

Accelerated microbial turnover but constant growth efficiency with warming in soil

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Rising temperatures are expected to reduce global soil carbon (C) stocks, driving a positive feedback to climate change¹⁻³. However, the mechanisms underlying this prediction are not well understood, including how temperature affects microbial enzyme kinetics, growth efficiency (MGE), and turnover^{4,5}. Here, in a laboratory study, we show that microbial turnover accelerates with warming and, along with enzyme kinetics, determines the response of microbial respiration to temperature change. In contrast, MGE, which is generally thought to decline with warming⁶⁻⁸, showed no temperature sensitivity. Using a microbial-enzyme model, we show that temperature-sensitive microbial turnover promotes soil C accumulation with warming, in contrast to reduced soil C predicted by traditional biogeochemical models. Furthermore, the effect of increased microbial turnover differs from the effects of reduced MGE, causing larger increases in soil C stocks. Our results demonstrate that the response of soil C to warming is affected by changes in microbial turnover. This control should be included in the next generation of models to improve prediction of soil C feedbacks to warming.

Many global C cycling models predict reductions in soil C with climate warming⁷. More recent models that include microbial controls over decomposition suggest a wider range of potential responses⁵. These models reproduce present soil C stocks more accurately than models that do not incorporate microbial dynamics⁹, but their ability to predict soil C responses to climate change is hampered by uncertainty in the temperature sensitivity of microbial processes⁴. There is an active debate in recent literature about which microbial mechanisms should be represented in soil C cycling models^{7,10-13}.

Warming increases kinetic energy, accelerating enzyme-requiring reactions¹ and stimulating C consumption by soil microbes. Microbial C consumption and respiration, the largest flux of C out of soil, is significantly affected by both the size and functioning of the soil microbial community^{3,6}. Warming may change the soil microbial biomass carbon (MBC) concentration and activities through two potentially concurrent mechanisms. First, warming can decrease MGE, which is the proportion of substrate C that is used for microbial growth relative to the total amount of substrate C consumed^{7,14}. Higher temperatures are generally expected to reduce MGE, as warming limits microbial growth by increasing the energy cost of maintaining existing biomass⁸. However, responses of MGE in soil microbial communities are equivocal, with studies reporting decreased MGE with temperature increase^{15,16}, no change¹⁴, or a variable response based on substrate

type¹⁷. It is unclear to what extent this variability is caused by the methods and procedures used for measuring MGE in soil⁸. Second, warming can affect microbial turnover rates¹⁸. Microbial turnover is determined by microbial cell production and cell death, which are processes that may be affected by temperature. Dead cells may either adhere to soil particles and join the pool of soil organic carbon (SOC) or be metabolized by living microbes¹⁹. Consequently accelerated turnover can increase respiration per unit of MBC even when MGE remains the same²⁰. However, most studies of MGE responses to warming do not account for the respiration and cell death that result from turnover¹⁵⁻¹⁷.

We determined the temperature sensitivity of MGE and turnover to examine the mechanisms controlling the response of soil C cycling processes to warming. We measured MGE and microbial turnover in mineral soil and organic soil from the Marcell Experimental Forest, Minnesota, after a one-week incubation at 5, 10, 15, or 20 °C. We used metabolic tracer probing to determine MGE (ref. 14). In this method, MGE is calculated from the fate of individual C-atoms in glucose and pyruvate. Unlike other methods¹⁵⁻¹⁷, the metabolic tracer probing method determines an MGE measurement almost entirely unaffected by microbial turnover because it can be done very quickly (1 h or less at room temperature) and calculates MGE based on metabolic modelling. We combined MGE measurements with measurements of microbial respiration and MBC to calculate microbial turnover rates.

We found that MGE was not sensitive to temperature (Fig. 1). Mean MGE was 0.72 (± 0.01 s.e.m., $n = 22$) in mineral soil and 0.71 (± 0.01 s.e.m., $n = 21$) in organic soil. Across all temperature treatments and replicates, MGE ranged between 0.67 and 0.75. These values for MGE are high relative to the average values observed in soils and other ecosystems^{7,8,21}. It is also higher than 0.6, an average maximum MGE value for pure culture studies^{8,22} (for further discussion on theoretical thermodynamic constraints of MGE, see Supplementary Note). This high value suggests that the active microbial community functions at high biochemical efficiency and microorganisms with relatively high maintenance costs contribute little to the total activity. High efficiency values may also indicate additional energy sources (for example, from oxalate or formate²³), or direct incorporation of large amounts of cellular compounds, such as amino acids¹⁴. However, what little information is available suggests that these effects will be only slightly affected by temperatures¹⁷.

Microbial growth efficiency is generally expected to decline as a result of increased microbial maintenance costs at higher temperatures^{6,7,24}. This effect of temperature on maintenance energy

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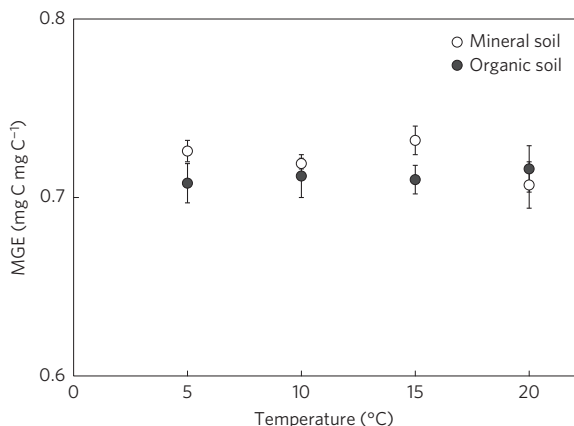


Figure 1 | Microbial growth efficiency (MGE) after a 7-day incubation at different temperatures for a mineral and an organic soil. Means and standard errors ($n=6$, except for mineral soil at 5, 10 °C and organic soil at 5 °C, where $n=5$). There was no significant effect of soil type ($p=0.21$) or temperature ($p=0.70$) on MGE.

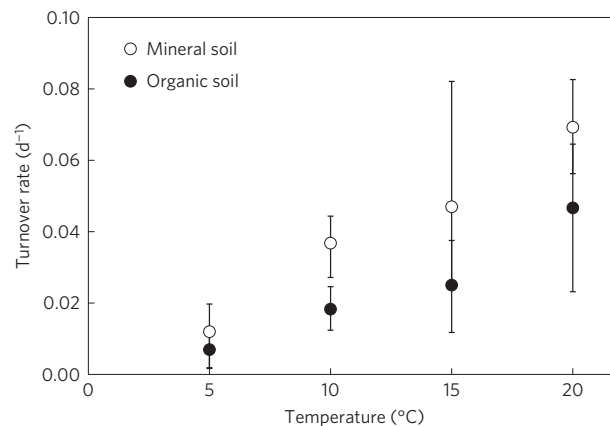


Figure 2 | Turnover rates (τ , d^{-1}) as a function of temperature for a mineral and an organic soil. The experimental values were resampled using a bootstrap method to calculate a 95% confidence interval (error bars). For each soil type, the turnover rate at 5 °C is significantly different from that at 20 °C.

Q.2

1 has been observed in a pure culture experiment²⁵, but may
2 not be observable in diverse soil communities, where growth
3 optimum temperatures can vary widely between microbial species¹¹.
4 If the composition of the active microbial community shifts,
5 higher maintenance costs might be avoided and MGE could
6 be unchanged. It is also possible that the microbial community
7 expresses physiological acclimation⁶.

8 Despite the constant MGE with temperature, higher
9 temperatures increased microbial respiration in the mineral
10 soil and organic soil by factors of nearly six and eight, respectively
11 (Supplementary Fig. 1). Across the same temperature range, specific
12 respiration rate ($\mu g CO_2-C mg^{-1} MBC h^{-1}$) increased by 540% in
13 the mineral soil and 630% in the organic soil. Because increased
14 respiration rates could not be explained by increased microbial
15 biomass, warming must have affected microbial C metabolism by
16 faster C consumption.

Q.3

17 Higher specific respiration rates and constant MGE with
18 increasing temperature indicate an increased production of new
19 microbial biomass. Warming significantly increased MBC gross
20 production rates ($0.97 \mu g MBC g^{-1} dry soil d^{-1} °C^{-1}$, $r^2 = 0.99$
21 in mineral soil and $3.63 \mu g MBC g^{-1} dry soil d^{-1} °C^{-1}$, $r^2 = 0.98$
22 in organic soil). However, temperature did not change the MBC
23 concentration ($p = 0.474$) in either soil (Supplementary Table 1).
24 Therefore, warming increased microbial turnover ($p = 0.02$) in both
25 soils—by $0.004 d^{-1} °C^{-1}$ in mineral soil and by $0.003 d^{-1} °C^{-1}$ in
26 organic soil (Fig. 2), compensating for increased MBC production.

27 Why did warming increase microbial turnover? One possibility
28 is that the abundance or activity of microbial predators and grazers
29 increased with temperature. However, the few studies examining
30 the effect of warming on microbial predator and grazer abundances
31 have found both increases and decreases in abundances after
32 several years of warming²⁶. Warming could cause a shift in the
33 microbial community composition that drives faster turnover.
34 Natural senescence of microbial cells may also be accelerated as
35 protein turnover is increased at higher temperatures¹⁸. Alternatively,
36 at higher temperatures and greater MBC productivity, activity
37 of viruses could increase cell death. Each of these mechanisms
38 may respond differently to temperature and could be important
39 to informing our understanding of responses of soil C fluxes to
40 temperature increases.

41 An increase in turnover with warming may partly explain the
42 generally observed decline in MGE with temperature. Previous
43 studies that suggest a decline in MGE did not separate the influences
44 of turnover and MGE on the residence time of carbon tracers in

the soil microbial biomass. Ideally, MGE is determined during a
very short period after the addition of ¹³C-labelled C compounds
(instantaneous MGE or MGE_i). Over time, microbial turnover
will cause some of the ¹³C initially incorporated into microbial
biomass to be released as CO_2 , resulting in an overestimation
of CO_2 production and an underestimation of microbial biomass
production and MGE (refs 16,21). This effect increases with
incubation duration and may cause differences in apparent MGE
(MGE_A), especially when microbial turnover rates differ between
treatments (as in this study, Fig. 2).

We modelled the effects of assay duration and temperature on
 MGE_A (Fig. 3a). Assuming an MGE_i of 0.72 for all temperatures
and microbial turnover rates, as determined in this study (Fig. 2),
we estimate that MGE_A declines by $0.005 °C^{-1}$ in mineral (Fig. 3b)
and $0.003 °C^{-1}$ in organic soil after a two-day incubation. Other
studies have found that MGE declines by $0.009 °C^{-1}$ (ref. 15) to
 $0.017 °C^{-1}$ (ref. 1) when measuring MGE over 24–48 h. These rates
of decline with temperature are greater than those in this study;
however, it remains unclear whether this is associated with higher
turnover rates in those studies or with genuine declines in MGE_i .
Studies that have used short-term assays (<6 h) reported no change
in MGE of soil microbial communities with warming^{14,17}, consistent
with results we report here (Fig. 1).

We found that microbial turnover rate is temperature sensitive,
but that MGE is not. These results were determined in a short-
term laboratory incubation, a controlled environment that provides
the best conditions to test mechanistic questions such as those in
this study. On a longer time scale, turnover rates and MGE could
be indirectly affected by temperature through nutrient limitation,
changes in community composition, and changes in soil moisture. It
is also likely that across a large spatial scale turnover rates will vary;
we saw differences in turnover rate between the two soils studied
here (Fig. 2). Other studies have found that warming decreases
MBC, indicating accelerated microbial turnover could be important
at time scales longer than in this study^{27,28}. However, accelerated
microbial turnover in response to warming is a mechanism that has
never been explicitly accounted for in soil carbon models.

To assess the implications of microbial turnover to soil
C predictions, we used the Allison–Wallenstein–Bradford (AWB)
model^{5,6}. The AWB model uses rates of microbial processes that
are based on the best estimate of steady-state conditions, which
allowed us to extrapolate the significance of our short-term results
to long-term steady-state C stocks. We simulated three different
scenarios. In the first scenario, neither MGE nor turnover was

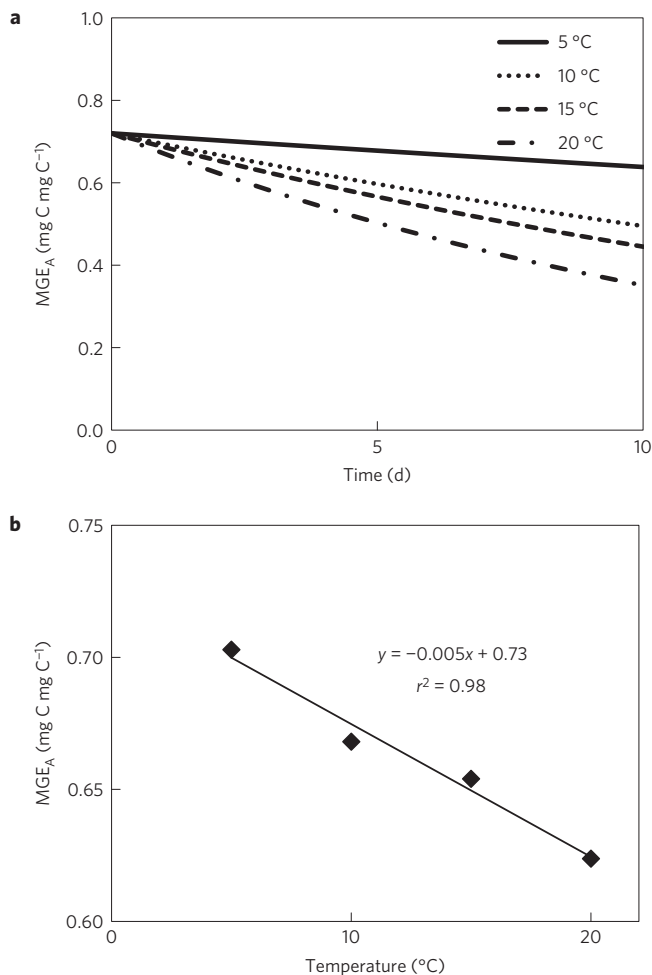


Figure 3 | Modelled effect of temperature and incubation duration on apparent MGE. **a**, The relationship between temperature and MGE_A over time was modelled using the microbial turnover rates for the mineral soil in our study (Fig. 2). **b**, The modelled relationship of MGE_A and temperature in mineral soil after two days.

altered by temperature and soil C decomposition was modelled with a first-order decay function and Michaelis–Menten enzyme kinetics, the current assumption in most biogeochemical models^{7,29}. In this scenario there was no change in MBC with warming and SOC declined as a result of accelerated enzymatic decomposition (Fig. 4). In the second scenario, MGE decreased by 0.016 °C⁻¹, as in previous theoretical studies⁶. Here, the reduction in MGE limited microbial growth at higher temperatures, resulting in a 5% decline of MBC °C⁻¹ averaged from 5 to 20 °C. As a result, SOC increased with temperature as decomposition became limited by MBC. The third scenario corresponded to our experimental observations of a constant MGE and accelerated microbial turnover with warming. Accelerated microbial turnover at higher temperature caused decreases in MBC and increases in SOC, which were larger than in the scenarios of constant turnover and declines in MGE. We conclude that accelerated microbial turnover is an alternative mechanism that can moderate the effects of temperature on soil C stocks, even when MGE does not decline. These model simulations suggest that temperature-sensitive microbial turnover produces an effect on MBC and SOC that is not accounted for in present biogeochemical or microbial models.

Our results show that accelerated enzyme kinetics and increased microbial turnover are the main mechanisms associated with an increased respiration at higher temperatures, and in model

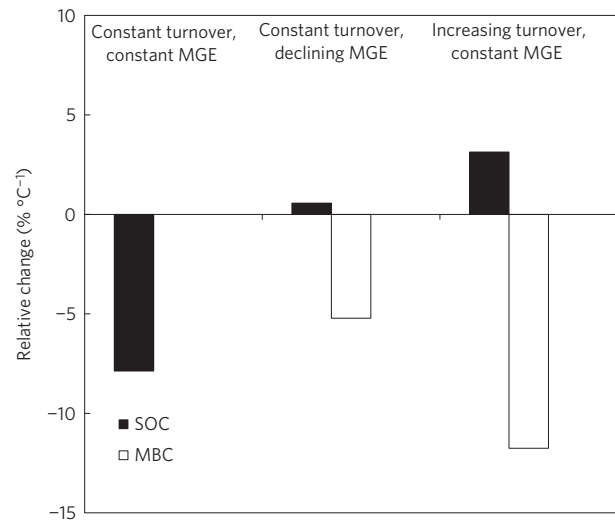


Figure 4 | The relative change in soil organic C (SOC) and microbial biomass C (MBC) from 5 to 20 °C under three scenarios using the AWB model. In the constant turnover, constant MGE scenario there is no change in MBC with temperature.

simulations lead to a small increase in SOC content under elevated temperatures. This effect on SOC is similar to those that have been predicted in models assuming a decline in MGE, but differs in direction from the predictions of traditional biogeochemical models. Consequently, soil microbial models should include a temperature-sensitive microbial turnover rate. The lack of temperature sensitivity in MGE, which is controlled at the cellular level, suggests that microbial biochemical efficiency is a weak control on soil C dynamics.

Methods

Soil samples were collected in October 2012 from the Marcell Experimental Forest in Grand Rapids, MN (MAT = 3 °C, MAP = 750 mm). Mineral soil samples were collected from the A horizon in a hardwood forest and organic soil samples were collected from an ombrotrophic peatland (top 40 cm after removing the living layer of moss). Soil samples were stored at 4 °C until the experiment began in April 2013. Replicates (*n* = 6) from both soils were randomly assigned to one of four incubators and incubated for seven days at 5, 10, 15, or 20 °C (Supplementary Section I).

After a seven-day incubation period, MGE was determined using two position-specific ¹³C-labelled isotopologues of glucose (U-¹³C and 1-¹³C) and two of pyruvate (1-¹³C and 2,3-¹³C) as metabolic tracers^{14,30}. We measured ¹³CO₂ accumulation in each jar three times over the course of 60, 90, 135, or 180 min at 20, 15, 10, or 5 °C, respectively. The ratios between ¹³CO₂ production rates from glucose and pyruvate isotopologues were calculated and used to model metabolic pathway activities and MGE (ref. 10; Supplementary Table 2). One complete replicate (that is, 4 temperatures × 2 soils × 4 isotopologues) was incubated and analysed each week. For more details and background information on metabolic probing and modelling, see Supplementary Section II and Supplementary Fig. 2.

Two weeks after the MGE measurements, another incubation was set up under identical conditions to measure respiration and MBC. Each of the four incubators was systematically assigned to one of the four treatment temperatures and both soils were incubated for seven days. After the seven-day incubation period, CO₂ concentrations were measured at 0 and 24 h. After the respiration measurement, MBC concentration was measured using chloroform fumigation-extraction (See Supplementary Section III and Supplementary Table 1).

We calculated microbial turnover using the experimentally measured respiration (*R*), MGE and MBC (Supplementary Section IV and Supplementary Fig. 3). We applied the assumptions that MBC was at steady state and that all turned-over MBC was released as CO₂. Our findings of temperature-sensitive turnover were not affected much by the non-steady state of MBC and whether C from turnover was released as CO₂ or added to the SOC pool (Supplementary Section V and Supplementary Figs 4 and 5).

The gross microbial production was calculated as

$$\Delta\text{MBC}_g = \text{MGE} \times R \tag{1}$$

1 and microbial turnover (τ) assuming steady state MBC pools and all C from
2 turnover going to CO₂ as follows

$$3 \quad \tau = \frac{\text{MGE} \times R}{\text{MBC}} \quad (2)$$

4 To calculate the effect of microbial turnover and incubation duration on MGE_A,
5 we used the following equation

$$6 \quad \text{MGE}_A = (1 - \tau)^n \times \text{MGE}_I \quad (3)$$

7 with n in days. In this calculation, MGE_I was set at 0.72 for all temperature
8 treatments, while turnover rates were those measured for mineral soil in this
9 experiment (Fig. 2). See Supplementary Section VI for more information.

10 We analysed all experimental data using a multifactor ANOVA with
11 temperature and soil type as the main factors. To calculate turnover from
12 experimental data, we used bootstrap resampling to calculate 95% confidence
13 intervals. Further details on all statistical analyses can be found in Supplementary
14 Section VII.

15 We modelled the consequences of accelerated microbial turnover with
16 warming, declining MGE with warming, and constant microbial turnover and
17 MGE using the AWB microbial model (Supplementary Section VIII and
18 Supplementary Table 3).

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21 References

- 22 1. Davidson, E. A. & Janssens, I. A. Temperature sensitivity of soil carbon
23 decomposition and feedbacks to climate change. *Nature* **440**,
24 165–173 (2006).
- 25 2. Cox, P. M., Betts, R. A., Jones, C. D., Spall, S. A. & Totterdell, I. J. Acceleration
26 of global warming due to carbon-cycle feedbacks in a coupled climate model.
27 *Nature* **408**, 184–187 (2000).
- 28 3. Schlesinger, W. H. & Andrews, J. A. Soil respiration and the global carbon cycle.
29 *Biogeochemistry* **48**, 7–20 (2000).
- 30 4. Ågren, G. I. & Wetterstedt, J. A. M. What determines the temperature
31 response of soil organic matter decomposition? *Soil Biol. Biochem.* **39**,
32 1794–1798 (2007).
- 33 5. Li, J. W., Wang, G. S., Allison, S. D., Mayes, M. A. & Luo, Y. Q. Soil carbon
34 sensitivity to temperature and carbon use efficiency compared across
35 microbial-ecosystem models of varying complexity. *Biogeochemistry* **119**,
36 67–84 (2014).
- 37 6. Allison, S. D., Wallenstein, M. D. & Bradford, M. A. Soil-carbon response to
38 warming dependent on microbial physiology. *Nature Geosci.* **3**, 336–340 (2010).
- 39 7. Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Ågren, G. I. Environmental
40 and stoichiometric controls on microbial carbon-use efficiency in soils. *New*
41 *Phytol.* **196**, 79–91 (2012).
- 42 8. Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L. & Richter, A. Carbon use
43 efficiency of microbial communities: Stoichiometry, methodology and
44 modelling. *Ecol. Lett.* **16**, 930–939 (2013).
- 45 9. Wieder, W. R., Bonan, G. B. & Allison, S. D. Global soil carbon projections
46 are improved by modelling microbial processes. *Nature Clim. Change* **3**,
47 909–912 (2013).
- 48 10. Schimel, J. P. & Schaeffer, S. M. Microbial control over carbon cycling in soil.
49 *Front. Microbiol.* **3**, <http://dx.doi.org/10.3389/fmicb.2012.00348> (2012).
- 50 11. Billings, S. A. & Ballantyne, F. How interactions between microbial resource
51 demands, soil organic matter stoichiometry, and substrate reactivity determine
52 the direction and magnitude of soil respiratory responses to warming.
53 *Glob. Change Biol.* **19**, 90–102 (2013).
- 54 12. Allison, S. D. A trait-based approach for modelling microbial litter
55 decomposition. *Ecol. Lett.* **15**, 1058–1070 (2012).
- 56 13. Manzoni, S., Schaeffer, S. M., Katul, G., Porporato, A. & Schimel, J. P. A
57 theoretical analysis of microbial eco-physiological and diffusion limitations to
58 carbon cycling in drying soils. *Soil Biol. Biochem.* **73**, 69–83 (2014).

14. Dijkstra, P. *et al.* Effect of temperature on metabolic activity of intact microbial
communities: Evidence for altered metabolic pathway activity but not for
increased maintenance respiration and reduced carbon use efficiency. *Soil Biol.*
Biochem. **43**, 2023–2031 (2011).
15. Steinweg, J. M., Plante, A. F., Conant, R. T., Paul, E. A. & Tanaka, D. L. Patterns
of substrate utilization during long-term incubations at different temperatures.
Soil Biol. Biochem. **40**, 2722–2728 (2008).
16. Tucker, C. L., Bell, J., Pendall, E. & Ogle, K. Does declining carbon-use
efficiency explain thermal acclimation of soil respiration with warming?
Glob. Change Biol. **19**, 252–263 (2013).
17. Frey, S. D., Lee, J., Melillo, J. M. & Six, J. The temperature response of soil
microbial efficiency and its feedback to climate. *Nature Clim. Change* **3**,
395–398 (2013).
18. Joergensen, R. G., Brookes, P. C. & Jenkinson, D. S. Survival of the soil microbial
biomass at elevated temperatures. *Soil Biol. Biochem.* **22**, 1129–1136 (1990).
19. McGill, W. B., Shields, J. A. & Paul, E. A. Relation between carbon and nitrogen
turnover in soil organic fractions of microbial origin. *Soil Biol. Biochem.* **7**,
57–63 (1975).
20. Kaiser, C., Franklin, O., Dieckmann, U. & Richter, A. Microbial community
dynamics alleviate stoichiometric constraints during litter decay. *Ecol. Lett.* **17**,
680–690 (2014).
21. Frey, S. D., Gupta, V., Elliott, E. T. & Paustian, K. Protozoan grazing affects
estimates of carbon utilization efficiency of the soil microbial community.
Soil Biol. Biochem. **33**, 1759–1768 (2001).
22. Roels, J. A. Application of macroscopic principles to microbial-metabolism.
Biotechnol. Bioeng. **22**, 2457–2514 (1980).
23. Gommers, P. J. F., Vanschie, B. J., Vandijken, J. P. & Kuenen, J. G. Biochemical
limits to microbial growth yields: An analysis of mixed substrate utilization.
Biotechnol. Bioeng. **32**, 86–94 (1988).
24. Van Bodegom, P. Microbial maintenance: A critical review on its
quantification. *Microb. Ecol.* **53**, 513–523 (2007).
25. Crowther, T. W. & Bradford, M. A. Thermal acclimation in widespread
heterotrophic soil microbes. *Ecol. Lett.* **16**, 469–477 (2013).
26. Blankinship, J. C., Niklaus, P. A. & Hungate, B. A. A meta-analysis of responses
of soil biota to global change. *Oecologia* **165**, 553–565 (2011).
27. Frey, S. D., Drijber, R., Smith, H. & Melillo, J. Microbial biomass, functional
capacity, and community structure after 12 years of soil warming. *Soil Biol.*
Biochem. **40**, 2904–2907 (2008).
28. Curtin, D., Beare, M. H. & Hernandez-Ramirez, G. Temperature and moisture
effects on microbial biomass and soil organic matter mineralization. *Soil Sci.*
Soc. Am. J. **76**, 2055–2067 (2012).
29. Manzoni, S. & Porporato, A. Soil carbon and nitrogen mineralization: Theory
and models across scales. *Soil Biol. Biochem.* **41**, 1355–1379 (2009).
30. Dijkstra, P. *et al.* Probing carbon flux patterns through soil microbial metabolic
networks using parallel position-specific tracer labeling. *Soil Biol. Biochem.* **43**,
126–132 (2011).

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Author contributions

S.B.H., P.D., E.S., B.A.H. and G.W.K. conceived the project, S.B.H. conducted the soil
incubation experiment and led the manuscript preparation. R.K.K. guided site selection
and provided the soils in the study. S.B.H., K.J.v.G. and P.D. contributed to data analysis
and interpretation. S.D.A. carried out the microbial-enzyme modelling. All authors
contributed to writing the final manuscript.

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Competing financial interests

The authors declare no competing financial interests.

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