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Description/ abstract:

The central objective of the proposed work was to develop a genomic approach (nucleic acid-based) that elucidates the mechanistic basis for the observed impacts of experimental soil warming on forest soil respiration. The need to understand the mechanistic basis arises from the importance of such information for developing effective adaptation strategies for dealing with projected climate change. Specifically, robust predictions of future climate will permit the tailoring of the most effective adaptation efforts. And one of the greatest uncertainties in current global climate models is whether there will be a net loss of carbon from soils to the atmosphere as climate warms. Given that soils contain approximately 2.5 times as much carbon as the atmosphere, a net loss could lead to runaway climate warming. Indeed, most ecosystem models predict that climate warming will stimulate microbial decomposition of soil carbon, producing such a positive feedback to rising global temperatures. Yet the Intergovernmental Panel on Climate Change highlights the uncertainty regarding this projected feedback. The uncertainty largely arises because although warming-experiments document an initial increase in the loss of carbon from soils, the increase in respiration is short-lived, declining to control levels within a few years. This attenuation response could result from changes in microbial physiological properties with increasing temperature. We explored such possible microbial responses to warming using experimental measures and conceptual modeling. Our work advances our understanding of how soil microbial communities and their activities are structured, and

generates insights into how soil carbon might respond to climate warming. Using genomic approaches, we show the importance or resource partitioning in structuring microbial communities. Specifically, we quantified the relative abundance of fungal taxa that proliferated following the addition of a variety of organic substrates to soil. We added glycine, sucrose, cellulose, lignin, or tannin-protein (in order of increasing resistance to decay) directly to soils in conjunction with 3-bromo-deoxyuridine (BrdU), a nucleotide analog. Active microbes absorb BrdU from the soil solution; if they multiply in response to substrate additions, they incorporate the BrdU into their newly synthesized DNA. After allowing soils to incubate for 48 h, we extracted BrdU-labeled DNA and sequenced the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) to identify fungal taxa to near-species resolution. In total, we analyzed 1,769 partial fungal ITS sequences. Fungal taxa that proliferated following a particular substrate addition were likely to be targeting the substrate as a resource for growth. We found that the structure of active fungal communities varied significantly among substrates. The active fungal community under the glycine treatment was significantly different from those under other conditions, while the active communities under sucrose and cellulose were marginally different from each other and the control. These data showed distinct groupings of the communities associated with glycine and sucrose. These results indicate that the overall community structure of active fungi was altered by the addition of glycine, sucrose, and cellulose and implies that some fungal taxa respond to changes in resource availability. We also found that the community composition of active fungi is altered by experimental warming. We found that glycine-users tended to increase under warming, while lignin-, tannin/protein-, and sucrose-users declined. The latter group of substrates requires extracellular enzyme activity for breakdown, but glycine does not. It is possible that warming selects for fungal species that target, in particular, labile substrates. Linking these changes in microbial communities and resource partitioning to soil carbon dynamics, we find that organic substrate mineralization rates are, in general, significantly lower in soils that have been exposed to long-term warming. This suggests that microbial processing of organic substrates is impaired by long-term warming. Yet the picture is complicated by the fact effects are dependent on the identity of substrate. That is, there are fundamental differences in the metabolic capabilities of the communities in the control and warmed soils. These differences might relate to the changes in microbial community composition, which appeared to be associated with groups specialized on different resources. We also find that functional responses are indicative of temperature acclimation at the microbial community-level. There are distinct seasonal patterns in the response of microbial community function to incubation temperature and long-term soil warming, with higher-temperature optima for soils exposed to warmer temperatures. To relate these changes within the microbial community to potential positive feedbacks between climate warming and soil respiration, we develop a microbial-enzyme model to simulate the responses of soil carbon to warming. We find that declines in microbial biomass and degradative enzymes can explain the observed attenuation of soil-carbon emissions in response to warming. Specifically, reduced carbon-use efficiency limits the biomass of microbial decomposers and mitigates loss of soil carbon. However, microbial adaptation or a change in microbial communities could lead to an upward adjustment of the efficiency of carbon use, counteracting the decline in microbial biomass and accelerating soil-carbon loss. We conclude that the soil-carbon response to climate warming depends on the efficiency of soil microbes in using carbon.

Intellectual property: Unlimited announcement

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Description of Project Objectives

The central objective of the proposed work was:

> **To develop a genomic approach (nucleic acid-based) that elucidates the mechanistic basis for the observed impacts of experimental soil warming on forest soil CO_2 efflux at the Prospect Hill and Barre Wood sites.**

The impacts of experimental, soil warming at these two sites is shown in Fig. 1. Briefly, the Prospect Hill soil warming study is the longest running field experiment of its kind and is located within an even-aged mixed hardwood forest stand in the Prospect Hill Tract at the Harvard Forest. The immediate impact of the warming treatment at this site was a stimulation of soil CO_2 efflux (Fig. 1a). The stimulation was greatest in the first year of warming and gradually declined across the following decade. By the tenth year of the study soil CO_2 efflux from warmed plots had returned to a rate equivalent to that of the controls (Fig. 1a). The Prospect Hill warming study is now in its seventeenth year and CO_2 efflux from control and warmed plots remains equivalent. We refer to the distinct phases in this temporal pattern of soil CO_2 efflux response to warming as the 'stimulation' and 'equalization' phases, the former referring to the initial positive response of soil CO_2 efflux to warming and the latter when rates in the warmed soils return to those observed for the non-warmed controls. The Barre Woods study, where warming was initiated in 2003, provided us with a unique opportunity to examine the two phases simultaneously: Barre Woods representing the stimulation phase (Fig. 1b) and Prospect Hill the equalization phase.

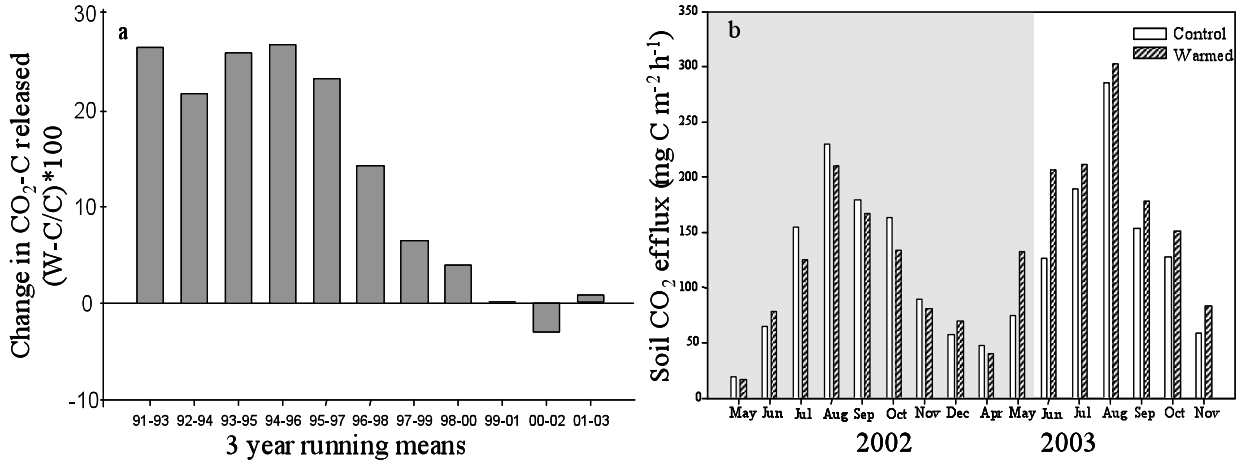


Fig. 1 (a) Percentage increase in the amount of soil CO_2 efflux from the warmed plots relative to the control plots (calculated as: [warmed rates – control rates]/control rates) for the Prospect Hill warming study. (b) Monthly soil CO_2 efflux from the Barre Woods warmed and control plots. The shaded area demarcates the pre-treatment period. An immediate stimulation of soil CO_2 efflux is observed when the warming treatment is initiated in May 2003.

To tackle the central objective of our initial, full proposal we designed a study based around four, specific research objectives (Fig. 2). These research objectives were centered round the application of Indicator Species Analysis (ISA). The purpose of ISA is to identify species that

have a high correlation and/or association with specific environmental conditions, such as soil pH, and then to use the occurrence and/or abundance of these 'Indicator Species' in other sites as estimators of these environmental conditions. Our proposal went a step further in that it proposed to associate indicator species with rates of ecosystem processes across space and time. In this way then, the Prospect Hill and Barre Woods sites permitted an ideal test-bed of the approach: development of indicators at one space and time (Prospect Hill, initial sampling), and then testing and application across space (Barre Woods) and time (seasonal sampling at both sites).

By May 2006, when we submitted our first progress report and from that received continuation of our funding into our third year, a body of theoretical work had been published that suggested that the temporal dynamics in response of soil CO_2 efflux to warming could be readily explained by depletion of fast-cycling, soil organic carbon (SOC) pools. One of the major conclusions from this work is that physiological and/or functional species shifts in soil microbial communities need not be invoked as a mechanism to explain variation in soil CO_2 efflux under warming. Undoubtedly, however, acclimation and other responses to climatic change do occur in biological systems but both the biotic responses themselves and their effects on ecosystem structure and function remain unclear: there is a need to investigate them. Through our modeling exercises it became apparent that for the Harvard Forest sites we would need to measure the size of labile and recalcitrant SOC pools to quantify their potential contribution to the measured dynamics in soil CO_2 efflux. This 'new-at-the-time', fifth objective (i.e. to quantify SOC fraction sizes in the warmed and control soils) was stated in our continuation, progress report. Undoubtedly, data on these pools are of great interest in terms of understanding and predicting potential carbon cycle feedbacks to climatic change, and in evaluating the theoretical work that explains soil CO_2 efflux responses to warming in the context of SOC pool size adjustment. However, in the context of our current and future work, they are most valuable because they provide data to evaluate competing hypotheses, established through our modeling, genomic and process work, which focus on phenomena that occur at microbial community- and organism-levels, and which then affect processes manifesting at the ecosystem scale.

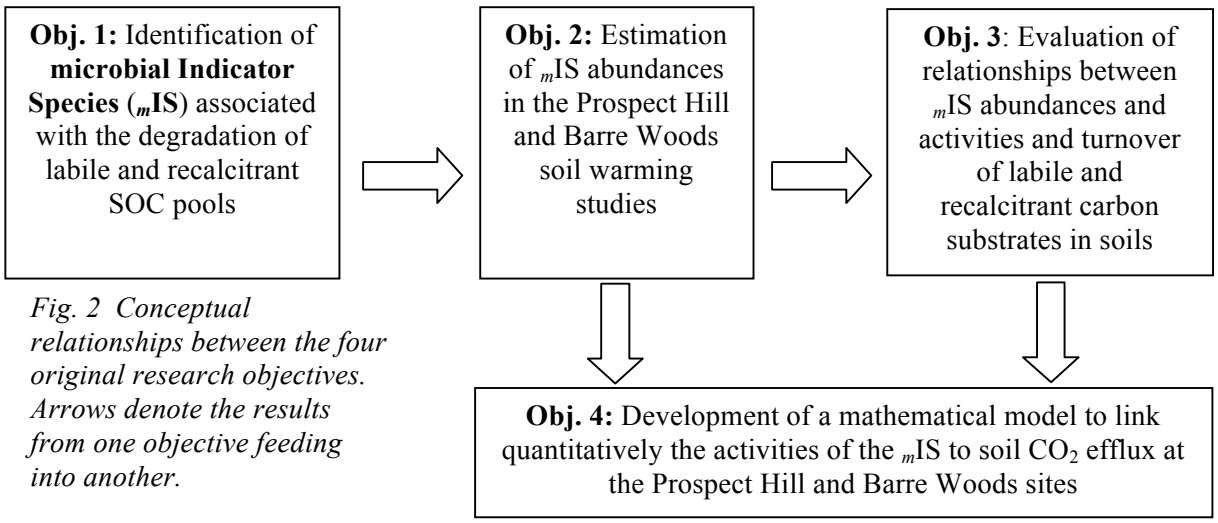


Fig. 2 Conceptual relationships between the four original research objectives. Arrows denote the results from one objective feeding into another.

Description of project achievements

Objective 1 - Identification of mIS: Soil microbes perform an immense range of metabolic functions. Indeed, they degrade almost every organic compound on Earth. If microbial decomposers vary in their abilities to use different resources for growth, then microbial diversity

may be maintained in part by the ability of species to exploit different resource niches (and our $_m$IS approach is then empirically valid). However, the importance or resource partitioning in structuring microbial communities within spatially heterogeneous soils remains unclear. Much of the uncertainty likely results from the difficulty in characterizing the metabolic or ecological functions of specific microbial taxa *in situ*. Culture-based experiments have delineated the functional potential of certain microbial species (*1, 15-17*) but few studies have linked the identities of microbial decomposers to specific resource use under natural conditions in soil. This is what we have done under Obj. 1 and results are summarized below and in the papers produced listed as Hanson et al. (2008) and Goldfarb et al. (in revision).

To examine the potential for resource partitioning among microbial decomposers we altered the availability of organic resources in the Harvard Forest soils. Specifically, we quantified the relative abundance of fungal taxa that proliferated following the addition of a variety of organic substrates to soil. We added glycine, sucrose, cellulose, lignin, or tannin-protein (in order of increasing resistance to decay) directly to soils in conjunction with 3-bromo-deoxyuridine (BrdU), a nucleotide analog. Active microbes absorb BrdU from the soil solution; if they multiply in response to substrate additions, they incorporate the BrdU into their newly synthesized DNA. After allowing soils to incubate for 48 h, we extracted BrdU-labeled DNA and sequenced the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) to identify fungal taxa to near-species resolution. In total, we analyzed 1,769 partial fungal ITS sequences. Fungal taxa that proliferated following a particular substrate addition were likely to be targeting the substrate as a resource for growth.

We found that the structure of active fungal communities varied significantly among substrates. According to Multi-Response Permutational Procedures (MRPP) analysis, the active fungal community under the glycine treatment was significantly different from those under cellulose, tannin-protein, and control conditions (P = 0.027, 0.030, 0.028, respectively), while the active communities under sucrose and cellulose were marginally different from each other (P = 0.058) and the control (P = 0.059, 0.054, respectively). An ordination plot produced by Nonmetric Multidimensional Scaling (NMS) analysis largely paralleled the MRPP analysis, and showed distinct groupings of the active communities associated with glycine and sucrose. The communities under cellulose also clustered together. These results indicate that the overall community structure of active fungi was altered by the addition of glycine, sucrose, and cellulose and implies that some fungal taxa respond to changes in resource availability.

Within these active fungal communities we identified 11 operational taxonomic units (OTUs, ≥97% sequence similarity level) that changed in relative abundance under different substrates (Fig. 3). The majority of these taxa responded positively to the addition of glycine (OTUs 5, 8, 17, 120) and sucrose (OTUs 5, 13, 18, 25, 52), which are labile compounds that are relatively abundant and easily metabolized by microbes. However, several taxa proliferated under the more complex and recalcitrant substrates: OTU 25 responded positively to cellulose, while OTUs 18 and 47 proliferated under lignin. Although some taxa had the capacity to respond to a diverse set of resources (OTUs 5, 8, and 18), others appeared to target a very specific resource, such as glycine (OTUs 17, 42, 120) or lignin (OTU 47). These results suggest that some fungal taxa are able to specialize in the breakdown of particular compounds in soil and thus occupy different resource niches.

While some taxa proliferated as a result of substrate additions, others declined. For example, all substrates reduced the relative abundance of OTU 77 as compared to the control (Fig. 3K), suggesting that superior competitors for these resources may have reduced the relative

activity of this fungal group. Additionally, the fraction of rare OTUs (abundance of 1 or 2 sequences within a treatment at ≥97% similarity) within the active fungal communities decreased upon the addition of all substrates: control (61%) > lignin (57%) > glycine (55%) > cellulose (46%) > sucrose (44%) > tannin-protein (35%). These patterns may be attributed to competitive exclusion, whereby fungi that specialize on the added substrates out-compete other taxa.

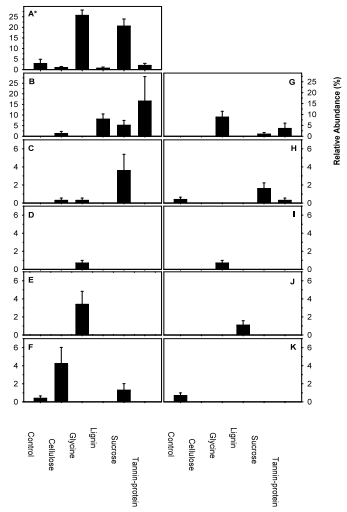
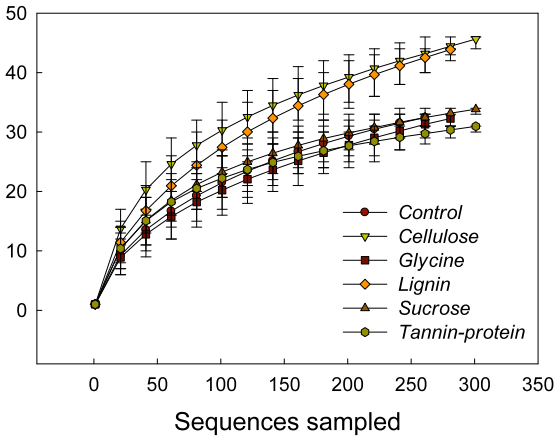


*Fig. 3 Relative abundances (% of sequences within a treatment) of fungal OTUs showing at least a marginally significant change in relative abundance (P<0.10, except * = P<0.05, Kruskal-Wallis tests) among substrate additions. (A) Zygomycete (Mortierellaceae), OTU 5. (B) Zygomycete (Mortierella), OTU 18. (C) Zygomycete (Mortierellaceae), OTU 13. (D) Zygomycete (Mortierellales), OTU 17. (E) Zygomycete (Umbelopsis), OTU 42. (F) Unknown fungus, OTU 25. (G) Unknown fungus, OTU 8. (H) Unknown fungus, OTU 52. (I) Unknown fungus, OTU 120. (J) Basidiomycete (Agaricales), OTU 47. (K) Basidiomycete (Lactarius), OTU 77. OTUs were determined by analysis of sequences at the ≥97% similarity level. Classification names in parentheses represent the best taxonomic designations that could be made by using the identification methodology. Bars indicate 1SE, n=4.*

The richness and diversity of active fungal communities also varied among substrate treatments. Specifically, cellulose and lignin additions elicited the greatest richness of OTUs (Fig. 4). These results imply that a more diverse assemblage of fungi may be responsible for the breakdown of these complex soil molecules compared to more labile compounds. This finding is consistent with the assumption that the decomposition of large, recalcitrant compounds such as cellulose and lignin requires a diverse suite of specific enzymes, each of which may act at different sites within these molecules. A relatively high diversity of

fungi may be supported if different taxa specialize in the degradation of different components of these compounds.

Fig. 4 Species-sample curves for active fungi from soils receiving various organic substrates. Taxa richness was greatest in soils receiving lignin and cellulose. Error bars represent 95% confidence intervals.



Estimates of total fungal abundance, as measured by quantitative polymerase chain reaction (qPCR) on total fungal DNA (active and inactive fungi), revealed no significant change in abundance among substrate additions. Nevertheless, total fungal abundance tended to increase with the addition of all substrates, particularly glycine. The trend toward greater fungal abundance under this substrate is likely explained by the notable prevalence of OTU 5, which represented over 25% of the active community under glycine (Fig. 3A).

Our results under Obj. 1 demonstrate that fungal taxa differ in their response to various resources and that some soil fungi specialize in the breakdown of particular organic compounds in soil. Therefore, differential resource utilization among microbial decomposers may be fundamental in structuring microbial niches and may provide an important mechanism by which microbial diversity is maintained in soils. Our results provide a strong basis for believing our $_m$IS approach will be effective as techniques develop (see below). The link between microbial communities and ecosystem functioning is especially relevant in light of recent findings that global change can alter microbial biomass, community composition, and activity. Our results imply that many soil microbial decomposers are not functionally redundant, contrary to previous suggestions. This finding has important consequences for ecosystem processes, as the loss of certain taxa may result in shifts in ecosystem processes under global change.

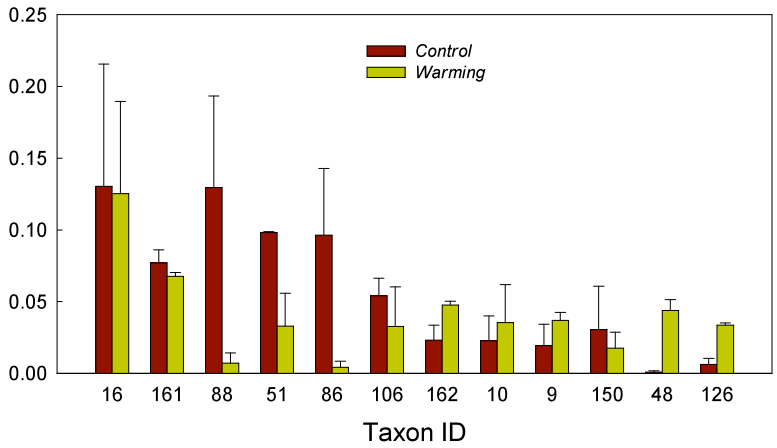


Fig. 5 Relative abundance of the 12 most common fungal taxa in soil samples from the Harvard Forest, as an example of community shifts under experimental warming. Taxon IDs were assigned randomly.

Objective 2 - Estimation of $_m$IS abundances/ activities: We identified a set of potential $_m$IS for each substrate. We also found that the community composition of active fungi is altered by experimental warming in the Harvard Forest (Fig. 5). As a preliminary exercise, we calculated the extent to which users of particular substrates responded to experimental warming. We found that glycine-users tended to increase under warming, while

lignin-, tannin/protein-, and sucrose-users declined. The latter group of substrates requires extracellular enzyme activity for breakdown, but glycine does not. It is possible that warming selects for fungal species that target, in particular, labile substrates. However, the strength of our results would have been improved through further collection dates (see below). Specifically, it is possible that microbial taxa will display temporal variability in their preference for substrates (as the mineralization data suggest: see later). We proposed to test for this possibility in a renewal year but unfortunately shortage of funds did not permit this work and indeed we did not manage to finally identify robust microbial indicators. In short, this was because the advances we had to make in the molecular work to understand microbial niche partitioning took considerably more time and resources than anticipated, and we would have needed additional funded years to meet fully this objective.

Objective 3 - Evaluation of relationships between $_m$IS abundances/ activities and mineralization activities: The central objective our proposal is to link genetic information garnered under Objs. 1 and 2 to ecosystem-level processes. To do this we needed to establish whether the nucleic acid abundances of the $_m$IS are related to the utilization rates of those organic substrates for which they were identified. After screening a range of ^{13}C-natural abundance and labeled compounds to identify suitable substrates and suitable concentrations, we worked on the sub-samples of the same field soils used under Objs. 1 and 2 to assay substrate mineralization rates. We collected data for the two field soil samplings in 2005 and for the four 2006 seasonal samplings. We expanded the proposed work in two ways. First, we worked on two soil horizons (organic and surface mineral). Originally we intended to pool across horizons but given the anticipated differences in the microbial communities between horizons, and our focus on linking microbial genomes to ecosystem function, we felt it necessary to quantify potential variation in response of these likely distinct microbial communities. Second, given the expectation that acclimation might be key to those dynamics we observed (both seasonally and in response to the warming treatment and that this phenotypic variation might not be captured at the phylogenetic level, we also incorporated multiple incubation temperatures into our lab assays. In 2005 we incubated all samples at 10°C and 20°C; in 2006 we repeated this and also incubated the sucrose assays at 15°C. These assays have provided important insights for the development of our modeling work (see below), where we evaluated the potential role acclimation plays in modifying ecosystem response to warming. Strictly speaking, temperature acclimation arises as a physiological response but at the level of microbial community function it is perceivable that it might also arise through evolutionary adaptation or species turnover. Our most notable findings under this objective are:

- Organic substrate mineralization rates are, in general, significantly lower in soils that have been exposed to long-term (i.e. 16-17 years) warming (Fig. 6). This suggests that microbial processing of organic substrates is impaired by long-term warming.
- The impacts of long-term soil warming on microbial community function, estimated by organic substrate mineralization rates, are dependent on the identity of the substrate (Fig. 6). That is, there are fundamental differences in the metabolic capabilities of the communities in the control and warmed soils. These differences cannot be simply accounted for by differences in overall microbial community activity.
- The relative difference in microbial community function, apparent from the significant interaction between long-term soil warming and substrate identity, is seasonally-dependent (Fig. 6).

- There are distinct seasonal patterns in the response of microbial community function to incubation temperature (Fig. 7a). In addition, there is a significant functional response to long-term soil warming, with higher-temperature optima for soils exposed to the warming treatment (Fig. 7b). Both responses are indicative of temperature acclimation at the community-level.
- Microbial communities in organic and mineral soil horizons, while showing qualitatively similar functional responses to long-term soil warming (e.g., reduced mineralization rates of organic substrates, after exposure to long-term soil warming), exhibit relative differences in their physiological temperature responses that are either seasonally or treatment (i.e. long-term soil warming) dependent.
- The functional responses of the microbial communities exposed to short-term warming (Barre Woods site) are qualitatively similar to those responses observed for communities exposed to long-term soil warming (Prospect Hill). This suggests that functional responses of soil microbial communities to a sustained increase in soil temperature of +5°C above ambient are relatively rapid (i.e. a few years).
- For soils collected in April 2006, the Q_{10} of enzyme activities was significantly lower for β-glucosidase (which degrades β-glucose polymers), xylosidase (which degrades hemi-cellulose), and cellulase (which degrades cellulose) in soils exposed to long-term warming. These results indicate that different isoenzymes, with lower average temperature sensitivities, are being expressed in the treatment soils. Furthermore, these data suggests that microbes are acclimating to soil warming either through community shifts or by using expressing alternative enzyme pathways. Notably, these differences were also detected in the Barre Woods experiment, which has only been actively warmed since 2003, suggesting that microbial acclimation may occur relatively rapidly in response to warming. These results are summarized in listed publications by Bradford et al (2008, 2009, 2010, in prep.).

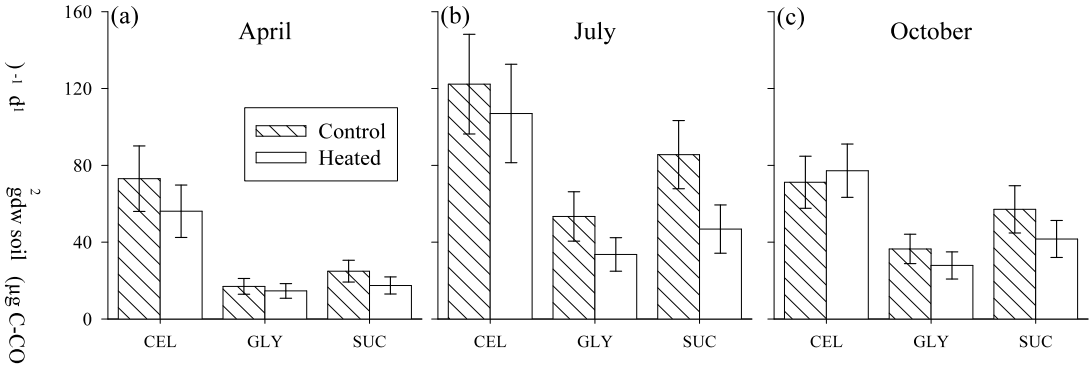


Fig. 6 Responses of microbial community function, estimated using organic substrate mineralization rates at 20°C, to long-term soil warming are dependent on both the substrate considered (shown are CEL: cellulose, GLY: glycine and SUC: sucrose) and the time of sampling in the growing season (warming treatment × substrate × date interaction: $P<0.01$). Within each sample date, the treatment effects on mineralization rates were substrate dependent (i.e. there were significant warming treatment × substrate interactions for each sample date). These data suggest that long-term soil warming results in functionally different soil microbial communities and that these communities (and importantly the treatment differences between them) functionally vary across the growing season. These effects were consistent across horizons (and so data are pooled by this factor).

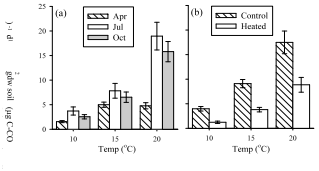


Fig. 7 Temperature responses of microbial community function, estimated using sucrose mineralization rates across different incubation temperatures, are dependent on season (a) and (b) long-term soil warming ($P<0.01$ for each 2-way interaction). Earlier in the growing season the temperature optima appears to be lower (note that in April the increase in mineralization rates, observed between 15 and 20°C in both July and October, is absent). In (b) the increase in mineralization rates across incubation temperatures is steeper in the long-term heated soils, which is indicative of temperature acclimation. Data shown are for the mineral horizon but the differences are qualitatively (if not relatively) similar for the organic horizon soils. Data in (a) are pooled across treatment and in (b) across date (i.e. these factors did not modify the interactions shown).

Objective 4 – Development of a mathematical model: Modeling work to link the microbial and process data, in the absence of robust $_m$IS, focused on examining both potential biotic and substrate mechanisms underpinning soil CO_2 efflux responses to warming. Specifically, in trying to understand climate warming-soil respiration feedbacks, which are a source of major uncertainty in climate prediction. Our model (Allison et al. 2010 – listed in publications) incorporated temperature sensitivity into a microbial-enzyme model to explore mechanisms that may explain the ephemeral increase in soil respiration with sustained warming. These mechanisms include depletion of SOC, thermal acclimation of microbial physiology, and altered plant C inputs. Based on positive empirical relationships between enzyme activities and microbial biomass, we assumed that enzyme production is directly proportional to microbial biomass in our model. We represented the temperature sensitivity of enzyme activity according to the Arrhenius relationship and established biochemical theory. Our model also incorporated temperature sensitivity of microbial carbon use efficiency (CUE). CUE may decline with temperature if respiration responds more positively to temperature than biomass production, thereby reducing allocation of assimilated C to growth. Empirical studies in soils suggest that microbial CUE declines by at least 0.009 per ºC, but in aquatic systems, the magnitude of the decline is uncertain. Therefore, we conducted model runs with and without temperature-sensitive CUE.

Soil warming models should be able to reproduce not only the ephemeral increase in soil respiration, but should also generate plausible changes in SOC, microbial biomass, and enzyme pools. For example, empirical studies suggest that microbial biomass and enzyme activity may decline with warming. The SOC response is less clear, but dramatic changes in SOC pools have not yet been reported, except in arctic systems. We therefore focused on parameter combinations that could generate these patterns. We also conducted preliminary runs to verify that model behaviours were consistent with theory and other empirical observations. For example, our model predicts that temperature sensitivity (Q_{10}) of respiration declines at higher temperature and lower substrate availability.

Our initial simulations allowed CUE to decline with temperature and examined the effects of 5ºC warming on soil respiration, SOC, DOC, microbial biomass, and enzymes. The

model predicted an initial increase in soil respiration due to the temperature sensitivity of enzyme activity. However, warming reduced CUE from 0.31 to 0.23, which reduced the amount of assimilated C that was allocated to microbial growth. Consequently, microbial biomass declined and soil respiration returned to control levels within a few years as the model approached steady state. Since enzyme production is linked to biomass, the decline in CUE ultimately limited the enzyme catalyst for SOC decomposition. At this level of temperature sensitivity for CUE, the SOC pool increased slightly after 30 years of warming. This increase contrasts with the depletion of SOC substrates predicted by models lacking an explicit coupling of microbial processes and SOC turnover.

Since some studies find that CUE is invariant with temperature, we also investigated warming effects with CUE held constant at 0.31. As with the temperature-sensitive CUE scenario, warming increased enzyme activity, but the CO_2 pulse and SOC losses were much greater. Because inputs must equal outputs at steady state, soil respiration ultimately returned to control values, but only after the SOC pool declined by >30%. These patterns were observed because enzymatic conversion of SOC to DOC initially stimulated microbial growth. Increased biomass led to more enzyme production, which fed back positively to SOC decomposition and respiration. With CUE held constant, SOC depletion ultimately constrained respiration because enzymes ran short of substrate. Notably, microbial biomass under warming constantly exceeded control values, which is inconsistent with evidence from field and laboratory experiments.

Thermal acclimation has also been proposed to explain the ephemeral increase in soil respiration with warming, so we examined whether acclimation could generate C cycling responses consistent with empirical observations. First, we simulated acclimation by reducing the temperature sensitivity of CUE. As a result, microbial biomass and enzyme pools increased (due to greater allocation of assimilated C to production), thereby stimulating SOC decomposition and CO_2 release. Next we invoked acclimation through a 50% reduction in the temperature sensitivity of maximal enzyme activity (Vmax) and a 50% increase for the half-saturation constant (Km). Ecological and evolutionary processes in the microbial community could reduce the temperature sensitivity of Vmax, and higher Km decreases the substrate affinity of enzymes. Both changes are consistent with thermal adaptation of respiratory enzymes. Overall, this form of acclimation reduced CO_2 losses, regardless of the CUE-temperature relationship, with peak soil respiration declining by 14-21%. Enzyme acclimation resulted in less SOC conversion to DOC, which constrained microbial biomass relative to no acclimation. As a result, SOC pools were 20-23% greater after 30 years relative to no enzyme acclimation. Notably, the enzyme acclimation scenario with adjusted CUE was consistent with empirical patterns showing an ephemeral increase in soil respiration and a decline in microbial biomass.

Several of our simulations show an attenuation of the soil respiration response to warming, which is expected because CO_2 losses must ultimately equal C inputs in a steady state model. However, the defining feature of our enzyme model is that microbial processes affect the integral under the soil respiration curve, resulting in a range of predictions for soil C storage. Allowing CUE to decline with temperature while increasing the DOC:SOC input ratio releases >15% of SOC. If we assume no change in C inputs but a lower (acclimated) temperature sensitivity for CUE, we observe a similar SOC loss. In contrast, higher temperature sensitivities for CUE cause little change in the SOC pool. For the scenarios predicting large SOC losses with warming, the soil respiration curves imply large and sustained CO_2 losses and a slow return to control respiration values, which is inconsistent with empirical data. However, additional studies

of microbial biomass, enzyme activity, and CUE responses to warming are needed to test among our model scenarios and refine predictions of the time scale and magnitude of SOC change.

Lastly, we tested whether conventional soil C models could reproduce the observed ephemeral augmentation of respiration with a decline in microbial biomass. Since conventional model structures vary and do not always include a microbial biomass pool, we constructed a second model with a biomass pool and a structure representative of many conventional models. Even though microbial processes were not explicitly coupled with soil C turnover, our conventional model predicted an ephemeral increase in respiration under warming accompanied by decreases in microbial biomass and DOC, whether we simulated a fixed or declining CUE. Yet in contrast to our enzyme model with temperature-sensitive CUE, warming caused a large net loss of SOC over 30 years. Therefore, conventional models without direct coupling between microbes and soil C turnover cannot simulate negative feedbacks on decomposition caused by reductions in microbial biomass and enzyme production. Our microbially-explicit modeling approach therefore shows that microbial biomass and enzyme activities may control feedbacks between climate warming and SOC loss.

The model characteristics are:

Model structure

The model was based on the conceptual framework developed by Schimel and Weintraub in 2003. To this framework we added temperature sensitivity of degradation processes, following established theory relating to soil respiration and biochemical responses to warming. The model starts by setting the soil organic carbon (SOC), dissolved organic carbon (DOC), microbial biomass, and enzyme pools to their initial values. Microbial biomass changes by the amount of DOC assimilated, times the carbon use (or microbial growth) efficiency, minus biomass death and enzyme production:

```
dMIC/dt = ASSIM*CUE - DEATH - EPROD
```

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

```
ASSIM = Vmax_uptake*MIC*DOC/(Km_uptake+DOC)
```

We assume that the cell surface area available for uptake will be directly proportional to the number of cells. Microbes may not assimilate more DOC than is available in the DOC pool. Microbial biomass death is modeled as a first-order process with a rate constant r_death:

```
DEATH = r_death*MIC
```

Enzyme production is modelled as a constant fraction (r_EnzProd) of microbial biomass:

```
EPROD = r_EnzProd*MIC
```

During uptake, the Vmax, Km, and CUE parameters are temperature sensitive. The model calculates a temperature-specific Vmax using the Arrhenius equation, where Vmax_uptake_0 is the pre-exponential coefficient, gas_const is the ideal gas constant, and Ea is the activation energy, or the amount of energy required to convert substrate into product:

```
Vmax_uptake = Vmax_uptake_0*exp(-Ea_uptake/(gas_const*(temp+273)))
```

SOC with higher Ea reacts more slowly, but the temperature sensitivity of the reaction is higher. Km values are calculated as a linear function of temperature between 0 and 50ºC:

```
Km_uptake = Km_uptake_slope*temp + Km_uptake_0
```

The carbon use efficiency is also a linear function of temperature:

```
CUE = CUE_slope*temp + CUE_0
```

CO_2 production is the fraction of DOC assimilated by microbes that is not allocated to biomass production:

```
CO2 = ASSIM*(1-CUE)
```

The enzyme pool increases with enzyme production and decreases with enzyme turnover:

```
dEnz/dt = EPROD - ELOSS
```

where enzyme turnover is modelled as a first-order process with a rate constant r_death:

```
ELOSS = r_EnzLoss*Enz
```

The SOC pool increases with external inputs and a fraction of dead microbial biomass (MIC_to_SOC) and decreases due to decomposition losses:

```
dSOC/dt = inputSOC + DEATH*MIC_to_SOC - DECOMP
```

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

```
DECOMP = Vmax*Enz*SOC/(Km+SOC)
```

The amount of SOC decomposed may not exceed the total SOC pool. The temperature sensitivity of decomposition is modelled in the same way as uptake, with temperature dependency built into the extracellular enzyme parameters Vmax and Km:

```
Vmax = Vmax_0*exp(-Ea/(gas_const*(temp+273)))
Km = Km_slope*temp + Km_0
```

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

```
dDOC/dt = inputDOC + DEATH*(1-MIC_to_SOC) + DECOMP + ELOSS - ASSIM
```

Objective 5 – Quantification of SOC pool sizes: Those SOC data we have collected, using a combination of physical, chemical and biological fractionation techniques, provide the first field

experiment evidence that long-term soil warming reduces the size of more labile SOC fractions. In addition, we have measured significant reductions in more recalcitrant SOC and N fractions, lending empirical data to the current debate on whether recalcitrant SOC pools are temperature-sensitive. While these data are significant for forecasting potential soil feedbacks to climate warming, they are of most relevance to our study because they permit us to investigate in detail to what extent microbial community responses (both physiological and structural) also contribute to the measured changes in soil CO_2 efflux. Work under Obj. 5 was published in Bradford et al. (2008).

List of products arising

Peer-reviewed manuscripts:

1. Allison, S. D., Treseder, K.K. (2008) Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* 14, 2898-2909.
2. Allison, S.D., Wallenstein, M.D., Bradford, M.A. (2010) Soil carbon response to warming is dependent on microbial physiology. *Nature Geoscience*, 3, 336-340. (featured in 'News and Views' of same edition)
3. Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J.E., Reynolds, J.F., Treseder, K.K., Wallenstein, M.D. (2008) Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, 11, 1316-1327.
4. Bradford, M.A., Watts, B.W., Davies, C.A. (2010) Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Global Change Biology*, 16, 1576-1588.
5. Bradford, M.A., Wallenstein, M.D., Allison, S.D., Treseder, K.K., Frey, S.D., Watts, B.W., Davies, C.A., Maddox, T.R., Melillo, J.M., Mohan, J.E., Reynolds, J.F. (2009) Decreased mass specific respiration under experimental warming is robust to the microbial biomass method employed. *Ecology Letters*, 12, E15-E18.
6. Bradford, M.A., Melillo, J.M. (In prep) Impacts of soil warming on the seasonal dynamics of microbial physiological and metabolic response to temperature and substrate variation.
7. Bradford, M.A., Melillo, J.M. (In prep) Labile carbon turnover is not accelerated in long-term, warmed soils.
8. Goldfarb, K.C., Karaoz, U., Hanson, C.A., Bradford, M.A., Treseder, K.K., Wallenstein, M.D., Brodie, E.L. (In revision) Resource partitioning of carbon substrates by bacterial communities in forest soil.
9. Hanson, C. A., Allison, S.D., Bradford, M.A., Wallenstein, M.D., Treseder K.K. (2008) Fungal taxa target different carbon sources in forest soil. *Ecosystems* 11:1157-1167.
10. Strickland, M.S., Callaham, M.A. Jr., Davies, C.A., Lauber, C.L., Ramirez, K., Richter, D.D. Jr., Fierer, N., Bradford, M.A. (2010) Rates of *in situ* carbon mineralization in relation to land-use, microbial community and edaphic characteristics. *Soil Biology & Biochemistry*, 42, 260-269.

11. Wallenstein, M.D., Allison, S., Ernakovich, J. Steinweg, J.M., Sinsabaugh, R. (In press) Controls on the temperature sensitivity of soil enzymes: A key driver of in-situ enzyme activity rates. In: "Soil Enzymology". Girish Shukla and Ajit Varma, eds.

12. Wallenstein, M.D., Weintraub, M. (2008) Emerging approaches for measuring and modeling in situ soil enzyme activities. *Soil Biology & Biochemistry* 40:2098-2106.

13. Watts, B.W., Bradford, M.A. (In prep) Substrate-limitation and temperature independently affect mass-specific microbial respiration.