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The temperature response of soil microbial efficiency and its feedback to climate

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Soils are the largest repository of organic carbon (C) in the terrestrial biosphere and represent an important source of carbon dioxide (CO_2) to the atmosphere, releasing 60–75 Pg C annually through microbial decomposition of organic materials[1,2]. A primary control on soil CO_2 flux is the efficiency with which the microbial community uses C. Despite its critical importance to soil–atmosphere CO_2 exchange, relatively few studies have examined the factors controlling soil microbial efficiency. Here, we measured the temperature response of microbial efficiency in soils amended with substrates varying in lability. We also examined the temperature sensitivity of microbial efficiency in response to chronic soil warming *in situ*. We find that the efficiency with which soil microorganisms use organic matter is dependent on both temperature and substrate quality, with efficiency declining with increasing temperatures for more recalcitrant substrates. However, the utilization efficiency of a more recalcitrant substrate increased at higher temperatures in soils exposed to almost two decades of warming 5 °C above ambient. Our work suggests that climate warming could alter the decay dynamics of more stable organic matter compounds, thereby having a positive feedback to climate that is attenuated by a shift towards a more efficient microbial community in the longer term.

Present carbon–climate models predict increased soil organic matter (SOM) decomposition as global climate warms, with higher than normal soil CO_2 fluxes to the atmosphere eliciting a positive feedback to climate[3,4]. However, results from several field studies demonstrate that although soil respiration is initially stimulated by warming, this effect often diminishes over time, with elevated soil respiration in chronically warmed soils returning to ambient levels within a few years[5–7]. Microbial decomposition of SOM is responsible for as much as half or more of the CO_2 released from soils[1], and so a thorough understanding of how soil microorganisms respond to temperature is needed to accurately predict how climate warming may alter soil CO_2 fluxes[8].

Microbial efficiency determines the partitioning of substrate C between microbial biomass and CO_2 production and is a key factor in determining the fate of C in soils[9,10]. Small changes in microbial efficiency could have profound effects on soil CO_2 flux[11], yet little is known about the factors controlling microbial efficiency in soils. Microbial efficiency estimates from aquatic systems generally suggest that efficiency declines with increasing temperature and decreasing substrate quality and nutrient availability[9,10,12,13]; however, determining the response to each of these variables independently is a challenge because they often co-vary across space and time in natural systems[9,10,14]. Soil-specific estimates of microbial efficiency in response to temperature, substrate quality and nutrient availability are needed to accurately parameterize SOM models and to better predict the response of SOM decay to these environmental controls.

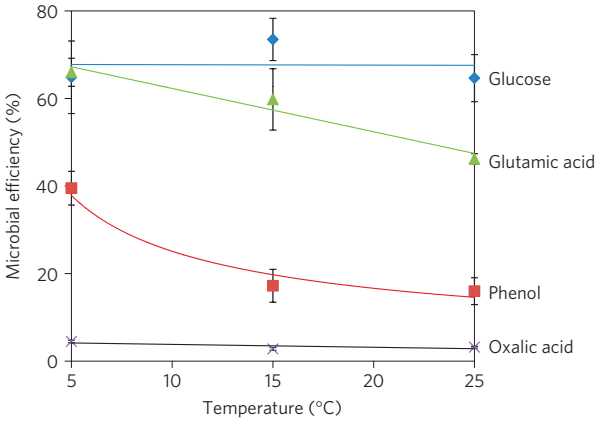

Figure 1 | Temperature response of microbial efficiency (%) in forest soil amended with substrates varying in lability. Soil samples were collected from control plots at two soil warming studies at the Harvard Forest LTER site, amended with one of four substrates (glucose, glutamic acid, oxalic acid or phenol) and incubated at 5, 15 or 25 °C. Error bars represent one standard error.

Several recent modelling studies have demonstrated that letting soil microbial efficiency vary with temperature results in an inverse relationship between temperature and efficiency[15] and that SOM dynamics are better predicted by a variable efficiency parameter that is temperature dependent[16]. However, experimental studies that have assessed the temperature sensitivity of soil microbial efficiency have reported mixed results, with some studies showing an inverse temperature–efficiency relationship[17,18] and others showing a limited response[19]. From a theoretical perspective, higher temperatures should increase maintenance costs, thereby increasing energy demand and decreasing efficiency[19]. We postulated that the discrepancy in the literature regarding the effects of temperature on efficiency could be explained if the temperature sensitivity were substrate dependent.

Here, we measured the temperature response of microbial efficiency for soils amended with substrates varying in lability. We found that the microbial utilization of glucose, which does

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not require extracellular enzymatic breakdown and is readily assimilated by most soil microorganisms, was temperature insensitive with respect to microbial efficiency ($P = 0.62$): efficiencies spanned a narrow range (70–75%) across the temperature gradient (Fig. 1 and Supplementary Table S1). The efficiency of oxalic acid utilization was also not significantly influenced by temperature ($P = 0.10$). Although oxalic acid amendment resulted in uptake rates similar to the other substrates, little of the oxalic acid added to the soil was incorporated into microbial biomass, yielding a consistently low efficiency (2.8–4.5%) regardless of temperature. Thus, most of the oxalic acid C assimilated by the microbial community was released as CO_2. In contrast to glucose and oxalic acid, the efficiency of glutamic acid and phenol utilization exhibited a strong temperature response, with glutamic-acid-amended soils showing a 30% drop in efficiency when incubated at 25 versus 5 °C ($P < 0.01$). Phenol-amended soils exhibited a 60% drop in efficiency with increasing incubation temperature ($P < 0.001$). These results are consistent with the kinetics of enzymatic reactions whereby the temperature sensitivity of decomposition increases with increasing molecular complexity owing to greater activation energies[20].

Unlike glucose, more complex substrates such as phenol require further extracellular enzymatic breakdown before they can be used by the microbial community; thus, increasing temperatures would be expected to increase respiration associated with enzyme production and excretion[10]. Hence, soil microbial efficiency is inversely related to temperature for some substrates but not for others, and more specifically, substrate complexity or inherent decomposability determines the degree of temperature sensitivity of microbial efficiency. Our work helps explain the inconsistent results reported in the literature, whereby studies report either little to no temperature response for soil microbial efficiency[19] or an inverse temperature–efficiency relationship[17,18]. No temperature response was observed when soils were amended with glucose[19], whereas a similar decline in efficiency as reported here (~1% ° C^{-1}) was observed when a less easily assimilated substrate, cellobiose, was used[17].

To determine whether long-term soil warming alters the temperature response function of microbial efficiency, we measured the efficiency of glucose or phenol utilization for soils collected from plots maintained under ambient versus elevated (5 °C above ambient) temperature conditions for 2 versus 18 years. Chronic soil warming for either 2 or 18 years did not alter the temperature sensitivity of microbial efficiency on glucose amendment (Supplementary Fig. S1). In contrast, the microbial efficiency measured in phenol-amended soils showed a strong and significant temperature response for control and warmed soils in both the 2- and 18-year soil warming study (Fig. 2). As for glucose, the temperature response pattern for phenol utilization was not significantly altered by two years of continuous soil warming. That is, soils from both the control and warmed plots showed the same degree of temperature sensitivity (that is, about a 50% decline in efficiency across the temperature gradient). However, for soils warmed continuously for 18 years, microbial efficiency was significantly higher in warmed when compared with control soils at higher incubation temperatures (15 and 25 °C).

The weaker decline in phenol utilization efficiency at greater temperatures following long-term (18 years) warming could have occurred either through changes in microbial community structure, temperature adaptation of the original microbial community, or soil nutrient status. Long-term soil warming at this site has previously been demonstrated to significantly alter the biomass, community structure and functional capacity of the microbial community[21]. The temperature sensitivity of heterotrophic soil respiration has also been shown to decline under long-term soil warming[22,23]. This apparent acclimation of the microbial community could be partially explained by a shift towards a microbial community that more efficiently uses the recalcitrant C

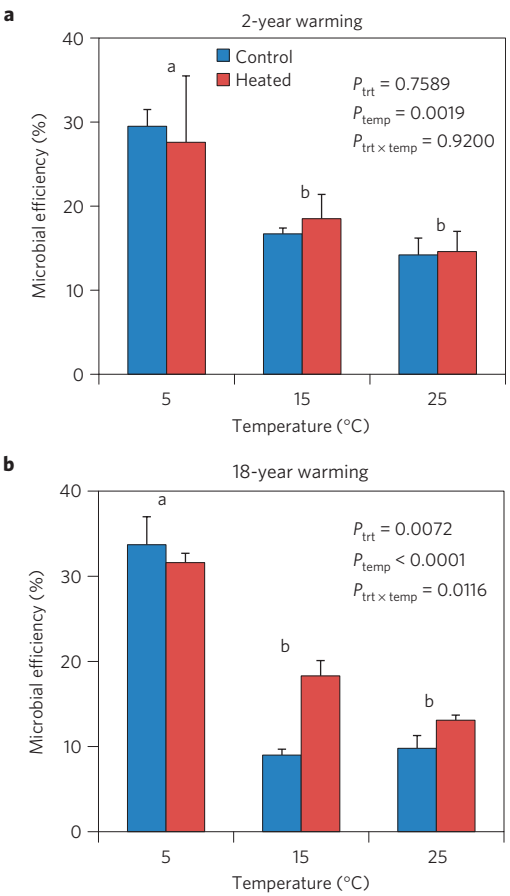

Figure 2 | Effect of chronic soil warming on microbial efficiency in control versus heated soils following amendment with phenol. a,**b**, The soils were collected from control or heated plots warmed continuously to 5 °C above ambient for 2 (**a**) or 18 (**b**) years. Error bars represent one standard error. The label trt indicates the experimental treatment (control versus heated). Different lowercase letters above the bars indicate a significant difference amongst incubation temperatures ($P < 0.05$). The data were analysed by non-parametric analysis of variance using SAS 9.3 (SAS Institute). Differences in efficiency between the control plots from the two warming experiments correspond to observed differences in other soil parameters (for example, microbial biomass, total organic C).

compounds remaining in the soil following long-term warming as more readily usable substrates are lost. Chronic soil warming also fundamentally alters nutrient cycling processes, most specifically by stimulating nitrogen (N) mineralization and thus increasing soil N availability. We estimate that 18 years of soil warming at Harvard Forest has increased N mineralization by about 60%, resulting in the release of ~25 kg N ha^{-1} yr^{-1} in the heated plots. Theoretical predictions indicate that microbial efficiency should decline as a function of nutrient availability[10] and empirical data, mostly from aquatic systems, suggest that efficiency often declines as nutrients become more limited[10,24]. However, efficiency estimates measured on soils collected from a long-term N addition experiment at Harvard Forest indicate that 22 years of N fertilization at two levels (50 and 150 kg N ha^{-1} yr^{-1}) has not altered microbial efficiency of glucose or phenol utilization when compared with control plots (S. Frey, unpublished data). This suggests that enhanced N availability in the heated plots is probably not the primary mechanism underlying the observed change in phenol utilization efficiency, although this needs to be more fully examined.

Using the temperature response functions obtained from the phenol data (presented in Fig. 2) and continuous soil temperature

data measured in the field plots, we estimated the efficiency of phenol utilization in control versus heated field plots across the growing season (April–November) when soil temperatures were within the range of our incubation temperatures (5–25 °C). For the two-year warming study, there was no significant difference in temperature response between treatments. Thus, at any given time under field conditions, the efficiency of phenol utilization, and potentially other complex substrates, was always lower in the heated plots when compared with the controls owing to the negative temperature response function for this substrate, possibly explaining in part the greater soil respiration observed in heated versus control soils during the early years of this and several other soil warming studies[5,7,22,25]. For soils continuously warmed for 18 years, the difference between control and heated plots in the efficiency of phenol utilization was inversely related to temperature and switched from being positive to negative at ~10 °C (Supplementary Fig. S2). That is, microbial efficiency was greater in the control when compared with heated plots only at lower soil temperatures (<10 °C). At higher soil temperatures (10–25 °C), which represent a significant portion of the growing season when soil respiration rates are highest, microbial efficiency was lower in the control when compared with heated plots. This observation is particularly relevant given that chronically warmed soils contain lower levels of readily available C (refs 21,23) and that the C being used by the microbial community is probably more recalcitrant. Our results can help explain the observation that elevated soil respiration in chronically warmed soils often returns to near ambient levels within a few years[5–7].

Microbial efficiency is a key parameter included in most ecosystem models used to simulate SOM dynamics; however, most commonly used models contain a fixed microbial efficiency parameter that is independent of ecosystem type, time or environmental conditions, including temperature and substrate quality[16,19]. Thus, a secondary objective of our work was to illustrate how sensitive such conventional SOM models might be to changes in microbial efficiency and, in particular, to illustrate how these changes in microbial efficiency might impact model predictions of soil C storage in the longer term. To address this objective, we used the DAYCENT model[26] to simulate the effects of soil warming on soil C storage at our site in response to altering the microbial efficiency parameter in the model (Supplementary Information). Relative increases of 10–50% in the values of microbial efficiency resulted in a gain in soil C of 89–427 g C m^{-2} (1–7% more soil C; Fig. 3). In comparison, soil C stocks declined by 89–375 g C m^{-2} at equilibrium when efficiency was decreased by 10–50%. Soil C losses were estimated to be slightly greater than soil C gains for the same degree of change in microbial efficiency owing to increased substrate decomposability.

Simulating soil warming of ~5 °C above ambient increased microbial respiration and resulted in soil C loss. However, a combination of soil warming and increases of 20–50% in the model's default efficiency values led initially (that is, over a 5–10 year time period) to soil C accumulation (Supplementary Fig. S3). This suggests that the soil may have a short-term potential to produce more microbial-derived C to be preferentially stabilized with increasing microbial efficiency. Nevertheless, 327–443 g C m^{-2} was lost under long-term warming when compared with no warming across the gradient of efficiency change and more importantly, the magnitude of the warming response by soil C was negatively related to microbial efficiency. Soil organic C loss under simulated warming was 0–288 g C m^{-2} (0–5%) when microbial efficiency was increased by 10–40%, compared with 496–819 g C m^{-2} (8–13%) when microbial efficiency was decreased by a comparable amount. Less soil C was lost at higher efficiency values than if the simulations had been conducted with the static efficiency values used at present in the working version of the model.

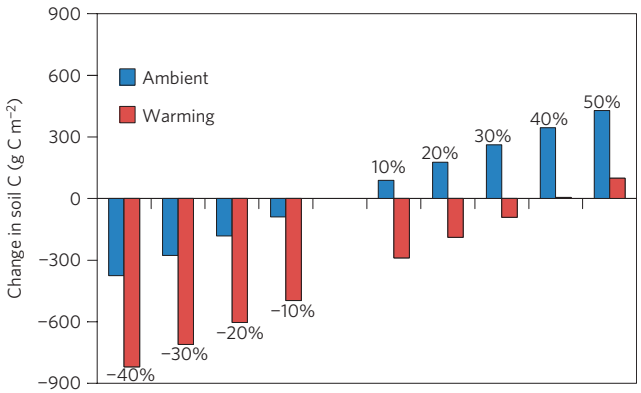

Figure 3 | Modelled changes in soil organic C content in response to changes in the microbial efficiency parameter for soils with and without warming. The equilibrium change in soil organic C content was simulated by running the DAYCENT model for 98 years under ambient versus warmed conditions and with changes in the default microbial efficiency parameter. The percentages above each bar represent the relative change in value of the efficiency parameter as set in the model.

The amount of soil C that was lost owing to warming was attenuated by 26, 52 and 77% by increasing the efficiency parameter by 10, 20 and 30%, respectively; however, the net effect was still a greater loss of soil C with warming relative to the ambient condition. Thus, our results suggest that climate warming may result in a positive feedback to climate that is counteracted to some degree, but not entirely, by a shift in the ability of the microbial community to more efficiently use recalcitrant C substrates.

Our modelling results illustrate that C stock estimates from commonly used, conventional SOM models, where the efficiency parameter is fixed, will increase or decrease in direct response to a change in that parameter. Thus, soil C loss is generally predicted to decline as the efficiency parameter increases. However, new models are emerging that challenge this view. For example, ref. 16 presents a model that directly couples soil C dynamics with microbial physiology, such that microbial biomass and extracellular enzyme production are linked to an efficiency parameter that is allowed to fluctuate in response to temperature. Results from this model suggest a more nuanced response to changes in microbial efficiency, whereby, a decline in efficiency (in response to higher soil temperatures, for example) would result in a decline in microbial biomass production over the long-term, which in turn would lead to reduced extracellular enzyme production, thus ultimately suppressing SOM decomposition and CO_2 loss from the system. This model structure assumes that microbial efficiency declines with temperature (including long-term soil warming) and does not account for potential shifts in microbial efficiency over time as microbial community structure changes in response to environmental perturbation such as chronic soil warming. Given our experimental results, there is a need for next-generation ecosystem models that incorporate an efficiency parameter that is allowed to fluctuate in response to temperature and substrate quality, but that also account for potential microbial community shifts that may induce changes in the efficiency of C use in the longer term.

Our experimental results demonstrate that microbial efficiency in a temperate forest soil is substrate dependent, with the utilization of a labile substrate such as glucose being temperature insensitive, whereas the utilization efficiency of other substrates (glutamic acid and phenol) is strongly temperature dependent. However, in soils exposed to long-term warming, the utilization efficiency of phenol varied over the temperature range exhibited in the warmed soils, such that microbial efficiency was significantly higher between the

temperature range of 10–25 °C. As microbial use of low-quality substrates may increase in chronically warmed soils where more labile substrates tend to be rapidly depleted[21,23], estimates of the temperature response for the utilization of recalcitrant substrates is particularly important. Given this, our work suggests that climate warming may lead to a change in the decay dynamics of these more stable SOM compounds. Our modelling results indicate that long-term warming leads to an overall loss of soil C, but the amount of C lost is attenuated when the microbial efficiency parameter for recalcitrant organic matter compounds in the model is increased to equivalent levels as found in our experimental work. Our results thus suggest that climate warming may ultimately lead to an increase in the decay of more stable SOM compounds, resulting in a positive feedback to climate that is, however, lessened with time by the shift towards a more efficient microbial community in the longer term.

Methods

Soil samples (0–10 cm mineral soil) were collected from three replicate control and heated plots at two soil warming studies located on the Prospect Hill Tract at the Harvard Forest Long-Term Ecological Research (LTER) site in Petersham, Massachusetts, USA. Soils in the heated plots had been continuously warmed to 5 °C above ambient using underground resistance cables for 2 or 18 years at the time of sampling (see Supplementary Information for further site and sampling details). We measured the temperature response of microbial efficiency for soils amended with one of four ^{13}C-labelled substrates varying in inherent decomposability (glucose, glutamic acid, oxalic acid or phenol) by incubating amended soils at 5, 15 or 25 °C and following the fate of the added ^{13}C into microbial biomass and labelled CO_2 according to the protocol of ref. 27 (see Supplementary Information). This approach measures the C utilization efficiency of added ^{13}C-labelled substrates and represents a proxy for microbial efficiency. Microbial efficiency was estimated as $dB_C/(dB_C + \Sigma CO_2\text{–}C)$, where dB_C is the amount of substrate C incorporated into microbial biomass and ΣCO_2–C is the cumulative substrate C lost during respiration[28].

We used DAYCENT to simulate the effects of soil warming on soil C storage at our site in response to altering the microbial efficiency parameter in the model. DAYCENT is a fully resolved biogeochemical model that simulates the main processes associated with dynamics of C, N and other nutrients, soil temperature and water dynamics[26] (see Supplementary Information for details on model structure, inputs, parameterization and calibration). The microbial efficiency parameter in the model is defined as the amount of C lost as CO_2 from the litter and SOM pools as a result of microbial respiration. Default model parameters were modified to alter microbial efficiency by ±10–50%, consistent with the temperature response we observed for the utilization of glutamic acid and phenol. Both ambient (no warming) and chronic soil warming conditions were considered. The chronic soil warming condition was achieved by increasing daily maximum and minimum temperatures by 5 °C year-round.

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Author contributions

S.D.F. and J.S. conceived the project. S.D.F. collected the soil samples, conducted the soil incubations, and led the data analysis and manuscript preparation. J.S. supervised the stable isotope analysis and modelling exercise and assisted with data interpretation. J.L. conducted the model runs. J.M.M. designed and conducted the long-term (18 year) soil warming experiment, made the *in situ* measurements of net nitrogen mineralization, and assisted with data interpretation. 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 S.D.F.

Competing financial interests

The authors declare no competing financial interests.