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1 **Long-term Pattern and Magnitude of Soil Carbon Feedback to the Climate System in a**
2 **Warming World**

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14 **Abstract:** In a 26-year soil warming experiment in a mid-latitude hardwood forest, we
15 documented changes in soil carbon cycling to investigate the potential consequences for the
16 climate system. We found that soil warming results in a four-phase pattern of soil organic matter
17 decay and carbon dioxide fluxes to the atmosphere, with phases of substantial soil carbon loss
18 alternating with phases of no detectable loss. Several factors combine to affect the timing,
19 magnitude, and thermal acclimation of soil carbon loss. These include depletion of microbially
20 accessible carbon pools, reductions in microbial biomass, a shift in microbial carbon use
21 efficiency, and changes in microbial community composition. Our results support projections of
22 a long-term, self-reinforcing carbon feedback from mid-latitude forests to the climate system as
23 the world warms.

24 **One Sentence Summary:** A 26-year soil warming experiment supports projections of a long-
25 term, self-reinforcing carbon feedback from forest soils to the climate system.

26 **Main Text:** A large and poorly understood component of global warming is the
27 terrestrial carbon cycle feedback to the climate system (1). Simulation experiments with fully

28 coupled, three-dimensional carbon-climate models suggest that carbon cycle feedbacks could
29 substantially accelerate or slow climate change over the 21st century (2–4). Both the sign and
30 magnitude of these feedbacks in the real Earth system are still highly uncertain because of gaps
31 in basic understanding of terrestrial ecosystem processes. For example, the potential switch of
32 the terrestrial biosphere from its current role as a carbon sink to a carbon source is critically
33 dependent on the long-term temperature sensitivity of soil organic matter (SOM) decay (5–7)
34 and complex carbon-nitrogen interactions that will likely occur in a warmer world (8–12).
35 However, without long-term field-based experiments, the sign of the feedback cannot be
36 determined, the complex mechanisms regulating that feedback cannot be quantified, and models
37 that incorporate the soil’s role in carbon feedbacks to the climate system cannot be tested.

38 Here, we present results from a long-term (26-year) soil-warming experiment designed to
39 explore these feedback issues in an ecosystem context. We started our soil warming study in
40 1991 in an even-aged mixed hardwood forest stand at the Harvard Forest in central
41 Massachusetts (42.54°N, 72.18°W), where the dominant tree species are red maple (*Acer rubrum*
42 L.) and black oak (*Quercus velutina* Lam.). The soil is a stony loam with a distinct organic
43 matter-rich forest floor. (See the supplementary materials for more information on the site’s
44 soils, climate, and land-use history.)

45 The field manipulation contains 18 plots, each 6 × 6 m, that are grouped into six blocks.
46 The three plots within each block are randomly assigned to one of three treatments: (i) heated
47 plots in which the average soil temperature is continuously elevated 5°C above ambient by the
48 use of buried heating cables; (ii) disturbance control plots that are identical to the heated plots
49 except that they receive no electrical power; and (iii) undisturbed control plots that have been left
50 in their natural state (no cables). The heating method works well under a variety of moisture and
51 temperature conditions (13). Here, we compared carbon dynamics measured in the heated plots
52 to those measured in the disturbance control plots, so as to isolate heating effects from the effects
53 of cable installation (e.g., root cutting and soil compaction) (14).

54 We used a static chamber technique (14) to measure soil CO₂ emission rates in the study
55 plots monthly between April and November each year for 26 years (see supplementary
56 materials). An ephemeral but significant soil respiration response to warming occurred over the
57 experiment’s first decade (Fig. 1), with soil respiration greater in the heated plots than in the

58 controls (phase I). In the next phase of the response, phase II (years 11 to 17), soil respiration
59 rates in the heated plots were generally equal to or less than those in the control plots. During
60 years 18 to 23 of the study (phase III), we observed a second trend reversal, with soil respiration
61 once again higher in the heated plots. In the three most recent years (phase IV) of the study, 2014
62 to 2016, soil respiration rates in the heated plots were again equal to or less than those in the
63 control plots.

64 We partitioned soil respiration into its two components, root and microbial respiration.
65 On the basis of field measurements of root respiration made between June and November 2009,
66 we developed a temperature-driven root respiration model that we used to estimate root
67 respiration over the course of the experiment (see supplementary materials). We calculated
68 microbial respiration by difference, with microbial respiration equaling soil respiration minus
69 root respiration (10). Our estimate is that two-thirds of the cumulative CO₂-C emitted from the
70 plots over the 26-year study has been microbial. By this analysis, we calculate a warming-
71 induced soil carbon loss from the full soil profile over the 26-year study of $1510 \pm 160 \text{ g C m}^{-2}$,
72 which is equivalent to a 17% loss of the soil carbon found in the top 60 cm of the soil at the start
73 of the experiment. With respect to timing, we estimate that about three-quarters of this soil
74 carbon loss occurred during phase I, and the remaining quarter during phase III. No measurable
75 carbon loss occurred during either phase II or phase IV (Fig. 2).

76 In both the control and heated plots, we made direct measurements of carbon stocks in
77 the upper horizons of the soil profile—the distinct, organic matter-rich surface horizon or forest
78 floor and the top 30 cm of the mineral soil just beneath the forest floor (fig. S1). We measured a
79 carbon loss from the forest floor in response to soil warming of $800 \pm 300 \text{ g C m}^{-2}$, which
80 represents a 31% reduction in forest floor carbon stock over the 26-year study. With our direct
81 measurements, we did not detect any statistically significant changes in the carbon stocks across
82 the top 30 cm of the mineral soil horizon. However, combining our estimate of carbon loss from
83 the full profile based on the respiration measurements with the carbon loss measured from the
84 forest floor, we estimate that the warming-induced carbon loss from the mineral soil in the full
85 soil profile over the study period was 710 g C m^{-2} . An in situ soil warming experiment in a
86 California forest also shows that warming increases the decay of subsoil organic matter (15).

87 We explored possible relationships between the multiphase pattern in soil respiration and
88 multiyear variations in climate, and found none (see supplementary materials and fig. S2, A and
89 B). We propose that several biogeochemical factors combine to affect the timing, magnitude, and
90 thermal acclimation of soil carbon loss. These include depletion of microbially accessible carbon
91 pools, reductions in microbial biomass, a shift in microbial carbon use efficiency, and changes in
92 microbial community composition. Thermal acclimation, a phenomenon observed in response to
93 soil warming in a grassland ecosystem (16), was evident in all four phases of our long-term soil
94 warming experiment, such that at a given temperature there was less respiration from the heated
95 plots than from the control plots (Fig. 3). During phases I and III, the acclimation was
96 insufficient to compensate for the 5°C increase in soil temperature, so that CO₂ emissions from
97 heated plots were greater than from control plots. During phases II and IV, however, acclimation
98 was large enough to compensate for the 5°C increase in soil temperature.

99 In this study, the importance of thermal acclimation of the soil respiration response
100 became evident when power to the heated plots was off for part of the year in 1995 and 2005 and
101 throughout 2010. The power shutdowns in 1995 and 2005 resulted when summer lightning strikes
102 damaged the system that controlled experimental heating. The power shutdown in 2010 was
103 planned and carried out to increase our understanding of the thermal acclimation response. In the
104 three instances, soil respiration rates in the powered down heated plots dropped below those of
105 the controls after the power was off, and they returned (within weeks) to rates above the controls
106 once the power was restored (fig. S3).

107 Our biogeochemical and molecular observations suggest that warming causes cycles of
108 soil carbon decay punctuated by periods of structural and functional changes in the microbial
109 community. Sustained reductions of microbial biomass over the course of the experiment [(17–
110 19) and this study] have been accompanied by several other changes, including (i) altered
111 respiratory and lipid profiles (17); (ii) changes in microbial community structure and function as
112 determined using small subunit ribosomal RNA analysis (20, 21), metagenomics (22), and
113 enzyme assays (19, 21); (iii) characterization of substrate utilization profiles of bacterial isolates
114 (22); and (iv) measurements of microbial carbon use efficiency (23).

115 We have integrated these observations (Table 1) to develop a conceptual model of the
116 time-varying (four-phase) effects of soil warming on feedback to climate. Phase I was a period of

117 substantial soil carbon loss, especially from the surface organic horizon. The rate of carbon loss
118 essentially followed an exponential decay pattern, rapid at first, slowing to near zero over the
119 experiment's first decade. In phase II, soil respiration rates in the heated plots were generally
120 equal to or less than those in the control plots. The transition from phase I to phase II was
121 characterized by a depletion of a labile C pool (18), which is considered to be the driver of
122 reduced microbial biomass (24).

123 Phase II appears to have been a period of microbial community reorganization, leading to
124 changes in structure and function. During this time, soil heating reduced the abundance of fungal
125 biomarkers and also caused a shift toward Gram-positive bacteria and especially actinobacteria
126 (17). Along with these structural changes, heating reduced the capacity of microbial community
127 to utilize simple C substrates (17).

128 The transition from phase II to phase III was characterized by a continued shift toward a
129 more oligotrophic microbial community with increased diversity due to increased evenness
130 (Pielou's J statistic), reduced microbial biomass, and reduced fungal dominance, as evidenced by
131 the same population size of bacteria and narrowed fungal/bacterial ratios for C-degrading genes
132 (20).

133 In phase III, soil respiration rates were higher in the heated plots than in the controls. This
134 third phase appears to have been a period when recalcitrant substrates such as lignin became an
135 important source of carbon for the microbial community. This is consistent with the evidence
136 that during phase III, relative to controls, there was an increase by a factor of 4 in potential
137 lignin-degrading enzyme (lignase) activity in surface soils from the heated plots (21).

138 As phase III transitioned toward phase IV, we observed a reduction in the relative
139 abundance of lignin in the soil C pool in the surface mineral horizon (19). At the same time, the
140 magnitude of the carbon loss to the atmosphere through decomposition during this period may
141 have been attenuated somewhat by a shift toward higher microbial carbon use efficiency for
142 recalcitrant substrates in warmed soils relative to control soils (23).

143 Our study is just 3 years into phase IV. This new phase may turn out to be another period
144 of microbial community reorganization that will eventually transition to yet another phase of
145 further carbon loss from decay of recalcitrant forms of SOM. Because recalcitrant SOM pools
146 make up a substantial fraction of global soil carbon stocks (25), small changes in the decay rates

147 of these pools could result in a large self-reinforcing feedback to the climate system over
148 multiple decades (26). As a preliminary test of global significance, extrapolating our results to
149 the world's forests, we estimate a global aggregate soil carbon loss from the upper 1 m of soil
150 (27) over the 21st century of ~190 Pg C. This does not account for possible future climate-driven
151 changes in plant-soil interactions that could affect the long term balance between the formation
152 and decomposition of SOM. Critical to this balance will be changes in the amount of fresh
153 carbon transferred from plants to the soils as the world warms. Inputs of this fresh carbon can
154 contribute to soil carbon sequestration, but they can also accelerate the decomposition of more
155 recalcitrant forms of SOM through biological priming mechanisms (28).

156 Our first-order estimate of a warming-induced loss of 190 Pg of soil carbon over the 21st
157 century is equivalent to the past two decades of carbon emissions from fossil fuel burning (29)
158 and is comparable in magnitude to the cumulative carbon losses to the atmosphere due to human
159 driven land use change during the past two centuries (30). A transfer of carbon of this magnitude
160 from forest soils to the atmosphere in response to warming would amplify the mitigation
161 challenge already faced by society. It is also important to recognize that a global-scale,
162 microbially mediated feedback could be very difficult, if not impossible, to halt.

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266
267 Datasets pertaining to this study are available on the Harvard Forest Online Data Archive
268 (<http://harvardforest.fas.harvard.edu/harvard-forest-data-archive>), datasets hf005 and hf171.

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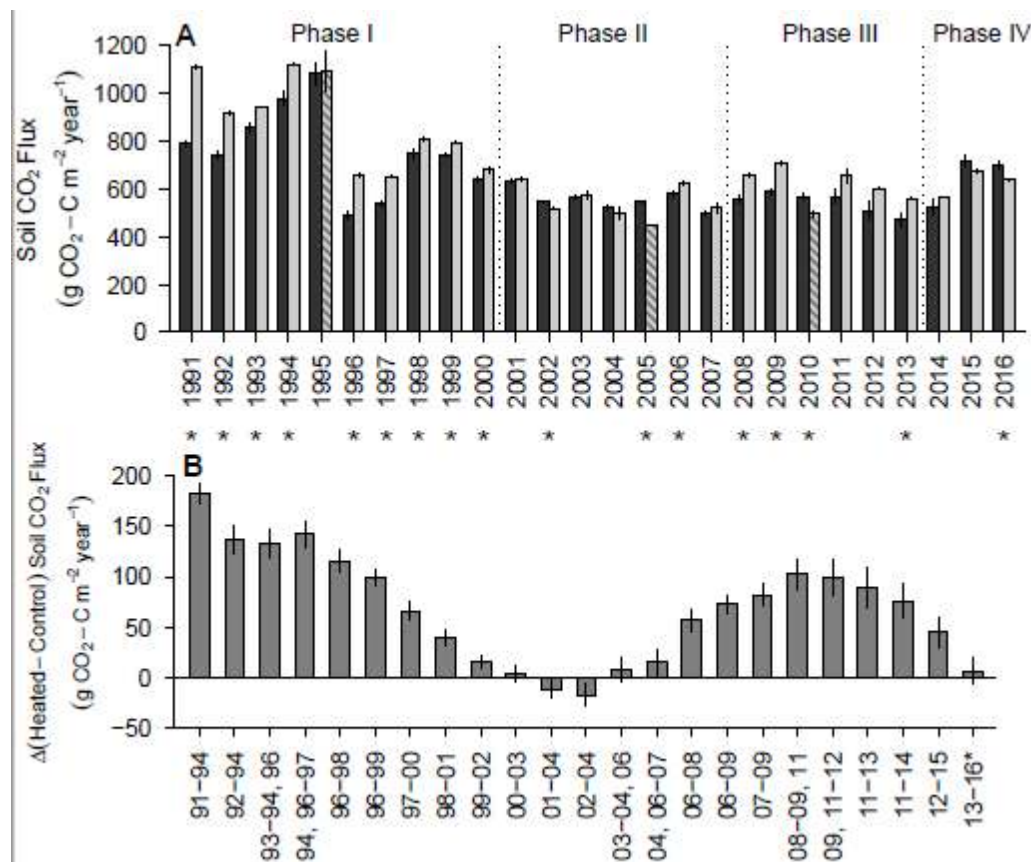
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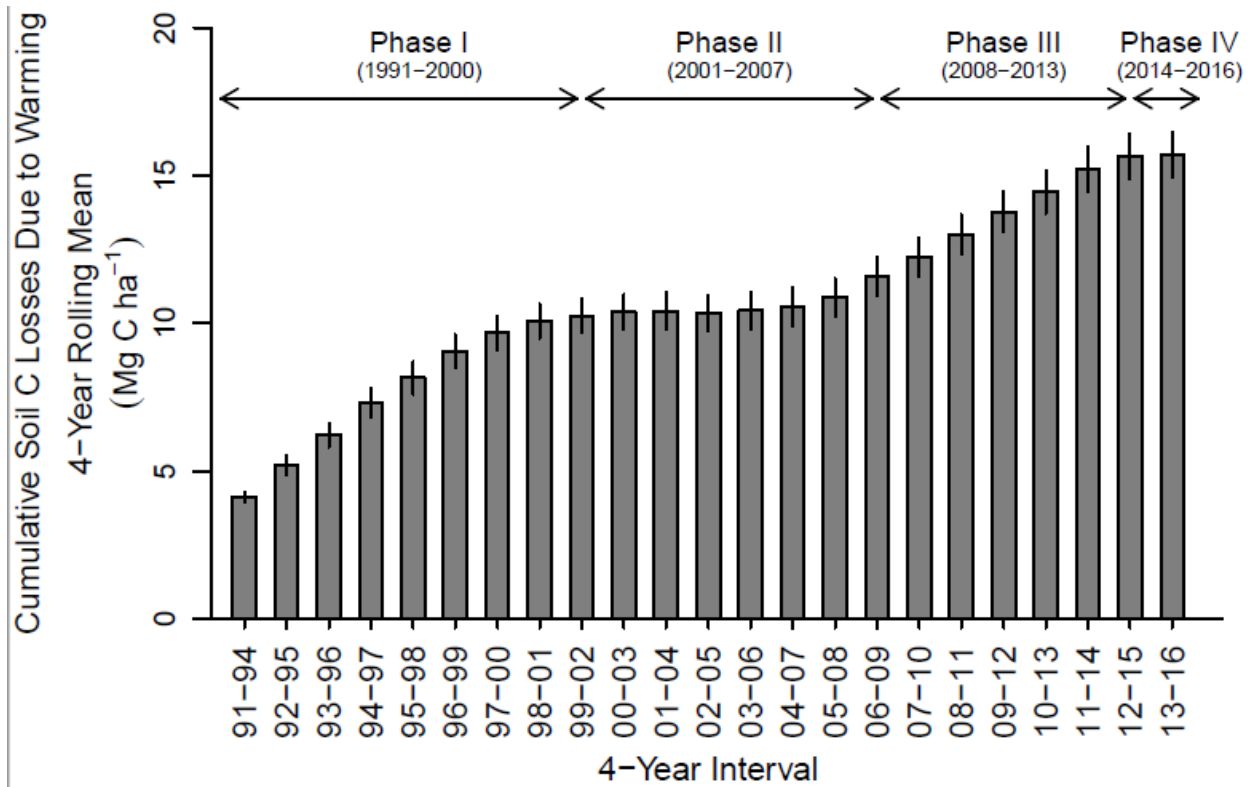
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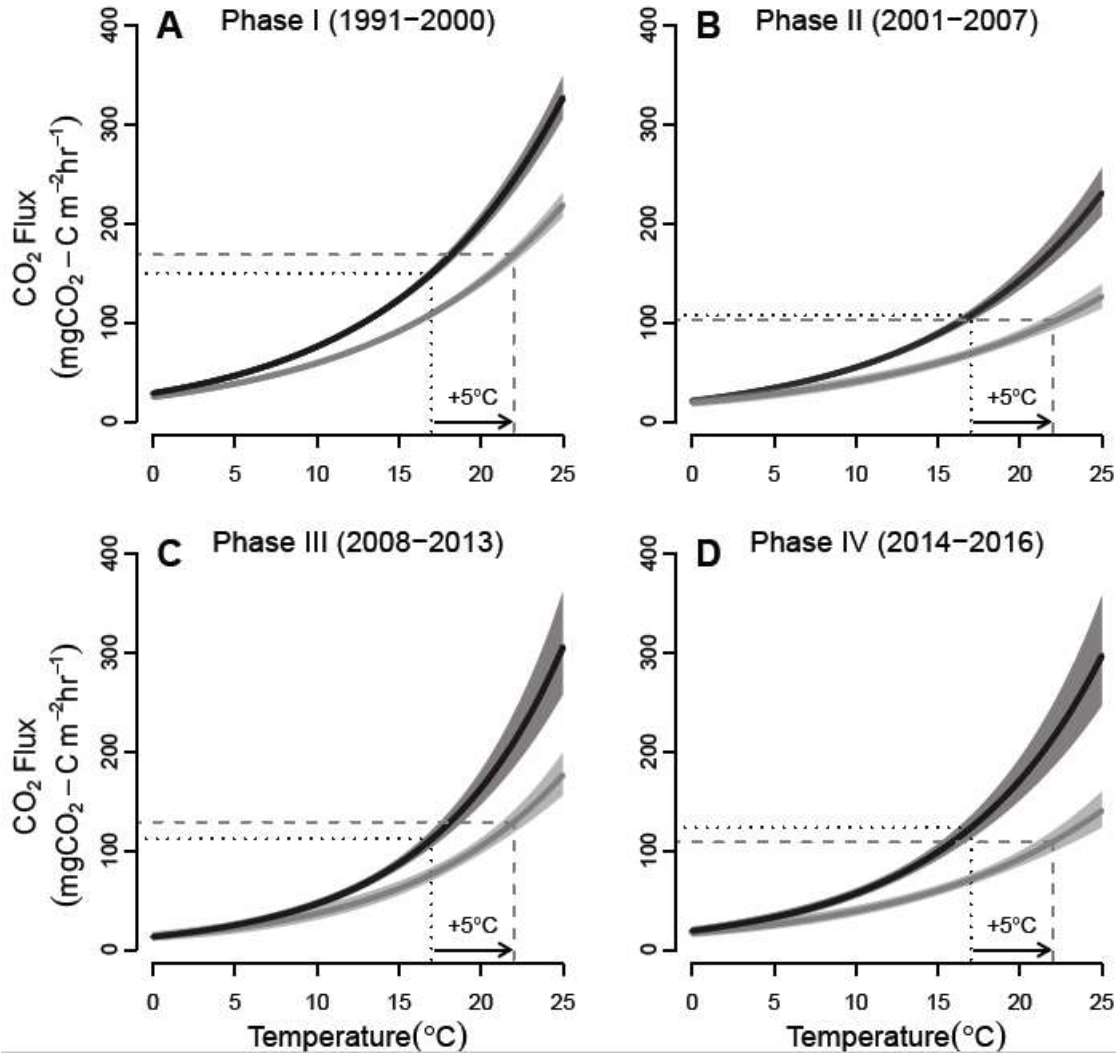
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 278 **Fig. 1. Effect of soil warming on soil respiration over 26 years. (A)** Annual soil CO₂ emissions
 279 from the control plots (black bars) and heated plots (gray bars). Asterisks denote years when
 280 the heated and control plots are significantly different (paired-sample t tests or Wilcoxon
 281 signed-rank tests as appropriate, n = (6, 6), *P < 0.05; see supplementary materials). Hatched
 282 bars denote years when the heating system was inactive for the majority of the growing
 283 season. Error bars denote SEM (n = 6). **(B)** Four-year rolling mean increase in soil CO₂ emissions
 284 in the heated plots relative to the control plots, excluding years when the heating system was
 285 inactive for the majority of the growing season. Error bars denote SEM derived from
 286 propagating SE estimates from (A) through the operations necessary to produce (B). See fig. S4
 287 for annual changes in soil CO₂ emissions in the heated plots relative to the control plots.
 288



289
 290 **Fig. 2. Four-year rolling mean cumulative modeled soil carbon losses from the full soil**
 291 **profile over 26 years of soil warming in the heated plots relative to the control plots.**
 292 Relative soil carbon losses are calculated as the difference in heterotrophic soil respiration
 293 between the heated plots and the control plots. Error bars denote SEM, calculated for the heated
 294 (n = 6) and control (n = 6) plots for each year and propagated through the necessary operations to
 295 produce this figure.



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Fig. 3. Relationship between soil respiration and soil temperature in the heated and control plots during each phase of the experiment. We modeled this relationship as an exponential function of soil temperature: $\text{respiration} = a[\exp(b \times \text{temperature})]$, where a is respiration rate at 0°C and b is temperature sensitivity of respiration. Solid dark gray curves represent control plot models; solid light gray curves represent heated plot models. For the purposes of this figure, each model was fitted to all respiration data collected within the phase. Shaded areas around each curve represent normal-based 95% confidence intervals. Dashed lines illustrate predicted soil respiration during each phase when the control plots are 17°C and the heated plots are 22°C, the respective summer modal temperatures of the control and heated plots over the course of the experiment.

Measurement	Organic		Mineral		Method (units)	Ref
	% change	Effect size	% change	Effect size		
Phase I (1991-2002)						
Phase II (2001-2007)						
Depletion of labile soil C pool	-25%	-3.3	-25%	-3.5	DOC ^a (ug C g ⁻¹ soil), seasonal median	18
Reduction in microbial use of simple C	-33%	-4.3	-57%	-5	SIR ^b (ug C g ⁻¹ soil day ⁻¹), seasonal median	18
Reduction in microbial use of simple C	-44%	-3.4	-44%	-3.4	SIR ^b (ug C g ⁻¹ soil day ⁻¹)	17
Reduction of microbial biomass	-74%	-3.6	-12%	-0.6	CFE ^c (ug C g ⁻¹ soil), seasonal median	18
Reduction of microbial biomass	-26%	-3.5	-27%	-6.1	Lipid P (nmol g ⁻¹ soil)	17
Relative loss of fungal biomass	-19%	-3.0	-28%	-3.8	Lipid P (nmol g ⁻¹ soil), fungi	17
Decline in fungal:bacterial ratio	-14%	-5.0	-23%	-8.5	Lipid P (nmol g ⁻¹ soil)	17
Phase III (2008-2013)						
Restructuring of microbial community	3.5%	2.1	3%	1.2	Phylogenetic diversity (Shannon's H)	20
Shift towards more oligotrophic community	-5.0%	-1.2	2%	0.4	rRNA copy number	20
Acceleration of microbial reduction of lignin	-6.0%	-0.1	372%	1.5	Oxidative EEA ^d (μmol cells ⁻¹ hr ⁻¹)	21
Reduction of microbial biomass	-23%	-1.6	-16%	-1.4	Lipid P (nmol g ⁻¹ soil)	20
Reduction of temperature sensitivity of microbial efficiency for recalcitrant substrates	<i>n.d.</i>	<i>n.d.</i>	-31%	-5.6	¹³ C-Phenol Utilization (C _{growth} C _{metabolized} ⁻¹ 9C ⁻¹)	23
Alteration of fungal:bacterial ratio of C-degrading genes	31%	1.0	-11%	-0.8	Fungal:bacterial CAZy gene ratio	22
Decreased lignin relative abundance	-24%	-1.2	-29%	-0.9	pyGCMS ^e (Lignin % fraction of SOM ^g)	TS ^h
Phase IV (2014-2016)						
Reduction in microbial biomass	-40%	*	-40%	*	DCE ^f (μg N g ⁻¹ soil)	TS ^h
Reduction in microbial biomass	-25%	-0.9	-20%	-0.7	Lipid P (ng g ⁻¹ soil), seasonal median	19
Decreased lignin relative abundance	-17%	-0.8	-53%	-1.3	pyGCMS ^e (Lignin % fraction of SOM ^g)	19

310

311 **Table 1. Changes observed in soil C pools and the microbial community in response to soil**

312 **warming of 5°C above ambient in phases II to IV of the 26-year experiment.** Percent change is

313 based on observed means in heated plots relative to controls. Effect size is calculated by

314 Cohen's D test using pooled standard deviation. Percent changes smaller than 10% and effect

315 sizes smaller than 2 (equivalent to a shift of two standard deviations) are in italics; n.d., not

316 determined. DOC, dissolved organic carbon; SIR, substrate-induced respiration; CFE, chloroform

317 fumigation extraction; EEA, extracellular enzyme activity; CAZy, carbohydrate-active enzymes;

318 pyGCMS, pyrolysis gas chromatography–mass spectrometry; DCE, direct chloroform extraction;

319 SOM, soil organic matter. For phases II to IV, “reduction in microbial biomass” refers to a

320 within-phase decrease in the microbial biomass in the heated soils relative to the control soils

321 measured during that phase. An asterisk indicates that within-treatment plot-to-plot variability

322 could not be measured and effect size could not be determined because measurements were

323 made on soil samples bulked by treatment. No measurements were made of changes in

324 microbial responses to warming during phase I.