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4 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 change¹³. However, the mechanisms underlying this prediction are not well understood, including how temperature affects microbial enzyme kinetics, growth efficiency (MGE), and turnover¹⁵. 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 warming^{6,8}, 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 warming³. More recent models that include microbial controls over decomposition suggest a wider range of potential responses⁵. These models reproduce current 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, and 20 °C. We used metabolic tracer probing to determine MGE¹⁴. In this method, MGE is calculated from the fate of individual C-atoms in glucose and pyruvate. Unlike other methods¹⁵⁻¹⁷, 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 modeling. 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 (Figure 1). Mean MGE was 0.72 ($\pm$ 0.01 SE, n = 22) in mineral soil and 0.71 ($\pm$ 0.01 SE, 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 a 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 has been observed in a pure culture experiment²⁵, but may not be observable in diverse soil communities where growth optimum temperatures can vary widely between microbial species¹¹. If the composition of the active microbial community shifts, higher maintenance costs might be avoided and MGE could be unchanged. It is also possible that the microbial community expresses physiological acclimation⁶.

Despite the constant MGE with temperature, higher temperatures increased microbial respiration in the mineral soil and organic soil by nearly 6-fold and 8-fold, respectively (Supplementary Figure S1). Across the same temperature range, specific respiration rate ($\mu\text{g CO}_2\text{-C mg}^{-1}\text{ MBC h}^{-1}$) increased by 540 % in the mineral soil and 630 % in the organic soil. Because increased respiration rates could not be explained by increased microbial biomass, warming must have affected microbial C metabolism by faster C consumption.

Higher specific respiration rates and constant MGE with increasing temperature indicate an increased production of new microbial biomass. Warming significantly increased MBC gross production rates ($0.97 \mu\text{g MBC g}^{-1}\text{ dry soil d}^{-1}\text{ }^{\circ}\text{C}^{-1}$, $r^2 = 0.99$ in mineral soil and $3.63 \mu\text{g MBC g}^{-1}\text{ dry soil d}^{-1}\text{ }^{\circ}\text{C}^{-1}$, $r^2 = 0.98$ in organic soil). However,

temperature did not change the MBC concentration ($p = 0.474$) in either soil (Supplementary Table S1). Therefore, warming increased microbial turnover ($p = 0.02$) in both soils by $0.004 \text{ d}^{-1} \text{ }^{\circ}\text{C}^{-1}$ in mineral soil and by $0.003 \text{ d}^{-1} \text{ }^{\circ}\text{C}^{-1}$ in organic soil (Figure 2), compensating for increased MBC production.

Why did warming increase microbial turnover? One possibility is that the abundance or activity of microbial predators and grazers increased with temperature. However, the few studies examining the effect of warming on microbial predator and grazer abundances have found both increases and decreases in abundances after several years of warming³⁶. Warming could cause a shift in the microbial community composition that drives faster turnover. Natural senescence of microbial cells may also be accelerated as protein turnover is increased at higher temperatures¹⁸. Alternatively, at higher temperatures and greater MBC productivity, activity of viruses could increase cell death. Each of these mechanisms may respond differently to temperature and could be important to informing our understanding of responses of soil C fluxes to temperature increases.

An increase in turnover with warming may partly explain the generally observed decline in MGE with temperature. Previous studies that suggest a decline in MGE did not separate the influences 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 addition of ^{13}C -labeled C compounds (instantaneous MGE or MGE_i). But over time, microbial turnover will cause some of the ^{13}C 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 $\text{MGE}^{16,21}$. 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, Figure 2).

We modeled the effects of assay duration and temperature on MGE_A (Figure 3a). Assuming an MGE_i of 0.72 for all temperatures and microbial turnover rates as determined in this study (Figure 2), we estimate that MGE_A declines by $0.005\text{ }^{\circ}\text{C}^{-1}$ in mineral (Figure 3b) and $0.003\text{ }^{\circ}\text{C}^{-1}$ in organic soil after a two-day incubation. Other studies have found that MGE declines by $0.009\text{ }^{\circ}\text{C}^{-1}$ (ref. 15) to $0.017\text{ }^{\circ}\text{C}^{-1}$ (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 (Figure 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 which provides the best conditions to test mechanistic questions like 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 (Figure 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.

In order to assess the implications of microbial turnover to soil C predictions, we used the Allison-Wallenstein-Bradford (AWB) model⁵⁶. 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 altered by temperature and soil C decomposition was modeled 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 (Figure 4). In the second scenario, MGE decreased by $0.016\text{ }^{\circ}\text{C}^{-1}$, as in prior theoretical studies⁶. Here, the reduction in MGE limited microbial growth at higher temperatures, resulting in a 5 % decline of $\text{MBC }^{\circ}\text{C}^{-1}$ averaged from 5 to $20\text{ }^{\circ}\text{C}$. 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 increase in SOC, which were larger than for the scenario of constant turnover and declines in MGE. We conclude that, although MGE did not decline, accelerated microbial turnover is an alternative mechanism that can moderate the effects of temperature on soil C stocks. These model simulations suggest that temperature-sensitive microbial turnover produces an effect on MBC and SOC that is not accounted for in current 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 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 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 (See Supplementary Methods Section I).

After a seven-day incubation period, MGE was determined using two position-specific ¹³C-labeled 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, and 5 °C respectively. The ratios between ¹³CO₂ production rates from glucose and pyruvate isotopologues were calculated and used to model metabolic pathway activities and MGE¹⁰ (Table S2). One complete replicate (i.e. 4 temperatures x 2 soils x 4 isotopologues) was incubated and

analyzed each week. For more details and background information on metabolic probing and modeling, see Supplementary Methods Section II and Figure S2.

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 Methods Section III, Table S1).

We calculated microbial turnover using the experimentally measured respiration (*R*), MGE, and MBC (Supplementary Methods Section IV, Figure S3). 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 non-steady state of MBC and whether C from turnover was released as CO₂ or added to the SOC pool (Supplementary Methods Section V, Figure S4 and S5).

The gross microbial production was calculated as

$$\Delta MBC_g = MGE * R,$$

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

$$\tau = \frac{MGE * R}{MBC}$$

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

221 $MGE_n = (1 - \tau)^n \times MGE_i$

222 with n in days. In this calculation, MGE_i was set at 0.72 for all temperature treatments,
223 while turnover rates were those measured for mineral soil in this experiment (Figure 2).
224 See supplementary methods section VI for more information.

225 We analyzed all experimental data using a multifactor ANOVA with temperature
226 and soil type as the main factors. To calculate turnover from experimental data, we used
227 bootstrap resampling to calculate 95 % confidence intervals. Additional details on all
228 statistical analyses can be found in Supplementary Methods Section VII.

229 We modeled the consequences of accelerated microbial turnover with warming,
230 declining MGE with warming, and constant microbial turnover and MGE using the
231 Allison-Wallenstein-Bradford microbial model (Supplementary Methods Section VIII,
232 Table S3).

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Author contributions

SBH, PD, ES, BAH, and GWK conceived the project, SBH conducted the soil incubation experiment and led the manuscript preparation. RK guided site selection and provided the soils in the study. SBH, KJvG, and PD contributed to data analysis and interpretation, and SDA did the microbial-enzyme modeling. All authors contributed to writing the final manuscript.

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348 **Additional information**

349 Supplementary information is available in the online version of the paper. Reprints
350 and permissions information is available online at www.nature.com/reprints.

351 Correspondence and requests for materials should be addressed to SBH.

352

353 **Competing financial interests**

354 The authors declare no competing financial interests

355

356

357 **Figure legends**

358 **Figure 1. Microbial Growth Efficiency (MGE) after a 7-day incubation at different**

359 **temperatures for a mineral and an organic soil.** Means and se (n = 6, except for

360 mineral soil at 5, 10 °C and organic soil at 5 °C, where n = 5). There was no significant

361 effect of soil type (p = 0.21) or temperature (p = 0.70) on MGE.

362

363 **Figure 2. Turnover rates (τ , d⁻¹) as a function of temperature for a mineral and an**

364 **organic soil.** The experimental values were resampled using bootstrap method in order to

365 calculate a 95 % confidence interval (error bars). For each soil type, the turnover rate at

366 5 °C is significantly different from that at 20 °C.

367

368 **Figure 3. Modeled effect of temperature and incubation duration on apparent**

369 **MGE.** a) The relationship between temperature and MGE_a over time was modeled using

the microbial turnover rates for the mineral soil in our study (Figure 2). b) The modeled relationship of MGE_a and temperature in mineral soil after two days.

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 Allison-Wallenstein-Bradford model. In the constant turnover, constant MGE scenario there is no change in MBC with temperature.

Temperature (°C)	MBC ($\mu\text{g g}^{-1}$ dry soil)		MBN ($\mu\text{g g}^{-1}$ dry soil)	
	Mineral Soil	Organic Soil	Mineral Soil	Organic Soil
5	276 $\pm$ 33	1,229 $\pm$ 117	37.7 $\pm$ 2.4	120 $\pm$ 9.4
10	236 $\pm$ 8.8	1,585 $\pm$ 200	35.8 $\pm$ 0.7	165 $\pm$ 32
15	282 $\pm$ 43	1,623 $\pm$ 170	36.9 $\pm$ 3.2	182 $\pm$ 16
20	263 $\pm$ 9.3	1,412 $\pm$ 81	35.0 $\pm$ 3.0	139 $\pm$ 7.7

Temperature (°C)	C _U /C ₁ Ratio of Glucose		C ₁ /C ₂₃ Ratio of Pyruvate	
	Mineral Soil	Organic Soil	Mineral Soil	Organic Soil
5	3.21 ± 0.31	2.92 ± 0.24	4.41 ± 0.40	3.56 ± 0.20
10	3.26 ± 0.15	2.91 ± 0.14	3.38 ± 0.20	3.73 ± 0.35
15	3.60 ± 0.39	2.94 ± 0.16	3.75 ± 0.30	3.51 ± 0.19
20	2.70 ± 0.12	3.15 ± 0.26	3.94 ± 0.46	3.61 ± 0.31

Parameter	Description	Value	Units
T_{ref}	Reference temperature	20	°C
MGE_{ref}	MGE at reference temperature	0.31	
m	MGE change with temperature	[0, -0.016]	°C ⁻¹
I_S	SOC input rate	0.00015	mg C g ⁻¹ soil h ⁻¹
I_D	DOC input rate	0.00001	mg C g ⁻¹ soil h ⁻¹
V_{ref}	SOC reference V_{max}	1	mg C mg ⁻¹ C h ⁻¹
$V_{U,ref}$	DOC uptake reference V_{max}	0.01	mg C mg ⁻¹ MBC h ⁻¹
K_{ref}	SOC reference K_m	250	mg C g ⁻¹ soil
$K_{U,ref}$	DOC uptake reference K_m	0.26	mg C g ⁻¹ soil
$r_{B,ref}$	Reference MBC turnover rate	0.00028	mg C mg ⁻¹ C h ⁻¹
Ea_V	SOC V_{max} activation energy	47	kJ mol ⁻¹ K ⁻¹
Ea_{VU}	Uptake V_{max} activation energy	47	kJ mol ⁻¹ K ⁻¹
Ea_K	SOC K_m activation energy	30	kJ mol ⁻¹ K ⁻¹
Ea_{KU}	Uptake K_m activation energy	30	kJ mol ⁻¹ K ⁻¹
Ea_r	MBC turnover activation energy	47	kJ mol ⁻¹ K ⁻¹
r_E	Enzyme production rate	5.6×10 ⁻⁶	mg C mg ⁻¹ MBC h ⁻¹

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5
6 **SUPPLEMENTARY METHODS**

7 ***I. Sample Collection and Incubation***

8 In October 2012, mineral and organic soil was collected from the Marcell
9 Experimental Forest near Grand Rapids, Minnesota on the same day. Organic soil
10 samples were taken from the top 40 cm of an ombrotrophic peatland (i.e. bog), dominated
11 by black spruce (*Picea mariana*) and covered with sphagnum moss (*Sphagnum* sp.). The
12 peat was a Greenwood peat (C content 54.5 %, N content 1.58 %). The mineral soil was a
13 Warba Fine Sandy Loam (C content 6.5 %, N content 0.34 %), collected from the A
14 horizon of a mixed hardwood forest dominated by aspen (*Populus* sp.), maple (*Acer* sp.),
15 and basswood (*Tilia americana*). Mean annual temperature at the site is 3 °C and mean
16 annual precipitation is 750 mm. Soil was shipped overnight on ice to Northern Arizona
17 University where the mineral soil was sieved (4 mm mesh). The organic soil was air-
18 dried to 400 % soil moisture content and live roots were removed by hand. Soil was
19 stored at 4 °C until the start of the experiment.

20 In April 2013, we incubated soil at four temperatures (5, 10, 15, and 20 °C) for
21 seven days before metabolic tracer addition in Precision™ Refrigerated Incubators
22 (Thermo Fisher Scientific Inc, Waltham, MA, USA). Each of the four incubators was
23 randomly assigned to one of four temperatures each week for six weeks, resulting in six

replicates of all soil x temperature x isotopologue combinations. For the organic soil incubated at 5 °C, one replicate was lost. Each replicate consisted of four jars, as is required for the metabolic tracer probing¹. Mineral soil (25 g dry weight, 66.5 % soil moisture content) and organic soil (4 g dry weight, 400 % soil moisture content) was incubated in specimen cups and placed in mason jars (473 mL). Mason jars were initially covered with SaranTM plastic wrap, to limit moisture loss but allow oxygen into the jars during the seven-day incubation period. We used iButton data loggers (Maxim Integrated, San Jose, CA, USA) to monitor soil temperature during the incubation and the metabolic tracer probing experiment.

II. Position-Specific ¹³C-Labeled Metabolic Tracer Experiment

After incubating the soil for one week, headspace was refreshed before the jar was closed using an airtight lid with a septum. Next, 10 ml of pure CO₂ was added to each jar in order to meet the CO₂ concentration and amount required by the Picarro G2201-*i* CO₂ cavity ring-down isotope spectrometer (Picarro Inc., Sunnyvale, CA, USA). Thirty minutes after addition of CO₂, we took a sample of the headspace (time=0). We then added one of four metabolic tracer isotopomers to each of the four parallel incubations per replicate, following the procedure in Dijkstra et al.¹². We used two glucose isotopologues (U-¹³C and 1-¹³C) and two pyruvate isotopologues (1-¹³C and 2,3-¹³C). All of the metabolic tracers were dissolved in deionized water at a concentration of 10.7 μmol C per mL. Two mL of tracer was added to each incubation, equivalent to 10.3 μg C g⁻¹ dry mineral soil and 64.3 μg C g⁻¹ dry organic soil. After tracer addition, 10 ml of headspace

was sampled three times at 20 min intervals in the 20 °C incubation, at 30 min intervals at 15 °C, at 45 min intervals at 10 °C, and at 60 min intervals at 5 °C.

All gas samples were analyzed on the Picarro G2201-*i* CO₂ cavity ringdown isotope spectrometer. The ratios of ¹³C production for each isotopologue pair were calculated as:

$$\frac{C_U}{C_1} \text{ ratio} = \frac{{}^{13}\text{CO}_2 \text{ production from U-}^{13}\text{C glucose}}{{}^{13}\text{CO}_2 \text{ production from 1-}^{13}\text{C glucose}} \quad (1)$$

and

$$\frac{C_1}{C_{2,3}} \text{ ratio} = \frac{{}^{13}\text{CO}_2 \text{ production from 1-}^{13}\text{C pyruvate}}{{}^{13}\text{CO}_2 \text{ production from 2,3-}^{13}\text{C pyruvate}} \quad (2)$$

The C_U/C_1 ratio for glucose and the $C_1/C_{2,3}$ ratio for pyruvate are determined by the characteristics of the central C metabolic network (i.e. glycolysis, citric acid cycle, and pentose phosphate pathway, Figure S2) which cause some C-atoms to be preferentially used for biosynthesis (for example in lipids and amino acids), while others are preferentially lost in decarboxylation reactions. If cells use substrate mainly for the production of ATP and very little for biosynthesis (substrate-limited microbial activity), the C_U/C_1 ratio for glucose will be close to 6:1 (all C-atoms are being released as CO₂), and the $C_1/C_{2,3}$ ratio of pyruvate be close to 1:2. Observed ratios (Table S2) for glucose and pyruvate were significantly different from the expected values for microbes without biosynthesis ($p < 0.05$).

The calculated ratios (Table S2) were used to model the metabolic flux patterns through the central C metabolic network as described in Dijkstra et al.¹ (Figure S2). It is assumed in this model that glucose is the only C substrate utilized by microbes. All model rates are expressed relative to glucose uptake (v_I), which is set at 100 moles. This

model has nine unknowns, seven of which are estimated using a known bacterial and fungal metabolite precursor demand². In this paper, we assumed a fungi : bacteria ratio of 50:50 for the modeled microbial community. Previous studies have shown that assumptions about microbial community composition do not much alter model MGE estimates^{2,3}, because the average precursor requirements are not that different between fungi and bacteria^{2,4}. The remaining two unknown model variables are then estimated using the observed isotopologue ratios of glucose and pyruvate. The Excel linear programming tool Solver was used to change the rates of $v14$ and $v10$ until modeled isotopomer ratios matched observed values. The final output of the model is relative rates for all 21 reactions of the central C metabolic network, which are used to calculate MGE. The MGE is calculated from the uptake rate and CO₂ producing reactions ($v10$, $v5$, $v7$ and $v8$) as:

$$MGE = \frac{6*v1 - \sum CO_2}{6*v1} \quad (3)$$

III. Respiration and Microbial Biomass Measurements

In a separate incubation two weeks after the final MGE measurement, we assessed the effects of temperature on respiration and microbial biomass C (MBC) concentration, and calculated the specific respiration rate ($\mu\text{g CO}_2\text{-C mg}^{-1}\text{ MBC h}^{-1}$)(n=4). In each incubator, we set up mason jars for each soil type following the same procedure that we used for the metabolic tracer incubation. After a one-week incubation, respiration rate was determined over 24 h using LICOR 6262 (LI-COR Biosciences, Lincoln, NE). Afterwards, we determined MBC using the chloroform-fumigation extraction method. Half of each sample was fumigated with chloroform for 7 days

(according to Haubensak et al.⁵) and extracted with 0.05 M K₂SO₄, while the other half was immediately extracted with K₂SO₄. The extracted salt solution was oven-dried at 60 °C until dry, and analyzed for %C and %N on an elemental analyzer with IRMS. The microbial biomass C and N were calculated as the difference between the fumigated and immediately extracted samples, expressed as mg C or N g⁻¹ dry soil (Table S1). We corrected microbial biomass C using an extraction efficiency (k_{EC}) of 0.45 for both soils⁶. We used the extraction efficiency for nitrogen (k_{EN}) of 0.54 proposed by Brookes et al.⁷.

IV. Calculation of Microbial Production and Turnover Rate.

In our calculations, we assumed that 1) the MBC pool was at steady state, so that net microbial growth was zero, and 2) all MBC that was turned over was turned into CO₂. In section V, we will assess the sensitivity of our results to these assumptions.

Total microbial respiration can be partitioned into

$$R = R_g + R_\tau \quad (4),$$

with R_g and R_τ as, the amount of C respired while making microbial biomass and C respired due to turnover (μg CO₂-C g⁻¹ soil d⁻¹) respectively.

New microbial biomass (ΔMBC_g; μg C g⁻¹ soil d⁻¹) is formed as follows,

$$\Delta MBC_g = \frac{MGE}{1-MGE} * R_g \quad (5).$$

Under steady state conditions for the microbial biomass pool, an equal amount of biomass is produced as is turned over and released as CO₂ (R_τ)

$$\Delta MBC_g = \tau * MBC = R_\tau \quad (6).$$

Where τ, is the proportion of the microbial community that is turned over (d⁻¹).

114 Therefore respiration from turnover (R_τ) is equal to:

$$115 \quad R_\tau = \frac{MGE}{1-MGE} * R_g \quad (7).$$

116 Combining equation 4 and equation 1, total respiration (R) is equal to:

$$117 \quad R = R_g + \frac{MGE}{1-MGE} * R_g \quad (8).$$

118 So respiration from creating new microbial biomass (R_g) can be calculated as:

$$119 \quad R_g = \frac{R}{1 + \frac{MGE}{1-MGE}} = R(1 - MGE) \quad (9).$$

120 And respiration from turnover (R_τ) is:

$$121 \quad R_\tau = R - R_g = R(1 - (1 - MGE)) = R * MGE \quad (10).$$

122 And turnover (d^{-1}) is calculated as flux of C out of MBC divided by MBC:

$$123 \quad \tau = \frac{R_\tau}{MBC} = \frac{R * MGE}{MBC} \quad (11).$$

124 A conceptual diagram of these equations is available in Figure S3.

125

126 ***V. Sensitivity of Turnover to Calculation Assumptions***

127 *1) Non-Steady State MBC Pool*

128 When the MBC pool is not at steady state, ΔMBC_g is divided over turnover

129 and net microbial growth. So, ΔMBC_g is calculated as before (eq. 2). A portion of this

130 C is added to the MBC pool (ΔMBC_n),

$$131 \quad \Delta MBC_n = \alpha * \Delta MBC_g \quad (12)$$

132 while the remainder is lost as CO_2 due to turnover:

$$133 \quad \Delta MBC_g - \Delta MBC_n = (1 - \alpha) \Delta MBC_g = \tau * MBC \quad (13).$$

134 When $\alpha=1$, all MBC produced is added to the existing MBC, and no C is

135 available for turnover. When $\alpha=0$, then all MBC that is produced is turned over

(steady state assumption described above). When $\alpha < 0$, a net decline in MBC occurs, and more C is available for turnover than is produced.

Under these conditions, R_τ is calculated as

$$\tau = \tau * MBC = (1 - \alpha) \Delta MBC_g \quad (14).$$

$$R_\tau = (1 - \alpha) \left(\frac{MGE}{1 - MGE} \right) * R_g \quad (15).$$

$$R = R_g + (1 - \alpha) \left(\frac{MGE}{1 - MGE} \right) * R_g \quad (16).$$

$$R_g = \frac{R}{1 + (1 - \alpha) \left(\frac{MGE}{1 - MGE} \right)} = \frac{R(1 - MGE)}{1 - \alpha * MGE} \quad (17).$$

$$R_\tau = R - R_g = R \left(1 - \frac{(1 - MGE)}{1 - \alpha * MGE} \right) \quad (18).$$

$$\tau = \frac{R_\tau}{MBC + 0.5 * DMBC_n} = \frac{R}{MBC + 0.5 * DMBC_n} \left(1 - \frac{1 - MGE}{1 - \alpha * MGE} \right) \quad (19).$$

We assessed the sensitivity of our results to the assumption that MBC was at steady state, by calculating turnover rate assuming that there had been a 20 % increase in MBC over our incubation period. This is the same as testing $\alpha = 0.2$. There was no significant difference between microbial turnover rate calculated with the assumption of steady state MBC and microbial turnover rate calculated with an assumed 20 % increase in MBC (Figure S4).

2) Fate of C from Turnover

In the following equations, MBC that is turned over is respired (R_τ) or added to the SOC pool (ΔSOC)

$$\tau * MBC = \Delta MBC_g = R_\tau + \Delta SOC \quad (20),$$

Introducing f as the fraction of microbial C that is being turned over into CO₂, and (1- f) as the fraction of microbial C that is turned over to dead SOC yields:

$$R_{\tau} = f * \tau * MBC = f * \Delta MBC_g \quad (21).$$

$$R_{\tau} = f \left(\frac{MGE}{1-MGE} \right) * R_g \quad (22).$$

$$R = R_g + f \left(\frac{MGE}{1-MGE} \right) * R_g \quad (23).$$

$$R_g = \frac{R}{1+f \left(\frac{MGE}{1-MGE} \right)} = \frac{R(1-MGE)}{1-(1-f)MGE} \quad (24).$$

$$R_{\tau} = R - R_g = R \left(1 - \frac{(1-MGE)}{1-(1-f)MGE} \right) \quad (25).$$

and

$$\tau = \frac{R_{\tau}}{f * MBC} = \frac{R}{f * MBC} \left(1 - \frac{(1-MGE)}{(1-(1-f)MGE)} \right) \quad (26).$$

We assessed the sensitivity of our results to the assumption that all turned over C is released as CO₂, by comparing calculated turnover rates under the “all C to CO₂” condition ($f=1$), with “all C to SOC pool” ($f=0$) and “C going for 50% to CO₂ and 50% to SOC” ($f=0.5$). When all or half the microbial turnover is directed to SOC, the calculated turnover rates are higher and the relationship with temperature is stronger (Figure S5). The assumption made in this experiment, that all turned over MBC goes to CO₂, represents the most conservative estimate of microbial turnover.

VI. Estimating Effects of Experiment Duration on MGE_A

In most studies⁸⁻¹⁰, MGE is determined by adding a stable or radioactive isotope labeled substrate, followed by measuring the incorporation of the label into microbial biomass. Here MGE is calculated as:

$$MGE = \frac{MBC}{S} \quad (27)$$

or

$$MGE = \frac{MBC}{MBC+R} \quad (28)$$

where MBC is the labeled microbial C produced from the substrate-C added (S), and R as the labeled C respired as CO₂. As pointed out by Frey et al.¹¹, the two definitions of MGE are similar, unless a portion of S remains in the soil solution, or if some of the initial labeled MBC ends up as dead organic matter, but is not released as CO₂. We make the assumption that all S is taken up and turned into MBC at t=0 with an instantaneous MGE (MGE_I) = 0.72. At time 0, MBC equals:

$$MBC_0 = MGE_I * S \quad (29)$$

However, as soon as new microbial biomass is produced, it becomes susceptible to turnover (either viruses, grazing or natural senescence). So MBC₁ at t=1 becomes

$$MBC_1 = MBC_0 - \tau * MBC_0 = (1 - \tau) * MBC_0 \quad (30)$$

with τ as the turnover rate (fraction of biomass that dies and is returned as CO₂ to the atmosphere and /or remains in the soil as dead organic matter). At t=2, MBC₂ becomes

$$MBC_2 = (1 - \tau) * MBC_1 = (1 - \tau)^2 * MBC_0 \quad (31)$$

So, at t=n

$$MBC_n = (1 - \tau)^n * MBC_0 = (1 - \tau)^n * MGE_I * S \quad (32)$$

and

$$MGE_A = \frac{((1-\tau)^n * MGE_I * S)}{S} = (1 - \tau)^n * MGE_I \quad (33)$$

197

198 ***VII. Statistical Analyses***

199 We performed a multifactor ANOVA on all experimental data using soil type and

incubation temperature as main factors. In two cases, the metabolic model could not find matches with the observed isotopomer ratios. This was the case with one of the replicates of mineral soil at 10 °C and mineral soil incubated at 15 °C, reducing the number of replicates to 5 for modeled metabolic rates and MGE for these treatments. Microbial biomass C and N data were log-transformed to meet the assumptions for ANOVA; the microbial biomass N data had one outlier that was excluded from statistics. The calculated microbial turnover and microbial production data were analyzed using a regression analyses on the means from the bootstrap resampling against temperature. Sensitivity analyses of assumptions used to calculate turnover were done using 95 % CI of calculated turnover rates within each soil x temperature combination and using an ANOVA on the regression of the mean turnover rates.

VIII. Microbial Enzyme Model

We made two modifications to the Allison-Wallenstein-Bradford (AWB) microbial model version in Li et al.¹². Instead of being constant, we made the microbial turnover rate (τ_B) an Arrhenius function of temperature:

$$\tau_B(T) = \tau_{B,ref} * \exp \left[\frac{-Ea_\tau}{R} * \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right] \quad (34)$$

where T is temperature (K), $\tau_{B,ref}$ is the microbial turnover rate at the reference temperature T_{ref} (20°C or 293 K), R is the ideal gas constant (8.314 J mol⁻¹ K⁻¹) and Ea_τ is the activation energy for microbial turnover (Table S3). The Arrhenius equation was used as we expect sensitivity of microbial turnover to temperature to be driven by biological or biochemical processes that do not usually respond linearly. The second modification was

to introduce a coefficient θ that determines the fraction of microbial turnover that enters soil carbon pools versus being respired to CO_2 . When $\theta = 1$, all dead microbial biomass enters soil carbon pools, and when $\theta = 0$, all dead biomass is respired to CO_2 . Although θ can vary, it was set to zero for all analyses reported here. The model equations are given below.

Microbial biomass (B) increases with DOC (D) uptake (F_U) times microbial growth efficiency (MGE) and declines with death (F_B) and enzyme production (F_E):

$$\frac{dB}{dt} = F_U * MGE - F_B - F_E \quad (35)$$

where assimilation is a Michaelis-Menten function scaled to the microbial biomass pool size:

$$F_U = \frac{V_U * B * D}{K_U + D} \quad (36)$$

and where E_C is a linear function of temperature with intercept MGE_{ref} and slope m :

$$E_C(T) = MGE_{ref} + m * (T - T_{ref}) \quad (37)$$

Microbial biomass turnover is modeled as a first-order process with the temperature-sensitive rate constant τ_B :

$$F_B = \tau_B * B \quad (38)$$

Enzyme production is modeled as a constant fraction (τ_E) of microbial biomass:

$$F_E = \tau_E * B \quad (39)$$

234 Temperature sensitivities for V , V_U , K , and K_U follow the Arrhenius relationship as in Eq.
 235 34. CO_2 respiration is the fraction of DOC that is not assimilated into MBC plus the
 236 respired fraction of microbial biomass turnover:

$$C_R = F_U * (1 - MGE) + F_B * (1 - \theta) \quad (40)$$

237 The enzyme pool (E) increases with enzyme production and decreases with enzyme
 238 turnover:

$$\frac{dE}{dt} = F_E - F_L \quad (41)$$

239 where enzyme turnover is modeled as a first-order process with a rate constant τ_L :

$$F_L = \tau_L * E \quad (42)$$

240 The SOC pool (S) increases with external inputs and a fraction of dead microbial biomass
 241 ($a_{BS} * \theta$) and decreases due to decomposition losses:

$$\frac{dS}{dt} = I_S + F_B * a_{BS} * \theta - F_S \quad (43)$$

242 where decomposition of SOC is catalyzed according to Michaelis-Menten kinetics by the
 243 enzyme pool:

$$F_S = \frac{V * E * S}{K + S} \quad (44)$$

244 The DOC pool receives external inputs, a fraction of dead microbial biomass, the
 245 decomposition flux, and dead enzymes, while assimilation of DOC by microbial biomass
 246 is subtracted:

$$\frac{dD}{dt} = I_D + F_B * (1 - a_{BS}) * \theta + F_S + F_L - F_U \quad (45)$$

247 The steady-state analytical solutions for SOC, DOC, MBC, and ENZC are given here:

$$S = \frac{-K * \tau_L * (x_1 * \tau_E + x_2 * MGE * \tau_B * \theta - I_S * \tau_B)}{\tau_E * ((I_S + I_D) * MGE * V + x_1 * \tau_L) + \tau_L * \tau_B * (x_2 * MGE * \theta - I_S)} \quad (46)$$

248 where

$$x_1 = I_S * (MGE - 1) \text{ and } x_2 = I_S - a_{BS} * (I_D + I_S) \quad (47)$$

$$D = \frac{-K_U * (\tau_B + \tau_E)}{\tau_B + \tau_E - MGE * V_U} \quad (48)$$

$$B = \frac{MGE * (I_D + I_S)}{(1 - MGE) * \tau_E + \tau_B * (1 - MGE * \theta)} \quad (49)$$

$$E = \frac{B * \tau_E}{\tau_L} \quad (50)$$

249 To generate Figure 4, we calculated the relative change in steady-state solutions for SOC
 250 and MBC at from 0 to 20°C under three scenarios with $\theta = 0$, meaning that all microbial
 251 turnover is respired as CO₂. For the “constant turnover, constant MGE” scenario, $Ea_i = 0$
 252 kJ mol⁻¹ and $m = 0$. For the “constant microbial turnover, declining MGE” scenario, $Ea_i =$

253 0 kJ mol^{-1} and $m = -0.016 \text{ }^{\circ}\text{C}^{-1}$. For the “increasing microbial turnover, constant MGE”

254 $Ea_r = 47 \text{ kJ mol}^{-1}$ and $m = 0$.

SUPPLEMENTARY NOTE

Thermodynamic Limits to MGE.

The value of MGE in soil microbial communities is important for our understanding of soil C cycling processes. This value is explicitly or implicitly part of soil C cycling models, usually a constant value is used^{8,13,14} ranging from 0.15 to 0.60 (ref. 13).

Efforts to predict MGE from thermodynamic and chemical principles have been ongoing for several decades^{15,16}. Experimental data are mostly limited to pure culture studies where substrate availability is usually high relative to substrate availabilities in natural environments. MGE is limited by thermodynamic constraints¹⁴. The theoretical maximum value of MGE was calculated in several studies. The thermodynamic maximum yield can be predicted from the ratio between the degree of reduction of the substrate (e.g., glucose $\gamma_s = 4$ or formate $\gamma_s=2$) and product, (γ_p biomass ~ 4.2)¹⁷. The MGE_{max} is about 0.95 (ref. 13, 16). A second theoretical maximum is defined by the cost of making new biomass. This yields an MGE_{max} from glucose of about 0.88 (ref. 4). The observed MGE values in this study (MGE ranged from 0.67 to 0.75) were lower than the MGE_{max} values identified above, but higher than the average thermodynamic efficiency in pure culture studies^{13,14,16}.

Variability of yield (MGE) in culture studies spans almost two orders of magnitude from 0.01 to 0.8 (ref. 15-19) associated with species differences, substrate type and concentration, and environmental factors. Experimental values for MGE in pure culture studies are always lower than the theoretical maximal yield values described above. The ratio between experimentally observed MGE_{max} and the theoretical MGE_{max}

278 is called the thermodynamic efficiency¹³⁻¹⁶. This value is used as a “first approximation”
279 according to Roels 1980¹⁶, and should not be mistaken for a theoretical thermodynamic
280 maximal yield, as higher values have been observed in pure culture studies^{15,16,18,19} and
281 soil and aquatic ecosystems (ranging from close to zero to >0.8 for both environments¹³).

SUPPLEMENTARY TABLES

Table S1. Microbial biomass C (MBC) and microbial biomass N (MBN) means and standard error for four incubation temperatures and two soil types (n=4). Microbial biomass was calculated using $k_{EC}= 0.45$ and $k_{EN}= 0.54$.

Temperature (°C)	MBC ($\mu\text{g g}^{-1}$ dry soil)		MBN ($\mu\text{g g}^{-1}$ dry soil)	
	Mineral Soil	Organic Soil	Mineral Soil	Organic Soil
5	276 $\pm$ 33	1,229 $\pm$ 117	37.7 $\pm$ 2.4	120 $\pm$ 9.4
10	236 $\pm$ 8.8	1,585 $\pm$ 200	35.8 $\pm$ 0.7	165 $\pm$ 32
15	282 $\pm$ 43	1,623 $\pm$ 170	36.9 $\pm$ 3.2	182 $\pm$ 16
20	263 $\pm$ 9.3	1,412 $\pm$ 81	35.0 $\pm$ 3.0	139 $\pm$ 7.7

289 **Table S2.** Glucose and pyruvate isotopomer ratios for mineral and organic soil at 5, 10,
 290 15, and 20 °C (means ± standard error, n=6 except organic soil at 5 °C; n=5).

Temperature (°C)	C _U /C ₁ Ratio of Glucose		C ₁ /C ₂₃ Ratio of Pyruvate	
	Mineral Soil	Organic Soil	Mineral Soil	Organic Soil
5	3.21 ± 0.31	2.92 ± 0.24	4.41 ± 0.40	3.56 ± 0.20
10	3.26 ± 0.15	2.91 ± 0.14	3.38 ± 0.20	3.73 ± 0.35
15	3.60 ± 0.39	2.94 ± 0.16	3.75 ± 0.30	3.51 ± 0.19
20	2.70 ± 0.12	3.15 ± 0.26	3.94 ± 0.46	3.61 ± 0.31

291

292 **Table S3.** Microbial- enzyme model parameter descriptions, values, and units.

Parameter	Description	Value	Units
T_{ref}	Reference temperature	20	°C
MGE_{ref}	MGE at reference temperature	0.31	
m	MGE change with temperature	[0, -0.016]	°C ⁻¹
I_S	SOC input rate	0.00015	mg C g ⁻¹ soil h ⁻¹
I_D	DOC input rate	0.00001	mg C g ⁻¹ soil h ⁻¹
V_{ref}	SOC reference V_{max}	1	mg C mg ⁻¹ C h ⁻¹
$V_{U,ref}$	DOC uptake reference V_{max}	0.01	mg C mg ⁻¹ MBC h ⁻¹
K_{ref}	SOC reference K_m	250	mg C g ⁻¹ soil
$K_{U,ref}$	DOC uptake reference K_m	0.26	mg C g ⁻¹ soil
$\tau_{B,ref}$	Reference MBC turnover rate	0.00028	mg C mg ⁻¹ C h ⁻¹
Ea_V	SOC V_{max} activation energy	47	kJ mol ⁻¹ K ⁻¹
Ea_{VU}	Uptake V_{max} activation energy	47	kJ mol ⁻¹ K ⁻¹
Ea_K	SOC K_m activation energy	30	kJ mol ⁻¹ K ⁻¹
Ea_{KU}	Uptake K_m activation energy	30	kJ mol ⁻¹ K ⁻¹
Ea_τ	MBC turnover activation energy	47	kJ mol ⁻¹ K ⁻¹
τ_E	Enzyme production rate	5.6×10^{-6}	mg C mg ⁻¹ MBC h ⁻¹
τ_L	Enzyme loss rate	0.001	mg C mg ⁻¹ C h ⁻¹
a_{BS}	Fraction of dead MBC partitioned to SOC	0.5	
θ	Fraction of dead MBC transferred to soil pools	0	

293

Figure S1. Response of a) soil respiration rate and b) specific respiration rate of mineral and organic soil to temperature (means and se; some standard errors are smaller than data points).

Figure S2. Model for metabolic processes in soil microbial communities. Flux rates (v2-v21) are normalized relative to glucose uptake (v1, set at 100 moles). Insert depicts details of the pentose phosphate pathway. Abbreviations: G6P, glucose-6-phosphate; F1,6P, fructose-1,6-phosphate; GAP, glyceraldehyde-phosphate; PYR, pyruvate; ACCO, acetyl-CoA; ICIT, isocitrate; α KG, α -ketoglutarate; OAA, oxaloacetate; RU5P, ribulose-5-phosphate; S7P, sedoheptulose-7-phosphate; E4P, erythrose-4-phosphate. Reprinted from ref. 1.

Figure S3. Schematic showing relationships between measured (total respiration, MGE, and MBC pool) and calculated values (production rate and turnover rate). Total respiration rate is the sum of respiration from turnover and growth ($R = R_g + R_T$).

Figure S4. Effect of change in MBC on calculated microbial turnover rates at each temperature for a) mineral soil and b) organic soil (means and 95% CI).

319

320 **Figure S5.** Effect of changing the fate of turned over MBC on calculated microbial

321 turnover rates for a) mineral soil and b) organic soil (means and 95% CI).

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