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Effects of litter addition and warming on soil carbon, nutrient pools and microbial communities in a subarctic heath ecosystem

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ARTICLE INFO

Article history:
Received 4 April 2007
Received in revised form
19 December 2007
Accepted 21 December 2007

Keywords:
Arctic
Climate change
Microbial biomass
Phospholipid fatty acids
Nutrient pools
Near-infrared spectroscopy

ABSTRACT

Climatic warming leads to the expansion of deciduous shrubs and trees in the Arctic. This leads to higher leaf litter inputs, which together with warming may alter the rate of carbon and nutrient cycling in the arctic ecosystems. We assessed effects of factorial warming and additional litter on the soil ecosystem of a subarctic heath in a 7-year-long field experiment. Fine root biomass, dissolved organic carbon (DOC) and total C concentration increased in response to warming, which probably was a result of the increased vegetation cover. Litter addition increased the concentration of inorganic P in the uppermost 5 cm soil, while decreasing the pool of total P per unit area of the organic profile and having no significant effects on N concentrations or pools. Microbial biomass C and N were unaffected by the treatments, while the microbial biomass P increased significantly with litter addition. Soil ergosterol concentration was also slightly increased by the added litter in the uppermost soil, although not statistically significantly. According to a principal component analysis of the phospholipid fatty acid profiles, litter addition differed from the other treatments by increasing the relative proportion of biomarkers for Gram-positive bacteria. The combined warming plus litter addition treatment decreased the soil water content in the uppermost 5 cm soil, which was a likely reason for many interactions between the effects of warming and litter addition. The soil organic matter quality of the combined treatment was also clearly different from the control based on a near-infrared reflectance (NIR) spectroscopic analysis, implying that the treatment altered the composition of soil organic matter. However, it appears that the biological processes and the microbial community composition responded more to the soil and litter moisture conditions than to the change in the quality of the organic matter.

1. Introduction

Arctic and boreal ecosystems harbor more than one-third of the total global soil carbon pool (Post et al., 1982; Callaghan et al., 2004) and are currently exposed to strong changes in climate (ACIA, 2005). Within the next 100 years, the annual mean temperature in the arctic region is predicted to be 3–5 °C higher than today, although the increase will depend on

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0929-1393/$ – see front matter
doi:10.1016/j.apsoil.2007.12.014

political measures taken to limit the anthropogenic emissions and to manage the natural emissions of greenhouse gases (ACIA, 2005; Insam and Wett, 2008). The potential effects of climatic warming on the large soil carbon pool have concerned scientists for 25 years (Billings et al., 1982). The question of how the carbon pool will respond is important, because if the soil carbon is increasingly released to the atmosphere in response to warming, the emission could result in a significant positive feedback on climate change.

Climatic warming can impact the soil carbon not only directly by accelerating the C cycle, but also indirectly via changes in the vegetation cover. The abundance of deciduous shrubs is expected to increase in response to warming (van Wijk et al., 2004; Walker et al., 2006). In fact, remote sensing and the comparison of modern aerial photographs with historic ones have demonstrated that widespread expansion of deciduous shrubs has already taken place (Sturm et al., 2001; Stow et al., 2004). This increase in deciduous plants results in a higher litter input and altered litter quality.

Litter is not only the major source of soil organic matter, but it also affects the microclimate by buffering the soil against fluctuations in soil moisture and temperature (Sayer, 2006 and references therein). Litter addition does not consistently increase soil microbial biomass (Park and Matzner, 2003), and the knowledge of its impacts on microbial community structure is limited.

Our aim was to assess how climatic warming directly and indirectly via enhanced litter input affects the concentrations and pools of carbon, nitrogen and phosphorus in a subarctic heath soil. Furthermore, we aimed to reveal whether 7 years of warming and litter addition affect microbial biomass and microbial community composition in this soil. We hypothesized that warming would have few effects, because a similar warming experiment on another heath caused significant responses first more than 10 years after the initiation of the study (Rinnan et al., 2007a). In contrast, we expected that the experiment would have been of long enough duration for the litter addition to have supplied additional nutrients to the microbial community, which would translate into an increase in microbial biomass and nutrient concentrations. Litter addition was also expected to increase fungal biomass in the surface soil.

2. Materials and methods

2.1. Experimental site

The field experiment simulating direct and indirect effects of climatic warming was established at a wet dwarf shrub/graminoid heath close to the Abisko Scientific Research Station in northern Sweden (68°21′N, 18°49′E; 400 m a.s.l.) in 1999. There were 24 plots (120 cm × 120 cm), including unmanipulated control, warming treatment, litter addition treatment and their combination in a factorial design with each treatment replicated in six blocks.

The temperature was elevated with passive open-top greenhouses (dome-shaped plastic tents; Havström et al., 1993), which increase the air temperature by 3–4 °C. The soil temperature in the top 0–5 cm soil was measured using a pair of temperature integrator cells in each plot. These cells are filled with water-absorbing resin, and the weight they gain during the monitoring period is proportional to ambient temperature (Ambrose, 1980; Havström et al., 1993). During the growing season of 2005, the integrated soil temperature was (mean ± S.E.) 10.34 ± 0.34 °C in the control, 10.46 ± 0.31 °C in the litter addition treatment, 11.36 ± 0.17 °C in the warming treatment and 11.25 ± 0.33 °C in the combined warming and litter addition treatment. Thus, the soil temperature was significantly higher in the warmed plots compared to the plots without warming greenhouses ($P < 0.01$).

For the litter addition treatment, dried mountain birch (*Betula pubescens* ssp. *tortuosa*) litter, collected soon after leaf-fall, was supplied every autumn. The addition was 90 g m^{-2}, which corresponds to the annual leaf litter production of a nearby *B. pubescens* ssp. *tortuosa* forest with a leaf area index of 1.44 (Bylund and Nordell, 2001). The litter addition mimics the predicted changes in the litter type and quality, which are expected to follow from the higher proportion of deciduous species under warmer climate (Chapin et al., 1996). The addition of litter increases input of organically bound nutrients, which – contrary to inorganic nutrient additions, which have been traditionally used in climate change research (van Wijk et al., 2004) – must be processed by soil microbes before the nutrients are available for plants. The decomposition rate of the birch leaves at the site is probably between 0.2 and 0.3 g g^{-1} yr^{-1} according to earlier reported loss rates of birch leaf litter (Robinson et al., 1995) and mixed deciduous leaf litter, mainly from *Betula nana*, (Jonasson, 1983) in the Abisko region.

The vegetation at the heath was a mixture of various dwarf shrubs (e.g. *B. nana*, *Empetrum hermaphroditum*, *Vaccinium uliginosum*, *Andromeda polifolia* and *Rhododendron lapponicum*), herbs (e.g. *Tofieldia pusilla*, *Polygonum viviparum* and *Bartsia alpina*) and graminoids (*Carex vaginata*, *Carex parallela* and *Carex capillaris*). The moss layer was patchy, and the spots dominated by moss were avoided when sampling the soil.

The soil was highly organic and always moist due to a lateral ground water flow through the site. The soil pH of 6.9 had not been affected by the treatments.

2.2. Soil sampling and estimation of root biomass and vegetation cover

After 7 years of warming and six annual litter addition treatments, the soil (excluding intact litter on the surface) was collected from the organic soil as three 4-cm-diameter cores from each plot on 26 August 2005. The cores were split to a 0–5 cm and a 5–10 cm layer plus an additional layer that extended down to the bedrock or to coarse, stony mineral soil. The thickness of this layer varied between 3 and 15 cm. During the month preceding the sampling, there was 60 mm of precipitation of which 13 mm fell during the previous 7 days.

Roots were removed, and the soil was homogenized by hand for 10 min per sample. The roots were washed and sorted to fine (<1 mm diameter) and coarse (>1 mm) fractions, followed by ovendrying and weighing to get an estimate of the root biomass. The soil samples were stored at 5 °C until analyses.

Normalized differential vegetation index (NDVI), which is an estimate of total plant greenness, was measured using an

SKR 110 sensor (Skye Instruments, Powys, UK) with narrow band interference filters centered at 660 and 730 nm.

2.3. Determination of C, N and P in soil and microbial biomass

Microbial biomass was analyzed by the fumigation-extraction technique (Jenkinson and Powlson, 1976; Vance et al., 1987). Five-gram soil samples were extracted in 0.5 M K_2SO_4 for 1 h. Meanwhile, another set of 5 g subsamples was first fumigated with ethanol-free chloroform for 24 h and then extracted in a similar manner. A third subsample was used to determine the gravimetric soil water content and to estimate soil organic matter content as loss on ignition after 6 h at 550 °C. Part of the dried soil was used for analysis of total C, N, and P and for characterization of the SOM by near-infrared reflectance (NIR) spectral analysis by methods described below.

Both the unfumigated and fumigated soil extracts were filtered through Whatman GF/D filters and frozen until analysis of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and phosphorus concentrations. DOC concentration in the soil extracts was measured by a TOC-5000A total organic C analyzer (Shimadzu, Kyoto, Japan). Colorimetric analysis by a FIAstar 5000 flow injection analyzer (FOSS Tecator, Höganäs, Sweden) was used to determine inorganic P and DON (after decomposition of all N to NO_3^- by persulfate oxidation). In addition, the unfumigated extracts were analyzed for nitrate with the FIAstar 5000 and for ammonium by the indophenol blue method (Allen, 1989). The difference between the concentrations in the fumigated and unfumigated extracts is an estimate of the amount of C, N or P in the microbial biomass. To account for incomplete extractability, a correction factor of 0.45 was used for microbial C (Joergensen, 1996) and a factor of 0.40 for microbial N and P (Jonasson et al., 1996). For the soil profile data, soil and microbial C, N and P pools were calculated per square meter of the entire organic horizon.

2.4. Analysis of total soluble protein

Five-gram soil samples (0–5 cm and 5–10 cm soil depths) were extracted in 25 ml of 0.1 M $NaHCO_3$ (Ladd and Paul, 1973) for 1 h, filtered through Whatman GF/D filters and frozen until analysis. Protein concentration in the extracts was determined colorimetrically using the Coomassie (Bradford) dry protein assay plates (Pierce Biotechnology Inc., Rockford, IL). The absorbances of the plate wells were read with an EL 808 BioKinetics Reader (BioTek Instruments, Winooski, VT, USA) at 590 nm and converted to concentrations with the help of a standard curve obtained with Bovine serum albumin standard.

2.5. Ergosterol analysis

The amount of living fungal biomass in soil was estimated by analyzing the ergosterol content (Nylund and Wallander, 1992). Freeze-dried 0.2 g soil samples were extracted in 4 ml 10% KOH in methanol, sonicated and heated for 90 min at 70 °C. After the extraction, 1 ml H_2O and 3 ml cyclohexane were added followed by vortexing, after which the samples were centrifuged at 750 × *g* for 5 min. The upper (cyclohexane) phase was collected, and the cyclohexane wash was repeated. The two cyclohexane phases were combined and evaporated under air stream at 40 °C. The samples were subsequently dissolved in 1 ml methanol and filtered through 0.45 μm Teflon syringe filters (Millex LCR-4, Millipore, Billerica, USA). The ergosterol concentration was analyzed at 282 nm of the Waters 996 UV detector of a HPLC system (Waters, Milford, MA, USA) equipped with a C18 reverse-phase column (Nova-Pak, 3.9 mm × 150 mm, Waters) preceded by a C18 reverse-phase guard column (Nova-Pak, 3.9 mm × 22 mm).

2.6. Phospholipid fatty acid analysis

Soil microbial community composition was analyzed using phospholipid fatty acid (PLFA) analysis following Frostegård et al. (1993). Freeze-dried 0.25 g soil samples were extracted and fractionated to collect the PLFAs, which were then transmethylated to their fatty acid methyl esters by alkaline methanolysis. With methyl nonadecanoate fatty acid (19:0) as internal standard, the samples were analyzed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector. The PLFA nomenclature used is presented by Rinnan et al. (2007a). Gram-positive bacteria were considered to be comprised of PLFAs i-14:0, i-15:0, a-15:0, i-16:0, 10Me-16:0, br-17:0, i-17:0, a-17:0, 10Me-17:0 and br-18:0; Gram-negative bacteria of 16:1ω7c, 16:1ω7t, cy-17:0, 18:1ω7 and cy-19:0; and fungi of 18:2ω6.

2.7. Near-infrared reflectance spectroscopy

As a complex measure of organic matter quality, we analyzed near-infrared reflectance (NIR) spectra of the soil samples from 0–5 cm to 5–10 cm depths. The ovendried soil samples were analyzed twice with re-packing on a NIR Systems 6500 near-infrared reflectance spectrophotometer (Foss Analytical, Höganäs, Sweden) for a spectral range of 400–2500 nm at 2 nm intervals. Samples were presented in round sample cups with a quartz window, equipped with a ring (Microsample inserts, Part No. IH-0337) to decrease the sample area to half. Each spectrum with 1050 data points was derived as the average of 32 scans of the spinning sample. Reflectance was converted to absorbance (log 1/R) by the internal software.

2.8. Statistical analysis

Linear mixed models of SPSS 14.0 for Windows with depth, warming and litter addition as fixed factors and block as a random factor were used to test for main effects and interactions between fixed factors. Similar models without the depth factor were used to test for effects on pool sizes of the whole soil (0–25 cm).

The PLFA data were subjected to a principal component analysis (PCA) using the correlation matrix. The first derivative of the NIR spectral data were analyzed by PCA as in Rinnan and Rinnan (2007). These multivariate analyses were run on Simca-P 11.0 (Umetrics, Umeå, Sweden). Treatment effects on the extracted principal components were analyzed with linear mixed model as above.

3. Results

3.1. Soil properties

The depth of the organic soil profile was not affected by the treatments ($P > 0.4$), but it was significantly different between the blocks ($P < 0.05$, data not presented). The soil organic matter content was on average $92 \pm 1\%$ at the 0–5 cm depth and $85 \pm 2\%$ at the 5–10 cm depth, and there were no significant differences between the treatments ($P > 0.2$, Table 1). The pools of SOM were also unaffected by the treatments (Fig. 1a).

Soil water content was 26% (0–5 cm depth) and 9% (5–10 cm depth) lower in the combined warming and litter addition treatment than in the control, while warming and litter addition had no such effects alone (W × L interaction, Table 1). Bulk density was 62.1 ± 4.8 g d.w. dm^{-3} within the upper 5 cm depth and 107.5 ± 8.4 g d.w. dm^{-3} within the 5–10 cm depth, averaged across the treatments, and without significant treatment effects ($P > 0.3$, Table 1).

3.2. Root biomass and vegetation cover

The root biomass was highest in the uppermost 5 cm of the soil profile (Table 1). Warming increased the fine root biomass in this soil depth compared with the non-warmed plots (Table 1), whereas the biomass in the deeper soil was unaffected by the treatments. There were no treatment effects on the coarse root biomass.

Both warming and litter addition led to significant increases in total plant greenness, measured as the normalized differential vegetation index (NDVI, Table 1).

3.3. Carbon and nutrient pools in soil

The dissolved organic carbon concentration was higher under warming in the uppermost 5 cm, but unaffected by the treatments in the deeper soil (Table 2). The temperature effect on the DOC pool per unit area was not statistically significant (Fig. 1b). The total C concentration of the SOM was significantly increased by both the warming and the litter addition treatments, with the greatest increase when the treatments were combined (Table 2). However, the relative changes were small—at most a 2.8% increase in the warming plus litter addition treatment compared with the control at the 5–10 cm depth. The pools of total C were not significantly affected by the treatments (data not shown).

The ammonium concentration in the uppermost 5 cm of the soil profile was about half of that of the 5–10 cm soil depth (Table 2). The concentrations and the pools of ammonium were not significantly affected by warming or litter addition treatments (Table 2 and Fig. 1c). The nitrate concentration was below the detection limit of 0.25 μg per g SOM. The dissolved organic nitrogen pools were highest below the 10 cm depth (Fig. 1d). There was a tendency of reduced DON pool in response to the litter addition treatment in the deepest soil depth, although the treatment effects on the total DON pool were not statistically significant (Fig. 1d). In the 0–5 cm soil depth, the total N concentration was lower in the individual warming and litter addition treatments than in the control, but when the treatments were combined this difference disappeared (W × L interaction, Table 2). In the 5–10 cm soil depth, the trends were less clear. There were no treatment effects on the total N pool (data not shown). The total soil C-to-N ratio was increased by warming and litter addition alone, but it decreased when the treatments were combined (W × L interaction, Table 2). The DOC-to-DON ratio was increased by warming in the 0–5 cm depth, and by litter addition in the 5–10 cm depth (Table 2).

The soil inorganic phosphorus concentration responded differently to the treatments at different depths. In the 0–5 cm soil depth, litter addition increased phosphate concentration,

Table 1 – Soil characteristics and root biomass at 0–5 cm and 5–10 cm depth, and normalized differential vegetation index (NDVI) of the warming and litter addition experiment after seven growing seasons with treatments

Variable	Depth (cm)	Control	Litter	Warming	Warming + litter	Statistical significance
H_2O_{grav} (%)						L+, W+, W × L**
	0–5	360 (31)	362 (50)	381 (50)	267 (33)	
	5–10	357 (30)	375 (30)	365 (34)	326 (25)	
SOM (%)						D**
	0–5	89 (4)	93 (1)	92 (1)	92 (1)	
	5–10	83 (5)	88 (1)	84 (6)	85 (2)	
Bulk density (g dm^{-3})						D***
	0–5	74.7 (15.3)	68.7 (4.3)	67.3 (6.3)	81.8 (10.4)	
	5–10	112.5 (20.4)	100.1 (10.9)	121.6 (20.5)	125.1 (13.8)	
Fine roots (g dm^{-3})						D***, W*, D × W+
	0–5	1.05 (0.13)	1.03 (0.11)	1.32 (0.16)	1.46 (0.13)	
	5–10	0.95 (0.13)	0.65 (0.10)	0.85 (0.05)	0.84 (0.07)	
Coarse roots (g dm^{-3})						D**
	0–5	9.03 (1.88)	8.74 (2.23)	10.55 (1.43)	10.24 (1.66)	
	5–10	6.28 (1.20)	6.58 (1.06)	6.99 (1.30)	6.36 (1.57)	
NDVI		0.519 (0.015)	0.544 (0.014)	0.544 (0.010)	0.571 (0.009)	L*, W*

The values are means (S.E.) of six replicates. The statistical significances for main effects of depth (D), warming (W) and litter addition (L) and interactions are shown at +$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (linear mixed model with D, W and L as fixed and block as a random factor).

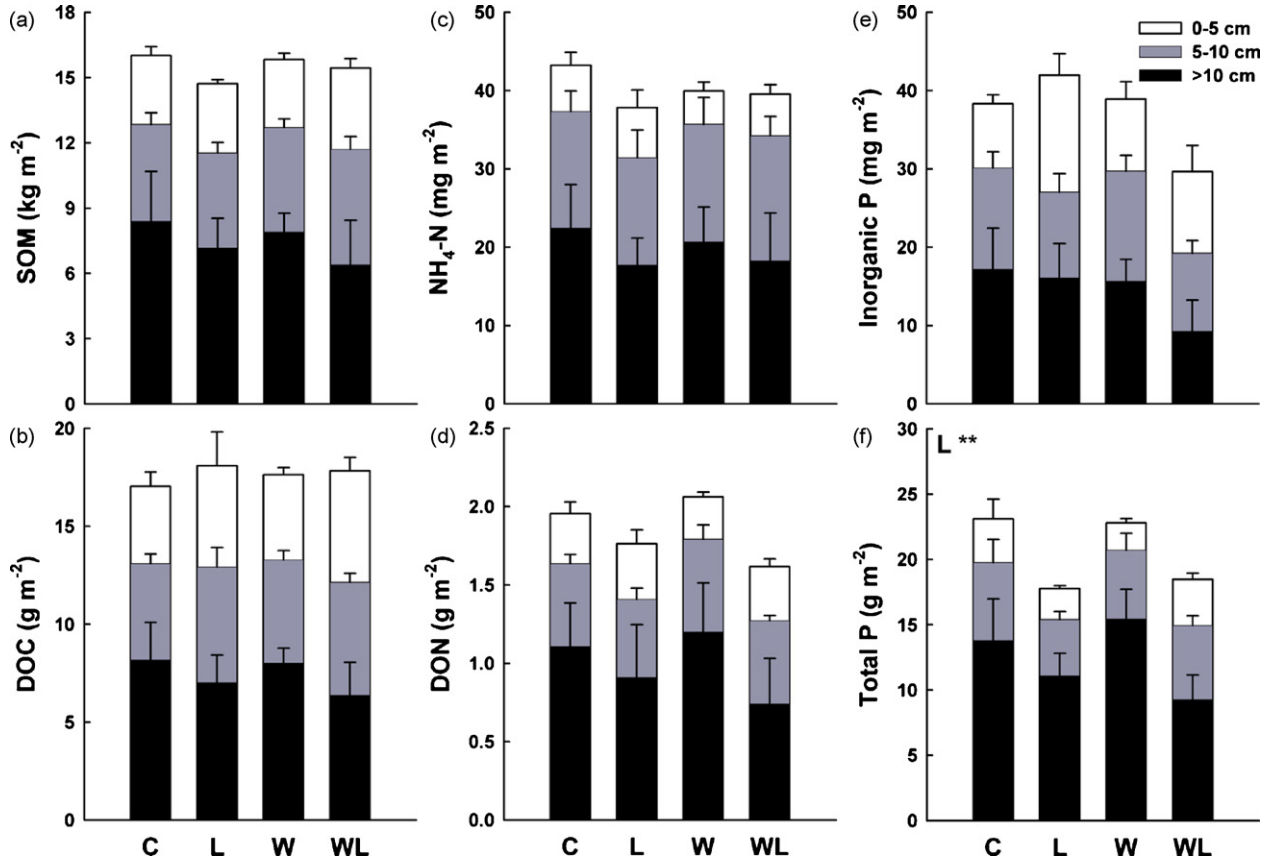

Fig. 1 – Pools of soil organic matter (a), dissolved organic carbon (DOC) (b), ammonium-N (c), dissolved organic nitrogen (DON) (d), inorganic P (e) and total P (f) at the soil depths of 0–5 cm, 5–10 cm and >10 cm in the control (C), warming (W), litter addition (L), and combined warming and litter addition (WL) treatments after seven growing seasons with treatments. The values are means (+S.E.) of six replicate plots. Statistical significances for the total pools are shown at *P < 0.05, **P < 0.01 (linear mixed model with W and L as fixed and block as a random factor).

but when combined with the warming treatment, this increase was not evidenced (W × L interaction, Table 2). In the 5–10 cm soil depth, litter addition rather decreased than increased the phosphate concentration. The pool size of inorganic P did not differ among treatments, except for a non-significant tendency of reduced inorganic phosphorus pool in the combined warming and litter addition treatment ($P = 0.14$, W × L interaction, Fig. 1e). Total P concentration of the 0–5 cm soil was reduced by both warming and litter addition alone, but it was not different from the control when these treatments were combined (W × L interaction, Table 2). In the 5–10 cm depth, the total P concentration was slightly lower than in the control also in the combined treatment. The total P pool of the whole organic soil layer was significantly reduced by litter addition (Fig. 1f).

3.4. *Microbial biomass and nutrient pools in microbial biomass*

The microbial biomass was highest in the top 10 cm of the soil, and there were no treatment effects on the concentrations or the pools of microbial biomass C (Table 2 and Fig. 2a). Neither the N concentration nor the N pool in the microbial biomass was significantly affected by warming or litter addition (Table 2 and Fig. 2b). The microbial biomass P was, however, significantly increased by litter addition both on per gram SOM basis (Table 2) and per square meter basis (Fig. 2c). The soil ergosterol concentration was highest in the uppermost soil (0–5 cm), and it was not significantly affected by the treatments (Table 2); the concentration was 11% higher in the litter addition treatment than in the plots without added litter, although this effect was only marginally significant ($P < 0.1$). A similar marginal increase under litter addition was observed for the pool of ergosterol in the top 5 cm ($P < 0.1$), but the total pools per unit area were not significantly affected by the treatments (Fig. 2d).

3.5. *Total soluble proteins*

The soluble protein concentration in the soil was significantly decreased by litter addition (Table 2) and contrastingly affected by warming depending on the soil depth (depth × warming interaction); it decreased the concentration in the top 5 cm, but increased it in the 5–10 cm depth. There was no increase when warming was combined with litter addition.

Table 2 – Chemical and microbial characteristics of the soil at 0–5 cm and 5–10 cm depth of the warming and litter addition experiment after seven growing seasons with treatments

Variable	Depth (cm)	Control	Litter	Warming	Warming + litter	Statistical significance
DOC (mg g^{-1} SOM)						D*, W+, D × W**
	0–5	1.21 (0.11)	1.10 (0.11)	1.42 (0.08)	1.52 (0.07)	
	5–10	1.13 (0.06)	1.30 (0.09)	1.10 (0.06)	1.15 (0.15)	
NH_4 (μg g^{-1} SOM)						D***
	0–5	1.75 (0.41)	1.53 (0.63)	1.46 (0.36)	1.38 (0.21)	
	5–10	3.35 (0.46)	3.10 (0.69)	3.03 (0.59)	3.10 (0.54)	
DON (μg g^{-1} SOM)						D***
	0–5	94.6 (12.6)	85.5 (11.4)	90.2 (10.8)	91.9 (4.2)	
	5–10	121.9 (8.4)	113.1 (8.2)	120.8 (11.8)	103.2 (6.9)	
Inorganic P (μg g^{-1} SOM)						D*, W × L*, D × L*
	0–5	2.57 (0.15)	4.63 (0.86)	3.08 (0.72)	2.62 (0.63)	
	5–10	3.03 (0.46)	2.34 (0.42)	2.91 (0.29)	1.90 (0.30)	
Total C (% of SOM)						L**, W*
	0–5	47.4 (0.2)	47.5 (0.4)	47.7 (0.4)	48.2 (0.2)	
	5–10	47.1 (0.3)	47.9 (0.3)	47.6 (0.5)	48.4 (0.4)	
Total N (mg g^{-1} SOM)						D**, W × L*
	0–5	15.9 (2.6)	13.8 (0.7)	13.4 (0.8)	17.3 (1.0)	
	5–10	20.5 (1.4)	15.1 (3.1)	19.6 (1.1)	21.5 (1.0)	
Total P (mg g^{-1} SOM)						D**, W × L*
	0–5	0.89 (0.25)	0.73 (0.04)	0.65 (0.07)	0.94 (0.04)	
	5–10	1.26 (0.27)	0.97 (0.06)	1.06 (0.17)	1.07 (0.11)	
C:N ratio						D***, W × L*
	0–5	32.8 (3.8)	34.8 (1.6)	36.2 (1.8)	28.4 (1.6)	
	5–10	23.6 (1.7)	26.8 (1.5)	24.5 (1.2)	22.8 (1.2)	
DOC:DON ratio						D***, W*
	0–5	14.0 (2.0)	14.1 (1.1)	17.0 (2.3)	16.9 (1.5)	
	5–10	9.4 (0.4)	11.8 (1.2)	9.4 (0.8)	11.2 (1.5)	
C_{mic} (mg g^{-1} SOM)						D***
	0–5	10.69 (0.65)	10.66 (0.36)	10.51 (0.61)	10.63 (0.40)	
	5–10	7.64 (0.82)	8.06 (0.26)	8.20 (0.72)	7.76(0.49)	
N_{mic} (mg g^{-1} SOM)						NS
	0–5	1.30 (0.06)	1.22 (0.11)	1.21 (0.10)	1.26 (0.07)	
	5–10	1.36 (0.15)	1.33 (0.05)	1.35 (0.12)	1.26 (0.12)	
P_{mic} (μg g^{-1} SOM)						D***, L*
	0–5	490 (19)	617 (49)	510 (30)	621 (62)	
	5–10	326 (48)	375 (50)	353 (42)	367 (52)	
C_{mic}:N_{mic} ratio						D***
	0–5	8.4 (0.9)	9.0 (1.0)	8.9 (0.6)	8.5 (0.3)	
	5–10	5.7 (0.3)	6.1 (0.2)	6.1 (0.3)	6.4 (0.6)	
Ergosterol (μg g^{-1} SOM)						D***
	0–5	169 (21)	199 (24)	177 (24)	188 (15)	
	5–10	98 (14)	122 (15)	116 (22)	108 (22)	
Proteins (mg g^{-1} SOM)						L*, D × W*
	0–5	2.3 (0.6)	1.2 (0.4)	1.2 (0.2)	0.9 (0.1)	
	5–10	1.1 (0.1)	1.0 (0.1)	1.4 (0.3)	1.0 (0.1)	

The values are means (S.E.) of six replicates. Statistics and abbreviations as in Table 1. NS = no significant effects.

3.6. *Microbial community composition*

There was a pronounced difference in the PLFA composition between the 0–5 cm and 5–10 cm soils as demonstrated by principal component analysis (PCA); the first two principal components (PCs, explained variances 36% and 21%) were related to the difference between the depths (data not shown). To further assess the lipid biomarkers, the data were subjected to separate PCAs for 0–5 cm and 5–10 cm depths (Fig. 3).

In the top 5 cm, the first and the second PC with explained variances of 48% and 13%, respectively, accounted for the differences between the blocks, while the third PC (9%) showed a significant warming × litter interaction effect ($P < 0.05$, Fig. 3). The litter addition treatment had high scores on PC 3, whereas the warming and the combined warming and litter addition did not differ from the control with low scores.

Based on the loadings of the PLFA variables (Fig. 3), the control treatment was characterized by relatively high amounts of fatty acids 18:1 and 18:1ω7. The amounts of cy-19:0 and 10Me-16:0b were high in the warmed plots. However, when warming was combined with litter addition, 16:1ω7, 19:1a and the indicator of actinomycetes, 10Me-18:0 (Frostegård et al., 1993) increased. Litter addition alone seemed to increase the abundance of Gram-positive bacteria indicated by

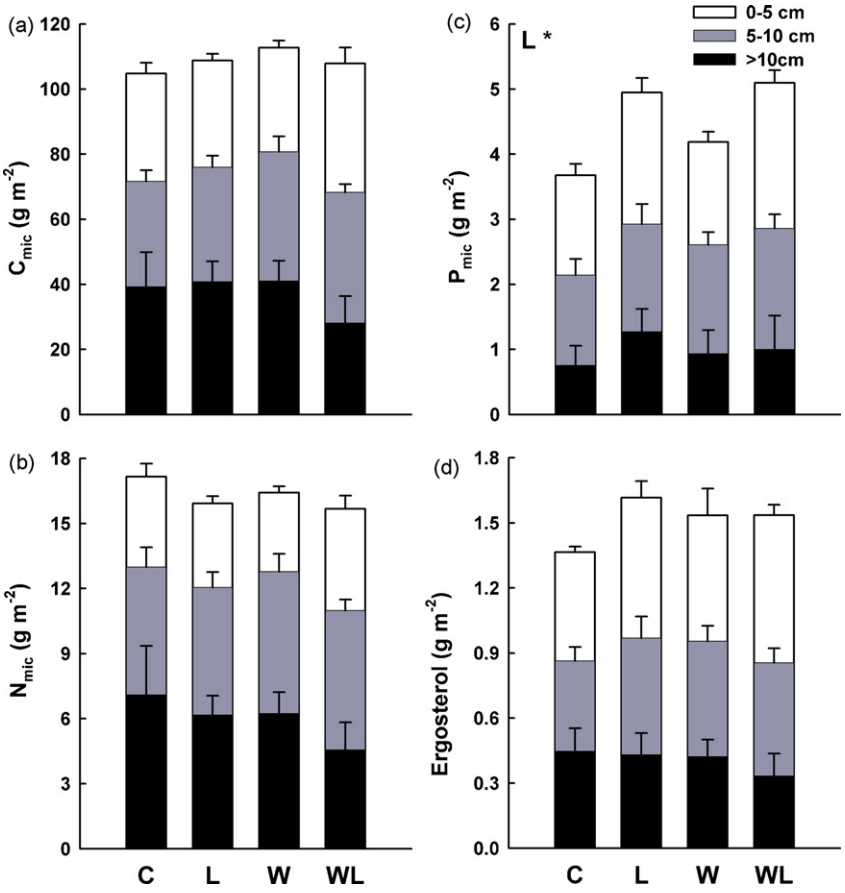

Fig. 2 – Pools of microbial biomass C (a), N (b), P (c) and ergosterol (d) at the soil depths of 0–5 cm, 5–10 cm and >10 cm of the warming and litter addition experiment after seven growing seasons with treatments. The symbols and statistics are as in Fig. 1.

relatively higher amounts of for example i-16:0, i-15:0, br-18:0 and 10Me-17:0. The amount of the fungal biomarker 18:2ω6 had little influence on the PCA model as it was located close to the origin.

There were no significant differences in the microbial communities between the treatments in the 5–10 cm soil depth (data not presented).

3.7. *Near-infrared reflectance spectra*

The soil organic matter quality, analyzed by NIR spectroscopy, was significantly altered by the treatments (Fig. 4). When analyzed separately for each soil depth, the first PC (explained variance 56% for the 0–5 cm depth and 54% for the 5–10 cm depth) demonstrated that the combined warming and litter addition treatment differed from the other plots. In the 5–10 cm depth, the control and the warming treatments had high scores and both the litter treatments had low scores on PC1 (litter effect, $P < 0.01$, Fig. 4b), while there was only a slight tendency to differences in the surface soil ($P = 0.16$, Fig. 4a). The third PC (11% and 12%, respectively) accounted for the difference between the control and the different treatments, with a nearly significant warming effect for the top 5 cm soil ($P = 0.069$).

4. Discussion

Seven years of elevated temperature simulating climatic warming had only slight effects on the carbon pools in the subarctic wet heath soil, and the nutrient pools in soil and microbes were largely unaffected. The slightness of responses to warming was likely due to the experimentally obtained soil temperature increase of only 1 °C. Increased litter input had stronger influence on the nutrient pools, and as hypothesized, it slightly altered microbial community composition and increased the fungal biomass in the top 5 cm soil.

The concentrations of both dissolved organic carbon and total C were higher in the warmed plots than in the plots under ambient temperature. Total C was also significantly increased by the litter addition treatment. Increased production of dissolved organic carbon in response to warming has frequently been observed in peaty soils (reviewed by Evans et al., 2006). However, it is more likely that the increase in soil C in our experiment was a result of higher plant biomass and thereby higher carbon inputs both in dissolved (root exudates) and particulate (litter) form. In fact, we observed a higher fine root biomass under elevated temperature, and both warming and litter addition led to significant increases in total plant greenness, measured as the normalized differential vegetation index (NDVI). The warmed plots with increased root

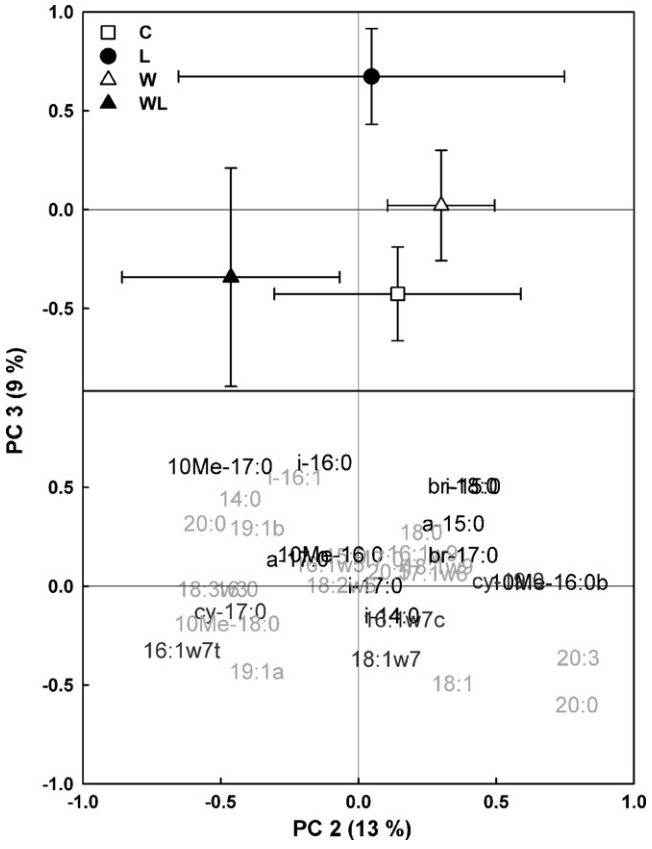

Fig. 3 – Principal component analysis of the phospholipid fatty acid (PLFA) profiles at the 0–5 cm soil depth in the control (C), warming (W), litter addition (L), and combined warming and litter addition (WL) treatments after seven growing seasons with treatments. The score plot shows mean ± S.E., $n = 6$. In the loading plot, PLFAs specific for Gram-positive bacteria are shown in black, those specific for Gram-negative bacteria in dark grey, and the rest in light grey. Explained variances are shown in parentheses for each principal component (PC).

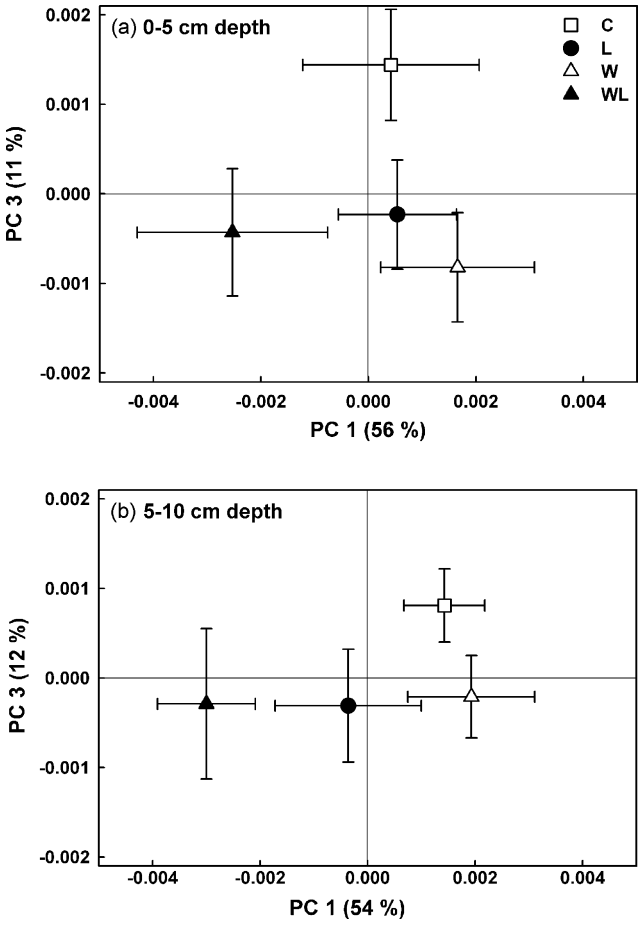

Fig. 4 – Score plots (mean ± S.E., $n = 6$) of the principal component analysis of the near-infrared reflectance (NIR) spectra after a first derivative-transformation for (a) 0–5 cm and (b) 5–10 cm soil depths in the control (C), warming (W), litter addition (L), and combined warming and litter addition (WL) treatments after seven growing seasons with treatments. Explained variances are shown in parentheses for each principal component (PC).

biomass also had an enhanced DOC-to-DON ratio, which corresponds to the higher DOC and lower DON content observed in the presence of plants (Khalid et al., 2007).

The additional litter with a *P* concentration of about 1.2 mg g^{-1} d.w. appeared to be an important source of phosphorus to the soil. Inorganic phosphorus concentrations and pools in the soil and in the microbial biomass were significantly increased by litter addition. However, the total soil P concentration decreased rather than increased in response to litter addition, probably because the major part of the P added with the litter was accumulated in the plant biomass. In 10 litter types from subarctic to cool temperate regions, Moore et al. (2006) observed that while P is usually immediately lost from litter, N is retained until about half of the original C content remains. As an example, 50–70% of P in *B. nana* litter disappears during the 1st year (Jonasson, 1983). Furthermore, the P content of the same litter as used in the present study has been reported to decrease to 50% during a 22-week-long incubation at 10–12 °C (Jonasson et al., 2004). Based on these P loss rates, it is likely that most of the P in the birch litter of the present study has been released during the 7 years the experiment has been running.

While the pools of dissolved inorganic and organic nitrogen were largely unaffected by the treatments, the concentration of soluble proteins, the predominant form of organic nitrogen in soils (Lipson and Näsholm, 2001), was significantly decreased by litter addition. It is plausible that humic substances and phenolics, which are abundant in subarctic birch leaves (Graglia et al., 2001), have been released from the additional litter causing enhanced binding and thereby reduced extractability of proteins (Lipson and Näsholm, 2001).

Although dark ecosystem respiration in the warmed plots was twice as high as in the plots without greenhouses (Michelsen et al., unpublished), the microbial biomass was not affected by the treatments. The lacking net effect may be due to top–down control of the microbial biomass by nematodes and other consumers (Ruess et al., 1999). While the microbial biomass was unaffected, bacterial growth rate

was significantly increased by warming (Rinnan et al., 2007b). This together with the higher fine root biomass and plant cover (NDVI) is probably causing the higher respiration.

Only the phosphorus content in the microbial biomass increased due to litter addition, indicating immobilization of the phosphate released from the additional litter. Generally, litter removal leads to reduced soil microbial biomass, while there are few data on effects of litter addition (Sayer, 2006). However, in an experiment with mesocosms from Abisko, litter addition decreased microbial biomass (Jonasson et al., 2004). Litter addition to a temperate deciduous forest had no clear effects on microbial biomass, although the release of DOC and DON from the organic horizons greatly increased (Park and Matzner, 2003). In another study, doubling the litter supply had no significant effects on DOC and DON concentrations in the soil water collected in lysimeters (Lajtha et al., 2005).

Although the differences in the microbial community composition based on the phospholipid fatty acid profiles were mostly accounted for by block effects, also differences among the treatments were evidenced. Litter addition increased the abundance of some biomarkers for Gram-positive bacteria. This is in agreement with the results from an experiment, in which substrate additions to forest soil increased the relative abundance of Gram-positive bacteria (Brant et al., 2006). However, several studies have reported an opposite response to substrate additions (Griffiths et al., 1998; Phillips et al., 2002; Waldrop and Firestone, 2004). Our response may result from the fertilization influence of the litter addition, as fertilizer additions to subarctic heath soils have been reported to increase the relative abundance of Gram-positive bacteria (Schmidt et al., 2000; Rinnan et al., 2007a).

The effects of the combined warming and litter addition treatment were often contrasting with the single-factor effects. It is possible that the open-top greenhouses decreased the litter decomposition by reducing soil moisture. In fact, the soil water content was significantly lower in the combined warming and litter addition plots than in the other treatments, including the warming without litter addition, possibly due to higher evapotranspiration from a higher plant biomass. In nearby plots at Abisko, warming increased the decomposition of fine roots in the soil, while graminoid litter decomposition at the surface was reduced due to drying of the litter (Robinson et al., 1997). Also, in a meta-analysis of manipulation studies investigating temperature effects on litter decomposition in boreal and arctic ecosystems, Aerts (2006) observed that while warming by infrared lamps increased decomposition, warming by open-top chambers significantly decreased it. This was due to moisture limitation of decomposition.

The reduced moisture content in the combined warming and litter addition treatment may also have limited microbial processes. Based on our NIR spectroscopic analysis, which measures the complex characteristics of the soil organic matter by the absorbance of near-infrared radiation by various organic compounds, the soil from the warming plus litter addition treatment clearly differed from the other treatments. This indicates that the combined treatment had led to changes in the soil organic matter quality, although microbial biomass and community composition did not significantly differ from the control soil. An analysis of soil from another site at Abisko showed that NIR spectra were associated, e.g. with SOM content, ergosterol content, microbial biomass C, microbial biomass P and phospholipid fatty acid content (Rinnan and Rinnan, 2007). The difference also coincided with the significantly higher C concentration of the SOM in the combined treatment as compared with the other treatments.

5. Conclusions

Vegetation changes following climatic warming are – at least in a 5–10 years' perspective – likely to have stronger effects on microbial processes and thereby carbon and nutrient pools in arctic soils than direct warming. Based on our results, the expected expansion of deciduous shrubs and trees, and the consequent increase in leaf litter fall will lead to enhanced phosphorus availability in the soil. Whether this in turn will increase soil microbial biomass and carbon turnover in the long-term cannot be predicted from the results of the present work. It appears, however, that changes in soil moisture content have a considerable influence on microbial processes, even in the studied heath with relatively high soil moisture content. For example, the observed increase in soil carbon under warming and litter addition was due to moisture limitation of litter decomposition at the soil surface and not due to effects of higher temperature or litter addition *per se*. The effects of warming should therefore be assessed with precise monitoring of soil moisture or preferably including precipitation manipulations in the experimental design.

Acknowledgements

This study was financially supported by the Academy of Finland (decision 108277) and the European Commission Marie Curie Intra-European fellowship to R. Rinnan. Logistical support and facilities were provided by the EU ATANS grant and Abisko Scientific Research Station. The Danish Natural Science Research Council supported the set-up and maintenance of the site. Gosha Sylvester, Esben Vedel Nielsen and Karna Heinsen assisted in chemical analysis.

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