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Fungi Benefit from Two Decades of Increased Nutrient Availability in Tundra Heath Soil

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Abstract

If microbial degradation of carbon substrates in arctic soil is stimulated by climatic warming, this would be a significant positive feedback on global change. With data from a climate change experiment in Northern Sweden we show that warming and enhanced soil nutrient availability, which is a predicted long-term consequence of climatic warming and mimicked by fertilization, both increase soil microbial biomass. However, while fertilization increased the relative abundance of fungi, warming caused only a minimal shift in the microbial community composition based on the phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) profiles. The function of the microbial community was also differently affected, as indicated by stable isotope probing of PLFA and NLFA. We demonstrate that two decades of fertilization have favored fungi relative to bacteria, and increased the turnover of complex organic compounds such as vanillin, while warming has had no such effects. Furthermore, the NLFA-to-PLFA ratio for 13C-incorporation from acetate increased in warmed plots but not in fertilized ones. Thus, fertilization cannot be used as a proxy for effects on warming in arctic tundra soils. Furthermore, the different functional responses suggest that the biomass increase found in both fertilized and warmed plots was mediated via different mechanisms.

Introduction

Microbial use of carbon substrates in soil is of critical importance when estimating impacts of climate change on the fate of soil carbon. Especially in the Arctic, where the amount of soil carbon exceeds the amount of carbon in aboveground plant biomass by an order of magnitude [1], microbial processes transforming the carbon compounds are crucial. In this region, climate change is predicted to increase the mean annual temperature by 2–9°C within this century [2].

Warming generally stimulates microbial growth [3] and thereby increases nutrient mineralization [4] in soil. As enhanced nutrient availability thus is an expected long-term consequence of warming, fertilization is often used as a long-term proxy for warming in experimental studies [5,6]. Both warming and enhanced nutrient availability affect turnover of soil carbon, directly through effects on microbial activity [3] and indirectly by increasing plant growth [7] and altering vegetation composition [8,9].

Changes in plant growth and vegetation composition influence carbon inputs to soil in several ways. In the short-term, the quantity and the chemical quality of root exudates may change as these differ among plant species [10]. Also, the distribution of these fresh carbon inputs in the soil horizon may change, as different plant groups have different rooting depths [6,11]. In the long-term, vegetation changes will influence litter input. For example, an increase in deciduous shrubs [12] would lead to higher leaf litter deposition each autumn, while an increase in graminoids [8] would add easily decomposable plant litter to soil [13]. Climate change may also alter the chemical composition of a plant, often increasing the concentration of secondary metabolites in the plant tissue [14].

We aimed to unravel how the composition of microbial communities and their use of carbon substrates in subarctic tundra soil respond to long-term warming and enhanced nutrient availability using SIP-PLFA (stable isotope probing of phospholipid fatty acids). Soil samples were taken from a field experiment which had been running for 18 years prior to the sampling on a mesic/dry heath just above the tree line near Abisko Scientific Research Station in Northern Sweden. The warming treatment, which was accomplished by open-top chambers, initially resulted in an increased biomass of deciduous shrubs [15], decreased abundance of bryophytes [8], and reduced soil microbial biomass [5]. After a decade, warming had thus reduced the frequency of mosses by 50% and that of lichens by 33%, but there was no effect on graminoids [8]. The NPK (nitrogen, phosphorous and potassium) fertilization treatment, which aimed at mimicking increased soil nutrient availability, had also increased vascular plant biomass at the expense of bryophyte biomass [15], but increased soil microbial biomass including fungal biomass [3]. After 14 years, both warming and fertilization had increased the biomass of deciduous shrubs, but the increase in graminoids was
still confined to the fertilized plots [15]. After 22 years, both warming and fertilization treatments had 35% higher vascular plant cover than the control [16].

In the laboratory, the soil samples were amended with a range of 13C-labelled substrates, and the uptake of these substrates into various fatty acids indicating different microbial groups and either growth (PLFAs) or storage (neutral lipid fatty acids, NLFAs) was followed. The selected substrates represented carbon sources present in soil. Glucose, acetic acid and glycine are simple compounds common in plant root exudates, and glycine is also a source of nitrogen for subarctic plants and microbes [11]. Starch is a very common polysaccharide in plant residues. Vanillin is a common product of lignin depolymerisation [17], containing a phenol ring, and is often used as a model substance indicating lignin degradation. Starch and vanillin are therefore examples of more complex substrates and supposedly more difficult to decompose.

We expected that, in line with the previous results from the field experiment [5], fertilization would increase and warming decrease microbial biomass. We hypothesized that fertilization would increase the uptake of carbon substrates into PLFA as compared to NLFA due to alleviated nutrient limitation. Furthermore, we expected that vanillin uptake would decrease with increased N availability, since it has earlier been shown that fertilization can suppress ligninolytic enzymes in forest soil [18–20]. Warming was expected to have the same effects as fertilization if this treatment would lead to increased nutrient availability.

**Results**

**Treatment Effects on Fatty Acid Concentrations**

Substrate additions did not cause any significant changes in the total PLFA or NLFA concentration, and therefore an average of each substrate is presented (Fig. 1). Fertilization and warming significantly increased the total PLFA concentration in soil by about 31% and 20%, respectively (Fig. 1; P<0.001), which indicates that these treatments increased microbial biomass. The total NLFA concentration was only increased by fertilization (P<0.001), although less when fertilization was combined with warming (Fig. 1; P<0.01 for warming×fertilization interaction). There were no significant differences between the two incubation times.

In the PCA of the PLFA profiles, the first PC (explained variance 32%) described treatment effects on the PLFA patterns (Fig. 2). The control soil had the highest values along this PC axis, followed by partly overlapping warming and warming + fertilization treatments close to the origin, and the fertilized soil had the lowest values (Fig. 2a; P<0.001 for effects of both warming and fertilization). The warming×fertilization interaction term was highly significant (P<0.001) because of the relatively similar PLFA patterns in the warming + fertilization treatments as compared to fertilization alone. The fertilized soil was characterized by relatively higher amounts of the fungal biomarker 18:2ω6ω9 than the unfertilized soil (Fig. 2b). The second PC (explained variance 15%) mainly accounted for the difference between the incubation times (Fig. 2a; P<0.001); the relative abundance of the straight-chained PLFAs 14:0, 15:0 and 16:0, and the markers for Gram-positive bacteria i14:0, i15:0, a15:0 and i16:0 increased from one to seven-day samples (Fig. 2b). The fungal biomarker 18:2ω6ω9 was not affected by the incubation time.

The PCA of the NLFA mole percentage profiles showed that while the 1-day incubation time produced relatively similar patterns except for the fertilization treatment, there was a large spread in the patterns for the 7-day-long incubation (Fig. 3a). As for PLFA, the first PC (explained variance 38%) accounted for the difference between the fertilized and the unfertilized soils, and showed a warming×fertilization interaction (Fig. 3a; P<0.001). As for PLFAs, the difference was mainly due to a higher abundance of the fungal markers, in this case the NLFA 18:2ω6ω9 and 18:1ω7ω9 (Fig. 3b). The second PC mainly separated the incubation times from each other (Fig. 3a; P<0.001).

**Treatment Effects on 13C-incorporation into PLFA**

There were no significant effects of treatments or incubation time on total 13C-incorporation from glucose, acetic acid, glycine (Table 1). Fertilization increased total 13C-incorporation from vanillin (P<0.001), but did not significantly affect incorporation from starch. The uptake of starch and vanillin increased from one to seven-day-long incubation (Table 1; P<0.01).

The PCAs for the 13C-incorporation patterns showed that for all substrate additions, the first two PCs separated the fertilized soil from the unfertilized soil (Fig. 4; P<0.001). A common explanation for this difference was an increased incorporation of 13C into the fungal biomarker PLFAs, 18:2ω6ω9 and 18:1ω7ω9 and a decreased incorporation into most of the bacterial biomarker PLFAs under fertilization (see Figs S1, S2, S3, S4, S5, S6, S8). As an exception, the Gram-negative bacteria indicated by cy17:0 and cy19:0 took up significantly more glycine, starch and vanillin in the fertilized than in the unfertilized soil (Fig. S3). In addition, utilization of starch was higher in the fertilized than in the unfertilized soil for Gram-positive bacteria containing i16:0 and the actinomycetes indicated by the methylated PLFA 10Me16:0, especially after incubation for seven days (Figs S6 and S7).

The substrate utilization patterns in the warming treatment were rather similar to those in the control samples, except for starch addition with a significant warming effect on the second PC (Fig. 4, P<0.01). The incorporation of 13C from starch into the PLFAs i16:0 and 10Me16:0 was higher in the warming treatment than in the control soil after incubation for seven days (Figs S6 and S7).
S8). Uptake of glucose by organisms containing the PLFA 18:1<sub>v</sub>9 was significantly reduced by warming (Fig. S2). The warming→fertilization treatment had relatively similar substrate utilization patterns as fertilization alone (Fig. 4), which led to warming→fertilization interactions in the incorporation to some individual PLFAs (Figs S1, S2, S3, S4, S5, S6, S7, S8).

A clear difference in the 13C-incorporation patterns between the two incubation times could be observed on the first two PCs for all substrates (Fig. 4; P<0.001). This owed mainly to the increased incorporation into the PLFAs cy17:0, cy19:0, 10Me16:0 and 10Me18:0 over time (Figs S3, S7, S8).

**Treatment Effects on 13C-incorporation into NLFA**

The incorporation of 13C to NLFA decreased over time for glucose, acetic acid, and glycine (Table 1; P<0.01). Fertilization increased the total incorporation of 13C from vanillin (P<0.01) but did not significantly affect incorporation from other substrates.

Incorporation of 13C to the fungal biomarker NLFA 18:2<sub>v</sub>6,9 and 18:1<sub>v</sub>9, was highest from acetic acid, followed by glucose and...
vanillin (Fig. 5a, b). Incorporation of $^{13}$C to these NLFAs from glycine and starch was minimal. The response patterns for 18:2\(\omega 6,9\) and 18:1\(\omega 9\) were similar, but because of the higher variance, only vanillin-amended samples showed significant differences at $P<0.01$ for 18:2\(\omega 6,9\); fertilization increased ($P<0.001$) and warming decreased ($P<0.01$) 13C-incorporation from vanillin (Fig. 5a). Similar responses were observed for 13C-incorporation from vanillin into the NLFA 18:1\(\omega 9\) (Fig. 5b; $P<0.001$ for both warming and fertilization). Fertilization also significantly increased 13C-incorporation from glucose, when not combined with warming treatment ($P<0.01$ for warming\times fertilization).

The NLFA-to-PLFA ratios for 13C-incorporation into 18:2\(\omega 6,9\) and 18:1\(\omega 9\) were not significantly affected by treatments for other substrates than acetic acid (data for glucose and acetic acid shown in Table 1). For acetic acid, the ratio for incorporation into 18:2\(\omega 6,9\) was significantly increased by warming after incubation for 7 days (with a similar insignificant trend for 18:1\(\omega 9\)), while it was unaffected by fertilization (Table 1).

Figure 3. Principal component analysis of the NLFA mole percentage profiles in tundra heath soil from a field experiment with long-term fertilization and warming treatments. (a) A score plot showing mean values for substrate additions after a 1-day-long incubation (small symbols drawn with black line) and after a 7-day-long incubation (large symbols drawn with grey line). The mean values for all substrates within each treatment (glucose, glycine, acetic acid, starch and vanillin) are marked with similar symbols since the substrate additions did not affect the NLFA pattern. (b) A loading plot showing the individual NLFAs. Variance explained by principal component (PC) 1 and 2 in parentheses. $P<0.001$ for effects of Fertilization, Time and Warming\times Fertilization on the PC scores (Linear Mixed Models with Warming, Fertilization and Time as fixed factors and Block as a random factor).

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**Table 1.** Total $^{13}$C-incorporation into phospholipid (PLFA) and neutral lipid fatty acids (NLFA) and NLFA/PLFA ratios for $^{13}$C-incorporation into fungal biomarkers (mean±SE, n=6) in tundra heath soil following incubation with added glucose, glycine, acetic acid, starch or vanillin for 1 or 7 days.

<table>
<thead>
<tr>
<th></th>
<th>1-day incubation</th>
<th>7-day incubation</th>
<th>Statistical significance</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>F</td>
<td>W</td>
</tr>
<tr>
<td>Total $^{13}$C-incorporation into PLFA (µg $^{13}$C per mg added $^{13}$C g⁻¹ soil)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1.48±0.13</td>
<td>1.35±0.06</td>
<td>1.19±0.12</td>
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<tr>
<td>Glycine</td>
<td>0.92±0.11</td>
<td>0.85±0.06</td>
<td>0.85±0.12</td>
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<td>Acetic acid</td>
<td>2.38±0.24</td>
<td>2.50±0.11</td>
<td>2.20±0.08</td>
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<tr>
<td>Starch</td>
<td>0.40±0.06</td>
<td>0.33±0.06</td>
<td>0.28±0.03</td>
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<tr>
<td>Vanillin</td>
<td>0.32±0.03</td>
<td>0.41±0.01</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>Total $^{13}$C-incorporation into NLFA (µg $^{13}$C per mg added $^{13}$C g⁻¹ soil)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>0.51±0.06</td>
<td>0.70±0.08</td>
<td>0.51±0.04</td>
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<tr>
<td>Glycine</td>
<td>0.15±0.02</td>
<td>0.14±0.02</td>
<td>0.23±0.05</td>
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<tr>
<td>Acetic acid</td>
<td>1.27±0.11</td>
<td>1.45±0.13</td>
<td>1.51±0.12</td>
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<td>Starch</td>
<td>0.06±0.01</td>
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<td>Vanillin</td>
<td>0.05±0.01</td>
<td>0.12±0.01</td>
<td>0.10±0.04</td>
</tr>
<tr>
<td>NLFA/PLFA for $^{13}$C-incorporation from glucose to 18:2ω6,9</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>18:2ω6,9</td>
<td>0.09±0.01</td>
<td>0.15±0.03</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>1.09±0.16</td>
<td>1.78±0.15</td>
<td>1.65±0.25</td>
</tr>
<tr>
<td>Acetic acid to 18:2ω6,9</td>
<td>0.16±0.01</td>
<td>0.12±0.02</td>
<td>0.15±0.02</td>
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<tr>
<td>Acetic acid to 18:1ω9</td>
<td>1.65±0.23</td>
<td>1.53±0.20</td>
<td>2.21±0.42</td>
</tr>
</tbody>
</table>

*C*, control; F, fertilization; W, warming; W+F, warming+fertilization.

Significant effects of factors warming (W), fertilizer addition (F), time (T) and their interactions are shown at **P<0.01, ***P<0.001.

**Figure 4.** Principal component analysis (PCA) of the relative $^{13}$C-incorporation of added $^{13}$C-labelled glucose, glycine, acetic acid, starch and vanillin into phospholipid fatty acids (PLFAs) of tundra heath soil. Scores (mean ± SE) of the principal components (PC) 1 and 2 showing effects of warming and fertilization, and differences between the incubation times (1day, small symbols drawn with black line; 7 days, large symbols drawn with grey line). The PCAs were run separately for each substrate. The explained variances were 33–46% for PC1 and 18–27% for PC2. For glucose, glycine, acetic acid and vanillin P<0.001 for Fertilization and Time effect on the PC scores (Linear Mixed Models with Warming, Fertilization and Time as fixed factors and Block as a random factor). For starch P<0.01 for Warming, P<0.001 for Fertilization and Time, and P<0.01 for Warming×Fertilization.

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Discussion

Fertilization of the subarctic heath for 18 years had increased the total PLFA concentration in soil by 31%, which indicates increased microbial biomass. This increase is in agreement with the results after 15 treatment years, when fertilization also had increased the total PLFA concentration, however only by 16% [5]. In contrast to the reduction of total PLFA by the warming treatment after 15 years [5], warming for the additional three years up to the sampling for the present study had led to a significantly increased total PLFA concentration. This implies that the total microbial biomass responses in the warming treatment now, for the first time, resemble those in the fertilization treatment [21]. The result probably reflects the long-term increase in the total cover of vascular vegetation. After 22 years, both warming and fertilization treatments had 35% higher vascular plant cover than the control [16], in contrast to previous measurements after 5 and 10 years, with varying responses in the two treatments [22]. Increased primary production typically leads to an increase in soil microbial biomass [23], thanks to enhanced substrate inputs.

Based on a PCA of the PLFA profiles, the microbial community composition in the fertilized soil was characterized by a significantly higher abundance of the fungal biomarker, 18:2ω6,9, as compared to the unfertilized soil, which was similar to the results after 15 treatment years [5]. The fertilization-induced increase in fungi was further evidenced by the significantly higher total concentration of NLFAs, which are mainly found in eukaryotic organisms and thus are indicative of...
fertilization for vanillin (both PLFA and NLFA) and starch (only NLFA). Allocation to fungal energy communities was mainly affected by fertilization, while warming as also suggested by [34]. Thus, although starch degradation is fairly common among microorganisms [35], a different subset of microorganisms appears to degrade starch as compared to the other studied substrates. Also, in contrast to the other substrates used in this study, utilization of starch was significantly affected by both fertilization and warming. More specifically, the incorporation of 13C from starch into indicators of Gram-positive bacteria (16:0) and actinomycetes (10Me16:0 for fertilization and 10Me18:0 for warming) increased by the treatments. It appears that the starch-degrading microorganisms were favored by the changes that had occurred both in the fertilized and warmed plots, possibly by the increase in deciduous shrubs and the concomitant increase in leaf litter [8,15].

Increased biomass and activity of soil fungi relative to those of bacteria in response to enhanced nutrient availability may lead to altered ecosystem functioning. Most importantly, an increased importance of fungi relative to bacteria has been suggested to result in an increased carbon sequestration in soil [36], but see also discussion by [37]). Because of the enormous size of the arctic soil carbon pool, even tiny changes in soil carbon processing have a potential to feed back on climate change. Effects of climate change on microbial use of carbon substrates would influence soil carbon storage both directly, as altered efflux of carbon from the soil, and indirectly, via priming effects (for a review, see [38]) that could lead to altered degradation of older carbon.

Several of our results indicate that – at least in the 18-year time horizon – warming does not result in similar effects as experimentally increased soil nutrient availability. Thus, climate warming will not increase carbon sequestration in tundra heaths via beneficial effects on soil fungi to the same degree as fertilization unless soil nutrient availability increases more than the increase in moderately warmer soil. As the N deposition rates in the region north of 60° N are low [39], a pronounced increase in nutrient availability via atmospheric deposition is, however, unlikely.
Materials and Methods

Experimental Design and Soil Sampling
A long-term climate change simulation experiment has been maintained on a tree-line heath at Abisko, northern Sweden (68°21′N, 18°49′E; 450 m a.s.l.), since 1989. The vegetation is dominated by ericoid dwarf shrubs (mainly *Cassiope tetragona* (L.) D. Don) and mosses, with deciduous shrubs and graminoids as subdominants. The experiment hosts various treatments (originally described in [21]) of which 1) warming, 2) fertilization, 3) the combination of warming and fertilization and 4) unmanipulated control plots were selected for the present study. For the warming treatment, 3–4°C air and 1–2°C soil temperature (4 cm depth; [40,41]) increases were achieved with the help of dome-shaped open-top plastic greenhouses, that were erected on the plots for the growing season from early June to late August. The fertilization treatment simulated enhanced nutrient availability and it was maintained by annual additions of 10 g m⁻² N, 2.6 g m⁻² P and 9 g m⁻² K as NH₄NO₃, KH₂PO₄ and KCl (except 1989, when half of this amount was applied, and 1993 and 1998, when no fertilization was performed). Each of the plots covered a 1.2×1.2 m area and was randomly distributed in six blocks, i.e. there were six replicate plots of each treatment. No specific permits were required for the described field studies, as the location is not privately-owned and not protected and the field studies did not involve endangered or protected species.

Three soil cores were taken from each field plot on August 24, 2006, i.e. after 18 years of treatments. The 5-cm-deep cores with a diameter of 4 cm were mixed into a single sample per treatment plot (total volume of 65 cm³), while removing roots and stones by hand sorting. The soil has a c. 15 cm deep organic layer with a pH of 7.1, which has not been affected by the treatments [41] and a soil organic matter content of 89% in the unfertilized and 92% in the fertilized soil in the top 5 cm [5]. The organic layer rests on rocky mineral soil without permafrost.

Incubation with ¹³C-labelled Substrates

Uptake of label from ¹³C-labelled substrates into fatty acids was determined by SIP-PLFA as described by [34]. Five different substrates were used: universally ¹³C-labelled glucose (99%), glycine (99%) and starch (98%), ring-labelled vanillin (99%) and glycolipids (phospholipids, the PLFA fraction). The NLFA 18:1 *v* and 18:2 *v* is mainly of fungal origin (see discussion in [47]), and the NLFA 18:1 *v* is of fungal origin, and often found to increase under excess of carbon [24].

Fatty Acid Analysis

Fatty acids were extracted from the freeze-dried soil following a modified Bligh and Dyer method according to [42] as described in [34]. Shortly, lipids were extracted and fractionated on silicic acid columns (Bond Elut, Varian Inc., Palo Alto, CA, USA) with chloroform (neutral lipids, the NLFA fraction), acetone (glycolipids) and methanol (phospholipids, the PLFA fraction). The NLFA and PLFA fractions were subjected to mild alkaline methanolysis, and the resulting fatty acid methyl esters were analyzed on a Hewlett-Packard 6890 gas chromatograph equipped with a 30-m HP5 capillary column (Hewlett-Packard, Palo Alto, CA, USA) and helium as the carrier gas. The GC was interfaced with a Europa 20/20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) used for determination of the ¹³C values [43]. Methyl nonadecanoate fatty acid (19:0) was used as an internal standard.

Isotope data was calculated following Böckstiegel [44]. The ¹³C values of each lipid were corrected for the methyl group added during methanolysis [45]. For each PLFA and NLFA, the absolute amount of ¹³C incorporated was calculated by relating the increase in the fraction ¹³C after labeling \[F = R/(R + R' + 1)\], where R is a ¹³C/¹²C-ratio to the fatty acid concentration and the amount of ¹³C in the added substrate.

The fatty acids are presented as the total number of carbon atoms followed by a colon and the number of double bonds. The prefixes a and i signify anteiso- and isobranching, respectively. The prefix cy indicates cyclopropyl fatty acids, while 10Me is a methyl group on the 10th carbon atom from the carboxyl end of the molecule. Terminally and mid-chain branched fatty acids (i15:0, a15:0, i16:0, 10Me16:0, i17:0, a17:0, 10Me17:0, 10Me18:0), cyclopropyl saturated (cy-17:0 and cy-19:0) and some monounsaturated (16:1o7 and 18:1o7) fatty acids were considered indicative of bacteria [46]. The fatty acid 18:2o6,9 was considered to represent fungi [46]. The PLFA 18:1o9 is mainly of fungal origin and the NLFA 18:1o9 is of fungal origin, and often found to increase under excess of carbon [24].

Statistical Analysis

The fatty acid concentrations and the total ¹³C-incorporation to fatty acids were analyzed for treatment effects by Linear Mixed Models of SPSS 14.0 for Windows with warming, fertilization, substrate and incubation time as fixed factors. The block variable from the field experiment was used as a random factor. As the differences between the ¹³C-labelled substrates were not of primary interest in the present study and have been described before [34], the analysis was run separately for each substrate. To reduce the chance for type I errors, the alpha-level of 0.01 was used to indicate statistical significance.

The unit-variance scaled and centered fatty acid mole percentage profiles and the patterns of ¹³C-incorporation were subjected to principal component analyses (PCA) using Simca-P 11.0 (Umetrics, Umeå, Sweden). The extracted principal components (PC) were analyzed for treatment effects using Linear Mixed Models in a similar manner as described above.

Supporting Information

Figure S1 Incorporation of ¹³C from glucose, glycine, acetic acid, starch and vanillin into the PLFA 18:2o6,9.

Tundra heath soils from a field experiment with long-term warming and fertilization treatments were incubated for 1 and 7 days. The bars represent mean±SE. Note different y-axis scales.

Significant effects of factors warming (W), fertilizer addition (F), time (T) and their interactions for each substrate are shown at **P<0.01**, ***P<0.001 (Linear Mixed Models with Warming, Fertilization and Time as fixed factors and Block as a random factor). (TIF)

Figure S2 Incorporation of ¹³C from glucose, glycine, acetic acid, starch and vanillin into the PLFA 18:1o9.

Tundra heath soils from a field experiment with long-term warming and fertilization treatments were incubated 1 and 7 days. The bars represent mean±SE. Note different y-axis scales.
Significant effects of factors warming (W), fertilizer addition (F), time (T) and their interactions for each substrate are shown at *P<0.01, **P<0.001 (Linear Mixed Models with Warming, Fertilization and Time as fixed factors and Block as a random factor).

(TIF)

Figure S3 Incorporation of $^{13}$C from glucose, glycine, acetic acid, starch and vanillin into the PLFA cy19:0.

Tundra heath soils from a field experiment with long-term warming and fertilization treatments were incubated 1 and 7 days. The bars represent mean±SE. Note different y-axis scales. Significant effects of factors warming (W), fertilizer addition (F), time (T) and their interactions for each substrate are shown at *P<0.01, **P<0.001 (Linear Mixed Models with Warming, Fertilization and Time as fixed factors and Block as a random factor).

(TIF)

Figure S4 Incorporation of $^{13}$C from glucose, glycine, acetic acid, starch and vanillin into the PLFA 18:1o7.

Tundra heath soils from a field experiment with long-term warming and fertilization treatments were incubated 1 and 7 days. The bars represent mean±SE. Note different y-axis scales. Significant effects of factors warming (W), fertilizer addition (F), time (T) and their interactions for each substrate are shown at *P<0.01, **P<0.001 (Linear Mixed Models with Warming, Fertilization and Time as fixed factors and Block as a random factor).

(TIF)

Figure S5 Incorporation of $^{13}$C from glucose, glycine, acetic acid, starch and vanillin into the PLFA 15:0.

Tundra heath soils from a field experiment with long-term warming and fertilization treatments were incubated 1 and 7 days. The bars represent mean±SE. Note different y-axis scales. Significant effects of factors warming (W), fertilizer addition (F), time (T) and their interactions for each substrate are shown at *P<0.01, **P<0.001 (Linear Mixed Models with Warming, Fertilization and Time as fixed factors and Block as a random factor).

(TIF)

Figure S6 Incorporation of $^{13}$C from glucose, glycine, acetic acid, starch and vanillin into the PLFA 16:0.

Tundra heath soils from a field experiment with long-term warming and fertilization treatments were incubated 1 and 7 days. The bars represent mean±SE. Note different y-axis scales. Significant effects of factors warming (W), fertilizer addition (F), time (T) and their interactions for each substrate are shown at *P<0.01, **P<0.001 (Linear Mixed Models with Warming, Fertilization and Time as fixed factors and Block as a random factor).

(TIF)

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**Author Contributions**

Conceived and designed the experiments: RR AM EB. Performed the experiments: RR. Analyzed the data: RR EB. Wrote the paper: RR AM EB.

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