LINKING MICROBIAL COMMUNITY FEATURES AND BIODEGRADABILITY OF ORGANIC MATTER IN SIBERIAN PERMAFROST AFFECTED SOILS

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Abstract

Boreal forest soils have globally relevant functions that affect atmospheric chemistry and climate. We investigated the microbial community structure and activity of Siberian sub-arctic region (N 64°, E 100°) regarding to features and biodegradability of soil organic matter stored in permafrost soils. Soil microbial biomass, number of copies of DNA fragments and heterotrophic microbial activity of soil in larch stands of Central Evenkia were studied. It was shown that soil heterotrophic activity is closely correlated to the labile fraction of soil organic matter while microbial pool depends on the proportion of stable fraction of soil organic matter.

Keywords: *Soil organic matter, Microbial biomass and activity, Active layer, Permafrost-affected soils, Boreal forest, Central Siberia*

1. INTRODUCTION

Permafrost-affected landscapes cover about one quarter of the land surface of the northern hemisphere (Zhang et al., 1999). In these landscapes, the permafrost is covered by a thin soil layer (active layer) which thaws during summer and facilitates vegetation growth. According to recent estimates, 352 Pg of organic carbon is stored in the uppermost 1 m of permafrost landscapes and 818 Pg down to a depth of 3 m (Zimov et al., 2006; Tarnocai et al., 2009). Comparing these values with the 1500 Pg organic carbon stored in the upper most 1 m of terrestrial soils (Jobbagy & Jackson, 2000) illustrates the global importance of organic carbon in permafrost.

The effects of current and future climate warming will be stronger in the Arctic than the global average (Trenberth et al., 2007). In response to Arctic warming the area of permafrost landscapes will decrease and the thickness of the active layer will increase (Anisimov, 2007; Schaefer et al., 2011). The organic carbon that has been preserved frozen in permafrost will then become accessible to microbial degradation resulting in the formation of carbon dioxide (CO_2) and methane (CH_4) (Wagner et al., 2007; Lee et al., 2012). Hence, permafrost thawing may provoke a positive feedback to climate warming by causing increased greenhouse gas emissions. Recently, a positive feedback of methanogenic communities to warmer periods in the Late Pleistocene and Holocene was shown for the Lena River Delta (Bischoff et al., 2013)

Multiyear measurements of trace gas production from northeastern Siberian permafrost demonstrate a significant amount of labile organic matter in the permafrost that can be readily mineralized after thawing. However, model simulations of aerobic and anaerobic CO_2 production over 100 years predict a substantially lower carbon release from thawing permafrost than currently available first-order estimates. One reason for these lower estimates is the dominance of the slowly degrading carbon pools in the studied permafrost deposits, which are characterized by turnover times of centuries. Another reason for the lower estimates is that the presented model confines microbial carbon degradation to the few months of the year when the soils in permafrost landscapes are not frozen and carbon mineralization takes place (Knoblauh et al., 2013).

Global warming has the potential to change permafrost landscapes from a carbon sink into a carbon source but the response of the Arctic carbon cycle to current changes in permafrost areas is still highly uncertain (McGuireet al., 2009). The strong interannual variability of carbon fluxes in Arctic ecosystems (Oechel et al., 2000; Groendahl et al., 2007; Schuur et al., 2009) and the lack of sufficient studies on multiannual greenhouse gas budgets still prevent a clear picture of the current source-sink term of the Arctic tundra. Recent studies indicate an increase in CO_2 release from permafrost-affected

landscapes, e.g., due to deeper permafrost thawing (Schuur et al., 2009). However, warming of arctic ecosystems may also increase the uptake of atmospheric CO_2 due to a longer growing season (Aurela et al., 2004).

Northern ecosystems are characterized by extreme climatic conditions and low productivity during the short growing seasons. Low soil temperature regimes and partially water saturation of large areas give rise to slight microbial activity and long turnover time for organic materials. As a result, accumulation of organic matter is favored in cold climate soils (Rodionow *et al.*, 2006; Meyer *et al.*, 2006).

The main obstacle for a better understanding of carbon dynamics in cryogenic soils that soil organic matter (SOM) is unevenly distributed within the soil, due to cryoturbation activities, making soil organic carbon (SOC) estimation based on simple upscaling/modelling very difficult (Schuur et al., 2008). There is evidence that the north ecosystems arctic carbon stock is bigger than previously thought, also because of underestimation of carbon stored in distorted, broken and warped horizons (Post et al., 1982). Knowledge of the linking microbial community features and SOM quality as a substrate for decomposing organisms is necessary to predict the magnitude and the time-scale at which C will get mobilized in permafrost soils at climate change (Khvorostyanov et al., 2008).

2. SITE DESCRIPTION AND METHODS

2.1. Study site

Study site is located in the homogeneous larch forests of the Central Evenkia (N 64°, E 100°). The investigated area is situated in borders of continuous permafrost zone, with a permafrost thickness up to 300 m and with the permafrost temperature of -3.5 °C. Soil cover is presented by Cryosols. Climate is continental, humid. Mean annual temperature is -8.9°C, temperature of January – -36 °C, July – +16 °C. Sum of the active temperatures above 10°C is equal to 1000 °C. Mean annual precipitation equal to 369 mm and evenly distributed between year seasons. Duration of vegetative period is about 70-80 days (Climatic atlas, 1960).

2.2. Soil sampling and preparation

Soil samples were taking within active layer profiles on the south-facing and north-facing slopes differing with intensity of solar radiation, a forest floor thickness, soil moisture and temperature, and the active layer thickness. Subsamples for heterotrophic respiration rate and microbial biomass estimation have been stored at 4ºC before analysis. Subsamples for DNA analysis were immediately frozen at -18°C.

2.3. Soil carbon and nitrogen determination

Evaluation of the accumulation of carbon and nitrogen in soil was done at Elemental Carbon and Nitrogen Analyzer (Vario EL III, Elementar).

Total organic carbon was determined by I.V. Tyurin's method (Arinushkina, 1970). Labile organic matter was extracted using serial daily extractions of a soil sample by distilled water and 0.1N NaOH solution, without preliminary decalcifying (Ponomareva and Plotnikov, 1975). The content of stable soil organic matter was determined by a difference between the content of the total organic carbon and carbon of the labile organic matter.

2.4. Microbial biomass and heterotrophic respiration rate

Microbial biomass was assessed by rehydration method (Methods…, 1991) and using substrate-induced respiration: by addition into substrate overdosage of D(+)glucose and $(NH_4)_2SO_4$. CO_2 released during first two hours was detected using gas chromatograph Agilent 6890N configured with FID and methanizer (Hewlet-Packard, USA) and converted into microbial carbon: μg CO_2 – C g $soil^{-1}$ h^{-1} (Anderson and Domch, 1978; Sparling, 1995).

Basal (heterotrophic) soil respiration was estimated from CO_2 emission rate from soil samples incubated at 23ºC and 60% moisture content.

2.5. DNA extraction and Quantitative PCR

Total genomic DNA was extracted using a Power Soil™ DNA Isolation Kit (Mo Bio aboratories Inc., Carlsbad, California, USA), according to the manufacturer's protocol. DNA templates for qPCR analyses were extracted from 3 biological replicates.

Quantitative PCR was performed on an iQ5 thermocycler (Bio-Rad Laboratories, Inc., Hercules, USA). Each reaction contained iQ Mastermix (10 µl; Bio-Rad Laboratories, Inc., Hercules, USA), PCR primers (1 µl containing 10 pmoles μl^{-1} each), sterile water (6 µl), SYBR® Green (0.2 µl per reaction of 100 × diluted from 10,000 × concentrate) and DNA template (5 µl) added to a final volume of 20 µl. Primers targeting *Bacteria* were used. The qPCR reactions comprised an initial denaturation (10 min at 95°C), followed by 39 cycles (for the bacterial primer pair) of 30 s at 95 C, 30 s at 57 C and 40 s at 72 C. The qPCR assays were calibrated using known amounts of PCR amplified and cloned 16S rRNA gene fragments from corresponding taxa (10^7 *Bacillus subtilis* standart).

Statistical comparisons between PCR efficiencies were done using t-tests implemented in Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Elemental carbon and nitrogen content in soils of investigated sites

The carbon concentration in the upper 0-5 cm layer of soil on the south-facing slope was 26 mg g^{-1} soil, and decreased to 2-4 mg g^{-1} soil at 40-50 cm soil depth. Soils on the north-facing slope differed from those at the south-facing slope by four folds higher carbon concentrations in the uppermost soil layer, i.e. 112 mg g^{-1} soil. Despite the narrow C/N ratio in mineral soil horizons, the soils both slopes characterised by small amount of nitrogen content (Table 1).

Table 1. Elemental carbon and nitrogen content in soils of investigated sites

Place of sampling	Soil depth, cm	TC, %	N, %	C/N
South-facing slope	Forest floor	42.22±0.11	0.63±0.07	67.34±7.58
	Litter	19.73±0.46	0.63±0.02	31.52±1.63
	0-5	2.63±0.06	0.12±0.01	22.65±0.33
	5-10	0.48±0.01	0.03±0.01	14.20±0.34
	10-15	0.30±0.03	0.03±0.01	9.59±2.89
	15-20	0.22±0.01	0.02±0.01	10.11±0.76
	20-30	0.44±0.01	0.04±0.01	12.34±2.57
	30-40	0.41±0.02	0.03±0.01	13.82±1.12
	40-50	0.24±0.02	0.02±0.01	11.96±1.99
	50-60	0.25±0.12	0.02±0.01	10.38±0.55
	60-70	0.41±0.01	0.03±0.01	15.20±1.20
	70-75	0.52±0.08	0.03±0.01	15.35±2.83
North-facing slope	Forest floor	43.78±0.09	0.86±0.04	51.16±2.12
	Litter	35.81±0.18	0.77±0.03	46.41±1.29
	0-5	1.49±0.02	0.09±0.01	17.02±0.45
	5-10	1.08±0.01	0.07±0.01	15.40±1.32
	10-15	1.20±0.03	0.08±0.01	15.16±0.20
	15-20	2.04±0.06	0.11±0.01	19.62±0.41
	20-30	1.74±0.03	0.11±0.02	16.17±0.04
	30-40	1.57±0.04	0.10±0.02	15.55±0.24
	40-48	2.16±0.01	0.12±0.01	17.41±0.64

The concentrations of both biogenic elements decreased with the soil depth. The carbon and nitrogen contents of mineral soils strongly depended on the location and the permafrost regime. This indicates that soils heterogeneity, exposition and permafrost regime can influence on soil organic matter storage in permafrost-affected soils, where the organic carbon storage shows a high spatial variability.

3.2. Soil organic matter and microbial biomass

The total organic carbon stock in the active soil layer was 5.4 and 2.1 kg m^{-2} on the north-facing and south-facing slopes, respectively, in spite of the thickness of this layer on the south-facing slope is 1.3 times as great as on the north-facing slope. The upper 0-20 cm soil layer of north-facing slope contained 2.3 times more carbon than soils on the south-facing slope. Carbon stock in soil profile on the both slopes showed gradual decrease down within the profile up to permafrost table (Table 2).

Table 2. Carbon stock in main fractions of soil organic matter

		Carbon stock, kg m^2			
Site	Depth, cm	C_{tot}	C_{lab}	C_{stab}	C_{MB}
North-facing slope	Moss	0,524	0,082	0,442	0,011
	Litter	1,001	0,109	0,892	0,013
	0-5	0,489	0,086	0,403	0,016
	5-10	0,677	0,120	0,557	0,022
	10-15	1,019	0,136	0,882	0,031
	15-20	1,103	0,136	0,967	0,021
	20-30	0,893	0,145	0,748	0,020
	30-40	0,693	0,105	0,589	0,007
	40-50	0,277	0,038	0,239	0,000
	50-60	0,299	0,056	0,243	0,000
	Total	6,975	1,013	5,962	0,142
South-facing slope	Moss	0,790	0,109	0,681	0,075
	Litter	1,424	0,320	1,104	0,060
	0-5	0,801	0,086	0,715	0,007
	5-10	0,166	0,034	0,131	0,000
	10-15	0,217	0,041	0,176	0,000
	15-20	0,241	0,029	0,212	0,000
	20-30	0,370	0,094	0,276	0,000
	30-40	0,070	0,055	0,015	0,000
	40-50	0,091	0,044	0,047	0,000
	50-60	0,066	0,032	0,034	0,000
	60-70	0,057	0,022	0,035	0,000
	70-80	0,056	0,040	0,016	0,000
	Total	4,350	0,906	3,443	0,143

Note: C_{tot} – total organic carbon, C_{lab} – labile organic carbon, C_{stab} – stable humus stock, C_{MB} – microbial biomass

In active layer of north-facing slope, the microbial biomass determined by a rehydration method accounted for 3% of total organic matter content. On south-facing slope, microbial biomass was determined only in the upper soil layer (0-5 cm) and its contribution to the total organic matter content was less that 0.9%. The main reason of low microbial biomass amount in deeper soil layers of the south-facing slope profile, probably, was critically low soil moisture -10-12% at the date of sampling in consequence of hot and dry summer (soil moisture of the north-facing slope in the same time was 19-42%).

The main part of accumulation of soil organic matter stocks on both slopes was stable humus (C_{stab}). C_{stab} accumulation in soil of north-facing slope took place up to permafrost table while those on south-facing slope indicated within mostly 0-30 cm layer. Labile carbon prevailed in soils of north-facing slope (Fig. 1). We assume that higher moisture conditions of a north-facing slope favored to migration of the labile organic matter down a soil profile and to accumulation of higher stocks, both the labile organic matter, and a stable humus, in the deep soil horizons.

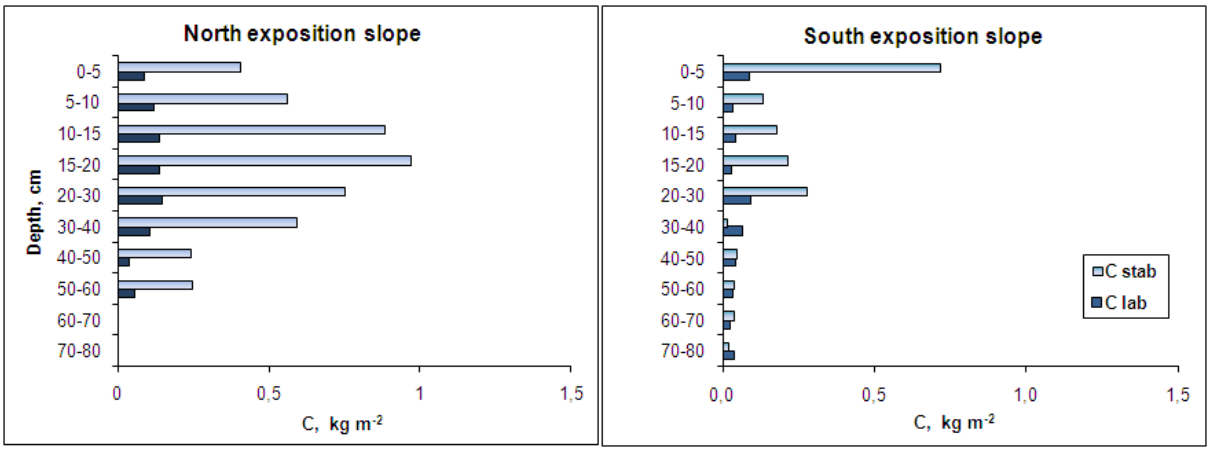

Fig. 1. Stable carbon (C_{stab}) and labile carbon (C_{lab}) distribution within soil profiles both slopes

3.3. Soil heterotrophic activity and number of DNA fragments copies

Basal respiration rate within active layer of soil profile both south-facing slope and north-facing slope decreased with the depth and strongly correlated with C-content (Fig. 2). Heterotrophic microbial biomass correlated with C-content as well. We complemented the data of microbial community abundance with information about DNA fragments copies within active layer of soil profiles, and found out that number of DNA fragments copies in turn correlated with soil microbial biomass (fig. 2, 3).

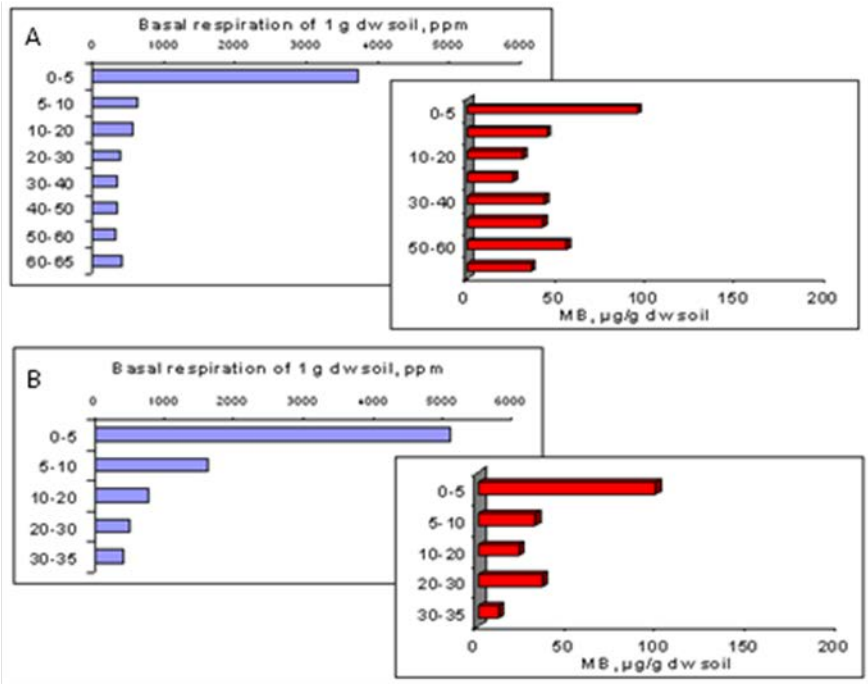

Fig. 2. Basal (heterotrophic) respiration rate and microbial biomass (MB) in soil on south-facing (A) and north-facing (B) slopes

The number of DNA fragments copies in the soil of the south-facing slope decreased down on a profile with sharp increase in the over-permafrost horizon while the found number of DNA fragments copies in the soil of the north-facing slope was rather evenly distributed within active layer profile, with a tendency to decrease in the over-permafrost horizon. Possibly, such distinction is bound to various soil moisture and its flushing regime on slopes. Also it was revealed that biomass of the heterotrophic microorganisms is distributed in the soil on slopes homologously to distribution of number of DNA fragments copies that, appealingly, testifies that the majority of a microbial pool in these soils is heterotrophic, although inactive – heterotrophic respiration of the underlying horizons in studied soils was found extremely low.

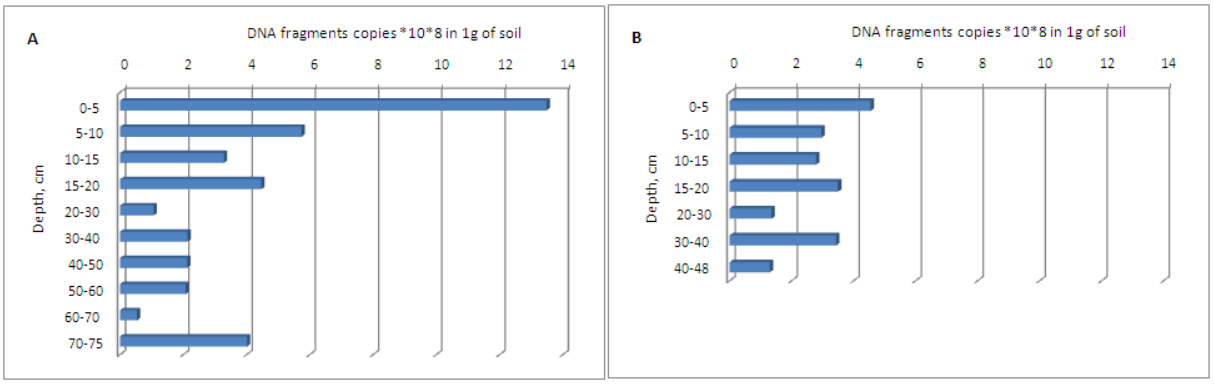

Fig. 3. DNA fragments copies within active layer soil profiles in south-facing slope (A) and north-facing slope (B)

Accordingly, at equivalent quantitative stocks of microbial fragments in the soil of both slopes, activity of soil microbial communities on the north-facing slope is higher that probably is a consequence of higher soil moisture. The majority of microbial communities in studied soils is heterotrophic. Soil heterotrophic activity is closely correlated to the labile fraction of soil organic

matter while microbial pool depends on the proportion of stable fraction of soil organic matter. Soil moisture promotes microbial activity and indirectly influences on transformation of the stable organic matter fraction in the labile. The increase in rate of organic matter mobilization in soils of the studied region will depend not so much on temperature increase, but on moisture increase, and also on ability of soils to retain moisture that in turn depends on a micro-relief and a flushing regime of soils.

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