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Reduced carbon use efficiency and increased microbial turnover with soil warming

Running head: soil warming experiment and data assimilation

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Abstract. Global soil carbon (C) stocks are expected to decline with warming, and changes in microbial processes are key to this projection. However, warming responses of critical microbial parameters such as carbon use efficiency (*CUE*) and biomass turnover (*rB*) are not

well understood. Here, we determine these parameters using a probabilistic inversion approach that integrates a microbial-enzyme model with 22 years of carbon cycling measurements at Harvard Forest. We find that increasing temperature reduces *CUE* but increases *rB*, and that two decades of soil warming increases the temperature sensitivities of *CUE* and *rB*. These temperature sensitivities, which are derived from decades-long field observations, contrast with values obtained from short-term laboratory experiments. We also show that long-term soil C flux and pool changes in response to warming are more dependent on the temperature sensitivity of *CUE* than that of *rB*. Using the inversion-derived parameters, we project that chronic soil warming at Harvard Forest over six decades will result in soil C gain of <1.0% on average (1st and 3rd quartiles: 3.0% loss and 10.5% gain) in the surface mineral horizon. Our results demonstrate that estimates of temperature sensitivity of microbial *CUE* and *rB* can be obtained and evaluated rigorously by integrating multi-decadal datasets. This approach can potentially be applied in broader spatiotemporal scales to improve long-term projections of soil C feedbacks to climate warming.

INTRODUCTION

Integration of microbial processes into carbon (C) cycle models can potentially improve simulations of soil C dynamics under climate warming (Wieder et al. 2013, Luo et al. 2016). Uncertainty in long-term soil C responses to climate change will likely be reduced with more realistic and accurate parameterizations of key microbial processes that regulate soil C stocks and respiratory C losses (Todd-Brown et al. 2012, Wieder et al. 2015, Luo et al. 2016). These key parameters include carbon use efficiency (hereafter *CUE*), defined as the fraction of C uptake allocated to growth (Allison et al. 2010, Geyer et al. 2016), and microbial biomass turnover rate (hereafter *rB*), i.e. the fraction of microbial biomass that leaves the microbial pool per unit of time (Hagerty et al. 2014). These two parameters are critical for modeling soil C change with warming (Hagerty et al. 2014, Li et al. 2014) but remain poorly quantified (Manzoni et al. 2017, Sinsabaugh et al. 2017, Xu et al. 2017). It is also unclear whether heterotrophic microbes might acclimate to long-term warming through reductions in the temperature sensitivities of *CUE* and *rB* (Allison et al. 2010, Frey et al. 2013, Wieder et al. 2013).

Rising soil temperatures are generally expected to reduce *CUE*, as warming limits microbial growth by increasing the energy cost of maintaining existing biomass (Manzoni et al. 2012, Sinsabaugh et al. 2013). Observed *CUE* of soil microbial communities, however,

has shown variable responses to rising temperature including increases, decreases, or no response (Steinweg et al. 2008, Frey et al. 2013, Sinsabaugh et al. 2013, Li et al. 2018), due to fundamentally different pathways of C allocation in assimilation, enzyme production, and respiration for biomass maintenance and enzyme production (Hagerty et al. 2018). In addition, warming can enhance *rB* if the cell-specific microbial death rate outpaces cell production (Joergensen et al. 1990). Dead microbial cells can be metabolized by living microbes, incorporated into the soil organic carbon (SOC) pool, or protected from decomposition by physicochemical occlusion in soil particles (Six et al. 2002, Lehmann and Kleber 2015).

Quantifying *CUE*, *rB*, and their temperature responses remains a major challenge. There are no techniques available to measure these quantities *in situ*, so prior studies have relied mainly on laboratory incubations with isotopic tracers. For example, Hagerty et al. (2014) showed increased *rB* but constant *CUE* with warming in a week-long soil incubation. Still, it remains unclear how these key microbial variables respond to warming over decadal time scales that are more relevant to climate change (Frey et al. 2013, Geyer et al. 2016). Traditionally, a sole value of a model parameter can be determined via least squares fitting between model output and observation (Luo et al. 2011). Probabilistic inversion techniques use data to inform model parameters and produce most probable values and uncertainties of parameters (Clark 2005, Luo et al. 2011). Probabilistic inversion thus offers an alternative to the deterministic modeling approach and direct empirical measurement of key microbial parameters, particularly for those not well quantified due to technical difficulty. With an inversion approach, observational data are used to constrain the model. Parameter values are discounted if they result in model outputs inconsistent with the data (Clark 2005, Xu et al. 2006, Luo et al. 2011). Previously, such approaches have been applied successfully in many contexts, including terrestrial carbon cycling (Niu et al. 2014, Hararuk et al. 2015).

Here we used a probabilistic inversion approach (i.e., the Bayesian inference) to estimate the apparent temperature sensitivities (hereafter referred to as temperature sensitivities) of *CUE* and *rB* under field conditions. We assembled 14 datasets that were collected from soil warming experiments at the Harvard Forest Long-term Ecological Research (LTER) site in Petersham, MA, USA, where soil temperature has been continuously elevated to ~5°C above ambient for 10 to 26 years (Melillo et al. 2017). We used Bayesian probabilistic inversion to obtain the temperature sensitivity coefficients of *CUE* and *rB* by assimilating data into the Microbial-ENzyme Decomposition (MEND) model. MEND was chosen because it has been validated previously, and it represents relevant microbial

processes and mineral interactions without excessive complexity (Wang et al. 2013, Li et al. 2014). To analyze the effects of temperature-sensitive *CUE* and *rB* on long-term soil C dynamics, posterior parameter values and forcing data obtained from the control and heated plots were implemented in long-term projections of soil carbon and respiratory responses over six decades.

MATERIALS AND METHODS

Data compilation from Harvard Forest

We assembled multiple observational datasets collected from several experimental soil warming studies at the Harvard Forest Long-term Ecological Research (LTER) site in Petersham, MA, USA (42°50′ N, 72°18′ W). The list of data sources is presented in Table 1. The climate at Harvard Forest is cool, temperate and humid, with mean annual precipitation and mean annual air temperature of 1080 mm and 7.0 ºC, respectively. Soils are of the Gloucester series (fine loamy, mixed, mesic, Typic Dystrochrepts) and dominant tree species are red oak (*Quercus rubra*) and red maple (*Acer rubrum*) (Peterjohn et al. 1993).
Data span the period of 1989-2010 and were obtained from published articles or the Harvard Forest online data archive (http://harvardforest.fas.harvard.edu/harvard-forest-data-archive). Data were collected from three soil warming experiments initiated at three different times (1991, 2001, and 2006). Site and experimental design information is described in Peterjohn et al. (1993), Melillo et al. (2002), and Contosta et al. (2011).

Briefly, soils in heated plots were continuously warmed 5 ºC above control plots using buried heating cables placed 10 cm below the soil surface and spaced 20 cm apart. Climate conditions, soil temperature and soil moisture were monitored continuously. Soil respiration was measured monthly between April and October. Datasets of soil temperature (Melillo et al. 1999, Arguez et al. 2010, Brzostek and Finzi 2011a), CO_2 efflux (Melillo et al. 1999), soil C (Nadelhoffer et al. 1990, Frey 2009), DOC (Compton et al. 2004, Bradford et al. 2008), MBC (Compton et al. 2004, Wallenstein et al. 2006, Frey et al. 2008), extracellular enzyme activity (EEA) (Brzostek and Finzi 2011a), and litterfall (Frey and Ollinger 1999), were also used for this modeling study.

Several assumptions were made to meet the requirements for MEND model input and the inversion analysis. Litter input C used for the model was assumed to be 48% of measured litter biomass (Schlesinger and Bernhardt 2013), and litter entered the SOC and DOC pool at a constant rate (i.e. 98% as particular organic carbon (POC) and 2% as DOC). SOC

concentrations were selected to represent the top 10-cm mineral soil depth (i.e. A horizon). Using an average value for specific enzyme activity (i.e. μmol min^{-1} mgC^{-1}) and a temperature normalization based on a measured Q_{10} value (Q_{10}=2) (Allison et al. 2018), extracellular enzyme data in each collection were converted to potential activity (i.e. μmol g^{-1} soil hr^{-1}) of labile substrate-acquiring enzymes (i.e. the sum of β-D-cellobiosidase, acid phosphatase, protease and β-1,4-N-Acetyl-glucosaminidase) and oxidase (i.e. the sum of peroxidase and phenol oxidase) that contribute to fast- and slow-cycling soil organic matter turnover, respectively. The sum of these potential activities is equivalent to the sum of enzyme activities for POC and mineral-associated organic carbon (MOC). Soil heterotrophic respiration was assumed to represent 67% of measured soil CO_2 efflux (Bowden et al. 1993, Sanderman 1998, Melillo et al. 2002). Daily soil temperature measurements at 4-cm depth (i.e. approximately at the middle of 10-cm soil depth) were available during 1991-2010 (Melillo et al. 1999).

We calculated hourly soil temperatures based on daily averages and the NCEP Climate Forecast System Reanalysis (CFSR) which provides hourly gridded soil temperature data at 5-cm soil depth (http://rda.ucar.edu/datasets/ds093.1/index.html). Scaled hourly variation of soil temperature at Harvard Forest from the CFSR data was added to the daily average station observation. A scaling factor, computed as the ratio of standard deviation of daily station observation to standard deviation of daily average CFSR data, was applied to the hourly variation of CFSR data. The daily station observation was derived from hourly observations in 2009 and 2010 (Brzostek and Finzi 2011a). The use of scaling factor is to account for the depth difference below the soil surface in the CFSR and station data. The available datasets are presented in Fig. S1.

Microbial-ENzyme Decomposition (MEND) model

MEND is a microbial ecosystem model that incorporates multiple soil and enzyme pools (Wang et al. 2013) and shows reasonable fit to soil C observations in response to perturbation (Li et al. 2014). The model structure is presented in Fig. S2, and the full list of governing equations can be found in Li et al. (2014). In MEND, the decomposition of particulate organic matter (POC) and mineral-associated organic matter (MOC), and the uptake of dissolved organic matter (DOC) are described by the Michaelis-Menten kinetics with a half-saturation constant (*K*) and maximum reaction rate (*V*). The kinetics parameters are temperature sensitive and represented by the Arrhenius equation (Wang et al. 2012). In

addition, the adsorption and desorption rates of DOC are also temperature dependent (Cornelissen et al. 1997, Wang et al. 2013). Following SOC decomposition and DOC uptake, C is lost through growth and maintenance respiration dependent on *CUE*. Note that the *CUE* parameter in MEND refers to the assimilation efficiency (Pirt 1965, Wang and Post 2012). Consistent with previous studies, the model assumes that carbon use efficiency (*CUE*, E_C) varies with temperature based on a linear relationship (Fieschko and Humphrey 1984, DeVêvre and Horwáth 2000, Steinweg et al. 2008, Frey et al. 2013, Tucker et al. 2013):

$$E_C(T) = E_{C,ref} + m \times (T - T_{ref}) \quad (1)$$

where $E_C(T)$, $E_{C,ref}$, and m denote the *CUE* at simulation temperature *T*, the reference temperature (T_{ref}), and the slope parameter ($°C^{-1}$), respectively.

In the model, microbial turnover rate (*rB*) also depends on temperature. The temperature sensitivity of the microbial turnover rate (*n*) is defined based on the following equation (Saggar et al. 1999, Malik et al. 2013, Hagerty et al. 2014):

$$rB(T) = rB_{ref} + n \times (T - T_{ref}) \quad (2)$$

where $rB(T)$, rB_{ref}, and n denote the *rB* at simulation temperature *T* (i.e., 5 °C), the reference temperature (20 °C), and the slope parameter (mg C mg^{-1} C h^{-1} $°C^{-1}$), respectively.

Data-model integration via a probabilistic inversion analysis

We used a Bayesian probabilistic inversion technique to constrain five key model parameters and seven initial pool sizes under the control and heated conditions, respectively. These parameters include the *CUE* at the reference temperature ($E_{C,ref}$), the temperature sensitivity of *CUE* (*m*), the temperature sensitivity of the microbial turnover rate (*n*), the fraction of decomposed POC entering DOC (*fD*), and the fraction of dead microbes becoming DOC (*gD*), as well as seven initial pool sizes (*iPOC*, *iMOC*, *iQOC*, *iMBC*, *iDOC*, *iEP* and *iEM*; Table 2). Default values of these and other model parameters are presented in Table S1.

Constructing the likelihood function -- According to the Bayes' theorem (Clark 2005), the posterior probability density function (PDF) $P(p|Z)$ of model parameters *p* can be estimated from the prior knowledge of parameters *p* (i.e., a prior PDF, $P(p)$) and the information contained in existing observations (i.e., a likelihood function $P(Z|p)$):

$$P(p|Z) \propto P(Z|p)P(p) \quad (4)$$

Assuming that errors between observed and modeled values follow Gaussian

distributions, the likelihood function $P(Z|p)$ can be expressed by:

$$P(Z|p) \propto \exp\left\{-\sum_{i=1}^{6}\sum_{t\in Z_i}\frac{[Z_i(t)-X_i(t)]^2}{2{\sigma_i}^2(t)}\right\} \quad (5)$$

where $Z(t)$ is measured value, $X(t)$ is model simulation, and σ is the standard deviation for each measurement. i = 1, 2, … 6, represents the available observations of hourly CO_2 efflux, daily CO_2 efflux, SOC, DOC, MBC and ENC (i.e. the sum of EP and EM). We adopt the Gaussian assumption for mathematical convenience in the absence of more precise information about the data-model error structure (Feyen et al. 2003, Luo et al. 2003, Luo and Zhou 2010).

Prior knowledge -- The prior PDF $P(p)$ is specified by giving a set of limiting intervals for parameters p with uniform distribution. We set the prior range of m to (-0.017, 0.017) and the prior range of n to (-4e-5, 4e-5) to reflect the range of values observed in the literature (Table 2). Despite negative values revealed in previous experiments (Fig. S3), the positive values of m were included according to Sinsabaugh et al. (2017), in which the microbial *CUE* increased weakly with mean annual temperature. The prior ranges of the five parameters and seven initial pool sizes were determined based on published values and presented in Table 2.

Posterior probability density function -- The posterior PDFs were then generated from prior PDFs $P(p)$ with observations Z by a Markov chain Monte Carlo (MCMC) sampling technique, using the Metropolis-Hastings (M-H) algorithm as the MCMC sampler (Xu et al. 2006). Specifically, the M-H algorithm was run by repeating two steps: a proposing step and a moving step. In each proposing step, the algorithm generated a new point p^{new} for a parameter vector p based on the previously accepted point p^{old} with a proposed distribution $P(p^{\text{new}}|p^{old})$:

$$p^{new} = p^{old} + \theta(p_{max} - p_{min}) \quad (6)$$

where $p_{\max}$ and $p_{\min}$ are the maximum and minimum values within the *prior* range of the given parameter. θ is a random variable between -0.5 and 0.5 with a uniform distribution. In each moving step, point p^{new} was tested to determine whether it should be accepted or not. Whether a new point p^{new} was accepted or not depends on the comparison of $R = \frac{P(p^{new}|Z)}{P(p^{old}|Z)}$ with a uniform random number U from 0 to 1. Only if $R \geq U$ is the new point accepted;

otherwise $p^{new}=p^{old}$.

Parameter selection and long-term projection

Five parallel runs of the MCMC algorithm started at dispersed initial points were conducted with each run iterated for 100,000 times. The acceptance rates for the newly generated samples were ~10% under control conditions and ~22% under heated conditions for each run, and all five runs passed the stability test prior to data analysis (Table S2). The initial samples (about 5000 and 11000 in the so called burn-in period) were discarded after the running means and standard deviations stabilized. The union of the samples of the five runs (about 25,000 and 55,000 samples in total) after their burn-in periods was used to derive and compare the posterior means and standard deviations of the target parameters for control and heated conditions. The model performance with inversion (i.e., calibration of parameters based on observations) and without inversion (i.e., relying on default parameterization) was compared based on model simulations given the default and posterior mean parameter values (R^2 presented). The means of posterior parameters (*m*, *n*) were compared based on the student-t test and the *p-values* were reported.

To examine effects of different *CUE* and *rB* parameterization on soil C stocks and CO_2 emissions as well as the associated uncertainties, the model was first run to reach equilibrium under constant forcing data (i.e. soil temperature and litterfall inputs averaged over 22 years under control conditions). Then, long-term model projections were conducted by running the model forward based on 3,000 pairs of *m* and *n* sampled from the inversion derived posterior distribution under both control and heated conditions. We simulated four different scenarios to analyze the consequences of variation in *m* and *n*. The four scenarios included no temperature sensitivities of *CUE* or *rB* (*m=0; n=0; Scenario I*), no temperature sensitivity of *CUE* but sampled posterior temperature sensitivity of *rB* (*m=0; varying n; Scenario II*), no temperature sensitivity of *rB* but sampled posterior temperature sensitivity of *CUE* (*n*= 0; *varying m; Scenario III*), and sampled posterior temperature sensitivities of *CUE* and *rB* (*varying m* and *n*; *Scenario IV*). In each scenario, model projections were conducted for 66 years which represents three repetitions of the original 22-year forcing data. The end-simulation SOC pool sizes and cumulative CO_2 emissions were obtained.

To further examine climate change effects on soil C stocks and CO_2 emissions, the model projections were also conducted under three different forcing conditions, i.e. 0ºC increase in soil temperature (W0), 5ºC increase in soil temperature (W5), and 5ºC increase in

soil temperature in addition to 9.6% increase in litterfall input, a value derived from the litterfall input averaged over 22 years under heated conditions (W5L). The end-simulation SOC pool sizes and cumulative CO_2 emissions was calculated under W0, W5 or W5L for each scenario (*I~IV*). For each projection, the relative changes in SOC stock and CO_2 emission with climate warming (5ºC) were calculated by comparisons between W5 and W0. Based on the 3,000 independent simulations, the means of relative changes were compared between treatments with control plot parameters and heated plot parameters based on the student-t test. A bar graph and a boxplot were also produced to display the mean, standard deviation, median, 1st and 3rd quartiles of these long-term projections.

RESULTS

Model performance

The accuracy of model simulations was significantly enhanced when parameters were estimated via our probabilistic inversion approach. For heterotrophic soil respiration, the coefficients of determination (R^2) increased from 0.26 without the inversion to 0.59 with inversion in the control soil, and from 0.14 without inversion to 0.75 with inversion in the heated soil (Fig. 1). The simulations of respiration, MBC, DOC, and SOC also better matched the observations using this inversion approach (Fig. S4). The posterior probability distributions of all target parameters in the inversion differed between the control and heated conditions (Figs. S5, S6).

Temperature sensitivity of microbial *CUE* and *rB*

The mean values of temperature sensitivity of *CUE* (i.e. the slope *m*) were -0.0101 $°C^{-1}$ under control conditions and -0.0117 $°C^{-1}$ under heated conditions, which differed significantly from each other ($P<0.001$; Fig. 2). The standard deviation of *m* was 0.0052 in both cases. The absolute value of slope *m* was 15.1% greater under heated conditions than that under control conditions. Given the mean value of *m* and observed soil temperatures, the average *CUE* was estimated at 0.42 with a range of 0.25–0.67 in the control conditions, and the average was 0.39 with a range of 0.19–0.66 in the heated conditions (Fig. S7).

The mean values of temperature sensitivity of *rB* (i.e. the slope *n*) were 1.58e-5 h^{-1} $°C^{-1}$ (i.e., 3.80e-4 d^{-1} $°C^{-1}$) under control conditions and 1.66e-5 h^{-1} $°C^{-1}$ (i.e., 3.99e-4 d^{-1} $°C^{-1}$) under heated conditions, which differed significantly from each other (Fig. 2). The slope *n* was 5.0% greater under heated conditions than under control conditions.

Temperature sensitivities of microbial *CUE* and *rB* on long-term projections

The simulated trajectory of SOC stocks and CO_2 emissions with warming was influenced by the temperature sensitivities of *CUE* and *rB* (Fig. 3 and Fig. S8). Assuming control-plot derived parameters, no temperature sensitivity of either *CUE* or *rB*, and a +5ºC temperature forcing, SOC stocks on average declined by 15.6%, and emissions of CO_2 increased by ~8.0% on average (blue bars, top and bottom panels in Fig. 3). With a temperature-sensitive (i.e., increasing) *rB* and a constant *CUE*, the results were nearly identical. With a temperature-sensitive (i.e., decreased) *CUE* and a constant *rB*, SOC stocks declined by ~2.1% and emissions of CO_2 increased by ~0.7% on average. When both *CUE* and *rB* were temperature sensitive, the results were very similar to when only *CUE* was temperature sensitive.

Assuming heated plot parameters, SOC and CO_2 trajectories under warming appeared significantly different from those under control plot parameters (compare red and blue bars in scenarios II, III and IV, $P<0.001$, Fig. 3). When there was no *CUE* temperature sensitivity, the difference between treatments appeared minor (compare red and blue bars in scenario II, Fig. 3). However, increasing the *CUE* temperature sensitivity (i.e., heated plot parameters vs. control plot parameters) resulted in SOC gains of 0.5% and 0.9% on average, respectively, which contrasted with SOC reductions (compare red and blue bars in scenarios III and IV, Fig. 3). The variations of the projected end-simulation pool sizes and respiration are presented in Fig. S8. When the effects of experimental warming and temperature sensitivities of both parameters were combined, uncertainty in the SOC projection ranged from a 3.0% loss to a 10.5% gain for the 1st and 3rd quartiles, or from a 12.2% loss to a 13.6% gain for the 5% and 95% quantiles (i.e., scenario IV, Fig. S8). We also found that elevated litter inputs with warming did not substantially affect SOC stock changes (Table S3).

DISCUSSION

Warmer temperature reduced *CUE* but decades-long warming elevated *CUE* temperature sensitivity

Given the inversions conducted in both control and heated conditions, the negative slope *m* indicates that increasing temperature reduced microbial *CUE* in field experimental conditions, which is consistent with many studies based on laboratory experiments (Manzoni et al. 2012, Sinsabaugh et al. 2013). Previous observations also have suggested a wide range

of *m* from -0.017 to -0.003 $°C^{-1}$ (DeVêvre and Horwáth 2000, Steinweg et al. 2008, Frey et al. 2013, Tucker et al. 2013), consistent with the negative effect of increasing temperature on maintenance energy observed in experiments with heterotrophic soil microbes (Crowther and Bradford 2013, Frey et al. 2013). Therefore soil warming, under either field or laboratory conditions, can generally lead to constraints on microbial metabolic activity due to greater energy cost for maintaining microbial biomass (del Giorgio and Cole 1998, Frey et al. 2013) or energy spilling (i.e., waste metabolism) (Bradford 2013).

We found no evidence that Harvard Forest microbes acclimate to warming by reducing the temperature sensitivity of *CUE*. The absence of microbial acclimation is consistent with a sustained increase in soil microbial activity in response to geothermal warming in a different study (Walker et al. 2018). Incubations with C-rich calcareous temperate forest soils subjected to 9 years of warming also showed no thermal adaptation of the microbial decomposer community (Schindlbacher et al. 2015). Based on our model inversion, *CUE* was more temperature sensitive with long-term soil warming (slope m = -0.0101 $°C^{-1}$ for control plot vs. -0.0117 $°C^{-1}$ for heated plot). Our results contrast with those of Frey et al. (2013) who found a decline in the temperature sensitivity of microbial *CUE* in Harvard Forest soils subjected to 18 years of warming. Although the reason for this discrepancy is uncertain, the temperature acclimation in Frey et al. (2013) was only observed for one of three added carbon substrates (i.e., phenol) in a laboratory assay and may not apply to the integrated *CUE* determined by our inversion analysis.

The greater temperature sensitivity of *CUE* under heated compared to control conditions could be driven by selection for microorganisms with higher maintenance costs (Frey et al. 2008, Zhou et al. 2012, DeAngelis et al. 2015). After 12 years of warming at Harvard Forest, relative abundances of fungal biomarkers declined whereas gram positive bacterial and actinobacterial biomarkers increased (Frey et al. 2008). Such community shifts may have overridden physiological acclimation of *CUE* within some microbial species (Allison 2014, DeAngelis et al. 2015, Melillo et al. 2017).

The inversion-derived averages (0.39 and 0.42 for the control and warming plots) and range of *CUE* (0.19–0.67) are similar to values reported previously for Harvard Forest soils subject to 2- and 18-year warming treatments (Frey et al. 2013) and also comparable to the average values (i.e. 0.3) observed in soils and aquatic ecosystems (Sinsabaugh et al. 2013). The inversion-derived maximal *CUE* value (0.67) is close to the thermodynamic efficiency of aerobic microbial growth (Roels 2009). However, the inversion-derived average and range

are much lower than 0.72–0.74, the values reported from a week-long lab incubation study with ^{13}C-labelled glucose in a forest soil (Hagerty et al. 2014), or 0.7–0.8 reported in a month-long incubation study with cellobiose amendment in a cropland soil (Steinweg et al. 2008).

The lower value of *CUE* determined here suggests that the active microbial community functions at low biochemical efficiency under field conditions, implying that microorganisms with relatively high maintenance costs dominate in field soils. Low *CUE* may also indicate reduced availability of labile substrates as energy sources (Knorr et al. 2005) or dominance of recalcitrant organic compounds in SOC (Frey et al. 2013). On the other hand, the higher value of measured *CUE* in incubation studies could be due to short measurement periods of hours to weeks; longer incubations yield lower effective *CUE* values (Hagerty et al. 2018).

The isotopic probing approach via ^{13}C-labelled substrate amendment used to quantify *CUE* in these incubation studies (Steinweg et al. 2008, Hagerty et al. 2014) may also have led to an overestimation of *CUE*. In short-term incubation studies, the re-use of ^{13}C in microbial necromass and microbial preference for ^{12}C for respiration could result in a relatively ^{13}C-enriched micobial biomass pool and relatively ^{13}C-depleted respiration, which were used to derive *CUE*. Furthermore, some *CUE* values (~0.8) reported for agricultural soils (Steinweg et al. 2008) exceeded the formerly reported maximal carbon conservation efficiency for microbial growth (Roels 2009), potentially due to more efficient C uptake induced by the labile substrate addition in agricultural soils.

Warmer temperature accelerated turnover and decades-long warming increased *rB* temperature sensitivity

Given the inversion results in this study, the positive slope *n* indicates that microbial turnover was faster with higher temperatures, which may be attributed to a shift in microbial community physiology, stimulated viral activity, and/or accelerated senescence of microbial cells (Joergensen et al. 1990). The same mechanisms may also explain the increased temperature sensitivity of turnover with warming (i.e., +5ºC) over decades.

This slope *n* is 3.80–3.99e-4 d^{-1} $°C^{-1}$ under control and heated conditions, which is about one order of magnitude lower than the value of 0.003–0.004 d^{-1} $°C^{-1}$ derived from the one-week lab incubation experiment described previously (Hagerty et al. 2014). Given the mean value of *n* and observed soil temperatures in our inversion study, *rB* derived at 20ºC is

only half the value observed at the same temperature in the one-week laboratory study (Hagerty et al. 2014).

These comparisons marked a major difference in the microbial biomass turnover rate estimated over time scales of days vs. decades. We speculate that given little change in microbial biomass, the high biomass turnover rate with warming over the short term may be driven by stronger microbial competition, thus leading to greater cell death (Kakumanu et al. 2013), greater formation of necromass (Crowther et al. 2015) and higher extracellular enzyme activities (Blankinship et al. 2014). Furthermore, the metabolic tracer probing method used in the short-term laboratory experiment can potentially overestimate the biomass turnover rate (Dijkstra et al. 2011). Temperature sensitivities of microbial biomass turnover that were one order of magnitude lower in our study may be associated with widespread microbial dormancy through which microbes acclimate to stress and reduce mortality (Lennon and Jones 2011).

Elevated temperature sensitivity of *CUE* reduced long-term soil C losses

The 66-year simulation results indicated that *rB* had minimal effects, but that *CUE* was important in determining CO_2 emissions and SOC stocks. Mechanistically speaking, the lower *CUE* at higher temperature resulted in fewer resources allocated to microbial biomass and associated enzyme pools given a constant uptake. These changes might reduce the decomposition rate (Li et al. 2014), thereby diminishing both SOC loss and CO_2 emissions.

A recent report indicates that 26 years of soil warming at the Harvard Forest resulted in a loss of about 8-17% of SOC in the upper 60cm of the soil (Melillo et al. 2017). Given the 12.2% loss to a 13.6% gain (5% and 95% quantiles) in SOC over six decades revealed in the inversion analysis, the MEND model may underestimate potential SOC losses from the full soil profile under warming, even when parameterized through an inversion approach with Harvard Forest data. Future incorporation of SOC stock changes into the model inversion would be useful for improving estimates of parameters, particularly *m* (*CUE* temperature sensitivity) which showed a broad distribution (Fig. 2). Our results suggest that lower magnitudes of *m* could result in MEND simulations more consistent with observed SOC losses under warming (Fig. 3).

Implications for soil warming experiments and data assimilation

Using Bayesian inversion approaches to combine emerging biogeochemical datasets with more advanced models should help improve confidence in predictions of carbon-climate feedbacks. Our inversion approach offered a tractable means of parameterizing the long-term response of *CUE* and turnover rate sensitivity to temperature based on available data. Still, we emphasize that our results could change as additional data, mechanisms, and feedbacks are incorporated into models like MEND. More soil C and microbial biomass measurements over years to decades would likely have substantially reduced the uncertainty of our parameter estimates. Furthermore, the MEND model used in this study lacks potentially important details about microbial community structure, moisture responses, and climate-driven feedbacks with the vegetation community that should be considered in future modeling efforts. To address potential experimental artefacts, future inversion analyses should also consider incorporating disturbance controls (i.e., heating cables installed but not turned on) if such data are available from field experiments.

We conclude that both *CUE* and microbial turnover are key parameters moderating SOC stocks and respiratory C losses at higher temperatures, but their inferred temperature sensitivities differ substantially depending on experimental duration and measurement approaches. Our simulations confirm that these parameters influence the decadal-scale predictions of SOC stock and CO_2 emission changes with warming. In particular, the temperature sensitivity of *CUE* induced a more pronounced effect on soil C dynamics than that of microbial turnover. Further, we did not find evidence that acclimation of microbial *CUE* or *rB* is likely to affect soil dynamics under warming. Our method could be applied to the increasing number of datasets on soil C cycle responses to perturbation at annual to decadal time scales, thereby incorporating key microbial functions into global ecosystem models and improving long-term projections of soil C changes and CO_2 emissions under environmental and climate changes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Datasets and their sources collected from the soil warming experiments at Harvard Forest Long-term Ecological Research (LTER) site, Massachusetts, USA.

No.	Variable	Frequency	Measurement period	References
1	Litterfall	Yearly	1989-2010	(Frey and Ollinger 1999)
2	Litterfall	Yearly	2001-2013	(Melillo et al. 2013)
3	Soil CO_2 efflux	Hourly, consecutive	1991-2010	(Melillo et al. 1999, Contosta et al. 2013)
4	SOC	certain days	1990, 1991, 1995, 2000	(Nadelhoffer et al. 1999)
5	DOC	certain days	1999, 2000, 2001	(Compton et al. 2004)
6	DOC	certain days	2005, 2006	(Bradford et al. 2008)
7	MBC	certain days	1999, 2000, 2001	(Compton et al. 2004)
8	MBC	certain days	2002	(Wallenstein et al. 2006)
9	MBC	certain days	2002	(Frey et al. 2008)
10	MBC	certain days	2005, 2006	(Bradford et al. 2008)
11	EEA	certain days	2008, 2009, 2010	(Brzostek and Finzi 2011b)
12	Soil temperature	Daily, consecutive	1991-2010	(Melillo et al. 1999)
13	Soil temperature	Hourly, consecutive	2009-2010	(Brzostek and Finzi 2011b)
14	Soil temperature	Hourly, consecutive	1989-1990	(Arguez et al. 2010)

SOC: soil organic carbon; DOC: dissolved organic carbon; MBC: microbial biomass carbon; EEA: extracellular enzyme activity.

Table 2. Parameters and their prior ranges included under control and heated conditions in the probabilistic inversion analysis.

Parameter	Description	Unit	Lower limit	Upper limit	Reference
$E_{C,\,ref}$	*CUE* at reference temperature	mg C mg^{-1} C	0	0.72	(Manzoni et al. 2012, Sinsabaugh et al. 2013)
m	Temperature sensitivity of *CUE*	mg C mg^{-1} C $°C^{-1}$	-0.017	0.017	See Fig. S3; (Sinsabaugh et al. 2016, Sinsabaugh et al. 2017)
n	Temperature sensitivity of *rB*	mg C mg^{-1} C h^{-1} $°C^{-1}$	-4e-5	4e-5	(Gregorich et al. 1991, Gregorich et al. 2000)
fD	Fraction of decomposed POC allocated to DOC	-	0.3	0.7	(Wang et al. 2012, Wang et al. 2013)
gD	Fraction of dead MBC transferred to SOC	-	0.3	0.7	(Pietikainen et al. 2005)
iPOC	Initial pool size of POC	mg C g^{-1} soil	1	23	(Nadelhoffer et al. 1999)
iMOC	Initial pool size of MOC	mg C g^{-1} soil	30	55	(Nadelhoffer et al. 1999)
iQOC	Initial pool size of QOC	mg C g^{-1} soil	0.1	1.9	(Nadelhoffer et al. 1999)
iMBC	Initial pool size of MBC	mg C g^{-1} soil	0.02	0.9	(Frey et al. 2008)
iDOC	Initial pool size of DOC	mg C g^{-1} soil	0.02	0.9	(Compton et al. 2004)
iEP	Initial pool size of EP	mg C g^{-1} soil	0.0001	0.007	(Brzostek and Finzi 2011a)
iEM	Initial pool size of EM	mg C g^{-1} soil	0.0001	0.007	(Brzostek and Finzi 2011a)

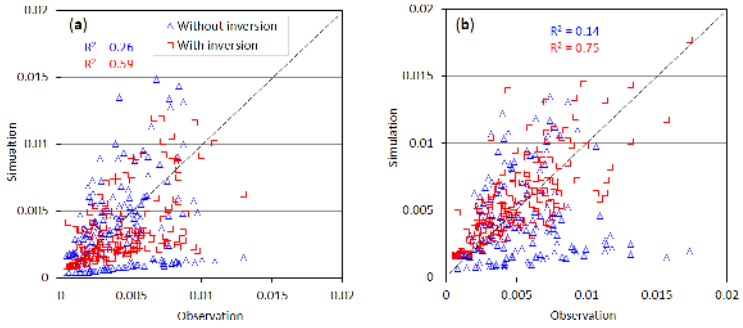
POC: particulate OC; MOC: mineral-associated OC; QOC: DOC associated with mineral surface; EP: enzymes for decomposition of POC; EM: enzymes for decomposition of MOC.

Figure captions

Figure 1. MEND model outputs of daily soil CO_2 efflux rate (mg C m^{-2} day^{-1}) at Harvard Forest better matched observational data with the inversion approach (red) compared to without the inversion (blue) in both control (a) and heated (b) conditions.

Figure 2. Boxplots of temperature sensitivities of *CUE* (above) and *rB* (bottom) in control and heated conditions. Boxplots show means (dot), medians (line), 1st and 3rd quartiles (box, interquartile range or *IQR*), upper and lower extremes (whiskers). The whiskers were determined as equal to or less extreme than 1.5 times *IQR* against 1st and 3rd quartiles, respectively. $P < 0.001$ denotes significant difference between means in control and heated conditions.

Figure 3. Mean (±SD) relative changes in percentage in SOC stock (top panel) and CO_2 emission (bottom panel) with warming (i.e. W5 vs. W0) based on 66-yr model projections using control and heated plot parameters under scenarios I~IV. Scenario details are presented in the *Method* section.



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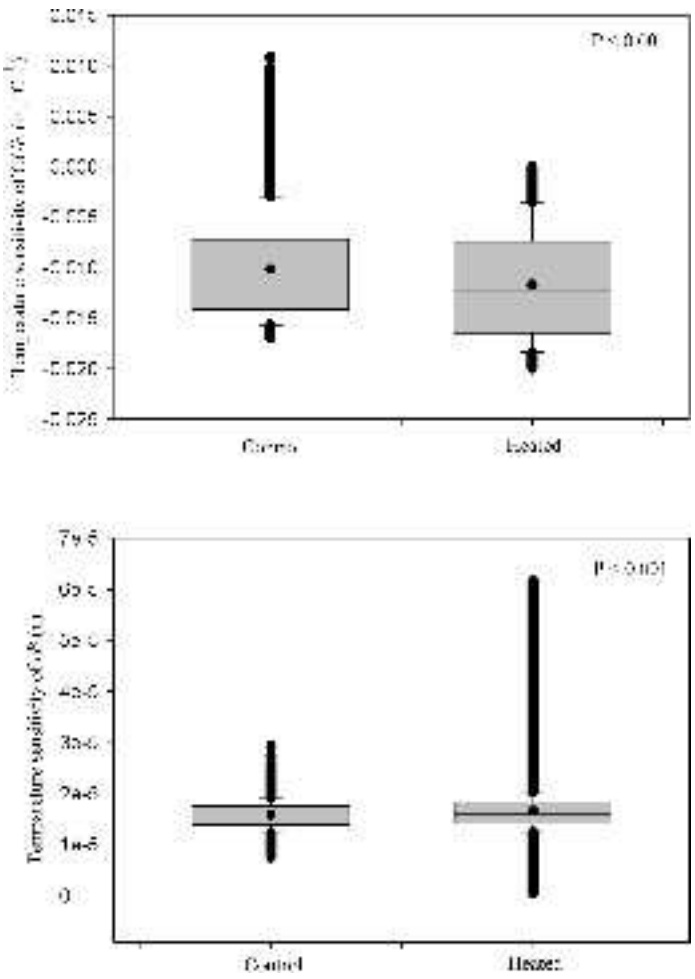

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