LETTER

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Long-term warming restructures Arctic tundra without changing net soil carbon storage

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High latitudes contain nearly half of global soil carbon, prompting interest in understanding how the Arctic terrestrial carbon balance will respond to rising temperatures[1,2]. Low temperatures suppress the activity of soil biota, retarding decomposition and nitrogen release, which limits plant and microbial growth[3]. Warming initially accelerates decomposition[4–6], increasing nitrogen availability, productivity and woody-plant dominance[3,7]. However, these responses may be transitory, because coupled abiotic–biotic feedback loops that alter soil-temperature dynamics and change the structure and activity of soil communities, can develop[8,9]. Here we report the results of a two-decade summer warming experiment in an Alaskan tundra ecosystem. Warming increased plant biomass and woody dominance, indirectly increased winter soil temperature, homogenized the soil trophic structure across horizons and suppressed surface-soil-decomposer activity, but did not change total soil carbon or nitrogen stocks, thereby increasing net ecosystem carbon storage. Notably, the strongest effects were in the mineral horizon, where warming increased decomposer activity and carbon stock: a 'biotic awakening' at depth.

The carbon balance of northern ecosystems will be regulated ultimately by the difference between carbon gains through greater primary productivity and losses through increased soil organic matter (SOM) decomposition[10]. The direct effects of warming on decomposers may be either mitigated or amplified by restructuring of the plant community, soil food web, and soil temperature regime. However, uncertainty regarding the strength of positive versus negative feedbacks that couple increased shrub dominance, decomposers and their shared soil environment complicates the ability to project future tundra carbon dynamics[11].

Warming promotes woody-shrub growth in Arctic systems[11,12]. Shrubs trap snow, creating warmer winter soil temperatures that stimulate decomposers and support shrub dominance further[3]. Shrubs also increase summer shade, thereby reducing decomposer activity by cooling soils[13]. Whether the winter-warming or summer-cooling effects will dominate temperature-driven changes in decomposition, or whether they will effectively neutralize each other is unknown. Shrub expansion also alters both the nature of carbon inputs and where in the soil profile they occur. Relatively labile shrub leaf litter[14,15] replaces slowly decomposing mosses at the soil surface, whereas greater root biomass may increase labile carbon inputs deeper in the soil[14,15]. Such changes in the nature and location of carbon inputs will alter the activity and community structure of decomposers further[9]. Labile, nitrogen-rich litter favours a rapidly cycling community dominated by bacteria that promotes nitrogen availability[16], whereas lignified woody litter selects for a more slowly cycling fungus-dominated food web[9].

As mineral interactions can protect soil carbon physically, control over decomposition in mineral soils is different to that in overlying organic soils[17]. Greater inputs of labile plant-derived carbon into the mineral horizon may stimulate decomposer communities[18,19], while increasing aggregate formation that can stabilize carbon and limit loss[20]. In parallel with these biotic feedbacks, increased thaw depth under warmer conditions will increase SOM availability in the mineral horizon, stimulating decomposers[2]. However, summer shade may ultimately reduce thaw depth, despite greater annual average soil temperatures[13,21]. The largest fraction of the permafrost soil carbon is contained in the mineral soil[1], and therefore these feedbacks can substantially alter tundra carbon storage over time.

We investigated the effects of two decades of summer warming on a moist acidic tussock (MAT) tundra ecosystem, to evaluate how warming-induced feedbacks affect Arctic carbon cycling. We sampled the longest-running whole-system tundra warming experiment: an *in situ* greenhouse experiment that was started in 1989 at the US Arctic Long Term Ecological Research site (68° 38′N, 149° 34′W). Twenty years of warming increased plant carbon storage and altered both plant community structure and the belowground food web; however, there was no net change in total soil carbon ($P = 0.5$) or nitrogen stocks

Table 1 | Effects of greenhouse warming on tundra carbon and nitrogen pools after 20 years of treatment

Soil characteristic	Surface organic soil		Deep organic soil		Mineral soil	
	Control	Greenhouse	Control	Greenhouse	Control	Greenhouse
Carbon ($g\,m^{-2}$)	1,026.9 ± 101.2	929.1 ± 101.8	4,547.2 ± 1064.4	3,906.6 ± 638.0	**6,381.7 ± 1411.1**	**8,342.3 ± 786.5**
Nitrogen ($g\,m^{-2}$)	26.5 ± 4.1	27.5 ± 3.1	163.5 ± 47.2	145.5 ± 18.1	318.0 ± 75.5	376.9 ± 29.3
Carbon:nitrogen ratio	**42.2 ± 1.7**	**35.8 ± 2.6**	31.5 ± 1.4	28.4 ± 1.3	**20. 4 ± 0.4**	**22.4 ± 0.6**
% Carbon	44.6 ± 1.5	38.2 ± 2.9	30.4 ± 2.5	28.3 ± 2.0	7.2 ± 1.5	8.7 ± 1.8
% Nitrogen	1.1 ± 0.04	1.1 ± 0.08	0.97 ± 0.067	1.1 ± 0.11	0.37 ± 0.083	0.39 ± 0.076
Bulk density (g soil per cm^3)	0.05 ± 0.006	0.06 ± 0.02	0.14 ± 0.04	0.2 ± 0.04	1.0 ± 0.11	0.8 ± 0.1
Sampling depth (cm)	5 ± 0	5 ± 0	10.1 ± 1.5	11.2 ± 1.2	17.0 ± 1.6	15.7 ± 0.8
Percentage soil moisture (g H_2O per g dry soil)	443.5 ± 40.5	531.6 ± 65.2	391.8 ± 27.4	446.1 ± 82.3	75.5 ± 14.9	107.9 ± 24.1

Numbers represent the mean ± 1 s.e. Soil pools include surface litter and fine roots. Surface organic soil, less than 5 cm in depth from the surface; deep organic soil, the soil between the surface organic and the mineral horizon. The mineral horizon was sampled to permafrost. Means that significantly differ from each other (within horizons, $n = 4$; $\alpha \leq 0.1$) are indicated in bold. Mineral-horizon soil carbon and nitrogen stocks were also analysed using a modified equivalent soil-mass method. The maximum sampled control mineral-horizon soil mass among the four replicates was defined as the initial soil mass; all greenhouse and control mineral-horizon soil-sample replicates were set as equivalent to that mass and soil carbon stocks were recalculated. The equivalent soil-mass greenhouse mineral-horizon carbon stock was approximately 28% greater than control (control average = 7451.0 ± 1043, greenhouse average = 9514.8 ± 996, $P = 0.06$).

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Table 2 | Effects of warming on plant community

(**a**), Effects of greenhouse warming on per cent cover of tundra plants and litter

Plant	Year	Treatment	Control (1998, 2008)	Greenhouse (1998, 2008)
Betula nana	*	*	13.5 ± 2.0, 16.0 ± 2.3	16.6 ± 3.3, 31.0 ± 6.8
Salix pulchra	NS	NS	3.7 ± 1.2, 3.8 ± 1.5	5.7 ± 1.4, 5.3 ± 1.6
Eriophorum vaginatum	NS	*	10 ± 1.9, 19 ± 2.5	11.2 ± 2.8, 15 ± 4.2
Carex spp.	NS	***	3.0 ± 0.74, 3.0 ± 0.72	0.5 ± 0.25, 1.0 ± 0.38
Vacinium vitis-idea	**	NS	14.0 ± 1.6, 11.3 ± 0.28	18.8 ± 0.4, 9.1 ± 2.3
Rhododendron tomentosum	NS	NS	8.3 ± 1.5, 10.6 ± 1.6	13.8 ± 2.0, 9.8 ± 0.86
Cassiope tetragona	*	***	7.0 ± 0.97, 5.7 ± 1.8	3.4 ± 1.2, 0.69 ± 0.23
Empetrum nigrum	**	NS	4.5 ± 0.26, 3.4 ± 0.6	4.6 ± 0.4, 2.3 ± 0.58
Rubus chamaemorus	NS	*	8.9 ± 2.9, 6.8 ± 1.6	13.1 ± 1.9, 11 ± 1.9
Pedicularis lapponica	NS	*	0.63 ± 0.26, 0.19 ± 0.05	0.12 ± 0.09, 0.013 ± 0.013
Lichen	NS	****	4.6 ± 1.2, 3 ± 0.27	0.075 ± 0.052, 0.036 ± 0.36
Moss	****	***	18.7 ± 2.9, 7.3 ± 0.98	8.2 ± 1.1, 2.7 ± 0.56
Litter	****	*	0.24 ± 0.14, 6.3 ± 0.8	2.6 ± 1.0, 8.8 ± 0.75
Deciduous shrub	*	*	17.2 ± 3.0, 20.4 ± 3.0	22.4 ± 3.3, 36.7 ± 7.0
Evergreen shrub	*	NS	34.1 ± 2.3, 31.2 ± 4.0	41.2 ± 3.7, 22.1 ± 3.7
Graminoid	*	*	13.4 ± 1.1, 21.9 ± 2.3	11.7 ± 2.9, 16.4 ± 4.5
Forb	NS	NS	10.9 ± 3.0, 7.8 ± 1.6	13.3 ± 1.9, 11.2 ± 1.9

(**b**), Effects of 14 years of greenhouse warming (2002 harvest) on tundra plant biomass

Plant biomass (g dry weight per m^2)	Treatment	Control	Greenhouse
Total aboveground (including mosses and lichens)	**	474.9 ± 32.6	748.7 ± 75.9
Vascular aboveground	***	369.5 ± 26.0	720.7 ± 85.9
Vascular belowground	*	438.3 ± 88.7	712.4 ± 70.6
Total aboveground and vascular belowground	**	913.1 ± 118.9	1,524.0 ± 112.6
Deciduous shrub	*	218.7 ± 51.8	551.2 ± 119.1
Evergreen shrub	*	324.5 ± 20.5	532.2 ± 80.8
Graminoid	NS	227.0 ± 57.5	179.0 ± 92.9
Forb	***	37.6 ± 12.3	170.7 ± 44.4
Litter and standing dead	*	569.3 ± 134	758.4 ± 171.4
Moss	**	75.5 ± 10.8	16.3 ± 4.4
Lichen	*	29.9 ± 6.4	11.8 ± 6.7

Numbers represent the mean ± 1 s.e. of plant percentage cover (sampled in 1998 and 2008), and plant biomass (sampled in 2002). *$P < 0.1$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$ for means that differ from each other significantly at $\alpha \leq 0.1$ ($n = 4$).

($P = 0.7$; Table 1 and Supplementary Fig. 1). Although warming significantly increased surface litter inputs (Table 2a) and probably also increased their quality[16], the greatest biogeochemical changes were at depth, in the mineral soil. The greenhouse treatment increased mineral soil carbon by 31% ($P = 0.06$) and its carbon:nitrogen ratio by 9.8% ($P = 0.05$), whereas the surface organic soil carbon:nitrogen ratio decreased by 15% ($P = 0.02$; Table 1).

The stability of the tundra soil's overall carbon and nitrogen stocks under sustained warming contrasted with the vegetation (Table 2) and soil trophic structure (Fig. 1, Table 3 and Supplementary Tables 1–3). The greenhouse treatment affected the plant community structure

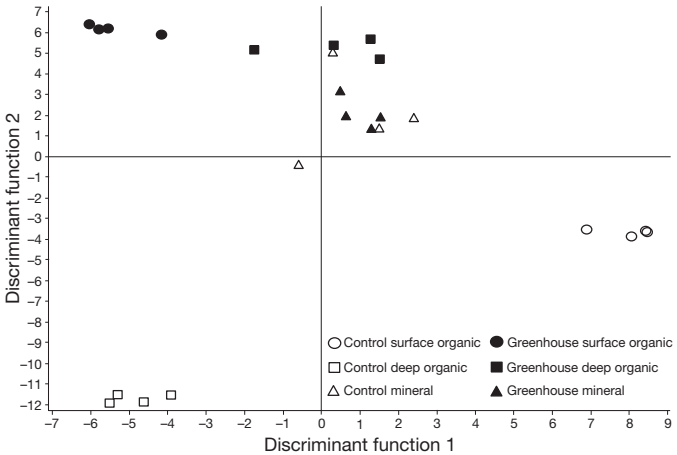

Figure 1 | A canonical discriminant analysis plot reveals the loss of trophic heterogeneity across soil horizons in the greenhouse treatment relative to control conditions. CDA biota groupings included total protozoa (amoebae, flagellates and ciliates were grouped) and did not include bacteria and fungi (which constitute a disproportionate majority of food-web biomass) (μg carbon per g soil; Wilks' lambda < 0.0001; $n = 4$; Table 3).

across all major MAT growth forms. The percentage cover of the deciduous shrub *Betula nana* increased by 94%, litter cover increased by 40%, and lichens and mosses declined by 99% and 63%, respectively. After 14 years of warming, greenhouse plant carbon stocks had increased by 50% (control = 446.5 ± 56.8 g C m^{-2}, greenhouse = 668.7 ± 39.9 g C m^{-2}; $P = 0.02$), whereas greenhouse plant nitrogen did not significantly respond (control = 10.1 ± 1.3 g N m^{-2}, greenhouse = 13.5 ± 0.84 g N m^{-2}; $P = 0.12$). The greenhouse effect on plant biomass was consistent with the percentage cover after 10 and 20 years of warming. The soil food web was also altered by the greenhouse treatment. The trophic structure of control MAT soil is dominated by fungi and is stratified by depth in biomass, diversity, connectance and linkage density, with each horizon possessing a distinct structure. The greenhouse treatment reduced the heterogeneity of the soil food web among horizons. In the control soil, food webs within each horizon differed from each other. Within the greenhouse treatment, only the soil communities within the surface organic (0 to 5 cm in depth) and mineral horizons differed (Fig. 1 and Table 3).

The surface organic soil carbon and nitrogen stocks were unaffected by warming although the carbon:nitrogen ratio declined (Table 1),

Table 3 | Mahalanobis distances by horizon and treatment

Horizon or treatment	Mahalanobis distances by horizon (within treatment)			Mahalanobis distances by treatment (within horizon)
	Surface organic versus deep organic	Surface organic versus mineral	Deep organic versus mineral	Control versus greenhouse
Control	**243.4****	**131****	**234.1****	-
Greenhouse	74.27	**115.4****	44.1	-
Surface organic	-	-	-	**275.6*****
Deep organic	-	-	-	**322.2*****
Mineral	-	-	-	0.1

CDA units are squared. **$P < 0.05$, ***$P < 0.01$.

Table 4 | Effects of greenhouse warming on tundra microbial biomass, activity and extractable nutrient pools during the growing season

Soil characteristic	Surface organic soil		Deep organic soil		Mineral soil	
	Control	Greenhouse	Control	Greenhouse	Control	Greenhouse
Microbial biomass carbon (μg C per g soil)	6,050 ± 1237	6,315 ± 1366	3,137 ± 496	3,810 ± 667	151 ± 44	176 ± 31
Microbial biomass nitrogen (μg N per g soil)	605 ± 106	563 ± 127	285 ± 63	307 ± 90	**5.78 ± 1.55**	**13.0 ± 3.86**
Fungal: bacterial biomass ratio	181 ± 53	220 ± 53	**858 ± 305**	**163 ± 69**	115 ± 37	76 ± 70
SIR active microbial biomass (μg C-CO_2 per g soil per day)	**6,011 ± 733**	**4,584 ± 664**	1,604 ± 434	1,848 ± 351	**59.9 ± 7.26**	**83.0 ± 4.28**
Carbon mineralization (14-day incubation; μg C-CO_2 per g soil per day)	31.83 ± 1.6	27.15 ± 11.6	12.37 ± 2.2	15.46 ± 3.4	**0.43 ± 0.05**	**0.64 ± 0.04**
Extractable organic nitrogen (mg m^{-2})*	3.93 ± 0.6	5.43 ± 1.3	25.16 ± 4.6	27.95 ± 8.8	84.31 ± 10.2	71.71 ± 9.8
Extractable organic carbon (mg m^{-2})*	111.07 ± 18.3	138.11 ± 37.1	610.94 ± 192.3	917.60 ± 57.2	**1,343.14 ± 160.2**	**1,092.11 ± 97.1**

* Scaling to m^{-2} uses average plot bulk density and volume. Numbers represent the mean ± 1 s.e. of soil microbial biomass, activity and extractable nutrient pools. Means that differ from each other significantly at $\alpha \leq 0.1$ (within horizons; $n = 4$) are indicated in bold. C-CO_2, carbon derived from or originating from CO_2.

reflecting a decline in carbon-rich mosses and lichens (Table 2) and increased inputs of leaf litter from *B. nana* and *Rubus chamaemorus* with lower carbon:nitrogen ratios[22]. We observed significant reductions in the greenhouse surface organic soil's root-feeding ($P = 0.07$) and predator nematode biomass ($P = 0.07$), as well as active microbial biomass (measured by substrate induced respiration (SIR); Table 4). This re-alignment of the trophic pathways and overall suppression of the surface organic decomposer food web activity highlights that changes in plant litter inputs alone do not account for soil-community responses to warming. It also challenges our hypothesis that soil nitrogen enrichment coupled with a decline in relatively recalcitrant matter correlates with more rapid carbon-cycling in the organic soil horizons. In the deep organic horizon (5 cm below the surface to mineral soil horizon), both bacterivores ($P = 0.09$) and the ratio of fungal to bacterial biomass ($P = 0.08$) declined, indicating that warming restricted both the root- and detritus-fed trophic pathways.

The altered soil-community structure and activity in the organic horizons may be driven in part by shifted greenhouse soil-temperature patterns, which are consistent with the snow-trap warming model of tundra shrub expansion (Fig. 2 and Supplementary Table 4). The greenhouse treatment initially warmed the soil so that its temperature was up to 2 °C higher than ambient temperature during the summer[23]. Although the summertime greenhouse soil warming declined over the course of the experiment[23], by 1996 a winter warming effect had developed in the mineral soil that consistently exceeded the direct effects on summer soil temperature (Supplementary Table 4). We propose that at the onset of the experiment, soil respiration increased, similar to an increase in soil respiration that was observed 3.5 years into another warming experiment nearby[5] and in meta-analyses of short-term tundra responses to warming[12]. Declining summertime organic soil respiration (the dominant period of tundra soil activity[24]), coupled with greater litter inputs, may have offset initial warming-driven carbon losses, causing no net change in organic horizon carbon stocks.

Unlike the overlying organic horizons, the greenhouse treatment stimulated mineral soil-decomposer activity; microbial biomass nitrogen more than doubled, SIR biomass increased by 39%, and incubation-based carbon mineralization was approximately 1.5 times greater than ambient levels, whereas extractable organic carbon was reduced by 19% (Table 4). This may reflect increased labile carbon consumption by the more active decomposer community. The greenhouse food web became more homogenized across depth relative to control conditions, owing to a vertical redistribution of functional groups, including the presence of phytophagous nematodes and oribatid mites (not found in the mineral soil under control conditions), and the loss of non-oribatid mites, in the greenhouse mineral soil (Fig. 1, Table 3, and Supplementary Tables 1 and 2). Thus, although the detritus-fed pathway underwent a transition, the root-derived trophic pathway expanded.

The stimulation of the greenhouse mineral soil food web is probably caused by increases in late August-thaw depth (Supplementary Fig. 2), in annual soil temperature and in the number of plant-derived resources at depth. Root-derived carbon is a primary resource for soil food webs[25] but roots (which in MAT extend into the mineral soil[15]) also contribute substantially to stable SOM pools[26]. Increases in the volume of litter that moves deeper owing to cryoturbation[3], and greater fine-root biomass at the interface of the organic and mineral horizons may have promoted the changes observed in the greenhouse mineral soil. Although we did not measure fine-root biomass in 2008, belowground vascular biomass had increased by 2002 (Table 2b), and *B. nana* root production is stimulated by warm conditions[14]. Furthermore, *B. nana* dominance increases overall rooting in the deep organic horizon[14]. We

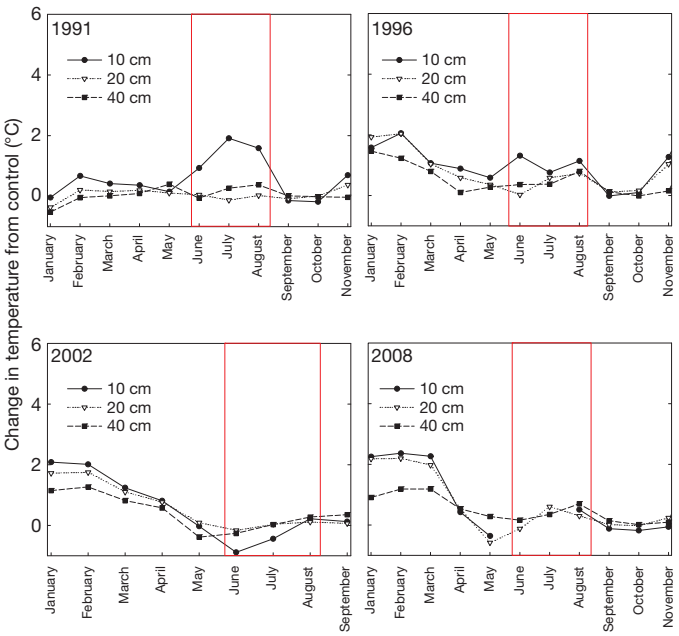

Figure 2 | A discontinuous record of the difference between greenhouse treatment and control soil. Temperature probes up to approximately 10, 20 and 40 cm in depth, from 1991 to 2008. Monthly averages of daily temperatures were used in each block. Vertical lines indicate when the greenhouse is actively warming. Only in 1991 and 1996 are summer greenhouse soil temperatures are significantly higher than control temperatures ($P < 0.1$; $n = 1$). Winter (January to March) temperatures do not initially differ between greenhouse and control; however, from 1996 to 2008, winter greenhouse soil temperatures exceeded ambient conditions in all horizons and exceeded the observed summer temperature differences ($P < 0.0001$; Supplementary Table 4).

propose that warming stimulated greater rhizome and root biomass in the greenhouses' deep organic and mineral soils, which increased the carbon-rich root exudates and leachates moving into the mineral horizon in these highly water-saturated soils.

Long-term tundra warming increased plant biomass carbon and the dominance of woody plants, altered the structure and activity of the soil food web, and changed both the vertical distribution and turnover of soil carbon without affecting overall soil carbon storage. We infer from this that 20 years of warming has promoted net ecosystem carbon storage. Our results also show that deeper mineral soils are susceptible to coupled biotic–abiotic effects driven by warming over decades. Although increased decomposer activity did not offset increased carbon inputs in the mineral soil, incubation studies suggest that labile carbon limits tundra mineral-soil-decomposer activity[19]. Thus, although greater carbon availability at depth may initially increase carbon storage, it remains uncertain whether the ecosystem response observed after 20 years of warming reflects a continued trajectory of increased net carbon storage or a transient state in which an activated decomposer system will ultimately outpace carbon inputs. As such, identifying the mechanisms under which warming stimulates and regulates tundra decomposer activity at depth—where the majority of permafrost soil carbon is stored—remains a pressing challenge.

METHODS SUMMARY

Site description. The study was established in 1989 in MAT near Toolik Lake, Alaska, the United States (68° 38′N, 149° 34′W), and is described in detail elsewhere[15,23]. MAT vegetation is similar to approximately 0.9×10^6 km^2 of tussock tundra found globally[27].

Experimental design. The study consists of four blocks containing control and greenhouse plots. The greenhouses are made of wooden frames ($2.5 \times 5 \times 1.5$ m) and 0.15-mm polyethylene sheets, and are erected yearly, immediately after snowmelt. In July 2008, we sampled four randomly selected subplots per plot ($n = 4$)[28]. Green litter was removed, and organic monoliths (8×8 cm) were collected and separated into surface organic (0–5 cm), deep organic (5 cm to mineral horizon) and mineral soil (sampled to the permafrost). Plant, soil and fauna traits were measured using standard methods[28–30].

Statistical analyses. Data were tested for normality. A blocked analysis of variance (ANOVA), repeated measures ANOVA followed by a Holm–Bonferroni correction for multiple comparisons across years, or a Wilcoxon signed-rank test was used to compare means with a two-tailed t-test at $\alpha \leq 0.1$; the fixed effects were treatment, year and their interaction (as appropriate), separated by horizon. Plant cover data were arcsine-square-root transformed and plant biomass data were natural-log-transformed. Food web data were log-transformed and analysed using ANOVA (comparing horizons across treatment and testing for a depth effect within treatment, and then using Tukey's honestly significant difference (HSD) for multiple comparisons) and canonical discriminant analysis (CDA). Food-web biomass estimates were the CDA interval variables; treatment and soil horizon combinations constituted the classification variables. The model was run with bacteria and fungi, with similar effects. A soil food web connectedness description based on functional groups was used to compare horizons within and across treatments.

Full Methods and any associated references are available in the online version of the paper.

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Supplementary Information is available in the online version of the paper.

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METHODS

Experimental treatment. The greenhouse treatment reduces photosynthetically active radiation and direct input from precipitation, but does not negatively influence plant growth or affect soil moisture (as far as it was possible to detect this), and uneven microtopography enables air circulation beneath the greenhouse bases[15,23]. The greenhouse air temperature is elevated by 2 °C on average, and soil temperature increased by more than 1 °C on average (up to 40 cm in depth)[23]. Soil temperature is measured at a depth of approximately 10, 20 and 40 cm using a Campbell CR21x data logger that recorded two profiles of soil temperature per treatment in one block with copper or constantin thermocouple wires. Soil-temperature data are patchy because of field-equipment failures and irreconcilable movement of the probes with freeze–thaw cycles. The probability of making a type I error ($\alpha \leq 0.1$) was chosen to balance the potential for making type II versus type I errors, given the study's small replicate number ($n = 4$).

Soil harvest and analyses of soil carbon and nitrogen pools. Live coarse roots were removed from soil subsamples by hand, and the volume and mass of rocks were determined and subtracted from the volume and mass of soil in the laboratory. The remaining soil was subsampled for gravimetric water content, and carbon and nitrogen concentrations. Soil subsamples were dried to a constant mass and carbon and nitrogen concentrations were determined using a Fisons NA1500 carbon and nitrogen analyser. The mass of carbon and nitrogen in each horizon (g carbon per m^2 and g nitrogen per m^2) was calculated by multiplying the percentage of carbon or nitrogen per subsample with the corresponding quadrat subsample bulk density and volume, and dividing this value by the subsample area. Treatment effects on the percentage of carbon or nitrogen in soil, and on the mass of carbon and nitrogen in horizons were analysed at the block level using the average of the four block subsamples. Subsamples from the four individual quadrats, separated by horizon, were composited (aggregated) at the block level by hand for further biogeochemical analyses at the block level[28].

Vegetative cover and biomass. Vegetative cover was quantified visually in eight permanent1-m^2 quadrats per plot. A plant harvest in July 2002 used four subplots per block, with vegetation (excluding roots) cut through the mineral-soil surface (including rhizomes that extended into the mineral horizon)[28].

Carbon mineralization and SIR microbial biomass. Composited soils (approximately 8 g wet weight for the surface organic soil and approximately 10 g wet weight for the deep organic and mineral horizons) were weighed out into airtight incubation vessels and incubated at approximately 5 °C (similar to *in situ* soil conditions) for 14 days to capture carbon-mineralization rates immediately after soil harvest. Cumulative carbon mineralization (CO_2 accumulation) was measured by drawing headspace air samples from the incubation vessels using airtight glass syringes and injecting each sample into an infrared gas analyser (IRGA; LI-COR 6252). The carbon-mineralization rate was calculated as the change in headspace CO_2 concentrations (in μg C-CO_2 (carbon originating or derived from CO_2)) per g soil (dry weight equivalent) per day over the 14-day incubation period. Microbial biomass at the end of the carbon-mineralization incubation period was estimated by SIR[31,32]. Samples were amended with autolysed yeast extract (a 10-ml solution of 3 g yeast extract dissolved into 250 ml milli-Q H_2O) in airtight tubes and placed horizontally on a shaker (100 r.p.m.) for 6 h. Headspace CO_2 concentrations were monitored after 3 and 6 h. The average respiration rate (μg C-CO_2 per g soil per h) over the incubation period is an index of the SIR-responsive microbial biomass.

Extractable microbial-biomass carbon and nitrogen. Chloroform-extractable microbial biomass was determined as described elsewhere[29] on approximately 8 g wet weight of surface organic and approximately 10 g wet weight of deep organic and mineral composited soil, immediately after soil collection. Microbial-biomass carbon or nitrogen (extractable plus non-extractable biomass) was calculated by dividing the measured extractable biomass values by correction factors of 0.45 for carbon[33] and 0.54 for nitrogen[34]. Extractable inorganic nitrogen was analysed using the 0.5 M K_2SO_4 extracts on a Lachat autoanalyzer (NH_4^+ using Lachat method no. 31-107-06-5-A; NO_3^- using Lachat method no. 12-107-04-1-B).

Direct counts of bacteria and fungi. Bacterial and fungal biomass were estimated using a direct count method[30,35]. All finished samples were stored at 4 °C until direct counts could be made. Bacterial direct counts were made using a confocal microscope at ×1,500 magnification. Biomass conversions were made assuming an average dry weight of 6.65×10^{-13} g per bacterial cell[36]. Fungal hyphal length was estimated using a confocal microscope at ×400 magnification by counting the number of times hyphae crossed an ocular lens grid. Hyphal length was estimated using the equation $R = \frac{\pi NA}{2H}$, where R is the total hyphal length, N is the number of times hyphae crossed the horizontal lines on the grid, A is the area of one slide well, and H is the total length of the horizontal grid lines. Biomass conversions were made assuming 2.3×10^{-6} g m^{-1} of hyphae.

Protozoan biomass. Protozoan densities were estimated using a most-probable-number (MPN) technique[37], with a starting serial dilution of 10 g of sieved soil in 90 ml of sterile de-ionized water, with tenfold dilutions to 10^{-6} ml. Four 0.5-ml subsamples were taken from each dilution in the series and individually transferred to a well in a standard 24-well plate. A 50-μl suspension of the bacterium *Escherichia coli* was transferred to each well. Plates were incubated at 26 °C for 3 days, after which individual wells were observed using an inverted compound microscope at ×100 magnification. Wells were scored for the presence or absence of 'ciliates', 'flagellates' and 'amoebae'. The densities of total protozoa, and the individual subgroupings were estimated from the frequency distribution of positive reads across the dilution series using the MPN estimation program provided by the US Environmental Protection Agency.

Invertebrate biomass. Soil fauna were isolated from fresh soils and enumerated as described previously[38]. Soil nematodes were isolated using Baermann funnels. Microfauna were heat extracted into a solution of 90% ethanol and 1% glycerine from soils using Tullgren funnels. The intact samples were wrapped individually in cheesecloth, weighed, placed over a funnel and heated with a 9-W incandescent light for 5 days. The dried soils were re-weighed, and the arthropods were sorted into taxonomic groupings and functional groups. Enchytraeids, ciliates, rotifers, tardigrades, nematodes and microarthropods (collembola, mites, copepods and insects) were counted from 5-g subsamples of sieved soil immersed in de-ionized water using a dissecting microscope.

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