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Substrate quality influences organic matter accumulation in the soil silt and clay fraction

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ABSTRACT

Substrate quality impacts the rate of microbial decomposition of soil organic matter (OM), with higher quality substrates leading to faster rates of decomposition. Since OM decomposition is the opposite of OM stabilization, one might presume higher quality substrates would lead to less OM stabilization. Yet, there is growing evidence that C stabilized in the soil silt and clay fractions is preferentially derived from microbial metabolites. We hypothesized that the decomposition of higher quality substrates would increase silt and clay-sized (S)OM pools despite higher initial mineralization rates. Soils low in initial organic carbon were incubated for 139 d with substrates spanning a range of quality/lability including: (a) bermudagrass forage cut after 14d, 21d, 28d, 35d, and 42d of re-growth, and (b) ruminal digesta produced from these forages. We then monitored the production of CO_2 as well as the carbon abundance and isotopic composition in the bulk, silt, and clay fractions. Undigested forage was respired at higher initial rates than ruminal digesta and resulted in more carbon (C) and nitrogen (N) in the clay fraction. Overall, substrate quality—assessed as the ratio of neutral detergent fiber (NDF-cellulose, hemicellulose, and lignin) to crude protein (CP)—was directly related to decomposition kinetics with higher substrate quality resulting in more silt and clay C. These findings provide evidence that substrate quality, as a driver of microbial response, can control the flow of C and N to silt and clay fractions where there is the potential for interactions with mineral surfaces to greatly increase C and N residence times. Incorporating this concept into numerical models of SOM generation and turnover will likely improve projections of carbon dynamics in global change models.

1. Introduction

Soil organic matter (SOM) represents the Earth's largest terrestrial carbon reservoir (Jobbágy and Jackson, 2000). Plant and animal debris are the main biological inputs to soil, comprising a molecularly complex mixture rich in polysaccharides and lignin (Kögel-Knabner, 2002), which are physically and chemically broken down by soil macro and micro-biota to form SOM. The breakdown of these inputs drives biological processes in soils (Janzen, 2006) that provide many ecosystem services. The abundance of SOM is a dynamic balance between the quantity of organic inputs, the rates and efficiency of biomass synthesis during decomposition, and the interaction of transformed organic molecules with soil mineral phases (Schlesinger and Bernhardt, 2013). These same processes influence the magnitude of soil CO_2 flux to the atmosphere, as well as the form of resulting SOM (Grandy and Neff, 2008).

Traditionally, scientists estimate litter decomposition rates based on the chemical composition of the organic substrate and climatic variables (Meentemeyer, 1978; Zhang et al., 2008; Prescott, 2010). The concept of substrate quality—indexed as lignin/N or C/N—has historically been implemented in soil carbon and nitrogen models as a means to partition new organic materials into pools of either rapidly or slowly decomposing organic matter, often with fixed first-order kinetic rate constants (i.e., *k* values) (Manzoni and Porporato, 2009). These approaches conceptually rely on the assumption that stable SOM is formed from more recalcitrant

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substrates (Prescott, 2010). However, recent observations have shifted the paradigm for explaining SOM persistence from one based on molecular recalcitrance alone (Kleber and Johnson, 2010; Dungait et al., 2012) to one driven by ecosystem properties, especially physiochemical interactions such as the stabilization of SOM via sorption to mineral surfaces (Schmidt et al., 2011; Kleber et al., 2015; Lehmann and Kleber, 2015).

While there is a large body of literature relating substrate chemistry to the initial decomposition rates, currently there is little data linking the quality of added substrates to changes in the more stable silt and clay fractions of soil (von Lützow et al., 2006; Rubino et al., 2007; Kleber et al., 2011; Wieder et al., 2013). Though absent from many models, overall substrate use efficiency (SUE) of the active microbial community—as influenced by physiological (Beardmore et al., 2011) and external constraints (Manzoni et al., 2012)—governs the supply of microbial metabolites to the fine mineral fraction. According to the proposed Microbial Efficiency-Matrix Stabilization Framework (MEMS) (Cotrufo et al., 2013), microbially-labile substrates (Lekkerkerk et al., 1990; Hobbie, 2005) should stimulate microbial growth and provide more C and N in the form of microbial exudates and necromass to the soil mineral fraction. Several field studies have shown that inorganic nutrient amendments (N, P, S) increase microbial carbon SUE as well as SOM formation and stabilization (Thiet et al., 2006; Kirkby et al., 2013). Litter C can be rapidly incorporated (<8 h) into silt and clay fractions (Angers et al., 1997; Vogel et al., 2014; Hatton et al., 2015) with many studies suggesting that this incorporation is linked to microbial growth (Miltner et al., 2012; Bradford et al., 2013) as well as substrate quality and community structure (Williams et al., 2006).

Missing from this revised paradigm is how the capacity of the soil mineral matrix influences the process of SOM formation (Castellano et al., 2015). The fine mineral fraction (<20 μm) is purported to have the capacity to protect a discrete mass of SOM based on the mineral surface area (Hassink, 1997) and composition (Six et al., 2002). SOM will thus accumulate within the fine mineral fraction only until this capacity is reached (i.e., the mineral surface becomes saturated). The importance of litter quality to C stabilization may thus depend on how close the soil is to its theoretical C saturation limit. Castellano et al. (2015) hypothesized that litter quality will only impact mineral-associated SOM stocks when there is a substantial saturation deficit (Stewart et al., 2007). This may be one reason evidence has been provided both for (Kirchmann et al., 2004; Bradford et al., 2013; Puttaso et al., 2013) and against (Voroney et al., 1989; Helfrich et al., 2008) substrate quality as a driver of the formation of mineral-associated SOM. Regardless, mineral stabilization of fresh litter inputs is already being introduced into the next generations of soil C models (Wieder et al., 2015).

We suggest the role of substrate quality on soil C stabilization could be best tested using low carbon soils that are far below their hypothetical C saturation (Six et al., 2002). We hypothesized that higher quality organic matter inputs would lead to more mineral associated C and N and test if *de novo* SOM was more or less biologically stable than the initial SOM. To test this we incubated plant-derived substrates of varying initial quality in soil with a high potential for C accumulation (Machmuller et al., 2015) under optimized environmental conditions. We then monitored C mineralization as well as C and N accumulation as a function of soil particle size.

2. Methods

2.1. Substrate preparation

The substrate addition treatments were designed to mimic residue inputs likely to occur in a management intensive grazing (MiG) dairy system, which typically receives forage and manure inputs with a range of quality (see Machmuller et al., 2015 for management details). A single grass species, bermudagrass, was chosen as the substrate source. Ruminal digestion was chosen as a treatment, as manure dominates aboveground carbon inputs in the MiG system (Pal et al., 2012) and ruminal digestion represents a significant microbial processing event that impacts substrate quality. The ratio of fibrous materials to protein present in the animal's diet will impact the chemical composition in deposited manure (Chadwick et al., 2000; van Vliet et al., 2007). For the purposes of this study, substrate quality is defined as the ratio of neutral detergent fiber (NDF) content (a measure of the structural components: cellulose, hemicellulose, and lignin) to crude protein (CP). The ratio of NDF/CP represents the relative proportion of structural plant cell components vs. more nitrogen rich components. Lower NDF/CP values indicate higher substrate quality. Forage maturity is a secondary treatment variable, as plant biochemical composition changes with age. Older forage tends to have lower protein content and higher lignin content (Waksman and Tenney, 1927; Hill et al., 1995). Thus, increasing maturity and digestion decrease substrate quality. A factorial combination of these treatments was used to design an incubation of substrates ranging in NDF/CP (Table 1, Fig. 1).

A field of bermudagrass ('Tifton 85', *Cynodon dactylon* (L.) Pers. X C. *nlemfuensis* Vanderyst) was grown under field conditions at the University of Georgia Plant Sciences Farm in Watkinsville, GA from July to October of 2011. Harvest was initiated on September 1st, 2012 by mowing to a height of 5 cm and fertilizing with 100 kg/ha N. The forage was cut after 14, 21, 28, 35, and 42 d of re-growth to a height of ~5 cm above the soil, representing a range of grazing periods within the MiG dairy system. All herbage samples were immediately frozen, lyophilized, ground through a 1 mm screen using a Wiley Mill.

An anaerobic digester under continuous culture conditions with mixed ruminal bacteria was used to generate ruminal digesta from herbage (Jenkins et al., 2014). Each forage treatment was divided into three, 30 g subsamples and digested for a 7 d, including 4 d of acclimation and 3 d of overflow sampling. Dried forage samples were analyzed for nutrient content by Cumberland Valley Analytical Laboratories (Maugansville, MD). Analyses included crude protein (CP) (Horwitz, 2000) and NDF via the α-amylase method (Van Soest et al., 1991). Carbon and nitrogen content and isotopic composition was measured at the UGA Stable Isotope Ecology Laboratory (Athens, GA). Results indicate the ruminal digesta has C/N ratios and NDF contents similar to what could be expected in manure produced from the respective feeds (van Vliet et al., 2007; Jost et al., 2013).

2.2. Site description and soil collection

Soil was collected from a MiG dairy located in Burke County, Georgia. The soil was a Fuquay loamy sand (Arenic Plinthic Kandiudult) and was located on a 1–5% slope within the coastal plain region of the state. Burke County has an average annual temperature of 19 °C and annual precipitation of 1224 mm yr^{-1}. The site had been converted from row-crop agriculture to MiG dairy management 4 years prior to sampling. A more detailed description of the land use regimes can be found in Machmuller et al. (2015).

Soil was collected in September 2013 from a 0–10 cm A horizon. Samples were immediately placed in Ziploc bags, and kept on ice during transport. All soil was passed through a 5 mm sieve at field moisture and any large, visible roots, or plant materials were removed. The homogenized soil was stored at 4 °C in the dark for 1 d prior to beginning the incubation. Subsamples from the