nature climate change

LETTERS

PUBLISHED ONLINE: 7 SEPTEMBER 2014 | DOI: 10.1038/NCLIMATE2361

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 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. A microbial-enzyme model suggests that such temperature sensitive microbial turnover would promote 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 present 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 by living microbes[19]. Consequently accelerated turnover can increase respiration per unit of MBC even when MGE remains the same[20]. However, most studies of MGE responses to warming do not account for respiration and cell death that result from turnover[15–17].

We determined the temperature sensitivity of MGE and turnover to examine the mechanisms controlling the response of soil C cycling processes to warming. We measured MGE and microbial turnover in mineral soil and organic soil from the Marcell Experimental Forest, Minnesota, after a one-week incubation at 5, 10, 15, or 20 °C. We used metabolic tracer probing to determine MGE (ref. 14). In this method, MGE is calculated from the fate of individual C-atoms in glucose and pyruvate using a metabolic model. Unlike other methods[15–17], the metabolic tracer probing method determines MGE measurement over a very short period of time (1 h or less at room temperature), making it less sensitive to microbial turnover. We combined MGE measurements with measurements of microbial respiration and MBC to calculate microbial turnover rates.

We found that MGE was not sensitive to temperature (Fig. 1). Mean MGE was 0.72 (±0.01 s.e.m., $n = 22$) in mineral soil and 0.71 (±0.01 s.e.m., $n = 21$) in organic soil. Across all temperature treatments and replicates, MGE ranged between 0.67 and 0.75. These values for MGE are high relative to the average values observed in soils and other ecosystems[7,8,21]. It is also higher than 0.6, an average maximum MGE value for pure culture studies[8,22] (for further discussion on theoretical thermodynamic constraints of MGE, see Supplementary Note). This high value suggests that the active microbial community functions at high biochemical efficiency and microorganisms with relatively high maintenance costs contribute little to the total activity. High efficiency values may also indicate additional energy sources (for example, from oxalate or formate[23]), or direct incorporation of large amounts of cellular compounds, such as amino acids[14]. However, what little information is available suggests that these effects will only be slightly affected by temperature[17].

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

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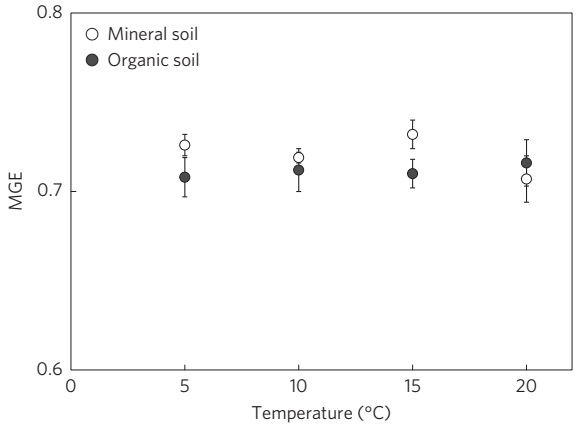

Figure 1 | Microbial growth efficiency (MGE) after a 7-day incubation at different temperatures for a mineral and an organic soil. Means and standard errors ($n=6$, except for mineral soil at 5, 10 °C and organic soil at 5 °C, where $n=5$). There was no significant effect of soil type ($p=0.21$) or temperature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

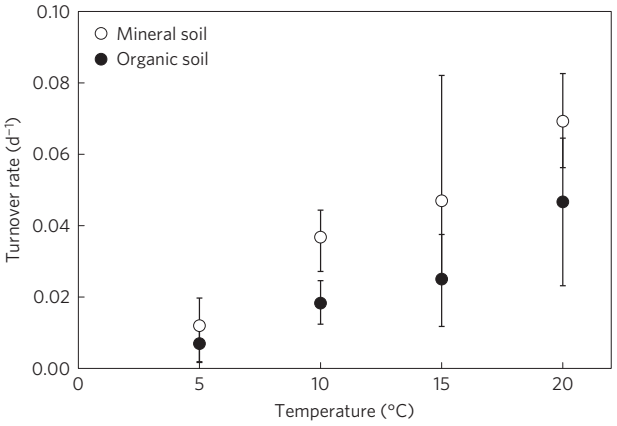

Figure 2 | Turnover rates (τ, d^{-1}) as a function of temperature for a mineral and an organic soil. The experimental values were resampled using a bootstrap method to calculate a 95% confidence interval (error bars). For each soil type, the turnover rate at 5 °C is significantly different from that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

has been observed in a pure culture experiment[25], but may not be observable in diverse soil communities, where optimum temperatures for growth can vary widely between microbial species[11]. 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 acclimation[6].

Despite the constant MGE with temperature, higher temperatures increased microbial respiration in the mineral soil and organic soil by factors of nearly six and eight, respectively (Supplementary Fig. 1). Across the same temperature range, specific respiration rate (μg CO_2–C mg^{-1} 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 μg MBC g^{-1} dry soil d^{-1} °C^{-1}, $r^2=0.99$ in mineral soil and 3.63 μg MBC g^{-1} dry soil d^{-1} °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 1). Therefore, warming increased microbial turnover ($p=0.02$) in both soils—by 0.004 d^{-1} °C^{-1} in mineral soil and by 0.003 d^{-1} °C^{-1} in organic soil (Fig. 2), compensating for increased gross 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 warming[26]. Warming could also 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 temperatures[18]. 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 of time after the addition of ^{13}C-labelled C compounds (instantaneous MGE or MGE_I). 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 MGE (refs 16,21). This effect increases with incubation duration and may cause differences in apparent MGE (MGE_A), especially when microbial turnover rates differ between treatments (as in this study, Fig. 2).

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

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

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

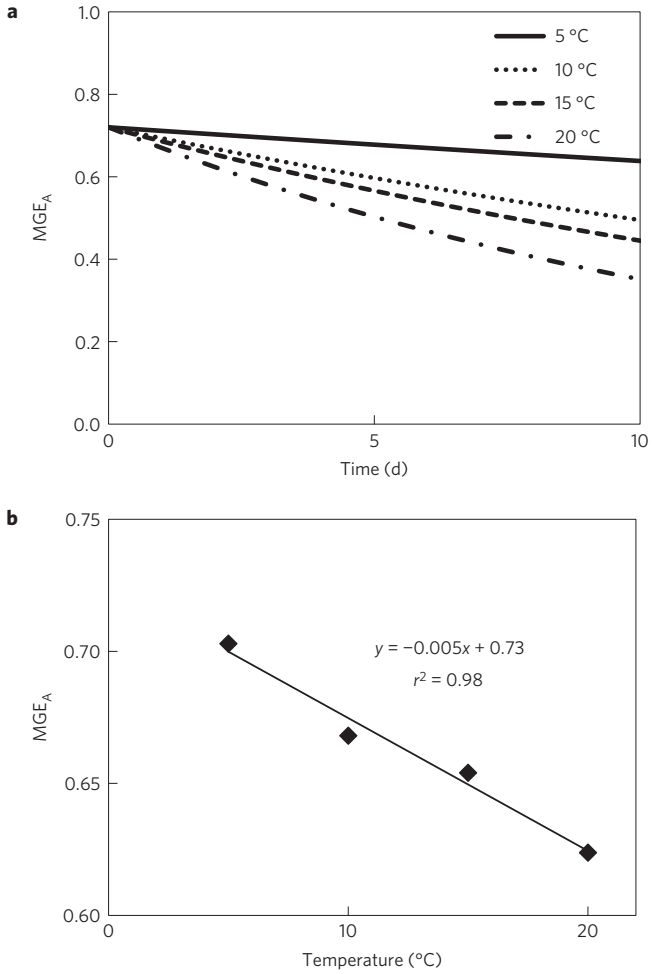

Figure 3 | Modelled effect of temperature and incubation duration on apparent microbial growth efficiency (MGE). a, The relationship between temperature and MGE_A over time was modelled using the microbial turnover rates for the mineral soil in our study (Fig. 2). **b**, The modelled relationship of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

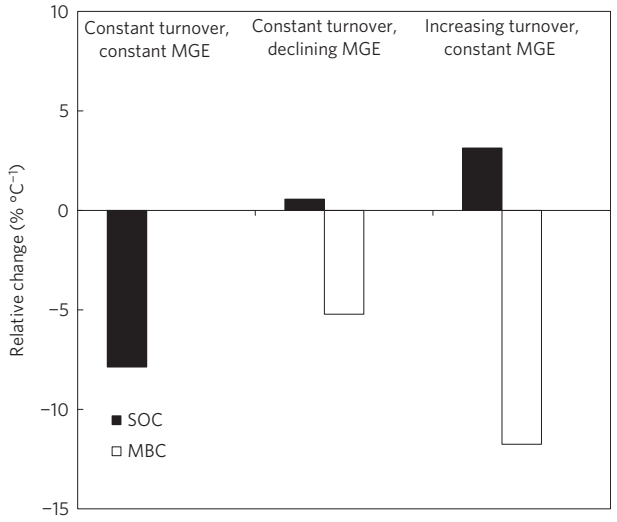

Figure 4 | The relative change in soil organic C (SOC) and microbial biomass C (MBC) from 5 to 20 °C under three scenarios using the AWB model. In the constant turnover, constant microbial growth efficiency (MGE) scenario there is no change in MBC with temperature.

altered by temperature and soil C decomposition was modelled with a first-order decay function and Michaelis–Menten enzyme kinetics, the current assumption in most biogeochemical models[7,29]. In this scenario there was no change in MBC with warming and SOC declined as a result of accelerated enzymatic decomposition (Fig. 4). In the second scenario, MGE decreased by 0.016 °C^{-1}, as in previous theoretical studies[6]. Here, the reduction in MGE limited microbial growth at higher temperatures, resulting in a 5% decline of MBC °C^{-1} averaged from 5 to 20 °C. As a result, SOC increased with temperature as decomposition became limited by MBC. The third scenario corresponded to our experimental observations of a constant MGE and accelerated microbial turnover with warming. Accelerated microbial turnover at higher temperatures caused decreases in MBC and increases in SOC, which were larger than in the scenarios of constant turnover and declining MGE. We conclude that accelerated microbial turnover is an alternative mechanism that can moderate the effects of temperature on soil C stocks, even when MGE does not decline. These model simulations suggest that temperature-sensitive microbial turnover produces an effect on MBC and SOC that is not accounted for in present biogeochemical or microbial models.

Our results show that accelerated enzyme kinetics and increased microbial turnover are the main mechanisms associated with 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 of traditional biogeochemical models. Consequently, soil microbial models should include a temperature-sensitive microbial turnover rate. The lack of temperature sensitivity in MGE, which is controlled at the cellular level, suggests that microbial biochemical efficiency is a weak control on soil C dynamics.

Methods

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

After a seven-day incubation period, MGE was determined using two position-specific ^{13}C-labelled isotopologues of glucose (U-^{13}C and 1-^{13}C) and two of pyruvate (1-^{13}C and 2,3-^{13}C) as metabolic tracers[14,30]. We measured $^{13}CO_2$ accumulation in each jar three times over the course of 60, 90, 135, or 180 min at 20, 15, 10, or 5 °C, respectively. The ratios between $^{13}CO_2$ production rates from glucose and pyruvate isotopologues were calculated and used to model metabolic pathway activities and MGE (ref. 10; Supplementary Table 2). One complete replicate (that is, 4 temperatures × 2 soils × 4 isotopologues) was incubated and analysed each week. For more details and background information on metabolic probing and modelling, see Supplementary Section II and Supplementary Fig. 2.

Two weeks after the MGE measurements, another incubation was set up under identical conditions to measure respiration and MBC. Each of the four incubators was systematically assigned to one of the four treatment temperatures and both soils were incubated for seven days. After the seven-day incubation period, CO_2 concentrations were measured at 0 and 24 h. After the respiration measurement, MBC concentration was measured using chloroform fumigation-extraction (See Supplementary Section III and Supplementary Table 1).

We calculated microbial turnover using the experimentally measured respiration (R), MGE and MBC (Supplementary Section IV and Supplementary Fig. 3). We applied the assumptions that MBC was at steady state and that all turned-over MBC was released as CO_2. Our findings of temperature-sensitive turnover were not affected much by the non-steady state of MBC and whether C from turnover was released as CO_2 or added to the SOC pool (Supplementary Section V and Supplementary Figs 4 and 5).

The gross microbial production was calculated as

$$\Delta MBC_g = MGE \times R$$

and microbial turnover (τ) assuming steady state MBC pools and all C from turnover going to CO_2 as follows

$$\tau = \frac{\mathrm{MGE} \times R}{\mathrm{MBC}}$$

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

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

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 (Fig. 2). See Supplementary Section VI for more information.

We analysed 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. Further details on all statistical analyses can be found in Supplementary Section VII.

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

Received 28 February 2014; accepted 4 August 2014; published online 7 September 2014

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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 P.D. and NSF MRI (DBI-0723250 and 1126840) to G.W.K. and T. Whitham.

Author contributions

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

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 P.D.

Competing financial interests

The authors declare no competing financial interests.