Decomposition of labile and recalcitrant soil organic matter of Gleyic Cryosols in permafrost region of Siberia

Olga Rusalimova and Pavel Barsukov*

Institute of Soil Science and Agrochemistry, Russian Academy of Sciences, Siberian Branch, Str. Sovetskaya 18, 630099 Novosibirsk, Russia

Corresponding author: pavel-b@inbox.ru

1. INTRODUCTION

Climate change models predict that the mean global surface air temperatures will increase by between 1.4 and 5.8°C until the end of the twenty-first century (IPCC, 2001). As a result, decomposition of soil organic matter (SOM) is expected to be accelerated, thereby releasing carbon (C) from the soil to the atmosphere (Trumbore et al., 1996). Much uncertainty remains, however, on the amount of C that may potentially be released from soils as a consequence of global warming. Although microbial activity and thus decomposition processes are known to be temperature-sensitive, the impact of warming on soil processes is currently under considerable debate.

This study is aimed at determining (1) whether the substrates utilized by arctic microbes change with temperature, and particularly whether the recalcitrant C stocks may be utilized at higher temperatures; (2) whether such changes are caused by a change in microbial community composition; and (3) whether substrate limitation is controlling the resource utilization patterns.

2. MATERIALS AND METHODS

Soils were taken from Gdansky-Peninsula, Siberia (69° 43.0'N, 74° 38.8'E, 74 m a.s.l.). The site is located in the arctic bioclimate subzone D, (CAVM Team, 2003) also called typical tundra subzone (Chernov, Matveyeva, 1997). Mean annual temperature was -9.88°C and mean annual precipitation 336 mm. One of the most common landscape forms within this area is frost-boil tundra, which consists of patches of bare soil surrounded by rims (small mounds of earth) and troughs (depressions). At rims dwarf shrubs (*Vaccinium vitisidea, V. minus, Dryas punctata*), graminoids (*Carex arctisibirica, Arctagrostis latifolia* and *Calamagrostis holmii*) and lichens (e.g. *Cetraria nivalis*), in troughs deciduous shrubs (*Betula nana, Salix lapponum, S. glauca*) and mosses (e.g. *Aulacomnium turgidum, Hylocomium splendens* and *Dicranum* sp.) are dominant.

The soils are classified as Gleyic Cryosols according to the World Reference Base for Soil Resources (WRB). Organic horizons (O and Ap) and mineral horizons (Ah) were on average 5.1 and 5.5 cm deep in rims, and 6.6 and 13.6 cm deep in troughs. The mean bulk density of organic and mineral soil was 0.19 and 0.84 g m^{-3}, respectively. Organic soils of troughs contained highest amounts of SOM (69.3% of dry matter), followed by organic soils of rims (44.1%). Mineral soils were considerably lower in SOM content (25.1% in troughs and 11.4% in rims). The C/N ratios ranged from 21.0 in organic soil to 15.2 in mineral soils, and pH was generally around 5. Mean soil temperature during July 2001 was 8.9 and 5.0°C (at the surface and in 5 cm depth, respectively), with minima of 1.1 and 2.8°C and maxima of 22.8 and 12.6°C, respectively.

Symptom of Environmental Change in Siberian Permafrost Region,
Eds. Hatano R and Guggenberger G, p 93-102, Hokkaido University Press, Sapporo, 2006

Three intact soil cores (approx. 25×25×25 cm) were collected each from rims and troughs within 1 ha plot. The soil cores were frozen at -20°C and transported to the laboratory within 2 days. The soil cores were kept frozen until the start of the experiment. After being allowed to thaw, the soil cores were subdivided into the two horizons (organic and mineral). Four cubes (approx. 10×10×10 cm) were cut for each horizon to incubate three of them at each temperature. One soil cube was used to determine SOM content and SOM fractions before incubation procedure. The soil cubes were incubated in the dark at 2, 12 and 24°C for 6 weeks. Moisture content was adjusted to 50% fresh weight for organic soils and 40% for mineral soils and kept constant by adding deionized water as necessary. After the termination of the incubation experiment, soils were analyzed as detailed below.

Total SOM content was determined by dry combustion (at 450°C). A chemical fractionation procedure was applied to separate soil organic carbon pools and to determine their isotopic signatures. Sequential extraction with cold water to dissolve simple sugars and acid hydrolysis to solubilize pectins and cellulose (leaving lignin and humic substances behind) was performed (Sollins et al., 1999). All soil samples and fractions were dried at 60°C and homogenized before analysis by elemental analyzer-isotope ratio mass spectrometry (EA-IRMS). The $\delta^{13}C$ value of the acid-soluble pool was calculated by the difference between soils before and after acid hydolysis according to the mass balance equation: $C_s \times \delta_s = C_{as} \times \delta_{as} + C_r \times \delta_r$, where $_s$ and $_{as}$ represent soil and acid-soluble fraction, and $_r$ the residue. C is the amount of carbon in each fraction and δ the corresponding $\delta^{13}C$ value.

Heterotrophic respiration was determined followed the alkali trap and titration method. Subsamples of approximately 5×5×5 cm (10-20 g soil fresh weight) were incubated with NaOH (10 mL, 0.5 M) for 8–10 days at various temperatures. The CO_2 produced and trapped in NaOH was precipitated by addition of 15mL 2M $SrCl_2$. Excess NaOH was titrated back with 0.2M HCl. Precipitated $SrCO_3$ was collected by centrifugation, after washing five times with deionized water and once with EtOH, and used for ^{13}C analysis (Harris et al., 1997). Aliquots of $SrCO_3$ (2.5 mg) were analyzed with vanadium pentoxide (5 mg) as a combustion reagent by EA-IRMS. Blanks without soil addition were included in all experiments.

The natural abundance of ^{13}C was expressed as parts per thousand relative to the international standard PDB using delta units (δ). The isotopic signature of CO_2 of soils incubated at 24°C was further determined at 12°C (´temperature reversal experiment´). Therefore, soils incubated at 24°C were measured at 12°C for 8 days to collect CO_2 as described above.

Effects of temperature on heterotrophic respiration are described by Q_{10} values:

$Q_{10} = R_1 / R_2^{10/(T2-T1)}$, where T1 and T2 represent the lower and the higher temperature, respectively, and R is the respiration rate at the particular temperature.

To rule out isotopic discrimination during absorption of CO_2 in alkali or during precipitation with $SrCl_2$, we incubated a known amount of CO_2 with a known isotopic composition at various temperatures for 8 days, and collected $SrCO_3$ as described above. Quantitative recovery of CO_2 was achieved and no isotopic fractionation occurred for amounts >2 mg C.

This corresponded to a minimum volume of all samples except for CO_2 produced in mineral soils incubated at 2°C. These isotopic data were therefore excluded. No differences in isotopic signature of CO_2 between the temperature treatments were observed.

For determination of microbial biomass C and $\delta^{13}C$ we made use of the chloroform-fumigation method (Vance et al., 1987) and a persulfate digestion technique (Paul et al.,1999). The dissolved C was oxidized by heating at 120°C for 2 h, and the evolved CO_2 was trapped in NaOH. The culture tubes were kept sealed for several hours to guarantee full absorption of CO_2. Blanks and standards (glycine) were digested simultaneously with samples. Absorbed CO_2 was precipitated as $SrCO_3$. Microbial biomass C of initial soils as part of the soil C fractions was

calculated as the difference between C concentrations in fumigated and non-fumigated samples, corrected by the extraction coefficient for C (k_{EC}=0.45) (Joergensen, 1996).

Collection of $SrCO_3$ for ^{13}C analysis of microbial biomass at each incubation temperature was performed as described above. A large, but constant (with respect to C content and temperature) isotope fractionation of -5.2‰ was associated with the persulfate digestion. Thus, the measured $\delta^{13}C$ values of microbial biomass were corrected to this value.

Phospholipid fatty acids (PLFAs) were analyzed as fatty acid methyl esters (FAMEs) by using a modified procedure described by Frostegard et al. (1991) PLFAs were identified by chromatographic retention time using a standard (bacterial acid methyl ester mix, Supelco). Total PLFA content was used as a measure of viable microbial biomass at different temperatures. The specific PLFAs were organized into five compound groups: the monounsaturated, straight-chain (16:1ω7c, 18:1ω9c, 18:1ω9t) and the cyclopropane (17:0cy9-10, 19:0cy9-10) PLFAs, which are typical for Gram-negative bacteria. Saturated, terminally branched PLFAs (i-15:0, a-15-:0, i-16:0, i-17:0) were used as markers for Gram-positive bacteria. For fungi, the polyunsaturated, straight-chain PLFA (18:2ω6) was used, while the long, saturated, straight-chain PLFA (20:00) was chosen to represent the plants and other eukaryotic cells. The straight chain, saturated PLFAs (14:0, 15:0, 16:0, 17:0, 18:0) are ubiquitous among bacteria and fungi.

3. RESULTS

3.1 Effects of temperature on the $\delta^{13}C$ of CO_2

The $\delta^{13}C$ values of CO_2 respired were negatively correlated with temperature in organic horizons ($p<0.05$, for both rims and troughs). They linearly decreased from -23.9‰ to -26.6‰

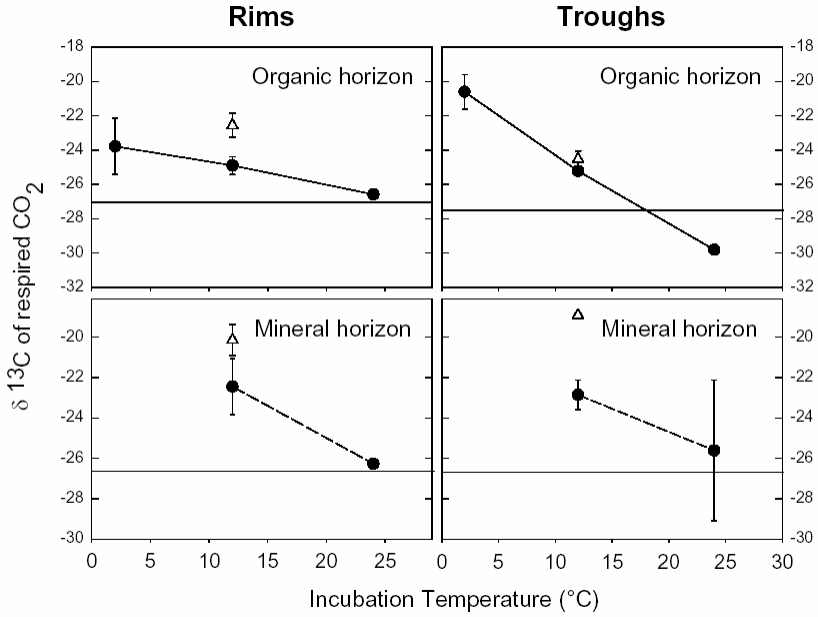

Fig. 1. $\delta^{13}C$ of CO_2 respired from arctic soils incubated for 6 weeks at 2°C, 12°C and 24°C. Black circles indicate $\delta^{13}C$ of CO_2 respired at incubation temperature, open triangles $\delta^{13}C$ of CO_2 respired at 12°C after incubation at 24°C ('temperature reversal experiment'). Values represent means ± SE (n=3). The horizontal lines indicate the isotopic signatures of the respective bulk soil.

in rims and from -20.6‰ to -29.8‰ in troughs between 2°C and 24°C (Fig. 1). In mineral horizons, the $\delta^{13}C$ of CO_2 respired also decreased from 12°C to 24°C.

When heterotrophic respiration of soils which had been incubated at 24°C was measured at 12°C (´temperature reversal experiment´), the $\delta^{13}C$ of CO_2 shifted to values which were close to those previously found at 12°C incubation.

Stable isotopic signatures of CO_2 were generally less negative in mineral than in organic horizons.

3.2 Effects of temperature on heterotrophic respiration

In organic horizons, respiration rates increased across the entire temperature range (mean Q_{10} of 2.6). Temperature sensitivity, however, was greater between 2°C and 12°C (Q_{10} of 3.8) than between 12°C and 24°C (Q_{10} of 1.3). Heterotrophic CO_2 production in mineral horizons of troughs at 2°C was below detection limits. Between 12°C and 24°C, however, respiration rates increased (Q_{10} of 2.6), while they slightly decreased in rims (Q_{10} of 0.8). Generally, CO_2 production rates were similar between the two microsites, and greater for organic than for mineral horizons (Fig. 2).

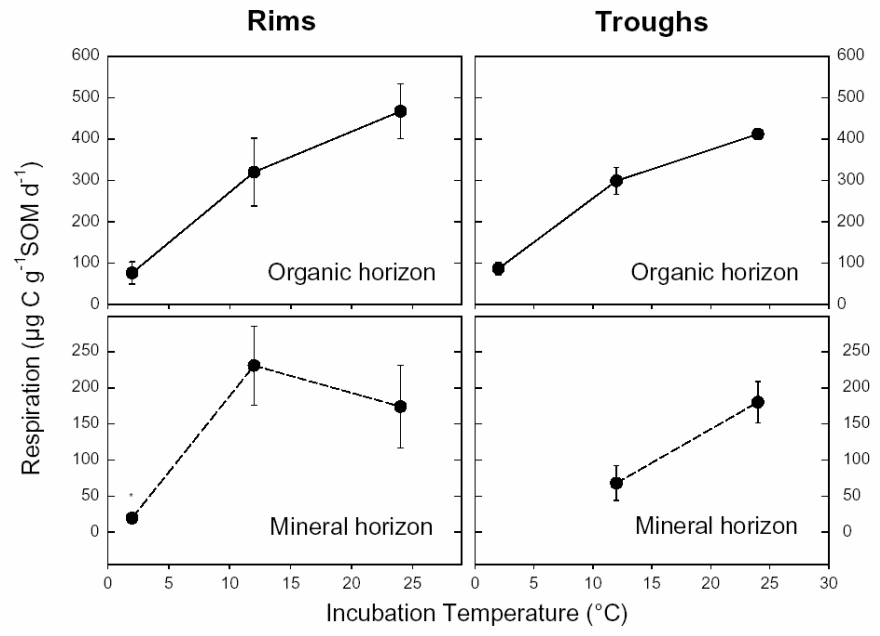

Fig. 2. Respiration rates of soils of frost-boil tundra after 6 weeks of incubation at 2°C, 12°C and 24°C. Values represent means ± SE (n=3).

3.3 Effects of temperature on microbial biomass and community structure

Total PLFAs as an indicator of microbial biomass ranged between 1.3 and 4.3 µmol PLFA g^{-1} SOM, and were greatest at 12°C (Table 1). The $\delta^{13}C$ values of microbial biomass did not differ significantly between the temperature regimes (Table 1). However, $\delta^{13}C$ values were generally less negative in mineral than in organic horizons. In both horizons, Gram-negative bacteria and fungi significantly decreased with warming, while Gram-positive bacteria and the saturated, straight-chain PLFAs (characteristic of both bacteria and fungi) significantly increased (Fig. 3). In general, PLFAs showed increasing saturation with increasing temperatures.

Table 1. Microbial biomass (MB) and $\delta^{13}C$ of microbial biomass of arctic soils incubated at 2°C, 12°C and 24°C for 6 weeks. Values represent means ± SE (*italics*) (n=3).

	Rims			Troughs		
	2°C	12°C	24°C	2°C	12°C	24°C
Organic horizon						
MB (µmol PLFAs g^{-1} SOM)	1.40	2.93	2.38	1.93	4.27	2.13
	0.11	*0.25*	*0.63*	*0.16*	*0.67*	*0.14*
$\delta^{13}C$ of MB	-25.94	-26.47	-25.96	-25.00	-24.97	-25.18
	0.11	*0.58*	*0.39*	*0.47*	*0.54*	*0.40*
Mineral horizon						
MB (µmol PLFAs g^{-1} SOM)	1.90	3.49	2.31	1.26	2.09	1.70
	0.21	*0.57*	*0.28*	*0.12*	*0.27*	*0.12*
$\delta^{13}C$ of MB	-24.00	-22.81	-23.71	-26.57	-25.55	-25.80
	0.48	*0.72*	*0.29*	*0.11*	*0.11*	*0.22*

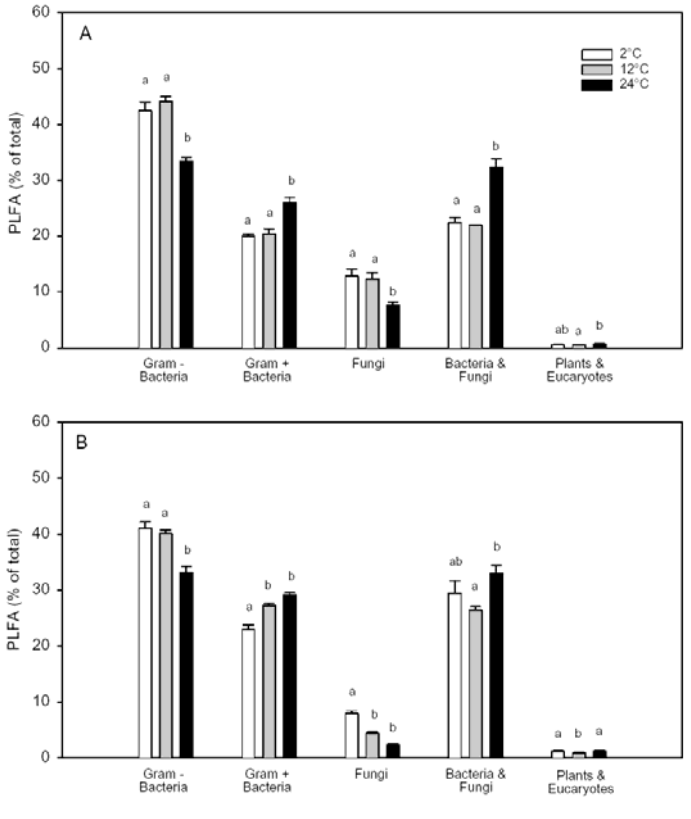

Fig. 3. PLFA composition (% of total; see Table 1 for details) of the microbial biomass of organic (A) and mineral (B) soils of a frost-boil tundra incubated at three different temperatures. 'Gram-negative bacteria' include monounsa-turated, straight-chain PLFAs and cyclopropane, 'Gram-positive bacteria' saturated, terminally branched PLFAs, 'Fungi' polyunsaturated, straight-chain PLFAs, 'Bacteria and Fungi' saturated, straight-chain PLFAs and 'Plants and Eukaryotes' long, saturated, straight-chain PLFAs. Values represent means ± SE (n=3). Within each group, different letters denote statistically significant differences (ANOVA, LSD, $p < 0.05$).

This change in PLFA composition was gradual across all temperature regimes in mineral horizons, but not in organic horizons, suggesting that thermoadaptation of specific PLFAs without a change in microbial community structure (Cooke, Whipps, 1993) was only of minor importance. No consistent temperature effect was found for the longer, saturated, straight-chain PLFAs which are representatives for plants and other eukaryotes. Since rims and troughs did not show any significant difference in specific PLFA composition, the data given (Fig. 3) are the average of both subsites.

3.4 Soil fractionation

The chemical soil fractionation revealed that distinct carbon pools exhibit distinct isotopic signatures. The pattern was generally similar for both rims and troughs, as well as for organic and mineral horizons (Table 2). The fraction with the most positive $\delta^{13}C$ values was the water-soluble fraction (-23.2 to -24.9‰) followed by microbial biomass (-24.9 to -26.6‰) and the acid-soluble fraction (-25.1 to -25.5‰).The acid-insoluble fraction had the most negative $\delta^{13}C$ values (-27.1 to -28.0‰). The bulk soil was thus ^{13}C-enriched relative to the acid-insoluble fraction and depleted relative to all other fractions.

Table 2. Amount and isotopic signatures of carbon pools of arctic soils after chemical fractionation. Values represent means ± SE (*italics*) (n=3)

Carbon fractions	Rims		Troughs	
	C (% of total)	$\delta^{13}C$ (%)	C (% of total)	$\delta^{13}C$ (%)
Organic horizon				
Microbial biomass	1.53	-25.89	1.62	-26.62
	0.14	*0.26*	*0.30*	*0.32*
Water soluble	1.80	-24.29	2.09	-24.94
	0.15	*0.10*	*0.46*	*0.12*
Acid soluble	43.74	-25.19	34.31	-25.13
	2.72	*0.45*	*3.43*	*0.45*
Acid insoluble	52.93	-27.62	61.97	-28.09
	7.24	*0.20*	*2.80*	*0.25*
Bulk soil	100.00	-26.50	100.00	-27.07
Mineral horizon				
Microbial biomass	1.99	-24.89	2.04	-25.30
	0.35	*0.41*	*0.61*	*0.91*
Water soluble	3.36	-23.25	3.12	-24.06
	1.23	*0.27*	*0.25*	*0.16*
Acid soluble	78.60	-25.54	38.71	-25.11
	40.06	*0.21*	*10.58*	*1.41*
Acid insoluble	16.04	-27.23	56.12	-27.05
	2.46	*0.26*	*6.03*	*0.87*
Bulk soil	100.00	-25.77	100.00	-26.20

Similarly to CO_2 respired and microbial biomass C, the $\delta^{13}C$ values of bulk soils and SOM fractions were less negative in mineral than in organic horizons. This is consistent with the frequently observed trend of ^{13}C enrichment with soil depth, and most likely results from a progressive incorporation of microbial components in the residual SOM (Ehleringer et al., 2000).

In all soils except the mineral soil of rims the acid-insoluble fraction made up the largest proportion of total organic C followed by the acid-soluble fraction (Table 2). In mineral soils of rims the acid-soluble fraction comprised the largest fraction followed by the acid-insoluble

fraction. The lowest C contents, however, were found in microbial biomass and in the water-soluble fraction.

4. DISCUSSION

The incubation temperature strongly affected the $\delta^{13}C$ values of CO_2 respired from arctic soils. At higher temperatures, the CO_2 was more depleted in ^{13}C, whereas, at lower temperatures, the CO_2 was more enriched in ^{13}C. Thus, the temperature regime affected the utilization of C sources with different $\delta^{13}C$ signatures by soil microbes or by microbial metabolisms leading to isotopic fractionation. Although the temperature itself is thought to cause no isotope effect on individual steps of enzymatic reactions (O´Leary et al., 1992), large isotope effects might be possible if metabolic pathways of microorganisms change (Santruckova et al., 2000). However, a possible shift in $\delta^{13}C$ values of CO_2 respired should be balanced by an opposite shift in $\delta^{13}C$ values of microbial biomass.

In our study, the isotopic signature of microbial biomass did not correlate negatively with the isotopic signature of CO_2 respired, making such metabolic changes unlikely. Our data therefore suggest that the observed decrease in $\delta^{13}C$ values of CO_2 respired with temperature is due to utilization of different C compounds by soil heterotrophs, i.e., that arctic microbes preferentially consume ^{13}C depleted compounds at higher temperatures.

These results are similar to forest ecosystems, where lower $\delta^{13}C$ values of CO_2 produced at 40°C and 22°C than at 4°C were found (Andrews et al. 2000). It was suggested that at higher temperatures more recalcitrant sources were decomposed by microbes, since literature data indicated that recalcitrant C sources are depleted in ^{13}C (Benner et al. 1987). In our study of arctic soils similar C isotopic signatures were found. The non-extractable and thus chemically most resistant fraction of the SOM, which mainly comprises lignin and humic substances, was most ^{13}C depleted, while the acid- and water-extractable fractions were relatively more enriched in ^{13}C. Since the $\delta^{13}C$ values of CO_2 respired at 24°C were most similar to the $\delta^{13}C$ values of the resistant fraction, we conclude that the recalcitrant C fractions are preferentially respired at 24°C. The $\delta^{13}C$ values of CO_2 respired at 12°C and 2°C were relatively more enriched in ^{13}C, indicating the utilization of the chemically less resistant compounds at lower temperatures. In soils of troughs, however, the $\delta^{13}C$ signal of CO_2 respired at 2°C was significantly more positive than the water-soluble fraction.

This may be explained by the fact that each SOM fraction itself consists of various chemically different substances, e.g., of soluble humic compounds and sugars (Gregorich et al., 2000), which may have very different isotopic signatures. It has been questioned, however, whether the utilization of recalcitrant compounds of the soil is induced by temperature increase or by depletion of more labile substrates (Leifeld 2003). In our study, a decrease of temperature to 12°C after incubation at 24°C resulted in an increase of $\delta^{13}C$ values of CO_2 respired, indicating that the more easily available substrates were not exhausted at 24°C.

Therefore, temperature rather than substrate limitation was responsible for selective substrate utilization. Temperature may increase the solubility of the recalcitrant substrates, or may destabilize these compounds (Waldrop, Firestone 2004). Temperature further may affect exoenzyme activity in soils thereby potentially increasing the availability of recalcitrant substrates at higher temperatures. Weintraub and Schimel (2003) demonstrated recently that tundra soils do not show a chemical limitation to decomposition. Instead they suggested that limitation may result from unfavorable environmental conditions like cold temperatures or high soil moisture, leading to accumulation of soil organic matter at high latitudes.

Assuming a constant Q_{10} value over the entire temperature range, respiration rates would increase exponentially with warming (Reichstein et al., 2000; Kirschbaum, Paul, 2002; Mertens

et al., 2001). In contrast to older studies (Nadelhoffer et al., 1991), our study, as well as many newer laboratory and field studies (Dalias et al., 2001; Kirschbaum 1995; Lloyd, Taylor 1994; Lenton, Huntingford 2003), demonstrated that Q_{10} values were not constant, but decreased with increasing temperature. This decrease was only slight, however, with the exception of mineral soils of rims. This is surprising for two reasons: first, arctic ecosystems are dominated by microorganisms adapted to low temperatures (Dalias et al., 2001). Second, our analysis of respired CO_2 suggests that more recalcitrant C compounds are utilized at higher temperatures. The recalcitrant compounds are often considered as 'passive', i.e., not contributing to heterotrophic respiration (Parton et al., 1987). Our data suggest, however, that the recalcitrant C pools may be efficiently respired. Thus, the substrate quality may only have a small influence on CO_2 production rates and the extent by which the respiration rates may slow down as a result of a shift in resource quality due to warming may be overestimated.

The utilization of labile vs. recalcitrant C compounds may be mediated by different microbial communities (Schimel, Gulledge, 1998; Waldrop, Firestone, 2004). We found indeed significant shifts in microbial community composition within the soils as temperature increased. While fungi and Gram-negative bacteria decreased, Gram-positive bacteria were relatively more abundant at higher temperatures.

There was also a significant increase among microorganisms with straight-chain, saturated PLFAs. The observed pattern is generally consistent with other studies on the development of below-ground communities as a result of warming (Waldrop, Firestone, 2004; Bardgett et al., 1999; Zogg et al., 1997). It seems that the slow-growing fungi are less competitive at higher temperatures, whereas the fast-growing Gram-positive bacteria are better adapted to such conditions. Accordingly, in arctic soils, the Gram-positive bacteria may be responsible for the decomposition of low quality substrates. These organisms might increase their ability to utilize the recalcitrant compounds by increasing their investment in exoenzymes (Schimel, Weintraub, 2003). Indeed, although microbial biomass peaked at 12°C, the highest exoenzyme activities (e.g., urease) were found in soils incubated at 24°C (data not shown). However, organic soils incubated at 2°C and 12°C did not exhibit different PLFA compositions, despite differences found in substrate utilization. This implies that microbial community structure may not have changed, but that microorganisms may have adapted their physiology, i.e., changed their resource use with temperature (Santruckova et al., 2000). The finding that the recalcitrant SOM is preferentially respired at higher temperatures may be important in predicting climate-change effects on C cycling in northern ecosystems. First, even the most resistant SOM fractions can be potentially mineralized by arctic microbes, and second, they may be mineralized at relatively high rates. The utilization of recalcitrant C compounds may be explained by a shift in microbial communities to organisms with higher temperature optima and preference for more recalcitrant substrates. However, it is the temperature and not depletion of readily available C compounds that induces the preferential decomposition of recalcitrant SOM fractions. Thus, the recalcitrant C stocks in the arctic biome may not be permanent and the expression 'resistant carbon compounds' may not be valid in a future warmer world.

5. CONCLUSION

Our results suggest that, at higher temperatures, recalcitrant compounds are preferentially respired by arctic microbes. When the isotopic signatures of respired CO_2 of soils which had been incubated at 24°C were measured at 12°C, the $\delta^{13}C$ values shifted to values found in soils incubated at 12°C, indicating the reversible use of more easily available substrates. Analysis of phospholipid fatty acid profiles showed significant differences in microbial community structure at various incubation temperatures indicating that microorganisms with preference for

more recalcitrant compounds establish as temperatures increase. In summary our results demonstrate that a large portion of tundra SOM is potentially mineralizable.

Acknowledgements. The authors gratefully acknowledge Prof Andreas Richer and his working group from Department of Chemical Ecology & Ecosystem Research, Faculty of Life Sciences, University of Vienna as all presented data were obtained in the course of joint research work with these Austrian colleagues.

REFERENCES

Andrews JA, Matamala R, Westover KM, Schlesinger WH (2000) Temperature effects on the diversity of soil heterotrophs and the delta ^{13}C of soil-respired CO_2. *Soil Biol. Biochem.* **32**, 699-706.

Bardgett RD, Kandeler E, Tscherko D, Hobbs PJ, Bezemer TM, Jones TH, Thompson LJ (1999) Below-ground microbial community development in a high temperature world. *Oikos* **85**, 193-203.

Benner R, Fogel ML, Sprague EK, Hodson RE (1987) Depletion of delta ^{13}C in lignin and implication for stable carbon isotope studies. *Nature* **329**, 708-710.

CAVM Team (2003) Circumpolar Arctic Vegetation Map. Scale 1:7,500,000.: Conservation of Arctic Flora and Fauna (CAFF) Map No. 1, U.S. Fish and Wildlife Service, Anchorage, Alaska.

Chernov YI, Matveyeva NV (1997) Arctic ecosystems in Russia. In: Wielgolaski FE (ed) *Ecosystems of the World: Polar and Alpine Tundra*, pp 361-507. Elsevier, Oslo, Norway.

Cooke RC, Whipps JM (1993) *Ecophysiology of fungi*. 337 pp. Blackwell Scientific Publications, London.

Dalias P, Anderson JM, Bottner P, Couteaux MM (2001) Temperature responses of carbon mineralization in conifer forests from different regional climates incubated under standard laboratory conditions. *Global Change Biol.* **7**, 181-192.

Ehleringer JR, Buchmann N, Flanagan LB (2000) Carbon isotope ratios in belowground carbon cycle processes. *Ecol. Appl.* **10**(2), 412-422.

Frostegard A, Tunlid A, Baath E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. In: *Microbiological Methods 14*, 151-163.

Gregorich EG, Liang BC, Drury CF, Mackenzie AF, McGill WB (2000) Elucidation of the source and turnover of watr soluble and microbial biomass carbon in agricultural soil. *Soil Biol. Biochem.* **32**, 581-587.

Harris D, Porter LK, Paul EA (1997) Continuous flow isotope ratio mass spectrometry of carbon dioxide trapped as strontium carbonate. *Communication in plant and soil analysis* **28**, 747-757.

IPCC (2001) Climate change 2001: The scientific basis, Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press.

Joergensen RG (1996) The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EC} value. *Soil Biol. Biochem.* **28**, 25-31.

Kirschbaum MUF (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biol.Biochem.* **27**, 753-760.

Kirschbaum MUF, Paul KI (2002) Modelling C and N dynamics in forest soils with a modified version of the CENTURY model. *Soil Biol.Biochem.* **34**, 341-354.

Leifeld J (2003) Comments on "Recalcitrant soil organic materials mineralize more efficiently at higher temperatures" by R. Bol, T. Bolger, R. Cully, and D. Little; Journal of Plant Nutrition and Soil Science 166, 300-307 (2003). *J. of Plant Nutr. Soil sci.* **166**, 777-778.

Lenton TM, Huntingford Ch (2003) Global terrestrial carbon storage and uncertainties in its temperature sensitivity examined with a simple model. *Global Change Biol.***9**, 1333-1352.

Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. *Functional Ecol.* **8**, 315-323.

Mertens S, Nijs I, Heuer M, Kockelbergh F, Beyens L, Van Kerckvoorde A, Impens I (2001) Influence of high temperature on end-of-season tundra CO_2 exchange. *Ecosystems* **4**, 226-236.

Nadelhoffer KJ, Giblin AE (1991) Effects of temperature and substrate quality on element mineralization in six arctic soils. *Ecology* **72**(1), 242-253.

O´Leary MH, Madhavan S, Paneth P (1992) Physical and chemical basis of carbon isotope fractionation in plants. *Plant, Cell Environ.* **15**, 1099-1104.

Parton WJ, Schimel DS, Cole CV, Ojima DS (1987) Analysis of factors controlling soil organic matter levels in Great Plains Grassland. *Soil Sci. Soc. Am. J.* **51**, 1173-1179.

Paul EA, Harris D, Klug MJ, Ruess RW (1999) The determination of microbial biomass. In: Robertson GP, Coleman DC, Bledsoe CS, Sollins P (eds) *Standard soil methods for long-term ecological research*, pp 291-317. Oxford University Press, Oxford.

Reichstein M, Bednorz F, Broll G, Kätterer T (2000) Temperature dependence of soil carbon mineralization during a long-term incubation of subalpine soil samples. *Soil Biol. Biochem.* **32**, 947-958.

Santruckova H, Bird MI, Frouz J, Sustr V, Tajovsky K (2000) Natural abundance of ^{13}C in leaf litter as related to feeding activity of soil invertebrates and microbial mineralisation. *Soil Biol. Biochem.* **32**, 1793-1797.

Santruckova H, Bird MI, Lloyd J (2000) Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. *Functional Ecol.* **14**, 108– 114.

Schimel JP, Gulledge J (1998) Microbial Community Structure and Global Trace Gases. *Global Change Biol.* **4**, 745-758.

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.* 35, 549-563.

Sollins P, Glassman C (1999) Soil Carbon and Nitrogen. Pools and Fractions. In: Robertson GP, Coleman DC, Bledsoe CS, Sollins P (eds) *Standard Soil Methods for Long-Term Ecological Research*, pp 89-105. Oxford University Press, Oxford.

Trumbore SE, Chadwick OA, Amundsen R (1996) Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. *Science* **272**, 393-396.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703-707.

Waldrop MP, Firestone MK (2004) Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. *Biogeochemistry* **67**, 235-248.

Waldrop MP, Firestone MK (2004) Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. *Oecologia* **138**, 275-284.

Weintraub MN, Schimel JP (2003) Interactions between carbon and nitrogen mineralisation and soil organic matter chemistry in Arctic Tundra soils. *Ecosystems* **6**, 129-143.

Zogg GP, Zak DR, Ringelberg DB, MacDonald NW, Pregitzer KS, White DC (1997) Compositional and functional shifts in microbial communities due to soil warming. *Soil Sci. Soc. Am. J.* **61**, 475-481.