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Soil respiration is not limited by reductions in microbial biomass during long-term soil incubations

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ABSTRACT

Declining rates of soil respiration are reliably observed during long-term laboratory incubations. However, the cause of this decline is uncertain. We explored different controls on soil respiration to elucidate the drivers of respiration rate declines during long-term soil incubations. Following a long-term (707 day) incubation (30 °C) of soils from two sites (a cultivated and a forested plot at Kellogg Biological Station, Hickory Corners, MI, USA), soils were significantly depleted of both soil carbon and microbial biomass. To test the ability of these carbon- and biomass-depleted ("incubation-depleted") soils to respire labile organic matter, we exposed soils to a second, 42 day incubation (30 °C) with and without an addition of plant residues. We controlled for soil carbon and microbial biomass depletion by incubating field fresh ("fresh") soils with and without an amendment of wheat and corn residues. Although respiration was consistently higher in the fresh versus incubation-depleted soil (2 and 1.2 times higher in the fresh cultivated and fresh forested soil, respectively), the ability to respire substrate did not differ between the fresh and incubation-depleted soils. Further, at the completion of the 42 day incubation, levels of microbial biomass in the incubation-depleted soils remained unchanged, while levels of microbial biomass in the field-fresh soil declined to levels similar to that of the incubation-depleted soils. Extra-cellular enzyme pools in the incubation-depleted soils were sometimes slightly reduced and did not respond to addition of labile substrate and did not limit soil respiration. Our results support the idea that available soil organic matter, rather than a lack microbial biomass and extracellular enzymes, limits soil respiration over the course of long-term incubations. That decomposition of both wheat and corn straw residues did not change after major changes in the soil biomass during extended incubation supports the omission of biomass values from biogeochemical models.

1. Introduction

Following cessation of labile carbon inputs, soil respiration rates decline (Kelly et al., 1997; Conant et al., 2011; Schmidt et al., 2011), whether in the field (as bare fallow experiments; Cambardella and Elliott, 1992; Six et al., 2002) or in laboratory incubations (Liu et al., 2006; Conen et al., 2008; Haile-Mariam et al., 2008; Plante et al., 2010; Creamer et al., 2011). If this decline in respiration is controlled by a reduction of the available soil organic matter (SOM) pool as labile inputs diminish or by a decreased microbial biomass remains uncertain.

The chief objective of this work is to disentangle various drivers that might limit the decomposition of SOM to CO_2 in a laboratory incubation setting. Specifically, this work seeks to understand whether (1) the size of the available SOM pool or (2) microbially mediated depolymerization and respiration of available SOM limits respiration during a long-term soil incubation. Here we define available SOM as organic compounds susceptible to enzymatic depolymerization that are not bound in aggregates or on mineral surfaces.

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In their 2008 paper, Kemmitt et al. reported that despite culling up to 90% of the native microbial biomass, there was no significant decrease in soil respiration when soils were incubated under controlled conditions. This was unexpected since the process of SOM respiration to CO_2 is known to be conducted by living microorganisms. The authors of the study suggested that abiotic mechanisms of physical stabilization in microaggregates and chemical associations with clay and silt particles (Six and Jastrow, 2002) release a slow "trickle" of SOM to the available pool. Once SOM enters the available pool, it is depolymerized, assimilated, and respired by an excess supply of microbial extracellular enzymes and biomass. The authors concluded that the rate of soil respiration during incubations is independent of the size of the microbial biomass pool and is instead limited by physico-chemical drivers.

This finding marks a departure from a body of literature that seeks to understand how changes in microbial activity are reflected in the processes they mediate (e.g. depolymerization and respiration of SOM), and how this in turn drives overall changes in soil respiration rates observed during laboratory incubations. For example, Kuzyakov (2000) described that some increases in soil respiration in response to substrate addition are unexplained by the amount of substrate addition alone. Rather, this additional respiration is the result of a "priming effect", whereby a labile substrate amendment stimulates a spike in microbial activity, yielding an immediate increase in older, less labile SOM decomposition (Neff et al., 2002; Kuzyakov, 2006; Kuzyakov et al., 2009; Kuzyakov, 2010; Blagodatskaya et al., 2011). Under the priming scenario, the interactions among microbiota, substrate and native carbon determines soil respiration rates.

It is from these divergent conceptualizations of SOM respiration controls that our research emerges. To distinguish the effects of available SOM limitation from reduced microbial biomass on respiration rates, we used soil from a long-term (707 day, 30 °C) incubation sufficient to significantly deplete soils of both labile SOM and microbial biomass. A subsequent, shorter-term (double 21-day, 30 °C) incubation was used to test the hypotheses that SOM decomposition rates are limited by substrate availability or microbial biomass. To assess whether substrate availability limits SOM decomposition rates, we alleviated potential substrate limitation by adding wheat and corn residues to soil with fresh and reduced microbial biomass. To test whether a reduction in microbial biomass limits respiration rates, we alleviated potential microbial constraints by resampling the same sites to compare the respiration from the biomass-depleted soils to that of field fresh soils with their field levels of microbial biomass and extra-cellular enzyme pools. This experimental design represents a novel approach to investigating potential mechanisms of soil respiration limitation during laboratory incubations.

2. Methods

2.1. Establishing different levels of microbial biomass and substrate availability

In late 2007, four soil samples were collected from a cultivated site and three soil samples from a forested site (20 cm-deep cores; dia. = 1.8 cm) at the Kellogg Biological Station Long-Term Ecological Research site (Robertson, 1991) (Table 1) for the initial, 707 day incubation (30 °C). The cultivated plot was converted from deciduous hardwood forest in the early 1900s and was cropped in a mixed small grain corn–soybean rotation for many years and under alfalfa just prior to 1993, when it was converted to current management: continuous, conventionally-tilled corn. The forested plot has been under forest since at least the late 1800s and is currently a late-successional deciduous hardwood forest. Each site was selected to be broadly representative of common land use types at the Kellogg Biological Station.

Table 1
Kellogg Biological Station site descriptions, including total % carbon (C) respired after 707 days of incubation at 30 °C. Percent carbon values are means ± standard error. Kellogg Biological Station's mean annual temperature and mean annual precipitation are 9.7 °C and 890 mm, respectively.

Site	Vegetation	Total C (%)	C:N	C respired after 707d (% of total soil C)
Cultivated	Corn crop	0.76 (±0.03)	12.8	20.34 (±0.64)
Forested	Deciduous forest	1.22 (±0.05)	8.5	18.02 (±1.61)

After collection, soil samples were transported to the laboratory and stored at 4 °C in sterile, plastic bags for roughly one week. Soils were then passed through a 2 mm-mesh sieve and large (>2 mm) surface and belowground plant matter was removed. Samples of sieved, air-dried soil were then analyzed for carbonates (none were detected, using a standard technique of 1M HCl drops to detect effervescence at ambient temperature) and total organic carbon and nitrogen was determined using a LECO CHN-1000 analyzer (LECO Corp., St. Joseph, MI).

The processed soils were then subjected to incubation under constant 30 °C temperature and moisture for 707 days. Soil samples (200 g) were placed in 250 mL un-covered glass beakers, which were placed within sealed, half-gallon sized jars. The soils were maintained at 50% water filled pore space throughout the incubation. Air samples from the headspace of the sealed mason jars were drawn through septa, transferred to evacuated vials, and CO_2 concentrations were measured using a Li- Cor LI-6252 (Li-COR Biosciences, Lincoln, NE) infrared gas analyzer.

Carbon dioxide in the headspace of each jar was measured every two to five days at the outset of the incubation, when respiration rates were at their highest. Between days 14–707, samples were taken every 7–28 days. Jars were flushed with CO_2–free air after every measurement and lost moisture and worn septa were replenished as needed, roughly six times throughout the incubation (modified from Follett et al., 1997; Haddix et al., 2011). Incubating the soils for 707 days depleted them of soil carbon (18–20% soil C lost) (Table 1) and reduced microbial biomass.

After 707 days of incubation, all soil samples were removed from the incubator, and subjected to further experimentation (hereafter referred to as "incubation-depleted"). In 2010, we resampled the forested and cultivated sites at the Kellogg Biological Station (hereafter referred to as "fresh" soil), processing the samples in the same manner as those collected in 2007.

2.2. Substrate amendment experiments

To assess respiration from soils with and without substrate constraints, we added substrate to both incubation-depleted and fresh soil samples (hereby referred to as "amended"). We assessed microbial biomass constraints on respiration from the amended and unamended incubation-depleted and fresh soil samples by measuring respiration concurrent with microbial biomass and enzyme activity midway (Day 21) and at the end (Day 42) of the short-term incubation.

Substrate amendments were added as 600 μg C g^{-1} dry soil of dried and finely ground aboveground wheat or corn tissue. This amount of substrate was equivalent to the amount of microbial biomass C of the fresh, non-depleted soil. Wheat or corn substrate was added to the forested soils, while wheat only was added to the cultivated soil in an attempt to use $\delta^{13}C$ to track whether or not substrate amendment elicited a microbial priming response, and to trace the origin of respired CO_2 from the substrate amendment versus native SOM.