Supporting Information

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SI Methods

Soil Samples. The materials examined in this investigation were samples of the organic horizons (O-horizons) of a Spodosol, the major type of forest soil in the boreal region, covering 2% of the total global ice-free landmass (1). Samples were collected from a site in the central boreal climate zone of northern Sweden (64°11′ N, 19°35′ E) at the end of October 2008. The site was dominated by spruce (*Picea abies, L.*) with sparse pine (*Pinus sylvestris, L.*), and an understory and bottom vegetation dominated by lingonberry (*Vaccinium vitis idea*), blueberry (*Vaccinium myrtillus*), and feather moss (*Pleurozium schreberi*). The soil type was Typic Haplocryods (1). The investigations were confined to the O-horizon because microbial activity occurs mainly in this horizon of boreal soils, and frost in the boreal region affects mainly the surface layers. The soil samples were pooled into a single composite sample to increase the representativeness. The soil was homogenized by passing through a cutting sieve (6 × 3.5 mm mesh), and coarse debris and fine roots were removed. Then the soil was mixed and pooled again. The soil was divided into subsamples and immediately placed in a freezer (−20 °C), except for samples (n = 3) used for determinations of dry weight (>24 h, 105 °C) and soil organic matter (SOM) content (6 h, 600 °C). These subsamples had an SOM content of 96 ± 0.05% SOM per unit dry weight soil (dw) and a water content of 467 ± 2.78% (weight) water per unit dw.

Soil Incubation. The soil was thawed from −20 °C to *ca.* +4 °C, and undried samples of soil (2.5 g for −9 °C incubation and 5 g for −4 °C, +4 °C, and +9 °C incubations) were transferred to glass bottles (60 mL for −9 °C and 130 mL for -4 °C, +4 °C, and +9 °C incubations). A [^{13}C]glucose solution (0.33 M D-glucose U13C6, 99%; Larodan Fine Chemicals) with nitrogen [$(NH_4)_2SO_4$] and phosphorus (K_2HPO_4) (1, 1/13, 1/18) was prepared and added to the soil samples in amounts corresponding to 40 mg [^{13}C]glucose/g dry SOM (2, 3), and the water content of the soil was adjusted to 550% H_2O/g SOM (4). These incubation conditions were chosen to correspond to reportedly optimal conditions for soil microbial activity (2–4). The glass bottles then were sealed with gas-tight butyl rubber septa, evacuated, and refilled with air to standardize the gas composition, and incubated at the selected temperature (−9 °C, −4 °C, +4 °C, or +9 °C).

Control samples to measure nonbiological [^{13}C]CO_2 production were obtained by sterilizing soil samples with 0.5% NaN_3 (corresponding to ∼7.7 mmol NaN_3/kg soil) before the addition of nutrient solution and incubation (n = 2). Headspace samples of nonbiological CO_2 were taken at the start and end of the incubation period, which varied depending on the incubation temperature (see below). No evidence of anabolic processes was detected after sterilization with NaN_3. Some [^{13}C]CO_2 production was detected, but at insignificant rates in comparison with those in unsterilized samples (corresponding to *ca.* 0.16–0.3% of total [^{13}C]CO_2 produced in the nonsterile soil samples at corresponding temperatures).

Headspace samples also were taken from the unsterilized samples, according to the following schedule. The samples incubated at +9 °C and +4 °C were allowed to equilibrate at the respective temperatures for 1–2 h before extraction of the first gas sample. We incubated these samples for 6 and 10 d, respectively, and headspace gas CO_2 samples were withdrawn from the glass bottles (n = 3 at each sampling) approximately one to three times per day. The samples incubated at −4 °C and −9 °C were allowed to equilibrate to the respective temperatures for 12 h before extraction of the first gas sample to ensure that they had frozen. We incubated these samples for 3.3 mo and 6 mo, respectively, and headspace gas CO_2 samples were taken approximately once a week (−4 °C, n = 3 at each sampling) and once a week to once a month (−9 °C, n = 1–2 at each sampling). Each headspace sample gas was transferred into an N_2-exchanged vial, and its [^{13}C]CO_2 content was analyzed by gas chromatography-isotopic ratio mass spectrometry as described below.

[^{13}C]CO_2 Analysis. The fraction of ^{13}C in CO_2 gas (A) (A = ^{13}C/C, where C = total CO_2 − C) was determined using an isotope ratio mass spectrometer (Model 2020 Analyzer; Europa Scientific Ltd.) interfaced to a sample preparation unit (Cryoprep; SerCon Ltd.). A known amount of standard CO_2 gas at natural ^{13}C abundance (C_{std} and A_{std}, respectively) was added to the sample using a syringe, and this gas mixture subsequently was diluted in helium, which yielded a sample mixture suitable for instrumental analysis in terms of total CO_2 concentration ($C_{mix} = C + C_{std}$) and fraction of ^{13}C (A_{mix}). After analysis, the fraction A was obtained from the mass balance according to: $A_{mix} \times C_{mix} = A \times C + A_{std} \times C_{std}$. The [^{13}C]$CO_2$ concentrations produced from the samples were determined by correcting the total [^{13}C]CO_2 signals for nonbiological [^{13}C]CO_2 derived from the incubations with additions of NaN_3.

^{13}C Magic-Angle Spinning NMR. All NMR spectra were acquired with a 500 spectrometer (Avance DRX; Bruker) operating at 500.13 MHz, 125.77 MHz, and 76.77 MHz for ^{1}H, ^{13}C, and ^{2}H, respectively. The synthesized anabolic compounds, formed after addition of [^{13}C]glucose, were studied using ^{13}C magic-angle spinning (MAS) NMR spectroscopy, as follows. After each CO_2 sampling, the microbial activity in three soil samples was terminated by adding 0.5% NaN_3. The samples then were placed immediately in a freezer (−20 °C) awaiting NMR analysis, for which wet samples (n = 3 for each temperature and sampling time) were packed in 4-mm rotors and spun at 4 kHz in a MAS probe. ^{13}C NMR spectra were obtained with direct polarization and ^{1}H wideband alternating-phase low-power technique for zero-residual splitting (WALTZ) decoupling during acquisition. The acquisition time was 67 ms, followed by a relaxation delay of 2.5 s. The number of scans was 5,200–88,000. The processed spectra were baseline corrected and compensated for number of scans before analysis. The signal of the β anomer of glucose, C1β, was used as an internal reference and set to 96.9 ppm for all spectra (5).

The area of C1β and C1α in the glucose spectra of the sterilized samples was used to set a reference for glucose. The fractions of glucose remaining in each spectrum of the unsterilized samples then were calculated. Spectra of the metabolites formed in the unsterilized samples were obtained by subtracting a glucose spectrum from them. After pure glucose spectra from spectra of the sterilized samples collected at both time 0 and the end of the incubation period were subtracted, no signals from ^{13}C-compounds were detected; that is, no transformation of glucose into other organic compounds was detected in the sterilized samples.

^{13}C-Solution NMR. A sample from the −4 °C incubation was analyzed by ^{13}C-solution NMR to identify a ^{13}C MAS peak at 63.6 ppm. About 0.5 g of soil (from the last sampling at −4 °C) was extracted with a solution of 250 mM NaOH and 50 mM EDTA in a mixture of H_2O and methanol. The sample was sonicated and centrifuged, and then the liquid phase was analyzed by ^{13}C-solution NMR [500 MHz spectrometer, Bruker Avance DRX; sample temperature 25 °C, pulse sequence with buildup of ^{13}C-(^{1}H)-NOE and ^{1}H

decoupling, 10,000 scans, 50° ^{13}C pulse, sweep width 230 ppm, acquisition time 300 ms, recycle delay 2.4 s].

Identification of Compounds Measured by ^{13}C MAS NMR. We defined the total microbial synthesized compounds as the ^{13}C-compounds synthesized from the added [^{13}C]glucose and measurable by NMR. Several references were used to identify the newly synthesized ^{13}C-compounds derived from microbial [^{13}C]glucose transformation (6–10). The chemical shifts of our spectra were compared with the chemical shifts in the references (see above) and identified with respect to chemical shifts, intensities, and coupling patterns. The chemical shifts of the acyl region of the lipids in the spectra we acquired showed that the formed fatty acids were ester bound to a backbone. We identified the NMR-measured lipids as membrane lipids because the chemical shifts of the acyl chain did not correspond to the chemical shift of free fatty acids (6, 10), but they did correspond well to those of the phospholipid dioleoylphosphatidylcholine (9). The chemical shifts of the acyl chain in the lipids were identified as ~14.9 ppm C18 (-CH_3), ~23.2 ppm C17, ~25.7 ppm C3, ~28.1 ppm C8 and ^{11}C, ~30.4 ppm C4–7 and C12–15 (alkyl carbon), ~32.9 ppm C16, ~34.6 ppm C2,~130 ppm C9–10, and ~172.5 ppm C1. The average degree of unsaturation of the membrane lipids and the average length of the acyl chains in the lipids were determined from the NMR data. The average intensity for 1 C in the fatty acids was calculated by taking the average for C_n (CH_3), C_{n-1}, and C_2. The average number of C = C bonds per chain (i.e., the degree of unsaturation) was calculated as:

$$(\text{Intensity C} = \text{C at} 130\,\text{ppm}/\text{Intensity 1 C})/2 \qquad \textbf{[S1]}$$

To calculate the average length of the acyl chains in the lipids, the total intensity for the fatty acid region was divided by the average intensity for 1 C (see definition above), which gave the average length of the chains.

The chemical shift emanating from the backbone of the lipid was hidden behind other compounds that were formed at ~50–85 ppm. Glycerol was identified by liquid NMR through its chemical shifts and the coupling patterns (doublet at ~63 ppm and triplet at 72.4 ppm, with an approximate intensity ratio of 2:1). Bacteria do not accumulate lipids as reserve material, but they contain considerable amounts of lipids in their membranes, especially phospholipids (11). Glycerol, as we detected, is central in the synthesis of phospholipid, and this production of glycerol conceivably may be related to phospholipid formation. Other NMR studies of substrate turnover in soil samples have identified fatty acyl chains in compounds such as triglycerols (12, 13). In the ^{13}C MAS spectra, glycerol was detected at 63.6 ppm. Glycogen yields chemical shifts in the region of ~60–100 ppm and a characteristic peak at ~100 ppm. Signals corresponding to this pattern were observed, especially in the final +9 °C spectrum, indicating that glycogen was formed as a storage compound. In some of the obtained spectra (especially from the samples incubated at +9 °C) we also detected a "bump" in the amide carbonyl region (~164–183 ppm), indicating proteins (8).

Data Evaluation. The [^{13}C]CO_2 production rates at each temperature were determined from linear regressions, which were considered significant if $P < 0.05$. It has been well established that microbial CO_2 production after substrate addition (carbon + nitrogen + phosphorus) can be divided into different phases (14, 15). Notably, the activity of the initial population capable of using the provided substrate increases in direct response to the addition (substrate-induced respiration); then, after a lag-phase, the microbial population may start to grow, and its growth is reflected in an increased rate of CO_2 production. Therefore, the rates of [^{13}C]CO_2 production were determined during two phases of the incubations at each of the temperatures (Fig. 1 and Table S1): the initial rate (phase 1) and the highest observed rate (phase 2). At +9 °C and −4 °C, clear two-phase behavior was observed. However, because of technical problems with the incubations at +4 °C and −9 °C (leakage from some bottles and low [^{13}C]CO_2 concentrations relative to the sampling error, respectively), this pattern was less obvious at these two incubation temperatures. Nevertheless, because it is known that such phase divisions can be applied to identify substrate-induced respiration (15), we judge that the rates obtained represent good estimates of the CO_2 production rates during this phase at each of the incubation temperatures.

Changes in the allocation of carbon between catabolic and anabolic processes over time at each investigated temperature were estimated by calculating the relative proportions (%) of [^{13}C]glucose, [^{13}C]CO_2, and ^{13}C-compounds formed from anabolic processes as a function of time. The added amount of glucose was used as a reference for 100%. The proportions of the synthesized ^{13}C-compounds measured by ^{13}C MAS NMR—glycerol only (including C1 and C3), acyl chains of phospholipids, polymeric carbohydrates (e.g., glycogen), protein compounds, and ethanol—were estimated at the times when 50% of the added glucose remained in the samples incubated at each of the temperatures. This time was determined by linear interpolation between values obtained at the closest measurement times when more than and less than 50% of the glucose remained (three points were used for +9 °C and −4 °C data, and two points were used for +4 °C data). Estimations of variances (reported as SEs) for the interpolated values included the effect of covariance between the data points. Carbon use efficiency (CUE) was calculated as the total amount of synthesized ^{13}C-compounds divided by the sum of the total amount of synthesized ^{13}C-compounds and the amount of [^{13}C]CO_2. The growth index was calculated as the ratio between the amount of phospholipids and the amount of [^{13}C]CO_2. CUE and growth indexes of the microbial populations incubated at each of the temperatures then were determined at the times when half of the glucose remained.

We roughly estimated the lowest temperature at which the membrane lipids in the samples incubated at −4 °C should remain fluid. If each 16-carbon tail of a phosphatidylcholine (PC-16:1/16:1) contains only one *cis* double bond, the melting of the lipid occurs at around −36 °C (16). At −4 °C, the average length of the lipid chains (L) in our samples was 16.6 C, and the average number of *cis* double bonds was 0.69. The average number of *cis* double bonds (Y) (i.e., the degree of unsaturation) was described as a function of a constant (A) multiplied by the ratio of L and the melting temperature (L/T_{melt}), i.e., $Y = A \times (L/T_{melt})$. Applying the data from ref. 16 in this equation gives a constant (A) of −0.0017. Using this constant and applying the equation to the data obtained from the samples incubated at −4 °C indicates that the membrane lipids in the samples would be transformed into a more rigid gel state at *ca*. −24 °C.

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Table S1. Production rates of [^{13}C]CO_2 for the different growth phases at each of the four incubation temperatures

Temperature* (°C)	Phase	Time period†	[^{13}C]CO_2‡ average	SE	Linear regression r^2	*P*
+9	1	0.2–2	439.6	60.8	0.97	0.01
+9	2	5–6	1821.7	136.4	0.99	< 0.001
+4	1	1–2	522.0	27.6	0.95	0.14
+4	2	2–5	1361.6	240.8	0.94	0.001
−4	1	0.5–55	5.5	0.52	0.82	0.001
−4	2	55–99	63.1	11.7	0.92	0.001
−9	1	1–77	0.2	§	0.22	0.2
−9	2	77–160	0.6	§	0.99	< 0.001

*Incubation temperature.
†Incubation time period (days).
‡Production rates (μg ^{13}C g SOM^{-1} ·d^{-1}).
§No replicate samples at −9 °C.

Table S2. Raw data used for determination of the carbon budget

		Total C (mg ^{13}C·g^{-1} SOM)†		Glucose†		[^{13}C]CO_2*,‡		Anabolic metabolism†		
Time (days)	Temperature (°C)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Measured C/ added C
0.04	9	16.8	0.9	15.2	0.8	0.0	0.0	1.6	0.5	1.0
2.23	9	20.0	1.1	10.5	0.4	1.0	0.3	8.5	1.3	1.2
3.06	9	15.2	1.1	4.5	0.2	2.5	0.1	8.2	1.2	0.9
3.23	9	20.6	0.4	3.7	0.6	3.1	0.3	13.7	0.5	1.2
6.04	9	20.0	0.7	0.9	0.2	5.9	0.5	13.2	1.1	1.2
0.09	4	17.8	1.7	16.8	2.2	0.1	0.0	0.9	0.4	1.1
2.14	4	14.3	0.5	10.2	2.1	1.2	0.5	2.8	0.0	0.9
3.26	4	22.9	2.3	5.2	3.8	3.7	0.7	12.7	2.4	1.4
7.36	4	19.7		0.8		4.5		14.4		1.2
10.06	4	15.6	1.6	0.9	0.1	6.1	0.2	8.7	2.0	0.9
0.46	−4	15.5	1.7	14.9	1.5	0.0	0.0	0.9	0.5	0.9
34.08	−4	13.8	1.8	11.4	1.9	0.1	0.0	2.3	0.1	0.8
62.91	−4	12.2	2.1	9.9	2.5	0.6	0.1	1.7	0.2	0.7
77.91	−4	14.9	2.6	10.1	2.3	1.3	0.2	3.5	1.1	0.9
84.89	−4	24.4	1.4	11.3	1.1	1.8	0.4	11.3	1.7	1.5
98.82	−4	20.6	3.6	7.9	0.1	2.9	0.2	9.7	3.3	1.2
0.60	−9	20.7		20.7		0.0		0.0		1.2
41.60	−9	12.1		11.8		0.0		0.3		0.7
89.60	−9	16.3		15.9		0.0		0.3		1.0
124.69	−9	15.3		13.3		0.1		2.0		0.9
159.60	−9	14.5		14.4		0.1		0.0		0.9

See Fig. 1 for all [^{13}C]CO_2 data.
*Temperature.
†mg ^{13}C g SOM^{-1}.
‡[^{13}C]CO_2 raw data used in the carbon budget.