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Review Paper

# Soil microbes and their response to experimental warming over time: A meta-analysis of field studies

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ABSTRACT

Numerous field studies have found changes in soil respiration and microbial abundance under experimental warming. Yet, it is uncertain whether the magnitude of these responses remains consistent over the long-term. We performed a meta-analysis on 25 field experiments to examine how warming effects on soil respiration, microbial biomass, and soil microbial C respond to the duration of warming. For each parameter, we hypothesized that effect sizes of warming would diminish as the duration of warming increased. In support of our hypothesis, warming initially increased soil respiration, but the magnitude of this effect declined significantly as warming progressed as evidenced by the two longest studies in our meta-analysis. In fact, after 10 years of warming, soil respiration in warmed treatments was similar to controls. In contrast, warming effect sizes for fungal biomass, bacterial biomass, and soil microbial C did not respond significantly to the duration of warming. Microbial acclimation, community shifts, adaptation, or reductions in labile C may have ameliorated warming effects on soil respiration in the long-term. Accordingly, long-term soil C losses might be smaller than those suggested by short-term warming studies.



## 1. Introduction

To predict the effects of global warming on ecosystems, researchers have manipulated soil and air temperatures in numerous field experiments (Carey et al., 2016). Although some warming experiments have lasted over a decade (Dorrepaal et al., 2009; Melillo et al., 2011, 2002; Rousk et al., 2013), the majority have been shorter. Therefore, the long-term effects of field experimental warming on ecosystem functions have been challenging to examine. Here we focus on microbial responses to warming, because their contributions to soil $CO_2$ respiration can influence future trajectories of climate change (Wieder et al., 2013). In an earlier meta-analysis, Rustad et al. (2001) noted that warming generally increased soil respiration across 16 field studies. Nevertheless, at that time, these studies represented relatively short warming periods of six years or less. Whether soil respiration remains elevated or returns to baseline levels under longer-term warming has been subject to debate. Some studies have reported a decrease in warming effects over time (Luo et al., 2001; Melillo et al., 2002), whereas others have documented no significant change (Schindlbacher et al., 2011). Thus, an examination of the temporal trends in responses of ecosystems to warming should shed light on long-term feedbacks between soils and climate (Allison and Treseder, 2011; Pold and DeAngelis, 2013).

Warming might initially stimulate decomposition by enhancing the metabolism of decomposers, provoking increases in microbial $CO_2$ production (Lloyd and Taylor, 1994). This could lead to soil C losses, higher soil respiration rates, and an overall positive feedback to global warming (Jenkinson et al., 1991). However, this response can be transient (Luo et al., 2001). For example, in Prospect Hill at Harvard Forest, soil respiration rates in warmed plots were higher than those in the controls for the first few years, but the warming effect declined over time and eventually became non-significant (Giasson et al., 2013; Melillo et al., 2002). Several mechanisms could drive this pattern by altering microbial C use as warming proceeds (Allison et al., 2010b; Bradford et al., 2008; Frey et al., 2013; Pritchard, 2011; Rousk et al., 2012; Sierra et al., 2010). These include acclimation of individual microbes (Allison et al., 2010b; Crowther and Bradford, 2013; Malcolm et al., 2008; Tucker et al., 2013; Yuste et al., 2010), shifts in microbial

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communities (Bárcenas-Moreno et al., 2009; Luo et al., 2014; Rousk et al., 2012; Treseder et al., 2016; Wei et al., 2014), and evolutionary adaptation of microbial populations to higher temperatures (Romero-Olivares et al., 2015). In addition, labile C pools in the soils could become depleted owing to higher microbial activity (Bradford et al., 2008; Eliasson et al., 2005; Kirschbaum, 2004; McHale et al., 1998). These mechanisms are non-exclusive, and their influence may vary among seasons (Contosta et al., 2015), ecosystems, and across time scales.

To improve predictions of long-term consequences on soil C, we must determine whether warming effect sizes on soil respiration and microbial abundance diminish over time, and how quickly this occurs. Meta-analysis is a rigorous statistical tool that can address these questions; it combines quantitative data from previously published studies to reach conclusions with greater statistical power. For example, several meta-analyses have determined that experimental warming generally increases soil respiration, soil microbial abundance, net N mineralization, decomposition, soil microbial C and N, net primary production, and photosynthesis (García-Palacios et al., 2015; Lu et al., 2013; Rustad et al., 2001; Zhang et al., 2015). A recent meta-analysis also showed that the temperature sensitivity of soil respiration does not change with experimental warming in many ecosystems (Carey et al., 2016). Although these meta-analyses have contributed greatly to our knowledge of the response of ecosystems to warming, none has focused on trends over time.

Toward this end, we used meta-analysis to analyze the effect of field experimental warming over time on soil respiration, fungal biomass, bacterial biomass, and soil microbial C. We chose these parameters because they govern large ecosystem-scale processes affected by global warming, such as $CO_2$ inputs to the atmosphere through soil C losses (Allison et al., 2010a; Šantručková and SiraŠicraba, 1991; Wang et al., 2003). We compiled data from field-based experimental warming studies that varied in duration from 1 to 15 years. We asked, how do warming effects change as duration of warming increases? We hypothesized that warming effects on each parameter would diminish as duration of warming increased.

## 2. Materials and methods

### 2.1. Literature survey

We searched the ISI Web of Science and Google Scholar for published papers reporting the response of soil fungal and bacterial biomass, soil respiration, and soil microbial C to experimentally warmed soils and its respective controls. We performed separate literature searches for each of the following terms: "soil microb* experimental warming", "soil fung* experimental warming", "soil bacter* experimental warming", "soil resp* experimental warming". In addition, we manually searched for papers published in previous meta-analyses (Arft et al., 1999; García-Palacios et al., 2015; Lu et al., 2013; Rustad et al., 2001; Wu et al., 2011; Zhang et al., 2015) and review papers (Allison and Treseder, 2011; Giasson et al., 2013; Pold and DeAngelis, 2013). To complete our data collection, we used the geographic coordinates of the experimental plots as search terms, to account for all published studies conducted in the same experimental plots but missed by our initial search terms. Our literature search included papers published (or accepted for publication) between January 1994 and July 2015. We excluded studies manipulating factors other than temperature, unless a split-plot design was used and a single subplot for the temperature effect was present.

A total of 52 studies met our search criteria, representing 25 field warming experiments across 11 different types of ecosystems, and a total duration of warming ranging from 1 to 15 years (Table 1). Measurements that were taken from the same unique set of field plots were considered as belonging to the same experiment.

### 2.2. Data acquisition

For each experiment, we recorded the mean, standard deviation (SD), standard error (SE), and sample size (n), of both warmed and control plots, for fungal and bacterial biomass, soil respiration, and soil microbial C. The data were extracted directly from tables, published supplementary material, and from graphs using Plot Digitizer 2.6.6 (http://plotdigitizer.sourceforge.net). In addition, we recorded the type of warming (e.g., infrared heater, open top chamber, closed top chamber, buried heating cables), the duration of warming, and other information such as type of ecosystem, mean annual temperature, mean annual precipitation, magnitude of soil warming, change in soil moisture, and geographic coordinates (Table 1). If SEs were presented instead of SDs, we used the formula $SD = SE\ (n^{1/2})$ to obtain SDs. Any unidentified error bars were assumed to represent SE (Peng et al., 2014).

#### 2.2.1. Soil respiration, fungal & bacterial biomass, and soil microbial C

Soil respiration was measured in all studies by an in situ $CO_2$ flux chamber, with one exception where authors used a gas headspace with isotope mass spectrometer. To measure fungal biomass, authors used a variety of techniques; total phospholipid fatty acids (PLFA) analysis was the most common (19 out of 21 experiments used this method). The remaining two experiments used either total fatty acids methyl esters (FAME) or microscopy (i.e. hyphal lengths). Similarly, bacterial biomass was quantified through PLFA, in all but one experiment where microscopy was the preferred quantification method. Moreover, soil microbial C was measured through chloroform fumigation extraction in all studies.

### 2.3. Statistics

We used meta-analysis to determine warming effects on soil respiration, fungal biomass, bacterial biomass, and soil microbial C. For each experiment and each response variable, we calculated the effect size as the natural logarithm of the response ratio (lnR). First, we averaged all sampling time points per year within each experimental plot, to remove seasonal-level variation. Then, with the averaged data, we calculated the response ratio of the mean of the treatment group (warmed) divided by the mean of the control group (unwarmed). An lnR of 0 indicates that warming had no effect on the response variables. We also calculated the variance ($V_R$) using the means, n, and SD of both treatments (Suppl. Table 1). To calculate lnR and $V_R$, we used MetaWin software (Rosenberg et al., 2001).

We tested our hypothesis for each soil parameter separately. In each case, we used a linear mixed-effects model fitted with a restricted maximal likelihood (REML) approach ("nlme" R package) (R Core Development Team, 2009) (Suppl. R code). This structure allowed us to account for non-independence of repeated measurements within experiments, by essentially nesting measurements within experiment. Experiments were defined as unique sets of field plots. For each test, warming effect size (lnR) of soil respiration (or fungal biomass, bacterial biomass, or microbial C) was the dependent variable, duration of warming was the independent variable, and experiment ID was a random effect. In separate analyses, we tested if the magnitude of soil warming (or change in soil moisture) also influenced the effect size of soil respiration. Specifically, we tested whether lnR (dependent variable) was significantly related to magnitude of warming, duration