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Substrate identity and amount overwhelm temperature effects on soil carbon formation

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ABSTRACT

The size of the soil carbon sink depends on the balance between soil organic matter (SOM) formation and decomposition. Our understanding of how SOM forms and is stabilized, however, is shifting. Traditional theory maintains the formation of SOM is due to chemical complexity: difficult to decompose plant inputs persist in the soil while easily decomposable inputs are respired as CO_2. However, consensus is now building around an alternative thesis, hypothesizing that the plant inputs most easily assimilated by soil decomposers are the ones stabilized as SOM because dead microbial biomass is now considered one of the primary components of stable SOM. As such, the efficiency with which the microbial community uses these plant inputs has direct implications for the amount and rate of SOM formation under both a constant and changing climate. Our study empirically tests and measures the effects of substrate quality, quantity, and temperature on SOM formation rates – a process that may have profound impact on carbon stocks. We used ^{13}C-labeled substrates representative of plant root exudates (simple sugars, amino acids, and organic acids) to determine the proportion of substrate retained within SOM, microbial biomass, dissolved organic carbon, or evolved as $^{13}CO_2$. We found that glucose, the substrate most efficiently assimilated by the microbial biomass, leads to the greatest amount of SOM formation compared to glycine and oxalic acid. In contrast to expectations, higher concentrations of substrate addition lead to proportionally less ^{13}C label retention than lower concentrations. Temperature had a negligible impact on SOM formation, with higher temperatures actually leading to slight increases in SOM formation. While substrate quality and quantity drove the largest differences in SOM formation rates, once metabolized by the microbial biomass, eventual incorporation of carbon into the mineral associated SOM pool (thought to be the most stable of the soil C pools), was effectively equivalent across treatments. Our data suggest that changing composition and amount of labile carbon substrates supplied to soils will likely be key determinants of SOM formation rates and, hence, potentially soil carbon stock sizes.

1. Introduction

Soil is the world's largest terrestrial carbon sink, helping mitigate increasing carbon dioxide levels in the atmosphere (Batjes, 1996). The size of the soil carbon sink depends on the balance between soil organic matter (SOM) decomposition and formation. Our understanding of how SOM forms and is stabilized against decay is, however, under-going a paradigm shift (Dungait et al., 2012; Lutzow et al., 2006; Marschner et al., 2008; Sutton and Sposito, 2005). The notion that the formation of SOM is largely a function of biochemical complexity (i.e. inherent recalcitrance) is being overturned for an emerging thesis that emphasizes the chemical lability of inputs, the importance of mineral sorption within the soil matrix, and the physiology of the microbes that decompose SOM (Cotrufo et al., 2013; Schmidt et al., 2011). This emerging theory maintains that one of the main drivers of SOM formation may be microbial growth that leads to the accumulation of microbial-C into SOM via biomass turnover (Kallenbach et al., 2016; Liang and Balser, 2008; Liang et al., 2017). Thus, carbon use efficiency (CUE) – the proportion of carbon that goes towards microbial growth versus respiration – has emerged as a key parameter in estimating rates of SOM formation (Kallenbach et al., 2016; Liang et al., 2017). Understanding how the interaction between available substrates, the microbial community, carbon use efficiency, and the soil matrix impacts SOM formation is critical to projecting what will happen to resulting SOM stocks under climate change.

While there has been much research related to the fate of carbon stocks under a warming climate (Bradford et al., 2016; Lu et al., 2013; Melillo et al., 2017), there is a lack of research exploring what will

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happen specifically to SOM formation under climate change. Some studies point to the vulnerability of soil carbon stocks, and often project alarming losses with rising temperatures. However, these studies fail to account for changes in plant-soil interactions, net primary productivity, or changes in microbial physiology that could impact the balance between SOM formation and decomposition (Crowther et al., 2016; Melillo et al., 2017). In contrast, modeled projections of what will happen to carbon stocks under warming with changes in microbial physiology and growth suggest more divergent SOM responses, with both projected increases and decreases in SOM formation and decomposition rates (Frey et al., 2013; Hagerty et al., 2014; Wieder et al., 2014). These contrasting projections arise from the different representation of variables that can shape SOM stocks. Therefore, a critical next step to refine knowledge related to SOM formation is to evaluate empirical outcomes when these variables are imposed.

Our study empirically tests and measures the effects of substrate quality, quantity, and temperature on SOM formation – variables assumed to have potentially large effects on SOM formation because of their influence on microbial physiology and growth. The broad objectives of our study were two-fold. First, we aimed to provide empirical data that could help test emerging ideas about SOM formation and stabilization. Second, we aimed to explore how climate change scenarios (increased carbon inputs and warming) would impact these two processes. We recognize SOM formation is a complex process involving inputs from both above- and below-ground and microbial transformations driven by both microbial catabolism and anabolism (Cotrufo et al., 2015; Liang et al., 2017). Our hypotheses focus specifically on microbial anabolism of below-ground inputs and how substrate quality, quantity, and temperature might impact the "microbial filter." That is, we focus on the idea that microbes are a key control on the flow of below-ground plant C into SOM through turnover and necromass accumulation (Cotrufo et al., 2013; Liang et al., 2017; Wickland et al., 2007), and hence of SOM formation rates.

We added ^{13}C labeled substrates representing the common classes of plant root exudates (sugar, amino acid, and organic acid) to soil mesocosms at two different concentrations crossed with two different temperatures. The use of stable isotopes allowed us to track the fate of these substrates in the soil – whether they were respired as CO_2, retained within microbial biomass, remained in soil solution as dissolved organic C, or were recovered in SOM pools. The chemical class of plant input (e.g. sugar, amino acid, organic acid) could profoundly influence SOM formation. For example, microbes grow more efficiently on sugars versus organic acids, which impacts the amount of microbial biomass and potentially SOM formation (Bradford et al., 2013; Geyer et al., 2016; van Hees et al., 2005). Therefore, we predicted a definitive substrate effect, expecting that glucose would form the greatest amount of SOM versus glycine and oxalic acid, based on an expected decline in the efficiency of growth from glucose to oxalic acid (Frey et al., 2013). It is often expected that greater inputs will lead to more formation. However, the rate of formation could be affected by the resulting impacts of increased substrate availability on the microbial community. For instance, greater substrate availability could shift the microbial community to a faster growing and less efficient community dominated by copiotrophs (Fierer et al., 2007), meaning that proportionally less SOM is formed for each unit increase in soil carbon input rate. As such, we predicted that the concentration of available substrate would impact resulting SOM formation rates, with a greater amount of inputs leading to more absolute formation but potentially a lower proportion of inputs going on to form SOM. Finally, temperature effects on SOM formation through the microbial biomass pathway are likely dependent on CUE, with lower efficiency of carbon assimilation leading to reduced SOM formation. Whereas some previous studies have been premised on declining CUE with warming (Allison et al., 2010), other studies have shown limited to no temperature effects on CUE (Dijkstra et al., 2011; Hagerty et al., 2014). Additionally, declines in CUE with temperature may be substrate dependent (Frey et al., 2013). Therefore, the effect of temperature on CUE – and subsequently SOM formation rates – remains highly uncertain. If temperature does lower microbial growth efficiencies – at least on some substrates – we expected that overall, higher temperatures would cause a decline in SOM formation rates all else being equal.

2. Methods

The overarching goal of our experiment was to determine the impact of substrate identity, substrate concentration, and temperature on SOM formation and stabilization. As such, our experiment (outlined in greater detail below) consisted of two stages: the addition phase and the stabilization phase. The addition phase consisted of weekly additions (28 weeks in total) of substrates added in a "cocktail" with one ^{13}C labeled substrate for each substrate treatment. At the end of 28 weeks, we assessed different soil C pools for the amount of ^{13}C label retained. The stabilization assay assessed the shorter-term stabilization of the ^{13}C against microbial decay. During the ensuing stabilization phase, we subjected a sub-sample of soil from the additions phase to higher temperatures (30 °C) for 60 days and then re-measured soil C pools to see what proportion of ^{13}C label was retained during the assay. We used the stabilization phase as a way to assess the degree to which ^{13}C label added during the additions phase was protected from microbial-mediated decomposition.

2.1. Experimental design

Soil for experimental mesocosms was collected from a temperate deciduous woodland within Yale-Myers Forest in northeastern Connecticut, USA (41°57′ 7.8″ N, 72° 7′ 29.1″ W) from the A horizon to a depth of 20 cm. The USDA soil classification is a Canton and Charlton fine sandy loam (mesic Typic Dystrudept) (NRCS, 2018). After collection, soil was then homogenized and hand sorted to remove any large stones (> 5 mm diameter), macrofauna, and large roots (> 2 mm). Each mesocosm (15 cm deep x 10 cm wide PVC piping fit with a watertight rubber base cap) then received the same volume (1 L of soil) and mass (800 g) of soil. Each soil mesocosm was covered with 5 g dry oak (*Quercus rubra* L.) litter (also collected from Yale-Myers), which helped prevent moisture loss from soils throughout the course of the experiment.

The experimental design was a fully factorial design, with twelve unique combinations of the following treatments: three substrates (glucose, glycine, and oxalic acid) representing the common classes of plant root exudates, (sugar, amino acid, organic acid, respectively), crossed with two substrate quantities (200 and 400 g C m^{-2} y^{-1}), crossed with two temperatures (20 °C and 25 °C). Each unique treatment combination had 5 replicates for a total of 60 experimental mesocosms. We also had three control mesocosms (that received water only) for each temperature treatment, for a total of six controls. For brevity, we refer to the treatments as follows: substrate is either glucose, glycine, or oxalic acid; concentration is either low or high; temperature is either ambient or elevated (Fig. 1).

The temperature treatments were chosen to stimulate microbial activity within a biologically favorable range, and also because biological activity increases markedly across this increment, which from a 'climate change' perspective is a large shift in mean temperature. Given that low molecular weight carbon compounds comprising root exudates fuel between 30 and 50% of heterotrophic soil respiration (Högberg and Read, 2006; van Hees et al., 2005), we used average respiration rates for northeastern temperate hardwood forests (Rustad et al., 2001) to derive the low and high substrate addition concentrations. Substrates were added in a "cocktail" with one ^{13}C labeled substrate for each substrate treatment (Fig. 1). The ratio of sugar:amino acid:organic acid was representative of root exudates, and based on published ratios in the literature (de Graaff et al., 2010). Due to concern over the highly acidic pH of oxalic acid, we decided against adding it to all soils as we