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Faculty of Science

AMOUNT AND TRANSFORMATION OF PHOSPHORUS IN PERMAFROST AFFECTED SOILS

Ph.D. Thesis

Mgr. Petr Čapek

Supervisor: Prof. Ing. Hana Šantrůčková, CSc.
Department of Ecosystem Biology, Faculty of Science,
University of South Bohemia in České Budějovice

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➢ Annotation

The combined effect of temperature, moisture and phosphorus availability on soil organic matter mineralization in permafrost affected soils of northern circumpolar region was investigated. This study was a part of research activities of the European project CryoCARB and it was primarily focused on the cryoturbated organic horizons of permafrost affected soils. During this study, the temperature sensitivity of the organic matter mineralization and its relation to the soil moisture and phosphorus availability was investigated using series of incubation experiments and field measurements.

➢ Declaration [in czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v nezkrácené podobě elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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Petr Čapek ……..

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Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

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➢ **Acknowledgment** [in czech]

Díky Bohu, že to mám za sebou.

➢ List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

I. Wild, B., Schnecker, J., Bárta, J., **Čapek, P.**, Guggenberger, G., Hofhansl, F., Kaiser, C., Lashchinsky, N., Mikutta, R., Mooshammer, M., Šantrůčková, H., Shibistova, O., Urich, T., Zimov, S.A., Richter, A., 2013. Nitrogen dynamics in Turbic Cryosols from Siberia and Greenland. Soil Biology and Biochemistry 67, 85-93. (IF = 3.93)

Petr Čapek quantified available soil phosphorus in soil samples used in this paper.

II. **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 = 3.93)

Petr Čapek run incubation experiment, analyzed soil samples and wrote the paper.

III. **Čapek P.,** Kotas P., Manzoni S., Šantrůčková H., 2016. Drivers of phosphorus limitation across soil microbial communities. Functional Ecology (in press) (IF = 5.21)

Petr Čapek collected and analyzed all data and wrote the paper.

IV. **Čapek P.,** Manzoni S., Kaštovská E., Diáková K., Bárta J., Wild B., Schnecker J., Biasi Ch., Martikainen P. J., Alves R. J. E., Guggenberger G., Gentsch N., Hugelius G., Lashchinsky N., Gittel A., Schleper Ch., Mikutta R., Palmtag J., Shibistova O., Urich T., Richter R., Šantrůčková H. Plant-microbial interaction: effect of variable stoichiometry – manuscript

Petr Čapek collected and analyzed data and wrote the paper.

Content

*(*Because supporting information for unpublished manuscript is not available online it is attached behind the main text of the paper. Location of supporting information for published papers is referred at the end of each paper. All published supporting information are available online and therefore they are not presented here.)*

1. GENERAL INTRODUCTION

1.1. MOTIVATION OF THE WORK

Only recently, permafrost affected soils of northern circumpolar region became an object of scientific interest. The soils are recognized by its high organic carbon (C) content, which accumulated here over centuries as a result of slow microbial decomposition of dead organic matter (OM). Nowadays, permafrost affected soils occupy ~8% of total land area (Fig. 1; Bockheim *et al.* 2006) and contain approximately 1300 Gt of organic C (Schuur *et al.* 2008; Tarnocai *et al.* 2009; Hugelius *et al.* 2014).

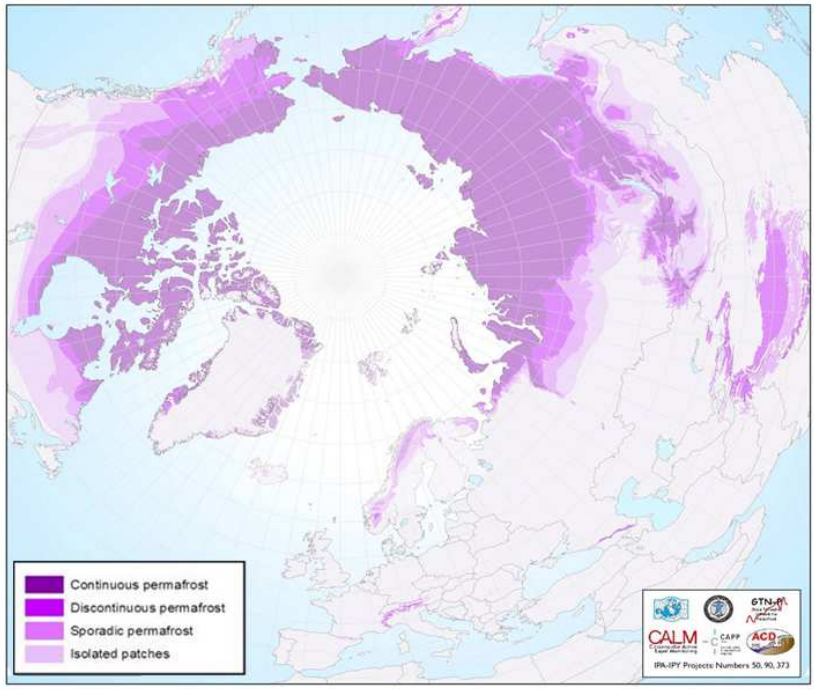

Fig. 1: *Distribution of permafrost in northern circumpolar region (International Permafrost Association, http://ipa.arcticportal.org/images/stories/permafrost%20map.jpg)*

In the circumpolar region, the OM decomposition is slowed down by the low temperature prevailing during the whole year. Similar to the well-defined chemical reactions, the OM decomposition is temperature sensitive (Zimov *et al.* 2006a; Zimov, Schuur & Chapin 2006b; Schuur *et al.* 2008; Koven *et al.* 2011; Hugelius *et al.* 2014). With the increasing temperature, the microbial decomposition generally increases. The decomposition is a process composed of a number of consecutive reactions. It is a complex process with three main consecutive steps: the enzymatic depolymerization, the OM uptake and the microbial metabolism. Reactions within

each step can differ in the temperature response and the slowest reaction obviously determines the rate of the overall decomposition. The temperature accelerates enzymatic depolymerization of a complex OM into molecules directly assimilable by soil microorganisms (Gershenson, Bader & Cheng 2009; German *et al.* 2012), it increases the rate at which a soil microbial community take up organic and inorganic molecules from the soil (Manzoni *et al.* 2012) and it increases the rate of the microbial metabolism (Brown *et al.* 2004; Price & Sowers 2004; Allen, Gillooly & Brown 2005; Yvon-Durocher *et al.* 2012). During the OM decomposition, organic C is depolymerized and further metabolized along with the organic or inorganic nitrogen (N), phosphorus (P) and micronutrients. Part of the metabolized organic C is transformed to either CO_2 under aerobic or CO_2 and methane under anaerobic soil conditions, and gasses are lost to the atmosphere. We refer this a specific process of the OM decomposition as organic C mineralization.

Because permafrost affected soils represent globally a significant C pool, whose origin and stability is linked to a low temperature, it was suggested that long term temperature driven changes of the microbial OM mineralization in permafrost affected soils (connected to changing climatic periods) partly control atmospheric CO_2 and methane concentrations (Zimov *et al.* 2006b; Koven *et al.* 2011; Köhler, Knorr & Bard 2014). For example, based on the ^{14}C data from Tahiti corals, it was hypothesized that a temperature driven increase of the microbial OM decomposition in permafrost affected soils was responsible for the increase of atmospheric CO_2 concentration by 10 ppm (equal to 125 Gt of mineralized organic C) 14,600 years ago (Bølling-Allerød interstadial; Köhler *et al.* 2014).

Another climate change is slowly approaching now. It is mostly visible in the circumpolar region. According to the IPCC (Intergovernmental Panel on Climate Change), the increase of the atmospheric mean annual temperature by 2°C was observed in the northern circumpolar region within the years 1901 and 2012, and a further temperature increase by up to 11°C is predicted to occur here before the year 2100 (IPCC 2014). The predicted temperature increase is highest for the circumpolar region compared to the rest of the planet, where the maximum temperature increase by 6°C is expected (IPCC 2014). In the light of the predicted temperature increase in the circumpolar region, there arises a question on how atmospheric CO_2 and methane concentration will be affected by the increased OM mineralization in permafrost affected soils in the future. Both gasses have a potential to strengthen the greenhouse effect and accelerate the present global warming. Hence this self-reinforcing warming cycle, so called a positive feedback, between the OM mineralization rate in permafrost affected soils, the temperature increase and the atmospheric CO_2 and methane concentration might occur (Fig. 2; Zimov *et al.* 2006b; Koven *et al.* 2011).

Recent studies have shown that permafrost affected soils are already loosing organic C as a result of an increased atmospheric temperature (Oechel *et al.* 1993; Mack *et al.* 2004; Schuur *et al.* 2009). The future development of organic C storage in permafrost affected soils is, however, subjected to a high uncertainty (Koven *et al.* 2011). There are many factors that interact with temperature, making the OM decomposition and the mineralization rates more or less sensitive to a temperature increase (Davidson & Janssens 2006). The most important ones are the water availability, followed by the oxygen availability, the OM complexity, pH, the soil microbial community structure and the **availability of nutrients** (Shaver *et al.* 1998; Dalias *et al.* 2001; Mack *et al.* 2004; Fang *et al.* 2005; Schädel *et al.* 2014; Gittel *et al.* 2014b; Treat *et al.* 2015). In the previous studies from the circumpolar region, much of the attention was paid to the effect of N availability on the OM decomposition and the mineralization under increased temperature (e.g. Chapin *et al.* 1995) but the effect of P has been marginalized so far. **Therefore, this work aimed to recognize and quantify the effect of P availability on the OM mineralization in permafrost affected soils.** In following chapters, I will *(i)* describe permafrost affected soils, *(ii)* review the main factors and their interactions that slow down the OM decomposition here, and *(iii)* based on the available knowledge, I will explain why the P availability should be included to our considerations about the development of organic C in the permafrost affected soils in the future.

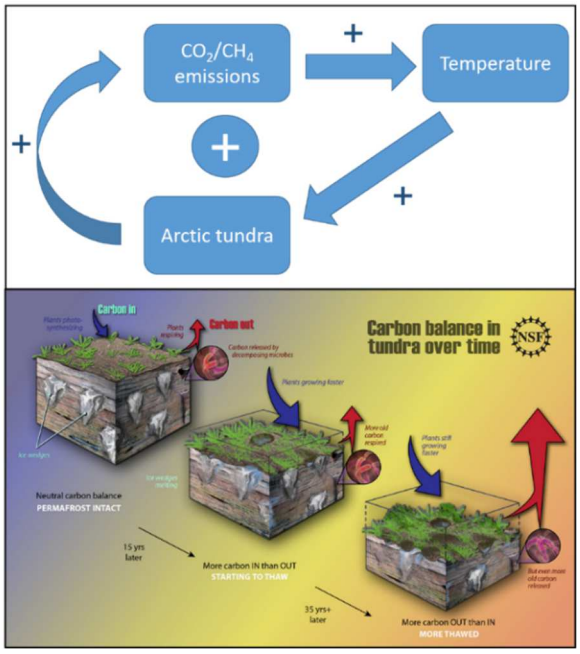

Fig. 2: *Schematic picture of the carbon balance in Arctic tundra areas under the warming climate conditions (Credit: Zina Deretsky, National Science Foundation).*

1.2. PERMAFROST AFFECTED SOILS

Permafrost affected soils are recognized by the presence of permanently frozen material (at least in two consecutive years; Tarnocai 2009; Fig. 3a,c), commonly known as **permafrost.** According to the definition, permafrost can only reach depths of up to two meters below the soil surface. In the US Soil Taxonomy and the World Reference Base, permafrost affected soils mainly correspond to "Gelisols" and "Cryosols", respectively. When the soil profile is not entirely occupied by permafrost, we recognize the **active soil layer**, the top layer of soil, which is thawing regularly during a short vegetation period between June and August.

The OM decomposition in permafrost is extremely slow due to the temperature below the freezing point, and can be assumed to be negligible (Price & Sowers 2004; Panikov & Sizova 2007). Nevertheless, an increase in the atmospheric temperature enough for permafrost to start thawing, the OM decomposition rate increases rapidly (Mack *et al.* 2004; Zimov *et al.* 2006a; Schuur *et al.* 2009). The thawed permafrost becomes a part of an active soil layer. Within the unfrozen active soil layer, the rate of the OM decomposition and mineralization is extremely variable in space. This fact limits the accuracy necessary for the prediction of organic C development under a future temperature increase. The OM decomposition usually decreases with the depth in all soils. However, beyond that, patches in soil with different soil conditions show an altered OM decomposition rate compared to the surrounding soil. So called cryogenic processes (the recurring freezing/thawing events in the active soil layer) are responsible for the creation of these patches (Fig. 3; Bockheim *et al.* 2006).

1.3. CRYOGENIC PROCESSES

Cryogenic processes are responsible for the ice segregation within the active soil layer, the soil compaction, the ice wedges formation and the cryoturbation (Fig. 3a; Tarnocai 2009). The **ice segregation and the ice wedge formation** cause a patterned growth, visible as a polygonal pattern or irregular surface elevations on a soil surface of permafrost affected soils (Fig. 3b). Water accumulates in the surface depressions (in the polygon center or between the elevations) and a local inundation might occur (Fig. 4). The inundation limits the oxygen diffusion towards soil microorganisms responsible for the OM decomposition. When a microbial community is limited by oxygen, the less efficient and slower anaerobic metabolism takes place. It is because the anaerobic metabolism uses different electron acceptors, such as NO_3^-, Fe^{2+}, Mn^{2+}, CO_2 etc., which are less prone to take electrons. This mechanism slows down the whole process of the OM decomposition and C mineralization. For example, compared to a well-drained polygon ridge, a wetter polygon depression will have two to four times lower C mineralization rate (Sommerkorn 2008).

The persisting local inundation leads towards a peatland formation (Fig. 4). Sphagnum moss starts to dominate the vegetation cover and the OM input to soil, hence the methanogenic metabolism, driven by the soil microorganisms transforming organic C into the methane usually prevails the C mineralization process (Treat *et al.* 2015). Therefore, more methane per unit of mineralized organic C is produced. The predicted temperature increase will be inevitably linked to the permafrost thawing and an increase in area of local inundation. This will lead to a higher frequency of anaerobic conditions in soil. This will have a great effect on

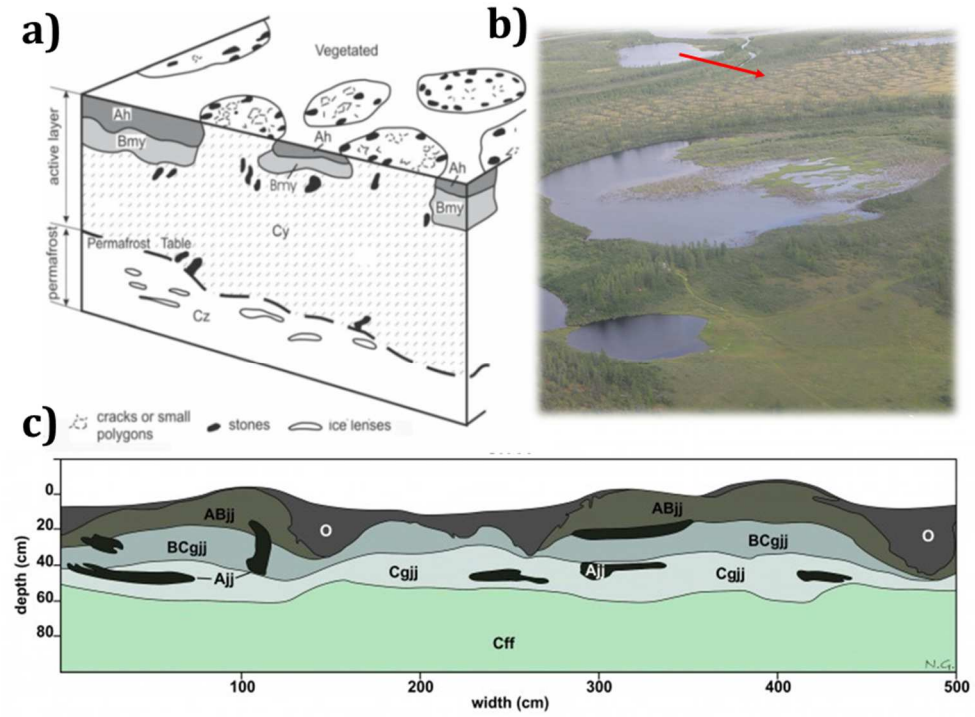

Figure 3: *a) Schematic diagram showing some of the physical features of permafrost affected soils (patterned growth, ice lenses, oriented stones) – modified original scheme of Tarnocai 2009. b) Polygonal structures on the soil ground. c) Schematic plot of soil profile with cryoturbated organic horizons (Ajj). Credit: Norman Gentsch.*

the future development of organic C concentration in permafrost affected soils and release of the greenhouse gasses into the atmosphere. Meta-analysis data from 25 incubation studies across the circumpolar region suggested, that the oxygen limitation of soil microorganisms has greater effect on the OM decomposition rate than an increase in temperature by 10°C (Schädel *et al.* 2016). Currently, ~300 Gt out of 1300 Gt of organic C is found in peatlands of the circumpolar region (Hugelius *et al.* 2014), and the area of peatland might increase as a result of increased precipitation in the future (IPCC 2014).

Permafrost affected soils are often alternatively referred to as cryoturbated soils (e.g. Xu *et al.* 2009; Klaminder, Giesler & Makoto 2013; Schnecker *et al.* 2014; Gentsch *et al.* 2015). Cryoturbation is a physical mixing of the soil profile by the action of frost. Cryoturbation is considered as a dominant soil formation process in the Arctic, according to which permafrost affected soils are sometimes being classified (Bockheim & Tarnocai 1998). It is primarily caused by the irregular ice segregation (Van Vliet-Lanoe 1998) and further magnified by a double-freezing of

Figure 4: *Picture of locally inundated soil in the center of the polygon (left side - Taymyr peninsula 2011) and created peatland with dominant cover of Sphagnum fuscum (right side – Seida study site 2011).*

the active soil layer - from the top and from the permafrost (Bockheim & Tarnocai 1998). This causes an increase of pressure in soil, followed by a soil compaction and movement. The soil movement mixes soil horizons resulting in regular soil horizons consisting frequently of material from other horizons (Fig. 3c). In respect to the soil organic C accumulation, the most visible and the most important is the incorporation of surface soil horizons with undecomposed or slightly decomposed OM into deeper soil layer (Figs. 3c, 5). Inside the mineral soil, there are patches (further in the text **as cryoturbated organic horizons**) different from other soil horizons in four respects: *i)* they have higher organic C content compared to the mineral soil but lower than surface soil horizons (Kaiser *et al.* 2007), *ii)* they have generally higher bulk density compared to the surface soil horizons (Kaiser *et al.* 2007), *iii)* they have altered water and thermal regime compared to the surface and the mineral soil horizons (Kaiser *et al.* 2007), and *iv)* they have altered soil microbial community compared to the surface and the mineral soil horizons (Gittel *et al.* 2014a).

Constraints of the OM decomposition in cryoturbated patches is indicated by the radiocarbon age of organic C in cryoturbated organic horizons that reaches several thousand years (Bockheim 2007; Kaiser *et al.* 2007; Hugelius *et al.* 2010; Palmtag *et al.* 2015). Generally, the mean residence time of organic C entering the soil is estimated to vary from 1 to 40 years depending on the nutrient concentration and temperature (Parshotam *et al.* 2000; Fröberg *et al.* 2010; Baisden *et al.* 2011;

Tian *et al.* 2015). When organic C includes physically and chemically stabilized OM, its mean residence time can increase up to 3000 years (Baisden *et al.* 2011).

Figure 5: *Signs of cryoturbation within the soil profile (Taymyr peninsula 2011). Cryoturbated organic horizons can be also found within the permafrost.*

However, such organic C is not very abundant in cryoturbated organic horizons (Gentsch *et al.* 2015b). Subducting of the OM down to the soil profile greatly decelerate the OM decomposition itself. This was further confirmed by comparing direct measurements of organic C mineralization rate in cryoturbated organic horizon with those in topsoil and mineral soil horizons (Kaiser *et al.* 2007).

Several factors have been hypothesized to be responsible for the slow microbial OM decomposition in cryoturbated organic horizons including altered microbial community structure (Gittel *et al.* 2014a), profound nutrient limitation of soil microbial community (Wild *et al.* 2013, 2014) or inefficient extracellular enzyme performance (Schnecker *et al.* 2014). Currently, ~400 Gt of organic C with unknown dynamics were found in cryoturbated organic horizons (Harden *et al.* 2012), which represents 25% of the total C storage in permafrost affected soils (Palmtag *et al.* 2015). It is hypothesized that the subduction of the surface OM may be the most important mechanism of organic C accumulation in permafrost affected soils (Tarnocai *et al.* 2009).

1.4 CryoCARB project

There is only few information about cryoturbated organic horizons. Therefore, the quantification of their organic C amount, its mineralization rate and

the vulnerability to a predicted temperature increase was the aim of the European project CryoCARB. This thesis represents a part of this project and thus it is mainly, but not entirely, focused on cryoturbated organic horizons. Within the CryoCARB project, five different individual projects were defined: IP_1 – Carbon storage in cryoturbated soils, IP_2 - Carbon Sequestration in Cryoturbated Soils of the Eurasian Arctic, IP_3 - Decomposition Processes in Cryoturbated Soil, IP_4 - Carbon Bioavailability and Microbial Transformations, and IP_5 - High-resolution Microbial Community Structure. This work was done as a part of the IP_4, but it uses data and information from all other individual projects.

The **vision** of the CryoCARB project is that "*one can build on gathered knowledge to improve the existing models to better predict the responses of cryoturbated soils to the future climate conditions*". In order to do so, it is first necessary to precisely quantify the sensitivity of the OM decomposition to the changing temperature across different soil horizons and different patches of permafrost affected soils. It is already known that the temperature sensitivity of the OM decomposition and organic C mineralization differ among different soils and soil horizons. Nevertheless, the understanding of the mechanisms, which cause this variability is still lacking (Davidson & Janssens 2006). Because of that, the temperature sensitivity of organic C mineralization is not very well captured in the current predictive models. For example, the Dynamic Global Vegetation Model ORCHIDEE, which was used to predict the positive feedback of permafrost affected soils to climate change, simply assume that organic C mineralization uniformly doubles with an increase of 10°C (Q_{10} quotient, see Chapter 1.5.) under aerobic soil conditions, and triples under anaerobic soil conditions (Koven *et al.* 2011). **In the next chapters, I will review general knowledge about the temperature sensitivity of organic C mineralization rate and show how the selected soil conditions (soil moisture and P availability) of permafrost affected soils may interact with the temperature affecting organic C mineralization and significantly changing the Q_{10} quotient.**

1.5. Temperature sensitivity of organic C mineralization

All mathematical models assume that the OM decomposition and organic C mineralization increase exponentially with increasing temperature, which is in accordance with majority of the published studies. Most of the studies use Q_{10} relationship, first developed by van't Hoff (Glinski & Stepniewski 1985; for review see Davidson & Janssens 2006). Q_{10} is defined as a relative increase of the reaction rate as a consequence of increasing temperature by 10°C. Thus, if the reaction rate doubles with temperature increase by 10°C, value of Q_{10} equals to two. When Q_{10} is one, the reaction rate stays the same. When it is less than one, the reaction rate decreases as temperature increases. It has long been employed a rule of thumb, that the rate of biological processes (the OM decomposition or organic C mineralization

rate in our case) generally doubles when temperature increases by 10°C (Davidson & Janssens 2006). Nowadays, Q_{10} parameter is commonly used to express the temperature sensitivity of soil processes regardless its original definition by van't Hoff. Thus, I will use Q_{10} further in the text to refer to the temperature sensitivity of the OM decomposition or organic C mineralization rate. Even though both processes, the OM decomposition and organic C mineralization, are ultimately connected, I will keep them separately as they are not the same.

In many studies, it has been shown that Q_{10} of organic C mineralization rate is not unique, but flexible (e.g. Knorr *et al.* 2005). Q_{10} is different for different soils at different soil conditions. For example, Treat et al. (2015) showed that Q_{10} of the anaerobic mineralization of organic C varies from 0.96 to 3.10 across the Arctic tundra. Even higher variability was observed under aerobic conditions, where Q_{10} of organic C mineralization in the Arctic tundra ranged from 0.7 to 34.6 (Moni *et al.* 2015). From these numbers it is evident that specific soil conditions might have stronger effect on organic C mineralization than the temperature itself.

Two basic theories exist that explain variability in Q_{10} among published studies – (i) the kinetic theory and (ii) the metabolic theory. Both of them are based on the Arrhenius theory, but their philosophy is different.

(i) **The kinetic theory** states that Q_{10} is inversely related to the OM quality (the abundance of easily and hardly decomposable OM, which microbial community growth on, e. g. amino acids or lignin-like compounds)(Fang *et al.* 2005; Conant *et al.* 2008). The background for this theory is the classical Arrhenius equation:

$$C_{MIN} = Ae^{\frac{-E_A}{RT}}, \quad [1]$$

in which C_{MIN} is organic C mineralization rate, A is the pre-exponential factor defining the proportion between reactants, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature (°K) and E_A is the activation energy (kJ mol (organic carbon)$^{-1}$); i.e. the energy that needs to be invested in a reaction to get it started. Based on this equation it can be shown that Q_{10}, i.e. a change of OM mineralization with temperature increase by 10°C (from T_1 to T_{1+10}) depends directly on the activation energy:

$$Q_{10} = Ae^{\frac{-E_A}{RT_{1+10}}} / Ae^{\frac{-E_A}{RT_1}} = e^{\frac{-E_A}{R}\left(\frac{1}{T_{1+10}} - \frac{1}{T_1}\right)}. \quad [2]$$

The reason why the temperature sensitivity of organic C mineralization should increase with the OM complexity is that the rate limiting step of organic C mineralization has always the highest activation energy and therefore it ultimately determines the temperature sensitivity of the whole process‡.

‡ For example, the mineralization of glucose, as a simple and directly assimilable organic C is supposed to have E_A = 30 kJ mol^{-1} (Davidson & Janssens 2006). When enough glucose is available in soil, Q_{10} of the OM mineralization rate is 1.5. When such a simple substrate is not directly available, it needs to be created extracellularly by depolymerization of a complex OM first. However, the complex OM represents less reactive compound than glucose and therefore, its depolymerization takes longer and requires higher activation energy - energy that needs to be invested to a reaction to get it started (e.g. tannin decomposition has E_A *= 70 kJ mol^{-1}; Davidson & Janssens 2006). In such case, the complex OM decomposition is the rate limiting step (soil microorganisms have to wait for depolymerization products), and according to the Eq. 2, its activation energy makes the whole process of C mineralization more temperature sensitive, Q_{10} is 2.8. The OM complexity usually increases in soil over time, because a microbial community first decomposes simple substrates like glucose, starch or amino acids. More complex OM is left behind. Thus, according to the kinetic theory, we can expect an increase of Q_{10} in older soils with more transformed and complex OM or in soil horizons without continuous supply of fresh easily available OM. Specifically, cryoturbated organic horizons of permafrost affected soils represent the case, where high temperature sensitivity of organic C mineralization rate can be expected due to restricted supply of fresh OM.*

However, empirical data do not always support kinetic theory (Fang *et al.* 2005; Thiessen *et al.* 2013). The study of Gershenson (2009) even showed the exact opposite effect of OM quality; Q_{10} of organic C mineralization increased after the addition of glucose to soil.

(ii) In contrast to the kinetic theory, the metabolic theory postulates that Q_{10} of organic C mineralization rate reflects activation energy of microbial metabolism, which is 65 kJ mol (organic carbon)$^{-1}$ (Gillooly *et al.* 2001; Brown *et al.* 2004; Allen *et al.* 2005; Yvon-Durocher *et al.* 2012). The metabolic theory has almost the same form as the Arrhenius equation, but the equation parameters have different meaning.

$$C_{MIN} = \rho_0 C_{MB} e^{\frac{-E_A}{k\,T}}. \qquad [3]$$

The pre-exponential factor A of the original Arrhenius equation is substituted by the microbial community biomass (C_{MB}) multiplied by the normalization constant ρ_0, which represents the rate of organic C mineralization by mol of C_{MB} at standard temperature (Allen *et al.* 2005). The activation energy has different units (electron volts; 1 eV = 104 kJ mol^{-1}) and therefore the universal gas constant is substituted by Boltzmann constant k (8.62 10^{-5} eV K^{-1}) to keep units consistent. The metabolic theory further includes body size dependent allometric term. This term can be

omitted when we assume that the OM mineralization is mediated by soil microorganisms with narrow variability of body size.

When we assume that the microbial community biomass does not change with temperature (C_{MB} is constant), Q_{10} of organic C mineralization rate can be defined as:

$$Q_{10} = \rho_0 C_{MB} e^{\frac{-E_A}{k T_{1+10}}} / \rho_0 C_{MB} e^{\frac{-E_A}{k T_1}} = e^{\frac{-E_A}{k}\left(\frac{1}{T_{1+10}} - \frac{1}{T_1}\right)}. \quad [4]$$

The form of this relationship corresponds to the Eq. 2, but E_A is not the activation energy of the rate limiting reaction as in the kinetic theory, but the activation energy of the microbial metabolism. In the metabolic theory, the rate of organic C mineralization is solely determined by the catabolic (energy yielding) reactions in cell during which CO_2 (or methane) is produced (CO_2 consumption by extracellular carboxylases is neglected) ##.

For example, the aerobic metabolism employs glycolysis connected to tricarboxylic acid (TCA) cycle to degrade glucose in order to get an energy in form of ATP with CO_2 as a side product. The overall reaction has following stoichiometric formula:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 38\ ATP$$

*Glucose and oxygen itself would react extremely slowly and the complex system of free and membrane bound enzymes has to be employed by the cell. On the pathway, enthalpy of this system continuously increases until the so called "**activated complex**" is formed. The activated complex represents the state of the enzymatic system and the reactants ($C_6H_{12}O_6$ and O_2), from which the whole system collapses to the spontaneous formation of products (CO_2, H_2O and ATP). The electrochemical potential on a cell membrane can be considered as an activated complex. When this potential is created, protons can spontaneously flow through the ATP synthase to produce corresponding number of ATP. E_A in the Eq. 4 corresponds to the enthalpy of the activated complex, i.e. a work (in eV) needed to be done to create an activated complex (=electrochemical potential). According to the metabolic theory, 0.65 eV is needed to do this work (Gillooly et al. 2001).*

According to the metabolic theory, change of the temperature sensitivity of C mineralization can result from a change of microbial biomass (C_{MB}), normalization constant (ρ_0) and/or rate of catabolic reactions (E_A). The theory was originally developed for organisms with the aerobic metabolism and thus E_A is set to 0.65 eV. However in soils, an anaerobic metabolism, which uses different final electron

acceptors than oxygen and different enzymatic systems for electron delivery from donors to acceptors (see Chapter 1.3.), can substitute an aerobic metabolism. Thus E_A in the Eq. 4 cannot be constant and should be set according to the type of the energy yielding reaction. In permafrost affected soils, the anaerobic conditions decrease Q_{10} of organic C mineralization rate (Schadel et al. 2015). Based on this observation, we would expect that the anaerobic metabolism has lower E_A than aerobic metabolism. E_A of different anaerobic pathways found in the literature and ordered decreasingly are given in Table 1. Estimated E_A are very variable and it is not possible to make any conclusion based on them. But in theory, the anaerobic pathways such as dissimilatory nitrate, manganese or iron reduction or substrate phosphorylation have less complex enzyme systems and thus we can expect less work needed to create an activated complex and lower E_A.

Table 1

Estimated activation energies (E_A) of various anaerobic metabolic pathways. The temperature sensitivity (Q_{10}) of particular metabolic pathway is calculated according to the Eq. 4 and listed in the table. Metabolic pathways are ordered decreasingly according to their Q_{10}. Reference Q_{10} of the aerobic metabolism is written in bold.

Metabolism	*E_A (eV)*	*Q_{10}*	*Reference*
Methanogenesis	1.2 - 2.7	5.7 - 49.8	Dunfield *et al.* 1993
Denitrification	1.42	7.8	Boulêtreau *et al.* 2012
Methanogenesis	0.96	4.2	Yvon-Durocher *et al.* 2014
Sulfate reduction	0.9	3.7	Knoblauch, Jørgensen & Harder 1999
Aerobic	**0.65**	**2.6**	**Gillooly *et al.* 2001**
Methanogenesis	0.65	2.6	Yao & Conrad 2000
Methanogenesis	0.61	2.4	Fey & Conrad 2000
Unspecified anaerobic	0.53	2.2	Tan *et al.* 1995
Fe(III) reduction	0.48	2	Yao and Conrad, 2000
Fermentation	0.39	1.8	Marques *et al.* 2009
Denitrification	0.38	1.7	Raven & Geider 1988

In contrast to the kinetic theory, an effect of the OM complexity on the temperature sensitivity of C mineralization is not explicitly included in the metabolic theory, its effect is implicitly expressed via **microbial biomass** (C_{MB}) and also via normalization constant (see later). Thus, the theory assumes that the effect of the OM complexity can have an impact on the temperature sensitivity of C mineralization only if the microbial biomass changes with temperature‡‡‡.

‡‡‡ *Organic C mineralization in catabolic reactions is running in cells in order to cover energy demand for production of cell components (DNA, phospholipids, cell walls etc.), to maintain the cellular integrity and to acquire organic C and nutrients from soil (extracellular enzymes production). If at given conditions the microorganisms are able to gain enough C and nutrients from soil to cover their nutrient and energy demands, the microbial biomass does not change. If C and nutrients are in excess, the microbial biomass grows, and in the opposite case, when C and nutrients are depleted, the microbial biomass decreases. The microbial energy demand depends on how harsh the soil conditions are and how complex the OM is. For example, main part of the extracellular cellulose degrading enzymes (cellulase), is composed of 35 amino acids (Kuhad, Gupta & Singh 2011). In order to create link between two amino acids, 17 kJ of energy is needed. One mol of ATP contains approximately 30 kJ. Thus, when soil microorganisms need to produce cellulase to gain glucose from the soil, they have to spend at least 20 molecules of ATP, which is equivalent to less than 1 molecule of glucose decomposed aerobically or 10 molecules of glucose decomposed anaerobically during fermentation. When nutrients availability decreases, or the organic matter in soil becomes more complex during decomposition, more enzymes and thus more energy from catabolic reactions is needed to maintain the unit of microbial biomass. If this energy demand becomes higher than energy supply by catabolic reactions (expenses are higher than gains), the microbial biomass cannot be maintained and decreases. This is the reason why organic C decomposition rate decreases during decomposition (Berg & McClaugherty 2003). In latter stages of decomposition, lignin-like compounds are mainly present in soil. Their decomposition requires many different enzymes, whose production requires more energy, than it is included in these compounds. Therefore, microbial biomass as well as microbial biomass to organic C ratio decreases (Witter & Kanal 1998).*

Incorporation of the change of the microbial biomass into the temperature sensitivity relationship modifies the Eq. 4 to the following form:

$$Q_{10} = \rho_0 C_{MB}^{T_{1+10}} e^{\frac{-E_A}{k\,T_{1+10}}} / \rho_0 C_{MB}^{T_1} e^{\frac{-E_A}{k\,T_1}} = \frac{C_{MB}^{\boldsymbol{T_{1+10}}}}{C_{MB}^{\boldsymbol{T_1}}}\; e^{\frac{-E_A}{k}\left(\frac{1}{T_{1+10}}-\frac{1}{T_1}\right)}. \qquad [5]$$

Compared to Eq. 4, Q_{10} given by the specific E_A is further multiplied by the relative difference in C_{MB} between two temperatures ($\frac{C_{MB}^{T_{1+10}}}{C_{MB}^{T_1}}$) in the Eq. 5. Here we assume constant ρ_0 (see later).

Increase of the microbial biomass with temperature may happen when *(i)* a more directly assimilable substrate becomes available in soil at higher temperature as a result of increased activity of previously produced extracellular enzymes

(German *et al.* 2012; Schindlbacher *et al.* 2015) or when *(ii)* an uptake rate increases under higher temperature, which allows microbial community to grow faster and make bigger biomass (Manzoni *et al.* 2012). Gershenson *et al.* (2009) amended soil with glucose and measured immediate increase of Q_{10} from approx. 2 to 2.25. Such an increase corresponds to approximately 11% increase of microbial biomass at higher temperature according to the Eq. 5. Note, that in this example of a particular situation, the kinetic and the metabolic theories are at odds and when the metabolic theory better corresponds with the measured data. The microbial biomass can also decrease with temperature when, e.g., extracellular enzymes or microbial cells denaturate as a result of too high temperature. In such case, Q_{10} would decrease.

Last, Q_{10} can further change when the **normalization constant** ρ_0 changes with temperature. The normalization constant expresses rate of organic C mineralization by unit of microbial biomass at a reference temperature, and it is supposed to be constant in the original metabolic theory (Allen *et al.* 2005). However, it is well documented that it is highly variable. At the same temperature, one mol of a microbial biomass can mineralize from 0.1 to 10 mol of organic C per hour depending on the physiological stage of microorganisms (for review see Mormile *et al.* 2013). In soil ecology, organic C mineralization rate expressed per unit of microbial biomass (C_{MIN}/C_{MB}) is called the **metabolic quotient (**qC_{MIN}**; Anderson & Domsch 1993)**. The metabolic quotient is a function of microbial physiology. Microbial cells are almost continuously taking up organic molecules. These molecules are distributed among catabolic reactions to cover the energy demand, and anabolic reactions to build and maintain cells and produce extracellular enzymes. Relative distribution between organic C anabolism ($C_{anabolic}$) and mineralization (C_{MIN}) is defined as the microbial/soil carbon use efficiency (CUE); i.e. the proportion of consumed C used for biomass and enzyme production:

$$CUE = \frac{C_{anabolic}}{C_{anabolic} + C_{MIN}}. \quad [6]$$

Functional relationship between CUE and qC_{MIN} is:

$$qC_{MIN} = \frac{C_{anabolic}}{C_{MB}} \left(\frac{1}{CUE} - 1\right). \quad [7]$$

The Eq. 7 can be further extended if we divide $C_{anabolic}$ by production of cell components (C_{CELL}) and extracellular enzymes (C_{ENZ}).

$$qC_{MIN} = C_{ENZ} \frac{C_{CELL}}{C_{MB}} \left(\frac{1}{CUE} - 1\right). \quad [8]$$

In the Eq. 8, the term C_{CELL}/C_{MB} expresses the microbial community turnover rate (the rate at which the standing microbial biomass rebuilds; given in units of time^{-1}).

The term qC_{MIN} in the Eq. 8 corresponds to the normalization constant ρ_0 from the Eq. 3. The Eq. 8 clearly documents that ρ_0 is a function of the microbial turnover rate, the extracellular enzyme production and $\left(\frac{1}{CUE}-1\right)$. The term $\left(\frac{1}{CUE}-1\right)$ corresponds to the amount of energy, in currency of organic C, which needs to be produced by catabolic reactions in order to allow microbial community to produce enzymes (C_{ENZ}) and cell components (C_{CELL}). This term is not constant, because different amount of energy is needed to produce phosphatases, and e.g, DNA. CUE has its theoretical physiological maximum - CUE_{MAX}, which is 0.6 (Roels 1980) and therefore $\left(\frac{1}{CUE_{MAX}}-1\right)$ is approximately 0.7. This number defines the least amount of energy needed to build one unit of biomass at optimal aerobic conditions (expressed relatively to C_{CELL} because the enzyme production is negligible compared to the cell components production under the optimal conditions). Since anaerobic pathways are less efficient in gaining energy from organic C decomposition, CUE decreases below CUE_{MAX} and $\left(\frac{1}{CUE_{MAX}}-1\right)$ become higher than 1. It means that under anaerobic conditions, disproportionally more organic C needs to be processed before unit of microbial biomass can be produced. Similarly, when the production of biomass or enzymes becomes more energetically demanding for any reason or the microbial community just spills the excess of available energy in futile cycles (see later), CUE decreases below CUE_{MAX} and the metabolic quotient increases.

Some experimental studies show that microbial community turnover rate increases with temperature until the temperature reaches the optimum value. Because of faster degradation of cell components caused by a higher temperature, the microbial community needs to rebuilt cell components more frequently (Hagerty *et al.* 2014). Turnover rate can increase from 0.02 d^{-1} to 0.04 d^{-1} when temperature rises from 10 to 20°C. This increases Q_{10} of organic C mineralization by a factor of two at constant CUE and C_{ENZ}. Diáková *et al.* (2016) showed that Q_{10} increases with the increasing enzyme production under increasing temperature across the Arctic tundra. In their experiments Q_{10} increased from 2.5 to nearly 3.5.

In conclusion, metabolic theory represents a better thermodynamic model for the organic C mineralization in soil because: *(i)* it is more flexible than the kinetic theory, *(ii)* it is more deterministic compared to the kinetic theory, which is more mechanistic, *(iii)* it has more solid theoretical backgrounds and it respects the effect of microbial activities, and *(iv)* it better corresponds to the published results. Therefore, I will refer it further when I will define how soil conditions in permafrost affected soils may affect organic C mineralization rate under predicted climate change.

1.6. Effect of changing soil conditions on organic C mineralization rate

In the previous chapters (1.2 and 1.3) I showed that the active layer of permafrost affected soils contains patches created by cryogenic processes at specific soil conditions. Slow organic C mineralization in these patches largely enhanced accumulation of the OM in these soils in the past. In the future, we expect temperature to increase. How it will affect organic C mineralization in these patches depends not only on the temperature itself, but also on specific environmental conditions. If we want to understand the effect of soil conditions on organic C mineralization rate, we need to understand their effect on soil microbial biomass, metabolism and physiology. The metabolic theory explained in the chapter 1.5 offers a convenient tool to do so. A deeper deterministic understanding would help to better predict the development of organic C in permafrost affected soils in the future. From all possible soil conditions, I will specifically aim to the soil moisture and the nutrient content of soil, which were hypothesized to be main factors responsible for slowing down organic C mineralization rate in cryoturbated organic horizons and inundated soils (Kaiser *et al.* 2007; Sommerkorn 2008; Wild *et al.* 2013).

Soil moisture affects oxygen diffusion towards soil microbial community. Therefore, it determines the type of the energy yielding reactions running in microbial cells. This is directly connected to the amount of energy, which soil microorganisms can gain from catabolic reactions, and thus to the total microbial biomass as explained in the chapter 1.5. When an aerobic soil becomes inundated and the oxygen diffusion becomes restricted, part of the aerobic microbial community will decrease. Only the microorganisms, which are able to temporarily live under anaerobic conditions, such as facultative anaerobes, will survive and obligatory anaerobes will start to develop. When anaerobic conditions persist, as in peatlands, also facultative anaerobes will vanish. At these conditions only obligate anaerobic microorganisms can stay active, but their biomass is largely restricted by energetically inefficient energy yielding reactions. Therefore, duration of anaerobic conditions in soils affects microbial biomass and type of the microbial metabolism. The least microbial biomass with the lowest E_A and therefore the lowest temperature sensitivity of organic C mineralization rate is expected in permanently inundated soils (Schädel *et al.* 2016). In the future, more frequent soil inundation might be expected because the soil surface will drop due to thawing of permafrost and precipitations are predicted to increase along with temperature (IPCC 2014). If the frequency of soil inundation will increase in the future, it will reduce the effect of the increased temperature on organic C mineralization. On the other hand, permafrost thaw can cause better soil drainage and development of well aerated soils. In that situation, we can expect strengthening of the temperature effect on organic C mineralization in currently inundated patches.

The nutrient availability is supposed to commonly limit organic C mineralization rate in permafrost affected soils. Nitrogen limitation of soil microbial

community was especially stressed (Hobbie & Gough 2002; Weintraub & Schimel 2005). When permafrost affected soils are fertilized, organic C mineralization rate doubles compared to the unfertilized soil in a long term (Mack *et al.* 2004). However, fertilization is usually done with NPK fertilizer in the experiments focused on the effect of N addition. Therefore, possible interaction of other nutrients with N is very likely in such experiments but it is not taken into account. Especially phosphorus (P) limitation can strongly affect organic C mineralization rate in permafrost affected soils according to some studies (Giblin *et al.* 1991; Arens, Sullivan & Welker 2008).

To estimate whether P limits organic C decomposition, P demand of soil microbial community and P availability in soil has to be known, however, such studies are still relatively rare. This is because of difficulties connected with P availability determination in soils. Determination of available soil P is lively discussed for a long time. P can create bonds of variable strength with different inorganic and organic compounds in soil and it is yet not clear which P forms are directly available to soil microorganisms and which are not. Many extraction methods were developed to estimate soil available P. Although not accurately, it is possible to estimate it. Contrarily to that, P demand by soil microbial community (see below) is completely unknown. This is rather surprising in a view of the fact that P significance in microbial metabolism is undeniable. P is the main macronutrient, which fulfill several important tasks. It is involved in cell energetics (as a functional part of the ATP and polyphosphates), production of macromolecules (in DNA and RNA), it binds to intermediate products of the cell metabolism as PO_4^{-III} and by doing so, it disables their transport via cell membranes, and it is functional part of phospholipids. Because P is so frequently used in microbial metabolism, microorganisms often create stores of P in form of polyphosphates (Kulaev, Vagabov & Kulakovskaya 1999), which are used when P availability in soil is low. According to the published studies, the insufficient amount of available P in soil may reduce microbial biomass growth rate or decrease CUE (Droop 1973; Cherif & Loreau 2010; Manzoni *et al.* 2012). **I will further show how the effect of P limitation on organic C mineralization rate can be theoretically quantified.** In order to do so, I will use two variables of the metabolic theory introduced in the chapter 1.5 - C_{MB} and ρ_0:

A) Microbial biomass (C_{MB})

Soil microbial community is not rigid but dynamic. Even though we do not observe a net change of microbial biomass, the microbial biomass is turning over continuously by rate of approximately 0.02 d^{-1} (Hagerty *et al.* 2014). During biomass turnover, a part of the microbial community is growing and a part is dying at the same time. To allow microbial growth, certain amount of P is needed. It was shown that microbial growth rate is a non-linear function of available substrate, usually modeled by Monod equation (Monod 1950; Droop 1973; Sinsabaugh, Shah & Follstad Shah 2012). The substrate has to have accurately defined composition to allow microbial growth. Although it would contain enough organic C to gain energy and subsequently produce inevitable cell components, it does not guarantee the

microbial growth until all the necessary nutrients including N, P and micronutrients are available (Droop 1973; Boer *et al.* 2009; Cherif & Loreau 2010). When P is in shortage with respect to the other nutrients and C, and in relation to the nutritional demand of microbial community, the microbial growth is directly driven by P availability according to the following equation:

$$\mu = \frac{V_{MAX}\, P}{K_M + P} + \mu_0, \qquad [9]$$

where μ is the microbial growth rate, V_{MAX} is the maximal growth rate, K_M is the affinity constant and μ_0 represents the microbial growth rate from microbial P storage compounds. Thus, when P availability decreases in soil, the microbial community growth rate decreases or even ceases. If the growth rate decreases to the extent that it does not balance the microbial death rate, net decrease of microbial biomass can be observed. Analogically, when organic C and N availability in soil increase to the extent at which the net microbial biomass increases, low P availability may restrict it.

B) Normalization constant (ρ_0)

The normalization constant or the metabolic quotient (qC_{MIN}) is a function of the microbial biomass turnover rate, in particular C_{MB}, enzyme production and energy demand to cover both. Mormile *et al.* (2013) showed that across all published studies, the metabolic quotient is negatively related to the microbial community C to P ratio. The microbial community with less P, and thus probably also limited by P, produces more CO_2 per unit of microbial biomass. This is very likely connected to the decrease of CUE, which can be theoretically expressed as (Manzoni *et al.* 2008, 2010, 2012; Sinsabaugh *et al.* 2013):

$$\frac{CUE}{CUE_{MAX}} = min\left[1, \frac{C{:}P_{CR}}{C{:}P_S \times CUE_{MAX}}\right]. \qquad [10]$$

In this equation, $C{:}P_{CR}$ is the critical C to P ratio of microbial community demand, $C{:}P_S$ is the substrate C to P ratio and CUE_{MAX} is the constant 0.6 (Roels 1980). The Eq. 10 shows that CUE decreases below its physiological maximum (CUE_{MAX}) when P limitation increases (high $C{:}P_S$ – low amount of P relative to C). The term $C{:}P_{CR}$ defines the relative amount of P from soil necessary for a soil microbial community to grow. When P limits microbial growth ($C{:}P_S \times CUE_{MAX} < C{:}P_{CR}$), relatively more organic C is mineralized; CUE decreases. However, the mechanism of this relationship is not clear. Two mechanisms can be speculated. First, low P availability causes higher production of extracellular enzymes (phosphatases), which need to be covered by energy from catabolic reactions producing more CO_2 (Diáková *et al.* 2016). Second, excess of C is released by soil microbial community in energy spilling reactions called futile cycles (Neijssel, Buurman & de Mattos 1990).

The Eqs. 9 and 10 imply that P limitation of a soil microbial community can either decrease organic C mineralization rate, when the microbial biomass decreases, or increase organic C mineralization rate, when the metabolic quotient increases. Both mechanisms have support in published literature. Whether any of the two mechanisms is occurring in permafrost affected soils in general or specifically in patches created by cryogenic processes, and to which extent it affects organic C mineralization rate, is not known. Therefore, **along the effect of soil moisture, the effect of P limitation on organic C mineralization rate was the aim of this work** and supplement the information gathered during the CryoCARB project.

2. AIM AND OBJECTIVES

The overall aim of this work was to quantify the OM decomposition rate in permafrost affected soils in relation to changing soil conditions. In order to meet the aim of this work and to meet aims of the CryoCARB project, four objectives were defined:

Objective 1. – To estimate the temperature sensitivity of organic C mineralization rate across different soil horizons under aerobic and anaerobic conditions.

Objective 2. – To quantify the amount of available soil P and its variability in permafrost affected soils.

Objective 3. – To determine drivers of soil microbial community demand for phosphorus.

Objective 4. – To determine the likelihood of P limitation of soil microbial community in permafrost affected soils and its consequence for OM decomposition.

3. RESULTS AND CONCLUSIONS

In order to meet the objective 1, we examined the effect of temperature on the OM decomposition in different soil horizons of permafrost affected soils (**paper II**). A four month incubation experiment was conducted, where a topsoil organic, a deep mineral soil and a cryoturbated organic horizon was incubated at 3 different temperatures (4, 12 and 20°C) with and without presence of oxygen. In this paper, we monitored organic C mineralization and defined it as total carbon loss (C_{LOSS}). Additionally, we measured its potential biotic and abiotic drivers, including the concentrations of available nutrients (both N and P), the microbial activity, the biomass and stoichiometry, and extracellular oxidative and hydrolytic enzyme pools. In accordance with the previous studies (Kaiser *et al.* 2007; Wild *et al.* 2013), organic C mineralization rate was slowest in cryoturbated organic horizon when normalized to the unit of the total soil C compared to the other two horizons. At the same time, the temperature sensitivity of organic C mineralization expressed as Q_{10} was 2.41 at a presence of oxygen, which is very close to value 2.48 predicted for a given temperature range by the metabolic theory at the steady state microbial biomass and CUE. Significantly lower Q_{10} was observed under anaerobic conditions, only 1.38.

No change in the microbial biomass was measured throughout the incubation. Multiple linear regression analysis followed by a ridge regression was used to identify the best predictors of organic C mineralization. As supposed by the metabolic theory, the microbial biomass was found to be the strongest predictor. Thus, it was concluded that the slow OM decomposition in cryoturbated organic horizons is connected to the low microbial biomass.

However, the reason for the low microbial biomass in cryoturbated organic horizons remained unclear. Stoichiometry of the microbial biomass indicated profound nutrient limitation but all extracellular enzymes decreased throughout the incubation in parallel, being unrelated to the nutrient availability or the microbial biomass stoichiometry. Based on these results, a theoretical concept explaining the observed patterns was proposed (Fig. 6). According to this concept, the microbial biomass cannot grow in cryoturbated organic horizons. The energy constrains do not allow production of extracellular enzymes, which would supply microbial communities with substrate essential for growth. Microbial communities largely rely on fluxes of allochthonous carbon and nutrients from the topsoil organic horizon. The flux of the allochthonous material creates a negative priming effect, which protects cryoturbated OM from the microbial decomposition. This hypothesized mechanism could be well responsible for the retarded cryoturbated OM decomposition. The more detailed data are shown in paper II.

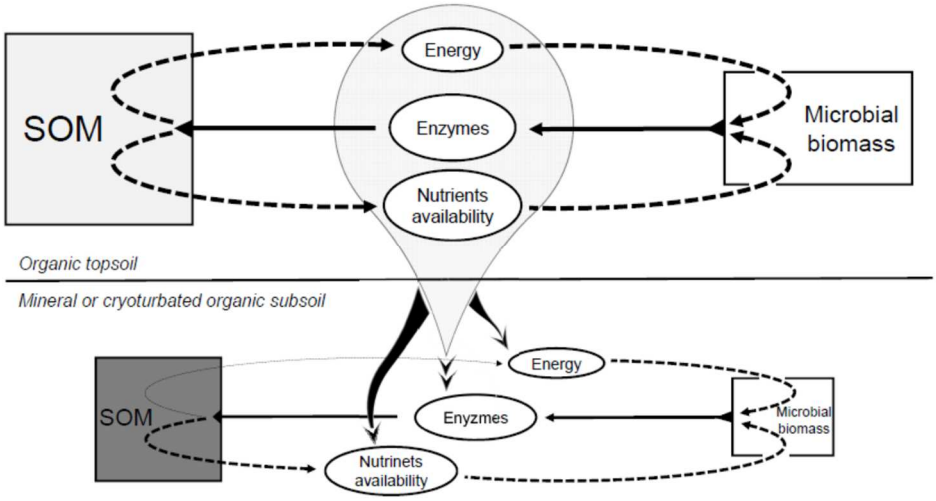

Fig. 6: *Conceptual diagram showing links between the microbial biomass, the soil organic matter (OM), the enzymatic production and the nutrient availability within and between soil horizons. The energy availability for the enzymatic production within each soil horizon is critical; since the low quality OM of subsoil horizons provides no energy, the enzymatic production in subsoil horizons is directly dependent on the fresh carbon flow from the topsoil organic horizon. Along with a fresh carbon, extracellular enzymes and nutrients are transported down the soil profile, thereby affecting enzymatic pools and nutrient availability of subsoil horizons.*

In respect to the second objective, $NaHCO_3$ extractable P was measured at ten different sites across the Arctic in different soil horizons and across different patches. Details about the sites are given in Table 2.

Table 2

Basic characteristics (position, land cover class, list of dominant species, present morphological features and active layer depth) of 10 sampling sites used in throughout this study.

Site	UTM coordinates	Land cover class	Dominant species	Morphological features, Size (cm)	Active layer depth (cm)
Cherskii	57W 0607781, 7706532	Shrubby grass tundra	*Betula exilis, Salix sphenophylla, Carex lugens, Calamagrostis holmii, Aulacomnium turgidum*	Frost boils (D 30-40)	30-70
Cherskii	57W 0606201, 7705516	Shrubby tussock tundra	*Eriophorum vaginatum, Carex lugens, Betula exilis, Salix pulchra., Aulacomnium turgidum*	Frost boils (D 30-40)	35-60

Cherskii	57W 0604930, 7628451	Shrubby lichen tundra	*Betula exilis, Vaccinium uligonosum, Flavocetraria nivalis , Flavocetraria cucullata*	Hummocks (H 30, D 200), barren patches	35-90
Ari Mass	47X 0589707, 8044925	Shrubby moss tundra	*Betula nana, Dryas punctata, Vaccinium uligonosum, Carex arctisibirica, Aulacomnium turgidum*	Polygonal cracks , frost boils (D 50-70), barren patches	60-85
Ari Mass	47X 0588873, 8045755	Shrubby moss tundra	*Cassiope tetragona, Carex arctisibirica, Aulacomnium turgidum*	Polygonal cracks, frost boils (D 50-60)	65-90
Logata	47X 0482624, 8147621	Dryas tundra	*Dryas punctata, Rhytidium rugosum , Hylocomium splendens*	Small hummocks (H 20-30, D 30-100)	35-70
Logata	47X 0479797, 8150507	Grassy moss tundra	*Betula nana, Carex arctisibirica, Hylocomium splendens, Tomentypnum nitens*	Small hummocks (H 25-40, D 30-100)	30-65
Vorkuta	41W 497824, 7436954	Unvegetated		Peat circles	40-70
Vorkuta	41W 497824, 7436954	Dwarf-shrub tundra	*Vaccinium spp., Empetrum hermaphroditum, Betula nana, Salix spp.*		40-70
Vorkuta	41W 497824, 7436954	Heath tundra	*Rubus chamaemorus, Betula nana, Vaccibium spp., Sphagnum spp.*	Peat plateau	40-70
Zackenberg	27X 513935, 8266016	Heath tundra	*Cassiope tetragona, Vaccinium uliginosum, Dryas octopetala, Salix arctica, Carex spp.*		47

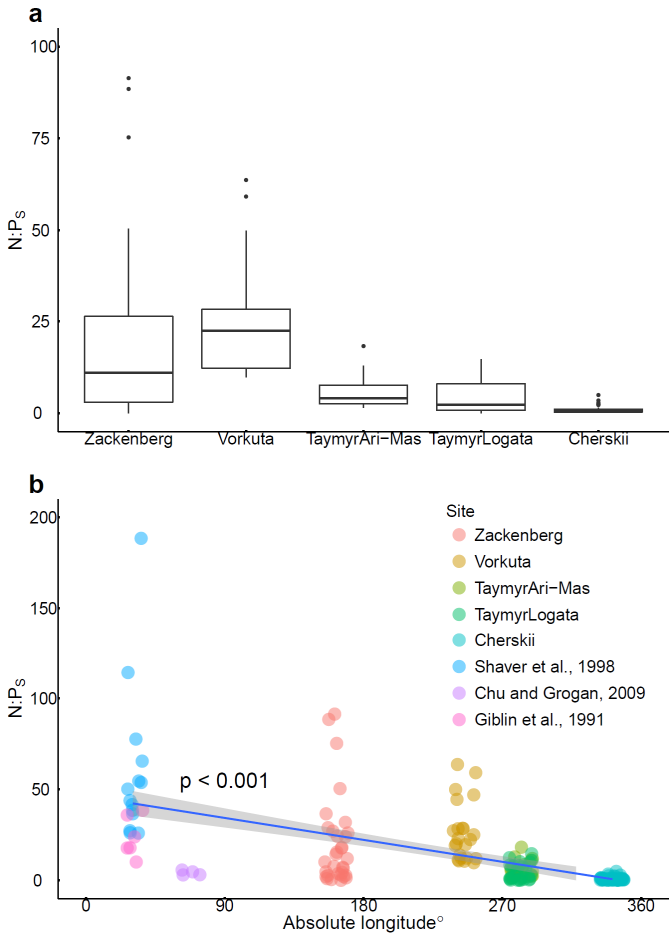

Fig. 7: *Box and whisker plot of available nitrogen to phosphorus ratio in soils from different sites (a). The middle line represents the median, the boxes comprise second and third quartiles, and the Whiskers show the lowest datum still within 1.5 IQR of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile, the outlier values are represented by points. Plot b shows increasing available nitrogen to phosphorus ratio along longitude (absolute longitude = longitude + 180). The linear regression with its probability is plotted (n = 173). The grey area along the regression line represents 95% confidence interval. Our data together with data found in already published literature was used.*

Phosphorus availability differed among different soil horizons at each site. Contrary to our expectations, we observed especially high P availability in cryoturbated organic horizons. Therefore, P limitation in the cryoturbated organic horizons was rare. The greatest difference was found between the different sites

when P availability was expressed as N to P ratio ($N:P_S$). The higher $N:P_S$, the lower P availability relative to N was.

A high P availability relative to N availability in soils in Eastern Siberia (Cherskii) is depicted in Fig. 7a. In contrast, soils in Western Siberia (Vorkuta) have low P relative to N availability and thus it might be expected, that P is likely to limit the soil microbial community (see later). Combining our data with data reported in the literature (Giblin *et al.* 1991; Shaver *et al.* 1998; Chu & Grogan 2009), we found statistically significant longitudinal gradient of the increasing soil P availability with the increasing absolute longitude (Fig 7b, $F_{1,173} = 19.5$, $p<0.001$). For the sake of simplicity, the absolute longitude is defined here as a longitude + 180 degrees (having the range 0 – 360). These data and found relationship were used in **paper IV** to estimate the likelihood of a soil microbial community P limitation in permafrost affected soils.

However, first we had to find out drivers of P limitation of soil microbial communities. This was the third objective of our work, which is covered by **paper III**. In this paper, we developed a method for measurement and calculation of a soil microbial community $C:P_{CR}$. $C:P_{CR,}$ defined as the optimal amount of available soil P relative to organic C, required by a soil microbial community to grow (see Eq. 10). Eighteen different soil samples from various geographic areas and ecosystems including a tundra ecosystem, and taken at different depths were used in this study to identify the potential variability and drivers of a microbial community demand for P ($C:P_{CR}$). This study showed, that $C:P_{CR}$ can range from 26.6 to 465.1 or from 20.9 to 740.7 when accounting for 95% confidence intervals. Unfortunately, $C:P_{CR}$ could not be simply predicted from the microbial community C:P or the available soil P as the stoichiometric theory suggests. $C:P_{CR}$ was only related to the relative abundance of phospholipid fatty acids (PLFA), which reflected simultaneously the microbial community structure and the physiology. Our results suggest complex controls over the microbial community $C:P_{CR}$, which need to be thoroughly tested in the future. Beside, we further found that microbial communities are able to growth on organic C when no P is available in the soil. Microbial communities can temporarily use P storage compounds inside their cells instead of an external available P. We speculate if using P storage compounds can represent a widely adopted strategies across soils that allow a microbial community to temporarily buffer variability in available P.

The objective 4 – determination of the likelihood of a P limitation of a microbial community in permafrost affected soils and its consequence for the OM decomposition, is covered by papers **I and IV**. We found an unexpected effect of P availability on nitrogen mineralization during the OM decomposition, however, only on the most western sampling sites (Vorkuta and Zackenberg, see also Fig. 7). In the paper I, we examined different soil horizons of permafrost affected soils at three sites (two Cherskii sites and Zackenberg site) for gross rates of protein depolymerization, amino acid uptake, N mineralization, NH_4^+ uptake and nitrification. The gross amino acid uptake and the gross N mineralization were used to calculate nitrogen use

efficiency (NUE) of a soil microbial community. NUE represents the relative amount of N incorporated in the microbial biomass, therefore it indicates N limitation of the soil microbial community. NUE is close to 1 when the microbial community is N limited and almost all N is incorporated in the microbial biomass. When NUE decreases below 1, less N is incorporated and more organic N is mineralized. This indicates N being in excess which means no N limited growth of the microbial community. We examined NUE, since we hypothesized from previous observations that the slow rate of the OM decomposition in the cryoturbated organic horizons (Kaiser *et al.* 2007) was partly connected to N limitation of the microbial community.

When all the gross processes were normalized to the unit of total soil N, their slowest rates were found in cryoturbated organic horizons across all sites. When NUE was further calculated, no difference between the horizons was detected, which contradicted to the hypothesized N-limitation of a microbial community in cryoturbated organic horizons. However, significant difference between Cherskii sites and Zackenberg site in NUE was found. It was hypothesized, that P limitation of a soil microbial community is responsible for the lower NUE at Zackenberg site. Unfortunately, we cannot acquire direct evidence for this in paper I. The data were reexamined later and the direct effect of P availability on NUE was confirmed (Fig. 8).

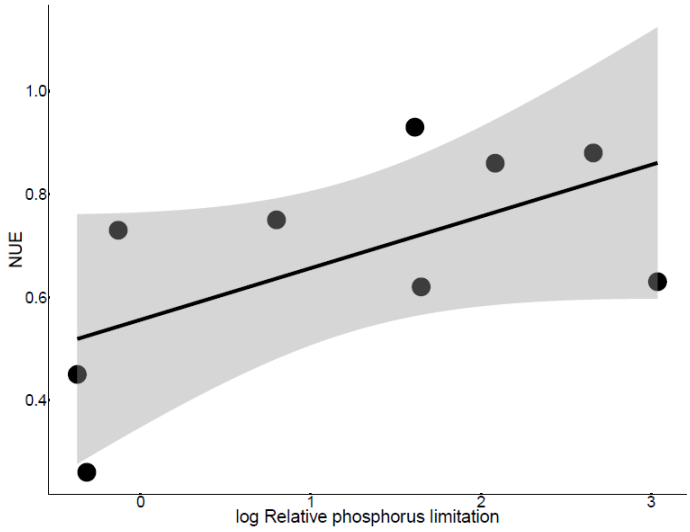

Fig. 8: *Linear relationship between NUE and log relative phosphorus limitation defined as* $N{:}P_{CR}/N{:}P_{sample}$ *across 3 different soil samples (top soil organic, cryoturbated organic and deep soil mineral horizon) taken at 3 different sites, one at Zackenberg site and at Cherskii site (see Tab. 2). For more details see Wild et al. 2013.*

This study was followed by the paper IV in which we quantified P limitation of soil microbial communities across permafrost affected soils listed in the Table 2. Therefore, we calculated nitrogen to phosphorus critical ratio ($N:P_{CR}$) and compared it with the ratio of a soil available N to P ratio. $C:P_{CR}$ estimated in the paper III was used to calculate $N:P_{CR}$. Across all soils, we confirmed the relationship between P availability and N mineralization, which was found in the paper I. A mechanistic explanation of this relationship was given. We further asked whether this relationship could be valid for the entire circumpolar region. The plant primary production is generally considered to be N limited here (McKane *et al.* 1997; Shaver *et al.* 1998; Oechel *et al.* 2000; Dormann & Woodin 2002; Schmidt *et al.* 2002; Weintraub & Schimel 2003; LeBauer & Treseder 2008). If a microbial community was simultaneously P limited, it would support the plant productivity by a mineral N. But this could only be possible if plants and microbes had different demands for P, precisely, the P demand of plants would need to be relatively lower when compared to the soil microbes. Combining our results with the results published in the literature, we showed that $N:P_{CR}$ of plants is higher than $N:P_{CR}$ of soil microbial communities. We than collected all data from all the factorial NP fertilization experiments across the Arctic and run a meta-analysis of the results. We assumed that if a P limited soil microbial community provides a mineral N to the N limited plants, a further addition of N fertilizer will support plants, whereas the addition of P fertilizer will have an opposite effect. It is because the addition of P fertilizer will balance the microbial P limitation upon which N mineralization will decrease and the competition for the available N between plants and microbes will occur.

The result of the meta-analysis supported this theory. P fertilization significantly decreased the plant growth and standing plant biomass, whereas N fertilization significantly increased the standing plant biomass and the plant growth. In terms of the plant N and P concentration, P fertilization had tendency to significantly decrease N to P ratio in plants, while N fertilization had the opposite effect. These results suggested that functional relationship between P availability and N mineralization rate is of great importance in the Arctic tundra areas.

4. FUTURE PROSPECTS

This work pointed out a significant effect of P limitation of soil microbial communities on N mineralization, which consequently affects the plant primary production in the western part of the circumpolar region. Since the results bring only indirect evidences, this effect should be further investigated and directly tested in situ. Our data showed a longitudinal gradient of the decreasing P availability from East Siberia towards Europe and Canada. The hypothesized effect of P availability on organic C decomposition rate and its temperature sensitivity could therefore follow this gradient. This was already suggested by Diáková *et al.* (2016), but it should be thoroughly examined in the future.

More general prospect for future work is to extend the metabolic theory to account for the anaerobic energy yielding reactions. This is a critical limitation of otherwise well-structured theory which restricts its general use for prediction of soil organic C turnover.

Beside these prospects, $C:P_{CR}$ and $N:P_{CR}$ estimation method should be further developed. More soil samples from a wider range of geographic areas would be needed to improve the quality of the dataset and to provide a robust method of $C:P_{CR}$ and $N:P_{CR}$ calculation. The last prospect of the work points to the environmental importance of P storage compounds in microbial cells, which would be further examined.

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Nitrogen dynamics in Turbic Cryosols from Siberia and Greenland

Birgit Wild [a,b,*], Jörg Schnecker [a,b], Jiří Bárta [c], Petr Čapek [c], Georg Guggenberger [d], Florian Hofhansl [a], Christina Kaiser [a,e], Nikolaj Lashchinsky [f], Robert Mikutta [d], Maria Mooshammer [a], Hana Šantrůčková [c], Olga Shibistova [d,g], Tim Urich [b,h,i], Sergey A. Zimov [j], Andreas Richter [a,b,**]

[a] University of Vienna, Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research, Althanstrasse 14, 1090 Vienna, Austria
[b] Austrian Polar Research Institute, 1090 Vienna, Austria
[c] University of South Bohemia, Department of Ecosystems Biology, Branišovská 31, 37005 České Budějovice, Czech Republic
[d] Leibniz Universität Hannover, Institut für Bodenkunde, Herrenhäuser Strasse 2, 30419 Hannover, Germany
[e] International Institute for Applied Systems Analysis (IIASA), Schlossplatz 1, 2361 Laxenburg, Austria
[f] Central Siberian Botanical Garden, Siberian Branch of Russian Academy of Sciences, St. Zolotodolinskaya 101, 630090 Novosibirsk, Russia
[g] VN Sukachev Institute of Forest, Siberian Branch of Russian Academy of Sciences, Akademgorodok, 660036 Krasnoyarsk, Russia
[h] University of Vienna, Department of Ecogenomics and Systems Biology, Althanstrasse 14, 1090 Vienna, Austria
[i] University of Bergen, Department of Biology/Centre for Geobiology, Allégaten 41, 5007 Bergen, Norway
[j] Northeast Scientific Station, Pacific Institute for Geography, Far-East Branch of Russian Academy of Sciences, 678830 Chersky, Republic of Sakha, Russia

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ABSTRACT

Turbic Cryosols (permafrost soils characterized by cryoturbation, i.e., by mixing of soil layers due to freezing and thawing) are widespread across the Arctic, and contain large amounts of poorly decomposed organic material buried in the subsoil. This cryoturbated organic matter exhibits retarded decomposition compared to organic material in the topsoil. Since soil organic matter (SOM) decomposition is known to be tightly linked to N availability, we investigated N transformation rates in different soil horizons of three tundra sites in north-eastern Siberia and Greenland. We measured gross rates of protein depolymerization, N mineralization (ammonification) and nitrification, as well as microbial uptake of amino acids and NH_4^+ using an array of ^{15}N pool dilution approaches. We found that all sites and horizons were characterized by low N availability, as indicated by low N mineralization compared to protein depolymerization rates (with gross N mineralization accounting on average for 14% of gross protein depolymerization). The proportion of organic N mineralized was significantly higher at the Greenland than at the Siberian sites, suggesting differences in N limitation. The proportion of organic N mineralized, however, did not differ significantly between soil horizons, pointing to a similar N demand of the microbial community of each horizon. In contrast, absolute N transformation rates were significantly lower in cryoturbated than in organic horizons, with cryoturbated horizons reaching not more than 32% of the transformation rates in organic horizons. Our results thus indicate a deceleration of the entire N cycle in cryoturbated soil horizons, especially strongly reduced rates of protein depolymerization (16% of organic horizons) which is considered the rate-limiting step in soil N cycling.

* Corresponding author. University of Vienna, Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research, Althanstrasse 14, 1090 Vienna, Austria. Tel.: +43 1 4277 76666.
** Corresponding author. University of Vienna, Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research, Althanstrasse 14, 1090 Vienna, Austria. Tel.: +43 1 4277 76660.
E-mail addresses: birgit.wild@univie.ac.at (B. Wild), andreas.richter@univie.ac.at (A. Richter).

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INTRODUCTION

Arctic soils are commonly affected by cryoturbation, i.e., by a mixing of soil layers due to freeze—thaw processes, and are thus often characterized by horizons of poorly decomposed matter subducted into and surrounded by mineral subsoil (Bockheim, 2007; Tarnocai et al., 2009). Cryoturbated soils are estimated to store 581 Gt of organic carbon that is currently protected from fast decomposition (Tarnocai et al., 2009). Although cryoturbated horizons (Ojj or Ajj) are chemically similar to organic (O) or mineral topsoil (A) horizons, they are usually several hundred to thousand years older (Xu et al., 2009; Hugelius et al., 2010), suggesting that C mineralization is slowed down in cryoturbated horizons. Likewise, N mineralization has been found to be lower in cryoturbated than in all other horizons, i.e., in organic (O), mineral topsoil (A), and mineral subsoil (B) horizons (Kaiser et al., 2007). Cryoturbation thus lead to retardation of soil organic matter (SOM) decomposition in general, and of N mineralization in particular, and this retardation cannot be explained by lower subsoil temperatures alone (Kaiser et al., 2007).

The rate limiting step for soil N cycling is the breakdown of N-rich, high molecular weight organic compounds by extracellular enzymes, especially the depolymerization of proteins (Schimel and Bennett, 2004; Geisseler et al, 2010; Jones and Kielland, 2012). The resulting oligopeptides and amino acids are taken up by microorganisms and are further mineralized to NH_4^+ which, in turn, is the substrate for nitrification (Jones and Kielland, 2012). Nitrogen mineralization is considered an overflow mechanism, i.e., only an excess of N that cannot be used to build up biomass (because another element is limiting) will be mineralized (Schimel and Bennett, 2004). Thus, gross N mineralization rates are expected to increase with increasing N availability, and, consequently, microbial N use efficiency (NUE; the proportion of N taken up that is incorporated into microbial biomass) will decrease.

Nitrogen availability in high latitude ecosystems is generally low (Schimel and Bennett, 2004), but is predicted to increase with global warming. Higher soil temperatures were shown to increase soil microbial activity, resulting in increased net N mineralization, and thus N availability (Nadelhoffer et al., 1991; Hobbie, 1996; Rustad et al., 2001). Additionally, the functional composition of tundra vegetation is expected to shift (Elmendorf et al., 2012), which is likely to alter the availability of N for soil microorganisms by changing patterns in plant N uptake, litter decomposability and competition for N in the rhizosphere (Wookey et al., 2009). An increase in N availability can stimulate plant and microbial growth (e.g., Hobbie et al., 2002; Sistla et al., 2012), and also SOM decomposition. In a long-term fertilization study in Alaskan tundra, Mack et al. (2004) observed that fertilization not only increased plant production, but also SOM decomposition, overall resulting in a net loss of C from the system. This suggests that the emission of CO_2 from Arctic soils could be amplified by increased N availability. The effect of N additions on SOM decomposition is, however, highly variable. Across sites, and across soil horizons,

both stimulation and inhibition of SOM decomposition by N addition have been observed (Lavoie et al., 2011). To predict the overall effects of N availability on SOM decomposition in Arctic soils in a future climate, an in-depth understanding of all steps of N cycling in the soil profile is necessary. So far most studies have exclusively focused on N mineralization and nitrification, but the depolymerization step of highmolecular weight organic N, which has been suggested to be rate-limiting, has not received much attention.

While many studies have investigated N dynamics in organic and mineral topsoil horizons, not much is known about mineral and cryoturbated horizons in the subsoil. In spite of the large amounts of poorly decomposed SOM in cryoturbated horizons, and in spite of the importance of N for SOM decomposition, we are only aware of one study investigating N dynamics in cryoturbated horizons. This study showed a reduction in N mineralization rates in cryoturbated compared to organic horizons (Kaiser et al., 2007). These low N mineralization rates could either have been the consequence of reduced protein depolymerization rates, or of a higher N demand of the microbial community. In the latter case, protein depolymerization would be similar in all horizons, but N mineralization would be lower in cryoturbated horizons, indicating that the microbial community needed a higher proportion of the available N for growth, and thus had a higher NUE.

We here report on microbial N transformations and NUE across different horizons in the active layer of tundra soil. We hypothesized that the observed reduction of gross N mineralization in cryoturbated horizons compared to organic horizons was due to a reduction in protein depolymerization, not due to a higher NUE of the microbial community. Since the capacity to depolymerize proteins constitutes a property of the microbial community, we further investigated the microbial community structure in different soil horizons, with the goal to identify groups that may be responsible for individual N transformations.

To achieve these goals, we sampled Turbic Cryosols (Turbels) at three sites in Siberia and Greenland and measured gross rates of protein depolymerization, microbial amino acid uptake, N mineralization, NH_4^+ uptake and nitrification using an array of ^{15}N pool dilution approaches. Additionally, we estimated the microbial community composition using phospholipid fatty acids (PLFAs) as biomarkers.

1. MATERIAL & METHODS

1.1. Sampling sites

We compared soils from three different sites; (1) The heath tundra site was located in eastern Greenland close to the Zackenberg Research Station (typical tundra subzone; 74°29' N, 20°32' W) on sedimentary bedrock (sandstone). It was dominated by *Cassiope tetragona, Vaccinium uliginosum, Dryas octopetala, Salix arctica,* and *Carex* sp., with lichens between the dwarf shrubs. (2) The shrub tundra

site was in north-eastern Siberia close to the town of Chersky (southern tundra subzone; 68°45' N, 161 °36' E) on aeolian late Pleistocene sediment. It was located in a shrubby moss lichen tundra dominated by *Betula exilis, Vaccinium uliginosum, Flavocetraria nivalis, Flavocetraria cucullata* and *Aulacomnium turgidum,* with sparse *Larix gmelinii* trees. (3) The tussock tundra site was approximately 80 km north of Chersky (southern tundra subzone; 69°26' N, 161°44' E) on aeolian late Pleistocene sediment. It was dominated by *Eriophorum vaginatum, Carex lugens, B. exilis, Vaccinium vitis-idaea, Aulacomnium turgidum,* and *Dicranum* sp.

All soils were classified as Turbic Cryosols according to the World Reference Base for Soil Resources (1USS Working Group WRB, 2007) or Turbels according to the US Soil Taxonomy (Soil Survey Staff, 1999). The active layer at the time of sampling was 47 cm (heath tundra, Greenland), 73 cm (shrub tundra, Siberia; under frost boils) and 72 cm (tussock tundra, Siberia; under frost boils).

At each site, we took samples from the active layer of three replicate soil pits. We sampled organic layers (O, including OA horizons) from the soil surface (further termed “organic horizon"). We then took samples from pockets of organic matter (Ojj) or mineral topsoil (Ajj) that was buried within the subsoil. Such buried horizons, caused by cryoturbations, i.e., by subduction of organic matter or mineral topsoil into the subsoil due to freeze—thaw processes (Bockheim, 2007), are common in permafrost soils. Sampled pockets of cryoturbated matter were between 20 and 50 cm from the soil surface (further termed “cryoturbated horizon”). We further sampled the mineral soil surrounding the cryoturbated pockets (A, AB, B or Cg horizons, at depths from 10 to 60 cm from the surface). These horizons are collectively termed “mineral horizon". Living roots were carefully removed and samples were kept cool until analysis.

All samplings took place in August 2010. We are aware of the fact that N cycling and N availability show seasonal variation in Arctic soils (e.g., Weintraub and Schimel, 2005a,b; Edwards et al., 2006). Data presented here do not reflect these variations, but represent N cycling in the late growing season.

1.2. Basic characterization and nutrient concentrations

Concentrations of ammonium and nitrate were determined photometrically in 1 M KCl extracts following Kandeler and Gerber (1988) and Miranda et al. (2001), respectively. Total dissolved nitrogen (TDN) was measured with a DOC/TN analyzer (Elementar LiquiToc II or Shimadzu TOC-$V_{CPH/CPN}$/TNM-1) in 0.5 M K_2SO_4 or 1 M KCl extracts (samples from Siberia and Greenland, respectively), and dissolved organic N (DON) was calculated by subtracting NH_4^+ and NO_3^- from TDN. Extraction with K_2SO_4 or KCl was previously shown to yield similar DON recovery (Jones and Willett, 2006). For determination of inorganic phosphate, soil samples were extracted with 0.5 M $NaHC0_3$ (Olsen et al., 1954) and measured photometrically with the molybdate-ascorbic acid method (Murphy and Riley, 1962). Contents of organic C and total N

were determined in dried and ground samples using elemental analysis-isotope ratio mass spectrometry (EA-IRMS), either with a CE Instrument EA 1110 elemental analyzer coupled to a Finnigan MAT DeltaPlus IRMS with a Finnigan MAT ConFlo II Interface, or with an Isoprime EA-IRMS system. Samples from Siberia contained traces of carbonate and were acidified in HCl atmosphere and neutralized over NaOH before EA-IRMS analysis. For determination of total P content, samples were amended with a mixture of concentrated $HClO_4$ and HNO_3 (1:4), heated stepwise to 160 ^{C}C and 220 °C for digestion, cooled to room temperature, filtered (Whatman 40 ashfree cellulose filter) and measured with inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer Optima 3000 XL) against external standards. Ratios of C, N and P were calculated as mass ratios. pH values were determined in suspensions of dried soil in de-ionized water (1:2.5; weight:volume).

1.3. Gross rates of N transformations

Gross rates of protein depolymerization and amino acid uptake were determined using a ^{15}N pool dilution assay as described by Wanek et al. (2010), with slight modifications to account for the low amino acid concentrations in soils. Briefly, a mixture of 20 ^{15}N labeled amino acids (>98 at%, Spectra and Cambridge Isotope Laboratories) was dissolved in 10 mM $CaSO_4$ and added to 2 g of field-moist soil in duplicates. Per sample, 500 µl solution containing 2.5 µg total amino acids were applied. After incubation for 10 or 30 min at 7 °C, activities were stopped with 19.5 ml of 10 mM $CaS0_4$ containing 3.7% of formaldehyde. Samples were extracted for 5 min and either allowed to settle for 10 min (both Siberian sites) or centrifuged for 5 min at 10 845 g (Greenland heath tundra site). Samples were filtered through synthetic wool and GF/C filters (Whatman), and loaded on cation exchange cartridges (OnGuard II H lcc cartridges, Dionex, cleaned with 3 M ammonia and re-generated with 1 M HCl before use). After application of the samples, cartridges were washed with 10 ml distilled water, stabilized with 5 ml 5% methanol and stored cool until elution. With each batch of samples, blanks and amino acid standards were processed to correct for losses due to ion exchange. After elution of the amino acids from the cartridges with 30 ml 3 M ammonia, an internal standard was added to the samples (1 µg of nor-valine, nor-leucine and para-chloro-phenylalanine each, Sigma—Aldrich). Samples were dried with rotary evaporation, re-dissolved in 1.5 ml 20% ethanol and dried in a SpeedVac system. Finally, samples were derivatized with chloroformate before analysis with a gas chromatography—mass spectrometry system (GC—MS), consisting of a CTC autosampler (CTC Analytics) and a Trace GC Ultra coupled to a quadrupole mass spectrometer (DSQ II; Thermo Scientific). Two µl of sample were injected in splitless mode (injector temperature 270 °C), separated on an Equity-1701 column (30 m x 0.25 mm x 1 µm; Sigma—Aldrich) with 1 ml min^{-1} He as carrier gas (GC method: 105 °C for 1 min, 6 °C min^{-1} to 135 °C, 3 °C min^{-1} to 180 °C, 20 °C min^{-1} to 260 °C, 260 ^{C}C for 35 min) and detected in Selected Ion

Monitoring mode. Concentrations of alanine, valine, leucine, isoleucine, proline, tryptophane, phenylalanine and tyrosine were calculated using calibrations against external standards, and the abundance of ^{15}N in each of these amino acids was calculated based on peak areas of fragments characteristic for ^{14}N and ^{15}N (for fragments see Wanek et al., 2010), using a calibration against standards of different ^{15}N abundance.

Gross rates of N mineralization (ammonification), NH_4^+ uptake and nitrification were determined as described by Kaiser et al. (2011) by adding 500 µl ^{15}N labeled $(NH_4)_2SO_4$ (N mineralization and NH_4^+ uptake) or KNO_3 (nitrification) to duplicates of 2 g of field-moist soil (0.125 mM, 10 at%. Sigma—Aldrich). Samples were incubated for 4 h and 24 h at 7 °C, extracted with 13 ml of 2 M KCl for 30 min, and filtered through ashfree filter paper (Whatman 40 ashfree cellulose filter). To stabilize the extracts, 20 µl 5 mM phenylmercuric acetate were added, and samples were frozen until further processing. For N mineralization and NH_4^+ uptake, NH_4^+ was diffused into acid traps and measured with an EA-IRMS system consisting of a CE Instrument EA 1110 elemental analyzer coupled to a Finnigan MAT DeltaPlus IRMS with a Finnigan MAT ConFlo II Interface. For nitrification, NH_4^+ was removed from the samples and NO_3^- converted to NH_4^+ before diffusion into acid traps and EA-IRMS analysis (Mooshammer et al., 2012). Gross rates were based on differences in concentration and isotopic composition of NH_4^+, NO_3^- or amino acids between two time points (e.g., 4 h and 24 h) and were calculated according to the equations described in Wanek et al. (2010).

As an indicator for microbial N limitation, we calculated the efficiency of microorganisms to use amino acid N for biomass growth (N use efficiency, NUE) by comparing gross rates of amino acid uptake and N mineralization:
NUE = (gross amino acid uptake - gross N mineralization)/gross amino acid uptake.

1.4. Phospholipid fatty acid (PLFA) analysis

For analysis of PLFAs, samples were stored frozen (Greenland heath tundra) or in RNAlater (both Siberian sites; Schnecker et al., 2012). Phospholipid fatty acids were extracted from 1 g of soil with chloroform/methanol/citric acid buffer and purified on silica columns (LC-Si SPE, Supelco) using chloroform, acetone and methanol (Frostegård et al., 1991; with the modifications described by Kaiser et al., 2010). After addition of methyl-nonadecanoate as internal standard, PLFAs were converted to fatty acid methyl esters (FAMEs) by alkaline methanolysis. Samples were analyzed on a Thermo Trace GC with FID detection: 1 µl per sample was injected in splitless mode (injector temperature 230 °C) and separated on a DB-23 column (Agilent; GC method:70 °C for 1.5 min, 30 °C min^{-1} to 150 °C, 150 °C for 1 min, 4 °C min^{-1} to 230 °C, 230 °C for 15 min) with 1.5 ml min^{-1} He as carrier. Individual FAMEs were identified using qualitative standard mixes (37 Comp. FAME Mix and Bacterial Acid Methyl Esters CP Mix, Supelco) and quantified by comparison with the

internal standard. We used 18:1ω9, 18:2ω6,9 and 18:3ω3,6,9 fatty acids as biomarkers for fungi, i15:0, a15:0, i16:0, i17:0 and a17:0 for gram positive bacteria, cy17:0 (9/10), cy19:0 (9/ 10), 16:1ω5, 16:lω7, 16:1ω9 and 18:1ω7 for gram negative bacteria, and 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, i14:0, 16:lω10, 16:1ω11, 17:1ω6 and 10Me16:0 as unspecific markers (Kaiser et al., 2010).

1.5. Statistics

To test for significant differences between sites and horizons, we applied two-way ANOVAs and Tukey HSD tests (after transformation, if necessary) or Kruskal—Wallis tests with unpaired Mann Whitney U tests as post-hoc tests if normal distribution and homoscedasticity could not be achieved. We additionally performed a Principal Component Analysis including gross N transformation rates (per g total N), pH values and the relative abundance of microbial groups. Samples with missing values were omitted from the Principal Component Analysis. Correlations were tested using Spearman's rank correlations. All statistics were performed in R 2.15 (R Development Core Team, 2012).

2. RESULTS

Organic C, total N, and total P, as well as the C/N ratio, decreased significantly from organic to cryoturbated and mineral horizons (Tables 1 and 2). pH-Values were in the range of 4.3—5.5 for organic horizons and significantly higher in cryoturbated and mineral horizons (5.1-6.1 and 5.3-6.4, respectively). Microbial biomass (estimated as the total amount of PLFAs per g DW) decreased significantly from organic to cryoturbated and mineral horizons (Supplementary Fig. 1, Table 2), mainly due to differences in SOM content.

In all horizons, the pool of dissolved N was dominated by organic N (Fig. 1). The relative contribution of DON to the TDN pool was similar in all horizons at each site, although absolute concentrations were significantly lower in mineral than in organic horizons (Table 2). We did, however, observe significant variations in the composition of the TDN pool between sites. Dissolved organic N accounted for 85 ± 3% (mean ± standard error of all horizons) and 86 ± 5% of the TDN pool at the heath tundra and tussock tundra sites, but only for 58 ± 7% at the shrub tundra site (Fig. 1). Also concentrations of inorganic phosphate varied significantly between sites. Inorganic phosphate was lower by a factor of ten at the heath tundra site in Greenland than at both Siberian sites, but did not show significant differences between horizons (Fig. 1).

Gross rates of protein depolymerization, amino acid uptake, N mineralization, NH_4^+ uptake and nitrification, expressed per g DW, were generally highest in organic horizons, and decreased significantly from organic to cryoturbated and mineral

Table 1
C, N and P content, C/N and N/P ratios (mass ratios), and pH values of organic, cryoturbated (cryot.) and mineral horizons of heath tundra (Greenland), tussock tundra (Siberia) and shrub tundra (Siberia). Values represent means (±standard error).

Site	Horizon	C (%)	N (%)	P (%)	C/N	N/P	pH
Heath	Organic	15.76 (0.86)	0.93 (0.06)	0.102 (0.006)	17.14 (1.95)	9.18 (0.39)	5.29 (0.10)
	Cryot.	9.35 (0.95)	0.65 (0.00)	0.112 (0.007)	14.46 (1.46)	5.82 (0.37)	6.02 (0.02)
	Mineral	3.94 (1.01)	0.29 (0.08)	0.074 (0.025)	13.81 (0.61)	4.86 (1.57)	6.16 (0.11)
Tussock	Organic	22.15 (1.57)	1.00 (0.05)	0.155 (0.019)	22.38 (2.18)	6.68 (1.09)	5.13 (0.05)
	Cryot.	15.30 (4.84)	0.81 (0.18)	0.112 (0.011)	18.23 (1.76)	7.36 (1.81)	5.57 (0.28)
	Mineral	2.08 (0.40)	0.14 (0.02)	0.046 (0.039)	14.33 (0.40)	9.40 (7.42)	5.50 (0.02)
Shrub	Organic	26.63 (5.98)	0.96 (0.18)	0.115 (0.005)	27.46 (1.61)	8.36 (1.57)	4.64 (0.23)
	Cryot.	9.69 (2.57)	0.64 (0.20)	0.121 (0.084)	15.46 (0.88)	8.01 (3.90)	5.80 (0.29)
	Mineral	2.50 (0.85)	0.16 (0.03)	0.061 (0.022)	14.66 (2.96)	4.55 (2.66)	5.54 (0.19)

horizons (Supplementary Fig. 2, Table 2). All N transformation rates were significantly and positively correlated with C and N content (Table 3). In order to assess horizon-specific differences in transformation rates that were not related to differences in SOM content, we calculated all gross N transformation rates per g total soil N. Cryoturbated horizons still exhibited significantly lower gross rates of protein depolymerization, amino acid uptake, N mineralization and nitrification per g total N than organic horizons, on average accounting for 16% (protein depolymerization), 32% (amino acid uptake), 27% (N mineralization) and 31% (nitrification) of the respective rates in organic horizons. In the case of N mineralization and nitrification, rates in cryoturbated horizons were even significantly lower than in mineral horizons (Fig. 2, Table 2).

In spite of the differences in absolute N transformation rates, NUE did not differ significantly between horizons (Table 2), and was not correlated with soil C/N ratio ($p = 0.130$, $R^2 = 0.10$). We did, however, observe significant differences in NUE between sites. NUE was highest at the tussock tundra site in Siberia, where 90% of amino acid N taken up was incorporated into microbial biomass, and only 10% were mineralized to NH_4^+. NUE was lower at both other sites, with 66% incorporation at the shrub tundra site in Siberia, and 51% at the heath tundra site in Greenland (Fig. 4).

To investigate possible relationships between N transformations and microbial community composition across horizons and sites, we performed a Principle Component Analysis, including gross N transformation rates (per g total N), the relative abundances of fungi, gram negative and gram positive bacteria

Table 2
Significance of differences between sites (heath tundra, Greenland; tussock tundra, Siberia; shrub tundra, Siberia) or between horizons (organic; cryoturbated; mineral), derived from two-way ANOVA with Tukey HSD test, or Kruskal—Wallis tests with Mann—Whitney U tests as post-hoc. Different letters indicate $p < 0.05$, with *"a"* denoting the highest values.

Parameter	Unit	Between sites				Between horizons			
		Sign.	Heath	Tussock	Shrub	Sign.	Organic	Cryot.	Mineral
C	%	n.s.				***	a	b	c
N	%	n.s.				***	a	a	b
P	%	n.s.				*	a	ab	b
C/N	g C g^{-1} N	n.s.				***	a	b	b
N/P	g N g^{-1} P	n.s.				n.s.			
pH		**	a	b	b	***	b	a	a
Total PLFAs	µmol g^{-1} DW	n.s.				***	a	b	c
TDN	µg N g^{-1} DW	n.s.				***	a	b	b
DON	µg N g^{-1} DW	n.s.				**	a	b	b
NH_4^+	µg N g^{-1} DW	*	b	ab	a	*	a	ab	b
NO_3^-	µg N g^{-1} DW	n.s.				n.s.			
DON	% of TDN	**	a	a	b	n.s.			
Inorganic phosphate	µg P g^{-1} DW	**	b	a	a	n.s.			
Gross protein depolymerization	µg N g^{-1} DW d^{-1}	n.s.				***	a	b	b
Gross amino acid uptake	µg N g^{-1} DW d^{-1}	n.s.				***	a	b	b
Gross N mineralization	µg N g^{-1} DW d^{-1}	***	a	b	a	***	a	b	b
Gross NH_4^+ uptake	µg N g^{-1} DW d^{-1}	*	ab	b	a	***	a	a	b
Gross nitrification	µg N g^{-1} DW d^{-1}	n.s.				**	a	b	b
Gross protein depolymerization	mg N g^{-1} N d^{-1}	*	b	ab	a	*	a	b	ab
Gross amino acid uptake	mg N g^{-1} N d^{-1}	n.s.				*	a	b	ab
Gross N mineralization	mg N g^{-1} N d^{-1}	***	a	b	a	***	a	b	a
Gross NH_4^+ uptake	mg N g^{-1} N d^{-1}	**	b	b	a	n.s.			
Gross nitrification	mg N g^{-1} N d^{-1}	**	b	ab	a	*	a	b	a
NUE		**	b	a	b	n.s.			
Principal Component 1		***	b	a	a	***	a	b	a
Principal Component 2		*	b	a	b	n.s.			

Levels of significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., not significant.

(in % of total PLFAs), and pH values (Fig. 3). Principal Component 1 accounted for 47% of the variation in the data set, and was positively connected to all gross N transformation rates (factor loadings 0.42 for protein depolymerization, 0.38 for amino acid uptake, 0.21 for N mineralization, 0.27 for NH_4^+ uptake and 0.38 for nitrification), and thus represents an aggregated parameter of N transformation activity. Principal Component 1 separated all cryoturbated horizons and the mineral horizons of the Greenland heath tundra site from organic and the Siberian mineral horizons. These findings were supported by a two-way ANOVA that showed significantly lower values of Principal Component 1 for cryoturbated horizons compared to organic and mineral horizons, and for the Greenland heath tundra site compared to both Siberian sites (Table 2). Principal Component 2 (16% of variation), in contrast, was positively connected to gross rates of protein depolymerization and microbial uptake of amino acids (factor loadings 0.13 and 0.19), but negatively connected to gross rates of N mineralization, NH_4^+ uptake and nitrification (-0.55, -0.43 and -0.31), thus separating organic from inorganic N transformation processes. Principal Component 2 was also significantly correlated with NUE which reflects the allocation of amino acid N to mineralization ($p = 0.004$, $R^2 = 0.34$).

The microbial groups of fungi, gram negative and gram positive bacteria contributed differently to Principal Components 1 and 2. Principal Component 1 was positively connected to the relative abundances of fungi and gram negative bacteria (factor loadings 0.37 and 0.27), and negatively to gram positive bacteria (-0.30). Principal Component 2 was positively connected to fungi (0.26) and negatively to both gram negative and gram positive bacteria (-0.27 and -0.35).

We further investigated possible relationships between individual N transformation processes and microbial groups using Spearman's rank correlations, and found that gross rates of protein depolymerization, amino acid uptake and nitrification (per g total N) were significantly correlated with the relative abundance of fungi, and that gross rates of amino acid uptake and nitrification were significantly correlated with gram negative bacteria (Table 3).

3. DISCUSSION

High-latitude systems are usually characterized by low N availability that limits both plant and microbial growth (Hobbie et al., 2002; Sistla et al., 2012). Plants and microorganisms rapidly immobilize all reactive N forms small enough for uptake, in particular amino acids (Jones and Kielland, 2002; Näsholm et al., 2009) and oligo-peptides (Hill et al., 2011; Farrell et al., 2013), and incorporate the N into their biomass, with a minimum of microbial overflow mineralization toNH_4^+, and further transformation to NO_3^-. Although organic N forms represent a major source of N for both plants and microorganisms in the Arctic, we know little about the steps controlling their availability, i.e., protein depolymerization.

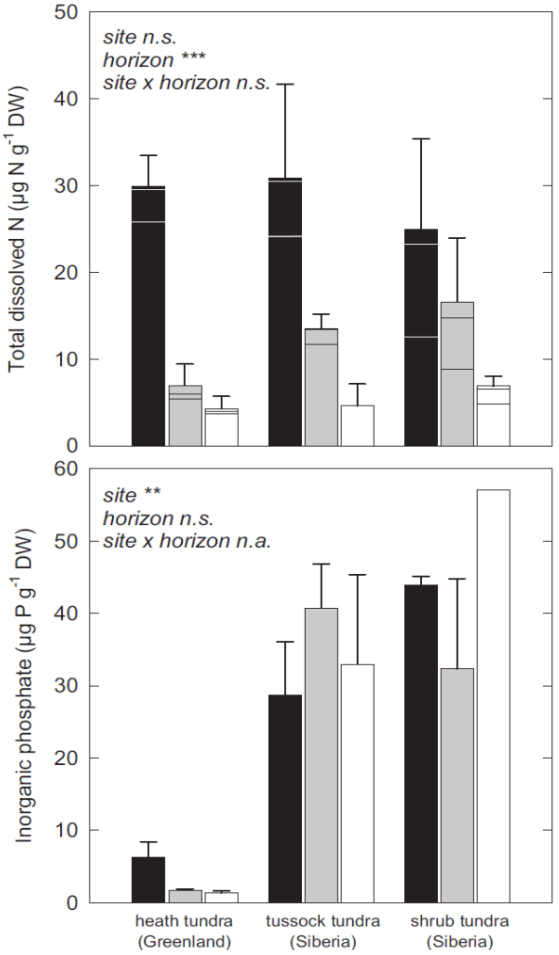


Fig. 1. Upper panel: Total dissolved N in soil extracts of organic (black bars), cryoturbated (gray bars) and mineral (white bars) horizons of three tundra sites. Bars are separated into DON (lowest part). $\mathbf{NH_4^+}$ (middle part) and $\mathbf{NO_3^-}$ (upper part). Concentrations of $\mathbf{NH_4^+}$ and DON could not be determined in the mineral horizons of the tussock tundra site. Lower Panel: Concentrations of inorganic phosphate in soil extracts of organic (black bars), cryoturbated (gray bars) and mineral (white bars) horizons of three tundra sites. All bars represent means ± standard error. Levels of significance: ***. $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., not significant; n.a., not analyzed (two-way ANOVA or Kruskal-Wallis test).

3.1. Nitrogen cycling in cryoturbated horizons

In the Arctic soils studied, gross rates of protein depolymerization, but also microbial amino acid uptake, N mineralization and nitrification were significantly lower in cryoturbated than in organic horizons, on average accounting for only 26% of the rates in organic horizons (Fig. 2). This corroborates our hypothesis that the whole sequence of N transformations, starting with the rate-limiting step of protein depolymerization, is decelerated in cryoturbated compared to organic horizons.

Protein depolymerization limits the amount of amino acids available for microbial uptake, and with it the potential for microbial growth and N mineralization. Severe N deficiency can even limit the production of extracellular enzymes that depolymerize complex organic compounds (Weintraub and Schimel, 2005a; Wallenstein et al., 2009; Sistla et al., 2012), including N-containing macromolecules such as proteins. While we did not directly estimate N availability in cryoturbated horizons, lower protein depolymerization rates compared to organic horizons point to a reduced N availability

in cryoturbated horizons. We therefore suggest that the slow decomposition of cryoturbated SOM (Kaiser et al., 2007; Xu et al., 2009; Hugelius et al., 2010) might be connected to N limitation of enzyme production. In this case, an increase in protein depolymerization with climate change (Weedon et al., 2011; Brzostek et al., 2012) could facilitate the decomposition of cryoturbated SOM, and lead to higher CO_2 emissions from Arctic soils.

3.2. Microbial communities and nitrogen transformations

The differences in N cycling across soil horizons and sites were likely caused, at least in part, by differences in composition and N demand of the microbial communities. The rate-limiting step in N cycling, protein depolymerization, requires specific enzymes, i.e., proteases, that are produced by a range of bacteria and fungi. We here found that gross protein depolymerization rates were significantly

Table 3
Correlation analysis of soil C and N content (in % of DW), microbial abundances (in % of total PLFAs) and gross rates of protein depolymerization (Protein depot), microbial amino acid uptake (AA uptake), N mineralization, microbial NH_4^+ uptake and nitrification, measured with a set of ^{15}N pool dilution approaches. For correlation with soil C and N content, gross N transformation rates were expressed in μg N g^{-1} DW d^{-1}. For correlation with microbial groups, and with each other, gross N transformation rates were corrected for the differences in SOM content between soil horizons and expressed in mg N g^{-1} N d^{-1} Significance of correlations and correlation coefficients were determined using Spearman's rank correlations.

	Protein depol.	AA uptake	N mineralization	NH_4^+ uptake	Nitrification
C	+0.577**	+0.621**	+0.445*	+0.565**	+0.639**
N	+0.519*	+0.541**	+0.418*	+0.539**	+0.650**
Fungi	+0.499*	+0.540**	+0.367	+0.176	+0.428*
Gram negative	+0.368	+0.483*	+0.304	+0.126	+0.528*
Gram positive	−0.305	−0.396	−0.166	−0.092	−0.276
AA uptake	+0.792***				
N mineralization	+0.237	+0.264			
NH_4^+ uptake	+0.356	+0.244	+0.532**		
Nitrification	+0.597**	+0.399	+0.424*	+0.394	

Levels of significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

correlated with the relative abundance of fungi (Table 3). Fungi are able to produce a wide range of extracellular enzymes (Baldrian et al., 2011; Schneider et al., 2012) and are involved in the degradation of many complex organic molecules including cellulose and lignin (de Boer et al., 2005; Strickland and Rousk, 2010). Protein breakdown in particular has been assigned to ecto- and ericoid mycorrhizal fungi in

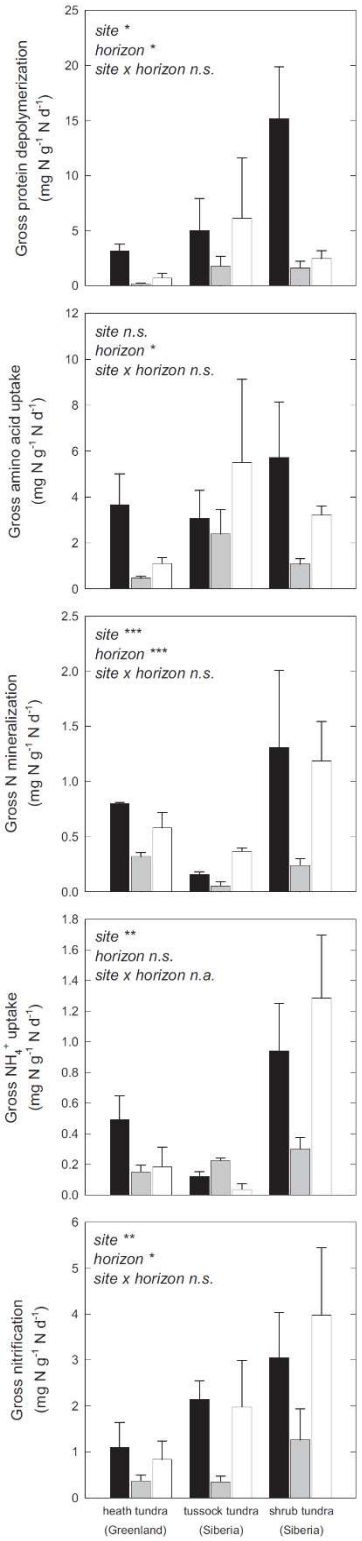
high latitude systems (Read and Perez-Moreno, 2003). A low abundance of fungi in cryoturbated horizons might thus not only contribute to the low protein depolymerization rates, but also generally to the retarded decomposition of cryoturbated SOM.

Protein depolymerization was closely correlated with microbial amino acid uptake (Table 3). We found a similar close correlation for N mineralization and NH_4^+ uptake. This suggests that the microbial uptake of amino acids and NH_4^+ was limited by the respective production rates (protein depolymerization and N mineralization), indicating a high demand of the microbial biomass for N. A tight coupling of production and consumption rates of both amino acids and NH_4^+ has already been demonstrated for decomposing beech leaf litter (Mooshammer et al., 2012). While N mineralization obviously limited the amount of NH_4^+ available for microbial uptake, N mineralization itself was not correlated with any upstream process such as protein depolymerization or amino acid uptake, or with any microbial group (Table 3). Nitrogen mineralization is the microbial de-amination of organic N and excretion of NH_4^+. The potential for N mineralization thus depends on the uptake of organic N by microorganisms. Microorganisms, however, can directly control the amount of N mineralized, and will only mineralize an excess of N that is not needed for growth or other cellular processes (Schimel and Bennett, 2004). Actual N mineralization rates therefore reflect both N availability and N demand for growth by the microbial community.

Nitrification, in contrast, was significantly correlated with the relative abundances of gram negative bacteria and fungi (Table 3). Nitrification requires a specific set of enzymes that oxidize ammonium over nitrite to nitrate, and is restricted to specific microbial groups. Autotrophic nitrification was found in few groups of archaea and gram negative bacteria (Hayatsu et al., 2008; Schleper, 2010; Alves et al., 2013). The abundance of gram negative bacteria might therefore influence nitrification rates, as already demonstrated for savanna and forest soils (Baiser and Firestone, 2005). Heterotrophic nitrification, in contrast, is more widespread among microorganisms, but was mainly connected to fungi, particularly in acidic soils (Hayatsu et al., 2008).

3.3. Nitrogen use efficiency of the microbial community

In addition to low protein depolymerization rates in cryoturbated horizons, the observed reduction in N mineralization may also have been caused by a higher allocation of the available N to growth, and thus by a higher NUE. Our results demonstrate, however, that microbial NUE did not differ significantly between horizons (Fig. 4), indicating that the microbial communities in all horizons, including the cryoturbated ones, had a similar demand for N. Furthermore, NUE was not



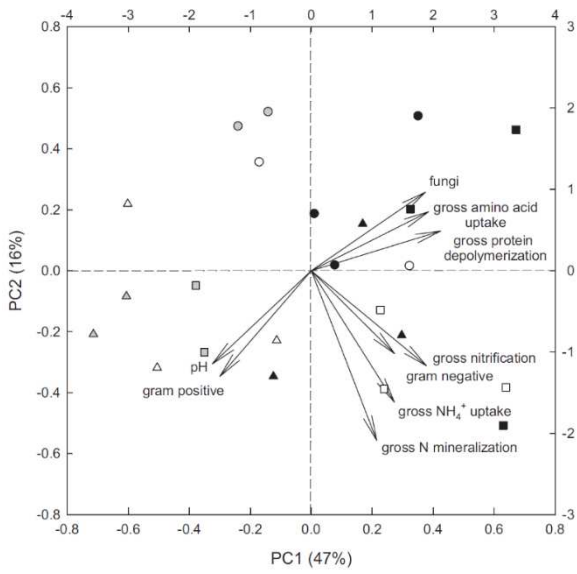


Fig. 3. Ordination of organic (black), cryoturbated (gray) and mineral (white) horizons of heath tundra (Greenland; triangles), tussock tundra (Siberia; circles) and shrub tundra (Siberia; squares) using Principal Component Analysis. Data include gross rates of protein depolymerization, microbial amino acid uptake, N mineralization, microbial $\mathbf{NH_4^+}$ uptake and nitrification (per g total N to correct for differences in SOM content between horizons), as well as pH values and the relative abundances of fungi, gram positive and gram negative bacteria (in % of total PLFAs).

Fig. 2. Gross rates of protein depolymerization, microbial amino acid uptake, N mineralization, microbial NH_4^+ uptake and nitrification (per g total N), of organic (black bars), cryoturbated (gray bars) and mineral (white bars) horizons of three tundra sites. Rates were measured using a set of ^{15}N pool dilution approaches. Bars represent means ± standard error. Levels of significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., not significant; n.a., not analyzed (two-way ANOVA or Kruskal-Wallis test).

correlated with soil C/N ratios, suggesting that the decrease in C/N ratio from organic to mineral horizons (Table 1) was likely offset by changes in C and N availability with soil depth. Montane et al. (2007) showed for a mountain grassland soil, while persistence of soil C was constant across the soil profile, persistence of soil that while persistence of soil C was constant across the soil profile, persistence of soil N increased with soil depth, probably due to differences in chemical composition or in binding to soil minerals (for a recent review on SOM persistence see Schmidt et al., 2011).

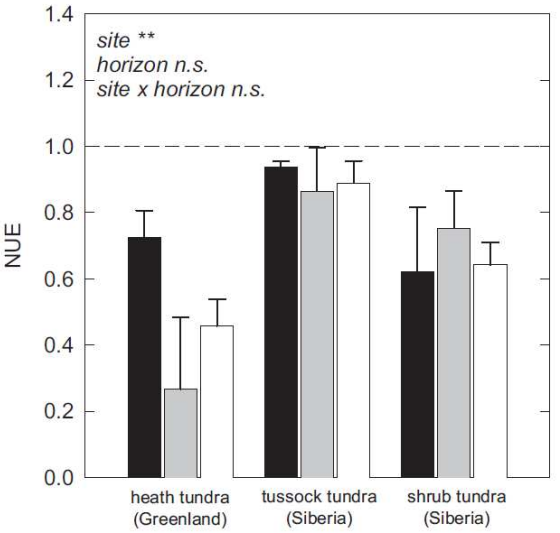


Fig. 4. Nitrogen use efficiency (NUE) of organic (black bars), cryoturbated (gray bars) and mineral (white bars) horizons of three tundra sites. NUE was calculated as the proportion of amino acid N taken up by microorganisms that was not mineralized to $\mathbf{NH_4^+}$. Bars represent means ± standard error. Levels of significance: ***, p < 0.001; **, *p* < 0.01; *, p < 0.05; n.s., not significant (two-way ANOVA).

NUE was generally rather high (as expected for N-limited systems), but differed significantly between sites (Fig. 4). NUE was significantly higher and gross N mineralization significantly lower at the tussock tundra site in Siberia than at the heath tundra site in Greenland, indicating that the microbial community at the tussock tundra site needed a higher proportion of the available N for growth. Size and composition of the TDN pool, as well as gross protein depolymerization and amino acid uptake rates were similar at both sites (Table 2), so it can be assumed that N availability for microorganisms was similar — pointing to the fact that N was not the main limiting element at the Greenland heath tundra site.

Since concentrations of inorganic phosphate were significantly lower at the Greenland heath tundra than at both Siberian sites (Fig. 1), we suggest that microbial growth at the Greenland heath tundra site could have been rather limited in P. Microorganisms have to maintain a rather constrained C:N:P ratio and have only limited capacities to store excess C, N or P in the biomass. Therefore, P limitation should lead to increased N mineralization as an overflow mechanism (Tezuka, 1990; Sterner and Elser, 2002). Although P limitation is usually considered characteristic for old, highly weathered soils, for example in the tropics (Cleveland et al., 2002),

there is increasing evidence that P is an important co-limiting and in some cases even the main limiting element in Arctic soils (Shaver and Chapin, 1995; Shaver et al., 1998; Giesler et al., 2002; Hartley et al., 2010; Giesler et al., 2012). In the case of the heath tundra site, we found that although concentrations of inorganic phosphate were significantly lower than at the Siberian sites, total P content was similar, indicating that differences in the sorption of P to mineral soil particles may have been responsible for the low P availability at this site.

3.4. Conclusions

We found significant differences in N transformation rates and microbial N use efficiency between sites and horizons, demonstrating that the respective microbial communities differed in nutrient limitation. Across all sites, N cycling was slower in cryoturbated compared to organic horizons, starting with protein depolymerization, which is rate-limiting in N cycling. Our results thus indicate that microbial communities have a lower capacity to break down proteins in cryoturbated compared to organic horizons, likely due to differences in community composition (e.g., a lower abundance of fungi). Overall, our study suggests that burial of organic matter by cryoturbation into the subsoil leads to changes in soil N transformations, which in turn may contribute to the observed retarded decomposition of cryoturbated SOM by altering N availability for microbial decomposers.

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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.2013.08.004.

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PAPER II

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

Petr Čapek [a, *], Kateřina Diáková [a], Jan-Erik Dickopp [b], Jiří Bárta [a], Birgit Wild [c, d, e], Jörg Schnecker [c, d, f], Ricardo Jorge Eloy Alves [d, g], Stefanie Aiglsdorfer [g], Georg Guggenberger [h], Norman Gentsch [h], Gustaf Hugelius [j], Nikolaj Lashchinsky [k], Antje Gittel [l, m], Christa Schleper [d, g, k], Robert Mikutta [h, i], Juri Palmtag [j], Olga Shibistova [h, n], Tim Urich [d, g, o], Andreas Richter [c, d], Hana Šantrůčková [a, **]

[a] University of South Bohemia, Department of Ecosystems Biology, Branišovská 31, České Budějovice, Czech Republic
[b] Institute of Systematic Botany and Ecology, University of Ulm, Albert-Einstein-Allee 11, D-89081 Ulm, Germany
[c] University of Vienna, Department of Microbiology and Ecosystem Research, Division of Terrestrial Ecosystem Research, Althanstrasse 14, 1090 Vienna, Austria
[d] Austrian Polar Research Institute, Althanstrasse 14, 1090 Vienna, Austria
[e] University of Gothenburg, Department of Earth Sciences, Guldhedsgatan 5A, 40530 Gothenburg, Sweden
[f] University of New Hampshire, Department of Natural Resources & the Environment, Durham, NH 03824, USA
[g] University of Vienna, Department of Ecogenomics and Systems Biology, Division of Archaea Biology and Ecogenomics, Vienna, Austria
[h] Leibniz Universität Hannover, Institute of Soil Science, Herrenhäuser Strasse 2, 30419 Hannover, Germany
[i] Martin-Luther-University Halle-Wittenberg, Soil Sciences, 06120 Halle, Germany
[j] University of Stockholm, Department of Physical Geography, 10691 Stockholm, Sweden
[k] Central Siberian Botanical Garden, Siberian Branch of the Russian Academy of Sciences, St. Zolotodolinskaya 101, 630090 Novosibirsk, Russian Federation
[l] University of Bergen, Department of Biology, Centre for Geobiology, Thormohlensgate 53B, 5020 Bergen, Norway
[m] Center for Geomicrobiology, Department of Bioscience, Ny Munkegade 114, 8000 Aarhus C, Denmark
[n] VN Sukachev, Institute of Forest, Siberian Branch of the Russian Academy of Sciences, Akademgorodok, 660036 Krasnoyarsk, Russian Federation
[o] University of Greifswald, Institute for Microbiology, 17489 Greifswald, Germany

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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.

* Corresponding author. Tel.: +420 387772347; fax: +420 387 772 368.
** Corresponding author. Tel.: +420 387772361; fax: +420 387 772 368.
E-mail addresses: petacapek@gmail.com (P. Čapek), hana.santruckova@prf.jcu.cz (H. Šantrůčková).

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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 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

Table 1
Basic physical (*BD* - bulk density, CEC - cation exchange capacity, ^{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} %	$\delta\ ^{13}C$ [‰] 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*

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 S ^{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 ß-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:

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

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:

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

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):

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

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}):

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

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

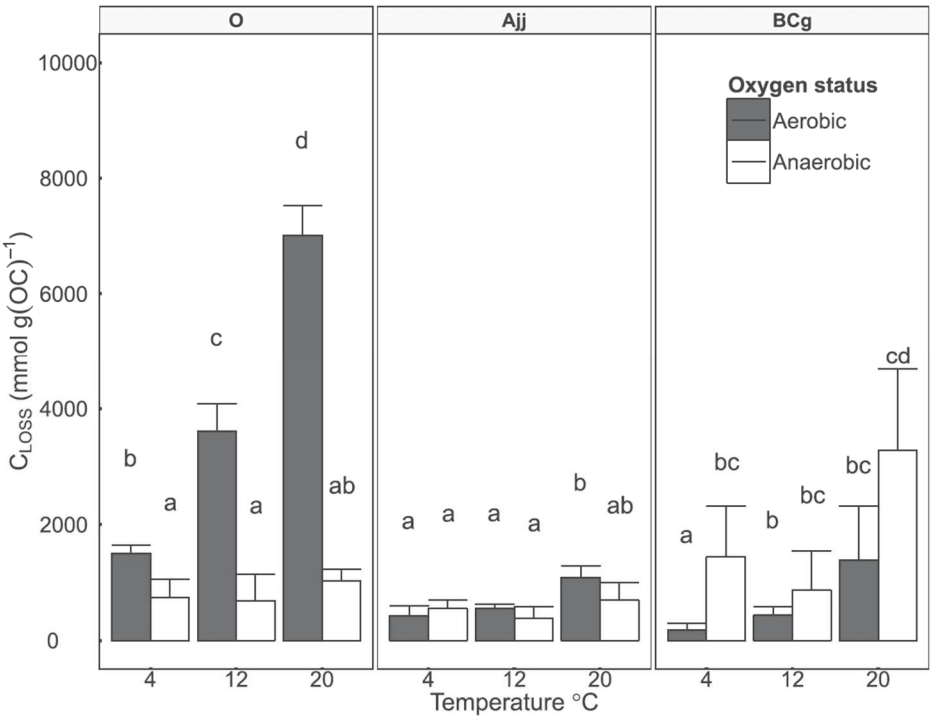

Fig. 1. Cumulative soil carbon loss (C_{LOSS}) from the organic (O), subducted organic (Ajj) and mineral (BCg) horizon, respectively, in 6 different incubation treatments (combinations of 3 temperatures and 2 oxygen levels). Filled bars represent aerobic treatments and open bars anaerobic treatments.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

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).

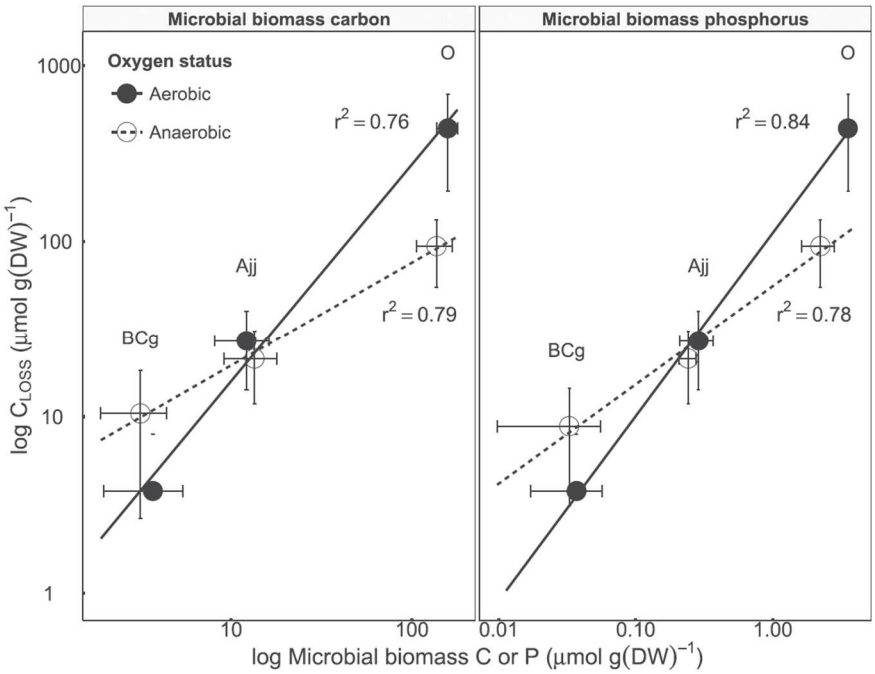

Fig. 2. Correlations between cumulative soil carbon loss (C_{LOSS}) and concentration of microbial biomass carbon and phosphorus. Open symbols show C_{LOSS} from the organic (O), subducted organic (Ajj) and mineral (BCg) horizon incubated anaerobically, averaged over 3 different temperature treatments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

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=l, 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). Tmax of O2 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

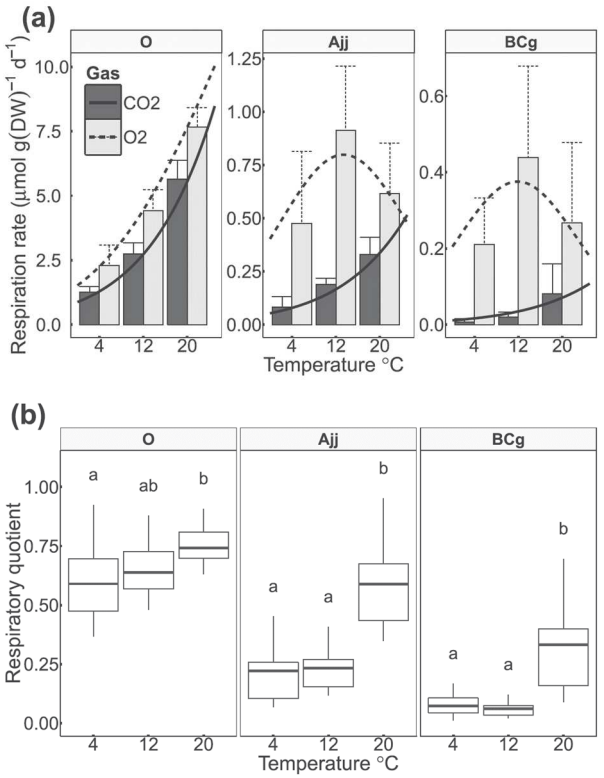

Fig. 3. (a): Respiration rate in aerobic conditions estimated from CO_2 production (gray bars) or O_2 consumption (black bars) in the organic (O). subducted organic (Ajj) and mineral (BCg) horizon in 3 different temperature treatments. Bar heights represent means and error bars standard deviations. Solid 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 1QR of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile.

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

3.1.2 Microbial biomass

The microbial biomass (C_{MB}) present at the start of the incubation was highest in the O horizon, with significantly and sequentially lower values in the Ajj and BCg horizons *(F=454.9, df=2, p< 0.001),* reflecting the trend observed for C_{EX} and nutrient contents (Table 1). Normalized to the amount of OC, C_{MB} was 4—5 times higher in the O horizon than in the BCg and Ajj horizons (Fig. 1). During the incubation, C_{MB} decreased at 20 °C in the O horizon (*F= 12.7, df=5,p < 0.001*; Fig. 4a) and increased at 12 °C in the Ajj horizon (*F=3.7, 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).*

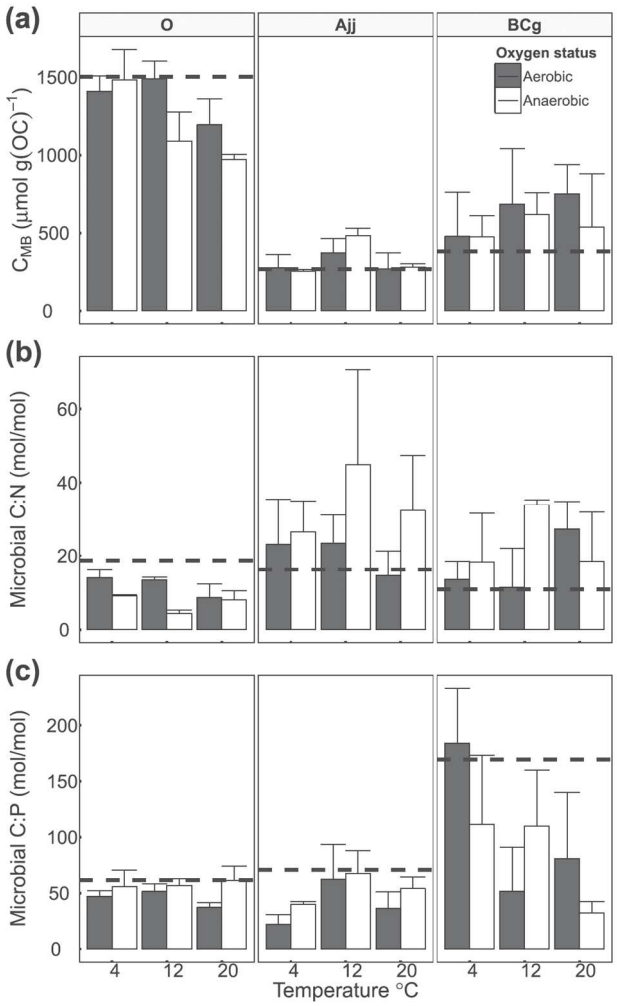

Fig. 4. Microbial biomass carbon (a), microbial biomass C:N (b) and C:P (c) in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard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

Microbial biomass stoichiometry (Fig. 4b,c; $C:N_{MB}$. $C:P_{MB}$) differed between horizons, with higher initial $C:N_{MB}$ in the O horizon, and sequentially lower values in the Ajj and BCg horizons ($F = 24.4$, *df=2, p <0.001)* (Table 1). By contrast, initial $C:P_{MB}$ was highest in the BCg horizon, followed by lower values in the Ajj and then the O horizon (*F = 522, 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.

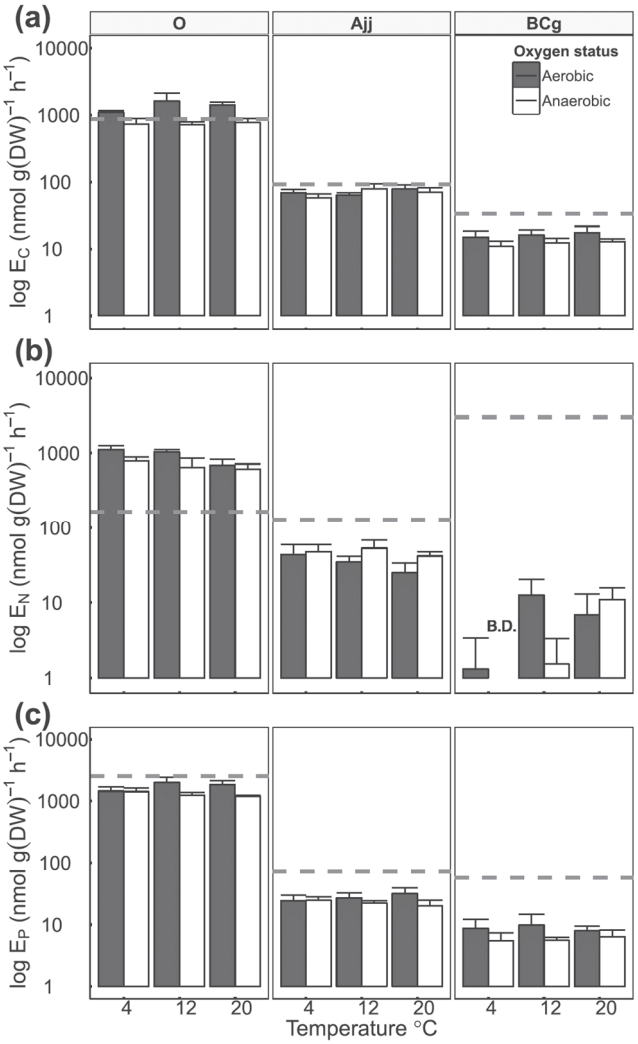

Fig. 5. E_c (a), E_N (b) and E_P (c) enzyme classes in the organic (0), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard deviations. Dashed horizontal lines show values at the start of the experiment. All enzyme classes are defined as sums of two different hydrolytic enzymes (Ec = *ß-glucosidase + cellobiosidase;* E_N = *alanin-aminopeptidase + leucine- aminopetpidase;* E_P = *phosphoesterase + phosphodiesterase).* Note that y-axes have logarithmic scale.

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 En pools decreased *(F= 30213, 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 = *25913, 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 ß- 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=25913, 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.

3.2. Anaerobic incubations

3.2.1 Microbial activity and soil carbon loss

As was the case under aerobic conditions, CO_2 production rates under anaerobic conditions were constant throughout the incubation at all temperatures and in all horizons (Fig. S2). The total amount of CO_2 produced by microbial activity during incubation represents the cumulative C_{LOSS} from the Ajj and BCg horizons, where no methane production was detected. In the O horizon, CH_4 production occurred from the 9th week to the end of incubation and thus C_{LOSS} was given by the sum of cumulative CO_2 and CH_4 production (Fig. 1). At the end of the incubation, CH_4 production represented 12, 39 and 42% of C_{LOSS} at 4,12 and 20 °C, respectively.

As under aerobic conditions, C_{LOSS} was higher at all temperatures in the O horizon, followed by the Ajj and then the BCg horizon (*F=44.4*, *df=2, p< 0.001),* although the differences were not as pronounced as under aerobic conditions. However, normalized to the amount of OC, C_{LOSS} was highest in the BCg, followed by the O and then Ajj horizon (Fig. 1). C_{LOSS} again strongly correlated with C_{MB} and P_{MB} (Fig. 2), a similar response as in aerobic conditions, although it increased more slowly with both C_{MB} and P_{MB}-

The temperature sensitivity of C_{LOSS} under anaerobic conditions was generally lower than under aerobic conditions (Fig. S4). C_{LOSS} did not increase between 4 °C and 12 °C and increased only slightly between 12 °C and 20 °C (Fig. 1). Temperature sensitivity was again statistically indistinguishable between horizons, regardless of the contribution of CH_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 = *122, df=1,p< 0.001*).

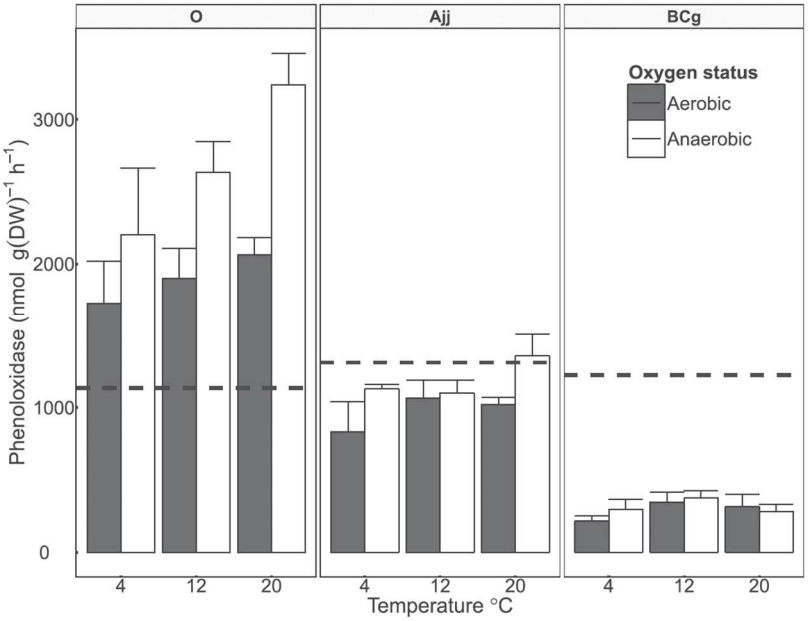

Fig. 6. Phenoloxidases in the organic (O), subducted organic (Ajj) and mineral (BCg) horizon in 6 different treatments. Filled bars represent aerobic treatments and open bars anaerobic treatments. Bar heights represent means and error bars standard deviations. Dashed horizontal lines show values at the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

3.2.3 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 Ec (*F=20092, 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.

3.2.4 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

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

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

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

C_{LOSS} showed lower temperature sensitivity than under aerobic conditions, but again it was the same in all soil horizons. In contrast to aerobic conditions, we found a significant decrease of microbial biomass in the 0 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,

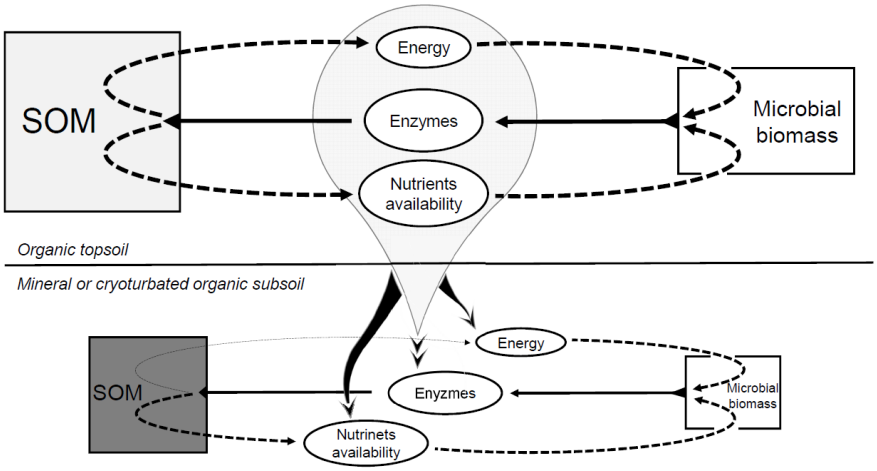

Fig. 7. Diagram of connections between microbial biomass, soil OC, enzymatic production and nutrient availability within and between soil horizons. The energy availability for enzymatic production within each soil horizon is critical; the low quality soil OC of subsoil horizons provides no energy and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

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^+, $N0_3^-$ and $P\text{-}PO_4$, Table S1). By contrast, the total enzyme pool slightly increased in the O horizon. Enzymes were negatively related to carbon and nutrient availability in this horizon. The greater nutrient availability was, the smaller was the enzyme pool, as microbes were provided with sufficient amounts of nutrients and enzyme production was not needed (Figs. S6 and S7). The enzyme pool was also related to microbial stoichiometry, indicating a direct link between microbes and enzyme activity. This was not seen in Ajj and BCg horizons.

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

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

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 III

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Drivers of phosphorus limitation across soil microbial communities

Petr Čapek*[,1], Petr Kotas[1], Stefano Manzoni[2,3] and Hana Šantrůčková[1]

[1]*Department of Ecosystem Biology, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic;* [2]*Department of Physical Geography, Stockholm University, SE-106 91 Stockholm, Sweden; and* [3]*Bolin Centre for Climate Research, Stockholm University, SE-106 91 Stockholm, Sweden*

Summary

1. Nutrient limitation of soil microbial communities controls the rates of plant litter and soil organic matter decomposition and nutrient mineralization, and as such, it is central to soil and ecosystem models.

2. According to ecological stoichiometry theory, when the carbon (C)-to-nutrient (E) ratio of resources used by a microbial community is higher than a critical ratio ($C : E_{CR}$), that nutrient is limiting. The C-to-phosphorus (P) critical ratio ($C : P_{CR}$) that determines P limitation is largely unknown for soils, and thus, it is the subject of our study.

3. Our results show that the $C : P_{CR}$ in widely different soils ranges from 26·6 to 465·1 or from 20·9 to 740·7 when accounting for 95% confidence intervals. Using constant or narrowly fluctuating $C : P_{CR}$ in ecosystem models is therefore inaccurate.

4. The $C : P_{CR}$ cannot be simply predicted from microbial community C : P or available soil P. $C : P_{CR}$ was only related to relative abundance of phospholipid fatty acids, which reflects microbial community structure and physiology. Our data suggest complex controls over microbial community $C : P_{CR}$.

5. We further propose that using P storage compounds that allow the microbial community to temporarily buffer variability in available P can represent a widely adopted strategies across soils.

Key-words: ecological stoichiometry, Monod equation, nutrient limitation, phosphorus mineralization and immobilization, phospholipid fatty acid, soil microbial community

Introduction

Since the 1990s, important ecosystem processes, such as plant litter and soil organic matter decomposition and nutrient mineralization, became widely framed in ecological stoichiometry theory (Enriquez, Duarte & Sandjensen 1993; Sterner & Elser 2002; Manzoni & Porporato 2009; Hall *et al.* 2010). One central paradigm of ecological stoichiometry is the constant or narrowly fluctuating (homeostatic) stoichiometry of three macronutrients in living organisms [carbon (C), nitrogen (N) and phosphorus (P), thereafter expressed on a molar basis]. In the soil, the degree of the microbial homeostasis is supposed to be strong (Makino *et al.* 2003; Cleveland & Liptzin 2007) and the variability in bulk microbial community stoichiometry is low ($6·3 < C : N < 8·3$, $31·6 < C : P < 130·7$; Xu, Thornton & Post 2013). Comparing to soil microbial communities, plants are less homeostatic (Elser *et al.* 2010) and therefore plant litter stoichiometry displays much higher variability ($5 < C : N < 100$, $250 < C : P$, 3500; Elser *et al.* 2010), primarily reflecting differences among plant organs (e.g. leaves are richer in P than wood), as well as species composition and stand conditions. The stoichiometry of soil organic matter also varies greatly ($10·5 < C : N < 31·4$, $6·6 < C : P < 1347·0$; Xu, Thornton & Post 2013), depending on plant litter input, edaphic conditions, geology, microbial transformations, etc. Thus, the variable stoichiometry of plant litter and to a lesser degree of soil organic matter often does not meet the nutritional requirements of homeostatic microbial communities and causes nutrient limitation of microbial growth. In turn, nutrient limitation may slow down the rate of plant litter decomposition and decrease nutrient mineralization rate (Enriquez, Duarte & Sandjensen 1993; Schade *et al.* 2005; Manzoni & Porporato 2009). Therefore, with abiotic factors and C availability, nutrient limitation is considered an

*Correspondence author. E-mail petacapek@gmail.com

INTRODUCTION

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Under controlled conditions, microbial growth is a nonlinear function of available substrate, usually modelled by Monod equation (Sinsabaugh, Shah & Follstad Shah 2012). This equation is hyperbolic; that is, it has saturating character and thus reflects substrate-saturated growth at high substrate concentration and substrate limited growth at low substrate concentration. Substrates are complex

mixtures of compounds including C, N and P and other nutrients including micronutrients. When one of these nutrients is in shortage with respect to the others and in relation to the decomposer nutritional requirements, microbial growth becomes limited by that nutrient and follows the linear part of the Monod equation (Schmidt 1992; Boer *et al.* 2009).

To determine the most limiting nutrients, which ultimately drive microbial growth rate, the principle of critical ratio was formulated - growth-limiting nutrients are those whose C-to-nutrient ratio is higher than the critical ratio. This concept has a simple mathematical origin (Bosatta & Berendse 1984; Ågren & Bosatta 1998; Sinsabaugh *et al.* 2013) it is the ratio at which the maximum rate of supply of a given nutrient is equal to the nutrient demand. For phosphorus, all P is supplied by an inorganic P pool because mineralization is extracellular process ensured by phosphatases (McGill & Cole 1981). It can be shown (see Appendix SI, Supporting information) that in this case the critical ratio of organic C-to-inorganic P can be approximated as,

$$(C{:}P_{CR}) = \frac{k_I}{k_D}\frac{(C{:}P_m)}{CUE} \qquad \text{eqn 1}$$

where k_I and k_D are kinetic constants for inorganic P and organic C uptake, respectively, $(C{:}P_m)$ is the microbial community C : P ratio, and CUE is the carbon use efficiency. When the C : P ratio of the resource is lower than C : P_{CR}, P is in excess of demand and is mineralized; otherwise, the rates of P immobilization and microbial growth become limited by inorganic P. The origin of C : P_{CR} as the breakpoint between C-limited and P-limited regimes is illustrated in Fig. 1a (dots). Figure 1b shows how C : P_{CR} varies as a function of the key stoichiometric traits $(C{:}P_m)$ (on the abscissa) and CUE (higher for the black curves), and the ratio of k_I to k_D, which indicates how effectively the microbial community takes up inorganic P vs. organic C.

Empirical studies estimated the C : N ratio above which microbes require net N immobilization. For terrestrial microbial communities, this threshold varies within ≈20 and 200 (Ågren *et al.* 2013; Zechmeister-Boltenstern *et al.* 2015), with higher values in N-poor litter and woody residues (Moore *et al.* 2006; Manzoni *et al.* 2010). These results suggest that microbial community C : N ratio and/or CUE might adapt

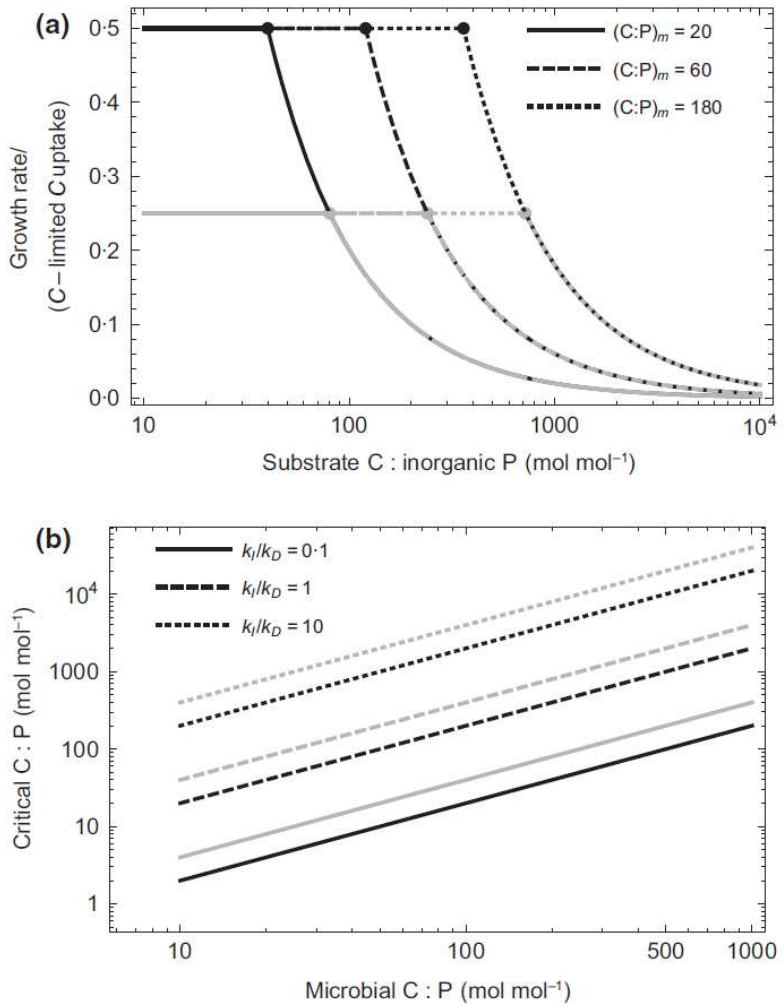

Fig. 1. Definition of critical C : P ratio and its variability as a function of microbial community characteristics, a) Microbial growth rate, normalized by the C uptake rate in C-limited conditions (eqn A4 in Appendix SI), as a function of organic C-to-inorganic P ratio, for different microbial C : P ratios [($\boldsymbol{C}$: $\boldsymbol{P_m}$), indicated by different dashing styles) and CUE (black curves, CUE = 0·5; grey curves, CUE = 0·25; $\boldsymbol{k_I/k_D}$ = 1], The critical C : P is the C : P value at which the microbial community switches from C-to-P limitation - that is, the breakpoints of the curves (dots), b) Critical C : P (eqn 1), as a function of ($\boldsymbol{C}$: $\boldsymbol{P_m}$), for different values of $\boldsymbol{k_I/k_D}$, where $\boldsymbol{k_I}$and $\boldsymbol{k_D}$ are the kinetic constants for inorganic P and organic C uptake, respectively (different dashing styles); CUE values are indicated as in panel a.

to the available substrates. However, the critical C : P ratio has not been estimated for soils yet, and thus, it is difficult to recognize when P limitation occurs and whether microbial communities can adapt to contrasting stoichiometric conditions.

It could be argued that the flexibility of critical ratios reflects an underlying strategy adopted by soil microbial communities to cope with the large variability of plant litter and soil organic matter stoichiometry and thus avoid nutrient limitation. Therefore, it is reasonable to expect that the C : P_{CR} increases with declining P availability, if microbial communities adapt their stoichiometric traits to alleviate P limitation. However, it could be also argued that the microbial community adapts to abiotic factors other than P limitation and thus their stoichiometric traits do not primarily reflect P limitation. In this case, the C : P_{CR} would not increase with declining P availability and strong effects of environmental and edaphic conditions could be expected.

The aim of the present study was to investigate the C : P_{CR} of soil microbial communities and its variability across ecosystems. We first present a methodological approach to calculate the C : P_{CR}**,** and then, we assess its variability among 18 soils from a variety of geographic areas and ecosystems and sampled at different depths. We expect the C : P_{CR} to vary widely among soils and generally increase in P-poor soils as a result of microbial community adaptation to P limitation. Microbial community can adapt by change in species composition [change of community level $(C: P_m)$] or physiology (change of CUE or k_I/k_D)- We further expect a direct link between C : P_{CR} and $(C: P_m)$ as result of prior community-level $(C: P_m)$ adaptation to P limitation.

MATERIALS AND METHODS

EXPERIMENTAL SET-UP

Soil samples collected from diverse localities and ecosystems were used in the experiment. Basic site and soil characteristics are given in Table SI; detailed methods are described in Appendix S2.

Soils were sieved on 3-mm mesh and stored wet at 4 °C in dark until start of the incubation. Before incubation, soils were conditioned for 1 week at 20 °C in the dark. Soils were then mixed with sterile C-free silica sand (sterilized by heating: 550 °C for 5 h) with grains <2 mm (25 : 1, w/w; sand : soil) to minimize the background effect of the original soil organic C and nutrients. All organic C and

nutrients were supplied to the mixture in basal medium. The mixture was moistened to 75% water holding capacity by supplying basal medium (8 g of sucrose, 0·1 g of yeast extract, 0·2 g of $MgSO_4.7H_2O$, 0·02 g of $FeSO_4$, 1 g of NH_4Cl, 0·02 g of CaCl in 500 mL of deionized H20; Veldkamp 1970). The amount of basal medium added was 1 mL to 5.2 g of the mixture and the medium concentration was appropriately manipulated to reach the concentration 20 mol of organic C per mol of soil microbial biomass C in all soils. N and other nutrients except for P were in excess. P was supplied in the form of K2HP04 to achieve concentrations from 0 to 5 g P per litre (11 concentration levels for each soil). Samples were incubated in 100 mL vials with oxitop heads on top (WTW Wissenschaftlich-Technische Werkstätten GmbH. Weilheim, Germany) for 7 days in the dark at 20 °C in three replicates for each treatment. Oxitop system was set to measure oxygen consumption continuously every 30 min during the whole incubation period.

The microbial growth rate was calculated from oxygen consumption. The slope of increase of O_2 consumption is directly related to microbial community growth rate (Hill & Robinson 1974). For data fitting, the R package GROFIT was employed (Fig. SI; Kahm et at. 2010; R Core Team 2014). When the slope of increase was statistically undistinguishable from 0, the microbial community was considered not growing.

MICROBIAL AND SOIL ANALYSIS

Microbial C (Vance, Brookes & Jenkinson 1987), N (Brookes et al. 1985) and P (Brookes, Powlson & Jenkinson 1982) were measured in 3 replicates in all soils by the chloroform fumigation extraction method before the start of the incubation. Soil available C, N and P were measured simultaneously with microbial C, N and P as 0·5 M K_2SO_4 extractable C and N and 0·5 M $NaHCO_3$ (pH = 8·5) extractable P. Details on the method are given in Appendix S2.

PHOSPHOLIPID FATTY ACID IDENTIFICATION AND QUANTIFICATION

The determination of phospholipid fatty acid (PLFA) was done in 6 representative soils with the low [Siberia (Ajj) and (BCg)], intermediate [Plesne, Čertovo, Siberia (O)] and high (Pop Ivan) C : P_{CR} (see Appendix S2) according to Frostegard, Baath & Tunlid (1993), with minor modifications. Soils were analysed in three replicates at two different stages of sample processing: (i) after a pre-incubation period before mixing with the sand and (ii) after 40 h of soil/sand mixture incubation (at the time of exponential growth of the microbial community). The analysis was done at the P supply value corresponding to incipient P limitation (i.e. at the critical C : P ratio, see Mathematical description). Such sampling design was chosen in order to answer two questions: (i) Does soil manipulation affect soil microbial community structure? (ii) What is the structure of growing microbial community at the critical C-to-P ratio? Details on the method are given in Appendix S2.

MATHEMATICAL DESCRIPTION

The response of microbial community growth rate to the amount of available P in the soil/sand mixture (for more details see Appendix S2) was described by a modified Monod equation.

$$\mu = \frac{V_{MAX}\, P}{K_M + P} + \mu_0, \qquad \text{eqn 2}$$

where μ is the biomass-specific growth rate of the bulk microbial community (h^{-1}; see also Appendix S2), P is the total amount of available phosphorus in the soil/sand mixture (μmol), K_M is the half saturation constant, and V_{MAX} is the maximum growth rate. In contrast to the classic Monod equation, equation (2) has an additional parameter μ_0 that represents the growth rate at zero P supply in the basal medium and shifts the curve along the y-axis (Fig. S2). When μ_0 is positive, the soil microbial community is able to temporarily grow without P supply, but solely relying on its own P storage compounds - as commonly observed among microbial species (Kulaev, Vagabov & Kulakovskaya 1999; Cotner, Makino & Biddanda 2006; Nikel et al. 2013, see below). Negative values mean instead that microbes cannot grow without external P supply and only respire. They do not grow until certain amount

of P is provided. The sum of μ_0 and V_{MAX} defines the asymptotic or maximum growth rate attainable by a microbial community at defined substrate-saturated conditions.

Equation (2) predicts a steep increase of growth rate with increasing amount of P followed by a slower approach to the asymptotic value (Fig. S2). These two 'stages' of growth rate response to P amount can be mathematically separated at a critical amount of P, at which the growth rate increase slows down. To calculate this critical P amount (P_{CR}), first the fraction of the asymptotic growth rate after all P storage has been consumed (R) is defined based on equation (2),

$$R = \frac{\mu - \mu_0}{V_{MAX}}. \qquad \text{eqn 3}$$

Combining equations (2) and (3) allows us rewriting R as a function of K_M and the P amount:

$$R = \frac{P}{P + K_M}. \qquad \text{eqn 4}$$

The critical value of P amount, P_{CR}, is chosen so that R = % (three-quarters of asymptotic growth rate), corresponding to the point where the sensitivity of μ to P availability starts decreasing. This is the point that most closely resembles the theoretical critical ratio when C and P limitation switch as described in Appendix SI. Rearranging equation (3), we obtain P_{CR} as a function of K_M,

$$P_{CR} = 3 \times K_M \qquad \text{eqn 5}$$

As nitrogen and other nutrients in the growth medium were in excess, the calculated P_{CR} is not affected by other nutrient limitations. Finally, the critical C : P ratio was calculated as the ratio of available organic C in the growth medium and P_{CR}.

STATISTICAL ANALYSIS

The relationship between microbial community stoichiometry and soil stoichiometry was tested using simple linear regression. To evaluate microbial community growth rate response to phosphorus availability, the modified Monod equation was fitted and equation parameters were estimated (with 95% confidence intervals) using nonlinear least square regression for each soil separately. Nonlinear mixed-effect models (using R package NLME; Pinhero et al. 2015) were used for further statistical evaluation. First, the null model was defined as nonlinear function of P availability according to modified Monod equation. Equation parameters were allowed to randomly vary across samples. Secondly, the null model was improved by forward selection of the best equation parameters predictors. The best predictors were chosen from all ancillary data we obtained. These included microbial and soil C, N, P, microbial and soil stoichiometric ratios (C : N, C : P, N : P), pH, mean annual temperature and precipitation, latitude, altitude, sampling depth, ecosystem type, land management and microbial community structure.

Because the characterization of the microbial community structure by relative abundance of PLFA markers in the sample lead to an extensive data set, this information was collapsed into vectors using principal components analysis (PCA). We retained the first three principal components and used scores for each soil on these axes as predictor variables in the nonlinear mixed-effect model. Before PCA analysis, data were normalized. Permutational multivariate analysis of variance (using R package VEGAN; Oksanen et al. 2015) was further used to check the difference between microbial community structure of each soil before and after manipulation. All statistical analyses were done using the statistical program R (R Core Team 2014).

RESULTS

SOIL AND MICROBIAL COMMUNITY STOICHIOMETRY

The C : N ratio of the soil microbial communities from our 18 samples was between 5·5 to 34·6 (Table SI). The $(C{:}P_m)$ ranged from 24·7 to 346·6 (Table SI). The stoichiometric ratios of available nutrients were more variable spanning more than one order in magnitude (C : N from 1·0 to 29·5; C ; P from 22·8 to 788·7, Table SI). There

was no linear relationship between soil and microbial stoichiometry (C : N and C : P, Fig. S3), and the slopes of both relationships were statistically undistinguishable from 0.

MICROBIAL COMMUNITY GROWTH RATE RESPONSE TO PHOSPHORUS

All microbial communities showed a clear trend of increasing microbial community growth rate with increasing amount of available P according to the modified Monod equation (Fig. 2), but parameters were distinctly different among soils (Table S2). Variations of the equation parameters from their mean values are interpreted as indicators of differences among soils. The highest variability occurred in the μ_0 parameter (mean value 0·53, coefficient of variation CV = 187%) and parameter, which defines P_{CR}, showed second highest variability (mean value 1·59, CV = 56%). P_{CR} for individual soils varied from 1·29 to 20·55 μmol (Table S2), resulting in a range of more than one order in magnitude for the critical C : P ratio (Fig. 3; 26·6-465·1). Accounting for uncertainty in K_M estimates using 95% confidence intervals, the range of C : P_{CR} increased (Fig. 3; 20·9-740·7).

MODIFIED MONOD EQUATION PARAMETERS

After quantifying the variability of the parameters in the modified Monod equation, we continued with the null regression model build up (Fig. 2).

μ_0 parameter

Growth rate at zero P concentration in the soil, μ_0, was inversely and significantly correlated with microbial C : P at the beginning of the incubation (Fig. 4a), except for deep soil horizons. This linear relationship was significant only for the topsoil horizons (F=31·6, d.f. = 1, P< 0·001); therefore, it did not lead to significant improvement of the whole nonlinear mixed-effect model (LR = 4·16, d.f. = 12, P = 0·125) as deep soil horizons fell out of the linear relationship.

Asymptotic growth rate

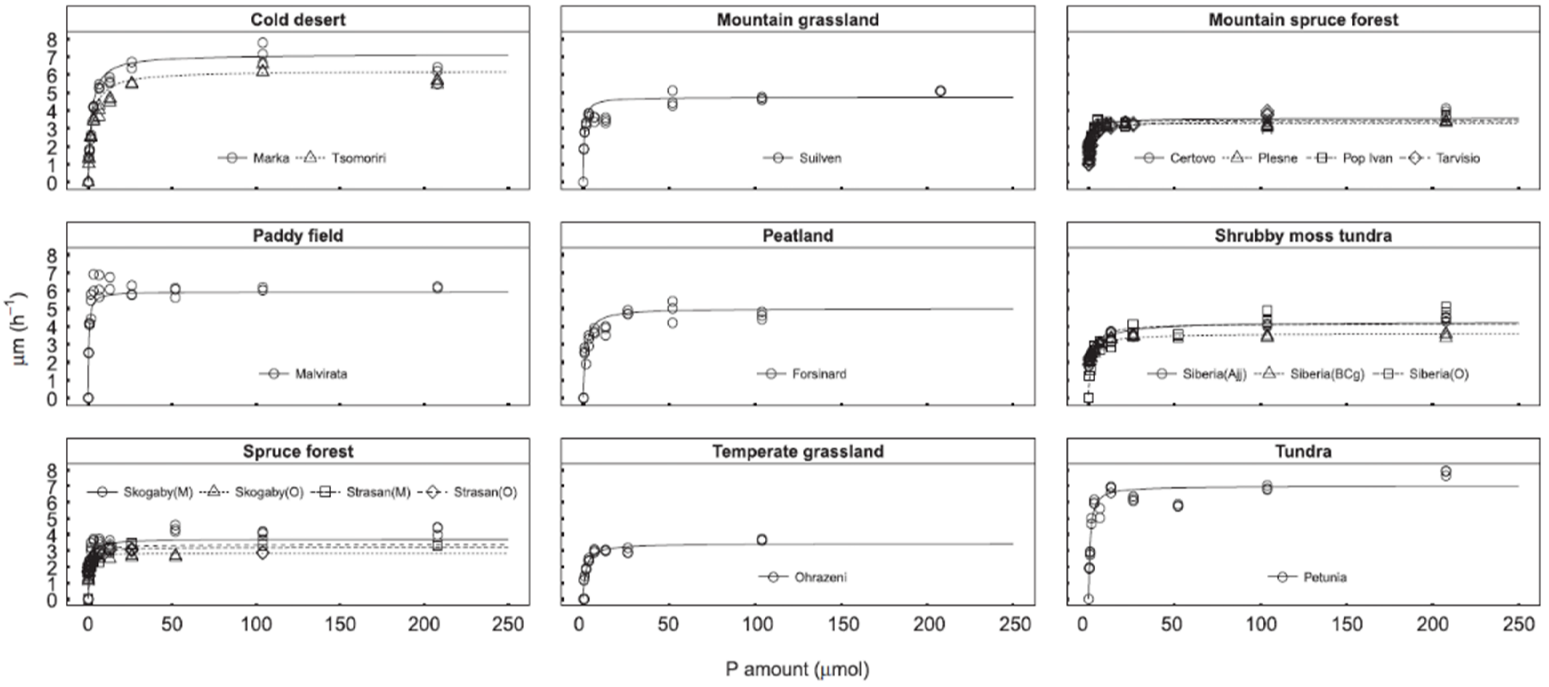

Fig. 2. Growth rate response of microbial communities to the amount of P in our 18 soils. Soils are grouped in the boxes according to ecosystem type. Open symbols represent raw data and curves represent the best fit of the modified Monod equation to the data. When more than one soil per ecosystem type is present, different soils are distinguished by different symbols and line dashing styles.

We found a significant linear relationship between microbial C : N and asymptotic growth rate (Fig. 4b, F = 8·8, d.f. = 1, P = 0·009). The lower was microbial C : N, and the higher was the asymptotic growth rate. Incorporation of this linear relationship into the mixed-effect model improved the model at 10% level of significance (LR = 2·84. d.f. = 13, P = 0·092).

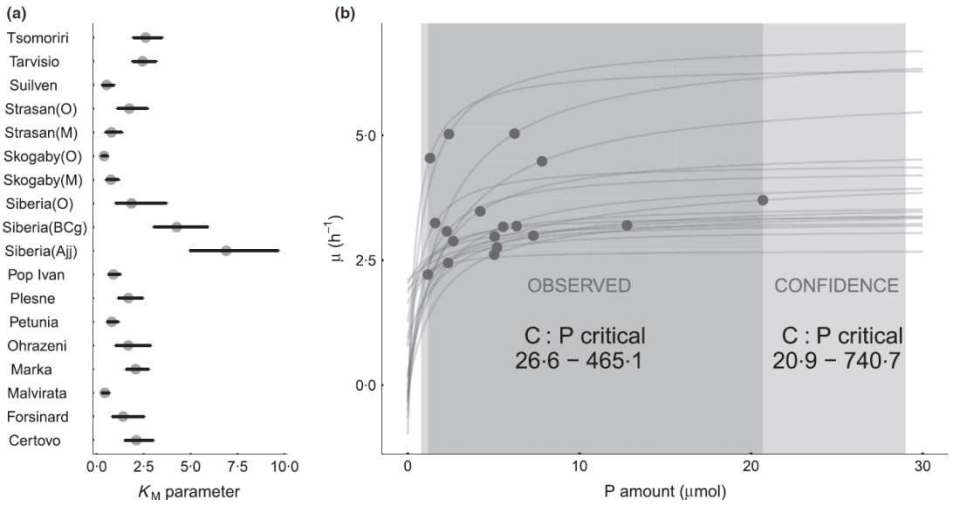

Fig. 3. (a) Estimates of the half saturation constant K_M for the 18 soils with its 95% confidence interval. (b) Mean values of the critical P amount (P_{CR} = 3 x K_M) are plotted as a dots on lines representing the fit of the modified Monod equation to the data. The critical carbon-to-phosphorus ratio (C : P_{CR}) is calculated as ratio between the amount of organic C and P_{CR}. Mean and 95% confidence of K_M estimates were used to calculate P_{CR} and C : P_{CR}.

K_M parameter

The half saturation constant, K_M, was driven by microbial community structure of the soil, as assessed by PLFA analysis on 6 representative soil samples. Microbial community structure explained most of the variability in K_M (78%; Fig. 5). The relationship between microbial species composition and K_M was significant (F=21·2, d.f. = 1, P < 0·001) and led to significant improvement of the nonlinear mixed-effect model (LR = 12·2, d.f. = 14, P = 0·015).

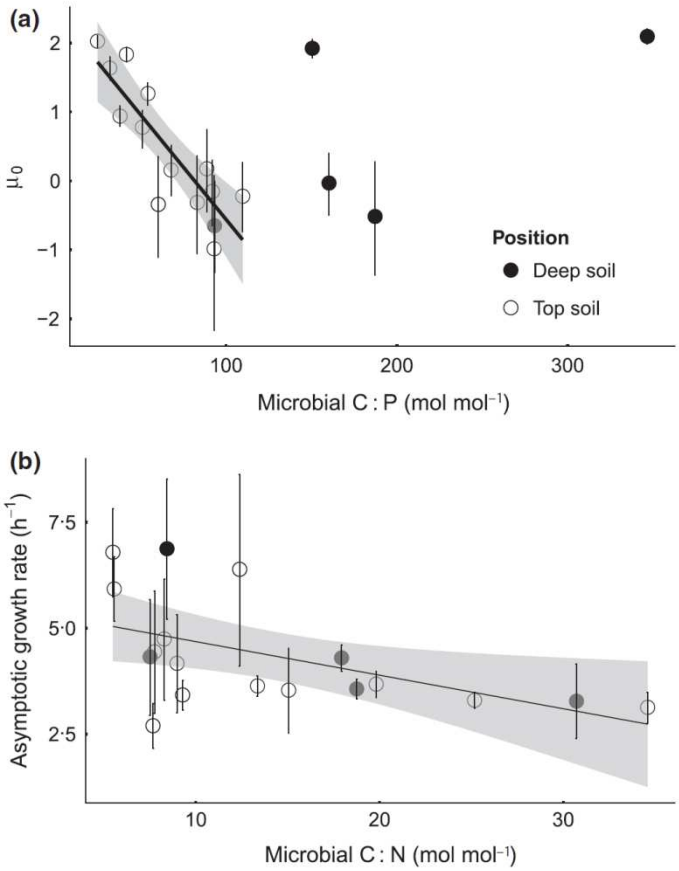

Fig. 4. Negative linear relationship between microbial community C : P and mean estimate of μ_0 parameter (a) and between microbial community C : N and asymptotic growth rate (b). Error bars represent 95% confidence intervals of parameter estimates. Open circles refer to topsoil horizons, whereas black dots refer to deep soil horizons. Solid lines represent linear regression fitted to the topsoil horizon data (a) or to all samples (b). The grey area along the solid lines shows the 95% confidence bands of the linear regression.

MICROBIAL PLFA COMPOSITION

Soil manipulation and incubation significantly affected microbial community structure of the original soil (Fig. S4; *pseudo F*= 194·9, d.f. = 1, $P < 0{\cdot}001$). Microbial community structure of all soils from spruce forest ecosystems (Certovo, Plesne and Pop Ivan), even though geographically distant, changed in the same way (Fig S4). The same was true for Siberia(Ajj) and Siberia(BCg) soil samples, which are

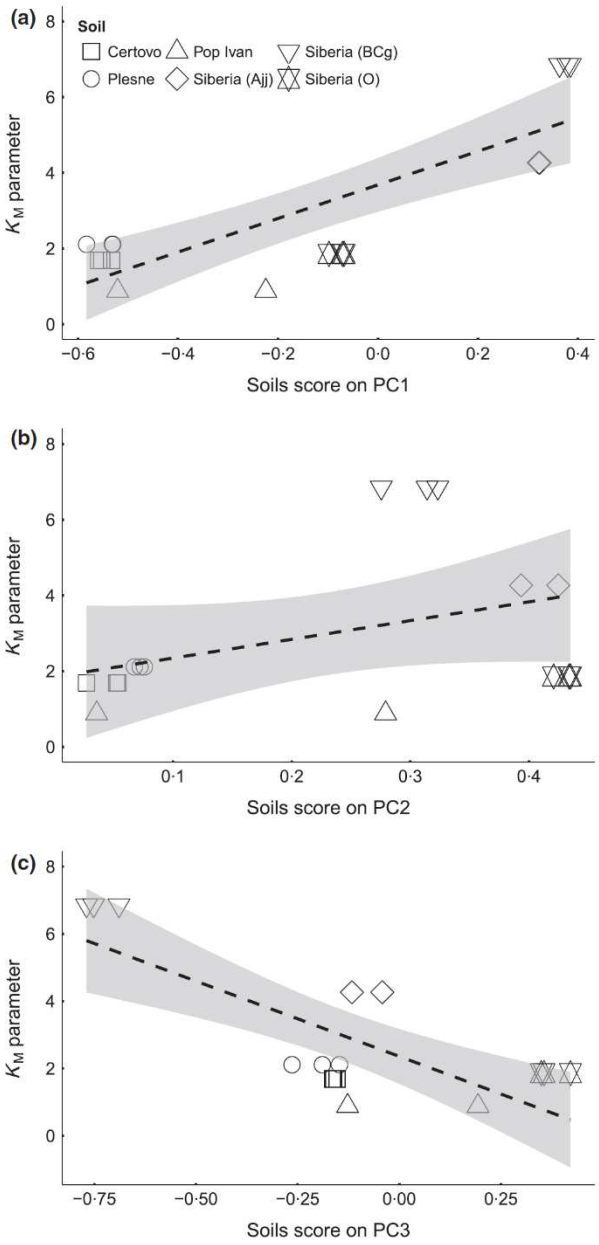

Fig. 5. Relationship between the half saturation constant K_M and principal components 1 (a), 2 (b) and 3 (c), which represent collapsed information from phospholipid fatty acid analysis done on 6 representative soil samples [Čertovo, Plesne, Pop Ivan, Siberia (Ajj), Siberia(BCg) and Siberia(O)]. Dashed lines represent the linear relationship between K_M and the score of particular soils on the PC axis. The grey area along the dashed lines shows the 95% confidence bands of the linear relationship.

next to each other in the soil profile. In spruce forest ecosystem soils, the shift of microbial community structure was mainly characterized by an increase of relative abundance of two fungal markers (18:1n9, 18:2n6,9) and parallel decrease of G+ and G- bacteria- specific markers (a15:0, 16:1n7, cy17 and 18:1n7; Fig. S4). The opposite was true for Siberia(Ajj) and Siberia(BCg) soil samples (Fig. S4). However, other G- and G+ bacteria-specific markers (cy19:0 and i16:0) decreased in both Siberian samples as well as actinobacterial and general bacterial markers (10Me16:0 and i15:0). Therefore, the

simple measure fungi to bacteria ratio could not characterize the shift of microbial community structure after incubation and thus was a poor predictor of K_M.

DISCUSSION

MODIFIED MONOD EQUATION PARAMETERS

μ_0 parameter

Among our soil samples, some had extremely low microbial community C : P, around or below 30 (Plesne, Strasan, Tarvisio). Those values are lower than the global average, which is according to different studies around 60 (Cleveland & Liptzin 2007), 88 (Mouginot *et al.* 2014) or 42 (Xu, Thornton & Post 2013). The high concentration of P in microbial cells relative to C can be reached either by increase of ribosomes in the cell (Sterner & Elser 2002; Elser *et al.* 2003) or by accumulation of P storage compounds, mainly polyphosphates (Kulaev, Vagabov & Kulakovskaya 1999; Cotner, Makino & Biddanda 2006; Nikel *et al.* 2013). While ribosomes can make up to 1·5% of cell dry weight (Elser *et al.* 2003), polyphosphates can make as much as 30% of cell dry weight (Kulaev, Vagabov & Kulakovskaya 1999) and thus might be responsible for observed variation in cellular P concentrations. Polyphosphates serve the microbes as an internal P source when external sources are scarce (Kulaev, Vagabov & Kulakovskaya 1999). The negative linear relationship we found between microbial community C : P and growth rate at zero P supply (μ_0) across all topsoil horizons (Fig. 4a) confirms this view. It suggests that P reserves stored as polyphosphates were utilized to allow growth of the microbial community in presence of abundant C, but at zero P supply. It might be argued that residual soil P could have also been used by the microbial community at zero P in the basal medium. However, we found no relationship between μ_0 and soil P. Moreover, the dilution rate of our soils by a sterilized sand was high enough to reduce the amount of residual soil P (see Appendix S2).

While the trend of decreasing μ_0 with increasing microbial C : P was strong across all surface soils, the deep soil horizons behaved differently. These soils were characterized by extremely high microbial community C : P ratios, almost two to four times higher than global average (Cleveland & Liptzin 2007; Xu, Thornton & Post

2013; Mouginot *et al.* 2014). However, the same communities also exhibited high μ_0 [especially Siberia(Ajj) - C : P = 150·5, μ_0 = 2·09; Siberia(BCg), C : P = 346·6, μ_0 = 1·92]. The two most extremes - the Siberian deep soil horizons - were taken from depths of 50 and 70 cm, respectively. We suggest that the reason why deep soil horizon falls out of the linear relationship is the high abundance of dormant microbial cells in their microbial community, which decouple microbial community C : P and μ_0:

1. It is documented that the microbial community of deep soil horizons is characterized by high abundance of dormant cells (up to 98%; Blagodatskaya & Kuzyakov 2013), and thus, it is expected that community C : P reflects the C : P of dormant cells. Microbial dormancy is accompanied by unique physiological changes (Herbert 1961; Oliver 1993; Lennon & Jones 2011) that increase cell C : P (Linder & Oliver 1989; Kieft *et al.* 1997; Wang & Bakken 1998; Sterner & Elser 2002; Kadouri *et al.* 2005). Assuming that the C : P of dormant cells is near the highest known level (409; Cleveland & Liptzin 2007), we can infer that the proportion of dormant cells needed to compensate the C : P of active cells [27·2 and 28·7 for Siberia(Ajj) and Siberia(BCg) soils, respectively, using surface values] has to be 87·6% and 96·5% for Siberia(Ajij) and Siberia(BCg) soil, respectively. Such an amount of dormant microbial cells in deep soil horizons is realistic and comparable to previous estimates (Blagodatskaya & Kuzyakov 2013). Thus, we surmise that the linear relationship between microbial C : P and μ_0 is generally valid for active microbial cells, which can store and use polyphosphates.
2. Dormant cells cannot start their reactivation and grow unless carbon and nutrients became available (Blagodatskaya & Kuzyakov 2013). At zero P amount, the only microbial cells that can grow are the already active ones with low C : P (dormant cells with high C : P cannot grow). However, at higher P amounts (and also around the critical P amount), the dormant cells reactivate and thus the growth response to P amount can be explained by the characteristics of the whole community.

These hypothesized mechanisms appear to explain the deviation of deep soil horizons from the behaviour of the top soils, but more data would be needed to properly test the mechanism.

Asymptotic growth rate

According to the modified Monod equation, the asymptotic growth rate is the maximum growth that the microbial community can reach under substrate-saturated conditions. This value was negatively related to microbial community C : N (Fig. 4b), in accordance with empirical evidence (Mouginot *et al.* 2014) and the theoretical predictions of Vrede *et al.* (2004), who showed that the maximum attainable growth rate increases with increasing total amount of catalytic macromolecules in the cell (mainly rRNA, see above) or with increasing ribosome-specific protein synthesis rate. The amount of catalytic macromolecules change cellular C : N or C : P, because they are N and P rich (Elser *et al.* 2003; Vrede *et al.* 2004). The higher the amount of catalytic macromolecules, the lower the cellular C : N and C : P, thus explaining the negative relationship between maximum attainable growth rate and cellular C : N or C : P. In our experiment, the relationship between asymptotic growth rate and microbial community C : N was statistically significant, but that was not the case for $(C:P)_m$. As we discussed above, microbial community C : P was affected by P storage compounds more than by the amount of catalytic macromolecules and thus the relationship was not found. There was also some residual variability in the negative linear relationship between asymptotic growth rate and microbial community C : N, possibly caused by different ribosome-specific protein synthesis rate, which is species specific (Vrede *et al.* 2004).

K_M parameter

The half saturation constant (K_M) was the most important parameter of the modified Monod equation, because it was shown to be proportional to the critical P amount, which in turn determines C : P critical (C : P_{CR})- In 6 representative soils, whose C : P_{CR} range from 26·6 to 227·2, nearly 80% of the variability in K_M could be explained by the first three principal components of the PLFA profiles (Fig. 5). The first principal component, which accounts for the most explained variability (66%),

was negatively correlated with fungal-specific markers (18:2n6,9, 18:1n9) and positively correlated with four G+ or G- bacteria-specific markers (al15:0, 16:1n7, cy17 and 18:1n7). The first principal component mainly separated spruce forest ecosystem soils (Certovo, Plesne and Pop Ivan) with C : P_{CR} around or above 100, from Siberia(Ajj) and Siberia(BCg) soils with C : P_{CR} below 30 (Fig. 5a). In this respect, our findings are partly in agreement with the general theory (eqn 1), which predicts higher C : P_{CR} of fungi compared to bacteria because of higher fungal C : P and therefore lower demand for P (Cleveland & Liptzin 2007; Mouginot *et al.* 2014). Nevertheless, we have no direct evidence to support a general relationship between cellular C : P and C : P_{CR} (Fig. S5a). This is in disagreement with our initial hypothesis. There was also no relationship between soil P and C : P_{CR} (Fig. S5b). We argue that $(C:P)_m$, soil P and C : P_{CR} are not necessarily connected, because each of them could be independently and differently affected by edaphic conditions.

1. *Microbial community C* : *P does not reflect demands of growing cells for P and thus it is not related to C : P_{CR}* - As we showed above, the concentration of P in microbial cells is modified by P storage compounds and it is also very likely affected by the microbial physiological status (active/dormant). Both formation of P storage compounds and dormant structures is not directly connected to growth. Therefore, across these widely different soils, the microbial community C : P ratio *per se* cannot be directly related to C : P_{CR}.
2. *C : P_{CR} is not related to available soil P because microbial communities do not adapt to avoid P limitation.* Usually, P availability is not the main driver of microbial community adaptation. One of the most important drivers is depth because temperature, substrate concentration, moisture, texture, etc. change along with increasing depth (e.g. Gittel *et al.* 2014b; Schnecker *et al.* 2014). Very often, P availability decreases with increasing depth (Kalcik & Santruckova 1994), which was also the case for soils from Siberia, Skogaby and Stråsan (Table SI). With decreasing P availability along increasing depth, C : P_{CR} decrease in Siberian and Skogaby soils, in contrast with our expectation. This unexpected trend could be explained by the fact that abundance of fungi as well as fungi to bacteria ratio generally decrease with increasing depth, independent of P availability (e.g. Gittel *et al.* 2014a,b).

With increasing abundance of bacteria with low C : P ratio, C : P_{CR} can decrease with depth (eqn 1).

Our data show that no simple predictor of C : P_{CR} exists. C : P_{CR} is very likely driven by many edaphic factors like substrate availability, P availability, pH, temperature, moisture, etc. and their mutual combinations. These factors modify C : P_{CR} via their effects on soil microbial community structure and physiology (CUE and k_I/k_D in eqn 1). The relative abundance of PLFA is a manifestation of effects of edaphic conditions on soil microbial community structure, explaining why PLFA is the only significant predictor of K_M and thus of C : P_{CR}.

C : P CRITICAL IN ECOSYSTEM MODELS

We showed that C : P_{CR} could vary from 26·6 to 465·1. Accounting for 95% confidence interval of K_M parameter estimate, the limits of C : P_{CR} are even wider - from 20·9 to 740·7. Using constant or narrowly fluctuating C : P_{CR} in ecosystem models is therefore inaccurate. However, implementation of variable C : P_{CR} into models can be problematic at present, because no simple predictor of C : P_{CR} could be found. C : P_{CR} does not reflect ecosystem type, biome, land management, microbial community C : P or P availability, but is related to community structure (Fig. 5). More data would be needed in future, especially from deep soil horizons, to develop the process-based understanding of variations in C : P_{CR} required for P cycle models.

Acknowledgements

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Data accessibility

All data used in this manuscript are present in the manuscript and supporting information.

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Paper IV

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PLANT-MICROBIAL INTERACTION: EFFECT OF VARIABLE STOICHIOMETRY

Petr Čapek, Stefano Manzoni, Eva Kaštovská, Kateřina Diáková, Jiří Bárta, Birgit Wild, Jörg Schnecker, Christina Biasi, Pertti J. Martikainen, Ricardo Jorge Eloy Alves, Georg Guggenberger, Norman Gentsch, Gustaf Hugelius, Nikolaj Lashchinsky, Peter Kuhry, Antje Gittel, Christa Schleper, Robert Mikutta, Juri Palmtag, Olga Shibistova, Tim Urich, Andreas Richter, Hana Šantrůčková

Abstract

Plants and soil microorganisms use nutrients from soil to subsist and grow. Availability of soil nitrogen (N) and phosphorus (P) is able to largely affect plant productivity and activity of soil microorganisms[1–4]. Since plants and soil microorganisms use the same pool of available nutrients, they are competitors. When both are limited by the same nutrient at the same time, their competition negatively affect one or the other [5,6]. However, when this situation occurs has not been defined yet. Here we show, that plants and soil microorganisms have different requirements for N and P (defined as N to P critical ratio ($N:P_{CR}$)[7,8]). Median of plant $N:P_{CR}$ is globally 32.4 and median microbial $N:P_{CR}$ is 6.8. Different plant and microbial $N:P_{CR}$ reflects their different stoichiometries. Because of different requirements, plants and soil microorganisms are both limited by N, when N to P ratio of available soil nutrients ($N:P_S$) drops below 6.8 and by P, when $N:P_S$ increase over 32.4. We demonstrate validity of our predictions and their consequences on meta-analysis of factorial N-P fertilization experiments from arctic tundra ecosystem, where $N:P_S$ can locally be between 6.8 and 32.4. Presented results offer new perspective of plant – microbial interaction. Its consequences for plant primary production or soil nutrient cycling should be thoroughly examined in future.

Both, plant primary productivity and activity of microbial decomposers often depends on availability of nutrients - nitrogen (N) and phosphorus (P), in soil[1–4]. Because plants and microbes have to share the same nutrients pool, they are competitors. When certain nutrient, for example N, exists in insufficient amount for both, the result of plant – microbial competition for N would favor the winner at the expense of the looser. Since microbes are particularly strong competitors for N and for P [5,6] they can reduce plant primary production in principle. However, the competition becomes important only if 1) nutrient exists in amount insufficient to cover demands of plants and microbes at the same time, and 2) microbial decomposers and plants are limited by the same nutrient at the same time and space.

Whereas the validity of the first condition is undeniable in many ecosystems, the second condition is less clear. Whether organisms become nutrient limited depends not only on nutrient availability, but also on organism demand for nutrients. Organism demand for nutrients can be expressed as N to P critical ratio ($N:P_{CR}$) [7–10].

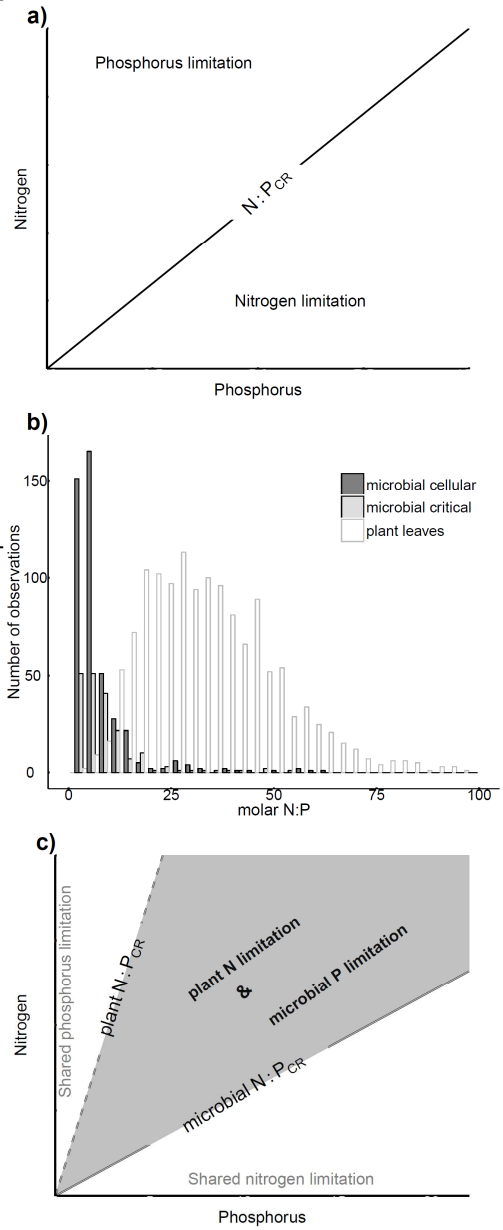

Fig. 1: (a) Concept of critical N to P ratio ($N:P_{CR}$ which is driven by biomass stoichiometry): organisms become N limited, when supply N to P ratio of available soil nutrients ($N:P_S$) drops below $N:P_{CR}$, In opposite case, organism become P limited. (b) histograms of N to P ratio of soil microbial biomass[12] (black bars), plant leaves[13] (white bars) and $N:P_{CR}$ calculated from available data (grey bars). Data suggests different $N:P_{CR}$ of plants and soil microorganism. (c) Conceptual model of plant – microbial interaction based on different plant and microbial $N:P_{CR}$. Plant demand for P (plant $N:P_{CR}$) is lower than microbial demand for P (microbial $N:P_{CR}$). Therefore plant N limitation and microbial P limitation may occur at particular N:P supply from soil (grey area).

Note that we only consider N as nutrient, not as a substrate for energy metabolism of certain soil microorganisms (e.g. during nitrification, denitrification or ammonia anaerobic oxidation). When supply N to P ratio of available nutrients ($N:P_S$) meets the critical ratio, organism has optimal or balanced nutrition (Fig. 1a). When $N:P_S$ is lower than the critical ratio, organism becomes N limited (Fig. 1a). In opposite case it becomes P limited (Fig. 1a). We have to, however, assume that no other factors like light in the case of plants or energy and carbon in the case of soil microbes limit organism.

$N:P_{CR}$ reflects organism stoichiometry and growth rate [7–9,11]. Two recent meta-analysis[12,13] show, that stoichiometry of plants and soil microorganisms ultimately differ (Fig. 1b). Thus, we can expect different plant and microbial $N:P_{CR}$

(Fig. 1b,c). Based on previous research[11], we directly estimated microbial $N:P_{CR}$ in soils collected across different ecosystems (see Supporting Information Appendix I). These data show, that the median critical N to P ratio for soil microorganisms across ecosystems is 6.8 (Fig. 1b). This number well corresponds with global average microbial N to P ratio 6 - 7 [12,14]. Plant critical N to P ratio is according to empirical studies given by N to P ratio of plants leaves. Plant leaves are the most homeostatic organ of plants, which runs photosynthesis and thus it is important parameter determining plant primary production [9,15]. Based on this assumption and the extensive dataset collected by Kerkhoff and collective[13], we estimated that median of plant $N:P_{CR}$ is 32.4 (Fig. 1b).

When $N:P_{CR}$ of plants and soil microorganisms differ, they become limited by the same nutrient only at particularly high (>32.4) or low (<6.8) $N:P_S$. Between 6.8 and 32.4, the competition should not occur. This can be directly tested on factorial N-P fertilization experiments. If our hypothesized model is valid, N fertilization but not P fertilization should support plant growth, biomass and increase plant N concentration when soil $N:P_{CR}$ is below 32.4 because plants are N limited. Nevertheless, soil microorganisms are already P limited, which restricts their growth and they leave N behind for the plants. When the soil is amended with P, $N:P_S$ decreases and after it drops below microbial $N:P_{CR}$ (6.8), microbial P limitation is relaxed and switches to microbial N limitation. Plants and soil microorganisms become both limited by N and their competition should let to decrease of plant growth and biomass.

In order to test hypothesized plant – microbial interaction model, we collected studies from factorial N, P fertilization experiments (13 primary research articles, Tab. 2) in the Arctic tundra areas. We choose arctic tundra area because we can, based on our and published soil data sets (i) demonstrate that $N:P_S$ ratio linearly increases from the East to West ($p = 0.002$) in the region where fertilization experiment were run, which enabled extrapolation of missing soil data (Figs 2c and S2, Supporting Information Appendix II), and (ii) estimate microbial N mineralization rates (Fig. 3).

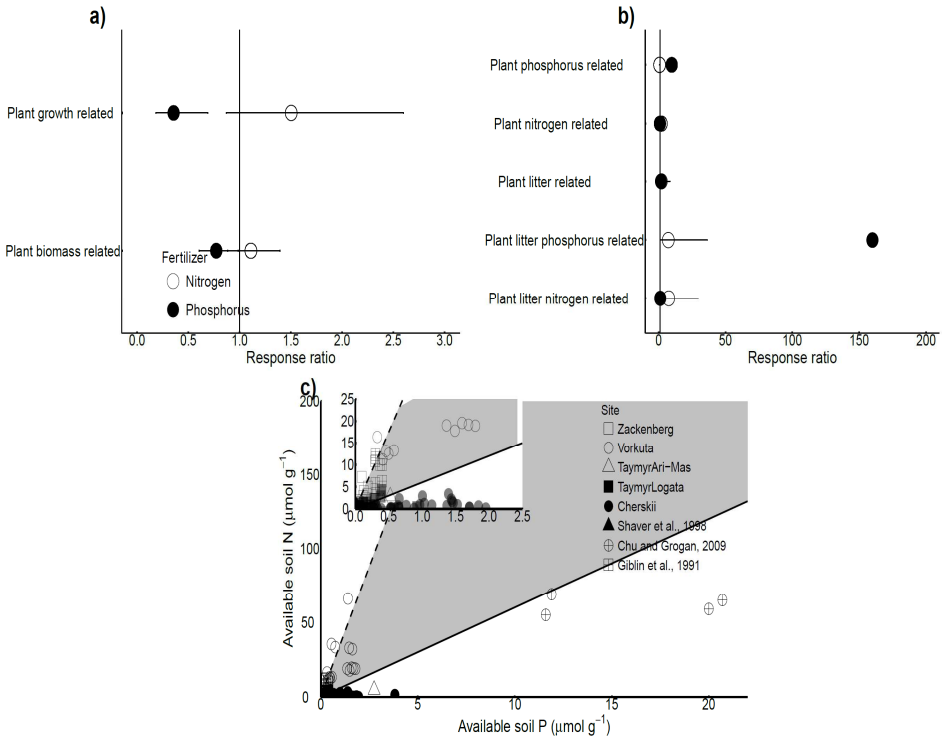

Fig. 2: Response ratios of a) plant growth, plant biomass, b) plant P, plant N, plant litter, plant litter P and plant litter N characteristics on N or P fertilization (for more details see Methods). Mean values with 95% confidence intervals are plotted. The full vertical line indicates response ratio 1, which denote no change. Response ratio less than 1 indicates net decrease, whereas response ratio higher than 1 indicates net increase. c) Concentrations of soil available nitrogen and phosphorus across different sites in arctic tundra. Our data with data found in literature[30,29,31] are combined. Different point shapes represent different sample sites (for closer information see Supporting Information Appendix II). Grey area in the plot represents combination of soil available nitrogen and phosphorus at which plants are N limited and soil microorganisms are P limited. Dashed line represents plant $N:P_{CR}$ (32.4) and solid line represents microbial $N:P_{CR}$ (6.8).

We extracted the data about plant biomass, plant N or P concentrations, plant growth, photosynthetic rate etc. (525 unique values in total) from studies referred in Tab. 2 and grouped these data into 7 categories according to their relation to plant growth, plant biomass, plant P, plant N, plant litter, plant litter P, or plant litter N (Tab. 3). All categories mirror plant response to changed nutrition. From 13 collected studies, all demonstrate positive effect of N fertilization (Fig. 2a) except of one[16], which shows positive effect of P fertilization on plant growth and almost no N fertilization effect. In this particular study, plants and microbes were probably both

P limited, because N:P_S was around 60. In other studies, however, N:P_S should be between 6.8 and 32.4 according to soil data extrapolation, thus within the range where plants are N and microorganisms P limited (see Supporting Information Appendix II) and where microorganisms should leave N behind for the plants. The data meta-analysis detected, that P fertilization had generally negative effect on all plant parameters and N fertilization had the positive effect (QM = 18.0, df=1, $p < 0.001$). After closer inspection we found, that P fertilization significantly decreased plant growth (RR = 0.36, $p < 0.001$) and biomass (RR = 0.77, $p<0.001$) related characteristics, whereas N fertilization significantly increased plant growth (RR = 1.50, $p < 0.001$) and biomass (RR = 1.11, $p<0.001$) related characteristics (Fig. 3). In terms of plant or litter N and P concentration, P fertilization tends to significantly decrease N to P ratio in plants and plant litter.

Published data support the hypothesized plant-microbial interaction model. Our data additionally indicates that observed negative effect of P fertilization on plant growth and biomass was not caused solely by stronger competition for N but also by disappearance of additional plant N source which was derived from microbial N mineralization occurring under microbial P limitation.

Control over organic N mineralization by P availability was first suggested by Wild et al. [16]. Extended data set shows that net N mineralization steeply increase when N:P_{CR} drops below N:P_S (*mic* N:P_{CR}:N:P_S < 1 = P limitation; Fig. 3). Piecewise linear regression[17,18] indicates significant breakpoint at *mic* N:P_{CR}:N:P_S = 0.8 (0.4 –

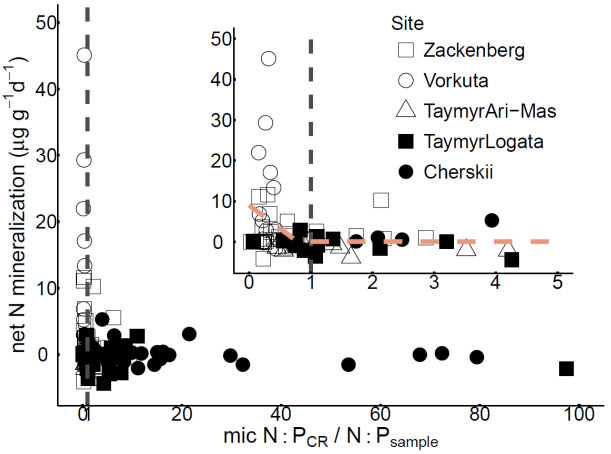

Fig. 3: "Broken stick" relationship between microbial P limitation net N mineralization rate. P limitation is defined as critical microbial N:P to soil extractable N:P ratio. When *mic*N:P_{CR}/N:P_S is one (black dashed line), microbial community have optimal nutrient supply. When *mic*N:P_{CR}/N:P_S < 1, microbial community is P limited and mineralize N in excess. Different point shapes represent different sample sites (Tab. 1). Red dashed line in nested graph represents fit of piecewise linear regression.

1.2 CI 90%) ($F_{2,93}$ = 8.5, p<0.001), which is statistically indistinguishable from theoretical value 1. When soil microorganisms are P limited, their N uptake decreases[19–21]. At the presence of N mineralization-immobilization-turnover pathway in soil[22], free or cell surface bound amino acid oxidases produced NH_4^+, which is not consumed by soil microorganisms and thus it is free for plant uptake. When soil is fertilized by P, N mineralization decreases. This might cause decrease of supply of soil available N to N limited plants even without competition.

It is important to note, that both plant and microbial $N:P_{CR}$ can vary depending on soil and climatic conditions [9,11,13]. Thus the range of $N:P_S$ in which plants are N limited and soil microorganisms are P limited can differ locally. Nevertheless, the present study offers new perspective of plant-microbial interaction and we suggest that presented theory is generally applicable. Its consequences for plant primary production or soil nutrient cycling should be thoroughly examined in future.

METHODS

Sampling sites

Soil samples were collected along the longitudinal gradient ranging from East Siberia (Cherskii) to Greenland (Zackenberg). Details about the sampling sites are given in Table 1 and also see references 23–26

Table 1
Basic characteristics (position, land cover class, list of dominant species, present morphological features and active layer depth) of 10 sampling sites used in throughout this study.

Site	UTM coordinates	Land cover class	Dominant species	Morphological features, Size (cm)	Active layer depth (cm)
Cherskii	57W 0607781, 7706532	Shrubby grass tundra	*Betula exilis, Salix sphenophylla, Carex lugens, Calamagrostis holmii, Aulacomnium turgidum*	Frost boils (D 30-40)	30-70
Cherskii	57W 0606201, 7705516	Shrubby tussock tundra	*Eriophorum vaginatum, Carex lugens, Betula exilis, Salix pulchra., Aulacomnium turgidum*	Frost boils (D 30-40)	35-60
Cherskii	57W 0604930, 7628451	Shrubby lichen tundra	*Betula exilis, Vaccinium uligonosum, Flavocetraria nivalis , Flavocetraria cucullata*	Hummocks (H 30, D 200), barren patches	35-90
Ari Mass	47X 0589707, 8044925	Shrubby moss tundra	*Betula nana, Dryas punctata, Vaccinium uligonosum, Carex arctisibirica, Aulacomnium turgidum*	Polygonal cracks , frost boils (D 50-70), barren patches	60-85
Ari Mass	47X 0588873, 8045755	Shrubby moss tundra	*Cassiope tetragona, Carex arctisibirica, Aulacomnium turgidum*	Polygonal cracks, frost boils (D 50-60)	65-90

Logata	47X 0482624, 8147621	Dryas tundra	*Dryas punctata, Rhytidium rugosum , Hylocomium splendens*	Small hummocks (H 20-30, D 30-100)	35-70
Logata	47X 0479797, 8150507	Grassy moss tundra	*Betula nana, Carex arctisibirica, Hylocomium splendens, Tomentypnum nitens*	Small hummocks (H 25-40, D 30-100)	30-65
Vorkuta	41W 497824, 7436954	Unvegetated		Peat circles	40-70
Vorkuta	41W 497824, 7436954	Dwarf-shrub tundra	*Vaccinium spp., Empetrum hermaphroditum, Betula nana, Salix spp.*		40-70
Vorkuta	41W 497824, 7436954	Heath tundra	*Rubus chamaemorus, Betula nana, Vaccibium spp., Sphagnum spp.*	Peat plateau	40-70
Zackenberg	27X 513935, 8266016	Heath tundra	*Cassiope tetragona, Vaccinium uliginosum, Dryas octopetala, Salix arctica, Carex spp.*		47

Soil profiles were excavated in at least three field replicates (28 profiles in total), with each replicate consisting of a 5×1m wide trench extending down to the permafrost table. The large dimension of the pro- files provided a representative cross section through microtopographic features (hummocks, patterned ground) and cryoturbation patterns. Diagnostic horizons, including subducted topsoil material, were sampled at various positions within the soil profile. The upper permafrost layer was cored (up to 30–40cm depth below the permafrost table) with a steel pipe at two positions in each profile: one directly underneath a hummock and

the other in between the hummocks. Directly after sampling, living roots and animals were removed.

Chemical and biological analyses

Bioavailable soil N and P was assessed as K_2SO_4 extractable N and $NaHCO_3$ extractable phosphate respectively. Total N concentration in extract was measured using TOC/TN analyzer (LiquicTOC II, Elementar, Germany) and soluble reactive phosphorus in extract was measured spectrophotometrically [27]. Net rate of N mineralization was determined as described by Kaiser et al. [28]. Total N content of soil was measured with an EA IRMS system (Isoprime) coupled to an Agilent Technology 7890A GC system. Critical nitrogen to phosphorus ratio was calculated from critical carbon to phosphorus ratio (see Supporting Information Appendix I), which was estimated from relative abundance of PLFA markers according to Capek et al. [11].

Published data compilation and treatment

Nitrogen and phosphorus concentrations in plant leaves and soil microorganisms were extracted from two meta-analyses, see reference 12 and 13.

Using Web of Science, we further search for all studies with experimental N, P fertilization in Arctic tundra areas. We were able to find 13 primary research articles, which are listed in table 2. All data presented in studies were directly extracted using Web Plot Digitizer. 525 unique values were extracted. Different characteristics were included in the articles such as plant biomass, apical growth, plant N concentration, litter amount etc.. In order to make statistical evaluation of the data across studies possible we grouped original data into 7 major vegetation characteristics - plant growth, plant biomass, plant P, plant N, plant litter, plant litter P and plant litter N characteristics (Tab. 3).

Three studies [29–31] also included data about available soil N and P. This data, together with our data set, were used to estimate available soil N and P in rest of the studies. Note, that this estimation is only possible because of the longitudinal gradient of $N:P_S$ and known coordinates of factorial N-P fertilization experiments (see Supporting Information Appendix II).

Table 2
List of studies used in the meta-analysis.

[1] Shaver et al., 1998, Ecological monographs
[2] Jonasson et al., 1999, Applied Soil Ecology
[3] Schmidt et al., 1997, Oecologia
[4] Arens et al., 2008, Journal of Geophysical Research
[5] Gough et al., 2002, Arctic, Antarctic and Alpine research
[6] Gough a Hobbie, 2003, Oikos
[7] Madan et al, 2007, Polar Biology
[8] Zamin et al, 2014, Journal of Ecology
[9] Weg et al., 2013, Plant Ecology
[10] Soudzilovskaia et al., 2007, Journal of Vegetation Science
[11] Bubier et al., 2007, Global Change Biology
[12] Shaver and Chapin, 1995, Ecography
[13] Shaver and Chapin, 1980, Ecology

Table 3
Different vegetation characteristics measured in particular studies grouped into 7 major characteristics - plant growth, plant biomass, plant P, plant N, plant litter, plant litter P and plant litter N.

Characteristic	Measured parameter	Study
Plant biomass related	Above ground biomass	[1] [5] [6] [8]
	Live plant biomass	[1]
	Total biomass	[1] [2]
	NDVI	[4]
	Below ground biomass	[5] [6]
	Plant cover	[7]
	Dead standing biomass	[7]
	Bare soil	[7]
	Leaf mass per plant	[12]
	Shoot mass	[13]
Plant growth related	NEP	[1] [4] [11]
	Apical growth	[5] [8]
	ANPP	[6]
	Photosynthetic rate	[11]
	Growth	[11]
Plant N related	Shoot N	[3]
	Root N	[3]
	Plant N	[3] [13]
	Leaf N	[9][12]

Plant P related	Shoot P	[3]
	Root P	[3]
	Plant P	[3] [13]
	Leaf P	[12]
Plant litter related	Litter left	[8]
	Litter mass	[10]
Plant litter nitrogen related	Litter N	[10]
Plant litter phosphorus related	Litter P	[10]

Statistical analysis

The significance of the relationship between $micN{:}P_{CR}/N{:}P_{Sample}$ and net N mineralization rate was tested using piecewise linear regression in statistical program R [17] using package segmented [18]. Mechanistic model described in Supporting Information Appendix II was fitted onto the date using generalized nonlinear models using package nlme [32]. Published data meta-analysis was done according to Lajeunesse [33]. Response ratios were calculated and effect sizes of N and P fertilization was estimated using package metafor [34]. Data are presented as response ratio without logarithmic transformation.

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Supporting Information

Plant-microbial interaction: Effect of variable stoichiometry

Appendix I

$N:P_{CR}$ calculation

$N:P_{CR}$ was calculated from $C:P_{CR}$. $C:P_{CR}$ was either directly estimated[1] or calculated from relative abundance of phospholipid fatty acid markers collapsed into 3 principal components[1]. We used combination of unpublished and published data[1–5]. Together we collected 210 unique estimates of $C:P_{CR}$ across wide range of ecosystmes[1]. The theory suggests that $C:P_{CR}$ is related to microbial community C to P ratio ($C:P_m$), carbon use efficiency (CUE) and kinetic constants of C and P uptake (k_C and k_P respectively):

$$C:P_{CR} = \frac{k_C}{k_P}\,\frac{C:P_m}{CUE} \qquad [1]$$

Similarly, we can define $C:N_{CR}$:

$$C:N_{CR} = \frac{k_C}{k_N}\,\frac{C:N_m}{CUE}\,. \qquad [2]$$

$N:P_{CR}$ is the ratio between $C:P_{CR}$ and $C:N_{CR}$:

$$N:P_{CR} = \frac{C:P_{CR}}{C:N_{CR}}\,. \qquad [3]$$

We can assume, that C and N are often bind together in organic molecules (e.g. amino acids), which are often taken up by microbes as an intact molecule (see later). We can further assume, that uptake of all N forms (organic N, NH_4^+, NO_3^-

) is similarly fast[6]. Therefore $\frac{k_C}{k_N}$ should be close to 1. Based on this assumption, combining Eq. 2 and 3 we get:

$$N{:}P_{CR} = C{:}P_{CR} \times \frac{CUE}{C{:}N_m}. \quad [4]$$

Because CUE is included inside $C{:}P_{CR}$ estimate, Eq. 4 simplifies to:

$$N{:}P_{CR} = C{:}P_{CR} \times \frac{1}{C{:}N_m}. \quad [5]$$

Both microbial C and N were directly measured in soil samples. Calculating $N{:}P_{CR}$ according to eq. 5, we get left skewed distributed $N{:}P_{CR}$ across all soil samples with median 6.8 (Fig. 1 in main text).

Appendix II

Microbial and plant nutrient limitation in Arctic tundra

It is generally accepted, that phosphorus availability often differs dramatically among different location and soil horizons within one soil [7]. This is also true for our data (Figs. S1 and S2). As a result, P limitation of soil microbial community also depends on locality (Fig. S1b).

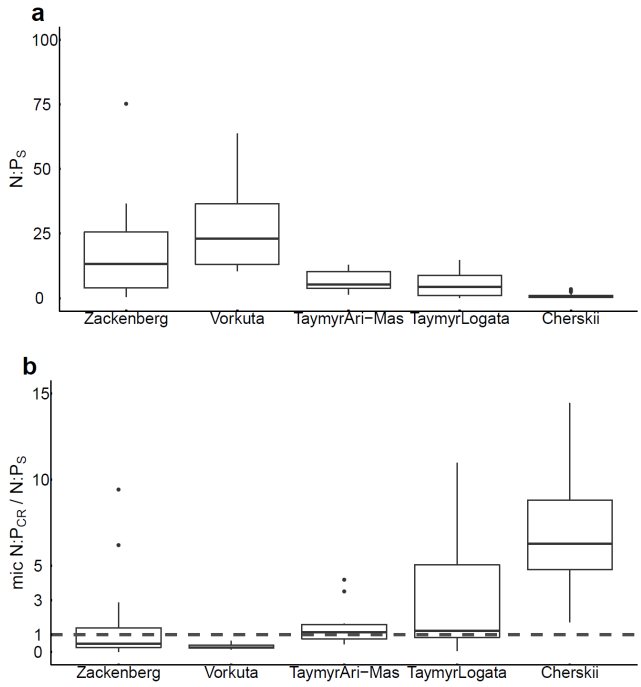

Fig. S1: a) Boxplot of amount of available soil P relative to available soil P at N ($N:P_S$) different localities across the Arctic (see Tab.1 in main text). b) Boxplot showing P limitation of soil microbial community at 5 different localities across the Arctic. Dashed line shows the threshold below which P limitation is likely to occur (where microbial demand is equal to supply *mic*$N:P_{CR}$ = $N:P_S$). 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. Points represent outlier values.

Figure 2a shows, that P availability in the arctic soils decreases from east (Cherskii) to west (Zackenberg). We combined our data with data published in literature [8–10] and found statistically significant longitudinal gradient of increasing soil P availability with increasing absolute longitude (Fig S3a, $F_{1,117}$ = 10.4, p=0.002). For the sake of simplicity, absolute longitude is defined here as longitude plus 180 degrees (getting a range from 0 to 360). If microbial $N:P_{CR}$ equal to 6.8, microbial P limitation is likely to occur west from 283° (103rd east meridian) in Arctic tundra.

This is critical for verifying our hypothesized plant-microbe interaction model (see the main text). All factorial fertilization experiments used in our

meta-analysis were done on localities located west from 283° (30 – 199°). Thus, P limitation of microbial community is likely to occur here. Except of one study [10], all the other studies reported positive effect of N fertilization on plant biomass at the same time. Therefore, microbial community is likely P limited on these locations, whereas plants are N limited at the same time. This is the main condition, which needs to be met to test our plant – microbe interaction. The one excepted study is study of Shaver et al. who found soil extractable N to P ratio around 60 (Fig S2), which is, above median plant N:P_{CR} 32.4 (see the main text) and thus, plants and microbes share P limitation here. Except of this study, all other studies from Arctic tundra represent ideal system for testing our hypothesis about plant microbial interaction. Available soil N to P naturally occur between hypothesized microbial and plant critical N to P ratio (6.8 – 32.4; Fig. S2b).

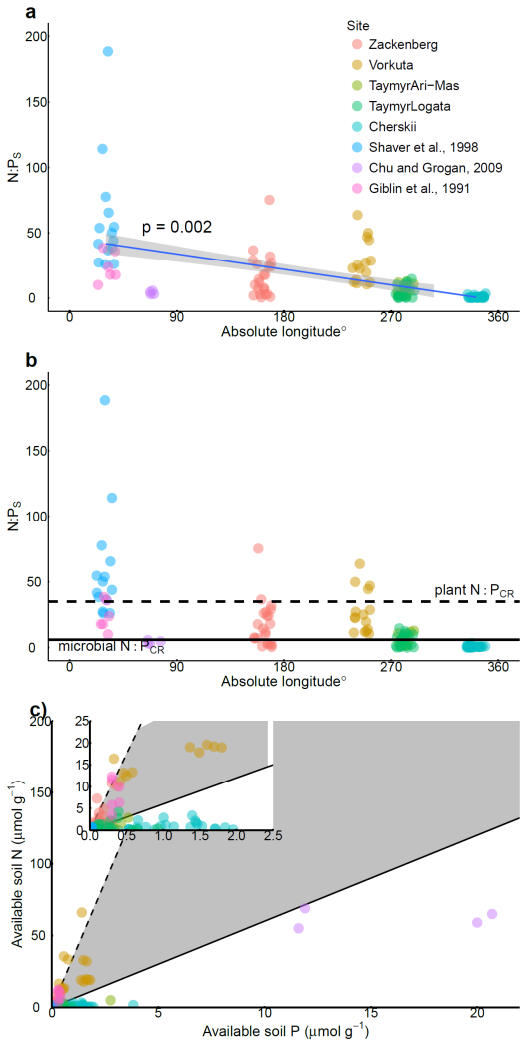

Fig. S2: a) Linear regression between absolute longitude (longitude + 180) and soil available P relative to soil available N on data collected from the literature[8–10] or directly measured. Grey area along the regression line represents 95% confidence interval (n = 173). b) Soil available P relative to soil available N ($N:P_S$) across longitudinal gradient in Arctic tundra. Dashed line represent plant $N:P_{CR}$ (35) (see the main text) and solid line represent microbial $N:P_{CR}$ (9.8) (see Supporting Information Appendix III). Data points lying between these two lines represent cases at which P availability is higher than plants demand but lower than microbes demand. c) Measured data within plant – microbial interaction model (see main text Fig. 1).

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7. APPENDIX

7.1. LIST OF ABBREVIATIONS

ρ_0	normalization constant of the metabolic theory
μ	microbial growth rate
μ_0	microbial growth rate at zero phosphorus concentration
A	pre-exponential factor of the Arrhenius equation
Ajj	cryoturbated organic horizon
Bcg	mineral soil horizon
C	carbon
$C_{anabolic}$	organic C anabolism
C_{CELL}	cell components production rate
C_{ENZ}	extracellular enzymes production rate
C_{EX}	extractable soil carbon
C_{MB}	microbial community biomass
C_{MIN}	organic C mineralization rate
C_{LOSS}	cumulative carbon loss
$C{:}N_{MB}$	carbon to nitrogen ratio of soil microbial biomass
$C{:}P_{CR}$	critical C to P ratio of microbial community demand
$C{:}P_{MB}$	carbon to phosphorus ratio of soil microbial biomass
$C{:}P_S$	substrate C to P ratio
CUE	carbon use efficiency
CUE_{MAX}	maximum carbon use efficiency (0.6)
E_A	activation energy
E_{CNP}	total soil enzymatic pool
E_C	carbon acquisition enzymes pool
E_N	nitrogen acquisition enzymes pool
E_P	phosphorus acquisition enzymes pool
k	Boltzmann constant ($8.62\ 10^{-5}$ eV K^{-1})
k_I	kinetic constant for inorganic P uptake
k_D	kinetic constant for organic C uptake
K_M	affinity constant
N	nitrogen
N_{EX}	extractable soil nitrogen
$N{:}P_{CR}$	critical N to P ratio of microbial community demand
$N{:}P_{MB}$	nitrogen to phosphorus ratio of soil microbial biomass
$N{:}P_S$	substrate N to P ratio
NUE	nitrogen use efficiency
O	topsoil organic horizon
OM	organic matter
P	phosphorus

P_{EX}	extractable soil phosphorus
Q_{10}	relative increase of reaction rate with 10°C temperature rise
qC_{MIN}	metabolic quotient
R	universal gas constant (8.314 J K^{-1} mol^{-1})
T	temperature
TCA	tricarboxylic acid cycle
V_{MAX}	maximal growth rate

7.2. CURRICULUM VITAE

Personal information

First name(s) / Surname(s)	**Petr Čapek**
Address(es)	Mokré 166, 370 01 Litvínovice, Czech Republic
Telephone(s)	+420 38 7772347 (office)
	Mobile: +420 723 259 416
E-mail	petacapek@gmail.com
	http://kbe.prf.jcu.cz/en/petr-capek
Nationality	Czech
Date of birth	02 October 1985
Gender	Male
University address	Department of Ecosystem Biology, Faculty of Science, University of South Bohemia Branišovská 1760 CZ – 370 05 České Budějovice Czech Republic

Education and training

2011 – present: Ph.D. program, University of South Bohemia, České Budějovice
(Faculty of Science, Department of Ecosystem Biology)
student of ecosystem biology
(supervisor: prof. Ing. Hana Šantrůčková, CSc.)

2008 – 2010: Master of Biology (Mgr.), University of South Bohemia, České Budějovice
(Faculty of Science, Department of Ecosystem Biology)
student of soil biology
(supervisor: prof. Ing. Hana Šantrůčková, CSc.)

2005 – 2008: Bachelor of Biology (Bc.), University of South Bohemia, České Budějovice
(Faculty of Science, Department of Ecosystem Biology)
student of soil biology

(supervisor: RNDr. Karolina Tahovská, Ph.D.)

Scientific focus
Importance of soil microorganisms in nutrient cycling, ecological stoichiometry

Work and science experience
Collaborator on the CryoCARB project (Long-term Carbon Storage in Cryoturbated Arctic Soils); http://www.univie.ac.at/cryocarb/the-cryocarb-project/

Member of joint sampling trip 2011 of CryoCARB project to Taymyr peninsula (Russia)

Member of a field works in the Himalaya, Ladakh, 2013 (collection and treatment of soil samples, field measurement of microbial activity)

Member of a field works in the Svalbard, 2014 (collection and treatment of soil samples, field measurement of microbial activity)
Other scientific skills Using stable isotopes in biological research
Using high level statistics and simple mathematical modelling in biological research with program R

Personal skills and competences
Mother tongue(s) Czech
Other language(s) English – proficient user
German – basic user

Other skills and competences
Toefl ITP, Open Source Academy certificate, Course of Decomposition of forest plant litter leading to carbon sequestration, Stable Isotope Course: an introduction to uses in ecology and plant physiology.

Driving licence
Holder of a driver's licence, category B vehicle.

Publications in peer-reviewed scientific journals
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.

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University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz