Title
Accelerated microbial turnover but constant growth efficiency with warming in soil

Journal
Nature Climate Change, 4(10)

ISSN
1758-678X

Authors
Hagerty, Shannon B
van Groenigen, Kees Jan
Allison, Steven D
et al.

Publication Date
2014-10-01

DOI
10.1038/nclimate2361

Peer reviewed

Title page

Title

Accelerated microbial turnover but constant growth efficiency with warming in soil

Author affiliations

Shannon B. Hagerty[1*], Kees Jan van Groenigen[1,2], Steven D. Allison[3], Bruce A. Hungate[1,2], Egbert Schwartz[1], George W. Koch[1,2], Randall K. Kolka[4], and Paul Dijkstra[1,2]

[1]Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011

[2] Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, Arizona 86011

[3] Department of Ecology and Evolutionary Biology and Department of Earth System Science, University of California, Irvine, California 92697

[4] USDA Forest Service Northern Research Station, Grand Rapids, Minnesota 55744

* email: sbhagerty@gmail.com

Rising temperatures are expected to reduce global soil carbon (C) stocks, driving a positive feedback to climate change[1-3]. 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 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 warming[2]. More recent models that include microbial controls over decomposition suggest a wider range of potential responses[5]. These models reproduce current soil C stocks more accurately than models that do not incorporate microbial dynamics[9], but their ability to predict soil C responses to climate change is hampered by uncertainty in the temperature sensitivity of microbial processes[4]. 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 reactions[1], 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 biomass[8]. However, responses of MGE in soil microbial communities are equivocal, with studies reporting decreased MGE with temperature increase[15,16], no change[14], or a variable response based on substrate type[17]. It is unclear to what extent this variability is caused by the methods and procedures used for measuring MGE in soil[8]. Second, warming can affect microbial turnover rates[18]. 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

61 by living microbes[19]. Consequently accelerated turnover can increase respiration per unit
62 of MBC even when MGE remains the same[20]. However, most studies of MGE responses
63 to warming do not account for the respiration and cell death that result from turnover[15-17]

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

75 We found that MGE was not sensitive to temperature (Figure 1). Mean MGE was
76 0.72 (± 0.01 SE, n = 22) in mineral soil and 0.71 (± 0.01 SE, n = 21) in organic soil.
77 Across all temperature treatments and replicates MGE ranged between 0.67 and 0.75.
78 These values for MGE are high relative to the average values observed in soils and other
79 ecosystems[7,8,21]. It is also higher than 0.6, an average maximum MGE value for pure
80 culture studies[8,22] (for further discussion on theoretical thermodynamic constraints of
81 MGE, see Supplementary Note). This high value suggests that the active microbial
82 community functions at a high biochemical efficiency and microorganisms with
83 relatively high maintenance costs contribute little to the total activity. High efficiency

84 values may also indicate additional energy sources (for example from oxalate or
85 formate[23]), or direct incorporation of large amounts of cellular compounds, such as amino
86 acids[14]. However, what little information is available suggests that these effects will be
87 only slightly affected by temperatures[17].
88 Microbial growth efficiency is generally expected to decline as a result of
89 increased microbial maintenance costs at higher temperatures[6,7,24]. This effect of
90 temperature on maintenance energy has been observed in a pure culture experiment[25], but
91 may not be observable in diverse soil communities where growth optimum temperatures
92 can vary widely between microbial species[11]. If the composition of the active microbial
93 community shifts, higher maintenance costs might be avoided and MGE could be
94 unchanged. It is also possible that the microbial community expresses physiological
95 acclimation[6].
96 Despite the constant MGE with temperature, higher temperatures increased
97 microbial respiration in the mineral soil and organic soil by nearly 6-fold and 8-fold,
98 respectively (Supplementary Figure S1). Across the same temperature range, specific
99 respiration rate (μg CO_2-C mg^{-1} MBC h^{-1}) increased by 540 % in the mineral soil and 630
100 % in the organic soil. Because increased respiration rates could not be explained by
101 increased microbial biomass, warming must have affected microbial C metabolism by
102 faster C consumption.
103 Higher specific respiration rates and constant MGE with increasing temperature
104 indicate an increased production of new microbial biomass. Warming significantly
105 increased MBC gross production rates (0.97 μg MBC g^{-1} dry soil d^{-1} $°C^{-1}$, $r^2 = 0.99$ in
106 mineral soil and 3.63 μg MBC g^{-1} dry soil d^{-1} $°C^{-1}$, $r^2 = 0.98$ in organic soil). However,

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

111 Why did warming increase microbial turnover? One possibility is that the
112 abundance or activity of microbial predators and grazers increased with temperature.
113 However, the few studies examining the effect of warming on microbial predator and
114 grazer abundances have found both increases and decreases in abundances after several
115 years of warming[26]. Warming could cause a shift in the microbial community composition
116 that drives faster turnover. Natural senescence of microbial cells may also be accelerated
117 as protein turnover is increased at higher temperatures[18]. Alternatively, at higher
118 temperatures and greater MBC productivity, activity of viruses could increase cell death.
119 Each of these mechanisms may respond differently to temperature and could be important
120 to informing our understanding of responses of soil C fluxes to temperature increases.

121 An increase in turnover with warming may partly explain the generally observed
122 decline in MGE with temperature. Previous studies that suggest a decline in MGE did not
123 separate the influences of turnover and MGE on the residence time of carbon tracers in
124 the soil microbial biomass. Ideally, MGE is determined during a very short period after
125 addition of ^{13}C-labeled C compounds (instantaneous MGE or MGE_i). But over time,
126 microbial turnover will cause some of the ^{13}C initially incorporated into microbial biomass
127 to be released as CO_2, resulting in an overestimation of CO_2 production and an
128 underestimation of microbial biomass production and MGE[16,21]. This effect increases with

129 incubation duration and may cause differences in apparent MGE (MGE_A), especially
130 when microbial turnover rates differ between treatments (as in this study, Figure 2).
131 We modeled the effects of assay duration and temperature on MGE_A (Figure 3a).
132 Assuming an MGE_I of 0.72 for all temperatures and microbial turnover rates as
133 determined in this study (Figure 2), we estimate that MGE_A declines by 0.005 $°C^{-1}$ in
134 mineral (Figure 3b) and 0.003 $°C^{-1}$ in organic soil after a two-day incubation. Other
135 studies have found that MGE declines by 0.009 $°C^{-1}$ (ref. 15) to 0.017 $°C^{-1}$ (ref. 1) when
136 measuring MGE over 24-48 h. These rates of decline with temperature are greater than
137 those in this study, however it remains unclear whether this is associated with higher
138 turnover rates in those studies or with genuine declines in MGE_I. Studies that have used
139 short-term assays (<6 h) reported no change in MGE of soil microbial communities with
140 warming[14,17], consistent with results we report here (Figure 1).
141 We found that microbial turnover rate is temperature sensitive, but that MGE is
142 not. These results were determined in a short-term laboratory incubation, a controlled
143 environment which provides the best conditions to test mechanistic questions like those
144 in this study. On a longer time scale, turnover rates and MGE could be indirectly
145 affected by temperature through nutrient limitation, changes in community composition,
146 and changes in soil moisture. It is also likely that across a large spatial scale turnover
147 rates will vary; we saw differences in turnover rate between the two soils studied here
148 (Figure 2). Other studies have found that warming decreases MBC, indicating
149 accelerated microbial turnover could be important at time scales longer than in this
150 study[27,28]. However, accelerated microbial turnover in response to warming is a mechanism
151 that has never been explicitly accounted for in soil carbon models.

152 In order to assess the implications of microbial turnover to soil C predictions, we
153 used the Allison-Wallenstein-Bradford (AWB) model[5,6]. The AWB model uses rates of
154 microbial processes that are based on the best estimate of steady state conditions, which
155 allowed us to extrapolate the significance of our short-term results to long-term steady-
156 state C stocks. We simulated three different scenarios. In the first scenario, neither MGE
157 nor turnover was altered by temperature and soil C decomposition was modeled with a
158 first-order decay function and Michaelis-Menten enzyme kinetics, the current assumption
159 in most biogeochemical models[7,29]. In this scenario there was no change in MBC with
160 warming and SOC declined as a result of accelerated enzymatic decomposition (Figure
161 4). In the second scenario, MGE decreased by 0.016 °C^{-1}, as in prior theoretical studies[6].
162 Here, the reduction in MGE limited microbial growth at higher temperatures, resulting in
163 a 5 % decline of MBC °C^{-1} averaged from 5 to 20 °C. As a result, SOC increased with
164 temperature as decomposition became limited by MBC. The third scenario corresponded
165 to our experimental observations of a constant MGE and accelerated microbial turnover
166 with warming. Accelerated microbial turnover at higher temperature caused decreases in
167 MBC and increase in SOC, which were larger than for the scenario of constant turnover
168 and declines in MGE. We conclude that, although MGE did not decline, accelerated
169 microbial turnover is an alternative mechanism that can moderate the effects of
170 temperature on soil C stocks. These model simulations suggest that temperature-sensitive
171 microbial turnover produces an effect on MBC and SOC that is not accounted for in
172 current biogeochemical or microbial models.
173 Our results show that accelerated enzyme kinetics and increased microbial
174 turnover are the main mechanisms associated with an increased respiration at higher

175 temperatures and, in model simulations, lead to a small increase in SOC content under
176 elevated temperatures. This effect on SOC is similar to those that have been predicted in
177 models assuming a decline in MGE, but differs in direction from the predictions
178 traditional biogeochemical models. Consequently, soil microbial models should include a
179 temperature-sensitive microbial turnover rate. The lack of temperature sensitivity in
180 MGE, which is controlled at the cellular level, suggests that microbial biochemical
181 efficiency is a weak control on soil C dynamics.
182
183 **Methods**
184 Soil samples were collected in October 2012 from the Marcell Experimental
185 Forest in Grand Rapids, MN (MAT = 3°C, MAP = 750 mm). Mineral soil samples were
186 collected from the A horizon in a hardwood forest and organic soil samples were
187 collected from an ombrotrophic peatland (top 40 cm after removing the living layer of
188 moss). Soil samples were stored at 4 °C until the experiment began in April 2013.
189 Replicates (n = 6) from both soils were randomly assigned to one of four incubators and
190 incubated for seven days at 5, 10, 15, or 20 °C (See Supplementary Methods Section I).
191 After a seven-day incubation period, MGE was determined using two position-
192 specific ^{13}C-labeled isotopologues of glucose (U-^{13}C and 1-^{13}C) and two of pyruvate (1-^{13}C
193 and 2,3-^{13}C) as metabolic tracers[14,30]. We measured $^{13}CO_2$ accumulation in each jar three
194 times over the course of 60, 90, 135, or 180 min at 20, 15, 10, and 5 °C respectively. The
195 ratios between $^{13}CO_2$ production rates from glucose and pyruvate isotopologues were
196 calculated and used to model metabolic pathway activities and MGE[10] (Table S2). One
197 complete replicate (i.e. 4 temperatures x 2 soils x 4 isotopologues) was incubated and

198 analyzed each week. For more details and background information on metabolic probing
199 and modeling, see Supplementary Methods Section II and Figure S2.
200 Two weeks after the MGE measurements, another incubation was set up under
201 identical conditions to measure respiration and MBC. Each of the four incubators was
202 systematically assigned to one of the four treatment temperatures and both soils were
203 incubated for seven days. After the seven-day incubation period, CO_2 concentrations
204 were measured at 0 and 24 h. After the respiration measurement, MBC concentration was
205 measured using chloroform fumigation-extraction (See Supplementary Methods Section
206 III, Table S1).
207 We calculated microbial turnover using the experimentally measured respiration
208 (R), MGE, and MBC (Supplementary Methods Section IV, Figure S3). We applied the
209 assumptions that MBC was at steady state and that all turned over MBC was released as
210 CO_2. Our findings of temperature-sensitive turnover were not affected much by non-
211 steady state of MBC and whether C from turnover was released as CO_2 or added to the
212 SOC pool (Supplementary Methods Section V, Figure S4 and S5).
213 The gross microbial production was calculated as
214 $$\Delta MBC_g = MGE * R,$$
215 and microbial turnover (τ) assuming steady state MBC pools and all C from turnover
216 going to CO_2 as follows
217 $$\tau = \frac{MGE*R}{MBC}$$
218
219 To calculate the effect of microbial turnover and incubation duration on MGE_A, we used
220 the following equation

$$MGE_A = (1-\tau)^n \times MGE_I$$

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

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

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

Literature Cited

1. Davidson, E. A. & Janssens, I. A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**, 165-173, doi:10.1038/nature04514 (2006).

2. Cox, P. M., Betts, R. A., Jones, C. D., Spall, S. A. & Totterdell, I. J. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* **408**, 184-187, doi:10.1038/35041539 (2000).

3. Schlesinger, W. H. & Andrews, J. A. Soil respiration and the global carbon cycle. *Biogeochemistry* **48**, 7-20, doi:10.1023/a:1006247623877 (2000).

4. Ågren, G. I. & Wetterstedt, J. A. M. What determines the temperature response of soil organic matter decomposition? *Soil Biology & Biochemistry* **39**, 1794-1798, doi:10.1016/j.soilbio.2007.02.007 (2007).

5. Li, J. W., Wang, G. S., Allison, S. D., Mayes, M. A. & Luo, Y. Q. Soil carbon sensitivity to temperature and carbon use efficiency compared across microbial-ecosystem models of varying complexity. *Biogeochemistry* **119**, 67-84, doi:10.1007/s10533-013-9948-8 (2014).

6. Allison, S. D., Wallenstein, M. D. & Bradford, M. A. Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience* **3**, 336-340, doi:10.1038/ngeo846 (2010).

7. Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Ågren, G. I. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist* **196**, 79-91, doi:10.1111/j.1469-8137.2012.04225.x (2012).

8. Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L. & Richter, A. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters* **16**, 930-939, doi:10.1111/ele.12113 (2013).

9. Wieder, W. R., Bonan, G. B. & Allison, S. D. Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change* **3**, 909-912, doi:10.1038/nclimate1951 (2013).

10. Schimel, J. P. & Schaeffer, S. M. Microbial control over carbon cycling in soil. *Frontiers in Microbiology* **3**, doi:10.3389/fmicb.2012.00348 (2012).

11. Billings, S. A. & Ballantyne, F. How interactions between microbial resource demands, soil organic matter stoichiometry, and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming. *Global Change Biology* **19**, 90-102, doi:10.1111/gcb.12029 (2013).

12. Allison, S. D. A trait-based approach for modelling microbial litter decomposition. *Ecology Letters* **15**, 1058-1070, doi:10.1111/j.1461-0248.2012.01807.x (2012).

13. Manzoni, S., Schaeffer, S. M., Katul, G., Porporato, A. & Schimel, J. P. A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. *Soil Biology & Biochemistry* **73**, 69-83, doi:10.1016/j.soilbio.2014.02.008 (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*

Biology & Biochemistry **43**, 2023-2031, doi:10.1016/j.soilbio.2011.05.018 (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 Biology & Biochemistry* **40**, 2722-2728, doi:10.1016/j.soilbio.2008.07.002 (2008).

16. Tucker, C. L., Bell, J., Pendall, E. & Ogle, K. Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? *Global Change Biology* **19**, 252-263, doi:10.1111/gcb.12036 (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 Climate Change* **3**, 395-398, doi:10.1038/nclimate1796 (2013).

18. Joergensen, R. G., Brookes, P. C. & Jenkinson, D. S. Survival of the soil microbial biomass at elevated-temperatures. *Soil Biology & Biochemistry* **22**, 1129-1136, doi:10.1016/0038-0717(90)90039-3 (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 Biology & Biochemistry* **7**, 57-63, doi:10.1016/0038-0717(75)90032-2 (1975).

20. Kaiser, C., Franklin, O., Dieckmann, U. & Richter, A. Microbial community dynamics alleviate stoichiometric constraints during litter decay. *Ecology Letters* **17**, 680-690, doi:10.1111/ele.12269 (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*

303 *Biology & Biochemistry* **33**, 1759-1768, doi:10.1016/s0038-0717(01)00101-
304 8 (2001).
305 22. Roels, J. A. Application of macroscopic principles to microbial-metabolism.
306 *Biotechnology and Bioengineering* **22**, 2457-2514,
307 doi:10.1002/bit.260221202 (1980).
308 23. Gommers, P. J. F., Vanschie, B. J., Vandijken, J. P. & Kuenen, J. G. Biochemical limits
309 to microbial-growth yields - an analysis of mixed substrate utilization.
310 *Biotechnology and Bioengineering* **32**, 86-94, doi:10.1002/bit.260320112
311 (1988).
312 24. van Bodegom, P. Microbial maintenance: A critical review on its quantification.
313 *Microbial Ecology* **53**, 513-523, doi:10.1007/s00248-006-9049-5 (2007).
314 25. Crowther, T. W. & Bradford, M. A. Thermal acclimation in widespread
315 heterotrophic soil microbes. *Ecology Letters* **16**, 469-477,
316 doi:10.1111/ele.12069 (2013).
317 26. Blankinship, J. C., Niklaus, P. A. & Hungate, B. A. A meta-analysis of responses of
318 soil biota to global change. *Oecologia* **165**, 553-565, doi:10.1007/s00442-
319 011-1909-0 (2011).
320 27. Frey, S. D., Drijber, R., Smith, H. & Melillo, J. Microbial biomass, functional
321 capacity, and community structure after 12 years of soil warming. *Soil*
322 *Biology & Biochemistry* **40**, 2904-2907, doi:10.1016/j.soilbio.2008.07.020
323 (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, doi:10.2136/sssaj2012.0011 (2012).

29. Manzoni, S. & Porporato, A. Soil carbon and nitrogen mineralization: Theory and models across scales. *Soil Biology & Biochemistry* **41**, 1355-1379, doi:10.1016/j.soilbio.2009.02.031 (2009).

30. Dijkstra, P. *et al.* Probing carbon flux patterns through soil microbial metabolic networks using parallel position-specific tracer labeling. *Soil Biology & Biochemistry* **43**, 126-132, doi:10.1016/j.soilbio.2010.09.022 (2011).

Acknowledgements

E. Miller contributed to experimental work and N. Aspelin assisted with soil sample collection. This research is supported by an NSF grant (DEB-1146449) to Paul Dijkstra and NSF MRI (DBI-0723250 and 1126840) to George Koch and Tom Whitham.

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.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to SBH.

Competing financial interests

The authors declare no competing financial interests

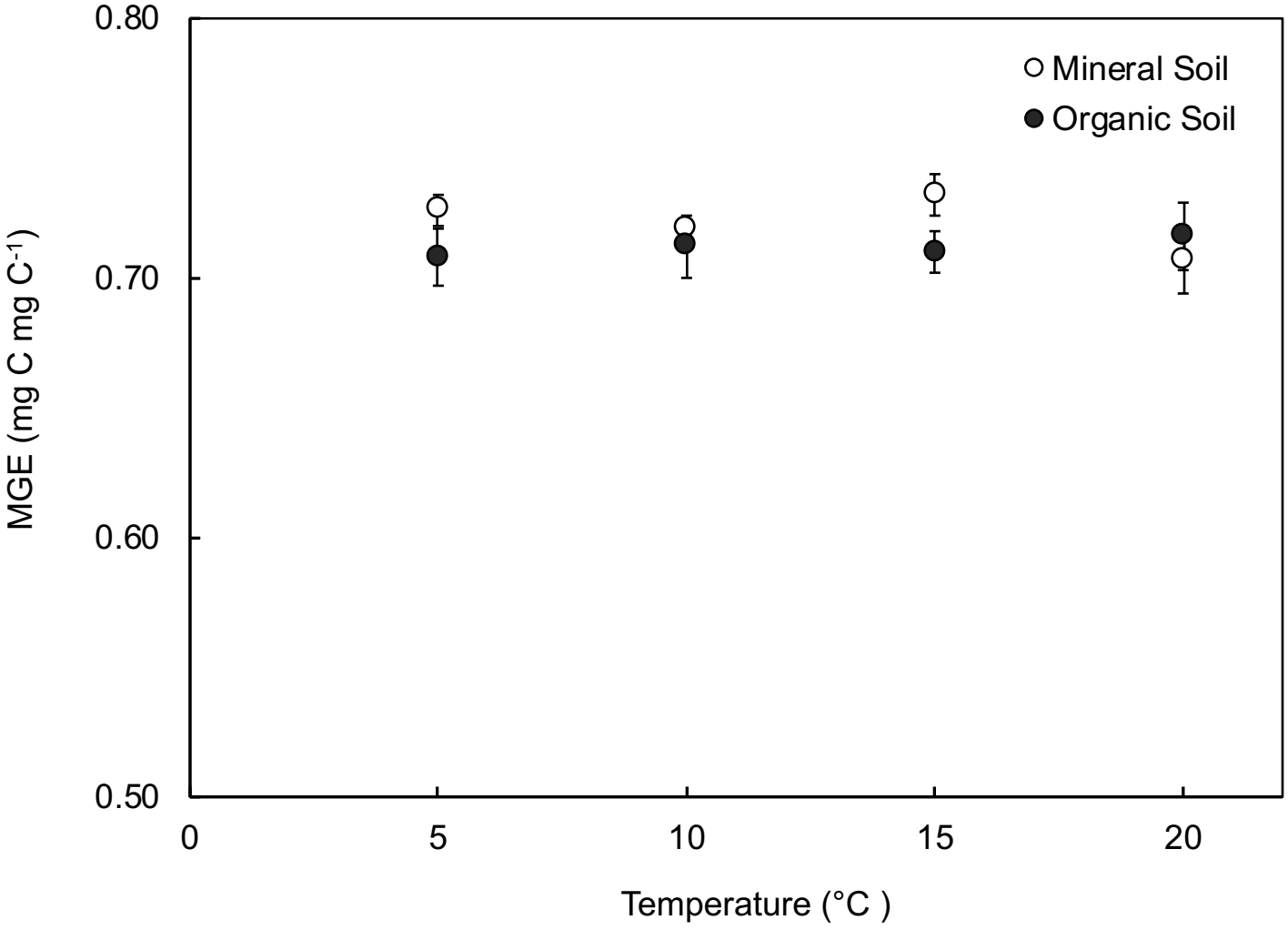
Figure legends

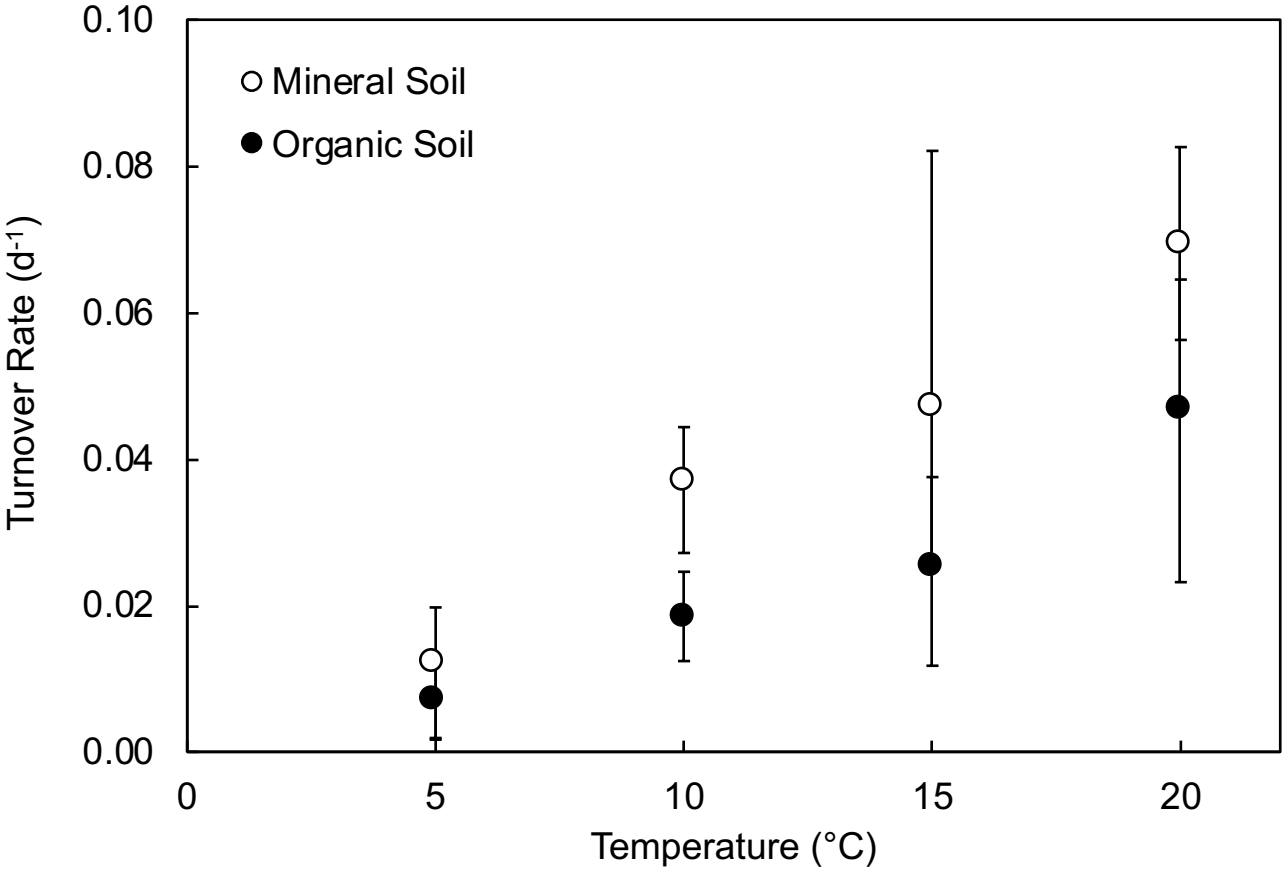
Figure 1. Microbial Growth Efficiency (MGE) after a 7-day incubation at different temperatures for a mineral and an organic soil. Means and se (n = 6, except for mineral soil at 5, 10 °C and organic soil at 5 °C, where n = 5). There was no significant effect of soil type ($p = 0.21$) or temperature ($p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

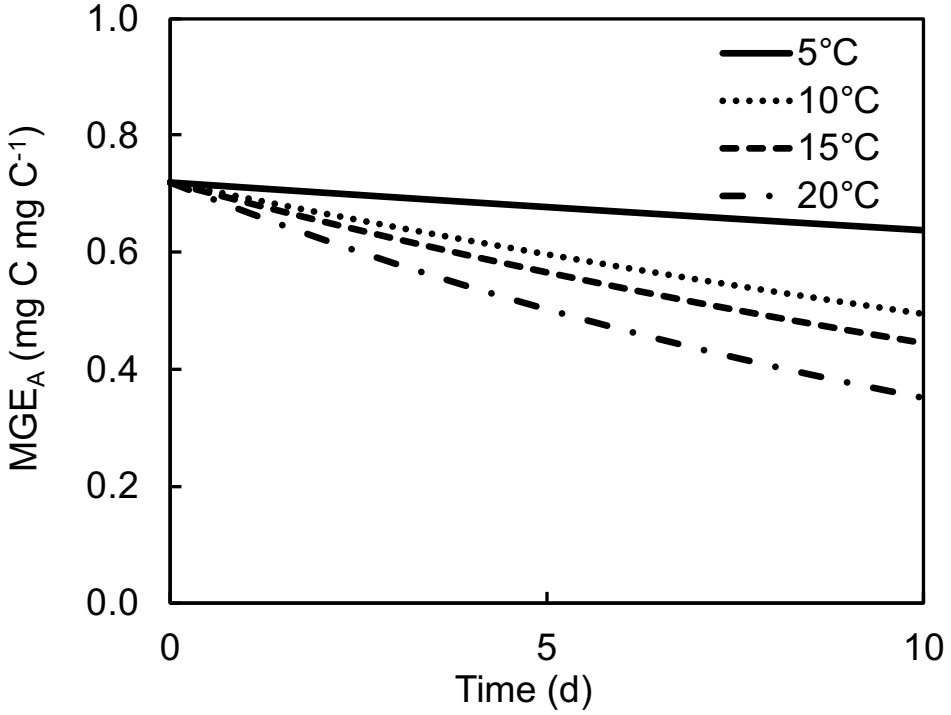
Figure 2. Turnover rates (τ, d^{-1}) as a function of temperature for a mineral and an organic soil. The experimental values were resampled using bootstrap method in order to calculate a 95 % confidence interval (error bars). For each soil type, the turnover rate at 5 °C is significantly different from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

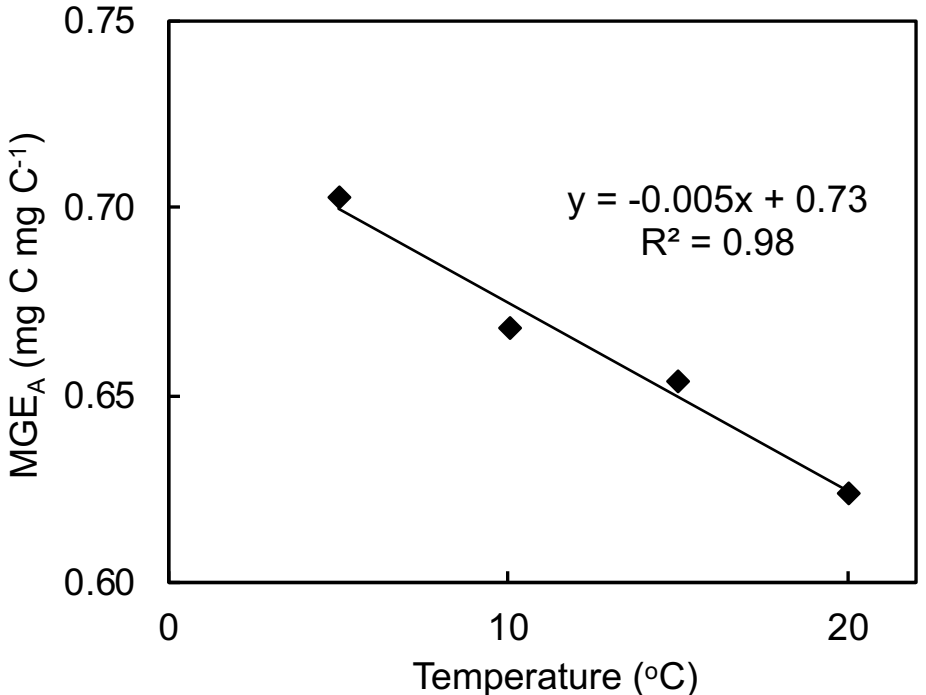
Figure 3. Modeled effect of temperature and incubation duration on apparent MGE. a) The relationship between temperature and MGE_A over time was modeled using

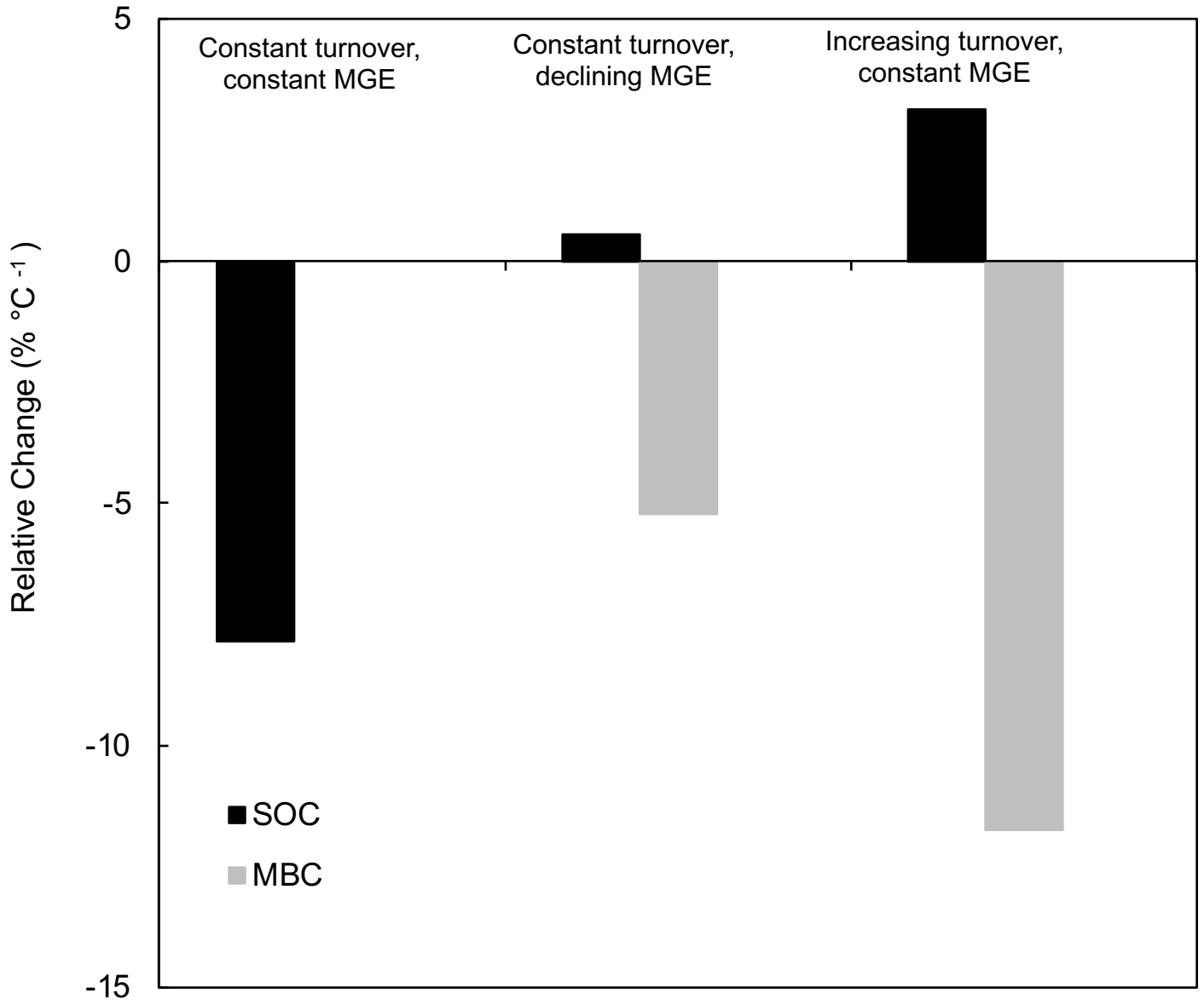
370 the microbial turnover rates for the mineral soil in our study (Figure 2). b) The modeled
371 relationship of MGE_A and temperature in mineral soil after two days.
372
373 **Figure 4. The relative change in soil organic C (SOC) and microbial biomass C**
374 **(MBC) from 5 to 20 °C under three scenarios using the Allison-Wallenstein-**
375 **Bradford model.** In the constant turnover, constant MGE scenario there is no change in
376 MBC with temperature.


0.80
0.70
0.60
0.50
MGE (mg C mg C-1)
Mineral Soil
Organic Soil
0
5
10
15
20
Temperature (°C)


0.10
0.08
0.06
0.04
0.02
0.00
Turnover Rate (d-1)
Mineral Soil
Organic Soil
0
5
10
15
20
Temperature (°C)


1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
Time (d)
MGE$_A$ (mg C mg C^{-1})
5°C
10°C
15°C
20°C

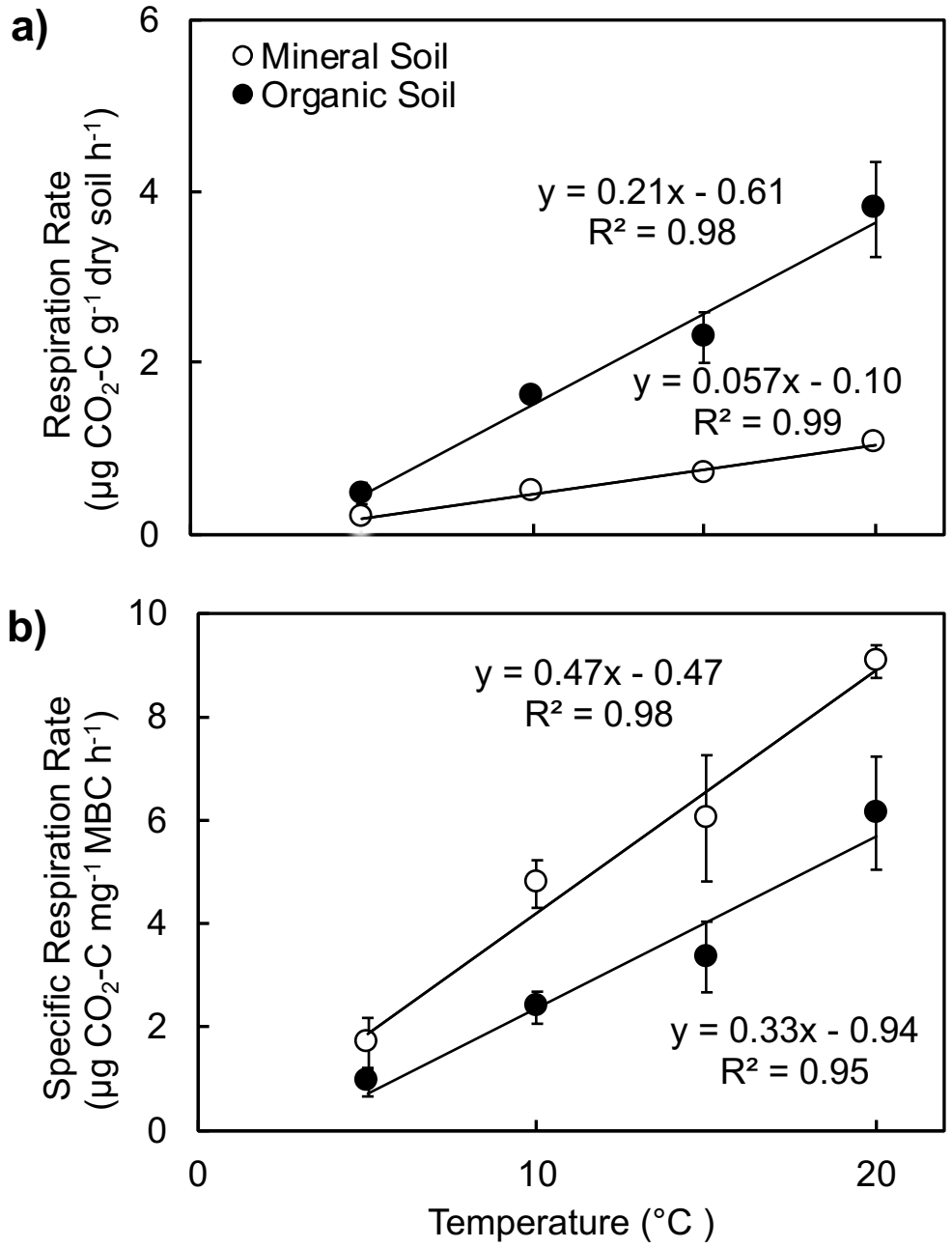

0.75
0.70
0.65
0.60
0
10
20
Temperature (°C)
MGE$_A$ (mg C mg C^{-1})
y = -0.005x + 0.73
R² = 0.98

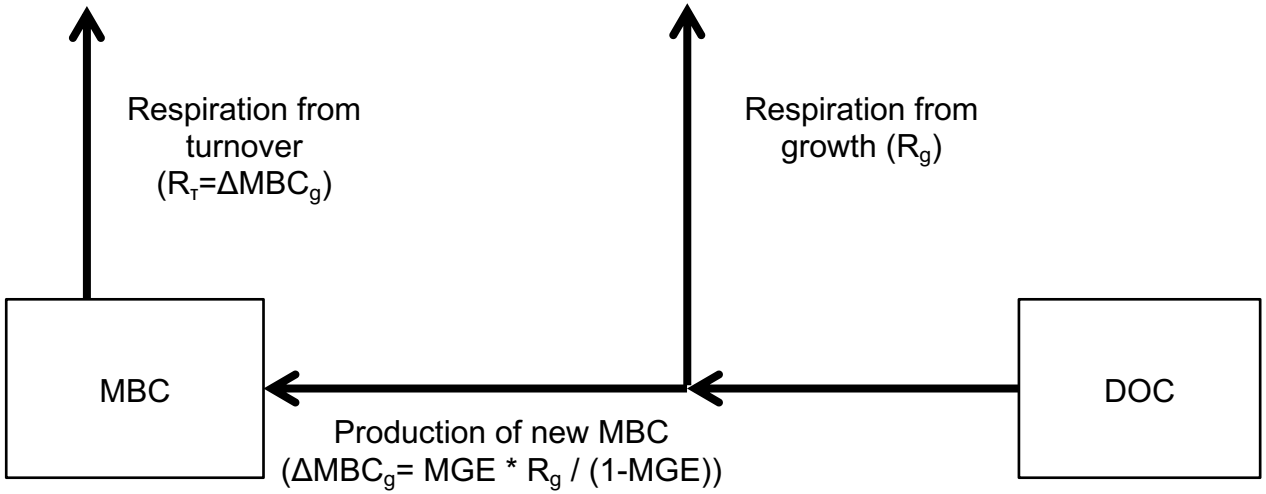

5
0
-5
-10
-15
Relative Change (% °C -1)
Constant turnover, constant MGE
Constant turnover, declining MGE
Increasing turnover, constant MGE
SOC
MBC

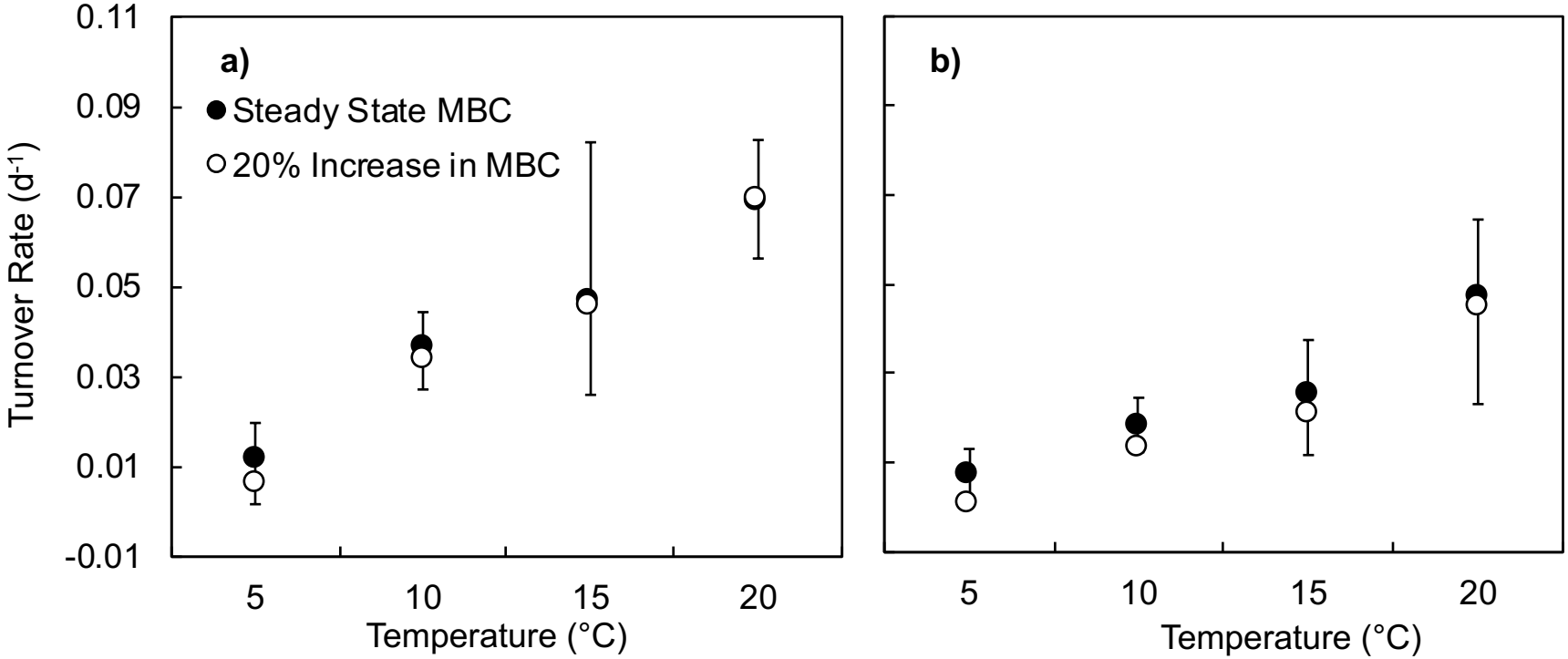
Temperature (°C)	MBC (μg g^{-1} dry soil)		MBN (μg g^{-1} dry soil)	
	Mineral Soil	Organic Soil	Mineral Soil	Organic Soil
5	276 ± 33	1,229 ± 117	37.7 ± 2.4	120 ± 9.4
10	236 ± 8.8	1,585 ± 200	35.8 ± 0.7	165 ± 32
15	282 ± 43	1,623 ± 170	36.9 ± 3.2	182 ± 16
20	263 ± 9.3	1,412 ± 81	35.0 ± 3.0	139 ± 7.7

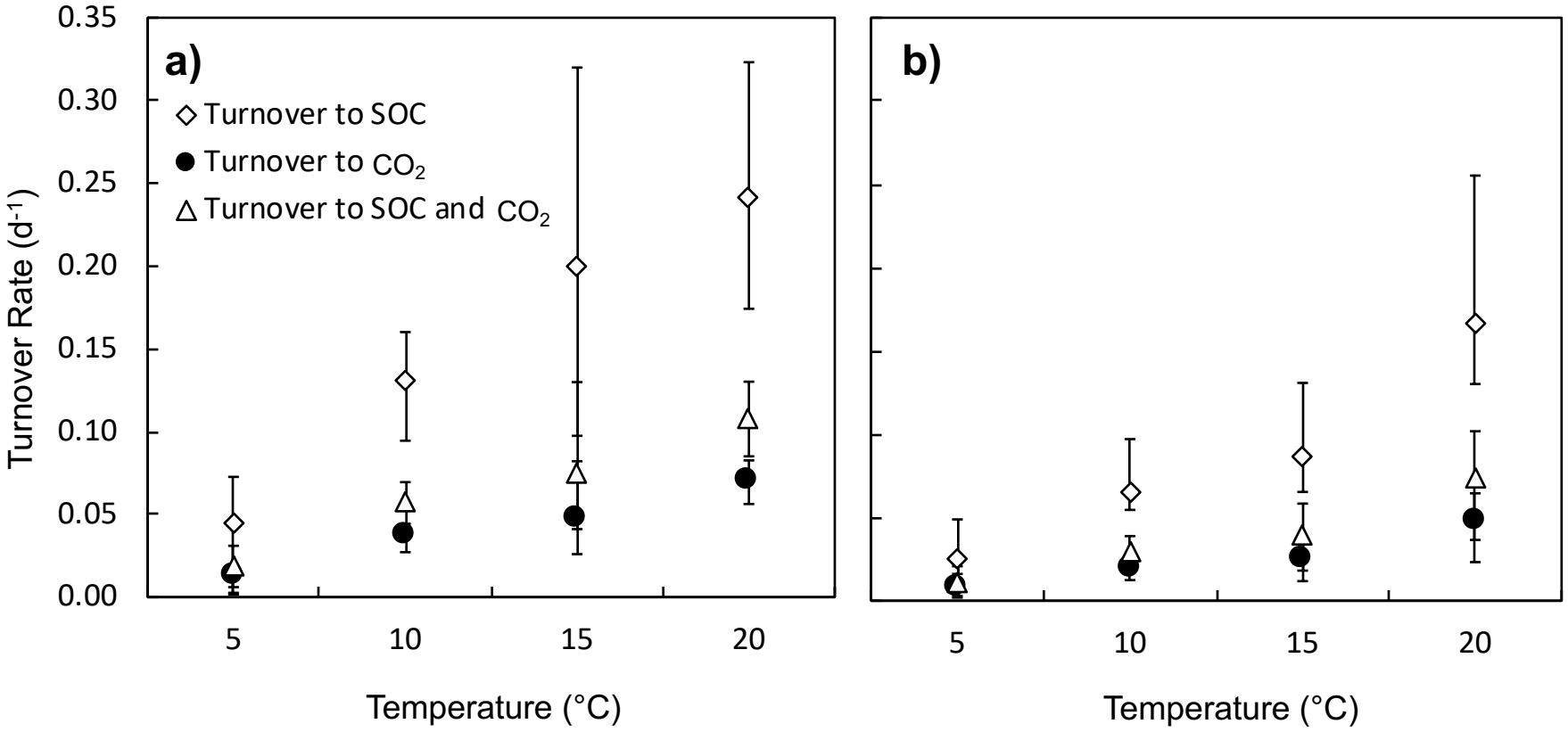
Temperature (°C)	C_U/C_1 Ratio of Glucose		C_1/C_{23} Ratio of Pyruvate	
	Mineral Soil	Organic Soil	Mineral Soil	Organic Soil
5	3.21 ± 0.31	2.92 ± 0.24	4.41 ± 0.40	3.56 ± 0.20
10	3.26 ± 0.15	2.91 ± 0.14	3.38 ± 0.20	3.73 ± 0.35
15	3.60 ± 0.39	2.94 ± 0.16	3.75 ± 0.30	3.51 ± 0.19
20	2.70 ± 0.12	3.15 ± 0.26	3.94 ± 0.46	3.61 ± 0.31

Parameter	Description	Value	Units
T_{ref}	Reference temperature	20	ºC
MGE_{ref}	MGE at reference temperature	0.31	
m	MGE change with temperature	[0, -0.016]	$ºC^{-1}$
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}


a)
Mineral Soil
Organic Soil
y = 0.21x - 0.61
R² = 0.98
y = 0.057x - 0.10
R² = 0.99
Respiration Rate
(µg CO2-C g-1 dry soil h-1)
0
2
4
6
b)
y = 0.47x - 0.47
R² = 0.98
y = 0.33x - 0.94
R² = 0.95
Specific Respiration Rate
(µg CO2-C mg-1 MBC h-1)
0
2
4
6
8
10
0
10
20
Temperature (°C)


Respiration from turnover (R_T=ΔMBC_g)
Respiration from growth (R_g)
MBC
DOC
Production of new MBC
(ΔMBC_g= MGE * R_g / (1-MGE))


a)
b)
Steady State MBC
20% Increase in MBC
Turnover Rate (d-1)
0.11
0.09
0.07
0.05
0.03
0.01
-0.01
5
10
15
20
Temperature (°C)
Temperature (°C)


a)
b)
Turnover to SOC
Turnover to CO_2
Turnover to SOC and CO_2
Turnover Rate (d^{-1})
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
5
10
15
20
Temperature (°C)

Accelerated microbial turnover but constant growth efficiency with warming in soil

Shannon B. Hagerty, Kees Jan van Groenigen,Steven D. Allison, Bruce A. Hungate, Egbert Schwartz, George W. Koch, Randall K. Kolka, and Paul Dijkstra

SUPPLEMENTARY METHODS

I. Sample Collection and Incubation

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

In April 2013, we incubated soil at four temperatures (5, 10, 15, and 20 °C) for seven days before metabolic tracer addition in Precision™ Refrigerated Incubators (Thermo Fisher Scientific Inc, Waltham, MA, USA). Each of the four incubators was 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 probing[1]. 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 Saran™ 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 ^{13}C-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 CO_2 was added to each jar in order to meet the CO_2 concentration and amount required by the Picarro G2201-*i* CO_2 cavity ring-down isotope spectrometer (Picarro Inc., Sunnyvale, CA, USA). Thirty minutes after addition of CO_2, 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-^{13}C and 1-^{13}C) and two pyruvate isotopologues (1-^{13}C and 2,3-^{13}C). 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* CO_2 cavity ringdown isotope spectrometer. The ratios of ^{13}C production for each isotopologue pair were calculated as:

$$\frac{C_U}{C_1}\text{ratio} = \frac{^{13}CO_2\ production\ from\ U-^{13}C\ glucose}{^{13}CO_2\ production\ from\ 1-^{13}C glucose} \quad (1)$$

and

$$\frac{C_1}{C_{2,3}}\text{ratio} = \frac{^{13}CO_2\ production\ from\ 1-^{13}C\ pyruvate}{^{13}CO_2\ production\ from\ 2,3-^{13}C\ pyruvate} \quad (2)$$

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 CO_2), 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 (*v1*), which is set at 100 moles. This

model has nine unknowns, seven of which are estimated using a known bacterial and fungal metabolite precursor demand[2]. 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 CO_2 producing reactions (*v10*, *v5*, *v7* and *v8*) as:

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

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 (μg CO_2-C mg^{-1} 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 K_2SO_4, while the other half was immediately extracted with K_2SO_4. 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 soils[6]. 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 CO_2. 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 CO_2-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 CO_2 (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}).

Therefore respiration from turnover (R_T) is equal to:

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

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

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

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

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

And respiration from turnover (R_T) is:

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

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

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

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

V. Sensitivity of Turnover to Calculation Assumptions

1) Non-Steady State MBC Pool

When the MBC pool is not at steady state, ΔMBC_g is divided over turnover and net microbial growth. So, ΔMBC_g is calculated as before (eq. 2). A portion of this C is added to the MBC pool (ΔMBC_n),

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

while the remainder is lost as CO_2 due to turnover:

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

When α=1, all MBC produced is added to the existing MBC, and no C is available for turnover. When α=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

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

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

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

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

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

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

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 CO_2, and (1-*f*) as the fraction of microbial C that is turned over to dead SOC yields:

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

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

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

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

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

and

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

We assessed the sensitivity of our results to the assumption that all turned over C is released as CO_2, by comparing calculated turnover rates under the "all C to CO_2" 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 CO_2, represents the most conservative estimate of microbial turnover.

VI. Estimating Effects of Experiment Duration on MGE_A

In most studies[8-10], 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 CO_2. 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 CO_2. 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 MBC_1 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 CO_2 to the atmosphere and /or remains in the soil as dead organic matter). At t=2, MBC_2 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

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

VII. Statistical Analyses

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} (20ºC 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

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

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

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

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

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

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

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

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

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

233 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:

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

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 20ºC under three scenarios with $\theta = 0$, meaning that all microbial
251 turnover is respired as CO_2. For the "constant turnover, constant MGE" scenario, $Ea_\tau = 0$
252 kJ mol^{-1} and $m = 0$. For the "constant microbial turnover, declining MGE" scenario, $Ea_\tau =$

253 0 kJ mol^{-1} and m = -0.016 $ºC^{-1}$. For the "increasing microbial turnover, constant MGE"

254 Ea_r = 47 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 constraints[14]. 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 efficiency[13-16]. This value is used as a "first approximation"
279 according to Roels 1980[16], 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 environments[13]).

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.

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

	C_U/C_1 Ratio of Glucose		C_1/C_{23} Ratio of Pyruvate	
Temperature (°C)	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	ºC
MGE_{ref}	MGE at reference temperature	0.31	
m	MGE change with temperature	[0, -0.016]	$ºC^{-1}$
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

SUPPLEMENTARY FIGURES

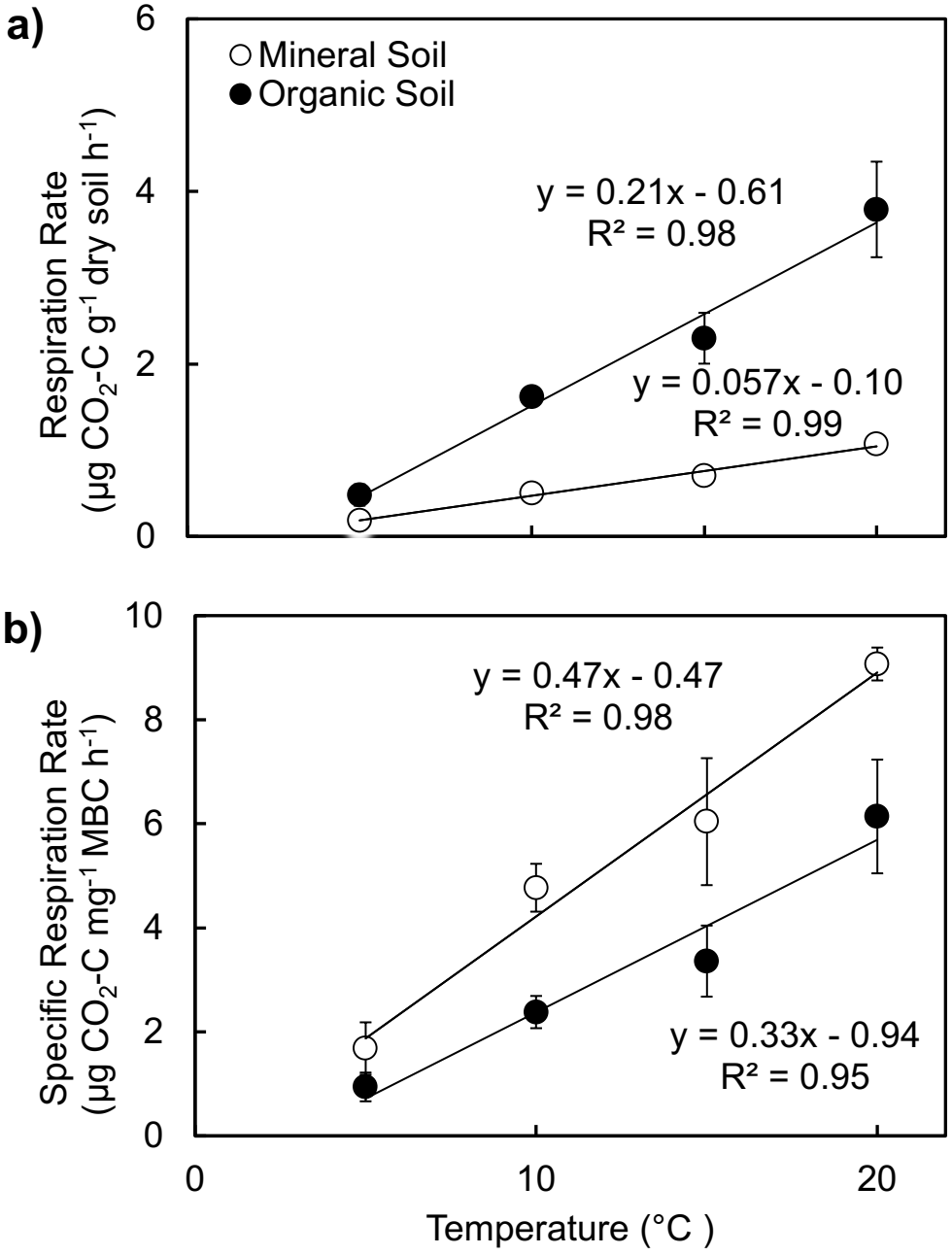
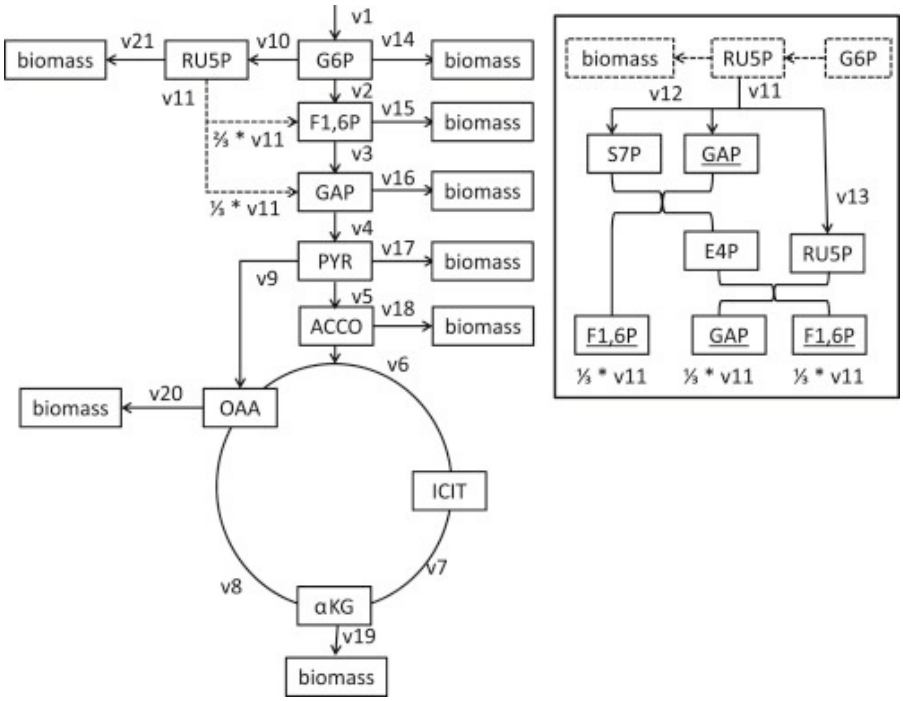


Figure S1. Response of a) soil respiration rate and b) specific respiration rate of mineral and organic soil to temperature (means and se; some standard errors are smaller than data points).



300

301 ***Figure S2.*** Model for metabolic processes in soil microbial communities. Flux rates (v2-
302 v21) are normalized relative to glucose uptake (v1, set at 100 moles). Insert depicts
303 details of the pentose phosphate pathway. Abbreviations: G6P, glucose-6-phosphate;
304 F1,6P, fructose-1,6-phosphate; GAP, glyceraldehyde-phosphate; PYR, pyruvate; ACCO,
305 acetyl-CoA; ICIT, isocitrate; αKG, α-ketoglutarate; OAA, oxaloacetate; RU5P, ribulose-
306 5-phosphate; S7P, sedoheptulose-7-phosphate; E4P, erythrose-4-phsophate. Reprinted
307 from ref. 1.

308

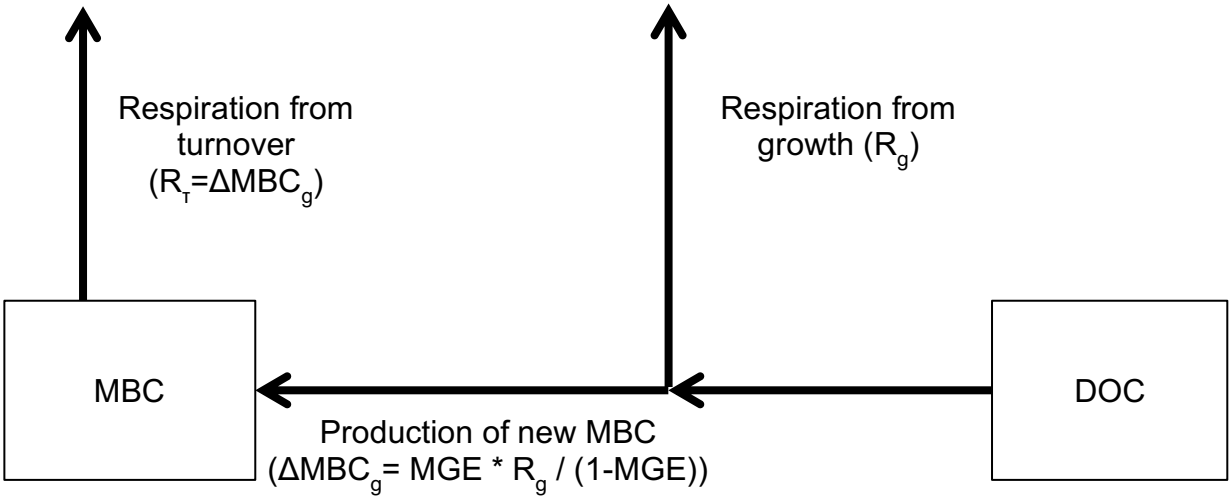
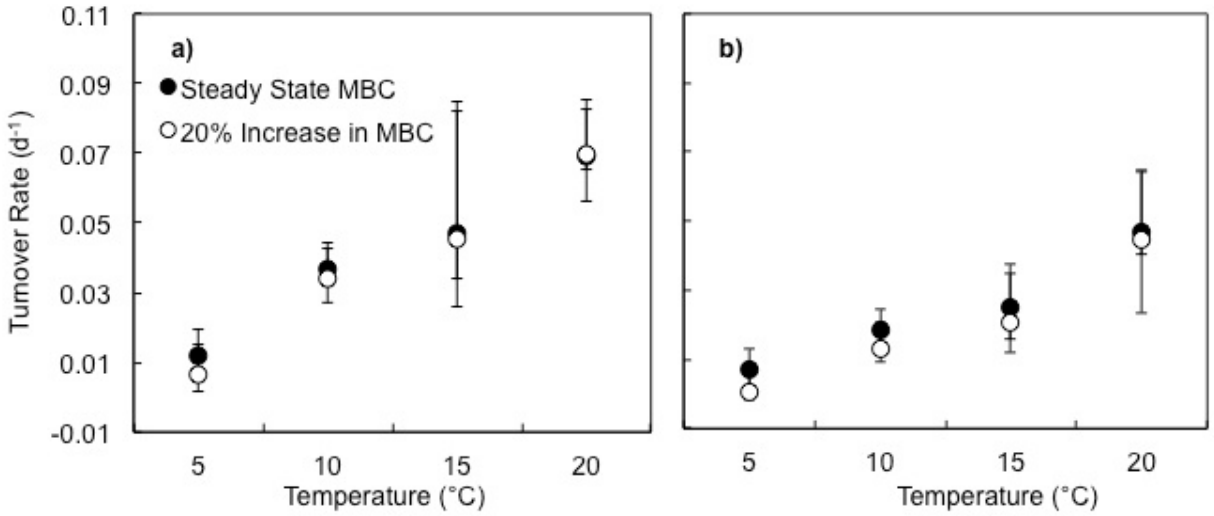


Figure S3. Schematic showing relationships between measured (total respiration, MGE, and MBC pool) and calculated values (production rate and turnover rate). Total respiration rate is the sum of respiration from turnover and growth ($R = R_g + R_\tau$).

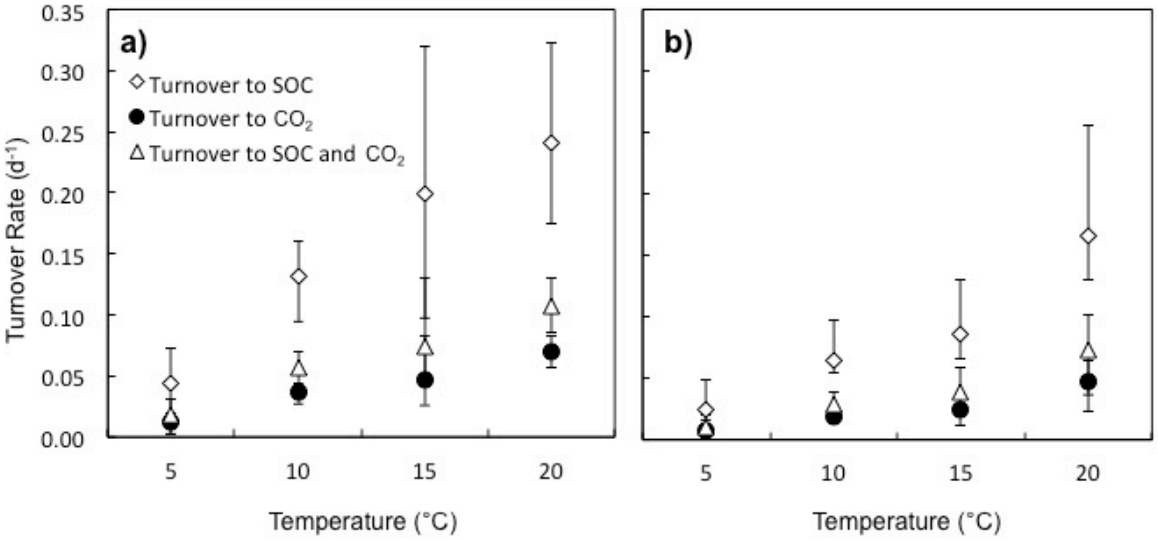


315

316 ***Figure S4.*** Effect of change in MBC on calculated microbial turnover rates at each

317 temperature for a) mineral soil and b) organic soil (means and 95% CI).

318



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).

SUPPLEMENTARY REFERENCES

1. 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 Biology & Biochemistry* **43**, 2023-2031, doi:10.1016/j.soilbio.2011.05.018 (2011).

2. Dijkstra, P. *et al.* Modeling soil metabolic processes using isotopologue pairs of position-specific C-13-labeled glucose and pyruvate. *Soil Biology & Biochemistry* **43**, 1848-1857, doi:10.1016/j.soilbio.2011.05.001 (2011).

3. van Groenigen, K. J. *et al.* Using metabolic tracer techniques to assess the impact of tillage and straw management on microbial carbon use efficiency in soil. *Soil Biology & Biochemistry* **66**, 139-145, doi:10.1016/j.soilbio.2013.07.002 (2013).

4. 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. *Biotechnology and Bioengineering* **32**, 86-94, doi:10.1002/bit.260320112 (1988).

5. Haubensak, K. A., Hart, S. C. & Stark, J. M. Influences of chloroform exposure time and soil water content on C and N release in forest soils. *Soil Biology & Biochemistry* **34**, 1549-1562, doi:10.1016/s0038-0717(02)00124-4 (2002).

6. Wu, J., Joergensen, R. G., Pommerening, B., Chaussod, R. & Brookes, P. C. Measurement of soil microbial biomass c by fumigation extraction - an

automated procedure. *Soil Biology & Biochemistry* **22**, 1167-1169, doi:10.1016/0038-0717(90)90046-3 (1990).

7. Brookes, P. C., Landman, A., Pruden, G. & Jenkinson, D. S. Chloroform fumigation and the release of soil-nitrogen - a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology & Biochemistry* **17**, 837-842, doi:10.1016/0038-0717(85)90144-0 (1985).

8. Frey, S. D., Lee, J., Melillo, J. M. & Six, J. The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change* **3**, 395-398, doi:10.1038/nclimate1796 (2013).

9. 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 Biology & Biochemistry* **40**, 2722-2728, doi:10.1016/j.soilbio.2008.07.002 (2008).

10. Tucker, C. L., Bell, J., Pendall, E. & Ogle, K. Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? *Global Change Biology* **19**, 252-263, doi:10.1111/gcb.12036 (2013).

11. Frey, S. D., Gupta, V., Elliott, E. T. & Paustian, K. Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. *Soil Biology & Biochemistry* **33**, 1759-1768, doi:10.1016/s0038-0717(01)00101-8 (2001).

12. Li, J. W., Wang, G. S., Allison, S. D., Mayes, M. A. & Luo, Y. Q. Soil carbon sensitivity to temperature and carbon use efficiency compared across microbial-

ecosystem models of varying complexity. *Biogeochemistry* **119**, 67-84, doi:10.1007/s10533-013-9948-8 (2014).

13. Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Agren, G. I. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist* **196**, 79-91, doi:10.1111/j.1469-8137.2012.04225.x (2012).

14. Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L. & Richter, A. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters* **16**, 930-939, doi:10.1111/ele.12113 (2013).

15. Heijnen, J. J. in *Biosystems Engineering Ii: Linking Cellular Networks and Bioprocesses* Vol. 121 *Advances in Biochemical Engineering-Biotechnology* (eds C. Wittmann & R. Krull) 139-162 (2010).

16. Roels, J. A. Application of macroscopic principles to microbial-metabolism. *Biotechnology and Bioengineering* **22**, 2457-2514, doi:10.1002/bit.260221202 (1980).

17. Xiao, J. H. & VanBriesen, J. M. Expanded thermodynamic model for microbial true yield prediction. *Biotechnology and Bioengineering* **93**, 110-121, doi:10.1002/bit.20700 (2006).

18. von Stockar, U., Vojinovic, V., Maskow, T. & Liu, J. Can microbial growth yield be estimated using simple thermodynamic analogies to technical processes? *Chemical Engineering and Processing* **47**, 980-990, doi:10.1016/j.cep.2007.02.016 (2008).

19. Heijnen, J. J. & Vandijken, J. P. In search of a thermodynamic description of biomass yields for the chemotropic growth of microorganisms - response.

390 *Biotechnology and Bioengineering* **42**, 1127-1130,
391 doi:10.1002/bit.260420916 (1993).
392