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# Mineralization and carbon turnover in subarctic heath soil as affected by warming and additional litter

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**Abstract**

Arctic soil carbon (C) stocks are threatened by the rapidly advancing global warming. In addition to temperature, increasing amounts of leaf litter fall following from the expansion of deciduous shrubs and trees in northern ecosystems may alter biogeochemical cycling of C and nutrients. Our aim was to assess how factorial warming and litter addition in a long-term field experiment on a subarctic heath affect resource limitation of soil microbial communities (measured by thymidine and leucine incorporation techniques), net growing-season mineralization of nitrogen (N) and phosphorus (P), and carbon turnover (measured as changes in the pools during a growing-season-long field incubation of soil cores *in situ*). The mainly N limited bacterial communities had shifted slightly towards limitation by C and P in response to seven growing seasons of warming. This and the significantly increased bacterial growth rate under warming may partly explain the observed higher C loss from the warmed soil. This is furthermore consistent with the less dramatic increase in the contents of dissolved organic carbon (DOC) and dissolved organic N (DON) in the warmed soil than in the soil from ambient temperature during the field incubation. The added litter did not affect the carbon content, but it was a source of nutrients to the soil, and it also tended to increase bacterial growth rate and net mineralization of P. The inorganic N pool decreased during the field incubation of soil cores, especially in the separate warming and litter addition treatments, while gross mineralized N was immobilized in the biomass of microbes and plants transplanted into the incubates soil cores, but without any significant effect of the treatments. The effects of warming plus litter addition on bacterial growth rates and of warming on C and N transformations during field incubation suggest that microbial activity is an important control on the carbon balance of arctic soils under climate change.


*Keywords:* Arctic; Climate change; Litter; Mineralization; Immobilization; Soil carbon; Bacterial growth; Thymidine; Leucine

## 1. Introduction

Soils of the arctic ecosystems contain approximately 14% of the global soil carbon (C) stock (Post et al., 1982). Even if the most recalcitrant fractions are excluded, the carbon amount per unit area is 50% higher than the global average (Jonasson et al., 2001). These soils also contain a large nitrogen (N) store, although the availability to plants is dependent on microbial mineralization and immobilization of nutrients (Jonasson et al., 2001).

The Arctic is experiencing rapidly advancing global warming, which can have a serious impact on the biogeochemical cycling of carbon and nutrients in the soil. If the predicted 3–5 °C increase in the annual mean temperature over the next 100 years (ACIA, 2004) leads to enhanced decomposition and mineralization of nutrients, higher availability of nutrients together with direct temperature effects can support higher plant biomass, but at the same time, the higher decomposition may lead to losses of carbon to the atmosphere (Mack et al., 2004).

Warmer temperatures are not only expected to increase plant biomass, but also to alter the plant community composition. It is already evident, that the abundance of deciduous shrubs is increasing (Sturm et al., 2001; Stow et al., 2004), and that the mountain birch tree-line has risen

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northwards and towards higher altitude (Kullman, 2003). These trends are predicted to further intensify in response to global warming (ACIA, 2004; van Wijk et al., 2004; Walker et al., 2006), which leads to higher amount of litter on the ground. It is not well known how the additional litter affects microbial processes in soil and whether it will increase or decrease the losses of soil C. Litter is the major source of soil organic matter, but an introduction of this additional substrate to soil may also induce a priming effect, that is, stimulated decomposition of the recalcitrant SOM fractions in soil (Kuzyakov et al., 2000). Evidence for a priming effect after litter addition has been observed in coniferous forests (Subke et al., 2004).

Warming itself, higher nutrient availability and the increased amount of litter can all potentially alter resource limitation of soil bacteria. Soil bacteria are in general considered to be C limited. However, recent evidence shows that this may not always be the case in the Arctic, where bacteria appear N or phosphorus (P) limited (Nordin et al., 2004; Rinnan et al., 2007). If the higher nutrient availability following warmer temperatures and more available litter induces a shift in resource limitation from N or P to C, increased consumption of the soil C stock and release of $CO_2$ can be expected. This would be a serious feedback on climate change.

In this work, we assessed how increasing temperatures in the Arctic directly and indirectly, via the increased litter inputs, affect the mineralization of N, P and C. This was done by a field incubation of soil modified from the traditional buried bag method (Eno, 1960) in a long-term field experiment with factorial warming and litter addition treatments. In addition, we determined whether warming and litter addition affect the soil bacterial growth rate by measuring incorporation of radioactive precursors of bacterial DNA (thymidine) and proteins (leucine) into bacterial cells (Bååth, 1992, 1994). The same technique was used to reveal how warming and litter addition affect the patterns of substrate limitation of bacterial growth.

We hypothesized that bacterial growth would be higher under elevated temperature, and that this would result in enhanced mineralization of nutrients. We also expected enhanced carbon losses from the warmed soil. The additional mountain birch litter was thought to serve as an extra source of energy and nutrients, and thus it was hypothesized to stimulate bacterial activity and to alter the resource limitation of the bacteria.

## 2. Materials and methods

### 2.1. Experimental site

Soil processes were investigated at a field experiment located close to the Abisko Scientific Research Station in subarctic Sweden (68°21′N, 18°49′E). The experiment has been running since 1999 with warming and litter addition as factorially applied treatments arranged in six blocks. The warming treatment was achieved with dome-shaped open-top plastic tents, which increased the air temperature by 3–4 °C and the soil temperature by approximately 1 °C. Plots in the litter addition treatment received $90\,g\,m^{-2}$ mountain birch (*Betula pubescens* ssp. *tortuosa*) litter every autumn. This treatment simulates predicted changes in the litter type and quality from the expected increased proportion of deciduous species (Chapin et al., 1996).

The experiment is on a mesic dwarf shrub/graminoid heath with a rich vegetation cover consisting of a mixture of dwarf shrubs, herbs and graminoids. The most dominant species include *Empetrum hermaphroditum*, *Vaccinium uliginosum*, *Andromeda polifolia*, *Carex vaginatum* and *Carex parallela*. The soil is highly organic, moist and has a pH of 6.9.

### 2.2. Field incubation experiment

#### 2.2.1. Soil and plant material

On June 15, 2005, an $8 \times 8\,cm^2$ soil block was cut with a knife to 5 cm depth and taken up from each plot of the warming and litter addition experiment. Green and dead standing aboveground plant biomass was removed, while litter was left untouched on the soil blocks.

Each block was split into four $4 \times 4 \times 5\,cm^3$ soil cores. One of these cores was used as a zero-sample in the analyses of carbon and nutrient concentrations in the soil and microbial biomass. Two cores were cut vertically to $\frac{2}{3}$ depth, and a *Carex capillaris* plant was transplanted into the formed groove.

The two *Carex* cores and the last core without a plant were inserted into plastic containers. The cores were watered to field capacity, i.e. water was added until a droplet formed at the bottom of the plastic container, and weighed. Then they were covered with a thin plastic film so that the *Carex* shoots were able to grow from between the two sheets of plastic overlapping in the middle of the core. Finally, the cores were put back to the sampling hole in the field. In the middle of the growing season, the cores were watered once to the initial weight while weighing with a portable balance.

After approximately 10 weeks of incubation in the field, the soil cores were collected on August 23, 2005. The *Carex* plants were carefully removed, divided into above- and belowground parts, oven-dried at $+70\,°C$ and weighed. This plant material and the *Carex* plants that were dried in the beginning of the mineralization experiment were analyzed for N and P by acid digestion as described below. The soil was analysed for carbon and nutrient concentrations and microbial biomass. The differences between the contents at the end and in the beginning were considered as an estimate of the amount mineralized or immobilized during the growing season.

The presence of plants has been suggested to improve estimates of nutrient transformations in buried bag experiments. Firstly, it lets soil and plants in concert affect decomposition and mineralization allowing rhizosphere processes to take place. Secondly, the modified method

reduces the possibility for soil microbes to absorb more nutrients than they would do when they compete for nutrients with plants, which would underestimate the mineralization. It has therefore been suggested that, particularly in nutrient-deficient soils, the increase of nutrients during incubation in both the transplanted plants and the soil inorganic pool (nutrient mobilization) reflects the mineralization better than the increase in the soil inorganic pool alone (Jonasson et al., 2004, 2006).

#### 2.2.2. Analyses of C, N and P

Directly after soil collection the roots were removed and the soil samples were homogenized by hand. Then one subsample was extracted in 2 M $K_2SO_4$ for 1 h, another was fumigated with ethanol-free chloroform for 24 h followed by $K_2SO_4$ extraction for microbial biomass analysis (Jenkinson and Powlson, 1976; Vance et al., 1987). A third subsample was used for gravimetric soil water content determination and soil organic matter content estimation as loss on ignition after 6 h at 550 °C. Chemical analyses on C, N and P fractions in the zero samples and end samples were conducted simultaneously in order to minimize systematic errors.

The soil extracts were analyzed for dissolved organic C (DOC), dissolved organic N (DON), ammonium + nitrate (only the non-fumigated soil extracts) and phosphate as in Rinnan et al. (2007). The oven-dried soil samples and the *C. capillaris* samples were analyzed for total N and total P by spectrophotometry after acid digestion (Allen, 1989). In addition, total C and total N in soil were analyzed by dry combustion. Roots removed from the soil were washed, sorted to fine (<1 mm diameter) and coarse (>1 mm) fractions, and oven-dried to get an estimate of the root biomass.

### 2.3. Laboratory assay to determine limiting factors

#### 2.3.1. Soil samples

A 4-cm-diameter corer was used to take soil samples down to 10 cm depth on August 26, 2005. Three cores were taken from each plot, divided into 0–5 and 5–10 cm deep profiles and the profiles were combined so that there was one sample per depth and per plot. Soil was homogenized by hand while removing the roots and refrigerated until the laboratory assay to determine limiting factors.

#### 2.3.2. Bacterial activity determination

Ten days after the soil sampling, thymidine and leucine incorporation technique (Bååth, 1992, 1994), as modified in Bååth et al. (2001), was used to determine bacterial activity. Bacteria were extracted from the 0.5-g (f.w.) soil samples by shaking in 20 ml sterile deionized water on a Multi Reax shaker (Heidolph Instruments, Schwabach, Germany) for 10 min. After centrifugation for 10 min at 1000*g*, 1.5 ml of the bacterial suspension was taken into a microcentrifuge tube.

These samples were incubated with a final concentration of 130 nM [methyl-$^3$H]thymidine (25 Ci mmol$^{-1}$, Amersham Biosciences, Little Chalfont, UK) and of 550 nM L-[U$^{14}$C]leucine (306 mCi mmol$^{-1}$, Amersham Biosciences) at 20 °C for 2 h. The incubation was terminated by adding 75 µl 100% trichloroacetic acid (TCA). Centrifugation, washing, solubilization of macromolecules and measurement of incorporated radioactivity were performed according to Bååth et al. (2001).

#### 2.3.3. Limiting nutrients for bacterial growth

To determine the short-term bacterial growth response to nutrient and carbon additions, eight 0.5-g f.w. subsamples of each of the 24 soil samples from 0 to 5 cm soil depth were weighed into 50 ml centrifuge tubes. Carbon, N and P was added in a full factorial design as in Aldén et al. (2001) but giving the additions diluted in water rather than dry. Carbon addition was 4.95 mg glucose, N addition 0.28 mg $NH_4NO_3$ and P addition 0.43 mg $KH_2PO_4$ per subsample, equivalent to 2 mg C, 0.01 mg N and 0.01 mg P. Each subsample received in total 150 µl of deionized water without any additions (the control indicated by 0) or with a single added substance or any combination of the substances (denoted C, N, P, CN, CP, NP and CNP). The liquid was carefully mixed into the soil. The samples were incubated for 3 days at 20 °C, and bacterial activity was determined by the thymidine and leucine incorporation technique as described above.

### 2.4. Data analysis

The experimental design was randomized complete block design with block as a random factor and warming and litter addition as fixed factors. When applicable, the core type (with or without plants) was also included as a fixed factor. In addition, the statistical model included all interactions between the fixed factors. The values for the two *Carex* cores per experimental plot were averaged prior to analysis to yield one value per plot. Linear Mixed Model procedure of SPSS for Windows 14.0 was used to test for differences between the treatments and the core types, and Levene's test was used to test for homogeneity of variances.

The data from the laboratory assay to determine limiting factors was standardized by dividing values for all substrate amendments in each experimental plot by the corresponding value with no substrate amendments. This was performed in order to minimize the differences in absolute thymidine and leucine incorporation rates due to effects of warming and litter addition so that the effects of nutrient amendments could be assessed. To extract the response pattern in bacterial activity to the substrate amendments, the relative thymidine and leucine incorporation values were subjected to a principal component analysis (PCA) with correlation matrix followed by a Linear Mixed Model on the principal component (PC) scores to compare the field treatment effects on the response patterns. In addition, we assessed the statistical

significances of the factorial substrate additions on the relative bacterial activity data within each field treatment by a Linear Mixed Model with block as a random factor and C, N and P amendment as fixed factors, including all interactions between C, N and P. To correct for multiple ANOVA tests, we applied the Bonferroni correction to the statistical significances.

## 3. Results

### 3.1. Field incubation experiment

#### 3.1.1. Soil characteristics

The soil characteristics after 6 years of warming manipulation during the growing season and yearly litter additions were mainly affected by litter addition (Table 1). Litter addition significantly increased the concentrations of DON ($P<0.01$), microbial biomass P ($P<0.05$) and total P ($P<0.05$).

There were no treatment effects on bulk density, water content, soil organic matter, root biomass, inorganic N and P, microbial biomass C and N, DOC, or total C and N (Table 1). The ratios of microbial biomass C-to-N and total C-to-N were also unaffected by treatments, and there were no significant warming × litter addition interaction effects on any of the measured variables. The main inorganic N pool was comprised of ammonium, as the nitrate concentration was below the detection limit.

#### 3.1.2. Plant growth during the mineralization experiment

During the incubation period, biomass of the transplanted *C. capillaris* plants increased on average by 16 mg, i.e. to twice their initial mass. Plant growth was significantly reduced by litter addition leading to 18% lower root biomass ($P<0.05$) and 45% lower leaf biomass ($P<0.01$) in the litter addition treatment compared with the control treatment (Table 2). For leaf biomass, there was also a significant $W\times L$ interaction, because both litter and warming decreased biomass while the combination did not decrease it further. Root-to-shoot ratio increased both in the litter addition and warming treatments, but the effect in the combined treatment was less ($P<0.05$ for $W\times L$ interaction, Table 2).

The absolute concentrations or changes in the N or P concentration in the plant tissue were not affected by warming or litter addition (Table 2). However, due to the combined effect of slightly lower N concentration and much lower plant growth, the increase in the plant N pool was significantly reduced by the litter addition ($P<0.05$, black bars in Fig. 1a). There were no significant effects on the change in the plant P pool (Fig. 1b).

#### 3.1.3. Changes in soil carbon and nutrient pools in field incubated soil cores

The extractable DOC pool in the control soil increased by about 35% (1.7 mg core$^{-1}$) during the incubation (Fig. 2a). Warming significantly decreased this amount ($P<0.01$ for main effect of warming), both in the cores with and without plants, while litter addition had no clear effects. The total C remained rather constant in the cores from the control and litter addition plots throughout the incubation, but warming led to a highly significant decrease in the total C pool ($P<0.001$ for main effect of warming, Fig. 2b), which, as for the DOC content, was consistent regardless of the litter treatment. As the SOM content exceeded 90% of the soil dry mass, the C loss did not, however, lead to a noticeable

Table 1
Soil and microbial variables at the 0–5 cm depth of the warming and litter addition experiment in the beginning of the field incubation, after 6 years of field treatment

| Variable | *C* | *L* | *W* | *WL* | Statistical significance[a] |
|---|---|---|---|---|---|
| Bulk density (kg dw m$^{-3}$) | 84.5 (11.5) | 92.0 (6.3) | 82.0 (7.3) | 109.3 (12.3) | NS |
| $H_2O_{grav}$ (%) | 379 (33.0) | 480 (99.6) | 388 (41.5) | 320 (29.1) | NS |
| SOM (%) | 92.6 (1.2) | 93.4 (0.7) | 93.5 (0.6) | 92.3 (1.4) | NS |
| Fine roots (g dw m$^{-2}$) | 245 (29) | 190 (38) | 288 (39) | 247 (37) | NS |
| Coarse roots (g dw m$^{-2}$) | 927 (145) | 1003 (208) | 1011 (104) | 860 (107) | NS |
| $C_{mic}$ (mg g$^{-1}$ SOM) | 19.9 (1.03) | 22.7 (3.16) | 19.2 (1.41) | 19.5 (1.44) | NS |
| $N_{mic}$ (mg g$^{-1}$ SOM) | 1.40 (0.09) | 1.55 (0.15) | 1.34 (0.10) | 1.49 (0.10) | NS |
| $P_{mic}$ (μg g$^{-1}$ SOM) | 486 (21.9) | 617 (32.7) | 522 (76.0) | 576 (57.2) | $L^*$ |
| $C_{mic}$:$N_{mic}$ ratio | 14.4 (0.9) | 14.7 (1.2) | 14.5 (0.7) | 13.2 (0.6) | NS |
| DOC (mg g$^{-1}$ SOM) | 1.07 (0.06) | 1.40 (0.24) | 1.21 (0.07) | 1.28 (0.08) | NS |
| DON (μg g$^{-1}$ SOM) | 94.4 (14.5) | 138.5 (21.3) | 114.8 (7.8) | 137.1 (22.1) | $L^{**}$ |
| $NH_4$ (μg g$^{-1}$ SOM) | 0.91 (0.31) | 1.53 (0.61) | 1.03 (0.26) | 1.33 (0.31) | NS |
| $PO_4$-P (μg g$^{-1}$ SOM) | 1.83 (0.64) | 2.29 (0.55) | 1.14 (0.28) | 1.76 (0.66) | NS |
| Total C (% of SOM) | 47.8 (0.4) | 47.4 (0.6) | 47.8 (0.2) | 47.9 (0.4) | NS |
| Total N (mg g$^{-1}$ SOM) | 12.7 (1.5) | 11.7 (0.9) | 11.9 (0.7) | 13.1 (1.0) | NS |
| Total P (mg g$^{-1}$ SOM) | 0.59 (0.06) | 0.67 (0.05) | 0.59 (0.05) | 0.70 (0.04) | $L^*$ |
| C:N ratio | 40.4 (4.4) | 41.5 (2.8) | 40.9 (2.3) | 37.7 (3.4) | NS |

The values are means (SE) of six replicates. Statistical significances are from Linear Mixed Model with warming (*W*) and litter addition (*L*) as fixed and block as a random factor, $^*P<0.05$, $^{**}P<0.01$. NS, no significant effects; *C*, control.

[a]There were no significant $W\times L$ interaction effects.

Table 2
Effects of warming and litter addition on biomass, root-to-shoot ratio and nutrient concentrations in *Carex capillaris* transplanted for one growing season

| | *C* | *L* | *W* | *WL* | Statistical significance |
|---|---|---|---|---|---|
| Leaf biomass (mg dw) | 11.3 (1.55) | 6.2 (0.53) | 8.3 (1.49) | 7.3 (0.67) | $L^{**}$, $W \times L^{*}$ |
| Root biomass (mg dw) | 25.5 (3.54) | 20.9 (1.16) | 27.2 (2.57) | 22.5 (2.12) | $L^{*}$ |
| Root-to-shoot ratio | 2.61 (0.42) | 3.65 (0.26) | 3.72 (0.46) | 3.22 (0.30) | $W \times L^{*}$ |
| N concentration (mg $g^{-1}$ dw) | 8.22 (0.59) | 7.50 (0.48) | 7.10 (0.43) | 7.72 (0.59) | NS |
| P concentration (mg $g^{-1}$ dw) | 1.34 (0.14) | 1.32 (0.12) | 1.05 (0.14) | 1.39 (0.20) | NS |

The values are means (SE) of six replicates. Statistical significances are from Linear Mixed Model with warming (*W*) and litter addition (*L*) as fixed and block as a random factor, $^{*}P<0.05$, $^{**}P<0.01$. NS, no significant effects; *C*, control.

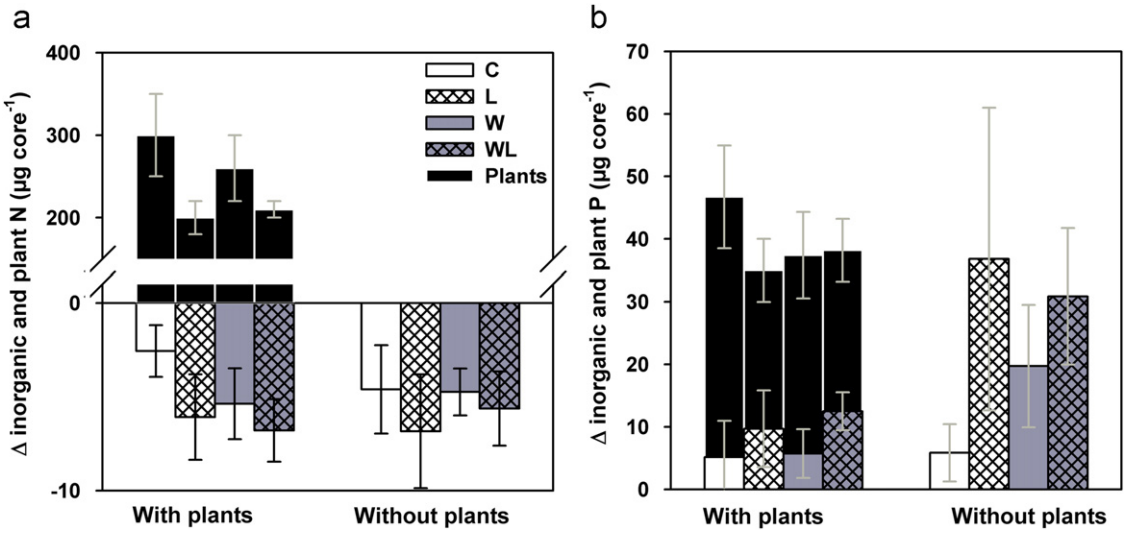


Fig. 1. Changes in the amount of nitrogen (a) and phosphorus (b) in the inorganic soil pool (patterned bars) and *Carex capillaris* plants (black bars) during a growing-season-long incubation in the warming and litter addition experiment. Bars represent means (±SE) of six replicates. *C* = control, *L* = litter addition, *W* = warming, *WL* = warming and litter addition. Cores were incubated with or without *C. capillaris* plants.

change in the C concentration. By the sampling in August, microbial biomass C had decreased to approximately 60% of the values in June without any significant treatment effects (data not shown). The presence of plants in the soil cores had no significant effects on the changes in C pools (Fig. 2).

The inorganic N pool in the soil decreased during the incubation (Figs. 1a and 3a), i.e. there was no net mineralization of N. Calculated as the relative change from the initial pool, the decrease in the inorganic N was 20–40% in the control plots, while it was up to 80% in the plots from the litter addition or warming treatment. However, the combined warming plus litter addition treatment was not different from the control soil ($P<0.01$ for $W \times L$ interaction). The changes in extractable DON, in contrast, varied from an increase from the pre-incubation level in the control to a significant decrease in the warmed plots ($P<0.05$ for main effect of warming), while litter addition had no significant effect (Fig. 3b).

While the inorganic N decreased, the pool of N in microbial biomass increased on average by 10% during the incubation, which indicates that mineralized N was immobilized by the microbes. The change in the microbial N pool was not significantly affected by the treatments, nor were there any significant treatment effects on the changes in the total N pool (data not presented). The presence of plants in the soil cores strongly increased mobilization of N (the plant N + inorganic N; $P<0.001$, Fig. 1a), but it had no significant effects on the changes in the soil N pools (Fig. 3).

The inorganic P pool increased during the incubation indicating net mineralization of P (Fig. 1b). Litter addition tended to increase P mineralization ($P<0.1$), but warming had no significant effects. The presence of plants strongly decreased the pool of inorganic P in the soil ($P<0.05$, Fig. 1b). It also stimulated the mobilization of P (the plant P + inorganic P) in the control, but across all the treatments the plant effect was not significant (Fig. 1b).

The relative changes in the P pools were not significantly affected by the treatments. The pool of inorganic P in the soil more than doubled, that of total P remained approximately constant, and that of microbial biomass P decreased to about 55% during the incubation (data not shown).

The ratio of microbial biomass C-to-N decreased from initial 14.2±0.3 (mean±SE averaged across the treatments) to 5.1±0.1 at the end of the incubation. The decrease was greater in the litter addition and warming treatments and lower in the combined litter addition and

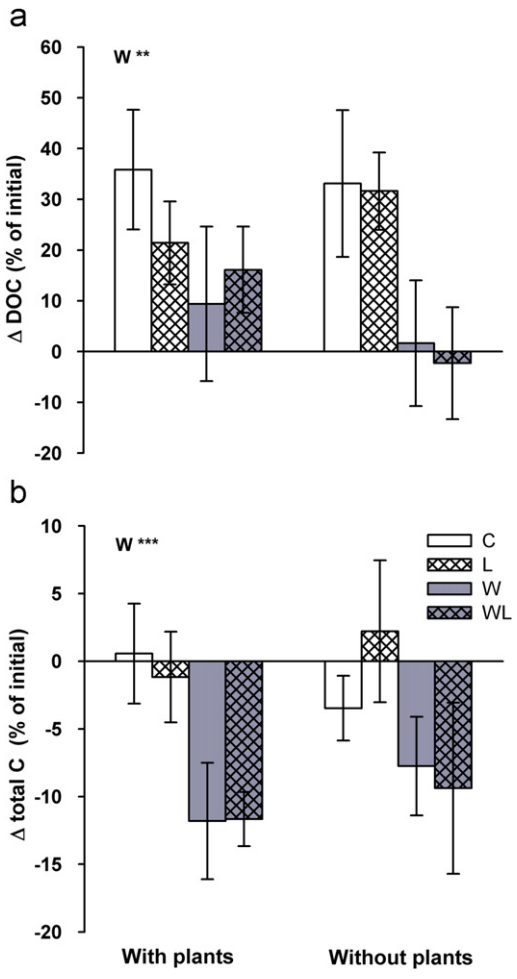


Fig. 2. Changes in the dissolved organic carbon (a) and total C (b) pools during a growing-season-long incubation in the warming and litter addition experiment. Bars represent means (±SE) of six replicates. $C$ = control, $L$ = litter addition, $W$ = warming, $WL$ = warming and litter addition. Cores were incubated with or without *C. capillaris* plants. Significant treatment effects are shown at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (Linear Mixed Model with warming ($W$) and litter addition ($L$) as fixed and block as a random factor).

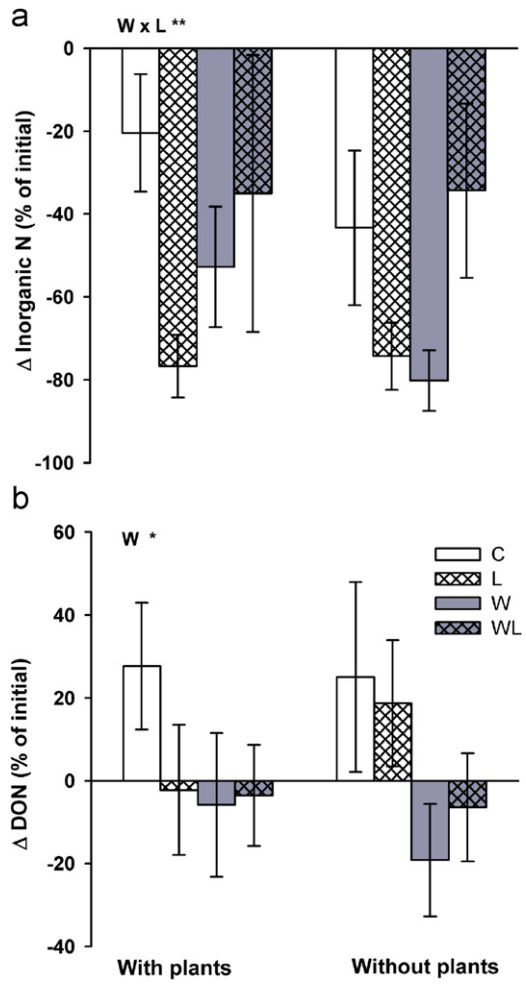


Fig. 3. Changes in inorganic N (a) and dissolved organic N (b) pools during a growing-season-long incubation in the warming and litter addition experiment. Symbols, treatments and statistics as in Fig. 2.

warming treatment compared with the control ($P<0.05$ for the interaction, data not presented). The presence of plants slightly reduced the decrease in the $C_{mic}$-to-$N_{mic}$ ratio ($P = 0.083$). The ratio of microbial C-to-P remained approximately constant during the incubation (data not presented).

### 3.2. Laboratory assay to determine limiting factors

#### 3.2.1. Bacterial activity in soil

Bacterial activity measured as incorporation of leucine and thymidine into macromolecules was about 65% lower at the 5–10 cm depth compared with the uppermost 5 cm in the soil profile in the untreated soil (Fig. 4).

Bacterial activity increased pronouncedly in the surface soil when warming was combined with litter addition, and less for each treatment alone (Fig. 4). For thymidine incorporation the warming effect was a non-significant tendency ($P = 0.110$), while it was statistically significant for leucine incorporation. The litter effect was a nearly significant tendency ($P = 0.081$ for thymidine and $P = 0.057$ for leucine incorporation).

#### 3.2.2. Factors limiting bacterial activity

Because the response patterns measured by thymidine and leucine incorporation techniques were similar, only results for thymidine incorporation are shown.

The PCA on the relative thymidine incorporation data shows the response patterns of the different field treatments to the factorial substrate amendments (Fig. 5a). The first PC, which explained 47.7% of the variance in data, accounted for the general responsiveness to all substrate amendments, which was strongest in the litter addition treatment (Fig. 5). The second PC (explained variance 14.5%) separated the control from the warming treatment (Fig. 5a; $P = 0.06$ for main effect of warming). Adding N had a small effect on the warmed soil in comparison with the control, which was located in the same direction as the N additions on the PC 2 (Fig. 5a). This indicates that

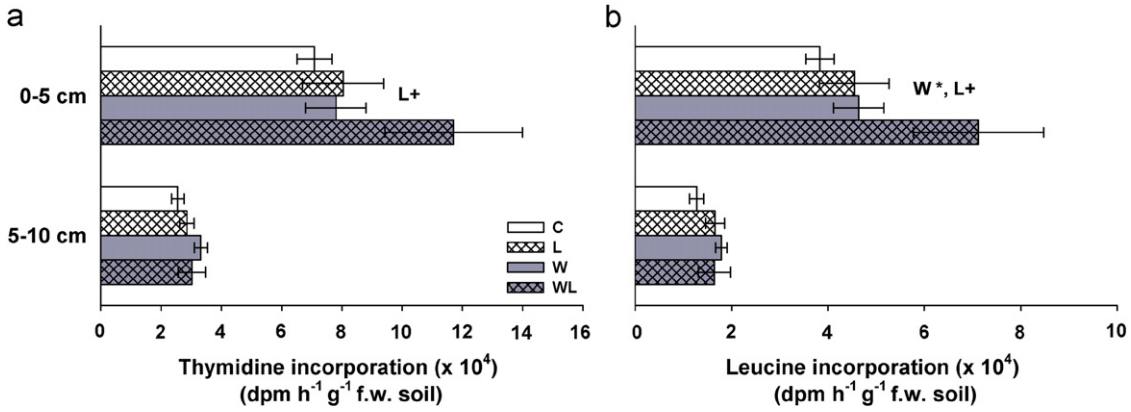


Fig. 4. Effects of warming and litter addition on soil bacterial activity measured as (a) thymidine and (b) leucine incorporation. The samples were from 0 to 5 cm and 5 to 10 cm soil depths. Symbols and treatments as in Fig. 2. Treatment effects are shown at $^{+}P<0.1$, $^{*}P<0.05$ (Linear Mixed Model with warming (W) and litter addition (L) as fixed and block as a random factor).

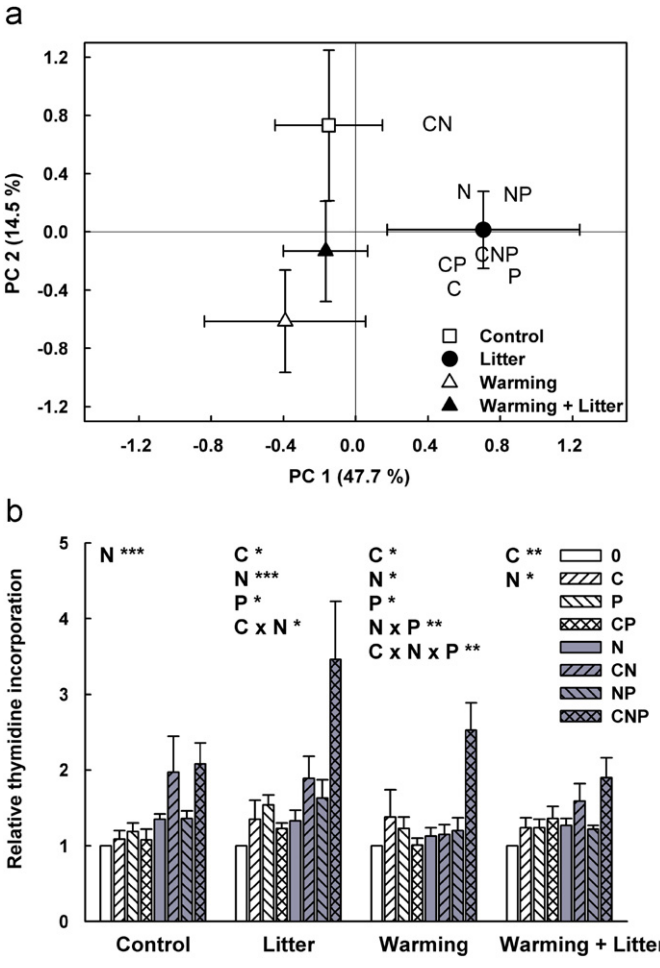


Fig. 5. (a) Principal component analysis biplot of the relative bacterial activity in response to factorial amendments of C, N and P and (b) relative bacterial activity as thymidine incorporation in control, warming, litter addition and warming + litter addition treatments. Activity of the samples without amendments (symbol 0 in B) was set to one. Bars/data points are means (±SE) of six replicates. Significant C, N and P amendment effects and interaction effects are shown at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (Linear Mixed Model separately for each field treatment followed by Bonferroni correction).

warming had switched the soil bacteria from being N-limited towards limitation by other substrates.

The actual responses to the substrate amendments in the field treatments are presented in Fig. 5b. The addition of individual substrates caused at maximum a 1.5-fold increase in bacterial activity, whereas the combination of all three substrates elicited a 2–3.5-fold increase. In the non-manipulated control plots, bacterial activity was significantly increased by N addition only (Fig. 5b, $P<0.001$ for main effect of N) showing that nitrogen was originally limiting bacterial growth in this heath soil. Addition of N and C in combination doubled the bacterial activity compared with addition of N alone, while adding P had no effect. This indicates that C was a second limiting substance when the N limitation was alleviated. In the plots that had received additional litter, bacterial activity significantly increased in response to all individual substrate amendments (Fig. 5b). However, N appeared to have the largest statistical effect ($P<0.001$ compared with $P<0.05$ for C and P). In the warmed plots, the responses to substrate additions were more complex, as demonstrated by the significant $C \times N \times P$ interaction term (Fig. 5b, $P<0.01$). Bacterial activity in the soil from the warming plus litter addition treatment only responded to C and N amendments, both alone and in combination ($P<0.01$ for main effect of C and $P<0.05$ for main effect of N).

## 4. Discussion

Realistic warming of ecosystem plots over six growing seasons had no effects on soil chemical and microbial characteristics when these were measured in June, after a long winter season without temperature manipulations. The lack of significant warming effects on microbial biomass is largely in agreement with previous results from a slightly drier nearby heath exposed to a similar period of warming (Jonasson et al., 1999) as well as with results from wet sedge and mesic tussock tundra in Alaska (Schmidt et al., 2002). A warming effect may, however, be masked by

a top-down regulation of the microbial biomass by consumers such as nematodes (Ruess et al., 1999). Ruess et al. (1999) observed that warming doubled the population density of nematodes at the same time as microbial biomass was unaffected.

As hypothesized, carbon loss from the soil was significantly increased by warming during the growing-season-long field incubation of soil cores. The C loss per unit area during incubation was equivalent to 23 g C $m^{-2}$ in the non-warmed soil and to 229 g C $m^{-2}$ in the warmed soil. This is 50% higher than the ecosystem respiration C loss from a subarctic heath under experimental warming as estimated by *in situ* $CO_2$ flux measurements (Illeris et al., 2004), and similar to the growing season C loss with ecosystem respiration from the warmed plots in the present experiment (Michelsen et al., unpublished). The high C loss may owe to the significantly higher bacterial growth rate in the warmed soil, although the growth rate was especially induced when warming was combined with litter addition. A study based on the isotopic signature of the respired $CO_2$ has shown that arctic soil microorganisms were able to use a higher proportion of recalcitrant carbon under 24 °C than under 12 or 2 °C (Biasi et al., 2005), which supports our suggestion of the stimulated carbon use by the more active microbial communities under warming. In our plots, the ecosystem respiration was twice as high in the warmed plots as in the controls across the growing season, while litter addition did not affect the ecosystem respiration (Michelsen et al., unpublished).

Due to the high SOM content of the soil, the higher C loss from the warmed soil during the growing season could not be seen as changes in the proportion of SOM or bulk density measured in the spring, after a winter season without warming manipulation. Moreover, a likely increased C input to the warmed field plots from the increased aboveground plant cover (Michelsen et al., unpublished) has at least partly compensated for the C loss from the soil outside the cores. Also, the impact of warming on the soil C may have been intensified by the incubation setup, in which the soil in the plastic containers may have had slightly higher temperature than soil outside of these containers. The extra warming effects by the containers may have been especially pronounced in the warmed plots, where the plastic tents reduced cooling by wind of the soil surface. Therefore, caution is needed in interpreting these results.

The higher bacterial growth rate in the warmed soil is not due to a direct stimulating effect of elevated temperature, since the measurements were made at one standard temperature. Also, it is unlikely due to an increase in carbon supply from roots, in that we detected no significant increase of fine root mass in response to warming. A slight but sometimes statistically non-significant increase in root biomass in response to warming (Hobbie and Chapin, 1998; Jonasson et al., 1999; Rinnan et al., 2007) is likely to be a specific response in arctic areas where temperature constrains plant production. In temperate and boreal forests, higher soil temperatures have instead been observed to increase root mortality (Hyvönen et al., 2007).

DOC concentration did not increase during the incubation in the warmed plots as it did in the plots from ambient temperature. This was possibly due to enhanced microbial activity and labile C consumption in the warmed soil. Enhanced temperature also decreased DOC production in a mesocosm experiment with arctic heath soil (Jonasson et al., 2004).

Contrary to our expectation, litter addition did not lead to significantly higher SOM content nor did it induce a priming effect resulting in enhanced C loss. However, litter appeared to be a source of nutrients to the soil. The measurements in the beginning of the field incubation experiment showed that 6 years of litter addition had brought extra N (now as DON) and P (as microbial P and total soil P) to the soil.

Litter addition tended to increase P mineralization, shown by the higher phosphate accumulation in the litter treatment during the field incubation. However, the inorganic N pool decreased more in the warmed and litter addition plots than in the control. This change in the small inorganic N pool was not, however, large enough to be detectable as uptake in the large microbial N pool, and, consequently, it did not lead to a significant change in the microbial N pool. The finding that litter addition reduced net N mineralization and increased P mineralization is in accordance with earlier results from the subarctic (Jonasson et al., 2006). In contrast, net N mineralization was unaffected by litter manipulations in the temperate forests of the USA and central Europe (Holub et al., 2005).

The net growing-season N mineralization rates estimated in this study fall among those commonly observed in arctic ecosystems; the rates are low or even negative (Hart and Gunther, 1989; Giblin et al., 1991; Schmidt et al., 2002). In the cores without plants, the absence of plant roots and the concomitant lack of supply of labile carbon as root exudates and the lack of plant nutrient uptake have been suggested as explanations for the low estimated mineralization rates (Jonasson et al., 2004, 2006).

In this work, the presence of plants increased mobilization of N and the plant uptake kept the P concentration at a low level. Mobilization of P was increased in the control soil without warming or litter addition. While the stimulation of N mobilization by plant transplants is in agreement, effects on transformations of P are in contrast with previous results (Jonasson et al., 2006). This discrepancy may be explained by the use of plant species with different preferences for lime in the habitat. While *Festuca vivipara* used in the experiment of Jonasson et al. (2006) is indifferent, *C. capillaris* chosen for this work is a calcicole species. Calcicole plants are known to exude high amounts of dicarboxylic and tricarboxylic acids that efficiently solubilize phosphate in the soil matrix (Ström, 1997). The idea that plant roots excrete phosphohydrolase in

proportion to their demand for phosphorus (McGill and Cole, 1981) may explain why P mobilization was only increased in the control soil and not where litter addition had provided extra P.

The reduction in microbial C-to-N ratio during the incubation from 14 to 5 may be due to that plants with ericoid and ectomycorrhizal fungi, which dominate these ecosystems, were excluded from the incubations and the extraradical mycelium present in the soil was degraded during the experiment, as also found in forest soil incubations (Bååth et al., 2004). The C-to-N ratio of ectomycorrhizal mycelium in tundra ecosystems is 15–19 (Clemmensen et al., 2006). The $C_{mic}$-to-$N_{mic}$ ratio outside of the incubation containers at the end of August was approximately 8.5 (Rinnan et al., unpublished), which also indicates a seasonal trend.

Bacterial growth rate was little affected by addition of potentially limiting substrates singly, compared with substrate combinations. This is in contrast with many other soils in which C addition alone often leads to at least two times higher growth rates (Aldén et al., 2001). It appears that arctic soil bacteria are close to being limited by several nutrients simultaneously. This was also earlier found by Demoling et al. (unpublished) using the same methodology with soil from an adjacent site at Abisko and by Sørensen et al. (2006) using a respiration based technique with soil from High Arctic Greenland. Despite the small effects of single substrate additions, our results confirm the earlier suggestion that arctic soil would differ from soils in most other ecosystems with respect to the main substrate limitation of bacterial growth. While soil microbes, for example in boreal forests are considered C limited (Aldén et al., 2001; Ekblad and Nordgren, 2002; Schröter et al., 2003), arctic soil microbes appear to be more N than C limited (Nordin et al., 2004; Demoling et al., unpublished). In the present work, only N addition elicited a statistically significant increase in bacterial growth of the unmanipulated control soil.

Warming and litter addition slightly altered the substrate limitation patterns of microbial growth so that N-limitation shifted towards C and to a lesser extent P limitation. The increased C limitation in the warmed plots may have contributed to the higher C loss from these plots. Since the extent of altered nutrient limitation could be of the utmost importance for the carbon balance in arctic soils, more studies on this aspect are needed.

Our work has shown that climatic warming and the higher litter fall from the increasing abundance of deciduous plant species (Sturm et al., 2001; Stow et al., 2004) is likely to increase bacterial growth rate in soil, which may threaten the large C stock of arctic soils. Although we were unable to show clear response patterns in the microbial nutrient transformation processes estimated by the buried bag technique (Eno, 1960), it appears that microbial activity is one of the central drivers determining the carbon balance of arctic soils under climate change.

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