 6 b).

[1] *„Priming efects are strong short-term changes in the turnover of SOM caused by comparatively moderate treatments of the soil. In the course of priming efects large amounts of C, N and other nutrients can be released or immobilized in soil in a very short time“* (Kuzyakov *et al.* 2000).

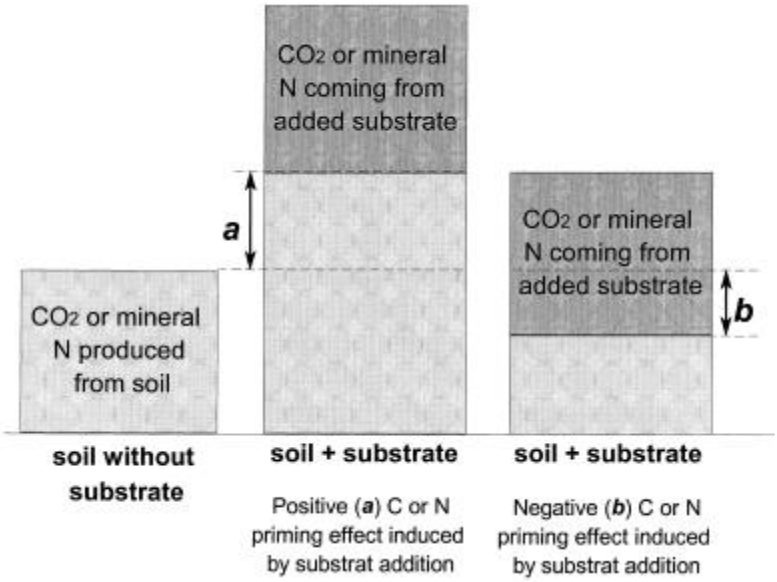

Figure 6*: Schematization of the priming effect, the non-additive interactions between mineralization of the added substrate and of SOM: (a) acceleration of SOM mineralization - positive priming effect; (b) retardation of SOM mineralization - negative priming effect (adopted from Kuzyakov et al. 2000).*

Increased plant production and different C and N allocation between soil and plants due to changes in vegetation composition (more abundant shrubs) and different distribution of plant material among aboveground and belowground biomass and root exudates has been observed in Arctic in experimental warming treatments (Hobbie & Chapin 1998, Van Wijk *et al.* 2003, Welker *et al.* 2004, Sistla *et al.* 2013, Natali *et al.* 2014, Hicks Pries *et al.* 2015, Salmon *et al.* 2016, Xue *et al.* 2016). Priming effect might significantly increase (Hartley *et al.* 2012) or decrease (Sistla *et al.* 2013) C_{LOSS} from tundra ecosystem. Positive priming effect could increase the C mineralization in soils beyond the expected temperature response of the microbial SOM mineralization if the nutrient and C surplus would be sufficient for microbes to invest into production of new enzymes. On the other hand, the negative priming would diminish the increase of microbial mineralization with temperature in case when lower substrate addition would cause only switch towards new substrate utilization and no production of new enyzmes would be initiated. There is an evidence from field and laboratory experiments that input of easily available plant material stimulates SOM degradation in arctic soils (Rinnan *et al.* 2007, Wild *et al.* 2014, Walker *et al.* 2016) but the response rate to a supply of

different substrates of varoius quality (root exudates or plant litter), can vary among different soil types and soil horizons (organic versus mineral). For example, microbial community in topsoils which compose of poorly decomposed, easily degradable organic compounds with high C:N ratio did not respond to addition of organic only-C substrate but amendment with organic N enhanced the SOM decomposition (Wild *et al.* 2014). Decomposition of SOM in tundra topsoils was stimulated also after inorganic N addition (Lavoie *et al.* 2011, Sistla *et al.* 2012, Nowinski *et al.* 2008), thus there is strong evidence of N-limited microbial community in the topsoils. Mineral subsoil with more processed SOM and low C:N ratio had a potential for higher C mineralization after addition of various energy-rich organic substrates but did not respond to additional N in substrate (Wild *et al.* 2014) likely because microbial community in subsoils was more energy- than N-limited (Fontaine *et al.* 2007). Decomposition in cryoturbated horizon positively responded to N-containing organic compounds, not to only-C organic substrate (Wild *et al.* 2014), likely due to mixed N and energy limitation. Experimental data suggest that the effect of plant material input to the soil will reflect stoichiometry of the SOM, nutritional demands of microbial community and composition of the fresh substrate. So far, the priming effect in the northern soils has received only little attention (Wild *et al.* 2014). There is an evidence that input of plant-derived organic compounds will inevitably increase under changing climate. Thus, better understanding of nutrient controls of the SOM decomposition and primig effects is needed in order to estimate the indirect effects of changes in plant production on the arctic C balance.

1.3 Ecosystem feedback to climate change

The permafrost region is the most vulnerable area to the climate change because the globally highest rise in mean annual temperature and increasing precipitation (IPCC 2013) present a threat to the frozen ground. Escalated thawing of permafrost leads to deepening of the active layer and release of SOM in the upper permafrost from the frost-protection. The ancient SOM from thawing upper permafrost becomes available to microbial decomposition (Schuur *et al.* 2008) and adds to the direct increase in SOM decomposition in response to increasing temperature. Permafrost layer can contain high amounts of relatively easily degradable SOM, nutrients and living microbial community (Uhlířová *et al.* 2007), which together may significantly contribute to increase of total C mineralization from tundra soils.

Ground-ice thaw is bringing changes in hydrological conditions and O_2 availability in soil profiles. It results either in drainage of active layer in well drained soils allowing better diffusion of ambient air to deeper layers (Swindles *et al.* 2015) or in surface subsidence and water-saturation of collapsing soil profiles leading to anaerobic conditions in the soils with poor drainage (Halsey *et al.* 1995, Jorgenson *et al.* 2001, Payette *et al.* 2004, Jorgenson & Osterkamp 2005; Fig. 7). The changes in arctic landscape due to permafrost degradation are rapid in the last decades and vary among landforms. For instance, area of permafrost peatlands, i.e. palsas and peat plateaus, decreased by half in northern Europe since the middle of the last century (Borge *et al.* 2017) while formation of new ice cores in northern peatlands (palsas) have been recently negligible (Zuidhoff & Kolstrup 2000). That suggests increased occurence of fully water-saturated peat soils, thermokarst lakes and exposed thermokarst walls (Sannel & Kuhry 2011). Upland tundra soils will likely experience drier conditions due to their elevated position and posibility of soil water run-off. Warming and sequential permafrost thaw can decrease water table in soil profile and allow better aeration of subsoils. Opposite to that, moisture will accumulate in poorly drained lowland mineral soils and support anaerobic conditions especially in subsoils but also in topsoils, depending on the level of water saturation. Warming is further likely to increase frequency of cryoturbation in the poorly drained mineral soils (Bockheim 2007) burying more topsoil organic material into subsoils. Therefore, it is relevant to assess potential C mineralization and GHGs emissions from arctic soils at both, presence and absence of O_2.

Warm and dry conditions can induce higher total C mineralization in tundra soils and higher contribution of older C from active layer (Natali *et al.* 2014, Dorrepaal *et al.* 2009) and permafrost (Schuur *et al.* 2009). Total C mineralization can be further accelerated by higher input of plant material which follows higher primary production in response to warming (see paragraph below). According to the estimates, 25–35 Pg C can potentially be lost from permafrost region by 2050 (Schädel *et al.* 2014) and up to 381–616 Pg C by 2300 if emissions of GHGs continue at present rates (Schuur *et al.* 2013) as a consequence of temperature rise and permafrost thaw. On the other hand, limited O_2 availability in water-saturated or inundated soil diminishes total C mineralization but supports CH_4 production (Schädel *et al.* 2016; Fig. 7). The relative ratio of CO_2 and CH_4 is crucial for identifying the feedback of arctic ecosystems to climate change (Schädel *et al.* 2016, Treat *et al.* 2015, Schuur *et al.* 2013) because both gasses have different long-term impact on

climate, defined as global warming potential (GWP[2]). Methane shows 28 times higher global warming potential than CO_2 considered on the 100-year time horizon (GWP_{100}, Tab. 1). Therefore CH_4 emissions from anaerobic soils might compensate for the lower C mineralization regarding the final GWP of aerobic versus anaerobic soils. Both gasses have further different pathways of their emission mitigation within the ecosystem. The CO_2 production from SOM decomposition can be counterbalanced to certain extend by plant CO_2 uptake where the CO_2 is incorporated into organic compounds of plant biomass (plant C sequestration, see paragraph below for ecosystem C balance). Production of CH_4 from water-saturated soils does not have so tight source-sink loop as CO_2. However, if CH_4 is produced in deeper layers of not completely water-saturated soil profile it can be oxidized to CO_2 when diffusing through drained aerobic topsoil if CH_4-oxidizing bacteria are present. Methane-oxidation would significantly reduce the final GWP per unit of mineralized C but the significance of this process in tundra soils will depend on hydrological regime of arctic CH_4-emitting soils.

Faster SOM decomposition brings along higher rates of nutrient mineralization, and thus increasing N abundance in soil (Salmon *et al.* 2016). Rising temperatures and higher N availability support higher plant productivity (Rustad *et al.* 2001, Hobbie *et al.* 2002, Sistla *et al.* 2013, Ju & Masek 2016) and plant material of higher quality. Lower C:N ratio of plant biomass is reflected in stoichiometry of plant-derived organic compounds entering to the soil and represent more balanced nutrition source for microbes. Carbon sequestration in plant biomass could potentially offset C mineralization from soils (Sistla *et al.* 2013) but many studies infer that soil C mineralization will dominate arctic C balance in the future (Natali *et al.* 2014, Abbott *et al.* 2016). The latter is supported by emerging understanding that the soil C mineralization might be further enhanced by positive priming by plant input (Schuur *et al.* 2013) enhancing mainly decomposability of OC in subsoils (Sistla *et al.* 2013, Wild *et al.* 2014) including cryoturbations. The process of cryoturbation itself represents C sequestration in the arctic ecosystem but the

[2] ***Global Warming Potential (GWP)***

"An index measuring the radiative forcing following an emission of a unit mass of a given substance, accumulated over a chosen time horizon, relative to that of the reference substance, CO_2. The GWP thus represents the combined effect of the differing times these substances remain in the atmosphere and their effectiveness in causing radiative forcing." (IPCC 2014)

Figure 7*: Scheme of climate-driven changes in arctic landscape and respective C-derived GHGs emissions. Increasing air temperature is entailed by permafrost thawing which enhances amounts of available SOM for microbial decomposition. According to the hydrological status of soils, organic C is lost from the soils to the atmosphere either as CO_2 when SOM is mineralized under aerobic conditions or as combination of CO_2 and CH_4 under anaerobic conditions. (Credit: Victor O. Leshyk / ECOSS)*

climate-driven changes in arctic soils can turn cryoturbated organic matter into significant source of CO_2 and CH_4, thus a contributor to positive feedback to arctic warming (Ping 2005). Therefore, the future C balance of this portion of arctic C will depend on the vulnerability of current cryoturbated OM and frequency of creation of new cryoturbated pockets. Complex understanding of vulnerability of OC in cryoturbations is thus crucial for future projections of arctic ecosystem response to climate change.

Although C-derived GHGs dominate the soil - atmosphere interactions, soils with high water content and substantial N turnover rates are also potential sources of N_2O. Indeed, current studies have documented also N_2O emissions from thawing permafrost (Elberling *et al.* 2010) and cryogenic bare surfaces of permafrost peatlands (Repo *et al.* 2009, Marushchak *et al.* 2011, Voigt *et al.* 2017). In the

hotspots, this strong GHG representing 265 higher GWP_{100} than CO_2 (Tab. 1) can be emitted at comparable rates as in tropical or agricultural soils. This represents significant contribution to positive climate-ecosystem feedback loop but until present, the underlying processes of the exceptional N_2O emissions are not well understood.

***Table 1**: Global warming potentials (GWP) for methane (CH_4) and nitrous oxide (N_2O) relative to CO_2 on the 20- and 100-year time horizon. Values adopted from AR5 (Myhre et al. 2013)*

	GWP	
	Cumulative forcing over 20 years (GWP_{20})	Cumulative forcing over 100 years (GWP_{100})
CO_2	1	1
CH_4	84	28
N_2O	264	265

To sum up, the climate-driven changes in arctic region can escalate the positive feedback to warming in two ways: i) surplus of C_{LOSS} from the soils which is beyond the simple temperature response of SOM decomposition or ii) increase in production of other, stronger GHGs; CH_4 and N_2O. The total C balance and GHGs emissions from arctic ecosystem are strongly affected by changes in nutrient availability and vegetation cover, and by priming effect.

1.4 MOTIVATION FOR THE WORK

Current research have refined soil OC and N inventories at both, regional and circumpolar scale (Tarnocai *et al.* 2009, Hugelius *et al.* 2014, Palmtag *et al.* 2016), and numerous field measurements of GHGs fluxes have provided estimates of regional C balance and apparent responses of microbial activity to changing conditions (Oberbauer *et al.* 1998, Grogan & Chapin 2000, Christensen *et al.* 2004, Groendahl *et al.* 2007, Oberbauer *et al.* 2007, Biasi *et al.* 2008, Schuur *et al.* 2009, Lamb *et al.* 2011, Natali *et al.* 2011, Marushchak *et al.* 2013, Fouché *et al.* 2014, Knoblauch *et al.* 2015, Voigt *et al.* 2016, D'Imperio *et al.* 2017). Observed C and N losses from soil surfaces in forms of GHGs are results of many sequential processes which need to be understood in order to explain the variability in space and time,

and the responses to impending climate changes (Schuur *et al.* 2013). Combination of field observations of climate-driven changes in landscape, *in situ* manipulative experiments and field measurements of GHGs fluxes, and targeted laboratory experiments is needed to achieve deeper mechanistic understanding of arctic SOM vulnerability, potential future GHGs fluxes and factors controlling microbial processes. Carbon and N dynamics studied *in situ* under natural environmental conditions reflect mainly the seasonal and daily variations in weather conditions and so can determine the responses of GHG fluxes to temperature, moisture and light conditions. The laboratory experiments under controlled conditions can specifically target potential C_{LOSS} and N transformations mediated by soil microbial community, reveal the biotic and abiotic drivers and functional responses.

Recent studies brought evidence on low degradability of abundant OC in cryoturbated organic horizons in mineral tundra soils and peat circles representing exposure of deep peat layers in permafrost peatlands. Yet, the OC and N processes, biochemical interactions and microbial communities in these soils rich in OC are not so well studied as in topsoils of mineral tundra and arctic peatlands. This study is focused on defining potential C mineralization and GHGs emissions from cryoturbated organic horizons (**paper I**) and peat circles (**paper II**) under various O_2 availability and temperatures. Temperature sensitivity of C mineralization presented here extends previous work of our research team summarized by Petr Čapek in his Ph.D. thesis (Čapek 2016). I search for reasons of different temperature sensitivity among various soils and attempt to find factors which regulate the temperature response of C mineralization among permafrost-affected soils. Further, this study intends to link C mineralization to OC quality, SOM stoichiometry and extracelular enyzme activity and reveal the main drivers of microbial SOM decomposition in permafrost-affected soils (**paper I** and **II**). I also attempted to understand the priming effect in arctic soils which will likely follow the rising input of plant-derived organic material into the soils (**paper III**). As a special interest, the study focused on N-rich peat circles which were previously identified as hot spots of N_2O emissions, the rare exception of N-poor arctic soils. The research focus here was to find out if biological N_2 fixation can contribute to the N abundance in the soil and thus support the specific N dynamics (**paper IV**).

2 AIMS AND OBJECTIVES

The overall aim of this work was to asses OC vulnerability in permafrost-affected arctic soils, specifically in cryoturbated upland tundra soils and permafrost peatlands, under various environmental conditions and specify factors modulating SOM decomposition and GHGs production. Special focus was aimed on understanding N dynamics which influence C cycling and GHGs emissions. Following five objectives were defined to meet the aims:

1. To quantify potential C mineralization in different horizons of mineral tundra soil and permafrost peatland, and determine factors controlling degradability of OC in cryoturbated organic horizon and peat circles.

2. To determine temperature sensitivity of C mineralization and to find factors which modify the temperature response among studied arctic soils.

3. To assess an effect of O_2 availability on C minealization and its temperature sensitivity and an impact on potential emissions of CO_2 and CH_4.

4. To define potential priming effect in different horizons of cryoturbated mineral tundra initiated by addition of plant-derived organic compounds and assess nutritional limitations in separate horizons.

5. To assess N availability in studied soils, examine the role of N_2 fixation in N dynamics of the soils from East-European subarctic tundra and identify microbial processes leading to high abundance of soil N and high N_2O emissions from peat circles.

3 RESULTS AND CONCLUSIONS

3.1 DEGRADABILITY OF OC IN PERMAFROST-AFFECTED ARCTIC SOILS

Three incubation experiments were designed to assess OC degradability in arctic soils affected by cryogenic processes. Carbon mineralization, i.e. C_{LOSS} from incubated soil in form of CO_2 and CH_4 was monitored over the incubation periods and the cumulative C_{LOSS} at the end of the incubation period normalized to OC content represented a measure of OC degradability. Representative soil horizons of cryoturbated upland tundra (organic topsoil, cryoturbated organic horizon and mineral subsoil from active layer) from Central Siberia were studied in the first experiment (**paper I**). Second experiment targeted diversity of surface soil habitats in East-European subarctic tundra including bare peat circles, topsoil of adjacent vegetated peat plateau as well as topsoil of upland tundra dominating the region (**paper II**). In both experiments, soils were incubated under three different temperatures (4, 12, 20°C) under aerobic and anaerobic conditions and chemical, biochemical and microbiological parameters were determined before and after the incubation. Third experiment included all horizons of cryoturbated upland tundra (organic topsoil, mineral topsoil, cryoturbated organic horizon, mineral subsoil of active layer and permafrost) collected from West, Central and East Siberia and besides the OC degradability focused also on assessment of priming effect induced by plant-derived organic compounds in these horizons (**paper III**).

The degradability of OC in cryoturbated organic horizons and bare peat circles was generally three- to fivefold lower than from topsoils of upland tundra (**paper I, III**) and vegetated peat plateau (**paper II**), respectively. The findings in presented studies add to previous evidence of retarded decomposition of SOM incorporated into subsoil in mineral tundra by cryoturbation and deep peat layers in permafrost peatlands which were drawn from the indices of OC chemical composition (Routh *et al.* 2014, Gentsch *et al.* 2015a, Pengerud *et al.* 2017), biochemical potential of microbial community (Schnecker *et al.* 2014, Wild *et al.* 2014) and microbial community composition (Gittel *et al.* 2014a, Gittel *et al.* 2014b, Palmer *et al.* 2012).

Organic C in cryoturbated organic horizons had lower degradability also as compared to mineral subsoil of active layer and permafrost (**paper I, III**) which was in line with data from earlier laboratory experiment by Kaiser *et al.* (2007), the first study that documented low rates of microbial processes and microbial biomass in C-

rich cryoturbated organic horizons. In permafrost subsoil, content of OC was low but the OC was characterized by high degradability comparable with OC in topsoil (**paper III**). Results of low OC degradability in peat circles, higher in topsoils of vegetated peat plateau and the highest degradability of OC in upland tundra topsoil (**paper II**) were in good agreement with earlier laboratory study by Biasi et al. (2014) which reported aerobic soil microbial respiration under one incubation temperature from same habitats of the same study site. Other literature sources on potential C mineralization of deeper layers of arctic peatlands or their exposures are scarce. For instance, Moni *et al.* (2015) detected slightly higher degradability of subsoil peat from 20-50cm depth of active layer of other permafrost peatlands as compared to bare peat in peatcircles, which likely reflected origin of peat circle material in greater depth and thus its higher age. Other data refer to comparably higher C mineralization in old peat in permafrost layers (Lee *et al.* 2012, Moni *et al.* 2015). Generally, permafrost layers accumulate labile OC leached down from topsoils (Dutta *et al.* 2006, Pengerud *et al.* 2017). After permafrost thaw, initial mineralization peak is attributed to utilization of this allochtonous fast-cycling C pool but if there is no continuous supply of topsoil leachates mineralization rates decrease with time due to forced substrate shift towards the more recalcitrant autochonouos resources (Dutta *et al.* 2006, Schädel *et al.* 2014, Moni *et al.* 2015).

Low C mineralization corresponded to low microbial biomass in both, cryoturbated organic horizon (**paper I, III**) and peat circles (**paper II**) and across all studied soil horizons and soil habitats, microbial biomass was evaluated as the strongest predictor of C mineralization among all the other environmental, microbiological and chemical parameters (**paper I, II**). While microbial biomass represented about 1% of total OC content in topsoils, cryoturbated horizons and peat circles harbored microbial community which constituted only about 0.2% OC. Several constraints imposed upon microbial community in deep layers of permafrost-affected soils were inferred from the presented results and related arctic research. These lines of evidence point towards multiple control of microbial activity and growth by combined effect of chemical, biological and physical constraints.

First, OC of low quality provides poor energy and OC supply for microbial growth. The OC in cryoturbated horizons and peat circles underwent a process of selective preservation of complex compounds such as less-degradable lignin forms (Routh *et al.* 2014) due to slow decomposition during long period in subsoil under unfavorable conditions. Moreover, the complex OC in cryoturbated horizon is associated with clay-sized minerals and coprecipitated with hydrolyzable Fe and Al species (Gentsch

et al. 2015a, Gentsch *et al.* 2015b) and such organo-mineral bounds require more energy from microbes to access the substrate.

Second, activity of soil microbial community in cryoturbated organic horizon and in bare soil of peat circles is impaired by disrupted common plant-soil interactions. Direct consequence of that is impeded input of easily degradable source of carbon, nutrients and energy in form of root exudates and plant litter. Thus, microbial communities were assumed to draw their resources from autochtonous complex SOM. This is entirely true for surface layers of peat circles (**paper II**). Due to loss of topsoil and reposition of deep peat layers towards the surface, microbial community in peat circles relies on autochtonous OC sources. Other habitats where deep peat layers come exposed in non-permafrost peatlands are harvested peatlands. Bare peat surface of harvested temperate peatland showed similar chemical composition and as low degradability and microbial biomass as found in peat circles (Glatzel *et al.* 2004, Basiliko *et al.* 2007). Constrained microbial degradation persisted also in abandoned sites which were still mainly bare after 30 years. Even though the time scale from peat circle formation to its natural cessation is still elusive, similar trend as in the abandoned harvested peatlands is apparent: long-term exposition of the deep peat layers to aerobic conditions does not initiate faster decomposition in the bare peat material. Different situation turned out to govern the SOM decomposition in the soils of cryoturbated upland tundra (**paper I**). Leaching of dissolved organic and inorganic compounds from topsoils represents important downward flux of nutrients in moist arctic conditions (Dutta *et al.* 2006, Lee *et al.* 2012, Voigt *et al.* 2016, Pengerud *et al.* 2017) and these resources reach microbial community in subsoils and cryoturbated horizons. Results of **paper I** implied that the microbial community in cryoturbated horizons preferably used allochtonous influx of dissolvable organic C, nutrients and extracellular enzymes to overcome shortage of available autochtonous resources but lacked energy to produce sufficient pool of own enzymes for efficient breakdown of autochtonous low-quality OC (Fig. 8). The allochtonous resources induced negative priming effect (see chapter 1.2.5) and ushered the cryoturbated SOM towards further preservation from decomposition.

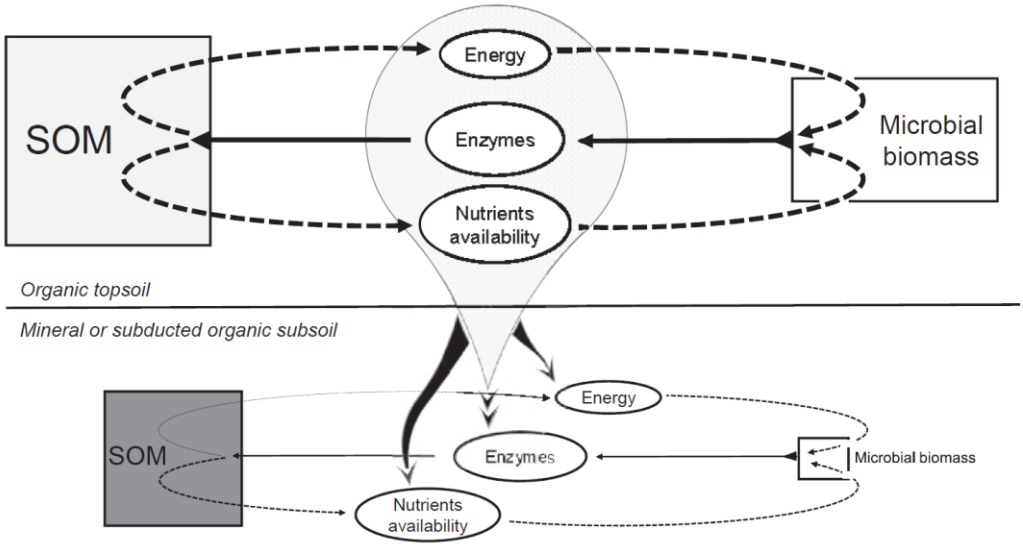

***Figure 8**: Scheme of energy, nutrient and extracellular enzyme flow between horizons of cryoturbated upland tundra soil profile. Labile OC and nutrients derived from faster SOM decomposition and extracellular enzymes production in topsoil are transported in the pore water downwards to mineral subsoil and cryoturbated organic horizons. Allochtonous sources of dissolved organic C, dissolved forms of nutrients and products of cleavage by allochtonous extracellular enzymes are preferably used by energy-limited microbial community in cryoturbated organic horizon and subsoil, which leads to preservation of autochtonous old SOM (scheme designed by Petr Čapek, **study I**).*

Third, low abundance of fungi within the microbial community in cryoturbated organic horizon (Gittel *et al.* 2014a; Fig. 9) and bare peat circles (**paper II**) might further restrain efficient decomposition of complex OC. Fungi are efficient decomposers and main producers of oxidative enzymes which are crucial for initial breakdown of phenolic and aliphatic macromolecules (Talbot *et al.* 2008). Lost connection with the plants detach fungal community from the advantage of easily degradable plant-derived C source which would support them in production of energetically demanding enzymes. Also unfavorable environmental conditions such as frequent subzero temperatures and high moisture add to growth limitations and low activity of fungi in arctic subsoils. Thus, low abundance of fungi can represents a limiting step in decomposition of complex forms of OC (Gittel *et al.* 2014a, Routh *et al.* 2014, Tveit *et al.* 2013). Instead, Actinobacteria came recently to attention as abundant frost-persistent (McMahon *et al.* 2011), oligotrophic (Fierer *et al.* 2003), apparently anaerobic (Tveit *et al.* 2013, DeAngelis *et al.* 2011) lignin decomposers

(Godden *et al.* 1992, Zimmermann 1990). Actinobacteria were found in high abundance in cryoturbated organic horizon (Gittel *et al.* 2014a) and in peat circles (Palmer *et al.* 2012) which indicated that they can fill the niche after disadvantaged fungi (Gittel *et al.* 2014a, Gittel *et al.* 2014b, De Boer *et al.* 2005). However, their degrading potential seems to lag behind the fungal decomposing activity (Gittel *et al.* 2014a). Therefore their presence might not fully substitute the role of fungi and thus undecomposed recalcitrant organic compounds might continue to cumulate in the soils.

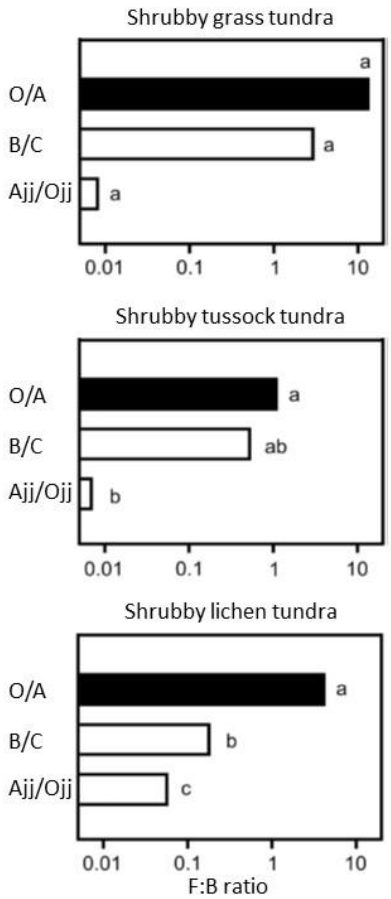

Figure 9*: Fungal-bacterial ratio in three cryoturbated upland tundra habitats from Eastern Siberia with different vegetation in soil horizons; O – organic topsoil, A – mineral topsoil, B and C – mineral subsoil, Ojj and Ajj - cryotubated organic horizons. Ratio was calculated from fungal and bacterial SSU rRNA gene copies* g^{-1} *dry soil. Small letters in the graphs indicate significant differences between soil horizons as determined by one-way ANOVA and Tukey's HSD test. (adopted from Gittel et al. 2014a).*

Fourth, subsidence of SOM into deep soil layers either by gradual peat accumulation or by cryoturbation caused soil compaction which resulted in high bulk density and reduced pore space with limited water flux and gas exchange. Cell dispersion and active microbial colonization of such soil matrix might be spatially limited (Nunan *et al.* 2002, Ekschmitt *et al.* 2008, Schmidt *et al.* 2011). The microscale habitat fractionation and physical disconnection between microbes and substrate can possibly add to all previous reasons for low ability of microbial community to decompose complex OM and multiply its numbers.

The presented studies emphasize that C mineralization in arctic soils does not show a simple correlation with OC content. Degradability of abundant OC in cryoturbated horizons and peat circles is low due to low microbial biomass harboring these soils. The growth of microbial biomass is probably restrained by combined effect of low OC quality and its availability, low abundance of fungi as effective primary decomposers, limited supply of easily decomposable plant-derived organic substrate and spatial disconnection between available organic compounds and microbial cell. It is important to note that the degradability of OC does not directly translate to surface C fluxes detected under field conditions on soil-volume or square-meter basis. For instance, Biasi *et al.* (2014) showed that even though the OC degradability of bare peat in peat circles is low, C mineralization on square-meter basis was similar or even higher than in vegetated peat plateau which reflected three- to fourfold higher bulk density of the bare peat than that of the topsoil of vegetated peat plateau. Therefore, the differences in soil bulk density need to be taken into account when drawing inferences from laboratory results into field conditions.

3.2 Temperature sensitivity of C mineralization

Temperature sensitivity of aerobic C mineralization from the cryoturbated horizon was invariant from the topsoils and the mineral subsoil of upland tundra (**paper I**). Similarly, temperature sensitivity of C mineralization in peat circles was comparable to vegetated peat plateau and adjacent upland tundra topsoils (**paper II**). Thus, temperature response of C mineralization in the laboratory experiments did not reflect the OC quality. Microbial biomass showed no significant change during the incubation period in all studied soils and thus did not influence the evident temperature sensitivity of C mineralization either in upland tundra soil horizons or in peat soils. Microbial biomass appeared as rather stable parameter in

the soils until the OC or N availability was artificially enhanced during the third experiment (**paper III**, see chapter 3.4). However, the temperature sensitivity varied between the two studies; an average Q_{10} = 2.41 for all studied soil horizons of cryoturbated upland tundra in Central Siberia; organic topsoil, cryoturbated organic horizon and mineral subsoil (Čapek 2016) was in agreement with physiological temperature response of aerobic microbial respiration suggested by metabolic theory (Brown *et al.* 2004, Allen *et al.* 2005, Allen & Gillooly 2009, Yvon-Durocher *et al.* 2012). In contrast, higher temperature sensitivity with an average Q_{10} = 3.68 was observed in all soil habitats of East-European subarctic tundra; in old bare peat, topsoil of vegetated peat plateau and topsoil of upland tundra (**paper II**). The basal C mineralization temperature function in all three soils was modified by changes in pool of hydrolytic enzymes during the incubation. All classes of hydrolytic enzymes involved in C, N and P mining increased in their abundance in soils which inferred active enzyme production over the incubation time. Correlation between temperature responses of C mineralization and pool of hydrolases at the end of the incubation period (Fig. 10) suggests that increased enzymatic pool can elevate evident temperature sensitivity of C mineralization, likely in two steps: i) more CO_2 is produced in oxidative phosphorylation when new enzymes are synthetized and ii) more substrate for microbial decomposers is available in a result of activity of the enzymatic surplus. In conclusion, temperature sensitivity of C mineralization is not only a function of physiological response of microbial C mineralization but can also reflect changes in enzymatic pool.

3.3 Effects of O_2 availability on C mineralization and C-derived GHGs emissions

Anaerobic conditions substantially decreased C mineralization as compared to aerobic conditions in the topsoils of upland tundra and vegetated peat plateau as well as in the cryoturbated horizons and bare peat circles (**paper I** and **II**). The data used for **paper I** and **II** were included in a dataset of existing laboratory incubations using permafrost soils (Schädel *et al.* 2016) where the authors searched for overall trends in aerobic versus anaerobic potential of C mineralization. The meta-analysis suggested that the ratio of aerobic to anaerobic C mineralization from high-latitude soils is invariant across soil types and across 0-20°C temperature range (Schädel *et al.* 2016). However, the **paper I** and **II** alone show results contrasting with the

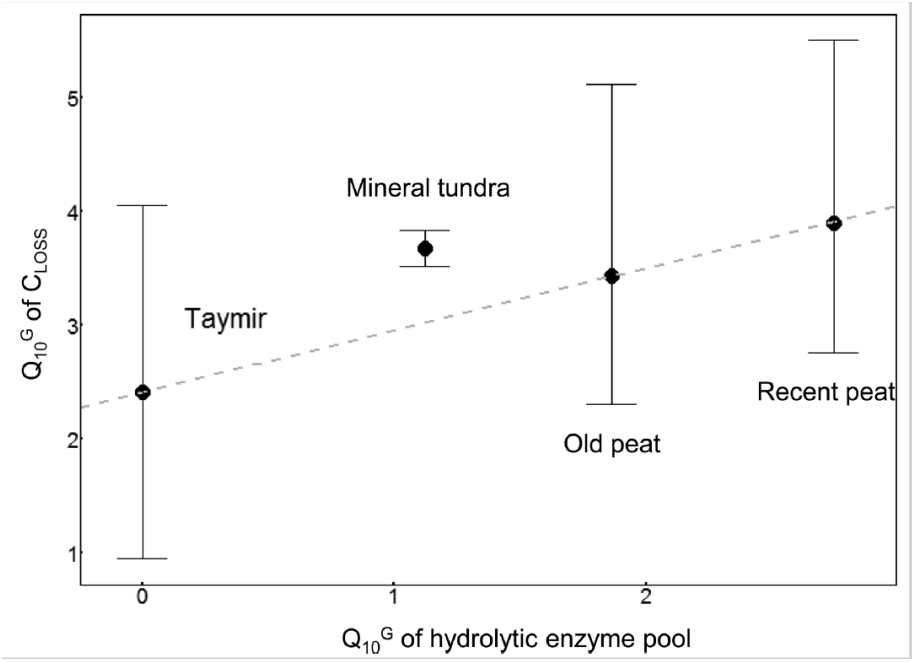

***Figure 10**: Correlation between temperature response of C mineralization (C_{LOSS}) and pool of hydrolytic enzymes under aerobic conditions expressed as Q_{10}. The temperature response was fitted by Gaussian equation and Q_{10}^{G} was calculated for the temperature interval 5 – 15 °C where all the fitted curves were in exponential phase. Lack of response of hydrolytic enzyme pool to temperature in soils of Central Siberia sets the C mineralization temperature sensitivity to the basal physiological response of microbial mineralization (**paper I**). On the other hand, increase in hydrolytic enzyme pool during the incubation period increased evident temperature sensitivity of C mineralization in soils from East European subarctic tundra; topsoil of vegetated peat plateau (recent peat), bare peat from peat circles (old peat) and topsoil of upland tundra (mineral tundra; **paper II**). At the latter, also other undefined factors play a role.*

general pattern. The difference between C mineralization from soils under contrasting O_2 regimes increased towards higher temperatures in all soil types and horizons because anaerobic C mineralization had lower temperature sensitivity. Thus, the ratio of aerobic to anaerobic C mineralization increased with temperature. Further, the lack of O_2 affected C mineralization in cryoturbated horizons and peat circles to a lesser extent than C mineralization in topsoils. The different OC degradability may play a role in varying effect of O_2 availability on C mineralization among the soils; the higher was the OC degradability, the greater was the difference between aerobic and anaerobic C mineralization (Fig. 11). Also, the different

response to O_2 availability may reflect adaptation of microbial community in cryoturbated horizons and peat circles to frequent anaerobic conditions in deeper layers of the soil profile where the soils have developed (Tveit *et al.* 2013) and ability of the microbes to utilize complex OC in presence as well as in absence of O_2. In fact, Actinobacteria have the potential to degrade complex compounds even under low O_2 availability (DeAngelis *et al.* 2011) so their high abundance and activity in cryoturbated horizons and peat circles (see above chapter 3.1) could explain the lower difference in aerobic and anaerobic C mineralization than in naturally aerobic topsoils. Interestingly, the small difference between aerobic and anaerobic C mineralization was maintained in the peat circles where the peat layers from deep were uplifted to the surface. This could be connected with the low abundance of fungi in the bare peat or with physical conditions in peat circle soil; the decomposed fine peat material with high bulk density forming aggregates may be providing stable anaerobic micro-environments and thus harbor anaerobic decomposers even in otherwise well-drained surface soil.

The highest potential for CH_4 production in arctic soils have been attributed to topsoils (Lee *et al.* 2012, Treat *et al.* 2015, Walz *et al.* 2017) and correlation with OC content have been suggested (Treat *et al.* 2015). In concordance with the earlier, methanogenesis evolved during the incubation only in topsoils of both studied upland tundra habitats; shrubby grass tundra from Central Siberia (**paper I**) and East-European subarctic tundra in Russia with vegetation composed of lichens, mosses, shrubs and graminoids (**paper II**). No CH_4 was emitted from subsoil and cryoturbated organic horizon. In contrast to the suggested link between CH_4 production and OC content (Treat *et al.* 2015), CH_4 production was detected neither in topsoil of peat plateau vegetated by Sphagnum mosses and shrubs nor in bare peat of peat circles which both composed of purely organic material. The results imply that methanogenesis is further closely linked with high OC degradability. Methanogenic activity is dependent on abundant source of labile OC substrate, mainly simple organic acids (Metje & Frenzel 2007, Herndon *et al.* 2015) which are scarce in peat circles, topsoil of peat plateau composed mainly of Sphagnum mosses, cryoturbated horizons and mineral subsoil.

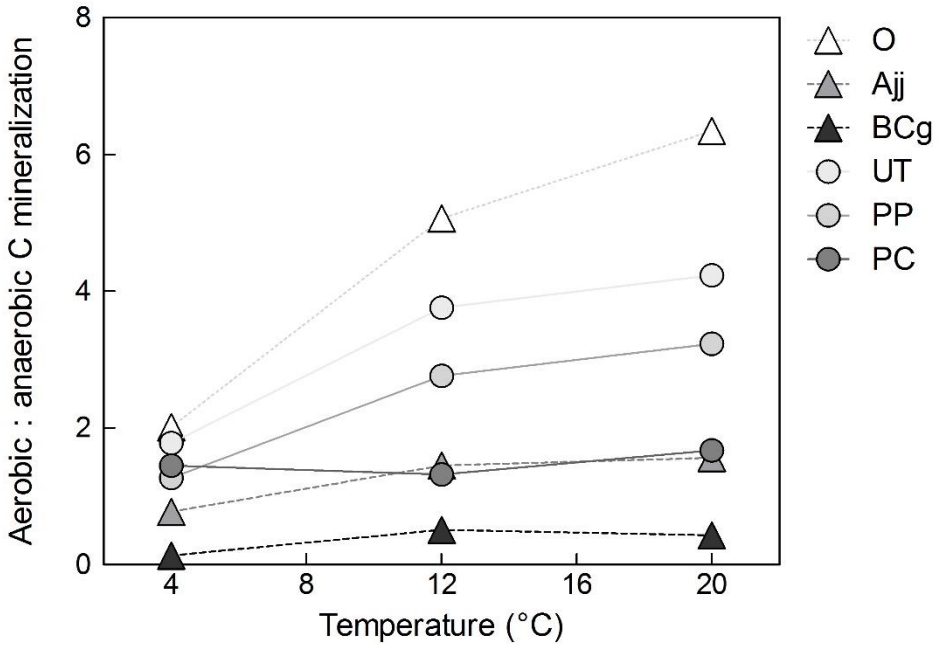

Figure 11*: Ratio of aerobic to anaerobic C mineralization in studied soils under different temperature. Data combined from* ***paper I*** *and* ***II*** *are shown. Lines connect points representing the same soil incubated under three different temperatures. Triangles with dashed lines show data from horizons of cryoturbated upland tundra from Central Siberia: O – organic topsoil, Ajj – cryoturbated horizon, BCg – mineral subsoil (data from* ***paper I****). Circles with solid line depict data points from habitats of East-European subarctic study site: PP – vegetated peat plateau, PC – peat circles, UT – upland tundra topsoil (data from* ***paper II****).*

Potential for CH_4 production in topsoils can be further modulated by dominating tundra vegetation because the OC quality of topsoil SOM is affected primarily by composition of plant species (Bellisario *et al.* 1999), e.g. herbaceous vegetation produces more degradable substrate and thus supports higher CH_4 production than bryophytes and shrubs (Moore & Dalva 1997, Treat *et al.* 2015). Thus, topsoil of upland tundra formed under vegetation with higher abundance of graminoids represented easily degradable substrate providing methanogens with adequate source of labile OC. Topsoil of vegetated peat plateau which formed under the bog vegetation and consisted mainly of Sphagnum mosses and also dwarf shrubs showed low anaerobic decomposition rates which indicated insufficient supply of OC substrate for methanogenesis (Nykänen *et al.* 1998). In addition, peat

soilscontain abundant sources of alternative, more efficient electron acceptors for anaerobic respiration which can delay onset of methanogenesis, e.g. nitrates were abundant in peat circles, and Fe^{3+} alone (Reiche *et al.* 2010) or in combination with humic substances (Lovley *et al.* 1996, Lipson *et al.* 2010) can play a role in peat soils. The results suggest that not only OC content regulates potential for methanogenesis but mainly positive relationship with OC degradability and negative correlation with abundance of oxidized forms of alternative electron acceptors are key controls of CH_4 production in arctic soils. In soils lacking the labile OC source, methanogenic community might not have developed (McCalley *et al.* 2014).

Methane can be oxidized in the topsoil of the vegetated peat plateau under aerobic condition (**paper II**), which is in concordance with an occasional function of the vegetated peat plateau as a CH_4 sink (Marushchak *et al.* 2016). Although the peat from peat circles and topsoil of vegetated peat plateau emitted no CH_4 during anaerobic laboratory incubation, both habitats were reported as CH_4 sources under field conditions with peat circles as a steady CH_4 source and vegetated peat plateau balancing between a source and a sink depending on temperature and moisture (Marushchak *et al.* 2016, Voigt *et al.* 2016). The observed surface CH_4 fluxes under natural conditions are result of balance between upward diffusion of CH_4 produced in deeper layers and oxidation in surface layers. Methane was likely produced in deeper layers of the peat soils (Tveit *et al.* 2013) where downward leaching of DOC from topsoils supplied degradable OC to methanogenic community (Voigt *et al.* 2016).

Methane oxidation activity was found also in the topsoil of East-European subarctic upland tundra during aerobic incubation (**paper II**). Indeed, the upland tundra habitat was identified mainly as a CH_4 sink in the natural environment where the decomposition in the topsoil was governed by aerobic conditions (Voigt *et al.* 2016). However, it has been documented that upland tundra habitat turned into a small CH_4 source during warmer seasons with higher precipitation (Marushchak *et al.* 2016) likely due to higher water saturation in topsoil and CH_4 production also from topsoil. Anaerobic incubation confirmed high CH_4 production potential in the topsoil which was linked to high OC degradability. The topsoil apparently harbors both, methanogenic and methanotrophic communities and the balance between their activities rules the final CO_2:CH_4 emission ratio.

The more frequent anaerobic conditions in tundra soils as a consequence of rising temperature and precipitation and permafrost degradation (IPCC 2013) can reduce C mineralization and total C_{LOSS} to the atmosphere (CO_2 and CH_4) mainly from inundated topsoils while this effect might be lower in subsoils and cryogenic soil

features. At the same time, however, potential for CH_4 production increases. The C mineralization rates and the portion of CO_2 and CH_4 emitted from anaerobic soils are affected by availability of easily degradable SOM, presence of other electron acceptors and methanogenes themselves.

3.4 Priming effect induced by plant-derived organic compounds

Addition of plant-derived organic material stimulated degradability of the autochtonous OC and enhanced microbial growth in all horizons of cryoturbated upland tundra. The effect was stronger in subsoils including the mineral subsoil of active layer, permafrost and cryoturbated horizons as compared to the organic and mineral topsoil. Microbial C mineralization increased in each horizon with individual response to addition of cellulose (source of easily degradable OC only), protein (source of easily available OC and N) or both. The response patterns reflected distinct stoichiometry and quality of bulk SOM in separate horizons (Fig. 1) and allowed to deduce the nutritional constraints of microbial activity; N, OC or energy limitation.

Organic topsoil was positively responsive only to protein addition while added cellulose did not promote mineralization of the soil OC (**paper III**). Together with evidence of high OC degradability and high C:N ratio (**paper I**), this suggested that microbial community in organic topsoils was limited by N availability and proteins were mainly used as N source, which was in agreement with previous studies (Wild *et al.* 2014, Sistla *et al.* 2012, Lavoie *et al.* 2011). The C mineralization in mineral subsoils from active layer and permafrost was stimulatedby both, protein and cellulose (**paper III**), and their effect was correlated suggesting limitation by low availability of OC. Sources of OC are scarce in the subsoil due to low OC content in the soil and low C:N of the bulk SOM (**paper I**). Obviously, the microbial community utilized both added organic compounds as OC source and preferentially used them to generate energy. Provided with the energy surplus, microbes could produce new enzymes necessary for more effective utilization of complex organic compounds and thus increase the C mineralization of autochtonous SOM (**paper III**).

Cryoturbated organic horizon and mineral topsoil were similar in chemical composition and stoichiometry of organic matter (Kaiser *et al.* 2007, Gentsch *et al.* 2015a). Protein and cellulose addition stimulated C mineralization and significantly increased microbial growth but no correlation between protein and cellulose effects was found (**paper III**). Samples reacted individually and the stimulating effects of

cellulose and protein were independent which implied intermingled C and N limitation. As mentioned above, microbial community in cryoturbated horizons lacked energy to produce extracellular enzymes (**paper I**) which was in line with evidence of low ability of microbes to access N bound in polymeric compounds (Wild *et al.* 2013). Indeed, the response to protein was higher than to cellulose (**paper III**). Also earlier study showed that addition of N-containing monomeric and polymeric organic substrate lead to substrate-derived OC incorporation into microbial biomass, increased investment into production of extracellular enzymes and stimulation of autochtonous SOM decomposition (Wild *et al.* 2014). Thus, combination of both, plant-derived C and N compounds have the potential to promote microbial decomposition of OC in cryoturbated horizons. Under current conditions with no or only negligible input of fresh plant material, SOM in cryoturbated horizons is preserved by the negative priming: decomposers lack energy to break down complex organic matter and N to produce extracellular enzymes. Microbial growth and activity relay to great extend on allochtonous sources of energy, nutrients and enzymes leached down the profile from organic topsoil. At low influx from topsoil, the microbial community has low potential to degrade the autochtonous SOM and to maintain sufficient pool of extracellular enzymes, and uses the allochtonous sources only to cover their fundamental metabolic energy demand (**paper I**). However, when higher amount of degradable organic compounds becomes available, e.g. as a result of increasing plant productivity in response to climate warming, both plant-derived OC and N can be invested by microbes also into production of new extracellular enzymes and can stimulate autochtonous SOM decomposition (**paper III**).

Nutritional limitations of C mineralization differ among separate horizons of upland tundra soils and are linked to gradual shift in degree of SOM decomposition which is accompanied by variable OC availability and C:N ratio along the soil profile. Microbial decomposition in the organic topsoil with poorly decomposed SOM is primarily N limited while decomposition in the OC-poor mineral subsoils with more decomposed SOM is limited by OC and energy. Microbial decomposition in cryoturbated horizons is limited by low availability of OC and N as well as by low energy. Only sufficient input of plant-derived organic compounds will likely have the positive priming effect on C mineralization in cryoturbated SOM while low additions might lead to negative priming and preservation of old SOM.

3.5 N TRANSFORMATIONS IN SOILS WITH VARYING N AVALABILITY

The fourth study presented in **paper IV** comprised of field measurements and complementary laboratory experiments targeting N transformations in the peat circles from the same soil habitats of East-European subarctic tundra as in the **paper II** with a specific focus on processes underlying N_2O emissions. Summary of main N transformations rates allowed to assess N availability in peat circles, vegetated peat plateau and upland tundra. Nutritional limitations of microbial decomposition in the soils could be then inferred by combining evidence of N availability together with the data on OC degradability (**paper II**).

High gross and net N mineralization was found in the peat circles (**paper IV**) which documeted surprisingly fast N turnover and high N availability under the conditions of low OC degradability and low OC quality (see chapter 3.1, **paper II**). Together these lines of evidence suggest that microbial activity and growth in peat circles are at least partly limited by OC availability. Microbes decompose old peat with low C:N in order to quarry for OC. Due to complex nature of SOM, energy yield is low and provides weak support to microbial growth. Excessive amount of organic N is mineralized and only a portion is assimilated by microbes into their biomass which leads to cumulation of mineral N species in the soil. Contrary to our hypothesis, diazotrophic activity was absent in peat circles (**paper IV**). Neither were autotrophic diazotrophs (cyanobacteria) detected in the soil using microscope screening or cultivation on media nor did heterotrophic N_2 fixation occur naturally or after OC amendment. Therefore, only autochtonous complex SOM, not biologically fixed N, was the source for the fast microbial N mineralization producing inorganic N species which accumulated in the bare peat of peat circles. Both forms of mineral N, NH_4^+ as a product of SOM mineralization and NO_3^- as a result of NH_4^+ oxidation mainly by nitrifying community, were abundant in the soil (**paper IV**, Repo *et al.* 2009, Marushchak *et al.* 2011). Nitrates in excess can be used as alternative electron acceptors by denitrifiers decomposing SOM under low access or absence of O_2. High water content and abundant anaerobic mirco-environments in the bare peat with high bulk density can support denitrification even when OC substrate availability is low. Indeed, high metabolic potential for denitrification and specific denitrifier community was found in peat circles (Palmer *et al.* 2012). Very low C:N ratio and pH of the bare peat lead to incomplete denitrification (Repo *et al.* 2009, Marushchak *et al.* 2011, Voigt *et al.* 2016) and result in high rates of N_2O emissions.

Excessive amount of mineral N in the bare peat is also related to absence of plants. The surfaces of bare peat circles are unfavorable for vegetation development

because the uppermost layer is disturbed by wind, exposed to temperature extremes and annually disrupted by cryoturbation (Kaverin *et al.* 2016). Therefore there is no N uptake by plants and all mineralized N in the soil is available to microbes. Besides that, there is also no input of plant material. Fresh source of organic matter in form of root exudates and plant litter would bring easily degradable OC substrate into the soil, relieve the microbial community from lack of energy and low OC availability, and likely stimulate microbial growth and SOM decomposition (Walker *et al.* 2016). Impact of vegetation succession at the exposed bare peat surfaces on C mineralization and N_2O emissions from the soil has not been described yet and would deserve further research.

Deep peat layers get exposed also at bare palsa surfaces (Seppälä 1986, 2003) and at the walls of thermokarst lakes (Schuur *et al.* 2008). Earlier research showed that barren peat surfaces share similar qualities of OM such as high content of inorganic N species, low C:N and for arctic soils exceptionally high soil N transformations rates, for instance high denitrification rates and N_2O emissions (Repo *et al.* 2009, Marushchak *et al.* 2011, Voigt *et al.* 2017). Due to the similarity in chemical composition of the bare grounds in arctic peatlands and their exposition to ambient conditions without vegetation cover, the current results on OC degradability (chapter 3.1) and N dynamics in peat circles (chapter 3.5) are relevant to the other bare peat surfaces in Arctic.

In contrast to peat circles, microbial activity in topsoil of vegetated peat plateau appeared to be limited by N availability. Poorly decomposed Sphagnum peat exhibited higher C:N ratio and OC degradability than bare peat (**paper II**) but low rates of N gross and net mineralization (**paper IV**). In N-poor environment, ability to fix atmospheric N_2 represents competitive advantage within the microbial community. Accordingly, we detected substantial N_2 fixation rates in the vegetated peat plateau and the active N_2-fixing community included moss-associated cyanobacteria as well as heterotrophic diazotrophs (**paper IV**). Autotrophs are often studied as main actors in N_2 fixation in tundra habitats (Liengen & Olsen 1997, Liengen 1999, Solheim *et al.* 2002, Sorensen *et al.* 2006, Stewart *et al.* 2011) but the results of **paper IV** suggested that also heterotrophs contributed by significant portion to the soil N budget in topsoil of vegetated peat plateau where their growth might have been supported by fresh plant material. The estimates of total annual N_2 fixation were one order of magnitude higher than net N mineralization and the sum of the N inputs together with atmospheric N deposition could not meet plant N demand for annual biomass production (**paper IV**). Similar N budget disbalance was reported by Vile *et al.* (2014) for Canadian boreal peatlands. The gap in N budget

might be caused by the fact that the estimates of N_2 fixation based on acetylene reduction assay frequently used especially under field conditions omitted N_2 fixed by methanotrophs. Indirect method of acetylene reduction assay cannot account for N_2 fixed by methanotrophs because these are inhibited by acetylene (Flett *et al.* 1975). According to recent findings, methanotrophs can play an important role in high-latitude peatlands not only as reducers of CH_4 emissions but also as contributors to N stocks in N-limited environment (Larmola *et al.* 2014, Vile *et al.* 2014, Knorr *et al.* 2015, Kox *et al.* 2018). Indeed, methanotrophic activity was confirmed in topsoil of vegetated peat plateau as discussed above (chapter 3.3). Thus, I presume that methanotrophic N_2 fixation could be the missing piece in the N budget of the vegetated peat plateau and thus future research could target this process in arctic peatlands.

The organic topsoil of upland tundra in East-European Subarctic showed high degradability of OC (**paper II**), fast N turnover but low N availability as indicated by high gross but low net N minerlization and plant N demand highly exceeding sum of net N mineralization and atmospheric N deposition (**paper IV**). Available N species are likely a subject of tight competition between plants and microbial community and can lead to N limitation of both, plants and microbial decomposers. The latter is in concordance with the previous evidence of N limitation in upland tundra topsoils collected across Siberia (**paper III**, chapter 3.4). Nitrogen fixation was not detected in upland tundra and thus the internal N cycling here probably represents the main source of N supply to microbes.

4 FUTURE PROSPECTS

This study contributed to mechanistic understanding of vulnerability of OC in permafrost-affected soils and factors influencing GHGs emissions in studied soils. From the collected studies, there came several points which would need to be further examined.

First, the lagging onset of methane production from anaerobic peat soils and cryoturbated organic horizons was not clearly understood during the incubation experiments. The hypothesized limitation of methanogenesis by OC quality, availability of electron acceptors or lack of methanogenes needs to be further investigated.

Second, priming effect was studied in horizons of cryoturbated tundra soil. **Paper I** and **III** brough evidence that addition of plant-derived organic substrate can either reduce or stimulate SOM mineralization depending on which resources are limiting the decomposition and how adequate is the fresh substrate supply to the lack of the resources. A study elucidating conditions under which priming in cryoturbated horizons is positive or negative is needed. Further, effects of higer input of plant-derived organic compounds to arctic peat soils has not been well studied yet. Similarly designed laboratory study as in **paper IV** could be set up to assess priming effect in soils of permafrost peatlands. Specifically bare grounds might be subjected to successive development of vegetation which will likely alter the OC degradability of old peat material.

Third, plant succession and thus competition between microbes and plants for N resources could diminish N_2O emission. Manipulative *in situ* experiments and field measurements could help to understand the future developments of N_2O hotspots.

Last but not least, it came to my attention that photodegradation as a pathway of physicochemical degradation of complex SOM could be an important source of more labile OM at the bare surfaces of arctic peatlands. This process has been studied mainly in water environments on dissolved organic matter (Porcal *et al.* 2015, 2018) but it has a potential to affect C mineralization and GHGs emission from solid organic matter, too (Rutledge *et al.* 2010, Mayer *et al.* 2012). Therefore it would be important to assess the significance of photochemical changes in bare peat soils.

Although the results of laboratory experiments cannot be directly translated into field conditions because the effects observed in soil isolated from its original

environments can be changed in field by interactions with plants, neighboring soil horizons, weather events etc., they can point at potential trends in responses of C mineralization to changes in temperature, hydrological stats and nutrient availability. Data on OC degradability linked to SOM stoichiometry, nutrient limitations of microbial community, extracellular enzyme pool and metabolic potential of microbial community determined in laboratory experiments under controlled conditions will help to set firm understanding of soil-based processes and the findings should be further investigated and verified *in situ*.

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6 ATTACHED PUBLICATIONS

Paper I

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

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

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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.

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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, 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, 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).

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

Table 1
Basic physical (*BD – bulk density, CEC – cation exchange capacity, δ ^{13}C – soil carbon isotopic signature*), chemical (pH_{H_2O} – 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, NH_4^+ – K_2SO_4 extractable ammonium, NO_3^- – 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	pH_{H_2O}	pH_{KCl}	BD g cm^{-3}	CEC meq kg^{-1}	OC %	N_{TOT} %	δ ^{13}C [‰] vs. PDB	C_{EX} μmol g^{-1}	C_{MB}
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*
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 μmol g^{-1}	NH_4^+	NO_3^-	N_{MB}	P_{EX}	P_{MB}	$C:N_{TOT}$ mol mol^{-1}	$C:N_{MB}$	$C:P_{MB}$
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*

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 $Al^{3+}{}_{ex}$ 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 δ ^{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, 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, 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:

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

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:

$$Q_{10} = e^{a \cdot 10},$$

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):

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

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}):

$$T_{MAX} = \frac{a}{-2 \cdot b}$$

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 – NO_3^-, ammonium ions – NH_4^+, 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}.

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.

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

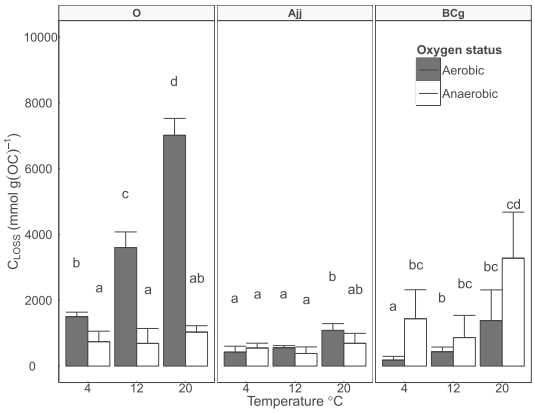

Fig. 1. Cumulative soil carbon loss (C_{LOSS}) from the organic (O), subducted organic (Ajj) and mineral (BCg) horizon, respectively, in 6 different incubation treatments (combinations of 3 temperatures and 2 oxygen levels). Filled bars represent aerobic treatments and open bars anaerobic treatments. 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.

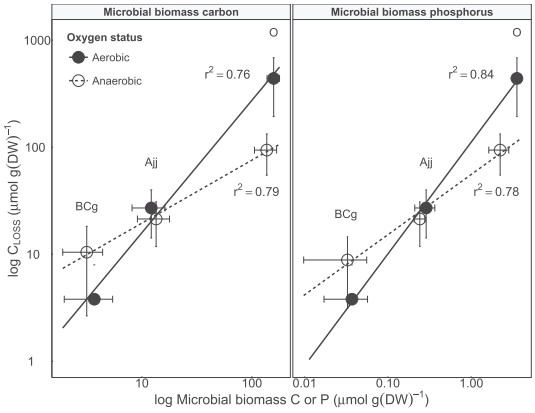

Fig. 2. Correlations between cumulative soil carbon loss (C_{LOSS}) and concentration of microbial biomass carbon and phosphorus. Open symbols show C_{LOSS} from the organic (O), subducted organic (Ajj) and mineral (BCg) horizon incubated anaerobically, averaged over 3 different temperature treatments (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.

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 ($F = 6.9$, $df = 2$, $p < 0.001$).

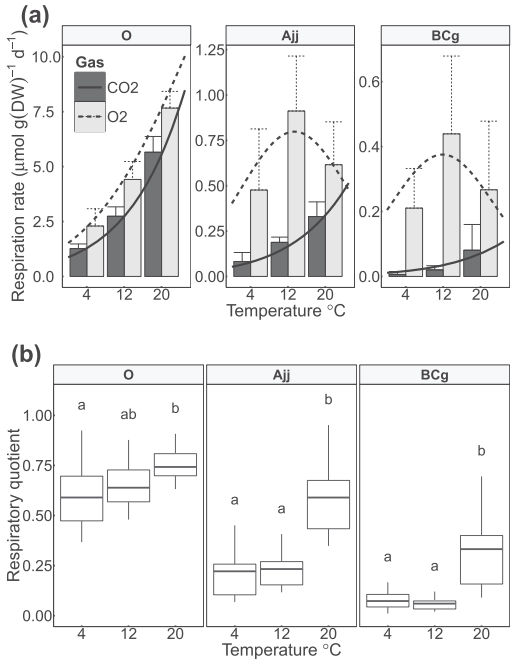

Fig. 3. (a): Respiration rate in aerobic conditions estimated from CO_2 production (gray bars) or O_2 consumption (black bars) in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 3 different temperature treatments. Bar heights represent means and error bars standard deviations. Solid 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.

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).

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.

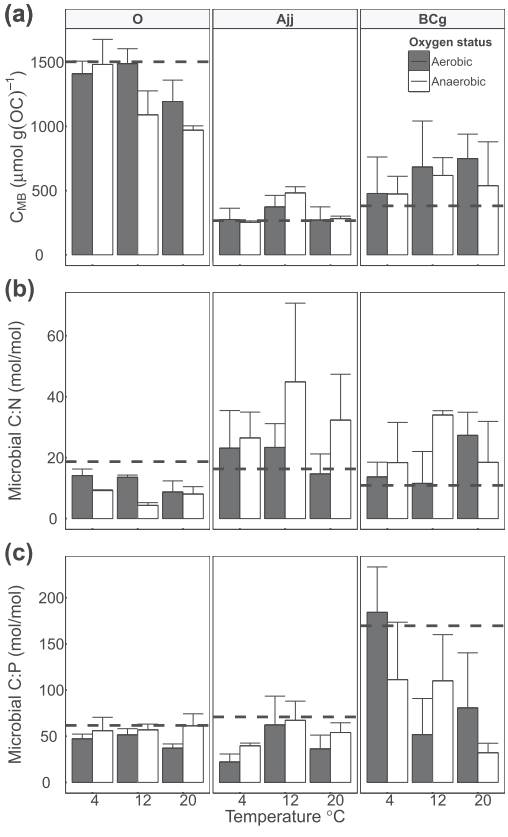

Fig. 4. Microbial biomass carbon (a), microbial biomass C:N (b) and C:P (c) in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard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

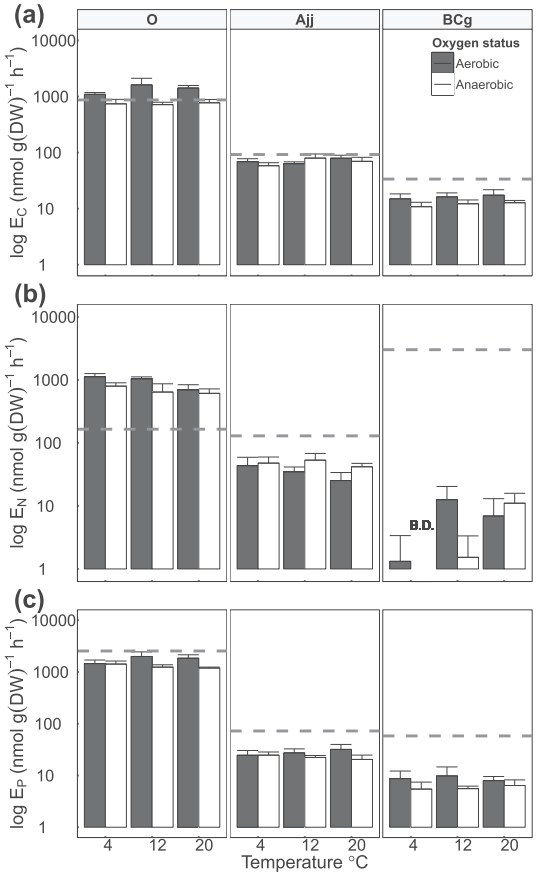

Fig. 5. E_C (a), E_N (b) and E_P (c) enzyme classes in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard deviations. Dashed horizontal 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.

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

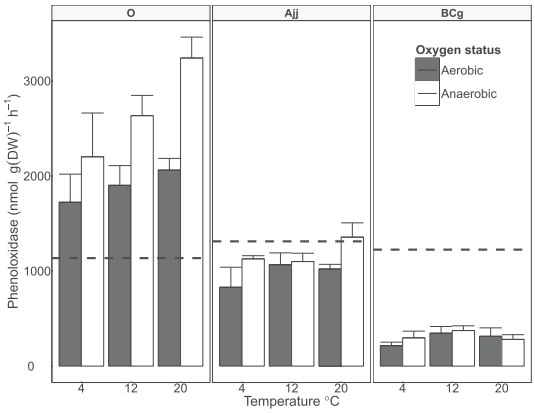

Fig. 6. Phenoloxidases in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard deviations. Dashed horizontal lines show values at the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

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_4 production 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.

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

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

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 S7 and 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 (NH_4^+, NO_3^- and P-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.

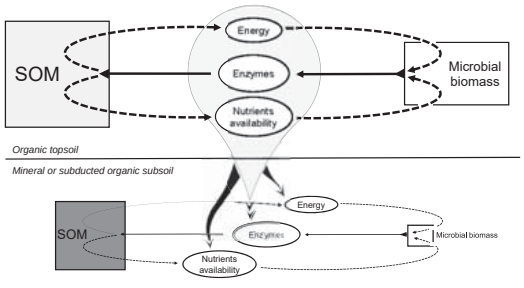

Fig. 7. Diagram of connections between microbial biomass, soil OC, enzymatic production and nutrient availability within and between soil horizons. The energy availability for enzymatic production within each soil horizon is critical; the low quality soil OC of subsoil horizons provides no energy and 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

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.07.013.

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Paper II

Heterogeneity of carbon loss and its temperature sensitivity in East-European subarctic tundra soils

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RESEARCH ARTICLE

Heterogeneity of carbon loss and its temperature sensitivity in East-European subarctic tundra soils

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One sentence summary: Decomposition of old organic deposits in arctic peatlands is slow due to scarcity of microorganisms there but is highly sensitive to temperature increase, even under anaerobic conditions.

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ABSTRACT

Arctic peatlands store large stocks of organic carbon which are vulnerable to the climate change but their fate is uncertain. There is increasing evidence that a part of it will be lost as a result of faster microbial mineralization. We studied the vulnerability of 3500–5900 years old bare peat uplifted from permafrost layers by cryogenic processes to the surface of an arctic peat plateau. We aimed to find biotic and abiotic drivers of C_{LOSS} from old peat and compare them with those of adjacent, young vegetated soils of the peat plateau and mineral tundra. The soils were incubated in laboratory at three temperatures (4°C, 12°C and 20°C) and two oxygen levels (aerobic, anaerobic). C_{LOSS} was monitored and soil parameters (organic carbon quality, nutrient availability, microbial activity, biomass and stoichiometry, and extracellular oxidative and hydrolytic enzyme pools) were determined. We found that C_{LOSS} from the old peat was constrained by low microbial biomass representing only 0.22% of organic carbon. C_{LOSS} was only slightly reduced by the absence of oxygen and exponentially increased with temperature, showing the same temperature sensitivity under both aerobic and anaerobic conditions. We conclude that carbon in the old bare peat is stabilized by a combination of physical, chemical and biological controls including soil compaction, organic carbon quality, low microbial biomass and the absence of plants.

Keywords: arctic peatlands; soil carbon loss; temperature; oxygen; microbial biomass; laboratory incubation

INTRODUCTION

The arctic region stores about a half of the terrestrial soil organic carbon (OC) pool in upland tundra, peatlands, loess and fluvial deposits, and thus represents a globally significant carbon reservoir (1300 Pg OC; Hugelius *et al.* 2014). The fate of this OC pool under the ongoing climate change is uncertain, but there is increasing evidence that at least a part of it will be released as a product of faster microbial OC mineralization in the form of CO_2 and/or CH_4 (Oechel *et al.* 1993; Shaver *et al.* 2006; Mueller *et al.* 2015; Natali *et al.* 2015). Rising emissions of these greenhouse gases from a vast area of arctic soils would contribute to their atmospheric concentration, increase radiative forcing and so trigger further warming by initiating a positive feedback loop to the climate (Friedlingstein *et al.* 2001; Schuur *et al.* 2008; Koven *et al.* 2011).

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In the Arctic, upland mineral soils dominate the landscape; however, a vast amount of OC is found in peatlands. Although peatlands cover only about 13% of the area of the circumpolar terrestrial region, their OC stocks of 300 Pg represent 29% of the total arctic soil OC pool (Hugelius *et al.* 2014). Peat deposits up to three meters deep have accumulated over thousands of years due to conditions which attenuate microbial OC decomposition: high water content, low availability of oxygen, low temperatures and presence of permafrost (Zoltai and Tarnocai 1975; Routh *et al.* 2014). The arctic landscape is highly dynamic because of cryogenic processes, which disturb the integrity of the soil profile, causing vertical movements of the soil. Thus, old peat deposits can experience a wide range of conditions under the changing climate. Old peat can be uplifted, due to deep-ice accretion, from the permafrost layer to the well-drained surface of palsa mire or peat plateau where it is exposed to ambient climatic conditions (Seppälä 2003; Payette *et al.* 2004; Kuhry 2008; Marushchak *et al.* 2011). These bare ground features experience high temperatures in the summer season due to the dark color of peat and a lack of shading by vegetation, which affects the active layer depth (Shur and Jorgenson 2007). In addition, the input of fresh OC from plant litter and root exudates, which can stimulate the development of microbial community and facilitate microbial mineralization of complex compounds (Wild *et al.* 2014), is missing. The annual temperature increase and precipitation patterns projected under future climate change in this region (Kirtman *et al.* 2013) support formation of inundated soils, or thermokarst (Sannel and Kuhry 2011). As a result, previously aerobic surface layers of arctic peatlands experience anaerobic conditions and the organic soils turn into a methane source (Berestovskaya *et al.* 2005; Treat *et al.* 2014; Turetsky *et al.* 2014). Higher temperature and changes in oxygen availability are expected to have a substantial impact on the microbial community (Deslippe *et al.* 2012; Männistö *et al.* 2013; Tveit *et al.* 2013; Peltoniemi *et al.* 2015). However, the controlling factors of microbial decomposition of the old peat are still not well understood. Little is known about changes in OC and N mineralization, intrinsic temperature sensitivity and decomposer community functions in response to a changing environment.

Generally, microbial OC mineralization is controlled by abiotic factors such as temperature and moisture content and by biotic factors such as microbial community structure and functioning. Temperature sensitivity is often soil specific and is modulated by other factors such as oxygen availability, substrate quality and quantity, nutrient limitation or the activity of extracellular enzymes (Allison, Wallenstein and Bradford 2010). According to the kinetic theory, temperature sensitivity is indirectly related to OC quality; the lower the OC quality, i.e. the more recalcitrant organic compounds, the higher the temperature sensitivity (Mikan, Schimel and Doyle 2002; Knorr *et al.* 2005; Davidson and Janssens 2006; Conant *et al.* 2008; Wetterstedt, Persson and Agren 2010). However, experimental data often show the opposite: a lower temperature sensitivity of more recalcitrant OC compared to that of more labile OC (Biasi *et al.* 2005; Gershenson, Bader and Cheng 2009). This is of major concern since the old peat deposits, in spite of having been preserved in anaerobic or subzero conditions for the most of their development (Routh *et al.* 2014; Treat *et al.* 2014), exhibit higher humification and decomposition degrees compared to recent soil OC (Hodgkins *et al.* 2014), which indicates low substrate quality for microbial decomposition. Further, soil water content and oxygen availability play a critical role in OC mineralization. Lower OC mineralization and its temperature response under anaerobic compared to aerobic conditions were shown by Čapek *et al.* (2015), but results of other studies are equivocal (Inglett *et al.* 2012; Hilasvuori *et al.* 2013).

To gain better understanding of mechanisms underlying OC mineralization and temperature and oxygen responses of arctic soils, incubation experiments are increasingly carried out (e.g. Dutta *et al.* 2006; Shaver *et al.* 2006; Waldrop *et al.* 2010; Lee *et al.* 2012; Knoblauch *et al.* 2013; Treat *et al.* 2014, 2015; Čapek *et al.* 2015; Moni *et al.* 2015). These experiments have brought recent progress in quantitative and qualitative understanding of the fate of OC stored in arctic soils; however, it has been emphasized recently that there is still a significant lack of laboratory data on decomposability of permafrost-affected soils, particularly peat, and its temperature response (O'Donnell *et al.* 2012; Moni *et al.* 2015; Schuur *et al.* 2015). In the presented incubation experiment, we studied decomposability of old bare peat, which had been uplifted from the permafrost layer to the surface of the peat plateau. We aimed to find biotic and abiotic drivers of carbon loss (C_{LOSS}) from the old peat and compare them with those of soils of adjacent vegetated habitats characterized as 'recent' peat plateau and mineral tundra. We hypothesized that (i) soil C_{LOSS} is a function of microbial biomass, (ii) microbial activity in old peat responds to the temperature with higher sensitivity compared to the adjacent soils due to the complex soil OC and (iii) C_{LOSS} and temperature sensitivity is lower under anaerobic than aerobic conditions in all soils.

MATERIALS AND METHODS

Study site and soil sampling

Samples were collected from the Seida study site, which lies within the East-European discontinuous permafrost zone in subarctic, southern tundra. The Seida study site (62°57′E, 67°03′N) is located about 70 km southwest of the town of Vorkuta, Komi Republic, Russia (Fig. 1A). Mean annual temperature in this region is −5.6°C and mean annual precipitation is 501 mm. The mosaic of the landscape consists of peat plateau complexes uplifted by permafrost formation, mineral upland tundra and other habitats such as fens and thermokarst lakes (Fig. 1B and C; Hugelius *et al.* 2011; Marushchak *et al.* 2013). Peat plateau with a shallow permafrost table 40–70 cm deep is composed of deep cryic histosols and covered by a typical tundra bog vegetation with abundant *Sphagnum* moss (Fig. 1D-1). The age of the uppermost peat layers in the vegetated peat plateau is about 50 years (Biasi *et al.* 2014) and thus of a recent origin. Throughout this study, we refer to the peat collected there as the 'recent peat'. The peat plateau is spotted with bare ground features, peat circles (Fig. 1D-2), which were previously identified as significant sources of CO_2 (Biasi *et al.* 2014) and hotspots for N_2O emissions (Repo *et al.* 2009) and thus became a focus of a further investigation of the C and N dynamics in the Arctic. The peat circles represent about 4% of the peat plateau in the studied area. The surface layer consists of 3500–5900 years old peat and the age gradually increases with depth (Biasi *et al.* 2014; Ronkainen *et al.* 2015); in the following text, we refer to this peat as the 'old peat'. The developmental history of these bare features is not yet completely understood but cryogenic processes associated with ground uplifting and subsequent erosion of surface layers seem to play a major role (Kaverin and Pastukhov 2013; Ronkainen *et al.* 2015). According to a chronosequence of a complete soil profile from vegetated peat plateau at the Seida study site (Routh *et al.* 2014), the age of the peat circle surface corresponds to adjacent peat deposits about 1–2 m below surface. This evidence

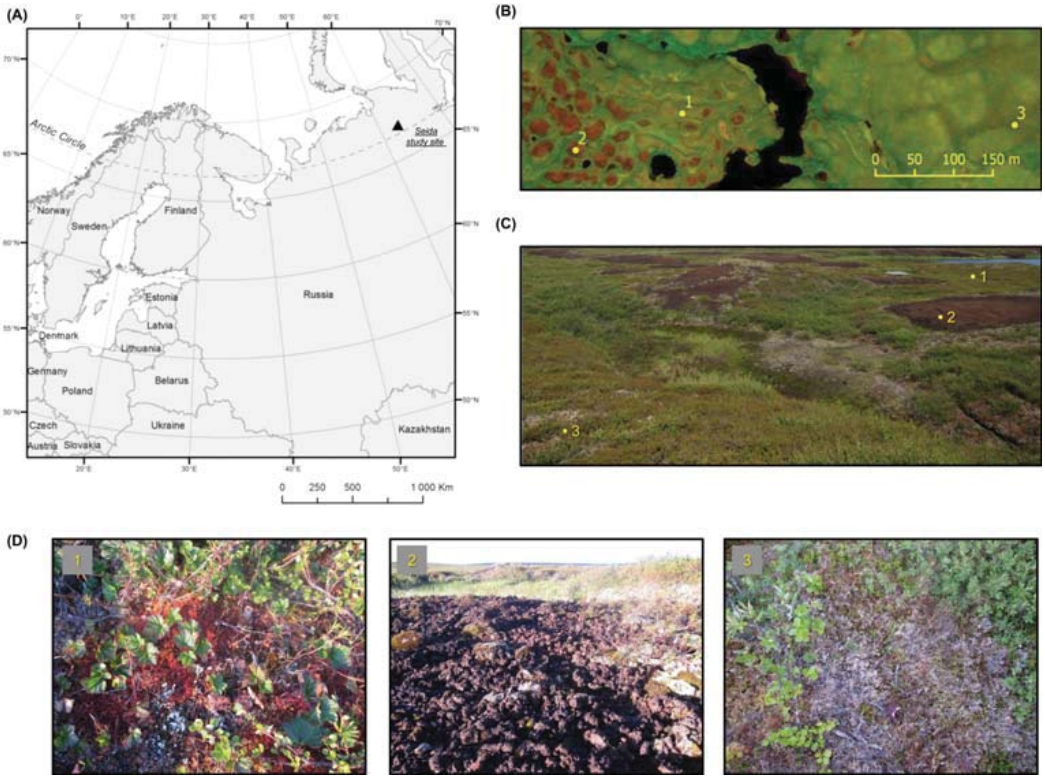

Figure 1. Seida study site shown as (**A**) location on a geographical map, (**B**) satellite image adopted from Map data ©2016 Google, DigitalGlobe, (**C**) landscape photo and (**D**) land cover photos for each habitat which the soils samples were taken from; 1: vegetated peat plateau, the sampling site for recent peat, 2: peat circle, the sampling site for old peat, 3: tundra heath, the sampling site for mineral tundra soil. Maps produced by Stanislav Grill.

implies that the old peat was uplifted from permafrost layer of the peat plateau.

Mineral tundra covers the majority of the subarctic tundra in this region (about 70%) and is characterized by tundra heath vegetation (Fig. 1D-3) formed on cryosols with the active layer deeper than 120 cm. More details about the habitats can be found in earlier studies conducted at the Seida study site (Repo *et al.* 2009; Hugelius *et al.* 2011; Marushchak *et al.* 2011, 2013; Biasi *et al.* 2014). The soil was collected within an area of 10 000 m^2 from each habitat (Fig. 1B and C) in three field replicates as a complete layer of 2–15 cm depth. This layer reflects seasonal and daily temperature changes and thus represents the most active pool of microbial processes during the summer period. The soil sample was composed of a pure organic (peat) soil for the habitats on the peat plateau, the recent peat and the old peat, while for the mineral tundra, this sample included mostly organic soil but occasionally also a few centimeters of upper mineral soil. Immediately after the soil sampling, visible, living roots were separated from the soil if present. The soil was stored at 4°C for 4 months to allow the labile substrate originating from vegetation (e.g. residual fine roots, root exudation) to be depleted from the soil. Bulk soil was homogenized before the start of the experiment and assessed for basic chemical and microbiological characteristics.

Experimental setup

Homogenized bulk sample of each soil was divided into three parts. The first part was analyzed for physical, chemical, microbial and biochemical parameters (see below, for scheme of analyses performed; see Fig. S1, Supporting Information). The other two parts were used for the incubation experiment (aerobic, anaerobic treatment). Their water content was adjusted to 80% or 100% water holding capacity (WHC) for aerobic and anaerobic treatment, respectively. Aerobic and anaerobic samples were incubated for 23 weeks at three different temperatures (4°C, 12°C and 20°C), which represent the range of field thermal conditions in the surface soil layer during the growing season at the Seida site. Each treatment was performed in four replicates and aerobic and anaerobic incubation vessels without soil were incubated as controls at each temperature. This resulted in the total number of 78 incubation vessels. After adjusting the WHC, 50 g of soil was placed into gastight 1000 mL incubation vessels (Schott, Mainz, Germany). A detailed description of the incubation setup and the experimental conditions was given by Čapek *et al.* (2015). In the current experiment, we followed this procedure without changes during aerobic incubation and with the following modification for anaerobic incubation: oxygen-free artificial atmosphere consisted of 99% He and 1% CO_2.

Gas analyses

Fluxes of CO_2 and CH_4 were quantified weekly during the first month and biweekly during the rest of the incubation (13 times in total). Aerobic vessels including controls were flushed after each sampling period with ambient air in order to sustain sufficient oxygen supply, while anaerobic vessels were flushed by a mixture of He and CO_2. Gas sampling procedures follow the protocol described earlier in Čapek *et al.* (2015). Briefly, concentration of CO_2 and CH_4 in the headspace of the incubation vessels was analyzed at the beginning and at the end of each sampling period using a gas chromatograph (Agilent 7820A GC, Agilent Technologies, Santa Clara, USA) equipped with thermal conductivity and flame ionization detectors.

Chemical soil parameters and microbial biomass

Chemical analyses were performed with soil dried at 60°C and ground with a ball mill (Mixer Mill MM 20, Retsch, Germany). Soil pH was determined in soil (dry) water suspension (1:10 m/v). Total OC and N and their isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) were determined using an elemental analyzer (Vario micro 209 cube, Elementar Analysensysteme GmbH, Germany) coupled to an isotope ratio mass spectrometer (IRMS DELTA plus XL, Finnigan, Germany). The quality of dissolved organic carbon (DOC) in filtered soil water extracts (1:20 m/v) was described by specific UV absorbance which is defined as absorptivity at a wavelength of 254 nm normalized to concentration of DOC ($SUVA_{254}$; L mg C^{-1} m^{-1}). Aromaticity, the portion of aromatic compounds in DOC, was calculated according to Weishaar *et al.* (2003) using equation (1.1).

$$\text{Aromaticity (\% DOC)} = 6.52 \,.\, SUVA_{254} + 3.63 \qquad (1.1)$$

The contents of extractable nutrients were determined before and after the incubation, as described in Čapek *et al.* (2015). Briefly, extractable carbon, nitrogen and phosphorus (C_{EX}, N_{EX}, P_{EX}), nitrate (NO_3^-), ammonium (NH_4^+) and dissolved organic nitrogen (DON) were analyzed in 0.5M K_2SO_4 extracts. Chloroform fumigation extraction method was used to assess carbon, nitrogen and phosphorus content in microbial biomass (C_{MB}, N_{MB} and P_{MB}, respectively) before and after the incubation. Nutrient content in microbial biomass was calculated as a difference between fumigated and non-fumigated sample. The yield of the fumigation method was corrected to incomplete extraction of microbial C ($K_{ec} = 0.38$; Vance, Brookes and Jenkinson 1987), microbial N ($K_{en} = 0.4$; Brookes *et al.* 1985) and microbial P ($K_{ep} = 0.4$; Brookes, Powlson and Jenkinson 1982). Microbial stoichiometry, i.e. the proportion of elements in microbial biomass, was expressed here as $C:N_{MB}$ and $C:P_{MB}$.

Extracellular enzymes

Potential activity of extracellular enzymes was determined by microplate fluorometric and photometric assays under standard conditions. To determine hydrolytic enzyme activities, 1 g of soil was suspended in 100 mL of distilled water and sonicated for 4 min to disrupt soil particles. Soil suspension (200 μL) was then added to 50 μL methylumbelliferyl substrate solution for β-glucosidase, cellobiosidase, phosphatase or chitinase determination, or to 7-aminomethyl-4-coumarin substrate solution for leu-aminopeptidase determination (Marx, Wood and Jarvis 2001). The plates were incubated at 20°C for 120 min. Fluorescence was quantified at an excitation wavelength of 365 nm and emission wavelength of 450 nm using INFINITE F200 microplate reader (TECAN, Germany). Activities of oxidative enzymes (phenoloxidase, peroxidase) were determined photometrically in buffered soil extracts using the l-3,4-dihydroxyphenylalanin (L-DOPA) substrate as described in Bárta *et al.* (2010). Briefly, 200 μL of filtered soil suspension was mixed with 50 μL of acetate buffer (pH = 5.0) and 50 μL of 25 mM L-DOPA solution. For peroxidase determination, 10 uL of 0.3% H_2O_2 (v/v) was added. Absorbance was measured after 18 h at a wavelength of 460 nm.

The sum of all measured enzyme activities (E_{CNP}) represents an estimate of the total enzyme pool in the soil. In order to relate enzymes with the acquisition of a certain nutrient, we grouped hydrolytic enzymes according to their main function: C-sequestering enzymes E_C (β-glucosidase, cellobiosidase), N-sequestering enzymes E_N (leu-aminopeptidase, chitinase) and P-sequestering enzymes E_P (phosphatase). Phenoloxidases and peroxidases were summed in a group of oxidases as nutrient non-specific enzymes.

N transformations

Gross ammonification and gross nitrification rates were determined at the end of the incubation period using pool dilution technique (Kirkham and Bartholomew 1954, 1955). All gross N processes were assayed under ambient oxygen level. Four aliquots of 3 g of fresh soil were separated from each incubation sample. Two of those were used for gross ammonification and the other two for the gross nitrification assay. An aliquot of 500 μL ^{15}N-enriched labeling solution (0.25 mM) was added to each aliquot in the form of $^{15}NH_4Cl$ (10 at%) for gross ammonification and $K^{15}NO_3$ (10 at%) for gross nitrification experiments. All four labeled aliquots were kept at the respective temperature of the former incubation. One aliquot from each set was harvested after 4 h, the other one after 24 h and the soil was immediately extracted with 15 mL of 0.5 M K_2SO_4. The microdiffusion technique was used to prepare the extracts for the determination of $^{15}N/^{14}N$ ratio in NH_4^+ or NO_3^- (Brooks *et al.* 1989). The procedure described by Marushchak *et al.* (2011) was adopted and the same analytical instrumentation and calculation formulas were used. Briefly, acid traps prepared from two acidified filter paper disks were added to 10 mL of the extract together with 0.1 g of MgO and incubated at 35°C in a rotary shaker for 5 days. Filter paper disks for determining gross ammonification rates were dried and stored for isotopic analysis. Teflon traps from the gross nitrification assay were discarded. A new acid trap together with 0.05 g of reducing agent (Devarda's alloy) was added to the solution, incubated under the same conditions, and filter paper disks from the second round of incubation were used for isotopic analysis. Net N mineralization rates were calculated as the sum of accumulated $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ during the incubation period divided by the number of incubation days.

Data evaluation and statistical analyses

The loss of OC initially present in the soils (C_{LOSS}) was defined as CO_2 and CH_4 production rate. For anaerobic treatments, C_{LOSS} represented the sum of CO_2 and CH_4 production while only CO_2 production was evident and thus used for these calculations in aerobic treatments. Dependence of C_{LOSS} on temperature was described using two approaches. For each model, the coefficient Q_{10} as a relative change of C_{LOSS} with 10°C temperature increase was determined to express C_{LOSS} temperature sensitivity.

First, data were fitted to a simple exponential model (equation 1.2), which is commonly used to describe microbial

activity as a function of temperature (e.g. Fierer, Schimel and Holden 2003; Dutta *et al.* 2006; Gershenson, Bader and Cheng 2009; Wetterstedt, Persson and Agren 2010; Hamdi *et al.* 2013; Moni *et al.* 2015).

$$C_{LOSS}{}^{E} = R_1.e^{a_1.T} \quad (1.2)$$

where T is temperature and R_1 and a_1 are parameters fitted to equation 1.2. Parameter a_1 was then used to calculate Q_{10} derived from the exponential function ($Q_{10}{}^{E}$):

$$Q_{10}{}^{E} = e^{10.\ a_1} \quad (1.3)$$

Coefficient Q_{10} calculated according to equation (1.3) is a widely reported measure of temperature sensitivity and allows direct comparison among studies. The simple exponential function predicts a continual increase of microbial activity with temperature and a constant Q_{10} over the whole temperature range. This assumption is valid for a short temperature range; however, Q_{10} is generally negatively correlated with temperature (Tuomi *et al.* 2008; Hamdi *et al.* 2013) and so the exponential model is biased when used on a wider temperature range.

Therefore, as a second option, we used the Gaussian model to describe our data with higher accuracy (Fig. S4, Supporting Information) as suggested by Tuomi *et al.* (2008) and to reveal intrinsic temperature responses of each studied soil along the whole temperature range using equations (1.4) and (1.5):

$$C_{LOSS}{}^{G} = R_2.\ e^{a_2.T+b.T^2} \quad (1.4)$$

where T is temperature and R_2, a_2 and b are fitted parameters of equation (1.4). These were used to determine the $Q_{10}{}^{G}$ for C_{LOSS} using equation (1.5) derived from the Gaussian function:

$$Q_{10}{}^{G} = e^{\left(a_2.T_2+b.T_2{}^2\right)-\left(a_2.T_1+b.T_1{}^2\right)} \quad (1.5)$$

$Q_{10}{}^{G}$ was estimated for temperature interval between $T_1 = 5$°C and $T_2 = 15$°C where Gaussian model predicted exponential increase of C_{LOSS} in all soils. Goodness of fit of the two models to the experimental data was compared using F-test and the Akaike information criterion (AIC).

Similarly, the dependence of hydrolytic enzyme pool on temperature was assessed using the Gaussian model with equations (1.4) and (1.5). Specific CO_2 production was defined as actual CO_2 production rate relative to microbial biomass (C_{MB}). Cumulative C_{LOSS} from the soil was used as a measure of OC decomposability and was expressed as the sum of CO_2 and CH_4 production integrated over the incubation period.

Absolute differences in C_{LOSS} among soils, temperatures and oxygen levels were evaluated by factorial ANOVA followed by post-hoc Tukey HSD test. Data on C_{LOSS} were log-transformed and checked for normality by the Shapiro-Wilk test. Because the differences in C_{LOSS} among the soils were greater than the effects of the environmental variables (temperature and oxygen status), a multiple linear regression model with stepwise selection was applied to find the best predictor of C_{LOSS} among all measured soil parameters, i.e. chemical and microbial parameters, and enzyme potential activities, using the program R (R Core Team 2014). Multicollinearity is common among soil parameters. Therefore, ridge regression was performed using the package MASS in R to test the stability of chosen predictors. Chemical, microbial and enzymatic parameters were evaluated for changes between initial and final measurements using t-test and differences among soils and treatments were tested using factorial ANOVA.

RESULTS

Initial soil properties

The old peat had a similar content of OC as the recent peat but contained a higher amount of N_{tot} which resulted in lower C:N_{tot}. The mineral tundra soil contained a considerably lower amount of OC than both peat soils and had intermediate C:N_{tot} (Table 1). The extractable C (C_{EX}) expressed on a gram of OC basis (μmol gOC^{-1}) differed among the soils ($F = 7.098$, $df = 2$, $P = 0.014$) and was the highest in the old peat, the lowest in the recent peat and intermediate in the mineral tundra soil. DOC quality also varied significantly ($F = 53.93$, $df = 2$, $P < 0.001$): the old peat was characterized by the highest DOC aromaticity, the mineral tundra soil by intermediate and the recent peat by the lowest (Table 1). The old peat exhibited the highest concentrations of $NO_3{}^{-}$, DON and P_{EX}, while these were intermediate in the recent peat and the lowest in the mineral tundra soil. The initial $NH_4{}^{+}$ concentration was negligible in all soils. Due to a high concentration of N_{EX} and P_{EX}, the old peat exhibited the lowest C:N_{EX} and C:P_{EX} ratios among the soils. The old peat had a higher bulk density than the recent peat but lower than the mineral tundra soil (Table 1).

Aerobic incubations

Soil C_{LOSS}

The cumulative C_{LOSS} in the form of CO_2 increased linearly during the incubation period in the old and recent peat soils reflecting constant C_{LOSS} rates. On the other hand, the cumulative C_{LOSS} in the mineral tundra soil grew exponentially due to gradually increasing C_{LOSS} rates during the incubation period (Fig. S2, Supporting Information). Cumulative C_{LOSS} relative to OC integrated over the incubation period was the lowest in the old peat, intermediate in the recent peat and the highest in the mineral tundra (Fig. 2). A total of 0.7% of OC was lost from the old peat over the 23 weeks at 20°C, while 3.1% of OC was lost from the recent peat and 14.6% of OC from the mineral tundra soil. Soil type thus accounted for 37% of the variability in C_{LOSS}, while temperature and oxygen status had a secondary effect on C_{LOSS}. From all determined soil properties, microbial biomass was the strongest and the most stable predictor of C_{LOSS}. The strongest correlation of C_{LOSS} was found with P_{MB}, while the correlation with N_{MB} or C_{MB} was weaker (Table 2, Fig. S5, Supporting Information). C_{LOSS} increased with temperature in all soils. A comparison of two models (Fig. S4, Supporting Information) describing temperature dependence showed that the Gaussian model fitted the aerobic C_{LOSS} with higher precision than the exponential model in the case of the recent peat ($F = 24.82$, $df = 1$, $P < 0.001$; $dAIC = 21.5$) and the mineral tundra soil ($F = 6.93$, $df = 1$, $P = 0.009$, $dAIC = 4.9$), while both models were equally good for the old peat ($F = 0.0006$, $df = 1$, $P = 0.981$; $dAIC = 2.0$). Temperature sensitivity predicted by the exponential model ($Q_{10}{}^{E}$) was the highest for the old peat ($3.52 \pm 0{,}22$), lower for the mineral tundra soil (2.56 ± 0.16) and the lowest for the recent peat (2.10 ± 0.15). The Gaussian model predicted exponential increase of C_{LOSS} in the temperature range below 15°C for all soils. Temperature sensitivity for the temperature interval 5°C –15°C calculated from the Gaussian model ($Q_{10}{}^{G}$) was equivalent among soils with an overall mean value 3.68 (Fig. 3). In the aerobically incubated old peat, no significant CH_4 consumption was detected. In contrast, CH_4 at ambient concentrations was steadily consumed from the

Table 1. Basic physical (pH, BD—bulk density, $\delta^{13}C$ and $\delta^{15}N$–isotopic signature of soil carbon and nitrogen, respectively), chemical (OC—total soil organic carbon, N_{tot}—total soil nitrogen, aromaticity of dissolved organic carbon, C_{EX}—K_2SO_4 extractable carbon, NH_4^+—K_2SO_4 extractable ammonium, NO_3^-—K_2SO_4 extractable nitrate, DON—K_2SO_4 extractable dissolved organic nitrogen, P_{EX}—$NaHCO_3$ extractable phosphorus) and microbial (C_{MB}—microbial carbon) characteristics as well as stoichiometric parameters ($C:N_{tot}$—total carbon to nitrogen ratio of the bulk soil, $C:N_{EX}$ and $C:P_{EX}$—extractable carbon to nitrogen and carbon to phosphorus ratio, $C:N_{MB}$ and $C:P_{MB}$—microbial carbon to nitrogen and carbon to phosphorus ratio) in the uppermost 15 cm layer from the recent peat of age about 50 years, old peat of age 3500–5900 and from the mineral tundra soil. Parameter values are shown as mean with standard deviation in italics ($n = 4$).

	pH	BD ($g\ cm^{-3}$)	OC (%)	N_{tot} (%)	$C:N_{tot}$ ($mol\ mol^{-1}$)	$\delta^{13}C$ ([‰] vs. PDB)	$\delta^{15}N$ ([‰] vs. AT-Air)	Aromaticity (% DOC)
Recent peat	4.09	0.22	42.69	1.42	30.0	−26.88	3.09	15.72
	0.08	*0.04*	*0.05*	*0.01*	*0.3*	*0.05*	*0.05*	*0.95*
Old peat	3.99	0.42	46.02	2.55	18.1	−28.37	1.65	25.50
	0.01	*0.04*	*0.06*	*0.01*	*0.1*	*0.04*	*0.21*	*2.12*
Mineral tundra	4.80	0.95	6.11	0.28	22.0	−27.17	2.08	19.50
	0.08	*0.14*	*0.08*	*0.01*	*0.3*	*0.11*	*0.16*	*0.95*

	C_{EX} ($\mu mol\ gOC^{-1}$)	NH_4^+ ($\mu mol\ gOC^{-1}$)	NO_3^- ($\mu mol\ gOC^{-1}$)	DON ($\mu mol\ gOC^{-1}$)	$C:N_{EX}$ ($mol\ mol^{-1}$)	$C:P_{EX}$	C_{MB} ($\mu mol\ gOC^{-1}$)	$C:N_{MB}$	$C:P_{MB}$ ($mol\ mol^{-1}$)
Recent peat	233.9	0.96	0.94	14.65	14.2	123.6	842.5	9.5	39.1
	8.6	*0.41*	*0.47*	*1.96*	*1.0*	*20.6*	*101.2*	*0.5*	*16.3*
Old peat	291.3	0.03	28.40	22.82	5.7	89.2	185.1	11.6	42.2
	27.0	*0.07*	*1.74*	*4.19*	*0.3*	*16.9*	*61.3*	*5.6*	*16.2*
Mineral tundra	255.6	0.06	0.97	13.81	18.8	111.3	784.0	7.3	25.9
	24.9	*0.11*	*0.39*	*6.43*	*5.2*	*11.0*	*87.3*	*1.1*	*8.0*

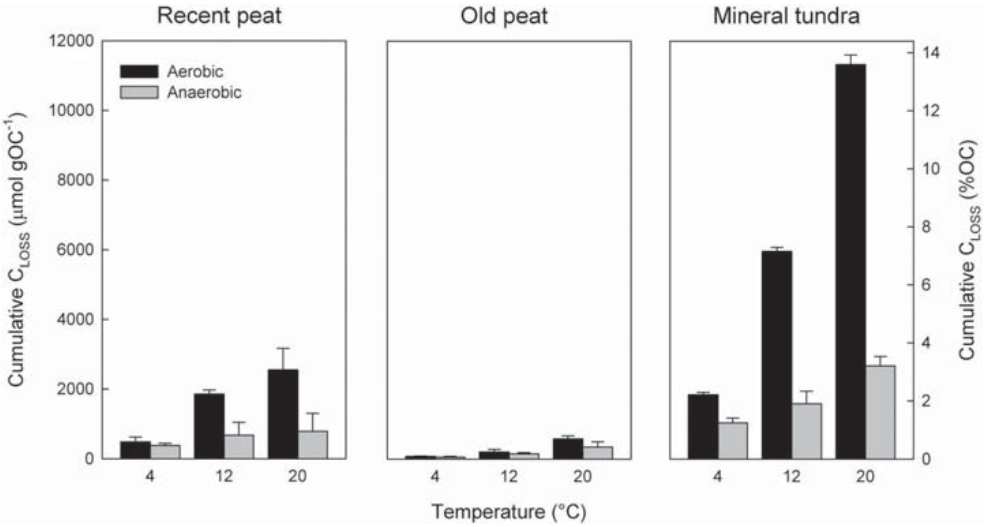

Figure 2. Cumulative C_{LOSS} integrated over the 23-week experimental period for recent peat, old peat and mineral tundra soil incubated under aerobic (black columns) and anaerobic (gray columns) conditions expressed on gram OC basis (left *y* axis) and as a portion of the total soil OC (right *y* axis) at each incubation temperature (*x* axis). Columns represent mean value and vertical bars standard deviation ($n = 4$).

headspace of aerobic incubation vessels in the recent peat and mineral tundra soil (Fig. S3B, Supporting Information).

Microbial biomass

Microbial biomass (C_{MB}) relative to OC was the lowest in the old peat ($df = 6$, $P < 0.001$), representing only 0.22% of OC, while it was similar between the two recent soils, the recent peat and the mineral tundra soil ($df = 6$, $P = 0.819$), representing 1.01% and 0.94% of OC, respectively. Accordingly, total PLFA concentration was lower in the old peat than in the recent peat (Table S1, Supporting Information). The stoichiometry of microbial biomass ($C:N_{MB}$ and $C:P_{MB}$) was equivalent among the soils (Table 1). The microbial biomass remained unchanged during the aerobic incubation in the peat soils (Fig. 4A). In the mineral tundra soil, C_{MB} substantially increased, irrespective of the temperature, which was accompanied by an increase in $C:N_{MB}$.

Table 2. Linear regression parameters between cumulative C_{LOSS} and different microbial parameters across the soils (C_{MB}—microbial carbon, N_{MB}—microbial nitrogen, P_{MB}—microbial phosphorus) for aerobic and anaerobic conditions. The table shows values for adjusted R^2, slope and P value of linear regression. The cumulative C_{LOSS} as a function of microbial biomass is depicted in Fig. S4 (Supporting Information).

Oxygen status	C_{LOSS} vs.	Regression parameters		
		Adj.R^2	Slope	P
Aerobic	PMB	0.502	78.5	<0.001
	NMB	0.450	16.5	<0.001
	CMB	0.414	1.96	<0.001
Anaerobic	NMB	0.390	7.43	<0.001
	PMB	0.364	41.3	<0.001
	CMB	0.200	0.73	0.004

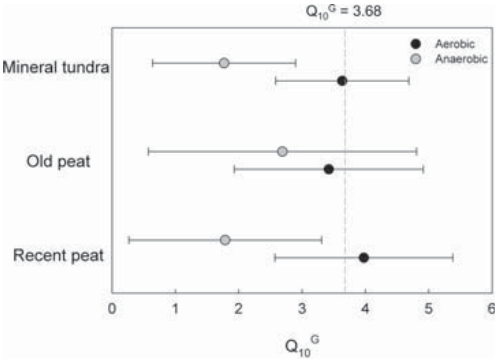

Figure 3. Temperature sensitivity of the C_{LOSS} expressed from Gaussian model as Q_{10}^G for temperature interval 5°C–15°C. Data points represent mean values and horizontal bars denote standard errors. Dashed line shows mean value of Q_{10}^G for aerobic C_{LOSS} across all three soils.

Otherwise, there was no significant change in microbial biomass stoichiometry over the incubation period (Fig. 4B and C). Specific CO_2 production, equivalent to the amount of C released by a unit of microbial biomass per day, was lower in the old peat than in the recent peat and the highest activity was detected in the mineral tundra soil (Fig. 5). This was in concordance with the cumulative C_{LOSS} (Fig. 2). Specific CO_2 production remained unchanged over the incubation period in the old and recent peat soils but significantly increased toward the end of the incubation in the mineral tundra soil.

Soil enzymes

Total enzyme pool E_{CNP} relative to OC was comparable in the old peat and the recent peat at the beginning of the incubation. The E_{CNP} in mineral tundra soil was higher than that in both peat soils. Pools of hydrolytic enzymes increased significantly over the incubation period in all soils and showed a positive effect of temperature (Fig. 6A). In the old and the recent peat, hydrolases showed a substantial increase with increasing temperature while the pools of oxidases were insensitive to the temperature increase in both soils (Fig. 6B). In the mineral tundra soil, neither enzyme pool differed between 4°C and 12°C, while both the pools of hydrolytic and oxidative enzymes increased substantially at 20°C. Hydrolases doubled and oxidases showed a 6-fold increase at 20°C compared to the initial pool. The increase in the pool of hydrolytic enzymes with temperature was expressed as Q_{10}^G and was correlated with temperature response of C_{LOSS} (Fig. 7).

N transformations

Gross ammonification and net mineralization rates in the old peat were low compared to the recent peat with no clear temperature response observed (Fig. 8A and C). In the recent peat, both of the processes positively responded to temperature and gross ammonification was about one order of magnitude higher than net mineralization. The highest net N mineralization in the recent peat suggests that N mineralization exceeded microbial N demand. The same was implied by a low but still substantial net mineralization in the old peat at 20°C. The mineral tundra soil showed the highest gross ammonification with no significant effect of temperature (Fig. 8A). Only a negligible net N mineralization rate was found in the mineral tundra soil suggesting complete immobilization of mineral nitrogen forms by microbes (Fig. 8C). Gross nitrification rates in both peat soils were highly variable and the measured values of a majority of the replicates were below the detection limit of the method (Fig. 8B). Gross nitrification in the mineral tundra soil exceeded the rates in peat soils, positively responded to temperature and was of the same order of magnitude as the gross ammonification (Fig. 8B).

Anaerobic incubations

Soil C_{LOSS}

Anaerobic C_{LOSS} was lower and more variable compared to aerobic treatment in all soils (Fig. S2, Supporting Information). Cumulative C_{LOSS} under anaerobic conditions represented the sum of CO_2 and CH_4 produced. Similarly to the aerobic conditions, the lowest cumulative C_{LOSS} was found in the old peat, intermediate in the recent peat and the highest in the mineral tundra soil (Fig. 2). The microbial biomass was again the best predictor of cumulative C_{LOSS} but unlike in the aerobic conditions, N_{MB} showed the strongest correlation with anaerobic cumulative C_{LOSS} (Table 2; Fig. S5, Supporting Information). C_{LOSS} increased with temperature and there was no significant difference in goodness of fit between the exponential and the Gaussian model for the recent peat ($F = 1.78$, $df = 1$, $P = 0.184$; $dAIC = 0.2$), old peat ($F = 0.073$, $df = 1$, $P = 0.787$; $dAIC = 1.9$) and mineral tundra soil ($F = 0.31$, $df = 1$, $P = 0.579$; $dAIC = 1.7$). The temperature sensitivity estimated by the exponential model (Q_{10}^E) was the highest in the old peat (2.47 ± 0.44), but rather low in the mineral tundra soil (1.79 ± 0.18) and the recent peat (1.57 ± 0.24). The Q_{10}^E was generally lower in comparison to aerobic conditions. According to the Gaussian model, temperature sensitivity of anaerobic C_{LOSS} from the old peat was comparable to the aerobic conditions ($Q_{10}^G = 2.69 \pm 1.49$) while it was reduced by anaerobic conditions in the recent peat and the mineral tundra soil with Q_{10}^G of 1.79 ± 1.52 and 1.77 ± 1.13, respectively (Fig. 3). Consistently with the aerobic treatment, the results of the Gaussian model are used in the discussion. The C_{LOSS} from the old peat decreased by 24.5%–40.1% as compared to aerobic conditions, regardless of the temperature. Anaerobic conditions decreased the C_{LOSS} from the recent peat and the mineral tundra soil to a greater extent by 21.6%–69.1% and 43.8%–76.4% in comparison to aerobic conditions, respectively, and this was positively affected by temperature in both soils. Production of CH_4 was not detected in the old and the recent peat soils for the whole incubation period. On the contrary, CH_4 contributed to C_{LOSS} from the mineral tundra soil with a positive effect of temperature on the CH_4 production (Fig. S3A, Supporting Information). The lag

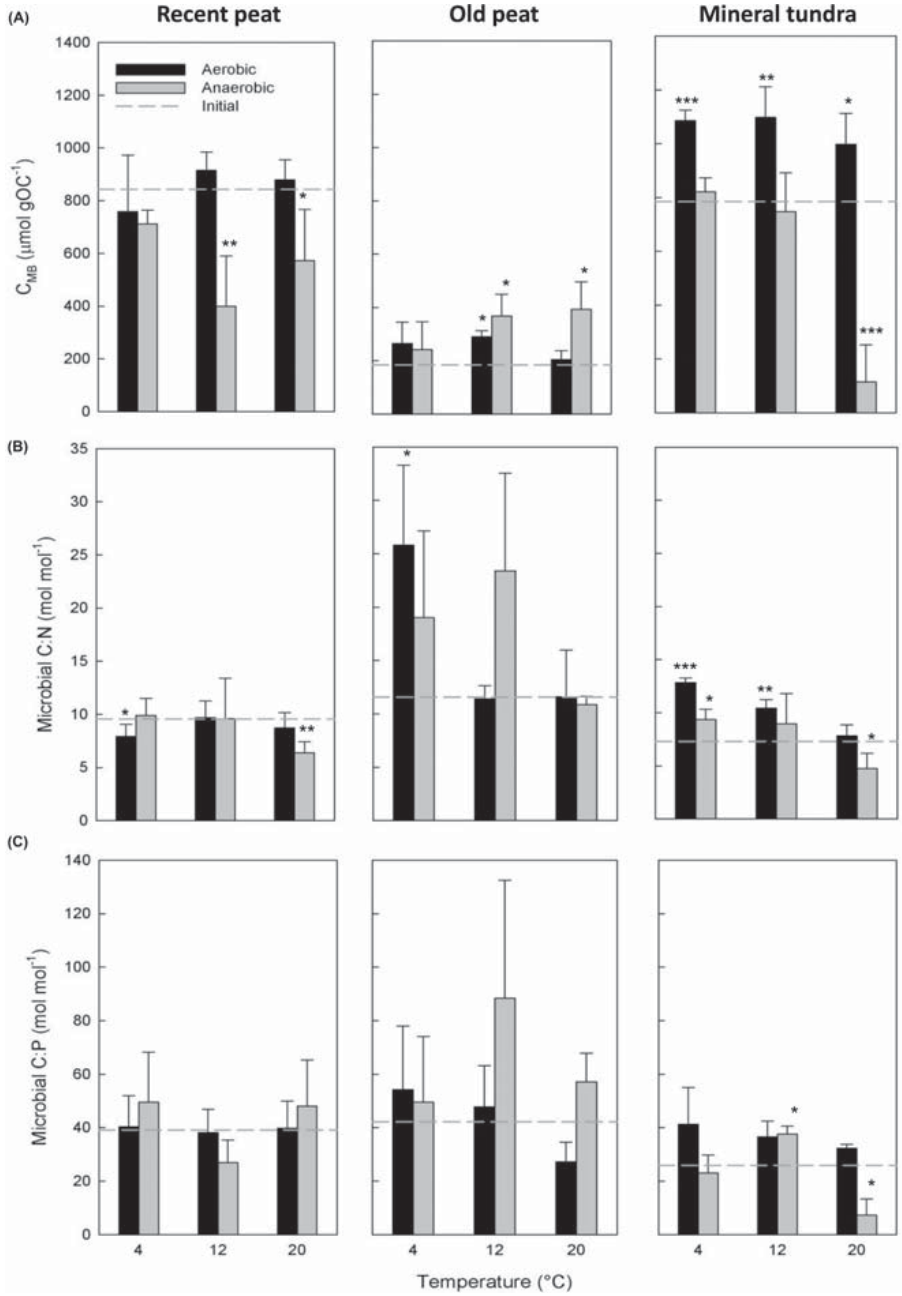

Figure 4. Parameters of microbial biomass as (**A**) concentration of carbon in microbial biomass (C_{MB}) in soil relative to OC and molar ratios of elements in microbial biomass (**B**) carbon to nitrogen ratio in microbial biomass ($C:N_{MB}$) and (**C**) carbon to phosphorus ratio in microbial biomass ($C:P_{MB}$); dashed horizontal lines show values of the parameters at the beginning of the incubation (initial), columns represent mean values in aerobic (black) and anaerobic (gray) samples and vertical bars show standard deviation ($n = 4$). Asterisks denote significance level of difference between the final and the initial values of each parameter: * $\alpha = 0.05$, ** $\alpha = 0.01$, *** $\alpha = 0.001$.

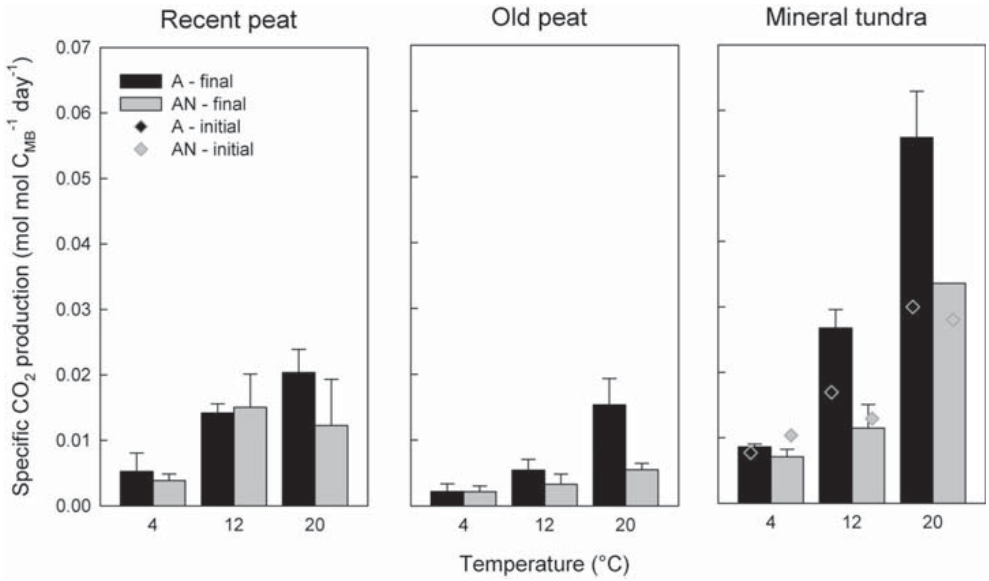

Figure 5. Specific CO_2 production expressed as CO_2 produced per mol of microbial biomass (C_{MB}) per day in aerobic (black, A) and anaerobic (gray, AN) samples. Columns represent mean values at the end of the incubation (final) and vertical bars show standard deviation (n = 4). Mean values of specific CO_2 production at the beginning of the incubation (initial, diamond symbols, $n = 4$) are depicted for mineral tundra soil because they significantly differed from the final values. The initial values of specific CO_2 production were identical to the final values in recent peat and old peat and therefore are not shown.

time of methanogenesis was 134 days and 107 days for 12°C and 20°C, respectively, while no CH_4 production was detected at 4°C during the whole incubation period. In the last week of the incubation, methanogenesis represented up to 8.4% and 28.4% of C_{LOSS} from the mineral tundra soil at 12°C and 20°C, respectively.

Microbial biomass

Microbial biomass increased in the old peat during the incubation, while it decreased in the recent peat in the whole temperature range (Fig. 4A). The microbial biomass in the mineral tundra soil substantially decreased at 20°C, which was accompanied by a decrease in $C:N_{MB}$ and $C:P_{MB}$ (Fig. 4B and C). Anaerobic-specific CO_2 production in the old peat was lower than in the recent peat and the value in the mineral tundra soil was higher than both (Fig. 5). Compared to aerobic conditions, the anaerobic-specific CO_2 production was equal in the recent peat, but lower in the old peat and the mineral tundra soil.

Soil enzymes

The E_{CNP} relative to OC reached the maximum at 12°C in the old and the recent peat and was comparably lower at 4°C and 20°C. The E_{CNP} in the mineral tundra soil was similar at all temperatures (Fig. 6). Compared to the initial pool, hydrolases in aerobic conditions showed a lower increase than those in anaerobic conditions in the old and the recent peat (Fig. 6A). The pool of oxidases in anaerobic conditions exceeded the aerobic pool at 12°C in both soils (Fig. 6B). There were no significant changes in the pool of enzymes in the mineral tundra soil.

N transformations

The potential gross ammonification rates in anaerobic samples were similar to those of aerobic conditions (Fig. 8A); similarly, net N mineralization rates did not differ from the aerobic ones in any soil (Fig. 8C).

DISCUSSION

We have studied C_{LOSS} from a permafrost-affected old peat recently exposed to ambient climatic conditions by cryogenic processes and searched for biotic and abiotic drivers of C_{LOSS} in comparison with two adjacent vegetated soils. We have found that C_{LOSS} is closely linked to variation in microbial biomass among the soils. The old bare peat was characterized by the lowest C_{LOSS} which correlated with extraordinarily low microbial biomass with low biomass-specific CO_2 production. We show that the biomass-specific CO_2 production is a function of OC quality and nutrient stoichiometry. We further argue that the microbial community in the old peat is adjusted to anaerobic conditions. The temperature sensitivity of the C_{LOSS} from the old peat was the same under aerobic and anaerobic conditions. In turn, the absence of oxygen reduced the C_{LOSS} temperature sensitivity by factor of 2 in the recent peat and the mineral tundra soil.

Link between C_{LOSS} and microbial biomass and its activity

A multiple regression analysis showed that microbial biomass was the strongest and the most stable predictor of C_{LOSS} in different temperature and oxygen regimes. Previous studies brought mixed evidence on microbial controls. Although several studies found that OC quality is the primary control of C_{LOSS} from arctic soils (Lee *et al.* 2012; Treat *et al.* 2014) while the impact of microbial control is minor, our results rather correspond to an earlier study on arctic soils from Taymir peninsula (Čapek *et al.* 2015)

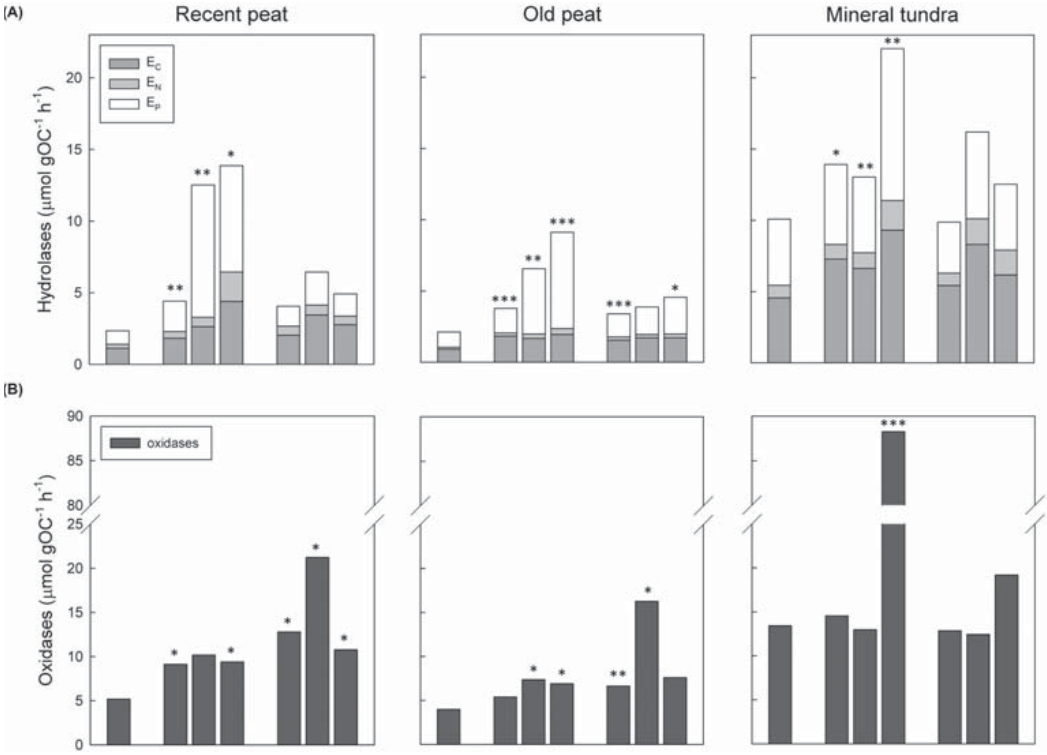

Figure 6. Total pool of hydrolytic enzymes (full column height) with relative proportions of carbon-sequestering enzymes (E_C), nitrogen-sequestering enzymes (E_N) and phosphorus-sequestering enzymes (E_P) in upper panel (**A**), and a pool of oxidative enzymes in the lower panel (**B**) at the start (initial) and at the end of the incubation in aerobic and anaerobic samples under all temperature treatments. Columns represent mean value ($n = 4$). Asterisks denote statistically significant change in hydrolytic or oxidative enzyme pools as compared to initial value: * $\alpha = 0.05$, ** $\alpha = 0.01$, *** $\alpha = 0.001$.

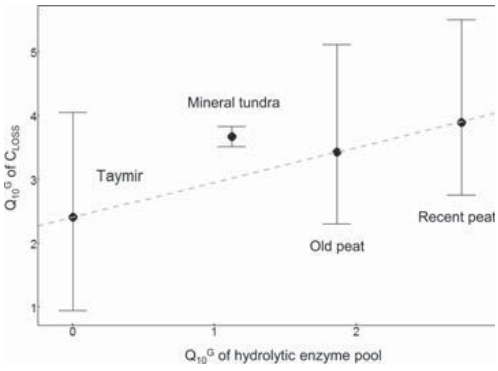

Figure 7. Relationship between temperature sensitivity ($Q_{10}{}^G$) of C_{LOSS} and pool of hydrolytic enzymes. The figure depicts all three soils from this study and a sum of soils from Taymir peninsula published by Čapek *et al.* (2015) refered to as 'Taymir'. The relationship was described by linear regression with following parameters: slope = 0.49, intercept = 2.65 and adjusted $R^2 = 0.64$. Full circles show mean values, and vertical bars represent standard errors.

and are in accordance with general metabolic theory (Brown *et al.* 2004; Allen, Gillooly and Brown 2005; Allen and Gillooly 2009; Yvon-Durocher *et al.* 2012): higher microbial biomass generates higher C_{LOSS}.

The microbial biomass in the old peat was only 0.22% of OC which was more than four times lower than microbial biomass of about 1% of OC in both recent peat and mineral tundra soil. Therefore, the C_{LOSS} per OC was the lowest here. Microbial biomass in non-permafrost soils usually represents about 1% of soil OC or more, even in deeper soil layers (Fierer, Schimel and Holden 2003; Yakutin, Anopchenko and Conen 2016). However, the permafrost layer of boreal and arctic peatlands is inhabited by a relatively lower microbial biomass than the active layer (Treat *et al.* 2014). Similarly, low microbial biomass as in the old peat was found in subducted organic horizon of cryoturbated soils from Taymir peninsula (Čapek *et al.* 2015). This evidence implies that perennially frozen organic soils are generally low in microbial biomass and this characteristic persists even after the soil has been uplifted back to the surface. Possible reasons are discussed in section 'Possible constraints on microbial biomass in cryogenic organic soils'.

The effect of low microbial biomass on C_{LOSS} from the old peat was further multiplied by low biomass-specific CO_2 production. This was again the lowest among the studied soils, while

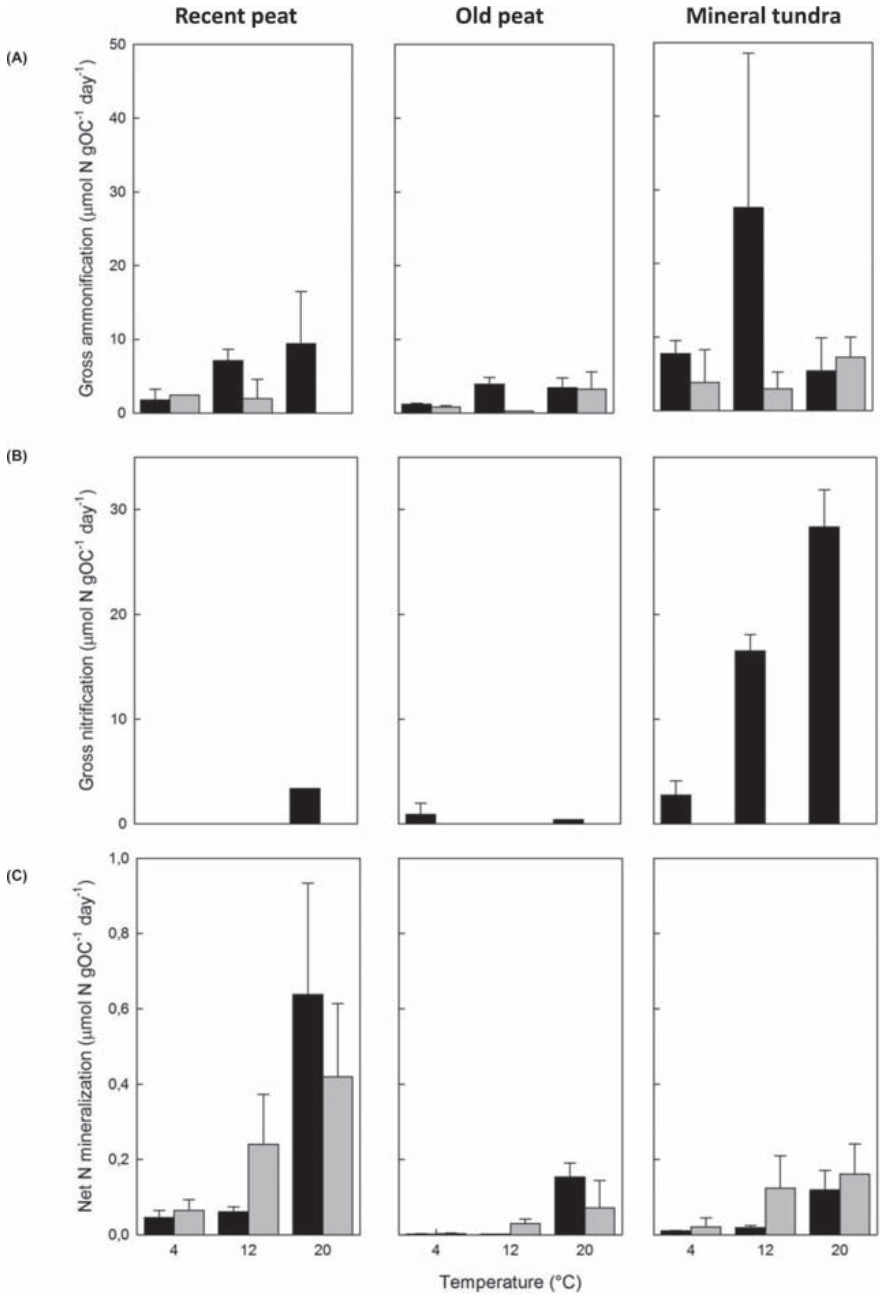

Figure 8. Nitrogen transformations in studied soils in aerobic (black) and anaerobic (gray) samples. Gross ammonification (**A**) and gross nitrification (**B**) rates were assayed at the end of the incubation. Net N mineralization rates (**C**) were determined over the incubation period. Columns represent mean values, and vertical bars show standard deviations ($n = 4$).

intermediate activity was detected in the recent peat and the highest in the mineral tundra soil. As a result, a maximum of 0.7% of OC was lost from the old peat over the incubation period of 23 weeks, while up to 3.1 and 14.6% of OC was lost from the recent peat and the mineral tundra soil, respectively. The biomass-specific CO_2 production expresses the activity of microbial community. The activity can be modulated by various edaphic factors. In the studied soils, we found that the biomass-specific CO_2 production was linked to the quality of OC and to nutrient stoichiometry.

Link between microbial activity and OC quality

The old peat from the surface of bare ground cryogenic formations, called peat circles, developed about 3500–5900 years BP from the litter of a rich fen vegetation (Biasi *et al.* 2014; Ronkainen *et al.* 2015). During its development, the old peat had experienced anaerobic conditions in the wet fen, peat plateau development while perennially frozen in permafrost, interims with permafrost degradation and cryogenic drift from the permafrost zone to the well-drained surface layer (Routh *et al.* 2014; Ronkainen *et al.* 2015). Although the OC decomposition had been limited by anaerobic conditions and subzero temperatures which can lead to preservation of labile OC in permafrost layers (Michaelson and Ping 2003; Dutta *et al.* 2006; Waldrop *et al.* 2010), the old peat from peat circles shows a higher level of degradation (Ronkainen *et al.* 2015) than the recent peat. Poor OC quality was indicated by a higher aromaticity of the bulk soil, higher proportion of hydrophobic compounds and higher residual carbon after acid hydrolysis compared to the recent peat (Pengerud 2013). The quality of bulk soil OC is reflected in OC availability for microbial metabolism. Indeed, we also detected higher aromaticity of DOC in the old peat compared to the other soils (Table 1). Generally, lower OC availability correlates with a lower CO_2 production (Gershenson, Bader and Cheng 2009). The link between microbial activity and OC quality is apparent from the comparison of the old peat and the recent peat. The quality of OC influences biomass-specific CO_2 production (Fig. 5) and thus modulates the C_{LOSS} (Fig. 2). However, OC quality cannot solely explain the variability in the biomass-specific CO_2 production in all studied soils.

Link between microbial activity and nutrient stoichiometry

Mineral tundra soil showed the highest biomass-specific CO_2 production even though it was characterized by intermediate DOC aromaticity. However, mineral tundra soil had the highest available C:nutrient ratio ($C:N_{EX}$, $C:P_{EX}$; Table 1), which contrasted with the lowest C:nutrient ratio in microbial biomass ($C:N_{MB}$, $C:P_{MB}$; Table 1). It was shown that microbial nutrient demand is closely linked to the stoichiometry of microbial biomass (Sinsabaugh *et al.* 2013); the lower the C:nutrient ratio of microbial biomass, the more nutrients relative to carbon (i.e. a lower C:nutrient ratio) are needed in the consumed substrate for optimal nutrition. When the substrate C:nutrient ratio is higher than the optimum, the excess carbon is mineralized in the so-called overflow metabolism (Tempest and Neijssel 1992; Spohn 2015) and limiting nutrients are fully immobilized into microbial biomass. Accordingly, the mineral tundra soil samples exhibited the highest biomass-specific CO_2 production, virtually no net N mineralization as well as the highest gross N mineralization rates (i.e. sum of gross ammonification and gross nitrification; Fig. 8). Clearly, all mineralized N was immobilized into microbial biomass during the incubation, suggesting that N was the limiting nutrient. Similarly low net N mineralization and high gross N mineralization rates were observed also in a field experiments in the mineral tundra soil at the same study site (Marushchak *et al.* 2011; Diáková *et al.* 2016). Our findings are in concordance with Briones *et al.* (2014), who showed that nutrient stoichiometry is one of the main controls of microbial activity in peatlands and boreal soils.

Temperature sensitivity of C_{LOSS}

C_{LOSS} was positively affected by temperature in all studied soils. Temperature sensitivity of aerobic C_{LOSS} below 15°C was the same among the soils and averaged at 3.68. This was similar to mean temperature sensitivity for arctic soils $Q_{10} = 3.4$ reported by Hamdi *et al.* (2013) from a collection of incubation studies, and within the range of collected Q_{10}s for permafrost-affected soils by Moni *et al.* (2015). However, our value is higher than that previously found in a similar laboratory experiment with soils from Taymir peninsula (Čapek *et al.* 2015). We found that this difference was linked to temperature sensitivity of hydrolytic enzymes production. In the Taymir soils, the pool of hydrolytic enzymes did not respond to the temperature. Therefore, the temperature sensitivity of C_{LOSS} was controlled primarily by physiological temperature response of basal microbial metabolism there (Brown *et al.* 2004; Allen, Gillooly and Brown 2005; Allen and Gillooly 2009; Yvon-Durocher *et al.* 2012). On the contrary, the microbial communities in all the soils in this study reacted to higher temperature with an increased production of hydrolytic enzymes. The enzyme production could raise the temperature sensitivity of C_{LOSS} over the basic physiological response (Fig. 7) on two levels. First, enzyme synthesis requires high energy in the form of ATP, which is formed during oxidative phosphorylation while producing CO_2. Therefore, increased enzyme production at higher temperatures enhances CO_2 production above the basal metabolic rate. Second, when the hydrolytic enzyme pool is higher at higher temperatures, microbial community is supplied with more available substrate, again reinforcing CO_2 production above the basal metabolic rate.

In the old peat, temperature sensitivity of C_{LOSS} was constant throughout the examined temperature range. On the other hand, the recent peat and the mineral tundra soil exhibited a decrease in temperature response in the higher portion of the range (Fig. S4, Supporting Information). It seems that microbial activities in the recent peat and the mineral tundra soil are slowly approaching their temperature optima, while the microbial activity in the old peat has a potential to increase exponentially further beyond the incubation temperature range. These functional responses might be reflecting adaptations of the microbial communities to field conditions: the bare old peat is exposed to higher temperature fluctuation and can experience overheating due to the lack of shading while the vegetation cover at the surfaces of the peat plateau and mineral tundra mitigates these temperature extremes.

Effect of oxygen availability on C_{LOSS}

C_{LOSS} was lower under anaerobic than under aerobic conditions in all studied soils. However, the difference between aerobic and anaerobic C_{LOSS} was the lowest in the old peat (Fig. 2), which agrees with a similar trend detected in the same soil type by Pengerud (2013), and it corresponds to the minor response to oxygen availability in the subducted organic horizon of the Taymir soil (Čapek *et al.* 2015). In addition, the microbial biomass in the old peat increased under anaerobic conditions (Fig. 4) and there was no difference in temperature sensitivity of C_{LOSS} under

anaerobic and aerobic conditions (Fig. 3). All these lines of evidence indicate that at least a part of an existing microbial community in the old peat thrived in anaerobic conditions and their metabolism was adjusted to the absence of oxygen. Microbes with a slow anaerobic metabolism could increase in biomass under anaerobic conditions, whereas under aerobic conditions more efficient aerobes would suppress their growth. Anaerobes in the microbial community of the old peat could have survived from the time when the old peat was subducted in the inundated layers of the soil profile. Cryopreservation of microbial community in permafrost layers has been documented, e.g. by Uhlířová, Šantrůčková and Davidov (2007). In turn, the response of cumulative C_{LOSS} to absence of oxygen was larger in the recent peat and the mineral tundra soil, and corresponded with response ratios in other arctic soils (Schädel *et al.* accepted). The difference between aerobic and anaerobic C_{LOSS} can indicate here the adaptation of the microbial community to aerobic conditions in well-drained surface soils. In support, microbial biomass in the recent peat and the mineral tundra soil was negatively affected by anaerobic conditions at higher temperatures (Fig. 4). An increase in extractable carbon and nutrients under these conditions suggests that lysis of microbial cells had taken place, confirming our assumption (Table 3). Also, the temperature sensitivity of anaerobic C_{LOSS} in the recent peat and the mineral tundra soil was lower than under aerobic conditions, which was in accordance with the results from the Taymir soils (Čapek *et al.* 2015).

Anaerobic C_{LOSS} occurred solely as CO_2 production in the recent and the old peat (Fig. S3A Supporting Information), which indicates that anaerobic respiration and fermentation were the major metabolic pathways and the development of a methanogenic community was slow. The lag phase of methanogenesis, i.e. the time from establishing anaerobic conditions to the start of the process, is partly affected by disturbances during sampling and soil processing and can take from weeks to over a year in arctic soils (Treat *et al.* 2015). However, the length of the lag phase at the higher extreme is rather rare in literature; the onset of methanogenesis is most commonly reported to lie within the range of several weeks (Treat *et al.* 2014; Herndon *et al.* 2015). Treat *et al.* (2015) have also shown that the lag phase of methanogenesis is shorter in organic soils than in mineral soils. Thus, the absence of methanogenesis in both the old and recent peat was unexpected. We argue that the onset of methanogenesis can be most likely delayed by the presence of alternative, more efficient electron acceptors. The old peat was exceptionally rich in nitrate at the beginning of the incubation (Table 1). Nitrate was depleted from the soil during the anaerobic incubation while none of the other N pools (NH_4^+, DON or N_{MB}) increased (Table 3) indicating that nitrate was utilized as an alternative electron acceptor in anaerobic respiration, i.e. denitrification, and transformed into gaseous N forms. Indeed, an abundant oligotrophic denitrifier community adapted to a complex OC substrate was described in the old peat from the same study site (Palmer, Biasi and Horn 2011; Pengerud 2013). Pengerud (2013) also indicated other undefined alternative acceptors present in the old peat but lacking in the recent peat. Possibly, humic substances in combination with oxidized metal forms could play the role here (Lovley *et al.* 1996). Therefore, we suggest that other anaerobic respiratory pathways such as denitrification or Fe reduction could have suppressed the development of a methanogenic community in the old peat (Palmer, Biasi and Horn 2011; Herndon *et al.* 2015). This is supported by the fact that the old peat in peat circles emits high amounts of N_2O (Repo *et al.* 2009; Marushchak *et al.* 2011). Another reason for the absence of methanogenesis could be the lack of a suitable substrate for methanogens or an absence of methanogenic community. A suitable substrate for methanogens was likely scarce due to high OC complexity in the old peat (Pengerud 2013; Ronkainen *et al.* 2015). In the recent peat, the initial nitrate concentration was low so denitrification could not represent the major anaerobic respiratory pathway. The reasons for a long lag phase in the recent peat could be thus an undeveloped methanogenic community in the well-aerated surface peat soil with low bulk density. Methanogenesis contributed to anaerobic C_{LOSS} solely in the mineral tundra soil (Fig. S3A, Supporting Information). The development of the methanogenic community was probably supported by the most labile OC substrate in the mineral tundra soil.

Possible constraints on microbial biomass in cryogenic organic soils

Our results on C_{LOSS} connection to microbial biomass and activity and the effect of temperature and oxygen availability fit with generally accepted theories, namely the metabolic theory and the ecological stoichiometry theory. Nevertheless, the reason for the exceptionally low microbial biomass that constrains the C_{LOSS} from the old peat remains unresolved. Based on our arctic research presented here and that on subducted organic soils from Taymir peninsula (Gittel *et al.* 2014; Schnecker *et al.* 2014; Wild *et al.* 2014; Čapek *et al.* 2015; Gentsch *et al.* 2015), we suggest that physical constraints of microbial cells dispersion, permafrost history and a lack of input of fresh organic material can be the main reasons. The old peat and the subducted organic soils were both affected by cryogenic vertical movement along the soil profile. Both soils got compressed when positioned in subsurface layers and the free pore space was reduced. Indeed, the old peat had 2 to 5-fold higher bulk density as compared to the recent peat (Table 1; Repo *et al.* 2009; Marushchak *et al.* 2011; Biasi *et al.* 2014) and the subducted organic horizon had a higher bulk density than a surface organic horizon (Čapek *et al.* 2015). This compression caused organic particles to cluster into small firm aggregates with small pores and a limited water flux and gas exchange. Thus, spatial separation and limited dispersion of microbial cells (Nunan *et al.* 2002; Ekschmitt *et al.* 2008; Schmidt *et al.* 2011) could have restricted the development of the microbial community. Old OC represents a complex substrate for microbial degradation. The complex OC is further protected from decay by low abundance of fungi as primary decomposers in both the old peat (Table S1, Supporting Information) and the subducted organic horizon (Gittel *et al.* 2014). Similarly, the input of plant material to the soil, which could prime the mineralization of complex OC and allow the development of the microbial community (Kuzyakov, Friedel and Stahr 2000; Wild *et al.* 2014), was limited in the old bare peat as well as in the subducted organic horizon. The vegetation succession on bare surfaces and the projected overall greening of the Arctic as a result of climate change might release the constraints of microbial development in the old organic soils by introducing fresh plant material to the soils. This could trigger increased decomposition of old organic deposits currently preserved in permafrost-affected soils.

CONCLUSIONS

The fate of the substantial OC stored in permafrost-affected peat deposits of arctic soils is uncertain under the changing climate, and its biotic and abiotic controls are not yet well understood. The presence of permafrost in the arctic peatland induces vertical shifts within the peat profile and so modifies the environmental conditions for microbial activity and C_{LOSS}

Table 3. Concentrations of available C, N and P in μmol gdw^{-1} (C_{EX}—K_2SO_4 extractable carbon, NH_4^+—K_2SO_4 extractable ammonium, NO_3^-—K_2SO_4 extractable nitrate, DON—K_2SO_4 extractable dissolved organic nitrogen, P_{EX}—$NaHCO_3$ extractable phosphorus) in all treatments of the studied soils. Chemical parameters are presented as mean values with standard deviation in italics ($n = 4$). The Δ values represent change relative to the initial value of parameter at the beginning of the incubation. Asterisks denote significance level of the difference: * $\alpha = 0.05$, ** $\alpha = 0.01$, *** $\alpha = 0.001$.

	Aerobic									Aerobic								
	Recent peat			Old peat			Mineral tundra			Recent peat			Old peat			Mineral tundra		
	4°C	12°C	20°C	4°C	12°C	20°C	4°C	12°C	20°C	4°C	12°C	20°C	4°C	12°C	20°C	4°C	12°C	20°C
C_{EX}	143.9	110.3	138.7	123.3	121.2	136.4	12.9	13.4	14.4	145.5	247.5	281.1	124.0	146.8	134.6	25.1	12.2	20.5
SD	*25.3*	*3.1*	*9.0*	*13.3*	*3.9*	*2.9*	*2.2*	*1.4*	*1.8*	*26.0*	*73.8*	*125.8*	*3.0*	*19.5*	*46.0*	*30.7*	*3.2*	*4.8*
Δ C_{EX} (%)	*44.1**	*10.5***	*38.9****	*−8.0*	*−9.6*	*1.7*	*−5.3*	*−1.5*	*5.6*	*45.7**	*147.9***	*181.5**	*−7.5*	*9.5*	*0.4*	*84.0***	*−10.1*	*50.7*
NH_4^+	1.90	2.36	24.14	0.10	0.09	0.17	0.06	0.09	0.67	2.89	9.72	16.70	0.14	1.24	3.01	0.11	0.69	0.90
SD	*0.61*	*0.46*	*11.00*	*0.03*	*0.01*	*0.04*	*0.01*	*0.03*	*0.28*	*1.17*	*5.15*	*7.56*	*0.05*	*0.51*	*3.03*	*0.13*	*0.47*	*0.44*
Δ NH_4^+ (%)	*363***	*476****	*5804***	*518***	*444***	*936****	*1563****	*2585***	*19403***	*607***	*2277**	*3984***	*746***	*7636***	*18720*	*3162*	*20223**	*26123***
NO_3^-	0.69	0.80	1.46	8.36	9.65	19.35	0.05	0.07	0.04	0.34	0.31	0.31	1.61	0.24	0.89	0.05	0.05	0.05
SD	*0.17*	*0.10*	*0.54*	*0.39*	*0.52*	*1.50*	*0.01*	*0.03*	*0.01*	*0.16*	*0.09*	*0.07*	*0.57*	*0.10*	*0.87*	*0.04*	*0.01*	*0.01*
Δ NO_3^- (%)	*71.9*	*99.6**	*264.9**	*−36.1****	*−26.1****	*48.0****	*−9.8*	*14.5*	*−35.0*	*−16.3*	*−23.2*	*−22.1*	*−87.7****	*−98.2****	*−93.2****	*−15.4*	*−11.2*	*−16.0*
DON	10.70	9.17	18.02	10.71	10.07	16.30	0.80	1.02	1.53	9.07	13.44	12.83	9.26	9.25	10.94	2.13	0.74	1.57
SD	*1.81*	*0.58*	*3.92*	*1.19*	*1.12*	*0.35*	*0.22*	*0.10*	*0.29*	*1.16*	*3.52*	*3.61*	*0.67*	*0.72*	*3.71*	*3.09*	*0.27*	*0.34*
Δ DON (%)	*71.2***	*46.7***	*188.1***	*2.0*	*−4.1*	*55.3**	*61.4*	*104.2*	*208.5**	*45.0***	*115.0***	*105.2**	*−11.8*	*−11.9*	*4.2*	*327.8*	*49.2*	*216.0**
P_{EX}	0.46	0.46	0.85	1.69	1.60	1.67	0.10	0.08	0.09	1.67	1.87	2.00	1.91	1.86	1.72	0.18	0.29	0.27
SD	*0.09*	*0.08*	*0.38*	*0.14*	*0.16*	*0.17*	*0.00*	*0.00*	*0.01*	*1.00*	*0.57*	*0.42*	*0.11*	*0.10*	*0.40*	*0.05*	*0.02*	*0.01*
Δ P_{EX} (%)	*−43.8***	*−43.8***	*2.3*	*10.3*	*4.1*	*9.2*	*−27.8***	*−40.8****	*−35.2***	*102.4*	*126.1**	*142.6***	*24.5**	*21.1**	*11.9*	*30.4*	*104.9****	*92.7****

in the old peat deposits. We found that low C_{LOSS} from old peat uplifted to the surface by cryogenic processes is a function of low microbial biomass with low activity, which mirrors low OC quality and nutrient stoichiometry. Thus, the effects of microbial biomass and OC quality are ultimately linked and cannot be separated from each other in principle. In turn, opposite to our expectation, temperature sensitivity of C_{LOSS} from the old peat does not reflect the OC quality under aerobic conditions and is enhanced by increased enzymatic activity at higher temperatures. C_{LOSS} and its temperature sensitivity in the old peat were only little affected by oxygen absence, which implies that the microbial community was adjusted to anaerobic conditions. Together with the low microbial biomass, these might be relict features of permafrost soil persisting even after the soil has been exposed to ambient climatic conditions. Even though the vulnerability of OC stocks stored in old peat deposits is lower than in the other soils, present and future changes in environmental conditions will likely support their diminution.

Our results acquired by the incubation approach provide valuable information about potential C_{LOSS} as a measure of OC vulnerability. However, stable laboratory conditions lack diurnal or seasonal climatic fluctuations, and the effect of vegetation. These data therefore cannot be directly upscaled to field conditions. Still, functional responses of microbial community and their driving factors can be thoroughly examined only under controlled laboratory conditions. We suggest that more incubation studies are needed to better understand the role of microbial communities in carbon dynamics in arctic soils, especially in deep organic deposits.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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Paper III

Plant-derived compounds stimulate the decomposition of organic matter in arctic permafrost soils

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Plant-derived compounds stimulate the decomposition of organic matter in arctic permafrost soils

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Arctic ecosystems are warming rapidly, which is expected to promote soil organic matter (SOM) decomposition. In addition to the direct warming effect, decomposition can also be indirectly stimulated via increased plant productivity and plant-soil C allocation, and this so called "priming effect" might significantly alter the ecosystem C balance. In this study, we provide first mechanistic insights into the susceptibility of SOM decomposition in arctic permafrost soils to priming. By comparing 119 soils from four locations across the Siberian Arctic that cover all horizons of active layer and upper permafrost, we found that an increased availability of plant-derived organic C particularly stimulated decomposition in subsoil horizons where most of the arctic soil carbon is located. Considering the 1,035 Pg of arctic soil carbon, such an additional stimulation of decomposition beyond the direct temperature effect can accelerate net ecosystem C losses, and amplify the positive feedback to global warming.

Plant productivity in the Arctic is stimulated by rising temperatures[1,2], which implies not only an increased uptake of CO_2 from the atmosphere by plants, but also an increased transfer of organic compounds from plants to the soil, e.g., as root exudates and root litter[3]. Such an increased input of plant-derived compounds can reduce the microbial decomposition of native SOM by providing soil microorganisms with additional, easily degradable C and N sources that thus decrease the microbial dependence on the more complex substrates of native SOM ("negative priming")[4]. On the other hand, increased C and N availability can also stimulate SOM decomposition ("positive priming"), since additional N may promote the synthesis of extracellular enzymes that break down polymeric compounds of SOM[5], whereas additional C may provide microorganisms with energy that facilitates the decomposition of energy-poor SOM compounds[6,7]. Additional C can also stimulate microbial growth in general, and thus lead to higher microbial N demand and higher microbial N mining, i.e., to a higher microbial decomposition of SOM to get access to N (refs. 8,9).

Studies on an ecosystem level suggest that an increased allocation of plant-derived organic compounds into the soil with warming can indeed stimulate the decomposition of native SOM. For instance in the European sub-Arctic, significantly smaller soil organic C (SOC) stocks have been observed in a forest than in an adjacent

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Figure 1. Map of sampling sites across the Siberian Arctic. The dotted line indicates the polar circle. The map was created in R using the packages sp and rworldmap[47,49,50].

tundra, indicating that the transition from tundra to forest with warming can lead to a net loss of C from the soil, which in this case even exceeded the higher plant C stocks in the forest[10]. In contrast, in an Alaskan tussock tundra, ten years of warming stimulated plant primary production, but did not lead to a net change in SOC stocks[11]. This variability in the effect of plant-derived compounds on SOM decomposition might be related to differences in the distribution of SOM through the soil profile, and in the susceptibility of its decomposition to changes in organic C and N availability.

For instance, SOM in the top soil layer (further termed "organic topsoil") mainly consists of poorly decomposed plant material with a high content of easily degradable C sources such as cellulose, but a comparatively low content of N. The decomposition of organic topsoil material is therefore expected to be rather insensitive to an increased input of organic C, but might be strongly affected by changes in the availability of organic or inorganic N. In line with a predominant N control on SOM decomposition in the organic topsoil, inorganic N addition has been found to stimulate the decomposition of organic topsoil material[12,13], whereas organic C addition had hardly any effect[14,15]. In contrast to the organic topsoil, SOM in the subsoil is partly bound to soil minerals, has been repeatedly processed by soil microorganisms, and is characterized by low C/N ratios. The decomposition of mineral subsoil material has been found to strongly respond to the addition of organic C, with more than a doubling of SOC mineralization rates[15]. Consequently, particularly pronounced effects of increased plant C input on the decomposition of SOM in mineral subsoil horizons of arctic permafrost soils have been suggested[15].

Furthermore, arctic permafrost soils are often characterized by a mixing of soil horizons due to freeze-thaw processes that lead to the burial of poorly decomposed organic material from the topsoil into the mineral subsoil ("cryoturbation"; for a recent review see ref. 16). Cryoturbated material shows particularly low decomposition rates[15,17], and although it is located in the subsoil, decomposition rates might depend not on C, but rather on N availability, as indicated by a stimulation of decomposition after addition of organic N, but not of C alone[15]. Since arctic soils store about 1,035 Pg of organic C, with more than 80% of that in horizons deeper than 30 cm (ref. 18), understanding the controls over SOM decomposition and its response to changes in C and N availability across soil horizons is crucial for predicting C losses from arctic ecosystems in a future climate.

In this study, we provide first mechanistic insight into the susceptibility of SOM decomposition in arctic permafrost soils to an increased input of plant-derived compounds, such as by enhanced root litter production in a warmer climate. For 119 individual soil samples derived from four locations across the Siberian Arctic (Fig. 1, Table 1) and from five soil horizon categories (organic topsoil, mineral topsoil, mineral subsoil and cryoturbated material from the active layer, and mineral subsoil material from the upper permafrost), we simulated such an increased input of plant-derived compounds in a laboratory experiment, by amending soil samples with ^{13}C-labelled plant polymers, either cellulose or protein. Expecting a transition from N to C limitation of the microbial community with progressing SOM decomposition, we hypothesized that (1) SOM mineralization in organic topsoils and cryoturbated material would be affected by organic N, but not organic C, and would hence be stimulated by protein, but not by cellulose, and that (2) SOM mineralization in mineral soil horizons would be affected by organic C irrespective of the N content of the substrate, and would hence be stimulated by both cellulose and protein. After the addition of the respective substrate, we incubated the soil samples for 25 weeks and monitored soil respiration, distinguishing between substrate-derived (i.e., ^{13}C-enriched) and SOC-derived (i.e., non-enriched) CO_2. At the end of the incubation, we finally determined microbial biomass and microbial substrate use efficiency.

Results

Characterization of Soil Organic Matter. From organic to mineral topsoils and further to mineral subsoils, organic C content, total N content, as well as C/N ratios decreased, and δ^{13}C values increased, reflecting the proceeding decomposition state of SOM with depth (Table 2). Cryoturbated material was sampled at a similar depth as the mineral subsoil, but was characterized by more abundant and less decomposed SOM, with organic

	Coordinates	MAT (°C)	MAP (mm)	Vegetation type	Soil type	Active layer (cm)	Dominant plant species
Cherskiy	69°26'N, 161°44'E	−12.7	160	Shrubby grass tundra	Ruptic-Histic Aquiturbel	30–70	*Betula exilis*, *Salix sphenophylla*, *Carex lugens*, *Calamagrostis holmii*, *Aulacomnium turgidum*
				Shrubby tussock tundra	Ruptic-Histic Aquiturbel	35–60	*Eriophorum vaginatum*, *Carex lugens*, *Betula exilis*, *Salix pulchra*, *Aulacomnium turgidum*
Ari-Mas	72°29'N, 101°40'E	−13.7	280	Shrubby moss tundra	Typic Aquiturbel	60–85	*Betula nana*, *Dryas punctata*, *Vaccinium uligonosum*, *Carex arctisibirica*, *Aulacomnium turgidum*
				Shrubby moss tundra	Typic Aquiturbel	65–90	*Cassiope tetragona*, *Carex arctisibirica*, *Aulacomnium turgidum*
Logata	73°26'N, 98°25'E	−13.5	270	Dryas tundra	Typic Aquiturbel	35–70	*Dryas punctata*, *Rhytidium rugosum*, *Hylocomium splendens*
				Grassy moss tundra	Typic Aquiturbel	30–65	*Betula nana*, *Carex arctisibirica*, *Hylocomium splendens*, *Tomentypnum nitens*
Tazovskiy	67°10'N, 78°55'E	−_8.2	454	Shrubby lichen tundra	Typic Aquiturbel	100–120	*Empetrum nigrum*, *Ledum palustre*, *Betula nana*, *Cladonia rangiferina*, *Cladonia stellaris*
				Forest tundra	Typic Aquiturbel	130–150	*Larix sibirica*, *Ledum palustre*, *Betula nana*, *Vaccinium uligonosum*, *Cladonia rangiferina*, *Cladonia stellaris*

Table 1. Characterization of sampling sites. Soil samples were taken from two representative vegetation types at each site. Mean annual temperature (MAT) and mean annual precipitation (MAP) were derived from the WorldClim database[51]; soil description follows the USDA Soil Taxonomy[52]. Active layer depth was determined at the time of sampling in the late growing season, the variation is due to small-scale differences in surface morphology.

	Number of samples	Depth (cm)	Organic C (%)	N (%)	C/N	$\delta^{13}C$ (‰)
Organic topsoils	18	10.0 ± 1.4	21.39 ± 1.46 *a*	0.87 ± 0.05 *a*	25.58 ± 1.88 *a*	−27.45 ± 0.17 *c*
Mineral topsoils	23	12.7 ± 1.5	4.17 ± 0.56 *b*	0.26 ± 0.03 *b*	15.50 ± 0.62 *b*	−27.09 ± 0.21 *c*
Mineral subsoils	29	40.6 ± 4.0	1.27 ± 0.17 *c*	0.10 ± 0.01 *c*	11.89 ± 0.49 *c*	−26.08 ± 0.27 *b*
Cryoturbated	27	46.4 ± 3.6	6.48 ± 0.92 *b*	0.37 ± 0.04 *b*	16.53 ± 0.66 *b*	−27.12 ± 0.17 *c*
Permafrost	22	89.3 ± 6.2	1.43 ± 0.44 *c*	0.09 ± 0.02 *c*	12.02 ± 1.30 *c*	−24.75 ± 0.52 *a*

Table 2. Characterization of the sampled soil horizons. Different letters indicate significant differences between horizon classes at $p < 0.05$. For data on individual sampling sites see Supplementary Table S1.

C and total N contents, C/N ratios and $\delta^{13}C$ values in the range of the mineral topsoil. Mineral subsoils from the permafrost were similar to mineral subsoils from the active layer in terms of organic C, total N and C/N ratios, but had significantly higher $\delta^{13}C$ values.

Soil Organic Carbon Mineralization. Based on cumulative SOC-derived respiration (Supplementary Fig. S1), we calculated the amount of SOC lost by mineralization during the 25 weeks of incubation. In the control samples,

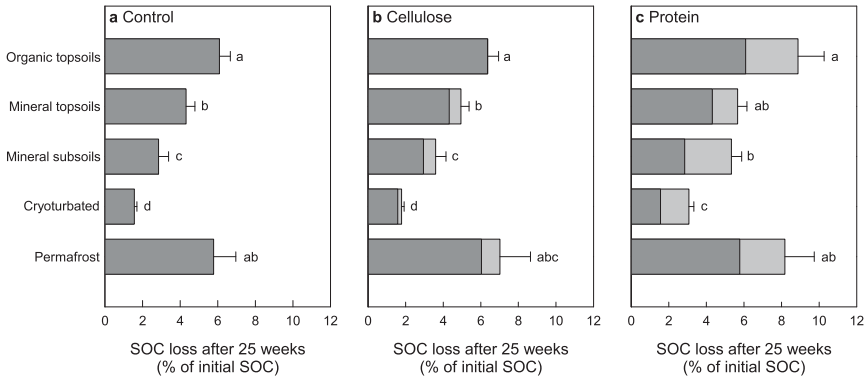

Figure 2. Losses of native SOC from different horizons of arctic permafrost soils after 25 weeks of incubation (dark grey bars). Losses induced by the addition of cellulose or protein in comparison to control samples are indicated in light grey. Bars represent means with standard errors, different letters indicate significant differences between horizons at $p < 0.05$. See Supplementary Fig. S1 for the development of SOC- and substrate-derived respiration over time.

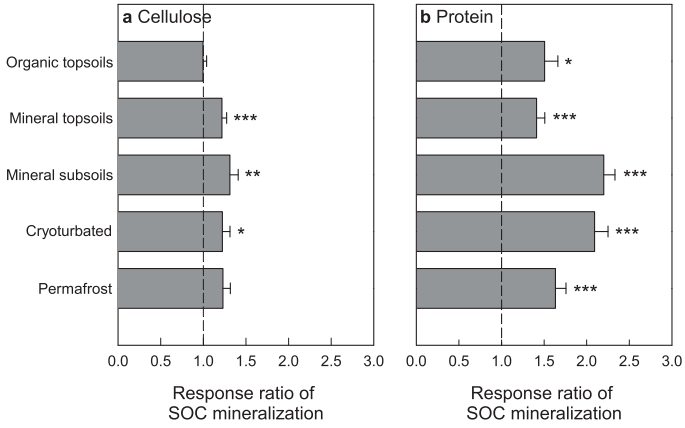

Figure 3. Response of cumulative SOC mineralization in different horizons of arctic permafrost soils to addition of cellulose or protein. Response ratios were calculated as ratios of samples amended with cellulose or protein over control samples. Bars represent means with standard errors, significant differences in SOC mineralization between amended and control samples are indicated (Welch's paired t-tests; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). For response ratios at individual sampling sites see Supplementary Table S5.

on average $6.1 \pm 0.6\%$ (mean $\pm$ standard error) of SOC were lost from organic topsoils, $4.3 \pm 0.5\%$ from mineral topsoils, and $2.8 \pm 0.5\%$ from mineral subsoils (Fig. 2). Hence, SOC loss decreased with increasing decomposition state of SOM. Although cryoturbated material was similar to mineral topsoil material in terms of organic C and total N content, C/N ratio and $\delta^{13}C$, it showed particularly low C mineralization rates, with only $1.6 \pm 0.1\%$ SOC lost during the incubation. Permafrost samples, in contrast, were characterized by SOC losses in the range of the organic topsoil ($5.8 \pm 1.2\%$).

Addition of cellulose or protein generally stimulated SOM decomposition, as indicated by an increase in the mineralization of native SOC (Fig. 3). Addition of cellulose had no significant effect in organic topsoils, whereas addition of protein led to a significant increase in SOC mineralization (response ratio (RR) = 1.51, corresponding to an average increase by 51%). A decrease in SOC mineralization was only observed in few of the organic topsoil samples, with response ratios of less than 0.80 in 18% (cellulose) and 11% (protein) of the incubated samples. This effect was likely due to a switch of microorganisms from SOM to cellulose or protein as substrate. Substrate replacement was negligible in the other horizons (Supplementary Table S2).

In mineral topsoils, mineral subsoils, and cryoturbated material, cellulose addition significantly increased SOC mineralization (RR = 1.22, 1.31, and 1.22, respectively), whereas the stimulation in permafrost material was not significant (RR = 1.23; Fig. 3). Effects of protein were stronger than of cellulose and significant in all horizons,

	RR (cellulose) vs. RR (protein): SOC mineralization	RR (cellulose) vs. RR (protein): Microbial biomass
Organic topsoils	n.s.	n.s.
Mineral topsoils	n.s.	n.s.
Mineral subsoils	0.524	0.577
Cryoturbated	n.s.	n.s.
Permafrost	0.462	0.724
All horizons	0.258	0.402

Table 3. Correlations between responses to addition of cellulose versus protein, for SOC mineralization and microbial biomass. Response ratios (RR) were calculated as ratios of amended over control samples. Given values are Spearman's rho of correlations significant at $p < 0.05$ (n.s., not significant).

	Temperature (°C)	Loss of native SOC (% of SOC)		
		No substrate amendment	Additional SOC loss induced by cellulose	Additional SOC loss induced by protein
Organic topsoils	8.0	2.23 ± 0.22	+ 0.00 ± 0.08	+ 0.89 ± 0.37
Mineral topsoils	7.5	1.65 ± 0.16	+ 0.26 ± 0.07	+ 0.55 ± 0.17
Mineral subsoils	4.5	1.33 ± 0.23	+ 0.27 ± 0.09	+ 1.13 ± 0.14
Cryoturbated	4.0	0.65 ± 0.09	+ 0.08 ± 0.04	+ 0.60 ± 0.09
Permafrost	1.0	2.67 ± 0.50	+ 0.58 ± 0.38	+ 1.09 ± 0.24

Table 4. Losses of native SOC without substrate amendment, and additional losses induced by cellulose or protein input, estimated for a growing-season of four months. Values derived from the incubation at 15 °C were adjusted for typical growing-season soil temperatures using Q_{10} values.

with response ratios of 1.41 and 2.20 in mineral topsoils and mineral subsoils, 2.09 in cryoturbated material and 1.63 in permafrost material. The amount of SOC additionally mineralized after addition of cellulose or protein exceeded the respective biomass C pools by average factors of 2.0 (cellulose; organic topsoil not included) and 8.3 (protein), indicating that the additional SOC mineralization was not caused by an accelerated turnover of the microbial biomass ("apparent priming"), but by an enhanced decomposition of SOM ("real priming")[7].

In mineral subsoils from the active layer and the permafrost, we further found significant correlations between the responses of SOC mineralization to cellulose and protein addition (Table 3), i.e., samples that responded to cellulose also responded to protein. Similar correlations were not observed in the other horizons.

Over the 25 weeks of incubation at 15 °C, the stimulation of SOM decomposition by cellulose and protein resulted in additional losses of native SOC of up to 1.0% SOC (cellulose) and 1.3–2.8% SOC (protein), depending on soil horizon (Fig. 2). Based on this incubation, we estimated SOC mineralization across a whole growing-season. Assuming a four-month season where soils are thawed[19] and plants are productive[20], as well as soil temperatures as measured in the field during sampling, the results of our laboratory experiment correspond to SOC losses of 0.7–2.7% without additional input of substrates. An input of cellulose or protein as simulated in our experiment would thus induce additional losses of up to 0.6% (cellulose) and 0.5–1.1% (protein) of native SOC (Table 4).

Microbial Growth and Substrate Utilization. In the control samples, microbial biomass decreased with soil depth following the decrease in SOM content. Calculated per unit SOC, microbial biomass was significantly lower in cryoturbated material than in the other soil horizons (Supplementary Table S3). The addition of cellulose led to a significant increase in microbial biomass in mineral topsoils (RR = 1.64), and the addition of protein had a similar effect in mineral topsoils (RR = 1.57), as well as in cryoturbated material (RR = 1.48; Fig. 4). In the other cases, substrate additions did not induce significant changes in microbial biomass.

Similar to the responses in SOC mineralization, we found significant correlations between the effects of cellulose addition and protein addition on microbial biomass in mineral subsoils of the active layer and the permafrost (Table 3). We further tested if responses of microbial biomass were connected to responses of SOC mineralization, but did not find any significant correlation, neither for cellulose nor for protein, and neither for individual horizons nor across all samples.

Since microbial biomass was only measured at the end of the incubation, our data do not consider potential transient peaks in microbial biomass, e.g., shortly after substrate addition. However, even at the end of the incubation after 25 weeks, we found ongoing mineralization of substrate-derived C (Supplementary Fig. S1) that could have stimulated microbial growth. Considering the lack of significant substrate effects on microbial biomass in mineral subsoils of active layer and permafrost, and the lack of correlation between responses of biomass and decomposition, we argue that the observed increase in SOM decomposition was likely not linked to an increase in microbial biomass.

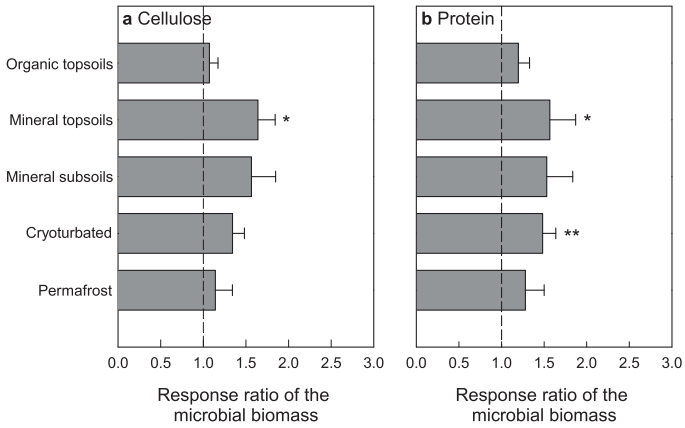

Figure 4. Response of the microbial biomass in different horizons of arctic permafrost soils to addition of cellulose or protein. Response ratios were calculated as ratios of samples amended with cellulose or protein over control samples. Bars represent means with standard errors, significant differences in microbial biomass between amended and control samples are indicated (Welch's paired t-tests; $^{**}p < 0.01$; $^{*}p < 0.05$). For microbial biomass in control samples see Supplementary Table S3, and for response ratios at individual sampling sites see Supplementary Table S5.

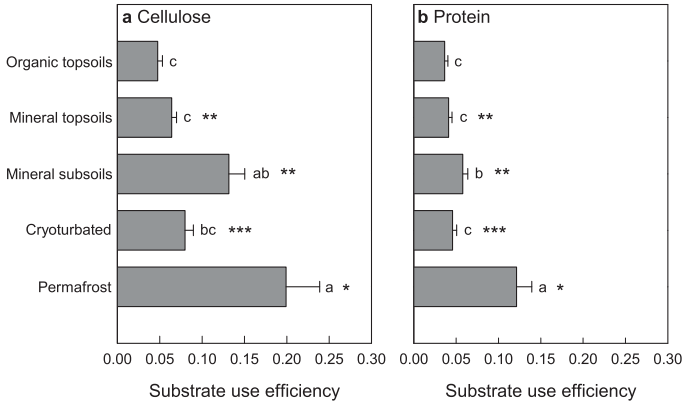

Figure 5. Microbial substrate use efficiency of cellulose- or protein-derived C in different horizons of arctic permafrost soils. Substrate use efficiency was calculated as the ratio of substrate-derived C in microbial biomass over substrate-derived C in biomass and cumulative respiration after 25 weeks of incubation. Bars represent means with standard errors. Significant differences between cellulose and protein treatments are asterisked ($^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$), significant differences between horizons for cellulose or protein are indicated by different letters ($p < 0.05$). For data on individual sampling sites see Supplementary Table S6.

Based on cellulose- or protein-derived C in cumulative respiration and microbial biomass, we calculated microbial substrate use efficiencies for cellulose and protein over the incubation time. Substrate use efficiencies were generally low, likely due to repeated turnover of the microbial biomass over the long incubation time, but still showed significant differences between substrates and horizons (Fig. 5). For all horizons except the organic topsoils, substrate use efficiencies were significantly higher for cellulose than for protein, and in both treatments, substrate use efficiencies were highest in mineral subsoils from the active layer and the permafrost, i.e., microorganisms allocated more substrate-derived C to growth and less to respiration than in the other horizons. Substrate use efficiencies for cellulose and protein were significantly correlated with the C/N ratios of SOM when calculated across all horizons, with high substrate use efficiency at low C/N (cellulose: $p < 0.001$, $rho = -0.481$; protein: $p < 0.001$, $rho = -0.343$; Supplementary Table S4). In contrast, we found no significant correlation between SOM stoichiometry or microbial substrate use efficiency and the response of SOC mineralization to substrate addition (Supplementary Table S4).

Discussion

Arctic soils contain about 1,035 Pg of organic C (ref. 18), more C than in today's atmosphere. According to recent model estimates, about 15% of this SOC will be lost as CO_2 or CH_4 until 2100 as a consequence of rising temperatures and permafrost thaw[21]. Our findings suggest that in addition to these direct temperature effects, SOC losses from arctic soils can be further promoted by changes in the availability of organic C or N, for example due to enhanced root litter production. Our findings further point to mechanistic differences between soil horizons: We found a high susceptibility (1) of organic topsoils to changes in organic N availability, (2) of mineral subsoils in active layer and permafrost to changes in organic C availability, and (3) of mineral topsoils and cryoturbated material to both.

In organic topsoils, the decomposition of native SOM was significantly altered by protein, but not by cellulose (Fig. 3), and we suggest that this effect was linked to protein-derived N. Previous studies on organic topsoil horizons of arctic soils have shown high microbial N use efficiency[22,23] and strong effects of inorganic N additions, including a stimulation of microbial growth[13] and SOM decomposition[12,13], as well as changes in microbial enzyme production[13,24]. Taken together, these studies indicate predominant N limitation of the microbial decomposer community in organic topsoil horizons of arctic permafrost soils and suggest a high susceptibility to changes in the availability of both inorganic and organic N. Increased organic N availability in organic topsoils can result from an increased input of root and leaf litter-derived proteins with rising temperatures[25], as simulated in our study. Warming additionally promotes a more active decomposer community[26] and increases the efficiency of extracellular enzymes[27], and might thus facilitate the depolymerization of proteins and other N-containing polymers into smaller units that can then be taken up by microorganisms and plants. Although plant N demand is also expected to increase with warming, several field studies show higher net N mineralization at higher temperatures and thus point to an overall increase in topsoil N availability[28,29]. In our study, an increase in protein availability overall stimulated SOM decomposition in the organic topsoil (Fig. 3), but we also observed a significant reduction at one of the four sites, suggesting that the direction of the response can differ between individual sites (Supplementary Table S5). A similar site-dependency in the response of SOM decomposition has also been observed for inorganic N additions[12].

We hypothesized that in deeper, mineral soil horizons, the microbial decomposer community would be increasingly limited by low C availability, and our findings support this hypothesis. Below a threshold of about 10% SOC and a C/N ratio of 20, not only protein, but also cellulose addition led to an overall stimulation of SOM decomposition (Supplementary Fig. S2). For mineral subsoils from active layer and permafrost, we further found significant correlations between priming effects by cellulose and protein (Table 3), as well as an efficient incorporation of cellulose- and protein-derived C into the microbial biomass (Fig. 5) that points at high microbial C use efficiency. These findings suggest that not only the effect of cellulose, but also of protein on SOM decomposition was at least partly induced by increased C availability. However, the effects of protein on SOM decomposition in general exceeded those of cellulose. This might have been due to a higher bio-availability of protein- than of cellulose-derived C, or due to the additional N contained in proteins that might have facilitated the synthesis of extracellular enzymes that break down SOM. Considering the low C/N ratio of proteins, such an additional N fertilization effect seems likely also in mineral subsoil horizons of active layer and permafrost.

For mineral topsoils and cryoturbated material, we did not find patterns that would suggest a distinct susceptibility of SOM decomposition to changes in either organic C or N availability, given the independent stimulating effects of both cellulose and protein (Fig. 3, Table 3). Mineral topsoil and cryoturbated material are at an intermediate state of decomposition compared to organic topsoil and mineral subsoil material (Table 2; see also ref. 30), and microbial C versus N limitation might differ strongly between individual soil samples. This variability was not connected to differences in bulk SOM stoichiometry (Supplementary Table S4). For cryoturbated material in particular, previous studies have suggested low bio-availability of the N present[22], and have shown a strong stimulation of SOM decomposition after addition of organic N, but not organic C alone[15]. Also in this study, effects of protein were by far stronger than those of cellulose. However, our findings suggest that not only low N availability, but also low C availability can constrain the decomposition of cryoturbated material.

With rising temperatures in the Arctic, increases in belowground plant biomass[11], in root production[31], and in plant belowground C transfer[32] have been observed. Our findings indicate that an increased transfer of organic compounds from plants to the soil (e.g., via root litter) can stimulate the decomposition of native SOM especially in deeper soil horizons, i.e., below the organic topsoil. Although the decomposition of SOM in these horizons is constrained by its low quality and by low temperatures, deeper soil horizons showed not only the highest relative increases in SOC mineralization after addition of cellulose or protein (Fig. 3), but also absolute increases in SOC mineralization within the range observed for organic topsoils (Fig. 2). This pattern prevailed even when we considered the decrease in soil temperature with depth under field conditions (Table 4). With the majority of arctic SOC in horizons below 30 cm (ref. 18), an enhanced mineralization of this SOC might strongly affect the C balance of arctic ecosystems, and amplify the positive feedback between permafrost CO_2 emissions and global warming (e.g., ref. 33). Mineral and cryoturbated horizons of the active layer are estimated to store 360 Pg of organic C (ref. 34), and the projected thawing of the upper permafrost table (active layer deepening) is expected to increase the stocks available for SOM decomposition by another 109 Pg until 2100 (mineral permafrost soil expected to thaw under moderate radiative forcing[34]). Although results from laboratory incubation experiments cannot be directly translated to the field, it is still interesting to note that additional losses of native SOC induced by cellulose or protein input in our experiment would correspond to additional SOC losses in the order of 1.2 Pg (cellulose) or 3.8 Pg (protein) across a four-month growing-season, demonstrating the potential for changes in C cycling by priming in permafrost soils. By comparison, CO_2 production from fossil fuels accounts for approximately 7.8 Pg C per year[35].

We emphasize that our extrapolated values likely represent the upper limit for potential additional SOC losses, given the favourable conditions for decomposition and the ample supply of plant-derived compounds

in our experiment. The extent to which these potential losses will be realized will depend on abiotic constraints on decomposition (e.g., anoxic conditions, protection by soil aggregates) as well as on quantity and quality of additional plant-derived organic compounds. For instance, deep active layer and current permafrost were most susceptible to an increased availability of plant-derived compounds in our experiment, but are hardly affected by plant roots under field conditions, given that more than 90% of plant roots are currently located in the top 30 cm of the soil[36]. However, as permafrost soils get warmer and the active layer deepens, plants might increasingly access deeper soil horizons to take up nutrients. Our findings suggest that the extent of such changes in plant rooting depth will strongly determine the magnitude of additional SOC losses induced by priming.

While our findings show that a higher availability of plant-derived organic compounds can considerably stimulate SOC mineralization in deeper soil horizons, the consequences for the ecosystem C budget will depend on the balance between additional plant primary production and additional C mineralization, including the direct stimulation of SOM decomposition by soil warming, the stimulation induced by increased plant-soil C allocation, and the decomposition of the additionally produced plant litter itself. Recent studies on an ecosystem scale suggest that both net C sequestration and net C losses might be observed: (1) Net ecosystem C sequestration will then occur where the enhanced CO_2 fixation by plants exceeds the additional losses by SOC mineralization. Such a case has been observed in a tussock tundra, where ten years of warming promoted belowground plant biomass and microbial activity in the mineral soil horizon, and overall increased organic C storage in this horizon, and in the entire ecosystem[11]. (2) Net C losses from the ecosystem will occur where the additional C mineralization exceeds the additional CO_2 fixation. Such a case has been suggested for the tree line in the European sub-Arctic, where ecosystem C stocks were lower in a forest than in an adjacent tundra, and where this difference has been specifically attributed to a stimulation of SOC mineralization in the forest due to higher plant-soil C allocation[10]. Also a range of other studies have observed a decrease in arctic ecosystem C storage due to warming[37,38], but contributions of direct stimulating effects on SOM decomposition, effects mediated by increased N availability and effects mediated by increased plant-soil C allocation have not been distinguished. Whether the stimulation of SOM decomposition will overall only reduce the ecosystem C sink strength, or even induce net ecosystem C losses, might thus depend on functional properties of the initially dominant plants and on changes in plant species composition with warming, for instance related to quantity and quality of root litter and root exudates, to rooting depths, or to mycorrhizal associations[10,39].

Material and Methods

Soil Sampling. Soils for the incubation experiment were sampled at four sites across the Siberian Arctic, in the areas of Cherskiy (Eastern Siberian Arctic), Ari-Mas, Logata (both Central Siberian Arctic), and Tazovskiy (Western Siberian Arctic; Fig. 1). All sampling sites were underlain by continuous permafrost, and samples were taken in the late growing-season at the maximum thaw depth. Sites are described briefly in Table 1, and in detail in ref. 30.

At each study site, we identified two zonal upland tundra vegetation types that were representative for the respective landscape. For each vegetation type, we excavated three soil profiles of 5 m length down to the permafrost table, and sampled soil horizons from these profiles. We additionally sampled the upper part of the permafrost using a steel corer, to a maximum depth of 30 cm from the permafrost table. Soil samples were grouped into five categories: Organic topsoils were O horizons (1), mineral topsoils comprised OA, A and AB horizons (2), and mineral subsoils BC and C horizons from the active layer (3). We further identified pockets of cryoturbated material (Ojj and Ajj) in the active layer that were characterized by a higher SOM content than the surrounding mineral soil; these samples formed a separate category (4). Finally, samples of mineral subsoil from the upper permafrost were classified separately (5). Unless specified otherwise, we refer to mineral subsoils from active layer as "mineral subsoils" and to mineral subsoils from the permafrost as "permafrost". Directly after sampling, living roots were removed, samples were air-dried, and stored in a dark, cool, and dry place. Air-drying of the soil samples was a prerequisite for sample transport from the remote field sites to the lab, but might have introduced a certain bias by reducing the active microbial community. In order to re-activate the microbial community, we therefore allowed for a pre-incubation period. Two weeks before the start of the experiment, we weighed triplicates of 2.5 g (O and OA horizons), 5 g (Ojj and Ajj horizons) or 10 g soil (A, AB, BC, and C horizons of the active layer, as well as permafrost samples) into glass bottles (headspace 100–130 ml), adjusted water contents to 60% water holding capacity, and loosely plugged the bottles with polyethylene wool. Samples were pre-incubated in the dark at 15 °C. Respiration rates measured after two weeks were similar to rates in fresh samples of organic topsoil, mineral subsoil and cryoturbated material from the Siberian Arctic measured in a previous experiment[15], and we therefore consider the two week pre-incubation sufficient for the re-establishment of an active native microbial community. This is supported by previous studies showing minor long-term effects of a single drying-rewetting event on microbial community composition and decomposition processes[40–43].

Carbon and N content were determined in dried and ground samples with EA-IRMS (Elementar vario MICRO cube EA, Elementar Analysensysteme GmbH, Germany, and Elementar IsoPrime 100 IRMS, IsoPrime Ltd., UK). Sample numbers, sampling depths, average contents of organic C and total N, C/N ratios, and C isotope values are given in Table 2 and Supplementary Table S1.

Setup of the Incubation Experiment. ^{13}C-labelled cellulose (Isolife *Cichorium intybus* cellulose, U^{13}C, >97 at%) and ^{13}C-labelled protein (Sigma-Aldrich algal crude protein extract, U^{13}C, 98 at%) were diluted with unlabelled cellulose or protein, respectively, to reach 5 at% ^{13}C. Triplicates of the pre-incubated samples were assigned to the three treatments (control, cellulose, protein), and samples assigned for cellulose or protein treatments were amended with 5 at% ^{13}C cellulose or ^{13}C protein in amounts equivalent to 4% SOC by mixing the dry powder into the soil. Neither cellulose nor protein significantly affected the pH of the soil.

In contrast to monomeric compounds such as glucose and amino acids that can be immediately taken up by soil microorganisms, cellulose and protein have to be broken down by extracellular enzymes before uptake. The addition of cellulose or protein, even of rather high amounts as in this study, therefore leads to only a slight, but persistent increase in organic C and N availability for soil microorganisms, as indicated by the ongoing respiration of cellulose- and protein-derived C even after 25 weeks of incubation (Supplementary Fig. S1), and thus more closely mimics the long-term mobilization of organic C and N during root litter decomposition.

After substrate addition, samples were again plugged with polyethylene wool and incubated at 15 °C for 25 weeks. During the course of the incubation, samples were weighed weekly and water lost by evaporation was replaced with ultrapure water.

Respiration Measurements. Respiration rates were measured immediately after substrate addition, and after 7, 14, 21, 28, 42, 56, 84, 112 and 175 days. For gas sampling, bottles were closed with rubber plugs and flushed with CO_2-free air. Gas samples were taken after 24–48 h incubation at 15 °C with gas tight syringes and analysed with gas chromatography (Cherskiy and Tazovsky: Agilent 7820A GC, Agilent Technologies; Ari-Mas and Logata: Shimadzu GC 2014, Shimadzu). CO_2 concentrations were corrected for the amount of CO_2 dissociated in the soil solution[44]. To determine the C isotope composition of respired CO_2, additional gas samples were taken on days 9, 44, 86, and 177 as described above and analysed with a GasBench II system coupled to a Delta V Advantage IRMS (Thermo Scientific). Based on the C isotope composition of respired CO_2, we distinguished between CO_2 derived from the added substrate and from SOC using the equations

$$C_{SOC} = C_{Total} \times (at\%_{Total} - at\%_{Substrate})/(at\%_{SOC} - at\%_{Substrate}) \text{ and} \quad (1)$$

$$C_{Substrate} = C_{Total} - C_{SOC}. \quad (2)$$

C_{Total}, C_{SOC} and $C_{Substrate}$ are total, SOC-derived and substrate-derived CO_2, and $at\%_{Total}$, $at\%_{SOC}$ and $at\%_{Substrate}$ are isotopic compositions (in at% ^{13}C) of total, SOC-derived and substrate-derived CO_2, respectively. The isotopic composition of SOC-derived CO_2 was set to the respective value for unamended control samples. Based on the contributions of substrate-derived and SOC-derived CO_2 to total respiration at these timepoints, we interpolated these values for the other timepoints where $^{13}CO_2$ data were not available, and calculated the cumulative amounts of CO_2 released from the added substrate and from SOC over the course of the incubation. Throughout the text, we use the term "SOC mineralization" exclusively for CO_2 production from native, unlabelled SOC, excluding C mineralized from added, ^{13}C-labelled cellulose or protein.

Since mineral, cryoturbated and permafrost horizons are not likely to experience temperatures of 15 °C under field conditions, we additionally estimated SOC mineralization for soil temperatures typical for the growing-season. Based on field measurements during sampling, we estimated growing-season soil temperatures as 8 °C (organic topsoil), 7.5 °C (mineral topsoil), 4.5 °C (mineral subsoil), and 4.1 °C (cryoturbated material). Permafrost material was frozen at the time of sampling, but since our experiment simulated the exposure of recently thawed permafrost to increased cellulose or protein input, we assumed a temperature of 1.0 °C. We finally estimated SOC mineralization rates and additional SOC losses induced by cellulose and protein for typical growing-season temperatures using Q_{10} values determined for the same set of soil samples in a second incubation experiment (Gentsch *et al.*, unpublished).

Microbial Biomass and Substrate Use Efficiency. Content and isotopic composition of microbial C were estimated with chloroform-fumigation-extraction[15,45]. Soil samples fumigated with chloroform, as well as non-fumigated samples, were extracted with 0.5 M K_2SO_4 and dissolved organic C was measured using an HPLC-IRMS system in direct injection mode against sucrose standards[46]. Microbial C content was calculated as the difference in dissolved organic C between fumigated and non-fumigated samples, and isotopic composition of microbial C was calculated using the equation

$$at\%_{Mic} = (C_{Fum} \times at\%_{Fum} - C_{Non-Fum} \times at\%_{Non-Fum})/C_{Mic}, \quad (3)$$

with C_{Fum} and $at\%_{Fum}$ representing content and isotopic composition of dissolved organic C in fumigated samples, and $C_{Non\text{-}Fum}$ and $at\%_{Non\text{-}Fum}$ in non-fumigated samples. C_{Mic} and $at\%_{Mic}$ represent content and isotopic composition of microbial C. Substrate- and SOC-derived microbial C were distinguished using equations (1) and (2), replacing respired C by microbial C. The microbial efficiency to incorporate substrate-derived C was calculated by comparing substrate-derived C in the microbial biomass ($MC_{Substrate}$) and in cumulative CO_2 respired during the course of the incubation ($RC_{Substrate}$) as

$$\text{Substrate use efficiency} = MC_{Substrate}/(MC_{Substrate} + RC_{Substrate}). \quad (4)$$

Statistical Analyses. Statistical analyses were performed in R 2.15.0 (ref. 47), with the additional package GenABEL[48]. We applied Mann-Whitney-U tests to test for differences between horizons and Welch's paired t-tests to test for differences between control and cellulose, control and protein, as well as cellulose and protein treatments, respectively. For paired t-tests, data were log- or rank-transformed if necessary to achieve normal distribution. We further performed Spearman's rank sum correlations that test for a monotonous relationship between two parameters, and describe the closeness of this relationship using Spearman's correlation coefficient rho. Differences and correlations were considered significant at $p < 0.05$.

Changes in SOC mineralization and microbial biomass are presented as response ratios (RR), calculated as ratios between samples amended with cellulose or protein and the respective control samples.

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Author Contributions

B.W., N.G., P.Č., K.D. and M.W. conducted the experiment and analysed samples; B.W. and A.R. led data interpretation and manuscript preparation. B.W., N.G., P.Č., R.J. E.A., J.B., A.G., G.H., A.K., P.K., N.L., R.M., J.P., J.S., O.S., M.T., T.U., H.Š., G.G. and A.R. contributed to soil sampling, and together with K.D., C.S. and V.L.T. to experimental design and writing.

Additional Information

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Paper IV

Variation in N_2 fixation in subarctic tundra in relation to landscape position and nitrogen pools and fluxes

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Variation in N_2 fixation in subarctic tundra in relation to landscape position and nitrogen pools and fluxes

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A B S T R A C T

Biological N_2 fixation in high-latitude ecosystems usually exhibits low rates but can significantly contribute to the local N budget. We studied N_2 fixation in three habitats of East European subarctic tundra differing in soil N stocks and fluxes: N-limited vegetated peat plateau (PP), frost formations of bare peat called "peat circles" (PC) with high availability of soil N, and vegetated upland tundra (UT) with low to intermediate N-availability. Nitrogen fixation was measured at field conditions twice during summer 2011 by acetylene reduction assay, and N_2 fixation rates were verified by $^{15}N_2$ fixation assay. Response to variation in nutrients, carbon, and temperature was studied in complementary laboratory experiments. Further, we aimed to link N_2 fixation rates to N deposition and major N transformation rates (gross and net mineralization, plant N uptake) including high N_2O emissions recently found from PC. We hypothesized that N_2O emissions in PC were fueled partly by biologically fixed N. Contrary to that hypothesis, N_2 fixation was found solely in PP (0.01–0.76 mg N m^{-2} d^{-1}), where N_2 was fixed by moss-associated cyanobacteria and heterotrophic soil bacteria. The low N and high P availability corresponded with the occurrence of N_2 fixation in these soils. Nitrogen fixation represented only a small portion of plant N uptake in PP. Conversely, bare PC (as well as vegetated UT) lacked N_2 fixation and thus N_2O efflux is most likely fueled by release of mineral N to the soil through internal nutrient cycling.

Introduction

The productivity of high-latitude ecosystems is constrained by low availability of nutrients and by unfavorable climatic conditions for plant growth and microbial activity (Shaver and Chapin, 1986; Chapin et al., 1995; Mikan et al., 2002; Sistla et al., 2012). Nitrogen is frequently the nutrient limiting primary production in tundra. Thus, N input through biological N_2 fixation (i.e., the activity of nitrogenase enzyme) might be critical to the N budget of Arctic ecosystems. Low nitrogenase activities have been reported from Arctic regions compared to other ecosystems due to the combined effect of low temperature, limited energy sources, and insufficient availability of other nutrients (Chapin and Bledsoe, 1992). Reported N_2 fixation rates from subarctic and Arctic tundra usually range from 0.15 to 6.45 mg N m^{-2} d^{-1} (Chapin and Bledsoe, 1992; Hobara et al., 2006; Sorensen and Michelsen, 2011; Stewart et al., 2011a; Stewart et al., 2011b), but can be as high as 1206 mg N m^{-2} d^{-1}

in biological soil crusts (Liengen, 1999a). Despite these generally low rates, N_2 fixation can account for as much as 50%–90% of total annual N input to tundra ecosystem (Hobara et al., 2006; Solheim et al., 2006). However, information on N_2 fixation as a portion of annual N turnover or local N stock is scarce in literature and little is known on the spatial variability and factors controlling N_2 fixation rates in heterogeneous tundra landscapes.

Nitrogen fixing microorganisms (diazotrophs) in tundra habitats occur in various forms; biological soil crusts on bare surfaces, cyanobacteria as phycobionts in lichens, free-living or moss-associated cyanobacteria, and free-living or rhizosphere-associated heterotrophic bacteria. Leguminous symbiosis can also play a role in Arctic region (Bordeleau and Prévost, 1994). The energy needed for N_2 fixation by heterotrophic diazotrophs is acquired from readily available carbon (C) provided via soil organic matter mineralization or by host plants. On the other hand, light has a direct positive effect on autotrophic diazotrophs. Photosynthesis constitutes the more abundant source of energy in tundra compared to easily decomposable organic compounds and therefore the majority of studies on N_2 fixation in high-latitude ecosystems are focused on autotrophic N_2 fixation by cyanobacteria (Liengen and Olsen, 1997; Liengen, 1999b; Solheim et al., 2002; Sorensen et al., 2006; Stewart et al., 2011a), while the contribution of heterotrophic bacteria has been considered insignificant. However, recent studies presented evidence that also diazotrophic soil bacteria can substantially contribute to the local soil N pool by fixing atmospheric N_2 (Nosko et al., 1994; Hara et al., 2009; Zadorina et al., 2009). As such, there is a paucity of studies on N_2 fixation in high-latitude ecosystems and our understanding of the role of diazotrophs for N budgets in tundra is poor.

The major components of East European subarctic tundra are represented by upland tundra (UT) and raised permafrost peat plateaus (PP) covering the most dominant parts of this area besides fens, water bodies, and forests (Virtanen and Ek, 2014). Patterned ground features called *peat circles* (PC), which are bare, round in shape, and characterized by relatively high water content and bulk density, occur on the peat plateaus (Repo et al., 2009, Biasi et al., 2014). The origin of the PCs is still unclear, but it is suggested that permafrost action and associated uplifting of deeper soil layers to the surface are responsible for their development (Repo et al., 2009). They are characterized by different availabilities of C and nutrients and dissimilar C and N transformation pathways in soil compared to adjacent vegetated areas underlain by peat and mineral soils (Repo et al., 2009; Marushchak et al., 2011, 2013; Biasi et al., 2014). Large nitrous oxide (N_2O) emissions were recently observed in PC (Repo et al., 2009). The discovery of these high N_2O fluxes in tundra was surprising since rates of denitrification, the main source of N_2O, are generally low in Arctic soils due to low nutrient status and high competition for N between plants and microbes (Ludwig et al., 2006; Siciliano et al., 2009). In PC lacking plant cover, however, a sufficient amount of mineral N is readily available for N_2O production (Repo et al., 2009; Marushchak et al., 2011). The origin of such abundant nitrate/nitrite sources in PC, the substrate for denitrification, has remained unrevealed. Even though N_2 fixation is often inhibited by high N concentration (Chapin and Bledsoe, 1992), nitrogenase activity has never been measured in cold Arctic soils wthat exhibit N_2O emissions as high as tropical soils (Repo et al., 2009). It is likely that not only internal cycling supplies N for this process. Cleveland et al. (1999) and Stewart et al. (2013) suggested that high denitrification rates can be linked to active N_2 fixation. Fixed N in the form of ammonia can enter a sequence of further biochemical transformations such as nitrification and denitrification and so can be eventually turned into N_2 and N_2O. Peat circles with dark bare surfaces could provide favorable thermal environments for diazotrophs. Higher temperatures and high light levels are likely to support biological crust formation (Liengen and Olsen, 1997; Zielke et al., 2002; Stewart et al., 2011b). Indeed, thin moss layers were found on PC with varying coverage. Moss-crust surfaces are often inhabited with cyanobacteria responsible for N_2 fixation (Evans and Johansen, 1999). In addition, PC are rich in available C (Biasi et al., 2014), and therefore we expect that also heterotrophic N_2 fixation can be important to the N budget.

The major aim of this study was to investigate the magnitude and spatial patterns of N_2 fixation rates in variable tundra habitats differing in environmental conditions, C and N stocks, as well as transformation rates. Specifically, we wanted to assess the impact of environmental variables (C and

nutrient concentration in soil, temperature) on nitrogenase activity and to determine the role of autotrophs and heterotrophs in N_2 fixation. Our particular objective was to relate N_2 fixation rates to major N transformation processes—for example, gross and net N mineralization, plant N uptake, and N_2O emissions (the latter only relevant for PC where N_2O emissions occur)—and compare to atmospheric N deposition. These data were concurrently measured or available from the site. Such a survey is rare in the literature on N cycling in Arctic ecosystems. We hypothesized that N_2-fixing biological crust boosts high denitrification rates in PC by supplying additional N, resulting in high soil-N concentration and high N_2O emissions. Thereby, we aimed to test the traditional view that N_2 fixation is limited in N-rich soils. Nitrogen fixation rates were studied in three habitats of East European tundra: PP, PC, and UT. We conducted field measurements to characterize nitrogenase activity under natural light conditions and under dark, which included dark N_2 fixation by cyanobacteria and heterotrophic diazotrophs. In laboratory experiments, we examined factors controlling N_2 fixation by heterotrophic soil bacteria to better understand the factors that control the process. The acetylene reduction assay (ARA) used to detect N_2 fixation rates was calibrated with a $^{15}N_2$ assay. Seasonal N input via N_2 fixation was estimated for each habitat using observed temperature variation.

Materials and Methods

Study Site

The study site is located 70 km southwest of the city of Vorkuta, Komi Republic, Russia (67°03′N, 62°57′E, 100 m a.s.l.) and falls within the discontinuous permafrost zone of subarctic tundra. Prevailing habitats are UT on mineral soil with thin organic layer (<10 cm) and raised PP with deep organic deposits spreading over 50% and 16% of the study site area, respectively (Hugelius et al., 2011). Upland tundra has typical tundra heath vegetation (*Betula nana*, *Salix* sp., *V. uliginosum*, and *Vaccinium vitis-idaea*), while PP is covered by tundra bog vegetation (*Ledum decumbens*, *Rubus chamaemorus*, *Vaccinium uliginosum*, and *Sphagnum* mosses). Distinct dark spots of bare peat soil, referred to as PC (round in shape, about 20 m diameter on average), cover about 4% of the PP (Repo et al., 2009). Detailed description of the study site, including climate, topography, vegetation composition, and general soil properties are given in previous studies conducted at this site (Repo et al., 2009; Marushchak et al., 2011). All examined soils were highly acidic with pH values in a range from 3.1 to 3.5. No legumes were present in the vegetation cover of both PP and MT, possibly due to the low pH (Bordeleau and Prévost, 1994). Ambient air and soil temperatures were monitored continuously over the vegetation season of 2011, when this study was conducted, using S-THA-M006 and S-TMB-M006 sensors (Onset Computer Corporation, Bourne, Massachusetts, U.S.A.), respectively, in conjunction with a HOBO Micro Station Data Logger H21-002 (Onset Computer Corporation, Bourne, Massachusetts, U.S.A.).

Experimental Design

Nitrogen fixation assays were divided into measurements with intact soil cores performed immediately after sampling, under field conditions (later referred to as *field measurements*), and experiments with homogenized soil conducted in the laboratory under controlled environmental conditions (*laboratory experiments*).

Total N_2 fixation under field conditions was measured twice during the 2011 summer season; the first sampling period took place on 20 July when the day length was 21 h 35 min and included measurements in all three habitats (PP, PC, UT). The second sampling period was carried out on 18 August 2011 with day length 16 h 55 min. The number of replicates were doubled for the PP site because of high variability among samples in July. Due to negligible nitrogenase activity in the first measurement, UT was not included again. In order to better understand the spatial variation in N_2 fixation, PC with differing amounts of small moss-species cover were chosen for the second measurement. Compared to PC selected in the first measurement, they represented early succession on the bare surface with more pronounced biological soil crust, which was potentially favorable for associated diazotrophs. Intact soil cores were collected immediately before start of the field measurements. Each of the examined habitats was represented by three field replicates (unless noted otherwise). The

sampling sites were chosen as typical of the respective habitat. Four intact soil samples of the 5-cm-thick top layer were collected using a polypropylene soil core of 5 cm diameter from each sampling site. Vascular plants were cut from the surface of soil cores, but the moss layer remained intact.

Laboratory experiments were designed to examine heterotrophic N_2 fixation and the potential controlling factors; response to carbon availability, effect of N and phosphorus (P) addition and temperature dependence were assayed. Thereafter, a stable isotope technique using $^{15}N_2$ was used to convert nitrogenase activity measured as acetylene reduction into amount of fixed N. Soil samples for laboratory experiments were collected in the same manner as for field measurements on 23 August 2011. Soil cores were kept in dark at 4 °C until processing (for a few weeks). Storage conditions were unfavorable for autotrophic organisms, and so we assumed that contribution of cyanobacteria to nitrogenase activity was negligible here. Prior to laboratory experiments, soil samples for each habitat were mixed and homogenized.

Nitrogen Fixation

Field Measurements

Nitrogen fixation rates in field measurements were assayed using acetylene reduction assay (ARA) modified from Černá et al. (2009). Intact cores were inserted into glass jars with final headspace of 350 mL. Jars were closed by screw-lids equipped with three-way stopcock for gas sampling, and soil cores were incubated in ambient air with 10% (v/v) acetylene (C_2H_2). At the end of a 48-hour incubation, 10 mL of headspace was withdrawn by airtight syringe from incubation flasks and the ethylene concentration was measured (see Analytical Methods). Control samples without acetylene and several blanks were also incubated and analyzed for endogenous ethylene production and ethylene traces in the acetylene gas used. The difference between the sample and blank ethylene concentrations in headspace was used to calculate the field acetylene reduction rate.

Samples from each sampling site were incubated under the natural light conditions as well as in the dark. Two mL of 0.05 M sucrose solution were added only into dark samples in order to detect potential light-independent N_2 fixation by cyanobacteria and heterotrophic soil bacteria. The temperature inside three dark and three light jars was monitored by inserting small temperature data loggers into the jars (i-button DS1921G, Maxim, Sunnyvale, California, U.S.A.). Mosses and liverworts covering the soil cores were identified, and cyanobacteria and their abundance in the uppermost layer of soil cores were determined after incubation. The soil cores were dried and N_2 fixation was expressed on dry weight basis (gdw^{-1}). Dry weight ranged between 0.02 and 0.50 gdw gfw^{-1}.

Laboratory Experiments

For each of the laboratory experiments, 40 mL glass vials were filled with 2.5 g of fresh soil. Samples were preincubated for 24 hours at relevant incubation temperature. All laboratory experiments were carried out in the dark and each treatment was performed in triplicate ($n = 3$). The ARA followed the same protocol described above except that gas samples were taken three times over an incubation period of six days (on the 2nd, 3rd, and 6th day).

I. *Kinetic of nitrogenase activity (C substrate addition).* Soils were amended with different amounts of a labile C source (sucrose) simulating plant root exudates, in order to determine the dependence of nitrogenase activity on additional energy source (Černá et al., 2009). Sucrose was added in 1.0 mL of solution; final carbon addition accounted for 0, 0.5, 1, 2, 5, 10, 20, and 30 mg C gdw^{-1}. Samples were incubated at 12 °C.
II. *Carbon and nutrient manipulation.* The responses of nitrogenase activity to increased concentrations of available P and N were examined at 20 °C, at optimal temperature, based on results of a temperature dependence experiment (see below). Soil samples were amended with 1.0 mL of a solution that contained optimal C concentrations (30 mg C gdw^{-1}) in one of three different nutrient treatments; carbon-only addition (C), carbon-phosphorus (C+P), and carbon-nitrogen (C+N) addition. Control samples with the same amount of distilled water were included. Phosphorus was added in the form of K_2HPO_4 solution and N as NH_4NO_3 solution, with the final concentration of added nutrient

in soil 40 μg PO_4-P gdw^{-1}, 30 μg NH_4-N gdw^{-1} and 30 μg NO_3-N gdw^{-1}, respectively, which corresponded to the highest measured nutrient levels in sampling area.

III. *Temperature dependence.* Nitrogenase activity was measured at four temperatures within a range of natural local conditions during the vegetation period (4, 12, 20, and 25 °C). Optimal C concentration, evaluated in the first experiment (30 mg C gdw^{-1}), was applied to all samples.

Acetylene reduction rates were calculated over the incubation period as a slope of the linear regression. The starting point was always represented by blanks. All data based on ARA were recalculated to N_2 fixation rates based on the results from the laboratory experiment with $^{15}N_2$ fixation (see below).

Additional Experiments

Occurrence of cyanobacteria was assessed in the soil cores used in field ARA measurements to verify contribution of autotrophic diazotrophs to N_2 fixation. Cyanobacteria were isolated from the uppermost 2 cm layer and cultivated in liquid BG-11 medium for three weeks at 20 °C, day-night mode 10/14 h with light intensity PAR = 100 μmol m^{-2} s^{-1}. Species of cyanobacteria present were then determined under a microscope Nikon Eclipse 80i at magnifications ×300, 675, or 1000. Sample scanning was repeated five times.

Net N mineralization was assayed under field conditions to quantify potentially plant available inorganic N in the soil. The buried bag technique was used (Knoepp and Swank, 1995; Holub and Záhora, 2008) and field triplicates were established for each habitat in July 2011. The uppermost soil layer of 5 cm excluding vegetation was sampled from PP and PC while organic horizon was taken from UT using a soil corer of 5 cm diameter; always in field triplicates. Soil cores were closed in polypropylene bags and reburied in the soil for 25 days to incubate at ambient temperatures. Ammonium (NH_4^+) and nitrates (NO_3^-) were extracted immediately at the beginning of the experiment and right after the in situ 25-days incubation, and the soil extracts were kept frozen until analysis. Extractions and analyses followed the protocol described below. Net N mineralization rates were calculated from the difference in N-NH_4^+ plus N-NO_3^- concentrations during the incubation period. Gross N mineralization was determined monthly during a period June–August 2008 (n = 3) using pool dilution method (for more details see Marushchak et al., 2011).

The $^{15}N_2$ fixation assay was conducted in the laboratory to calibrate fixation rates acquired by the ARA method. Two nutrient manipulations (C and C+P similar to experiment III) were included to test the sensitivity of both methods to changes in nitrogenase activity. Soil samples for the $^{15}N_2$ fixation assay were incubated in a similar way to the ARA laboratory experiments. However, the incubation vials were filled with an artificial aerobic atmosphere consisting of 80% $^{15}N_2$ (98 at%, Cambridge Isotope Laboratories, Andover, Massachusetts, U.S.A.) and 20% pure O_2 (Messer Technogas, Prague, Czech Republic) and incubated for six days at 20 °C to achieve sufficient amounts of ^{15}N label in soil (Šantrůčková et al., 2010). Enrichment of soil labeled by $^{15}N_2$ was elevated by 20‰ to 70‰. Control samples with the same nutrient treatment assayed by ARA (experiment II) provided data on acetylene reduction rates and natural abundance of ^{15}N. At the end of the experiment, soils from ^{15}N-labeled samples and controls were frozen, freeze-dried, and ground by a ball mill (Mixer Mill MM 200, Retsch, Germany). The ratio of stable isotopes ^{15}N:^{14}N in soil samples related to a standard (atmospheric N_2), denoted as $\delta^{15}N$, was then analyzed (see below) and the final N_2 fixation rate was calculated using the equation

$$nmol\ N\ fixed\ gdw^{-1}\ hr^{-1} = (nmol\ Ntot\ gdw^{-1} \times tracer\ fraction)\ t^{-1} \quad (1)$$

where total N amount in ^{15}N-labeled soil sample (*nmol Ntot* gdw^{-1}) was calculated with respect to a mass ratio ^{15}N:^{14}N in the sample and t was incubation time (hours). *Tracer fraction* expressed the distribution of the ^{15}N label between the gas phase and soil in incubation vial of ^{15}N-labeled sample according to the equation

$$tracer\ fraction = (at\%\ ^{15}N soil\text{-}labeled - at\%\ ^{15}N soil\text{-}control)\ (at\%\ ^{15}N air\text{-}labeled - at\%\ ^{15}N air\text{-}control)^{-1} \quad (2)$$

where *at%* indicates molar ratio of ^{15}N to total N. Further, C_2H_2:N_2 ratio (i.e., conversion factor) was

expressed as nmol C_2H_2 reduced to nmol N_2 fixed in corresponding samples.

Analytical Methods

Ethylene concentrations from gas samples taken in ARA measurements were determined using an Agilent 6890N gas chromatograph (Agilent Technologies, U.S.A.) with a flame ionization detector and a 0.53 × 30 m GS-Alumina column at 45 °C. Total N and $\delta^{15}N$ of soil samples were analyzed using an elemental analyzer (Vario micro cube, Elementar Analysensystem GmbH, Germany) coupled to isotope ratio mass spectrometer (IR-MS DELTA plus XL, Finnigan, Germany). Nitrate and phosphate (PO_4^{3-}) concentrations were determined in distilled H_2O soil extracts by an ion chromatograph (DX 120, Dionex Corporation, U.S.A.). Ammonium ions were extracted by 1 M KCl from soil and extracts were analyzed using spectrophotometer (Ultrospec 300 Pro, Biochrom, U.K.) according to Maljanen et al. (2009). Dissolved organic carbon (DOC) and dissolved nitrogen (DN) in water extracts were measured on DOC/DN analyzer (LiquicTOC II, Elementar, Germany). Since the studied soils were highly acidic, available P for C:N:P ratio calculation was determined in oxalate extracts (P_{ox}, Kopáček et al., 2001).

Data Analyses

For recalculation of acetylene reduction rates to N_2 fixation rates, we used a conversion factor of 7.54 determined in our calibration experiment using $^{15}N_2$ (see above). The factor was not affected by nutrient manipulation ($t = 0.33$, $df = 6$, $P = 0.75$). The molar ratio of C_2H_2:N_2 reduced by nitrogenase enzyme in excess of acetylene is between 3 and 4 (Zechmeister-Boltenstern and Kinzel, 1990; Tate, 2000, respectively). However, a wide span of values ranging between 0.56 and 22 has been reported in studies from terrestrial ecosystems (summarized in Liengen, 1999a). Studies on N_2 fixation in northern ecosystems reported ratios 0.022–4.9 (Liengen, 1999a; DeLuca et al., 2002; Sorensen et al., 2006). The reason for the relatively high conversion factor in our soils can be explained by low nitrogenase activity, but remains inconclusive.

Temperature dependence of heterotrophic N_2 fixation measured under laboratory conditions (experiment III) was described using the Arrhenius equation:

$$k = A \times e^{\frac{-E_a}{R \times (T + 275.15)}} \quad (3)$$

where k indicates N_2 fixation rate (ng N gdw^{-1} h^{-1}), A and Ea ($J \cdot mol^{-1}$) were estimated equation parameters, R represents gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$), and T is temperature (°C). Parameters of the Arrhenius equation were acquired using GraphPad Prism 4 for Windows (GraphPad Software, 2003).

We attempted to assess the importance of N_2 fixation relative to other N transformations in the overall N budget at the sampling sites including also the amount of N bound annually in NPP (net primary production). Data on N_2 fixation and net N mineralization from the present study and data on gross N mineralization rates determined in summer 2008 under laboratory conditions at 15 °C (Marushchak et al., 2011) were used. Based on aboveground biomass data from Seida site presented by Hugelius et al. (2011), we approximated total plant biomass; moss biomass was subtracted from the total aboveground biomass to express the aboveground biomass of vascular plants. Belowground biomass of vascular plants was estimated using the root:shoot ratio specific to each land cover type (Hogan, Crittenden, and Virtanen; unpublished data). Total plant biomass was a sum of moss, vascular plant aboveground, and belowground biomass. Shaver and Chapin (1991) concluded that the productivity:biomass ratio and C:N in NPP were relatively stable in different tundra habitats. Accordingly, we estimated the annual N allocation in NPP using C:N ratios calculated from the data given by Shaver and Chapin (1991). Using these assumptions, the average daily N requirement of NPP over a 60-day growing season was calculated. The total wet atmospheric N deposition in our sampling region was estimated based on data provided by Walker (2003).

The difference in N_2 fixation between two sampling campaigns and the effect of light/dark treatment on nitrogenase activity under field conditions were tested by a factorial ANOVA. The effect of nutrient addition in laboratory experiments was examined using one-way ANOVA with Tukey honest significant difference (HSD) post-hoc test. Nitrogen fixation rates from field measurements

were log-transformed in order to meet the assumptions of the ANOVA. Linear regression was applied to test for an effect of moisture on the magnitude of N_2 fixation in field measurements and also to examine the dependence of nitrogenase activity on C availability under laboratory conditions.

Results

Moss Species and Abundance of Cyanobacteria

No cyanobacteria were detected on the mostly bare peat soil of PC as well as on the vegetated surface of mineral UT (Table 1). In contrast, cyanobacteria were found at PP surface. Heterocystous cyanobacteria of genus *Nostoc* or *Anabaena* were observed in all samples collected in July while they were absent in samples from August. Also, representatives of nonheterocystous genera (*Chroococcus*, *Gleothece,* and *Microcystis*) were found in PP, with more frequent occurrence in July than in August. The surface of PP was dominated by *Sphagnum* sp. with a low occurrence of other mosses and different field replicates had highly variable composition of species. Various bryophyte species, namely liverwort *Gymnocolea inflata* and moss *Dicranella cerviculata*, were found on PC in the early succession stage (Table 1).

Nitrogen Fixation—Field Measurements

No nitrogenase activity was found in the PC and UT soils lacking cyanobacteria, either in natural light conditions or after C addition in the dark (Fig. 1). Both soils displayed higher gross and net N mineralization rates than PP (Table 2). PC soil was rich in mineral N and poor in P while UT soil was poor in N and P (Table 3). In contrast, nitrogenase activity was detected in PP that exhibited low N mineralization rates and was poor in mineral N and rich in P (Tables 2 and 3). Nitrogenase activity significantly diverged between the two sampling campaigns ($F = 19.73$, $df = 1$, $P < 0.001$). The highest rates were detected on 20 July (124 and 44 ng N gdw^{-1} day^{-1} in light and dark treatment, respectively; Fig. 1), while much lower temperatures on 18 August were associated with lower N_2 fixation rates (5.0 and 2.0 ng N gdw^{-1} day^{-1} in light and dark, respectively; Fig. 1). The large spatial heterogeneity of PP vegetation cover represented by moss species composition and associated cyanobacterial assemblage (Table 1) was mirrored by the high variability of nitrogenase activity. No statistical difference in nitrogenase activity between the light and dark treatment was found either in July or in August ($F = 0.27$, $df = 1$, $P = 0.61$) because each replicate exhibited a different response to the light/dark treatment. The water content of the PP sampling subsites ranged from 0.56 to 0.98 g H_2O gfw^{-1} and had no effect on nitrogenase activity ($F = 0.53$, $df = 1$, $P = 0.48$).

Nitrogen Fixation—Laboratory Experiments

Nitrogen fixation in laboratory conditions was detected exclusively in the PP soil and only these data are referred to in this section.

I. *Kinetic of nitrogenase activity (C substrate addition).* Addition of C substrate positively affected diazotrophic activity in PP soil (Fig. 2). Nitrogenase activity increased linearly and doubled following C addition of 30 mg C gdw^{-1}. This addition was used in further experiments.
II. *Carbon and nutrient manipulation.* N_2 fixation in PP soil was significantly affected by nutrient manipulations ($F = 13.24$, $df = 3$, $P < 0.01$), which changed C:nutrient stoichiometry in the soil. Ratios of C:N and C:P substantially increased in all treatments (Table 4). Samples amended with C only (C-treatment) showed increased nitrogenase activity by 111% compared to control samples without C and nutrient addition. Nitrogen amendment as ammonium nitrate suppressed nitrogenase activity by 26% on average compared to the C-treatment, while P addition in form of monopotassium phosphate increased nitrogenase activity by 26% compared to the C-treatment.
III. *Temperature dependence.* Nitrogenase activity in laboratory conditions increased exponentially with increasing temperature and fit the Arrhenius model well ($R^2 = 0.904$, $df = 10$). Temperature dependence derived from the laboratory experiments was within the range of field data (Fig. 3). Therefore, the equation was used to estimate cumulative N_2 fixation in the field.

TABLE 1

Occurrence of mosses and cyanobacteria species in the uppermost layer of soil samples taken from subarctic tundra.

Sampling site	Sampling campaign	Replicate	Moss species	Cyanobacteria species
PP	20 July	1	*Sphagnum russowii*	*Nostoc* cf. *paludosum**
			Mylia anomala	*Chroococcus* sp.
				Microcystis sp.
		2	*Sphagnum fuscum*	*Nostoc paludosum**
			Mylia anomala	*Anabaena oscillarioides**
				Chroococcus minimus
				Gloeothece sp.
				Microcystis sp.
		3	*Dicranum leioneuron*	*Nostoc* sp.*
			Sphagnum sp. (Sect. Cuspidata)	
			Barbilophozia binsteadii	
			Cephalozia cf. *pleniceps*	
	18 August	1	*Sphagnum russowii*	—
			Mylia anomala	
		2	*Sphagnum fuscum*	—
			Mylia anomala	
		3	*Warnstorfia fluitans*	—
		4	*Pleurozium schreberi*	—
		5	*Dicranum leioneuron*	n.d.
			Barbilophozia binsteadii	
			Polytrichum strictum	
			Mylia anomala	
		6	*Cladonia* sp.	*Chroococcus minutus*
			Lophozia ventricosa s.l.	
PC	20 July	1	—	n.d.
		2	—	—
		3	—	—
	18 August	1	cf. *Dicranella cerviculata* (juv.)	—
		2	*Gymnocolea inflata*	—
		3	*Dicranella cerviculata*	—
UT	20 July	1	n.d.	—
		2	n.d.	—
		3	n.d.	—
	18 August		n.d.	n.d.

*Cyanobacterium species with heterocysts.
Abbreviations: PP—vegetated peat plateau; PC—peat circles, i.e. circular cryogenic formations of bare peat soil; UT—upland tundra; n.d.—not determined.

N Transformations and Estimated N Input

The input of N via heterotrophic N_2 fixation was estimated using the soil temperature records of the subsites and the temperature functions established during the laboratory experiments (Fig. 3). Because the N_2 fixation rates during laboratory measurements were lower than the field rates,

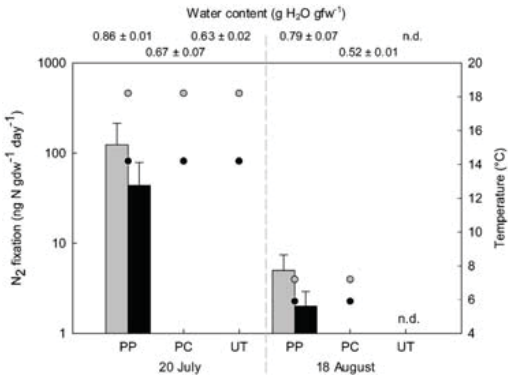

FIGURE 1. Nitrogen fixation rates of three main tundra habitats assayed in field measurements (20 July and 18 August 2011); mean incubation temperatures and water content of soil cores: N_2 fixation rates plotted as columns, temperature as points; gray—natural light conditions, black—dark treatment. N_2 fixation rates are means with standard errors (n = 3, except of PP-Aug where n = 6); water content given in numbers above the graph. For abbreviations, see legend of Table 1

our estimates are considered conservative. Estimated N input to PP via N_2 fixation over a 60-day growing season (July and August 2011) was 0.54 μg N gdw^{-1} (equivalent to 1.36 mg N m^{-2}). More N_2 was fixed during the warmer month of July (0.37 μg N gdw^{-1}) compared to the colder August (0.24 μg N gdw^{-1}).

Net N mineralization rates were generally the lowest in PP, intermediate in UT, and the highest in PC (Table 2). When daily rates were calculated for July, net N mineralization in PP was somewhat lower than N_2 fixation. Gross N mineralization was three orders of magnitude higher than net N mineralization in PP, while this difference was two orders of magnitude in UT and one order of magnitude in PC. Net N mineralization rates were roughly equivalent to the N in N_2O emissions in PC. Because the polyethylene bags are permeable for gases, denitrification should have occurred in the net mineralization experiment; thus, actual net mineralization rates should be at least two times larger in PC. The region received on average 0.69 mg N m^{-2} day^{-1} from atmospheric deposition (Walker, 2003), which was the only external input of N for UT and PC soils. The N_2 fixation rate in PP soil was in the same order of magnitude as N deposition (Table 2).

Mean plant N demand in UT (N in NPP, Table 2) was about three times lower than the mineral N delivered through gross N mineralization but much higher than net N mineralization. The N requirement for NPP in PP matched gross N mineralization and greatly exceeded net N mineralization. Similar as for UT, negligible N losses via N_2O emissions were observed in PP. PC soil with no vegetation cover exhibited high gross and net N mineralization, which occurred concurrently with high N_2O emissions (Table 2).

TABLE 2

A comparison of various N transformation processes in the peak season of a subarctic tundra landscape; in situ detected total N_2 fixation, an estimate of atmospheric N deposition, net and gross N mineralization in the uppermost 5 cm layer of soil, an estimate of N bound in NPP and N_2O emissions from surface.

N transformation processes					
mg N m^{-2} d^{-1}					
Sampling site	total N_2 fixation[a]	atmospheric N deposition[b]	net N mineralization[a]	gross N mineralization[c]	N in NPP[d]	N_2O emissions[c]
PP	0.30 ± 0.23	0.43–0.94	0.05 ± 0.07	50 ± 6	51	0.04 ± 0.03
PC	0.00 ± 0.00	0.43–0.94	9.25 ± 2.59	295 ± 73	0	5.16 ± 1.37
UT	0.00 ± 0.00	0.43–0.94	0.63 ± 0.33	160 ± 84	58	–0.01 ± 0.00

[a]Rates determined under natural field conditions in July 2011.
[b]Calculated from Walker (2003).
[c]Data from season 2008 published by Marushchak et al. (2011).
[d]Estimate based on biomass data from the study site provided by Hugelius et al. (2011).
Actual measured data ([a] and [c]) are given as means with standard errors (n = 3). For abbreviations, refer to the legend of Table 1.

TABLE 3

The concentration of nitrogen inorganic forms (DIN), phosphates, dissolved organic matter (DOC), and DOC:DIN ratio in tundra soils in July 2011 (concentration values are means with standard errors, n = 3). For abbreviations, see the legend of Table 1.

Sampling site	$N\text{-}NO_3^-$ (μg gdw^{-1})	$N\text{-}NH_4^+$ (μg gdw^{-1})	$P\text{-}PO_4^{3-}$ (μg gdw^{-1})	DOC (μg gdw^{-1})	DOC : DIN (mol mol^{-1})
PP	0.08 ± 0.13	0.88 ± 1.53	25.1 ± 22.0	613 ± 152	636
PC	15.9 ± 11.9	4.55 ± 3.29	0.33 ± 0.57	849 ± 234	42
UT	0.00 ± 0.00	1.38 ± 2.39	5.36 ± 1.30	630 ± 357	457

Discussion

We detected N_2 fixation only in the vegetated peat soil of PP. Corresponding with that finding, cyanobacteria were found solely in PP soil. Contrary to our hypothesis, the bare peat soil of PC did not show any detectable N_2 fixing activity. Nitrogen fixation by pioneer autotrophic organisms facilitates the early succession stage when plants are absent and nutrients are lacking (Hodkinson et al., 2003; Nemergut et al., 2007). Nevertheless, the bare soil of PC had higher mineral N concentration and much lower DOC:DIN ratio compared to the adjacent PP (Table 3). Therefore, N_2 fixation did not provide any advantage for colonizing species in PC. In addition to autotrophic N_2 fixation, heterotrophic diazotrophs can play an important role. However, nitrogenase activity in the PC soil was triggered neither by C addition under field conditions nor by C and P addition in laboratory experiments (results not shown), which indicates a lack of heterotrophic diazotrophs. The development of diazotrophs in the bare PC soil may have been restricted by combined effect of high mineral N and low P availability. According to our data, the traditional view that N_2 fixation is limited by high N availability holds true also for Arctic soils exhibiting high N_2O emissions. Therefore, the large N_2O emissions and high N availability in PC are not linked to high inputs of N via biological N_2 fixation.

Nitrogen fixation was also lacking in vegetated mineral soil of UT where no cyanobacteria were found. An active N_2-fixing moss-associated community has been reported from similar habitats of subarctic heath (Sorensen and Michelsen, 2011). It is possible that climatic conditions at our site were more favorable for soil microbial activity, resulting in a faster internal cycling of nutrients

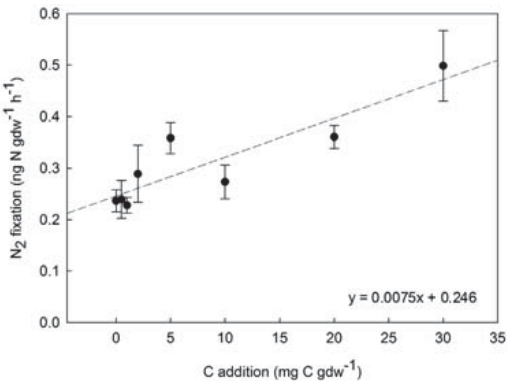

FIGURE 2. The response of heterotrophic nitrogenase activity in PP soil to C source addition (means with standard errors, n = 3). The correlation between N_2 fixation and C addition was significant (R^2 = 0.774, P = 0.002). The linear regression equation (dashed line) is shown in lower right-hand corner

TABLE 4

Nitrogen fixation rates in PP after different nutrient additions and the final C:N:P ratio expressed as DOC:DN:P_{ox}; N_2 fixation rates as means with standard errors (n = 3). Different letters denote statistically significant differences in nitrogenase activity among treatments (P < 0.05).

Nutrient treatment	DOC : DN : P_{ox}	N_2 fixation (ng N gdw^{-1} h^{-1})	
Control	13 : 0.2 : 1	1.03 ± 0.11	a
C	168 : 0.2 : 1	2.17 ± 0.19	cb
C+P	138 : 0.1 : 1	2.74 ± 0.11	b
C+N	168 : 0.5 : 1	1.60 ± 0.32	ca

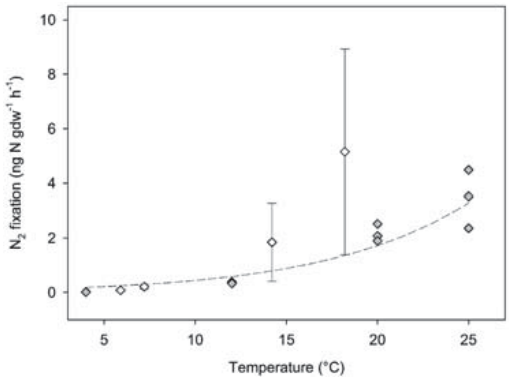

FIGURE 3. The temperature dependence of nitrogenase activity in PP soil acquired from data measured under laboratory conditions. N_2 fixation rates measured under field conditions are also depicted: gray symbols—individual laboratory measurements, solid line—Arrhenius T-dependence fit through laboratory data [$k = 9.42*10^{16}e^{-9.37*10\hat{}4/R*(T+273.15)}$, $R^2 = 0.880$, df = 10], white symbols—field data as mean for each incubation temperature with standard errors ($n = 3$ for July measurements at 14.2 and 18.2 °C, and $n = 6$ for August measurements at 5.9 and 7.2 °C).

and thus sufficient delivery of N to plants and microbes. Indeed, relatively high gross N mineralization rates suggested fast N turnover, and significant net N mineralization rates implied that microbial community had a surplus of available N (Table 2).

Only vegetated PP showed N_2 fixation among the studied habitats. There were favorable conditions for diazotrophic microorganisms due to low available N in soil and the highest DOC:DIN ratio and P availability among other habitats (Table 3). The regulating role of nutrients was confirmed in laboratory experiments (Table 4) and was significant within a short time period. These trends were consistent with other studies evaluating long-term fertilization experiments (Liengen, 1999b; Weiss et al., 2005; DeLuca et al., 2007; Šantrůčková et al., 2010). High soil concentration of ammonium as an end product of the enzymatic reaction process suppresses nitrogenase activity (Paul and Clark, 1996). Phosphorus is an essential element in ATP production for energy-demanding metabolism of diazotrophs, and P deficiency within soil can limit their activity (Chapin et al., 2002). Nitrogen fixation is related not only to the total amounts of individual nutrients, but mostly the ratios of nutrients regulate the process (Reed et al., 2007). High C:N and low N:P favored nitrogenase activity in PP soil in both field and laboratory experiments. With respect to the very low N:P ratio during the laboratory experiment (Table 4), it was remarkable that further addition of P still had positive effect.

Heterotrophic N_2-fixing bacteria require, besides available P, a labile source of C as a substrate for efficient respiratory metabolism—that is, simple sugars or organic acids (Burgmann et al., 2005). Heterotrophic N_2 fixation linearly increased with added C up to the highest addition rate of 30 mg C gdw^{-1} (Fig. 2). Neither substrate saturation nor inhibition was reached in the investigated range of C addition, unlike results reported by several other studies (Černá et al., 2009; Hara et al., 2010). Our results suggest that heterotrophic N_2 fixation in PP was at least partly limited by the source of available C.

We found that both autotrophic and heterotrophic diazotrophs were contributing to N_2 fixation in PP. The light/dark treatment indicated neither of the two groups dominated the N_2 fixation in the field sites (Fig. 1). It is presumed that cyanobacteria with heterocysts, the main autotrophic diazotrophs, fix N_2 during the day because of spatial separation of oxygen-sensitive nitrogenase and oxygenic photosynthesis (Fay, 1992; Liengen, 1999b), while nitrogenase activity at night is assumed in the case of nonheterocystous species (Fay, 1992; Bergman et al., 1997; Misra, 1999; Compaore and Stal, 2010). However, there is evidence that heterocystous cyanobacteria can also fix N_2 at night (Schell and Alexander, 1973; Liengen, 1999b). The heterocyst of the cyanobacteria has an incomplete photosynthetic pathway, so its respiratory metabolism is dependent on organic assimilates transported from neighboring green cells. Thus, heterocystous cyanobacteria can fix N_2 as long as available assimilated C is transported into the heterocysts (Fay, 1992; Bothe et al., 2010). Therefore, it is possible that they contributed to nitrogenase activity in both light and dark treatments during our 48-hour incubation under field conditions. This implies that N_2 fixation by autotrophs can take place not only during daytime but also during short nights in Arctic summers. However, the results do not exclude

participation of heterotophs in this process. In fact, when we compared total N_2 fixation values measured under field conditions and values of potential heterotrophic N_2 fixation from laboratory experiments (Fig. 3), it was evident that heterotrophic diazotrophs could account for a considerable part of total N_2 fixation in PP.

In our study, viable heterocystous cyanobacteria were detected on the PP surface in July, when generally higher nitrogenase activity was evident. However, they were completely absent in samples collected in August, which could have been a response to low temperatures and a shorter day period in the diurnal cycle (Fig. 1). No macroscopic cyanobacterial colonies were observed in PP, but cyanobacteria occurred in association with mosses. All soil samples where cyanobacteria were found were dominated by *Sphagnum* mosses, similar to a trend observed in a comparable habitat by Sorensen and Michelsen (2011). Many studies also documented an association of *Nostoc* sp. with *Pleurozium schreberi* (DeLuca et al., 2002; Houle et al., 2006; Gundale et al., 2012). However, soil cores covered with this moss species were the only ones from vegetated PP that did not exhibit any nitrogenase activity in our study.

Low pH and moisture can limit nitrogenase activity (Chapin and Bledsoe, 1992). Nevertheless, N_2 fixation was operating here even at a pH as low as 3.2–3.4. Furthermore, we do not assume moisture controls N_2 fixation in PP given that a sufficient amount of water was available in this tundra area over the whole vegetation season. Nitrogen fixation differed significantly between July and August as the latter month was colder by 8–11 °C (Fig. 1) and the day length shorter by 4 h 20 min. The air temperature in the summer months in this region frequently ranges from 5 to 25 °C. Heterotrophic nitrogenase activity determined within this temperature range in the laboratory showed an exponential increase with rising temperature and N_2 fixation rates were negligible at close-to-zero temperatures (Fig. 3). Temperature dependence measured under field conditions followed the same pattern, which suggested that the summer season remained the only period when there was a significant contribution of diazotrophs to the local N budget.

The N_2 fixation detected in PP (0.01– 0.76 mg N m^{-2} day^{-1}) was lower than rates reported from subarctic and Arctic tundra (Chapin and Bledsoe, 1992; Hobara et al., 2006; Sorensen and Michelsen, 2011; Stewart et al., 2011a; Stewart et al., 2011b). Zielke et al. (2002) reported N_2 fixation rates by moss-associated cyanobacteria in tundra mires that were similar to those reported in this study. Net and gross N mineralization determined at our Seida study site were in good agreement with other data from the scientific literature on N mineralization rate (Schmidt et al., 2002; Schimel et al., 2004; Kaiser et al., 2005; Pare and Bedard-Haughn, 2012; Wild et al., 2013).

In order to relate N_2 fixation rates to other N transformation processes in this ecosystem, we used measurements of gross N mineralization already conducted at this study site, estimates of N deposition, and estimates of N uptake in plant biomass. Because these data were collected at different times and estimated using laboratory measurements, the estimated N budget should be considered with caution. Nitrogen fixation rates measured in PP were in the same order of magnitude as N deposition and higher than net N mineralization rates. The low net mineralization rates were probably associated with high microbial immobilization rates (Schimel and Bennett, 2004). The estimated N demand by plants in PP was about two orders of magnitude higher than N_2 fixation rates. Nitrogen fixation, N deposition, and net N mineralization together (about 2% of N in NPP, Table 2) could not meet the annual plant N demand. This result agrees with previously published data from the Arctic (Atkin, 1996; Stieglitz et al., 2000). However, ARA method cannot quantify N_2 fixation by methanotrophs (Flett et al., 1975), which was recently shown to be of great importance in N budget of boreal peatlands (Larmola et al., 2014; Vile et al., 2014). This so-far omitted process would need further attention also in subarctic mires.

Relatively high net N mineralization rates in PC together with other favorable environmental conditions (high water content, higher temperature, low DOC:DIN) resulted most likely in high N_2O emissions despite low pH (Palmer et al., 2011). According to our approximation, a large portion of mineralized N in excess (net N mineralization) could be denitrified as N_2O in PC (Table 2). Thus, internal N cycling can fully fuel the large emissions of this strong greenhouse gas.

To sum up, N_2 fixation was a significant part of the N cycle solely in N-limited vegetated PP, while no nitrogenase activity was detected in bare PC and vegetated UT. We conclude that the N abundance in PC soil originated from internal N cycling (N mineralization) and currently external N inputs such as biological N_2 fixation do not play a significant role. Also, N deposition could not account for N emitted as N_2O. Thus, no relation between N_2O emissions and N inputs into soil was found, while the absence of plants and the low C:N ratio of the soil appeared to be the main reason for high N availability and supply in PC. The concentration and proportion of nutrients in soil as well as occurrence of diazotrophs explained, at least partly, the differences in nitrogenase activity in three habitats of subarctic tundra. Both autotrophic and heterotrophic N_2 fixation was proven present in PP. The diazotrophic community fixed N_2 under light as well as dark conditions, and activity varied with changing temperature during the season. Nitrogen fixation represented only a small portion of N built into NPP in PP, but was comparable to other inputs into the soil N pool such as N deposition and net N mineralization.

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7. CURRICULUM VITAE

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Education and Training

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(supervisor: Prof. Ing. Hana Šantrůčková, CSc.)

2014 six-month internship, Department of Environmental Sciences, University of Eastern Finland, Finland
(supervisor specialist: Assoc. Prof. Christina Biasi, Ph.D.)

2010 - 2011 six-month internship, Department of Environmental Sciences, University of Eastern Finland, Finland
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2010 defence of rigorous thesis

2007 - 2010 MSc in Applied Biology, Department of Ecosystem Biology, Faculty of Science, University of South Bohemia in České Budějovice
(supervisor: doc. RNDr. Jan Šíma, Ph.D.)

2008 - 2009 six-month internship under the program Erasmus, Department of Plant Biology, University of Århus, Denmark
(supervisor specialist: Prof. Hans Brix, Ph.D.)

2004 - 2007 BSc in Biology Department of Ecosystem Biology, Faculty of Science, University of South Bohemia in České Budějovice
(supervisor: doc. RNDr. Jan Šíma, Ph.D.)

Scientific Focus

Biological transformations of soil organic matter in arctic environment, carbon and nitrogen cycling between soil and atmosphere

Work and Science Experience

2016 – 2017 coordinator of international educational and research project Educational Network on Soil and Plant Ecology and Management
University of South Bohemia, Faculty of Science, Czech Republic

2016 member of field works in the Himalaya, Ladakh (collection of soil samples, *in situ* measurements of microbial activity)

2012 – 2014 coordinator of student teams in Czech educational project, Platform for education and reseach in forest ecosystems
University of South Bohemia, Faculty of Science, Czech Republic

Jun - Sep 2011 member of summer field works and sampling campaign in subarctic tundra at the Seida study side, Komi Republic, Russia, project CryoN
University of Eastern Finland, Finland

2010 – 2012 collaborator on the international CryoCARB project (Long-term Carbon Storage in Cryoturbated Arctic Soils)
http://www.univie.ac.at/cryocarb/the-cryocarb-project/
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International Courses and Certifications

Stable Isotope Course, Freising, Germany: an introduction to uses in ecology and plant physiology (2010), TOEFL ITP (2009), TOEIC L+R (2016)

Personal Skills and Competences

Native language:	Czech
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Driving license holder (B)

Publications in peer-reviewed scientific journals

Šantrůčková, H., Kotas, P., Bárta, J., Urich, T., Čapek, P., Palmtag, J., Diáková, K., … Richter, A. (2018). Significance of dark CO_2 fixation in arctic soils. *Soil Biology and Biochemistry*, ***119***. 11-21

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Šíma, J., Holcová, V., Dušek, J., & Diáková, K. (2006). Analytical approach to study of redox properties of constructed wetland. *Chemické Listy*, ***100***. 911−918

Participation in International Conferences

Diáková, K., Čapek, P., Kohoutová, I., Bárta, J., Biasi, C., Šantrůčková, H. Environmental controls on organic matter transformation in soils of East-European subarctic tundra (poster). Biogeomon 2014 - Bayreuth, Germany (13th – 17th July 2014)

Diáková, K., Čapek, P., Kohoutová, I., Bárta, J., Biasi, C., Šantrůčková, H. Microbial biomass as a key player in C losses from subarctic tundra soils in changing environment (poster). Polar and Alpine Microbiology 2015 – České Budějovice, Czech Republic (6th – 10th September 2017)

Lamprecht, R. E., Diáková, K., Voigt C., Biasi, C., Šantrůčková, H., Martikainen, P. J. SOM mineralization sensitivity to temperature and O_2 availability in deep peat profiles including permafrost interface (poster). Polar and Alpine Microbiology 2015 – České Budějovice, Czech Republic (6th – 10th September 2017)

Diáková, K., Čapek, P., Bárta, J., Biasi, C., Alves, R. J. E., Gentsch, N., Gittel, A., … Šantrůčková, H. Decomposability of old organic carbon in permafrost-affected arctic soils (oral presentation in English). Biogeomon 2017 – Litomyšl, Czech Republic (20th – 24th August 2017)

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