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Microbial activity of boreal forest soil in a cold climate

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Organic matter degrading microbial activities in economically managed, acid boreal Scots pine forest soils were analysed in different seasons. We observed Q_{10} values ranging from 2.3 to 2.8 for the production of CO_2 from endogenous detrital matter at close to *in situ* temperatures, when the soils were in natural state, immediately after sampling. The Q_{10} of methane oxidation, β-glucosidase, C2- and C4-esterases, exhibited values of 1.6 to 2.1 and the corresponding apparent activation energies were from 40 to 70 kJ mol^{-1}. Detrital decomposition extrapolated to zero activity at -7 ± 1 °C but the actual soil temperature under snow cover never dropped below –3 °C. The degrading activities towards 0.2 to 2 ppm of phenanthrene and of 2,4,5-trichlorophenol showed Q_{10} values of 2.0 to 4.4 in the fine roots and the rhizosphere fraction of aspen forest soil but there was no activity in the bulk soil. Our results show that the detritus degrading microbial activities in forest soil were only moderately temperature dependent and significant activity continued over the winter.

Introduction

The present annual average air temperature in Finland is from –0.3 °C (1997) to –2.0 °C (1998) in the north and +6.2 °C (1997) to +5.4 °C (1998) in the south (Anon. 1999). The surface soil may be frozen for many months in a year. The forests are mainly coniferous, and the soils are podzolised and acidic (pH 3 to 4.5). Mineralization of detrital organic matter is a complicated

succession of reactions, involving extracellular depolymerization of complex polymers and hydrolytic activity to liberate monomers, as well as oxidative, intracellular reactions finally leading to carbon dioxide. Many of these individual reactions and processes have been measured in boreal soils. However, the published studies have been performed in a warm season or in the laboratory, rendering it difficult to translate the results to *in situ* soil activities in the winter. In this paper, we show the rates and temperature dependence of reactions participating in detrital matter degradation in Finnish, economically managed forest both during warm and cold seasons, and demonstrate the intrinsic remediation potential of forests rhizosphere for low-level pollution with manmade chemicals. The studies were conducted at the forestry field station of the University of Helsinki at Hyytiälä, and the agricultural research farm of the University of Helsinki at Viikki.

Materials and methods

The study sites

The Hyytiälä forestry research station is located 61°48´N, 24°19´E, and the Viikki farm 60°11´N, 24°58´E. The former site is a Scots pine stand (sown after prescribed burning in 1962) on thin till soil, with ground vegetation of *Vaccinium vitis-idea* at 180 m a.s.l. The Hyytiälä soil properties are known in great detail (Ilvesniemi and Pumpanen 1997). A non-managed deciduous forest (silver birch with European aspen) soil and an agricultural soil were sampled from the farm property at Viikki (Helsinki), near the Baltic Sea coastal line (< 300 m). The Viikki farm in Helsinki belongs to the University of Helsinki, and has been used for agricultural education for over 60 years. The sampled farm soil has previously been limed to decrease acidity to pH > 6, and used for cultivation of rape (*Brassica campestris*) in recent years, fertilized in 1996 with lime (2300 kg), nitrogen (200 kg), phosphorus (46 kg) and treated with the herbicide trifluralin (1 kg ha^{-1}).

Sampling and analytical procedures

The soil temperature was continuously measured with thermocouples linked to a microcomputer via Nokeval data transmitters. Soils were sampled with a Westman corer (Westman 1995) at Hyytiälä, or a shovel (Viikki). Green matter was removed from the humus layer, and stones and the larger roots from all layers, but otherwise the soils were subjected to minimal mutilation.

Immediately after sampling, the cores were sliced into the humus (H, upper and lower), eluvial (E), illuvial (I) (upper and lower) and ground soil (GS), and the 2 to 5 cm slices from 3 to 5 cores were pooled carefully to avoid any disturbance of the sample. The biological activities were measured on the day of sampling at the natural pH and minimal storage (few hours, +7 °C) between the analyses. Endogenous carbon dioxide evolution was measured at +7 °C (24 h) with gas chromatography from the head space (100 ml) of 5 g of soil where 1 ml of H_2O was added to balance out the extremely dry weather on some of the sampling days. Methane oxidation was measured similarly, except that 200 ppm of methane substrate were injected into the head space to assure rapid assay (the soil air on-site contained 2 to 3 ppm). Q_{10} values were calculated from measurements performed at 4 temperatures from –2.5 °C to +12 °C.

The soil hydrolytic enzyme activities were measured with no other disturbance of the soils than adding the substrate (200 µl) to 0.1 cm^3 of soil in 5 parallel wells of 96-well microtiter plates. Fluorogenic synthetic surrogate substrates, methylumbelliferyl-β-D-glucoside, -β-D-xyloside, -α-D-glucoside, -N-acetylglucosamide, -phosphate, -acetate and -butyrate (all from Sigma, St Louis Mo, final concentration 1 mM) were used for assaying the hydrolytic cleavage activities essentially as described by Wittmann *et al*. (2000). An automated kinetic fluorometer (Fluoroskan Ascent, Labsystems, Finland) was used to measure the fluoresence of the methylumbelliferone deliberated in the assay. All activities showed zero order kinetics, i.e. were linear during the whole measurement peri-

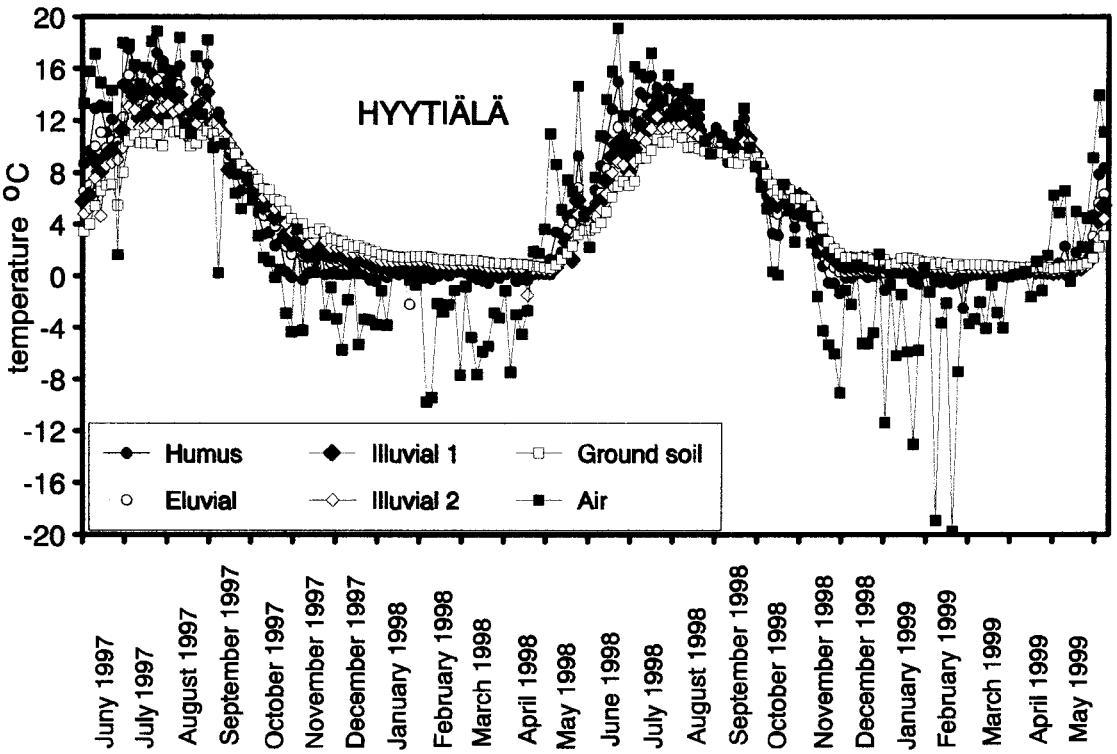

Fig. 1. Average daily soil temperatures over a 730 day period in air and the different layers of the Hyytiälä (61° 48´N, 24°19´E) Scots pine forest soil. Datapoints show the 24-h average temperatures at 4 day intervals.

od (3 to 30 min, depending on the activity). The activites were measured at +14 °C and 30 °C and extrapolated to +7 °C using separately assayed Q_{10} values based on measurements at 14 °C, 22 °C and 30 °C. The enzyme analyses were complete within 3 to 30 min from adding the substrate to the soils dispensed into the microtiter trays.

Mineralization of radiolabeled model pollutants, 9-^{14}C-phenanthrene (2.2×10^9 Bq mmol^{-1}) and U-^{14}C-labeled 2,4,5-trichlorophenol (2.04×10^8 Bq mmol^{-1}) (purity ≥ 95%, Sigma Chemicals St. Louis, Mo) was measured with microscale radiorespirometry using the method described by Fulthorpe *et al.* (1996). In this method, the evolving $^{14}CO_2$ from the reaction of 0.1 cm^3 soil (triplicates) with the ^{14}C-labeled substrate, was trapped into $Ba(OH)_2$ impregnated Whatman 3M sheet covering a 96-well microtiter plate. The $Ba^{14}CO_3$ accumulated on the sheets was quantitated by phosphoimaging at intervals of 7 days (BAS 1500, Fuji Tokyo Japan) with BAS-MP 20 40S imaging plate (Fuji, Tokyo, exposed for 5 h), and read with the BAS-Reader 2.9 program (Raytest Isotopengeräte, Straubenhardt, Germany). Serial dilutions of known concentrations of $NaH^{14}CO_3$ (3.7×10^8 Bq mmol^{-1}, CN Irvine USA) in 4 parallels were dispensed in two separate plates, and used for calibration (Kurola 1999). Nonlabeled phenanthrene and 2,4,5-trichlorophenol (Fluka, Buchs CH) were added to obtain higher substrate concentrations. The activation energies were calculated from the observed response of $^{14}CO_2$ evolution (7 weeks) measured of soil at temperatures stepwise increasing from –2.5 °C to +15 °C (step of 2.5 °C). Low temperature incubations were performed in a cooled incubator (Sanyo, Japan). The actual temperature was continously monitored with a calibrated datalogger (Tinytag, data loggers, Orion Group, Chichester UK) and found accurate within 0.5 °C.

Results

Physicochemical characterization of the study sites.

The soil temperature profiles of air and of the different layers of the podzol soil, measured during the study period in 1997–1999 at the Hyytiälä research station (Fig. 1) may be considered typical of the Finnish coniferous forest. The mean annual temperature at Hyytiälä is 2.9 °C. The temperature of all layers (Table 1) of soil remained mainly between –2 °C and +2 °C (November to April) even though the air temperature dropped in some periods below –20 °C. The summer temperature at the depths of ≤ 10 cm was above ca. 12 °C for about 6 to 8 weeks in 1997 and ca. 10 °C in the cold and wet summer 1998 (Fig. 1). The maximum temperatures detected in the humus layer were 16.6 °C and 14.5 °C, respectively, and wet precipitation (excl. snow) at Hyytiälä in the study years was 370 mm (1997) and 570 mm (1998).

The Hyytiälä forest soil is acidic (Table 1), with the water extractable acidity decreasing from pH of 4.4 in the humus to pH 5.3 in ground soil, and the KCl pH from 3.2 in humus to 4.2 in ground soil. The exchangeable acidity, protons bound to soil particles and aluminum ions, was very high in the humus layer and the eluvial layer (Table 1). The humus and the eluvial layers of the soil were approx. 1 mM in respect to exchangeable protons (1 mmol l^{-1} of soil) while the deeper layers were ca. 0.5 mM (Ilvesniemi and Pumpanen 1997). Also, there were downward decreasing gradients for total soil organic carbon (TOC, 100 fold) and for soil contained total nitrogen (30 fold) (Table 1). The Viikki deciduous forest surface soil (0 to 5 cm) was almost similar in acidity, TOC and total N to the Hyytiälä Scots pine humus layer, but the fraction containing fine roots was considerably less acidic (pH 6.0 and 5.4 with KCl) (Table 2).

Seasonal dependence of microbial activities in Scots pine soil towards detrital substrates

The rate of mineralization of detrital matter was measured in soil cores from the Hyytiälä Scots pine stand over two years in different seasons. The endogenous respiration in the whole soil column at actual, *in situ* temperature varied between 2.1 and 11.1 mmol CO_2 m^{-2} h^{-1} in the measurement period, while the monthly average soil temperature ranged from 0.1 to 13.3 °C (Table 3).The accurate estimation of the tempera-

Table 1. Quality of podzol soil of 38-year-old Scots pine stand at Hyytiälä. Measured in October 1997.

Soil layer	Depth to cm	TOC, mg g^{-1}	N_{tot},mg g^{-1}	pH (H_2O)	pH (KCl)
Humus	–4	315	8.2	4.4	3.2
Eluvial	–7	34	1.1	4.7	3.2
Illuvial up	–12	37	1.5	4.8	3.8
Illuvial low	–17	29	1.3	5.0	4.1
Ground soil	–23	5	0.3	5.3	4.2

Table 2. Quality of deciduous forest soil and agricultural soil at Viikki, Helsinki. Measured October 1996.

Soil type	Depth to cm	TOC, mg g^{-1}	N_{tot}, mg g^{-1}	pH (H_2O)	pH (KCl)
Agricultural	0–20	43	3	6.6	6.2
Agricultural	35–40	33	3	6.3	5.4
Deciduous forest, void of roots	0–5	183	8	4.7	4.3
Deciduous forest, aspen root fraction	0–5	Nd	Nd	6.0	5.4

Nd = not determined

ture dependence of the endogenous organic matter mineralization rate is most critical for the humus layer (Table 3), where the seasonal amplitude of daily average temperature was largest, from +16 °C to –2.5 °C (Fig. 1). The detrital carbon mineralizing activity in the boreal Scots pine humus was permanently adapted to low temperatures (–3 °C to 15 °C), with a Q_{10} of 2.3 to 2.8 and a rather stable energy of activation (60–80 kJ mol^{-1}) during all seasons of the year (Table 3). When the humus was analysed in the laboratory for activity at a temperature lower than the actual one in that season, zero activity was obtained (by extrapolation) at around –7.0 °C, independent of the season in which the humus was sampled and analysed.

The temperature responses of selected key enzymes for the individual layers of the soil column were measured (Table 4). The apparent Q_{10} values (1.6 to 1.9) and the activation energies (40 to 60 kJ mol^{-1}) of the three selected activities, two biomass-indicator enzymes (unspecific C2- and C4-esterases) and the indicator enzyme for cellulose degradation (β-glucosidase) were close to those found for detrital carbon mineralization (= endogenous soil respiration; Table 3).

Although the different soil layers responded to temperature very similarly (Table 4), the humus layer was slightly more responsive (average Q_{10} 2.1 for the three activities) than the deeper (eluvial, illuvial, ground soil) layers (average Q_{10} 1.8 for the three activities). The difference, however, is small and may be attributed to the fact that all soil activities were high in October

Table 3. Response of endogenous organic matter mineralization rates to temperature in Hyytiälä Scots pine forest soil. The activities are calculated for the podzol soil core comprising the humus, eluvial and illuvial layers (17 cm in total). The temperature given is the monthly average in the soil column.

Month/analysis parameter	Oct. 1997	Dec. 1997	Jan. 1998	Jul. 1998	Oct. 1998
The soil column (17 cm)					
Average soil temperature (°C)	4.2	0.4	0.1	13.3	4.8
Endogenous respiration corresponding to average soil temperature, mmol CO_2 m^{-2} h^{-1}	5.7	2.3	2.1	11.1	6.6
The humus layer					
Q_{10} respiration rate $(°C)^{-1}$		2.8	2.3	2.4	2.5
Extrapolated zero activity (°C)		–7.0	–7.6		–6.0
Activation energy (Ea, kJ mol^{-1})		71	62	78	70

Table 4. Temperature response of boreal forest soil detritus hydrolysing enzymes. The Q_{10} and activation energy (Ea) were calculated from activities measured with 1 mM surrogate substrate in Scots pine forest soil at Hyytiälä Station October 1998, when the soil temperature was 4.8 °C. The activities were calculated to +7 °C based on Q_{10} values separately measured.

Analysis parameter/horizon	Humus upper	Humus lower	Eluvial	Illuvial upper	Illuvial lower	Ground soil
Acetate-esterase, 150 mmol m^{-2} h^{-1} (combined)						
Q_{10} $(°C)^{-1}$	2.0	1.6	1.7	1.7	1.9	1.9
Activation energy (Ea, kJ mol^{-1})	63	40	47	48	53	57
Butyrate-esterase, 140 mmol m^{-2} h^{-1} (combined)						
Q_{10} $(°C)^{-1}$	2.1	1.9	1.9	1.7	1.7	1.7
Activation energy (Ea, kJ mol^{-1})	74	59	54	43	47	40
β-glucosidase, 27 mmol m^{-2} h^{-1} (combined)						
Q_{10} $(°C)^{-1}$	2.1	1.6	1.9	1.8	1.6	1.9
Activation energy (Ea, kJ mol^{-1})	71	61	63	54	36	53

Table 5. The degradation activities of the soil in the 38 year-old Scots pine stand at Hyytiälä forest station. The activities were measured separately from the different horizons listed in Table 1, within a few hours from sampling of the soil cores. The table shows the summed activities of the humus, eluvial and illuvial horizons (–17 cm; Table 1). The endogenous carbon dioxide production and the potential for methane oxidation (200 ppm CH_4) were measured at +7 °C. The hydrolytic enzyme activities were measured at 30 °C with 1 mM of surrogate substrate and calculated to 7 °C using the data on Q_{10} for the same soils (Table 4). The S.D. of activity measurements were 20% (respiration, methane oxidation) and 15% (enzyme assays).

Season	Endogen.	Potential activity*, mmol m^{-2} h^{-1} (7 °C)						
	Respiration mmol CO_2 m^{-2} h^{-1}	CH_4 oxidation	C4-esterase[a]	β-xylosidase[b]	β-glucosidase[c]	α-glucosidase[d]	N-acetyl glucosamidase[e]	Phospho-monoesterase
Jul. 1997	7.1	0.08	nd	10.6	5.9	1.0	12.8	18.2
Oct. 1997	7.4	0.10	nd	11.8	25.2	2.5	7.7	19.2
Dec. 1997	4.0	0.05	nd	1.8	6.0	0.6	2.5	10.8
Jan. 1998	4.3	0.04	nd	nd	nd	nd	nd	nd
Jul. 1998	6.4	0.14	nd	1.9	10.7	0.8	2.3	13.2
Oct. 1998	8.1	0.12	140	7.2	14.3	1.1	6.7	21.9
May 1999	5.5	0.09	75	2.8	11.4	0.8	3.3	9.4

* Measured as indicators for: unspecific esterase[a], hemicellulase[b], cellulase[c], starch degradation[d], chitinase[e], nd = not determined

(Tables 3 and 5) when the measurements were taken (Table 4).

Selected hydrolytic activities (Table 5), required for degradation of complex organic detritus, were analyzed during different seasons, and their activities compared with the downstream part (i.e. production of CO_2) of the decomposition process. To facilitate the comparison, all the activities were extrapolated to +7 °C irrespective of the sampling season. The upstream enzymes must deliver at least 1/5, 1/6 or 1/8 mmol m^{-2} h^{-1} of the 5-, 6- or 8-carbon building blocks (such as xylose, glucose, N-acetyl glucosamine) for each mmol m^{-2} h^{-1} of CO_2 to be generated by the downstream portion of the metabolic chain. The measured soil hydrolytic activity potentials (Table 5) show that they are not expected to be rate limiting for the downstream metabolism, even if 50% of the carbon flow is allowed for biomass synthesis. However, the actual soil concentrations of substrates may be lower than those of the synthethic substrates (1 mM calculated as monomer) used to measure the activity potentials shown in Table 5, and therefore, the actual soil activities may be lower than the potential activities.

The temperature-equalized activities of some enzymes (Table 5) showed more seasonal variation (e.g. β-xylosidase, β-glucosidase, chitinase, methane oxidation, 5–7 ×) than others (C4-esterase, phosphomonoesterase, ≤ 2 ×). This may relate to a season dependent change in the availability of different types of detrital substrates (hemicellulose, cellulose, chitin, methane) affecting the size of the degrader population, expressing that specific activity in soil.

Degrading activity of soil towards xenobiotic substrates

The substrates, phenanthrene and 2,4,5-trichlorophenol, were chosen as representatives for ubiquitous, long-distance transported organic pollutants to assess the soil activity towards xenobiotic substrates (Table 6). Radiorespirometry was used as the tool because it allows the use of low substrate concentrations, realistic in areas receiving distant pollution.

The apparent activation energies, and conse-

quently the Q_{10} values, were lower (75 to 97 kJ mol^{-1}) for low concentrations of substrate (≤ 5 ppm) simulating diffuse pollution than for high concentrations (50 ppm, 113–172 kJ mol^{-1}) simulating point source pollution (Table 6), both for 2,4,5-trichlorophenol and for phenanthrene. This may indicate that the microbial population in the natural soil was adapted to diffuse pollution. The bulk of the forest soil, from which the fine roots had been removed, showed no detectable mineralization activity towards phenanthrene or 2,4,5-trichlorophenol (Table 6) and also not towards other pollutants studied [pyrene, pentachlorophenol (not shown)] which were measurably mineralized by the fine roots fraction of the aspen forest soil and also by the agricultural soil. The results indicate an important role for the rhizosphere microorganisms as organic pollutant degraders.

The Q_{10} values obtained for mineralization of low concentration (0.2 to 2 ppm) of xenobiotic compounds in the Viikki forest soil (Table 6) were 2.0 to 4.4 (fine roots containing fraction), i.e. similar to or only moderately higher than those obtained for the Hyytiälä soil towards the natural detrital matter (1.6 to 2.8; Tables 3 and 4). This may indicate that the aspen forest soil, nonfertilized, not treated with pesticides (nature reserve) was naturally adapted to handling low levels of the ubiqutous pollutants.

Cold season microbial activities in soils

The rate of mineralization of the endogenous detritus ("soil respiration") and examples of selected biochemical potentials of the forest soils were measured over 4 seasons (Tables 3, 4 and 5). All the activities measured had linear kinetics, indicating the level of catalytic power at the time of sampling, i.e. no preadaptation. The total methane oxidation potential in the soil column was similar in July and in October (0.1 mmol CH_4 oxidized m^{-2} d^{-1}; Table 5), although the soil had experienced temperatures from +9 °C to +13 °C in the weeks before sampling in July and from +4 °C to +6 °C in October (Fig. 1). Potential activities of β-glucosidase and phosphomo-

Table 6. The rates of mineralization and apparent activation energies of ^{14}C-phenanthrene and ^{14}C-2,4,5-trichlorophenol by Viikki soils. Agricultural soil and deciduous forest (aspen) soil from Viikki farm area (Helsinki, Finland) were spiked with ^{14}C-labeled phenanthrene or 2,4,5-trichlorophenol (from 0.2 to 50 ppm). Mineralization activity was measured in October 1996 as the $^{14}CO_2$ evolution (7 d) at +7 °C.

Parameter	Soil (depth cm)	Phenanthrene (ppm)			2,4,5-trichlorophenol (ppm)		
		0.2	5	50	1.8	5	50
Rate of mineralization at 7 °C (μg kg^{-1} d^{-1})	agricultural (0–20 cm)	0.7	7.7	< 50	< 1.8	< 5	< 50
	agricultural (35–40 cm)	< 0.2	< 5	< 50	< 1.8	< 5	< 50
	aspen forest bulk soil* (0–5 cm)	< 0.3	< 8	< 80	< 2.9	< 8	< 80
	fine roots of aspen forest (0–5 cm)	1.2	18	< 80	3	8	81
Activation energy (Ea, kJ mol^{-1})	agricultural (0–20 cm)	91	133	171	nd	nd	nd
	fine roots of aspen forest (0–5 cm)	75	105	172	97	81	113
Q_{10} (°C)$^{-1}$	agricultural (0–20 cm)	4.5	12	nd	nd	nd	nd
	fine roots of aspen forest (0–5 cm)	2.0	7.6	nd	4.4	2.9	5.4

* void of fine roots; nd = not determined

noesterase (Table 5), β-xylosidase, cellobiosidase (not shown) and α-glucosidase were similar or higher in October than in July. The rate of CO_2 production from endogenous, natural detrital matter was also higher in October than in July, independently of the quality of the summer, dry and warm in 1997, or cool and wet in 1998 (Fig. 1). These findings indicate that the season for maximum microbial biocatalytic activity was not directly linked to the temperature, but maybe more related to the seasonal variation of the availability of substrate (litter fall in October) and the density of soil biota.

Discussion

Our study demonstrated that the microbial metabolic system of the acid soil of a Scots pine stand, a typical example of Finnish economically managed forests, was cold tolerant. We found that Q_{10} of endogenous biomineralization in the forest soil ranged from 2.0 to 2.8 irrespective of the season. Our values of Q_{10} were measured at –2.5 °C to +12 °C, i.e. the temperature relevant for the Hyytiälä forest soil (Fig. 1). The obtained Q_{10} values are significantly lower than the Q_{10} of near 8, 4.5 and 2.5 for soil organic matter decomposition, extrapolated for 0 °C, 10 °C and 20 °C, respectively, by Kirschbaum (1995) from literature data of various types of soil and geographic regions. Our values of Q_{10} are also lower than the range 3.1–6.2 measured at +5 °C to +10 °C for soil respiration by Niklinska *et al.* (1999) for the mildly acidic (pH 5.6–6.1) Scots pine forest humus from France, Poland and Sweden; sites located at 44°N to 66°N. Niklinska *et al*. (1999) found that Q_{10} decreased with increasing temperature, to values between 2.1 and 4.1 when measured at +10 °C to +15 °C, and to 1.4–1.9 at 15 °C to 20 °C. The Q_{10} values obtained in the present study for freshly sampled, minimally mutilated Scots pine forest soils at temperatures around +5 °C, were similar to those reported in other studies at around +20 °C. Our results suggest that the 10% loss of soil organic carbon as a response to 1 °C increase of temperature prognosed by Kirschbaum (1995) on the basis of a higher Q_{10}, does not apply to the permanently cool boreal forest soils studied by us. Thamdrup *et al.* (1998) reported a Q_{10} value as low as 1.8 for the coastal sediments in Denmark, also a permanently cool environment.

We also found in the Hyytiälä Scots pine forest soils a weak temperature dependence for the biomass indicator enzyme C 4-esterase (Kähkönen *et al*. 1999), Q_{10} values of 1.8 ± 0.1, and for the indicator enzyme of cellulose degradation, β-D-glucosidase (Tomme *et al*. 1995) a Q_{10} of 1.9 ± 0.2 (Table 4). These enzyme Q_{10} values are similar to those reported for other soil microbial processess, e.g. isoprene consumption in soil from Ithaca hardwood forest (42°N, Cleveland and Yavitt 1999) and many microbial enzymes (Atlas and Bartha 1993). Our enzyme data were, for technical reasons, obtained at 14 °C to +30 °C. The Q_{10} values tend to decrease towards higher temperatures (Kirschbaum 1995, Niklinska *et al*. 1999), thus the lower Q_{10} value found for the enzymes as compared with the soil respiration may be explained by the difference of the measurement range.

The discrepancies in the temperature dependence of Q_{10} data reported by these and other authors need explanation. Liski *et al*. 1999 proposed earlier that an explanation for this discrepancy could lie in the use of young litter in many decomposition studies, because the mineralization of young litter might be more temperature sensitive than that of old soil organic matter, present in the deeper layers of soil. The present study, however, showed that a weak temperature dependence (Q_{10} of 1.6 to 2.1; Table 4) was also observed for synthetic surrogate substrates, designed for measuring specific enzyme activities, so the age of the substrate carbon cannot be the sole explanation. Boone *et al.* (1998) measured temperature dependence of soil respiration in an 85-year-old, mixed hardwood forest (42°N, 72°W) during the growing season (late March to late October). They found Q_{10} values of 2.5 or 4.6, depending whether roots were absent or present, respectively. We also found higher Q_{10} values (5.4 and 7.6, Table 6) for the soil fraction with fine roots of aspen when 5 to 50 ppm phenanthrene or 2,4,5-trichlorophenol were added as the

substrate. Even higher Q_{10} values, 7 to 17, have been reported for similar concentrations of tri-, tetra- and pentachlorophenols in biofilm reactors fed with permanently cold Finnish ground water (Melin 1997). Therefore, the temperature response of biodegradation may be influenced not only by the degrader organism, but also by the quality and the concentration of substrate.

Most published reports do not indicate the season of sampling. A Siberian pine forest (61°N, 89°E) was shown to lose 77% of the carbon sequestered by the tree canopy in root and soil microbial respiration on July days, leaving the Siberian forest a modest sink for CO_2 (75 mmol m^{-2} d^{-1}) in summertime (Kelliher *et al*. 1999). The summer time soil microbial respiration of the boreal soil may mainly recycle the photosynthetic produce of trees into the atmosphere by root respiration with high Q_{10} values as compared with a bulk soil. Due to this, recycling the summertime activity may be of less relevance for the annual net efflux of carbon dioxide from the soil than the wintertime activity when there is little or no photosynthesis. We found Q_{10} values of 2.8 and 2.3 for the 38-year Scots pine forest soil at Hyytiälä (61°N) in December–January (Table 3). Our results show that the rate of soil respiration was only moderately sensitive to temperature, Q_{10} of 2.3 to 2.8, when measured in the laboratory with freshly sampled soils with no major conribution of roots. If the published studies, e.g. of Niklinska *et al.* (1999) and those compiled by Kirschbaum (1995), were mainly conducted in the growing season, the results may largely reflect the recycling of photosynthetic produce of the trees, and thus give a biased value for "soil" Q_{10}. Also, the results from laboratory measurements of extended duration, such as in the study of Niklinska *et al.* (1999), may not necessarily reflect the situation in the natural forest, because the soil microbial community structure is likely to change during extended laboratory incubation.

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