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Investigating microbial transformations of soil organic matter: synthesizing knowledge from disparate fields to guide new experimentation

S. A. Billings[1], L. K. Tiemann[2], F. Ballantyne IV[3], C. Lehmeier[1], and K. Min[1]

[1]Department of Ecology and Evolutionary Biology and the Kansas Biological Survey, University of Kansas, Lawrence, KS, USA
[2]Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI, USA
[3]Odum School of Ecology, University of Georgia, Athens, GA, USA

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Correspondence to: S. A. Billings (sharon.billings@ku.edu)

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Abstract

Investigators of soil organic matter (SOM) transformations struggle with a deceptively simple-sounding question: "Why does some SOM leave the soil profile relatively quickly, while other compounds, especially those at depth, appear to be retained on timescales ranging from the decadal to the millennial?" This question is important on both practical and academic levels, but addressing it is challenging for a multitude of reasons. Simultaneous with soil-specific advances, multiple other disciplines have enhanced their knowledge bases in ways potentially useful for future investigations of SOM decay. In this article, we highlight observations highly relevant for those investigating SOM decay and retention but often emanating from disparate fields and residing in literature seldom cited in SOM research. We focus on recent work in two key areas. First, we turn to experimental approaches using natural and artificial aquatic environments to investigate patterns of microbially-mediated OM transformations as environmental conditions change, and highlight how aquatic microbial responses to environmental change can reveal processes likely important to OM decay and retention in soils. Second, we emphasize the importance of establishing intrinsic patterns of decay kinetics for purified substrates commonly found in soils to develop baseline rates. These decay kinetics – which represent the upper limit of the reaction rates – can then be compared to substrate decay kinetics observed in natural samples, which integrate intrinsic decay reaction rates and edaphic factors essential to the site under study but absent in purified systems. That comparison permits the site-specific factors to be parsed from the fundamental decay kinetics, an important advance in our understanding of SOM decay (and thus persistence) in natural systems. We then suggest ways in which empirical observations from aquatic systems and purified enzyme-substrate reaction kinetics can be used to advance recent theoretical efforts in SOM-focused research. Finally, we suggest how the observations in aquatic and purified enzyme-substrate systems could be used to help unravel the puzzles presented by oft-observed

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patterns of SOM characteristics with depth, as one example of the many perplexing SOM-related problems.

1 Introduction

In spite of a multitude of studies exploring the drivers of soil organic matter (SOM) decay, investigators still struggle with a deceptively simple-sounding question: "Why does some SOM leave the soil profile relatively quickly, while other compounds, especially those at depth, appear to be retained on timescales ranging from the decadal to the millennial?" This question is important on a practical as well as academic level: understanding SOM retention over long time periods helps us predict soil fluxes of carbon (C) and thus Earth's atmospheric [CO_2], as well as fundamental features of ecosystem metabolism. However, addressing this question is challenging for a multitude of reasons. Most of the biogeochemical tools employed by those investigating SOM decay capture data of a very integrated nature, as they are influenced by many processes. As a result, such data are difficult to interpret. Respired CO_2, exo-enzyme activities, and changing availability of dissolved organic carbon (DOC), for example, integrate fluxes driven by the metabolically active subset of the whole living microbial community in a soil sample, where the proportion and the identity of the subset on the total number of microorganisms is not known. Furthermore, the organic substrates they transform into energy, biomass, exo-enzymes, or waste are typically of unknown identity. Of key interest for many scientists is how these fluxes (and hence the size of the pools those fluxes drain or augment) are modified with environmental factors such as temperature or moisture. Such knowledge remains elusive when we still struggle with attempts to measure and understand these processes in relatively stable environments. Further complicating our efforts, soil profiles are heterogeneous environments, with features such as physical and chemical protection of SOM and microbial community composition varying across spatial scales ranging from the molecular to the continental (Schimel and Schaeffer, 2012). Thus, one soil sample's SOM decay response to an

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environmental perturbation may not hold true for samples in close proximity, much less for different depths at the same location, or for soil types in distinct climate regimes.

Concerns about SOM destabilization with climate change have generated increased urgency within the discipline in recent decades (Kirschbaum, 1995; Bradford, 2013; Billings and Ballantyne, 2013). Soils-focused literature is now replete with papers empirically describing temperature, moisture or nutrient concentration effects on different SOM decay processes (e.g. Wagai et al., 2013; Manzoni et al., 2012b; Tiemann and Billings 2011a; Moyano et al., 2013). From these and related efforts, we have gained an appreciation for the apparent relevance of the carbon (C) quality hypothesis (Bosatta and Ågren, 1999) in many soils (Craine et al., 2010) but not in others (Laganiere et al., in review), the power of historic conditions as a driver of contemporary biogeochemical fluxes in soils (Evans and Wallenstein, 2012), the tremendous diversity and rapidly varying composition of soil microbial communities (Howe et al., 2014; Billings and Tiemann, 2014), and the apparent lack of inherent recalcitrance of many SOM pools previously thought to be relatively stable, particularly those at depth (Fontaine et al., 2007; Schmidt et al., 2011). Recent modeling efforts, particularly those focusing on temperature and nutrient availability as drivers of microbial behavior, also have enhanced our ability to identify key factors important to SOM fate in a changing environment (e.g. Manzoni et al., 2012). These and related works, as well as recent reviews (Trumbore, 2009; Conant et al., 2011; Rumpel and Kögel-Knabner, 2011; Schimel and Schaeffer, 2012), can help investigators refine their research focus to the parameters critical to evaluate to advance our understanding of the drivers of SOM transformations.

Simultaneous with these soil-specific advances, multiple other disciplines have enhanced their knowledge bases in ways potentially useful for future investigations of SOM decay. However, results of these efforts are reported in a widely-dispersed literature often not frequented by the SOM-focused community of scholars. For example, microbiologists have demonstrated how specific regions of heterotrophic bacterial transcriptomes derived from ocean water can exhibit diurnal fluctuations (Otteson et al., 2014), potentially challenging existing paradigms of the drivers of heterotrophic,

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microbial activities. Though some of the principles of organic matter decay in ocean systems are relevant to soils (Jiao et al., 2010), literature describing oceanic organic matter transformations are rarely cited in soil literature. Also rarely invoked by soil biogeochemists are laboratory experiments that study soil-relevant processes using reductionist, isolating approaches. For example, chemostat experiments are ideally suited to study fundamental physiological functioning of microbes. Such efforts recently revealed stoichiometric changes of microbial biomass exposed to different environmental conditions (Larsen et al., 1993; Payot et al., 1998; Chrzanowski and Grover, 2008; Simonds et al., 2010) and provide empirical data relevant to recent advances in ecological stoichiometric theory (Elser et al., 2000; Manzoni et al., 2012a). However, the relative paucity of linkages across disciplines exploring aquatic and terrestrial OM and microbiology makes it challenging to apply such results in a broader, ecological context.

In this article, we highlight observations highly relevant for those investigating SOM decay and retention but often emanating from disparate fields and residing in literature seldom cited in SOM research papers. We focus on recent work in two key areas. First, we turn to experimental approaches using natural and artificial aquatic environments to investigate patterns of microbially-mediated OM transformations as environmental conditions change. In 1997, John Hedges and John Oades made an elegant plea for investigators of OM decay in soils and aquatic environments to integrate their approaches and ideas to elucidate patterns and mechanisms common to both systems (Hedges and Oades, 1997). We echo this call by highlighting how some of the microbial responses to environmental change in aquatic environments can reveal processes likely important to OM decay and retention in soils. Second, we emphasize the importance of establishing intrinsic patterns of decay kinetics for purified substrates commonly found in soils to develop baseline rates. These decay kinetics can then be compared to substrate decay kinetics observed in natural samples, which integrate intrinsic decay reaction rates and edaphic factors essential to the site under study but absent in purified systems. That comparison permits the site-specific factors to be parsed from the fundamental decay kinetics, an important advance in our understanding of

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SOM decay (and thus persistence) in natural systems. We then suggest ways in which empirical observations from aquatic systems and purified enzyme-substrate reaction kinetics can be used to advance recent theoretical efforts in SOM-focused research. Finally, we suggest how the observations in aquatic and purified enzyme-substrate systems could be used to help unravel the puzzles presented by oft-observed patterns of SOM characteristics with depth, as one example of the many perplexing SOM-related problems.

2 Using well-mixed, natural and artificial systems to avoid challenges present in soils

One potential means of addressing some of the challenges in SOM research described above is to investigate the decay of organic substrates in liquid environments. Much ocean and freshwater OM decay proceeds via the same fundamental processes present in soil, via microbially produced exo-enzymes, and can be restricted via some of the same processes as well. For example, aggregate formation can protect ocean OM from decay (Jiao et al., 2010) much as it does in soils (Six and Paustian, 2013). As such, invoking knowledge derived from ocean and freshwater systems about the microbial processes relevant to aquatic OM decay, where substrate and enzymatic diffusion is far less limiting than in typical soil profiles, can provide valuable insight to the microbial processes driving SOM decay or retention.

Artificial aquatic systems in which environmental conditions and resident microbes can be strictly controlled are also useful for those investigating SOM decay and retention. Such systems represent conditions far removed from soil profiles, and at first glance appear foreign to SOM studies. Chemostats are well suited to support one, isolated microbial population (Monod, 1950), a feature indeed in sharp contrast with the complex communities found in natural systems. Chemostats also typically present the microorganisms they support with a constant substrate supply, and are subjected to manipulation of just one environmental parameter (Ferenci, 2008). As a result, we

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probably cannot consider absolute values of the size or composition of any resource pool or flux observed during such experiments as immediately comparable to those that would occur in soils. However, by largely relieving diffusional constraints on organic substrates, exo-enzymes, mineral nutrients, and the microorganisms themselves, these controlled environments mitigate at least one concern present in soil research: that results are relevant only for one particular soil profile due to heterogeneous conditions. Furthermore, experiments in artificial aquatic environments can offer proof-of-concept for physiological responses of microbes to a varying environment, and as such provide those who venture into natural soil environments with information about fundamental, baseline responses of microbes to changing conditions. That information, in turn, can provide a starting point for formulating predictions about how soil microorganisms may respond to environmental change.

In the following sections, we present advances from natural and artificial environments relevant to research on microbially-mediated SOM transformations, beginning with oceanic and lacustrine systems and then examining increasingly controlled environments.

2.1 Natural aquatic systems as well-mixed environments in which to explore drivers of C fluxes and microbial elemental composition

Investigations of microbial transformations of OM in the oceans provide important information for those interested in understanding the drivers of SOM transformations. For example, organic geochemists working in the ocean have appreciated the role of the "microbial loop" as a governing feature of ocean OM composition and availability for decades (Pomeroy, 1974; Azam et al., 1983; Pomeroy et al., 2007). Work in ocean waters has demonstrated the importance of microbial byproducts as contributors to the ocean's reservoirs of OM (Kawasaki and Benner, 2006; Kaiser and Benner, 2008) and, more specifically, to the ocean's slow-turnover OM pools (Jiao et al., 2010). Years ago, Hedges and Oades (1997) called for the integration of sedimentary and soil science perspectives for exploring OM transformations, and other investigators recently

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made a plea for geochemists and ecosystem ecologists to integrate their approaches to address questions about C cycle responses to climate change (Billings et al., 2010). These calls are slowly being heeded, as reflected in soils literature acknowledging the important role microorganisms appear to play as producers, not just consumers, of SOM (Simpson et al., 2007; Liang et al., 2010; Hobara et al., 2014), as has been elucidated in the ocean. The composition and transformations of aquatic C are increasingly being used to better understand the terrestrial systems from whence some fraction of aquatic C is derived. Indeed, Battin et al.'s "boundless C cycle" concept emphasizes the importance of aquatic C flows as essential to quantify if we wish to understand both terrestrial and aquatic C transformations (Battin et al., 2009), and yet more recent work highlights how OM composition in aquatic systems can help us understand both aquatic C fluxes and the terrestrial systems upstream (Marín-Spiotta et al., 2014).

The stoichiometry of resources and of microbial resource demand are both relevant to OM decay and retention because microbial stoichiometry governs the resources that can be used effectively and thus the stocks of OM (including microbial necromass) that are retained (Elser et al., 2000). Adding C to lake water, for example, can induce greater bacterial biomass and greater bacterial mass-specific uptake of phosphorus (P; Stets and Cotner, 2008). However, this effect is attenuated when grazing by organisms in higher tropic levels limits the pool size of bacterial biomass (Stets and Cotner, 2008). Thus, it seems important to investigate the extent to which soil food webs can provide a top-down limitation on the turnover of slow-turnover SOM after C additions. Knowledge of bacterial responses to C additions from the aquatic literature is also relevant to investigations of the distinctions between bulk soil SOM transformations and those in the rhizosphere, where C availability tends to be higher (Cheng et al., 2014), and can help us understand both lateral and vertical patterns of nutrient demand in soils.

Indeed, experiments in freshwater lakes also reveal that changes in bacterial stoichiometry with changing resource stoichiometry are dwarfed by the responses of biomass stoichiometry to changing growth rates (Makino et al., 2003). Stoichiometric plasticity of microorganisms, though acknowledged as a potentially important way in

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which microbes may respond to environmental change (Billings and Ballantyne, 2013), is rarely incorporated into conceptual or quantitative models of SOM transformations, in stark contrast to the aquatic literature (e.g. Klausmeier et al., 2004). The degree to which organisms exhibit stoichiometric flexibility appears to vary widely (Geider and Laroche, 2002), but in organisms exhibiting such plasticity, C : P can be many times more variable than C : N (Hessen et al., 2013). It is unknown how such variation may influence OM decay, whether in aquatic or soil environments, but because one or multiple resources ultimately limit growth and rates of decomposition, understanding the causes and consequences of microbial stoichiometry in soils is importance for modeling SOM degradation and associated respiratory C loss.

Aquatic scientists also have observed that increasing temperatures tend to result in increasing C : P and N : P of bacterial biomass (Cotner et al., 2006), and that some of these changes are driven by changes in community composition (Hall et al., 2008). Bacterial growth efficiency (analogous but not necessarily equivalent to CUE; delGiorgio and Cole, 1998; Thiet et al., 2006) appears to decline with warming in aquatic systems (Hall and Cotner, 2007) and to be lower in tropical compared to temperate lakes (Amado et al., 2013), though this warming response is not ubiquitous (delGiorgio and Cole, 1998). Lower respiratory C losses at a particular temperature from bacteria sampled from warmer environments compared to those sampled from colder environments are congruent with microbial acclimation to temperature regimes (Hall and Cotner, 2007). Currently, CUE is a key focus of SOM investigations, but aquatic literature suggests that variables like biomass pool size (driven by both bottom-up and top-down pressures, Amado et al., 2013) and stoichiometry (C : N : P) should not be neglected when studying the influence of environmental conditions on microbial CUE.

2.2 Chemostats as well-mixed, reductionist environments in which to explore drivers of microbial elemental composition

Chemostat experiments enable almost complete control over microbial growth dynamics, and thus are useful for exploring fundamental microbial responses to environmental

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variation. Scientists have used chemostats for decades to understand principles of microbial growth (Monod, 1950; Droop, 1974; Rhee and Gotham, 1981) and though far removed from soil profiles in form, chemostats provide investigators with the opportunity to control microbial growth rate (Table 1). This is because the dilution rate (D) is equivalent to the growth rate of the microbial population for chemostats operating in continuous culture mode (Monod, 1950; see Ferenci, 2008 for discussion). This feature is critical, given how difficult it is to know this parameter in soils and its importance for understanding microbial responses to environmental cues.

In recent years, chemostat studies have enjoyed a resurgence in popularity (e.g. Miller et al., 2013; Simonds et al., 2010), driven in part by investigations of bacterial responses to environmental change and associated patterns of gene expression (Ferenci, 2008). For example, components of recent models of SOM transformations such as the stoichiometric constraints on substrates, enzymes, and microbial biomass (Moorhead et al., 2012; Manzoni et al., 2012a; Allison, 2012; Ballantyne and Billings, 2015) are frequently investigated in chemostat studies. Though some models invoke plasticity of microbial stoichiometry as a potential response to environmental change, the extent to which biomass plasticity vs. homeostasis is realized, and under what conditions, remains unclear. While total soil microbial biomass C : N : P appears well-constrained to an average of 60 : 7 : 1 across multiple ecosystems and a wide range of nutrient availabilities (Cleveland and Liptzin, 2007), studies manipulating soil nutrients demonstrate that meaningful shifts in microbial stoichiometry are sometimes realized (Tiemann and Billings, 2011b). Where plastic biomass stoichiometry is observed, two key reasons make it difficult to understand the mechanisms underlying the phenomenon: (1) it is difficult to know if such shifts result from stoichiometric change in extant populations or from changing relative abundances of distinct populations, and (2) stoichiometric analyses of soil microbial biomass typically reflect total biomass, not just the active biomass (Table 1). Chemostats allow us to control for these challenges.

In a chemostat-derived microbial population, changes in stoichiometry provide evidence that microbial stoichiometric plasticity can be a consequence of environmental

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change, a conclusion difficult to formulate using soil in which we do not know the identity nor the abundance of the active microbial players. Stoichiometric plasticity of microbes can vary to a much greater extent than is what typically observed in SOM literature. For example, *Pseudomonas fluorescens* biomass C : N : P showed variation from 52 : 8 : 1 to 163 : 25 : 1, depending on whether P was abundant or scarce relative to N (Chrzanowski and Kyle, 1996). Chemostats also have revealed that some stoichiometric ratios (e.g. C : N) of actively metabolizing microorganisms can remain similar as nutrient availability changes, while others (e.g. N : P) vary only when a substrate stoichiometric threshold is surpassed. (Chrzanowski and Kyle, 1996). It remains unclear if stoichiometric plasticity is a coping mechanism of microbes responding to changing nutrient availability, or if it is a reflection of a microbial population's inability to regulate uptake and/or excretion. Regardless of the mechanism, changing microbial stoichiometry can influence both resource demand and, given the generation of microbial necromass, SOM composition.

Chemostats are also a key means of advancing our knowledge about microbial stoichiometry in different temperature regimes and at different growth rates, given that both of these parameters are easily manipulated in these tightly controlled environments. Chemostats inform us, with great clarity, that growth rate and (in some circumstances) temperature are key drivers of microbial stoichiometry. Growth rate appears to be a dominant driver of stoichiometric patterns in chemostat-raised organisms (Rhee and Gotham, 1981; Klausmeier et al., 2004; Chrzanowski and Grover, 2008), consistent with observations from lakes (Makino et al., 2003). Microbes growing at relatively fast rates tend to exhibit greater cellular P concentrations, consistent with observations from natural waters (Elser et al., 2003) and the growth rate hypothesis (GRH), which states that C : P and N : P ratios reflect changing organismal allocation to ribosomal RNA, a P-rich molecule, as growth rate varies (Elser et al., 2000). When nutrients are limiting, bacterial stoichiometry also appears to vary with temperature (Cotner et al., 2006). These observations have important implications for our understanding of the drivers of SOM transformations, because by integrating observations of microbial stoichiometry

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and associated resource demand in chemostats to our knowledge of spatial patterns of P- vs. N-limitation in terrestrial soils, we can formulate ideas of how changing temperature regimes may induce different patterns of microbial resource demand, and hence SOM decay vs. retention, among ecosystem types.

2.3 Chemostats as well-mixed, reductionist environments in which to explore C fluxes

In addition to the insights about microbial stoichiometry that chemostats can provide, chemostats can serve as important sources of information specific to soil organic C fate. A flurry of recent papers investigating microbial C flows with changing soil conditions highlights how microbial C fate dictates the magnitude of soil feedbacks to climate (Manzoni et al., 2012a; Wieder et al., 2013; Sinsabaugh et al., 2013), but without knowing the rate at which soil microorganisms are growing we cannot know the fraction of C uptake allocated to growth vs. respired CO_2 (typically expressed as the CUE). It follows that it is exceedingly difficult to assess how the propensity to generate biomass vs. CO_2 might change with environmental conditions (Table 1). Adding an isotopically labeled substrate can help us understand microbial uptake of a particular resource or suite of substrates (e.g. Ziegler et al., 2005; Li et al., 2012; Frey et al., 2013), but we must interpret resultant data with the knowledge that we have perturbed the natural system, and that recycling of the isotopic label through the microbial biomass is likely to confound inferences from such studies as the temporal extent of sampling increases.

Recently, Lehmeier et al. (2015) exploited the chemostat environment to investigate the consequences of changing temperature regime on C flux from OM substrate into microbial biomass, and then into respired CO_2. At a constant rate of growth, microorganisms experienced an increase in specific respiration and corresponding decline in CUE with increasing temperature. Though C exudation by bacterial cells was not quantified, the work substantiates inferences from other, soil-based studies that CUE declines with temperature (e.g. Frey et al., 2013). The CUE finding is critical for efforts

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to incorporate soil processes into Earth system models used to predict future atmospheric CO_2 concentrations (Wieder et al., 2013).

Second, this study also highlighted strong isotopic fractionations among substrate, biomass, and respired CO_2 pools that vary with temperature (Lehmeier et al., 2015). Apparent respiratory fractionation during fungal (Henn and Chapela, 2000) and bacterial (Blair, 1985) respiratory losses of CO_2 has been observed, but is difficult to interpret when microbial growth rate is not known and the system is not at steady state. Isotopic fractionation during CO_2-generating respiratory fluxes is rarely considered in studies that use δ^{13}C-CO_2 to infer mesocosm or ecosystem function, though the potential importance of this phenomenon in plant respiration across diverse scales has been noted (Pataki, 2005). Because of difficulties knowing which active microbial population produced measured CO_2, or the substrate from which it was derived, it is difficult to quantify isotopic fractionation effects among organic and inorganic C pools in soil-based studies. Lehmeier et al. (2015) demonstrate the importance of chemostat studies for avoiding these soil-based challenges and provide proof-of-concept for temperature dependence of a respiratory fractionation factor. In contrast to studies in which soil temperature is manipulated, chemostats demonstrate that isotopic variation in respired CO_2 can result even while holding constant substrate identity and availability, active microorganism identity, and microbial growth rate.

Importantly, other chemostat studies have demonstrated that microbial growth rate itself, in isolation from other conditions such as temperature or nutrient availability, appears to influence specific respiration rates (Larsen et al., 1993; Payot et al., 1998; Kayser et al., 2005). This is consistent with the GRH (Elser et al., 2000), given that a microbe experiencing a change in its population growth rate must change its resource allocation. However, soil biogeochemists and microbial ecologists typically presume that a combination of availability of resources and community composition determines the size and growth efficiency of a microbial community, which in turn influences the respiratory C efflux, and that changing environmental conditions (e.g. temperature) can induce changes in specific respiration rate. Chemostat studies, though, demonstrate

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that growth rate governs not only specific respiration (Kayser et al., 2005) but also the relative dominance of respiratory pathways that produce CO_2 (Nanchen et al., 2006). If growth rate is a driver of specific respiration in soil microbial communities, these data suggest an important and underappreciated mechanism driving microbially-mediated soil C fluxes.

2.4 Chemostats as well-mixed, reductionist environments in which to explore microbial gene expression

Chemostats can provide valuable information about microbial function and gene expression in controlled conditions that can be used to understand functional gene transcription and metatranscriptomes from more complex soil systems. Patterns of microbial gene expression are often considered the gold standard for understanding microbial community function in a multitude of environments (Otteson et al., 2014; Ofek-Lalzar et al., 2014), and microbial gene expression in soils is obviously of great relevance to questions of SOM decay and soil microbial ecology more generally (Baldrian and Lopez-Mondejar, 2014). However, as outlined by Schimel and Schaeffer (2012), using modern molecular tools to better understand SOM decay is challenging given the lack of specificity of decay-related genes; unlike processes like methanogenesis and methanotrophy or denitrification, SOM decay is governed by a relatively large number of genes residing in a greater diversity of organisms. Despite the seemingly daunting level of microbial genetic diversity, soil metagenomes can be mined for their annotated and functionally assigned genes, and then used to assess how potential metabolic pathways can shift with changes in the environment such as soil warming (Luo et al., 2014). New tools such as Functional Ontology Assignments for Metagenomes (FOAM, Prestat et al., 2014) are making it even easier to use metagenomic data to functionally group microbial communities based on broadly categorized metabolic processes. This is an important step forward, as it has been recently demonstrated that even inclusion of coarse, physiologically defined functional groupings, e.g. oligotrophs versus copitrophs, can improve models of litter and SOM decay (Wieder et al., 2014).

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Metatranscriptomes or quantification of functional gene RNA can move us one step closer to understanding function in soils by isolating only the actively transcribed genes at the time of sampling. However, there still remains the difficult task of linking metatranscriptomes or functional gene transcription to metagenomes and soil processes, because we lack a basic understanding of how environmental factors influence the regulation and rates of gene transcription. Additionally, patterns of gene expression in soils are driven by both bacterial growth rates (Ferenci, 1999) and the identity of any limiting nutrient (Hua et al., 2004) (Table 1). Thus, changes we observe in soil transcriptomes with environmental conditions may not be the direct result of, for example, a temperature change, but instead may result from altered growth rates and/or changes in relative nutrient availability as induced by the change in temperature. These gaps in our knowledge can be filled through the use of chemostats. In a controlled, chemostat environment where nutrient availability is constant and growth rates can be monitored, researchers can measure gene expression in response to isolated environmental stressors such as low water potential or temperature changes. For example, in a controlled, chemostat-like system, Gulez et al. (2012) examined gene expression in relation to stress induced by manipulating matric potential. Hebley et al. (2014) used a chemostat approach to quantify changes in gene transcription and physiology of *Saccharomyces cerevisiae* during cyclic 12 to 30 °C shifts in daily temperature, and demonstrate the importance of microbial acclimation to temperature at these short timescales. These studies are of direct relevance to SOM-related investigations of the influence of soil water stress and temperature on SOM transformations. As we increase our understanding of the environmental controls on gene expression and transcription networks we can begin to understand how the snap-shot of whole community gene transcription represented by a soil metatranscriptome is linked to changes in the physiology of the community, and observed changes in soil processes such as SOM decay. These research avenues are critical for formulating and parameterizing SOM decay models, discussed in Sect. 3.

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Both natural and artificial aquatic systems are useful for understanding how SOM decay may proceed; natural systems are increasingly viewed as relevant to soil studies (e.g. Marin-Spiotta et al., 2014), and we applaud such efforts. Though used in conjunction with natural aquatic environments (Sterner et al., 2008), chemostats are only just beginning to be explored in the context of soil-specific questions, and can provide knowledge about OM decay not feasible to obtain using natural soil profiles. In the next section, we explore another under-exploited concept relevant to SOM transformations – that of intrinsic vs. apparent exo-enzyme kinetics. Though different soils may exhibit different *apparent* E_a, we cannot know the extent to which *intrinsic* properties of a soil's substrates vs. other, soil-specific features govern apparent E_a. Parsing the individual drivers of different soils' unique apparent E_a values can provide valuable insight for interpreting differences among those values.

3 Intrinsic decay rates as baseline values for comparison with observed patterns of SOM decay

Multiple papers explore apparent activation energies (E_a; in KJ mol^{-1}) required for SOM decay to proceed, often in the context of investigating the temperature sensitivity of SOM decay. The E_a is one way to quantify the ease with which decay of compounds can proceed. A substrate with intrinsically higher E_a is more difficult to decay than one with lower E_a at a given temperature (Sierra, 2013) and, accordingly, the C quality-temperature hypothesis suggests that organic matter more resistant to decay should exhibit higher relative temperature sensitivity (Bosatta and Ågren, 1999; Davidson and Janssens, 2006). E_a thus represents one means of quantifying more qualitative terms like "recalcitrance" and "quality" that are difficult to interpret (Kleber, 2010; Kleber et al., 2010), and is an important feature to consider when investigating soil feedbacks to climate – in a warmer environment, SOM exhibiting long residency times may exhibit greater relative increases in decay rates than SOM that decays more rapidly. However, it is difficult to interpret why one soil's apparent E_a may be different from another's, for

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we cannot know if the substrates undergoing decay possessed different intrinsic E_a of decay, or if soil-specific factors such as texture or the identity of the active microbial community drove apparent E_a differences. Selecting ubiquitous substrates and some of the key biogeochemical reactions that induce their decay, and characterizing the kinetics of these reactions when isolated from other substrates and microbes themselves, represents an incremental movement towards addressing these questions. Such studies can provide values for these reaction rates and, potentially, the E_a of decay, that are as close to intrinsic values as is feasible, if they are conducted when neither enzyme nor substrate is limiting.

It is important to consider the drivers of differences among potential and observed reaction rates, and apparent and intrinsic E_a, for a specific decay reaction when interpreting decay reaction rates and apparent E_a values derived from the soil environment. Recalling that the slope of an Arrhenius plot is considered the E_a of a reaction, we first must note that the line defining intrinsic E_a should, in theory, always be above (have a higher Y-intercept than) any line defining apparent E_a. This follows from the assumption that a decay reaction rate quantified in purified, abiotic solutions when neither enzyme nor substrate is limiting represent the upper limit for that reaction at that temperature. This is a difficult hypothesis to test, because the units in which purified enzyme-substrate reaction rates are expressed must necessarily be different from the typical units employed in studies of exo-enzyme reactions in soils and sediments (e.g. Sinsabaugh et al., 2012), but its logic is difficult to challenge.

In spite of the difficulties directly comparing the Y-intercepts of lines defining intrinsic and apparent E_a, it is valuable to consider the multiple ways in which apparent E_a of decay reactions in soils exposed to different temperatures may vary relative to intrinsic E_a for those same reactions. Because the slope estimates (E_a in KJ mol^{-1}) are independent of the reaction rate units, they can be compared and yield meaningful interpretations across samples. In some soils, we may observe greater apparent E_a (steeper slope in an Arrhenius plot) with a lower Y-intercept, as application of the Arrhenius function to SOM decay would predict. However, it is feasible that some environmental

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samples may exhibit *lower* apparent E_a (a shallower slope), or *equivalent* E_a (parallel slope), also with a lower Y-intercept. Such scenarios may occur if, for example, cooler temperatures promote a competitive advantage for microbial populations that preferentially produce the exo-enzyme that catalyzes the reaction in question, boosting observed reaction rates to a greater extent than the direct influence of temperature on the purified reaction rate would predict. Alternatively, soil moisture may decrease with increasing temperature, constraining diffusion (Wang et al., 2014), or warming could affect plant inputs to soil in a multiple ways (Flury and Gessner, 2014). Either of these phenomena could alter microbial demand for substrates and thus modify exo-enzyme production, pushing observed reaction rates away from potential reaction rates differentially across a temperature range.

Lehmeier et al. (2013) determined reaction rates of β-D-cellobioside as catalyzed by β-glucosidase (BGase) and N-acetyl-β-D-glucosamine (NAG) as catalyzed by β-N-acetyl glucosiminidase (NAGase) in purified (and therefore non-confounding, ideal conditions) at temperatures between 5 and 25 °C and a pH of 6.5. These reactions are proxies for the cleaving of monomers from cellulose and chitin, respectively. Because they were conducted when neither enzyme nor substrate was limiting, the study provide E_a values of these compounds (31 KJ mol^{-1} for BG/BGase, 41 KJ mol^{-1} for NAG/NAGase), which are as close to intrinsic values as is feasible. Expanding on this study, Min et al. (2014) confirmed the values and explored how E_a of these reactions change when the pH was varied in a reasonable range for soil pH around the world. They report distinct pH optima for both BG/BGase (5.5–8.5) and NAG/NAGase (5.5–6.5), and a significant effect of pH on the temperature sensitivity of BGase but not NAGase (Fig. 1). Baseline, intrinsic properties of these reactions in multiple pH regimes helps us to develop biogeographically based predictions of the temperature response of cellulose and chitin decay.

Such baseline values for intrinsic E_a only represent conditions in which neither enzyme nor substrate is limiting, a scenario that only sometimes is relevant to soils. However, baseline values are nonetheless essential for comparisons with estimates

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of apparent E_a of cellulose and chitin decay derived from soil samples. For example, estimates for apparent E_a of the BGase/BG reaction derived from soil samples representing a variety of ecosystems appear either greater or less than intrinsic E_a values assessed in purified conditions (Fig. 1a). In contrast, apparent E_a for the NAGase/NAG reaction appears consistently higher than the corresponding intrinsic E_a (Fig. 1b). This suggests that soil-related factors confounding the intrinsic temperature response of the the NAGase/NAG reaction become relatively more influential at lower temperatures. In contrast, soil-related factors confounding intrinsic E_a for the BGase/BG reaction appear to both increase and decrease apparent E_a relative to intrinsic values. Assessing E_a values at the actual soil pH, not at an arbitrary buffer pH, may offer important insights too. For instance, Barta et al. (2014) demonstrated the BGase/BG reaction can proceed in soils at pH 3.5. This is in apparent contrast to Min et al. (2014), where BGase/BG activity at pH lower than 4.5 could not be detected in purified conditions. Reasons for this discrepancy remain unclear, but one possible explanation is microbial generation of distinct isozymes capable of inducing catalysis in low pH environments. This and related insights are impossible to generate without developing baseline data sets, of which only very few exist. Similar work on a diversity of substrate-enzyme pairings will provide an important knowledge base for future SOM decay research.

Values of intrinsic E_a of decay reported thus far suggest that the influence of temperature on exo-enzymes, even in isolation from all the other changes that temperature can impart on soils, is important for the relative availability of resources for microbial assimilation. Specifically, studies indicate how temperature alone can alter the relative availability of C and N liberated from substrates as they decay – the C : N flow ratio – if those substrates have distinct C : N ratios and E_a of decay (Billings and Ballantyne, 2013). Exo-enzyme age, too, appears to interact with temperature to influence the relative availability of C and N released during decay reactions; the catalytic rate of exo-enzymes and the temperature at which the enzyme ages prior to catalyzing decay reactions can influence the decay rate of cellobioside and N-acetylglucosamine differently (S. Billings, unpublished data). The C : N flow ratio is important because it

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represents the return on microbial investments in exo-enzymes, and how that return on investment may change with temperature in ways that have nothing to do with microbial responses to temperature per se. Because changing relative availability of microbial resources may influence microbial stoichiometry (see Sects. 2.1 and 2.2), and, in turn, decay of additional substrates, exploring additional drivers of changing C : N flow rates appears to be an important, complementary avenue of research.

4 Using experimental advances to enhance recent theoretical efforts to model SOM decay

Investigators have modeled SOM decay for decades. Though an exhaustive review of these advances is beyond the scope of this paper, we highlight recent advances and elucidate how these advances could benefit from some of the discoveries detailed above. Coarsely, models of SOM decay can be grouped into two categories: those that are spatially explicit, and those that implicitly treat the factors influencing SOM decay as spatially homogeneous. The first category comprises models such as reactive transport models, often invoked by engineers or hydrologists (Masse et al., 2007; Scheibe et al., 2009), while the second category is more familiar to ecologists (Schimel and Weintraub 2002, Allison 2005; Allison et al., 2010; Davidson et al., 2012; Manzoni et al., 2012a; Moorhead et al., 2012; Moyano et al., 2013; Ballantyne and Billings, 2015). Some efforts have incorporated space into ecologically focused models by considering diffusional constraints on exo-enzymes within the soil matrix (Allison, 2005; Allison et al., 2010), but realistic physics of diffusion are rarely incorporated, and thus it is difficult to know if the temporal and spatial scales invoked for modeled diffusion are appropriate. Comparing substrate usage in chemostats or natural aquatic environments to that in soils can be valuable for discerning the influence of diffusion constraints on OM transformations, given minimal diffusion limitation in liquid environments relative to that in soils. However, empirical measurements of enzyme flow in soil (e.g. Vetter et al., 1998) highlight how difficult it is to generate realistic enzyme movements in a

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diffusion-constraining matrix, and the challenges of integrating spatially distinct processes into ecologically focused process models. This category distinction is important because processes relevant to SOM decay occur at the fine scales typically envisioned by ecological modelers (Schimel and Schaeffer, 2012), but key goals of the community are to predict SOM decay and associated CO_2 release at far coarser scales (e.g. Wieder et al., 2013). Thus at its core, projecting decomposition of SOM processes relevant at the Earth system scale is an exercise in accurate physiological and physical modeling combined with up-scaling approaches.

Multiple modeling exercises have attempted to move us toward the goal of projecting large-scale SOM transformations from physiologically based models, and recent years have seen a proliferation of models describing SOM decay (Manzoni and Porporato, 2009). Only rarely have investigators tried to estimate both model parameter values and the variance in those estimates from empirically derived data (Davidson et al., 2012), and quantitative results are difficult to apply across diverse soil types, ecosystems, and climate regimes. As a result, most of the insights provided by SOM decay models are qualitative. These models attempt to model SOM transformations by incorporating factors known or thought to govern SOM decay rates and associated CO_2 efflux, such as microbial growth rates, CUE, allocation of C to enzyme production, and C uptake rates (Allison et al., 2010; Allison, 2012; Manzoni et al., 2014). However, many models assume fixed fractions of microbial C allocated to processes such as enzyme production and maintenance metabolism, contrasting with evidence from physiological experiments which indicate that allocation patterns shift with the interplay between microbial resource demand and availability (Larsson et al., 1993; Payot et al., 1998; Dauner et al., 2001; Dijkstra et al., 2011).

The omission of microbial physiological plasticity in these and related models is unfortunate, because it is the fundamental microbial physiology that shapes C flow through microbial biomass and associated CUE (Billings and Ballantyne, 2013). An important advance relates aggregate C fluxes through soil microbes to microbial CUE (Manzoni et al., 2012a), critical both because this term governs the propensity of SOC

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to remain in the soil profile vs. leaving as CO_2, and because CUE is a "tunable" parameter in multiple other models (e.g. Wieder et al., 2013). Importantly, though, CUE is not a parameter that microbes govern as an end goal; rather, CUE is a byproduct of the changing relative importance of anabolism and catabolism as metabolic resource demand and resource availability vary in response to environmental conditions. An important step forward will be to develop models that do not modify only CUE, but that reflect multiple changes in environmental conditions influencing microbial stoichiometry and metabolism, with CUE changing as a result. Chemostat data again become important for these modeling efforts, because they provide baseline values for biomass production and specific respiration rates under varying environmental conditions which, in turn, dictate CO_2 efflux.

Developing a theoretical scaffolding on which we can build physiologically mechanistic models that ultimately can be made spatially explicit, and thus relevant at the scale of the Earth system, will require two key advances. First, more physiological realism needs to be incorporated into our modeling frameworks. Enhancing the physiological realism of existing ecological models can take multiple forms. Regulatory-metabolic network models that reflect microbial decision making and metabolic constraints can be developed. Metabolic flux analysis can be an effective means of modeling in situ metabolic transformations in soils (e.g. Scheibe et al., 2009), but progress in this realm remains slow (but see Dijkstra et al., 2011). Interdisciplinary studies such as Tang et al. (2009), who highlight how ^{13}C and multiple "-omics" fields can be effectively integrated, represent large strides towards the development of this field. Importantly, chemostats represent ideal experiments from which to build such models. Gene expression and proteomics measured in chemostats under constant conditions provide the best chance for matching expression and network state to putative C transformations. Additionally, parameter values for microbial substrate uptake, mass of C per unit dry mass of microbial biomass, dry weight per cell, enzyme deactivation rate, and the microbial biomass fraction of N and P (e.g. Allison, 2012; Manzoni et al., 2014) are available for changing environmental conditions from chemostat studies (e.g.

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Chrzanowski and Kyle, 1996, Chrzanowski and Grover, 2008; Lehmeier et al., 2015). Though the absolute values from reductionist laboratory experiments may not be directly applied to soils, they are a great starting point for accurately parameterizing models. Values of E_a for SOM decay are typically treated as one aggregated value as a simplifying assumption (e.g. Allison et al., 2010), though we know this to be false. Estimates of intrinsic E_a values derived from purified, biogeochemically relevant enzymes (Lehmeier et al., 2013; Min et al., 2014) are analogous starting points for parameterizing decay kinetics, which result from regulatory-metabolic network driven allocation and feedback upon physiological state.

Second, we must develop accurate ways to average the spatial variability in SOM transformations occurring in soil profiles. To do this, investigators must develop the expertise to understand what features of SOM transformations can be "coarse grained" to permit extraction of important dynamics at scales appropriate for ecosystem modeling, for multiple ecosystems in different climate regimes. Spatially explicit model outputs can then be compared to non-spatial, ecological models. There are two approaches widely employed in other fields that could be applied. One is to start with individual dynamics, as in Masse et al. (2007), and then derive the dynamics of the aggregate or the whole profile from the individual level dynamics. Durrett and Levin (1994) refer to this as deriving a hydrodynamic limit because of the analogous derivation of Navier–Stokes equations from the mass transfer for individual parcels of liquid. From such limits, characteristic length scales can often be inferred. Another approach is to start again with individual-level dynamics, but with stochasticity, and then derive mean dynamics for a profile or site in terms of higher order moments. This gives rise to the problem of moment closure, but moment closure methods have been effectively applied to model the mean dynamics of spatially explicit ecological dynamics (Bolker and Pacala, 1997). Employing such analytical approaches would enable the contributions of spatial heterogeneity across the soil surface and heterogeneity with depth to be quantified, with the ultimate goal of extracting mean decomposition dynamics at scales relevant for Earth systems models.

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5 Applying these concepts to the puzzles presented by changing SOM characteristics with depth

We can apply some of the empirical and theoretical concepts described above to help address the question we posed in the introduction: "Why does some SOM leave the soil profile relatively quickly, while other compounds, especially those at depth, appear to be retained on timescales ranging from the decadal to the millennial?" In recent years, the community of scholars focused on SOM transformations has become increasingly appreciative of the importance of relatively deep SOM. Indeed, investigators are establishing Critical Zone Observatories around the globe to investigate whole-ecosystem function down to bedrock (Jordan et al., 2001), and are developing an increasing appreciation of the importance of deep metabolic processes for ecosystem functioning (Richter and Billings, 2015). It is difficult to define what is meant by "deep SOM"; certainly defining deep SOM as that residing below an absolute depth would be arbitrary and thus inappropriate for application across ecosystems. Using the plant rooting zone as an indicator of "shallow" horizons is challenging when we consider highly weathered profiles in which active plant roots can function tens of meters below the surface (Stone and Kalisz, 1991), surrounded by SOM we might otherwise consider to be "deep". However, general trends in SOM stability with depth are clear: with depth, SOM stability appears to increase, with mean residence times of millennia not uncommon (Trumbore, 2009; Schmidt et al., 2011, Fig. 2).

Though an estimated 21–46 % of global soil C stocks reside at depths > 100 cm (Jobbágy and Jackson, 2000), we understand very little about what controls the persistence or decay of deep SOM in comparison with our understanding of more surficial processes (Schmidt et al., 2011). Of course, it is not depth per se that governs SOM persistence or decay, but rather changes with depth in the relative dominance of variables that influence decomposition rates. The predominant state factors (Jenny, 1941) influencing SOM dynamics appear to change below surface horizons: climate becomes less dominant as an influence on SOM transformations with depth, and soil texture

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appears to assume a greater role (Jóbbagy and Jackson, 2000). Intriguingly, some studies demonstrate that decay of apparently stable, deep SOM can be initiated when sufficient energy is added to promote microbial activity (Fontaine et al., 2007; Wang et al., 2014). However, many mysteries remain about the drivers of variation in SOM characteristics with depth. In this section, we briefly describe our knowledge of physical features that can protect SOM from decay, and then attempt to describe how the microbially- and enzymatically-focused observations described in the above sections could be applied to address the puzzles presented by changing SOM characteristics with depth.

First, we must examine the physical and chemical features of soil known to be important drivers of SOM decay or retention. Though physical protection against decay occurs in relatively shallow soil horizons and is relatively well-studied (von Lützow et al., 2006; Jastrow et al., 2007; Six and Paustianm 2013), the phenomenon is less well understood deeper in the soil profile. There have been no systematic studies of aggregation processes with depth, or attempts made to link deep aggregate formation with the formation and stability of deep SOM. The aggregate formation hierarchy and trajectory proposed by Tisdall and Oades (1982) would suggest that deeper, and therefore likely older, SOM should have greater aggregate development and greater aggregate stability, with micro-aggregates and associated SOM protected inside macro-aggregates. In general, soils further along the aggregate formation trajectory, with a high proportion of stable macro-aggregates, provide greater physical protection for SOM.

This relationship between SOM stability and the size distribution and stability of aggregates remains untested in deep soils, but most studies that report aggregate size distributions at different depths show higher proportions of micro-aggregates, and their associated SOM, in deeper soils (Andruschkewitsch et al., 2014; Yang et al., 2014; Plaza-Bonilla et al., 2010; Bhattacharyya et al., 2009; Wright et al., 2009; Reuss et al., 2001, Fig. 2). Relatively fewer macro-aggregates in deeper soils may mean that the Tisdall and Oades aggregate formation model is not appropriate for application in deep soils. This model requires active root and fungal growth for “transient” binding

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of soil particles and micro-aggregates, and in deeper soils these biological factors are limited or non-existent. Thus it is perhaps not surprising that deeper soils do not develop macro-aggregates to the same extent as shallower soils, where biological activity is highest. The apparent dearth of more developed aggregates in deep soils, however, does not necessarily mean less protection for deep SOM. Indeed, the accumulation of many micro-aggregates can create fine-scale, relatively tortuous pore spaces promoting slower diffusive pathways for substrates and enzymes, or pores too small for microbes or enzymes (e.g. Horn, 1990), limiting the access of microbes or their enzymes to SOM. Furthermore, organic matter complexes with iron and aluminum can exhibit great resistance to decay (Kaiser and Zech, 2000; Kuzyakov, 2010; Rumpel and Kögel-Knabner, 2011; Buettner et al., 2014), and the reduced prominence of plant relative to mineral materials with depth increases the propensity for such complexes to become more dominant in function. Though it is difficult to incorporate accurate diffusive behaviors of enzymes and substrates into models of SOM decay, attempting to account for shallow vs. deeper horizon aggregate development and associated pore space tortuosity in models of SOM decay processes seems an important avenue of research. Given recent advances in our understanding of linkages between iron reduction and the mobilization of organic C in soils (Buettner et al., 2014), development of models that account for varying microbial access to SOM given varying concentrations and forms of soil minerals appears to be another low-hanging fruit for the research community.

In addition to appreciating how physical and chemical protection may function as a driver of deep SOM retention, we can apply knowledge gleaned from microbially-focused work in natural and artificial aquatic environments to formulate specific research foci important for investigating deep SOM transformations. For example, in addition to features of physical protection likely changing with depth in soil profiles, SOM composition also changes with depth in ways that either influence or reflect rates of SOM decay, and thus persistence (Fig. 2). A large proportion of deep SOM appears to have been subjected to a greater degree of microbial processing than SOM in shallower

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horizons. This is reflected in deep soils exhibiting relatively low C : N ratios, a higher abundance of lipids, polysaccharides and N-bearing compounds (including proteins), enrichment in ^{13}C and ^{15}N relative to ^{12}C and ^{14}N, respectively, and a greater proportion of apparently slow-to-decay compounds of microbial origin (e.g. Ehleringer et al., 2000; Billings and Richter, 2006; Fröberg et al., 2007; Rumpel and Kögel-Knabner, 2011). Deeper soils are likely to exhibit preferential sorption of compounds to mineral surfaces (Schrumpf et al., 2013), a chemical feature that can govern microbial access to substrates. Indeed, these organo-mineral complexes appear almost impervious to enzymatic attack (Schrumpf et al., 2013; Fontaine et al., 2007; Kögel-Knabner et al., 2008). This, combined with the well-processed nature of deep SOM molecules, results in deep SOM decay requiring a large energy investment by microbes to obtain resources from that decay. When the energy (i.e. organic C) required by microbes to produce enzymes exceeds the energy return, decomposition will not proceed (Schimel and Weintraub, 2002). For example, when microbes are given ideal conditions for decomposition, such as optimal soil water content, temperature and oxygen levels, decay rates of deep SOM appear much slower than decay rates of surface SOM (Fontaine et al., 2007; Wang et al., 2014), except when a labile form of organic C is added as an energy source, presumably because the energy limitation has been removed (Fontaine et al., 2007). It is this energy limitation that may be largely responsible for the apparent stability and persistence of deep SOM (Fontaine et al., 2007; Kuzyakov, 2010; Wang et al., 2014). Our growing understanding of this phenomenon – that old SOM is not necessarily intrinsically "recalcitrant" (Kleber, 2010; Kleber et al., 2010) – represents an important advance for our studies of deep SOM, as well.

Microbial characteristics also change with soil depth in ways likely important for SOM decay properties (Fig. 2). Changes in SOM chemistry and abiotic conditions with depth reduce microbial diversity and alter microbial community structure (Agnelli et al., 2004; Goberna et al., 2005; Fierer et al., 2003; Will et al., 2010; Gabor et al., 2014; Eilers et al., 2012). The byproducts of microbial communities appear to comprise a meaningful fraction of OM reservoirs, ranging from 40 to 80 % (Liang et al., 2010; Simpson et al.,

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2007), and can persist over long timescales (Voroney et al., 1989; Jiao et al., 2010; Six et al., 2006; Miltner et al., 2011; Liang et al., 2010; Grandy and Neff, 2008; Simpson et al., 2007; Hobara et al., 2013). Given that some microbial byproducts can exhibit relatively slow decay rates, we might expect SOM persistence to increase with depth as the relative dominance of plant relative to microbial inputs to the SOM pool decreases with depth. However, not all microbial byproducts exhibit slow decay rates (Throckmorton et al., 2012). Our growing appreciation of microbial contributions to SOM and the persistence of some of this material over relatively long timescales prompt calls for investigations into the relative dominance of microbial vs. plant inputs to deep SOM reservoirs, and for experiments designed to reveal how different microbial byproducts from distinct community compositions invite or resist decay.

These observations, and the changing C : N of SOM and soil temperature regime with depth, are directly connected to the knowledge obtained from aquatic environments about microbial transformations of OM, particularly when we consider interactions between substrate stoichiometry and temperature. For example, the observation that the relative availability of organic C (energy) can govern the ability of microbes to induce decay of slow-turnover SOM (Fontaine et al., 2007) is directly relevant to observations of substrate stoichiometry driving microbial biomass, and thus resource requirements, in natural and artificial aquatic environments. Furthermore, bacterial stoichiometry appears to vary in meaningful ways with temperature when nutrients are limiting (Cotner et al., 2006). We thus might predict that when energy (i.e. organic C) is more limiting, as is likely the case deep in a soil profile, temperature effects on microbial stoichiometry may be minimal. This prediction, if realized, has important implications for projecting the effect of temperature on deep SOM decay because it suggests that an increase in deep soil temperatures may not induce a large shift in the stoichiometry of resource demand of extant microbial populations, and that microbial responses to temperature will vary with substrate C : N, and thus with depth. The observed importance of substrate and microbial C : P and N : P ratios as drivers of OM flow in chemostat studies (Chrzanowski and Kyle, 1996) as temperature varies (Cotner et al., 2006) can also be

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applied to questions of SOM decay at depth, reminding us that the relative N vs. P limitation in terrestrial ecosystems likely will have an influence on each ecosystem's microbial response to temperature. Current models of SOM decay do not incorporate these ideas, but doing so will inform us about an important driver of SOM composition changes with depth: the composition of the material accessed by microbes and transformed into CO_2 and other, non-gaseous phase microbial byproducts.

We also can use purified enzyme-substrate reaction kinetics (Lehmeier et al., 2013; Min et al., 2014) to formulate additional research questions about increasing SOM persistence with depth, and how destabilization of deep SOM stocks may proceed in a warmer world. For example, pH optima for exo-enzymatic catalytic rates and well-characterized interactions between pH and E_a of decay for specific decay reactions (Min et al., 2014) are useful for predicting how these enzymatic-substrate reactions may proceed in different soil horizons, if we know how pH varies with depth in a soil of interest. We also can use changing C : N flow ratios as temperature varies (Lehmeier et al., 2013; Min et al., 2014) to predict how microbial resource availability may change with depth. We are far from knowing how C : N flow ratios change with temperature in natural environments at any depth, but we at least have a starting point derived from the two biogeochemically relevant substrate-enzyme pairings investigated in these works. Examining how divergence from purified reaction kinetics changes with depth in substrate-enzyme reaction rates will provide insight to the varying degree to which physical and chemical protection in the soil matrix, as well as microbial adaptation to temperature, govern depth patterns of SOM decay and retention. This research approach will permit us to address a critical question for understanding deep SOM retention: do deep-profile environmental factors drive greater divergence from intrinsic reaction kinetics than in more shallow horizons, and if so, which ones? Effectively addressing this question will promote a great number of studies that, if done well, can inform us about the environmental conditions that change with depth and transform well-characterized intrinsic decay rates to apparent decay rates. In so doing, we will

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gain a greater understanding of the environmental factors that govern deep SOM retention.

Finally, if a negative relationship between the E_a of decay and C : N ratio exists for many soil substrates, as has been hypothesized (Rumpel and Kögel-Knabner, 2011; Billings and Ballantyne, 2013), we can use purified enzyme-substrate reaction kinetics to develop concepts of how microbially available C and N may change with depth through a soil profile in a warming climate. This is feasible given known trends in C : N and E_a of aggregated substrate decay with depth, which decrease and increase with depth, respectively. It is also feasible to incorporate these concepts into current models of SOM decay: E_a of decay and stoichiometry are key features of multiple models currently invoked in the literature. If the temperature sensitivity of decay is greater for many substrates at depth, and many of these substrates possess low C : N, enzyme kinetics suggest that the relative availability of C relative to N may decline with warming, particularly at depth. Microbial communities must respond to any such change in resource availability, and in so doing can change either or both their community composition and their resource allocation. Investigators currently debate if microbial community composition matters for large scale phenomena like what substrates are decayed and how necromass may be retained over relatively long time periods (Throckmorton et al., 2012; Nemergut et al., 2014). If microbial community composition matters for these processes, changing community structure, be it via shifting relative abundances of distinct populations or the elemental composition of existing populations, can influence patterns of SOM decay and production via necromass formation, and hence retention.

6 Conclusions

1. There has been some effort in the literature to link the research communities that examine natural aquatic, sedimentary, and soil OM transformations (Hedges and Oades, 1997; Billings et al., 2010; Marín-Spiotta et al., 2014). In spite of these calls for integration, these disciplines have remained relatively distinct. We

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emphasize the great utility of employing knowledge from natural aquatic systems to better predict how SOM decay and retention will proceed in the future. Like soils, aquatic systems can reveal how both physical protection and microbially mediated processes govern OM transformations in changing environmental conditions. The concept of the microbial loop in the ocean (Pomeroy, 1974; Azam et al., 1983; Pomeroy et al., 2007) and the observation that microbial byproducts form a great fraction of oceanic OM (Kawasaki and Benner, 2006; Kaiser and Benner, 2008; Jiao et al., 2010) pushes soil scientists to test analogous hypotheses in terrestrial systems (Liang et al., 2010). We encourage further application of empirical observations in aquatic systems in terrestrial soils. In this way, we can develop the nascent concept of soil microbial communities functioning both as decomposers and generators of byproducts with potentially long residence times.

2. With the exception of a few investigators who work in both chemostats and natural aquatic environments (e.g. Elser, 2003), literature describing chemostats only rarely have been invoked by SOM-focused investigators (Lehmeier et al., 2015). Yet, chemostats have much to tell us about the importance of resource availability and temperature, for example, on microbial resource demand and resource allocation. Understanding these processes helps us to understand the characteristics of substrates *not* accessed by microbes, and thus features of SOM retention. This is especially relevant to questions of deep SOM, given the increase in SOM mean residence time deep in soil profiles. Chemostats also tell us that microbial growth rate has a direct influence on microbial stoichiometry and specific respiration rate, a phenomenon currently not appreciated by the modeling community. This, in turn, can govern CUE and resource demand – and thus the identity of substrates retained in the profile. An important step forward for the theoretical frameworks describing SOM transformations is to include these features in the models, and to explicitly model shallow versus deep SOM transformations. This can be accomplished if we recall that some chemostat experiments manipulate

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the very environmental features known to vary with soil depth, such as resource stoichiometry, E_a of decay, and temperature.

3. Developing baseline, upper values for substrate–exo-enzyme reaction kinetics is another important avenue of research for those interested in OM decay and retention. Baseline values derived from purified reaction kinetics allow parsing of intrinsic responses to top-down drivers of decay such as temperature from other, soil specific factors that may change with the environment. We also can use purified substrate–exo-enzyme reaction rates to develop estimates of intrinsic E_a of decay. Purified reactions do not generate an upper limit for temperature sensitivity, but deviations from intrinsic temperature sensitivities observed in the natural environment represent factors specific to the soil under study, which can be parsed from the known intrinsic E_a. Purified kinetics of biogeochemically relevant decay reactions thus provide baseline values to use in models of SOM decay, and differences among known biogeochemical reactions – their raw rates or E_a derived from them – give us a sense of E_a values appropriate for model use.

4. We highlight some additional, low-hanging fruit for the community of modelers focusing on SOM transformations in a changing climate, and within soil profiles across depth. For example, models that attempt to use soil physics and diffusive properties of enzymes and substrates to better predict OM transformations can expand their efforts to explicitly model shallow versus deep SOM. By altering diffusive parameters to better reflect the differences in relative abundances of macro – vs. micro – aggregate structure across soil depth, and the different agrees of tortuosity throughout a soil profile, we can gain a sense of the importance of these features as drivers of SOM protection at depth. Modelers also can use information from some natural aquatic environments and chemostats to better understand how microbial stoichiometry, resource access, elemental cell content, and specific respiration rates change with environmental conditions. Though absolute values of these parameters from chemostats are likely not appropriate for use in

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modeling soil profiles, chemostat values provide at least qualitative indications of how these parameters may change with environmental conditions, including those that vary with depth.

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Table 1. Parameters frequently of interest for empirical and theoretical investigations of SOM transformations (left column), typical challenges encountered when interpreting data derived from soil studies (middle column), and the benefits of employing chemostats (rows 1 through 3) and purified enzyme-substrate reactions (row 4, last column). Controlled environments where microbial populations and environmental conditions can be strictly monitored provide unique insights that can be used to develop hypotheses for soil-based studies or parameterize models of SOM transformations. See Sects. 2 and 3 for detailed explanation of all table cells.

Soil parameter of interest	Challenges for soil based studies	Benefits of chemostat-based studies (rows 1–3) Benefits of purified, abiotic studies (row 4)
Carbon use efficiency (CUE)	– Recycling of isotopic label through microbial biomass is likely across diverse timescales. – Growth rate is unknown.	– Growth rate is known. – Growth rate can be manipulated. – Isotopic fractionation can be quantified. – Fraction of dead cells is small.
Microbial stoichiometric plasticity	– Stoichiometric change may occur in extant populations, or from changing relative abundances of distinct populations. – Stoichiometric analyses of soil microbial biomass typically reflect total biomass, not just active biomass.	– The identity, pool size, and growth rates of the active microbes are all known.
Environmental controls on gene expression	– Metatranscriptomes or functional gene transcription are dependent on growth rates, nutrient availability, and environmental controls on transcription rates that are unknown.	– Growth rates are known, nutrient availability is constant, and gene expression can be monitored as individual environmental signals are manipulated.
E_a and associated temperature sensitivity of SOM decay	– Differences among soils in apparent Ea may result from different microbial physiology, microbial community structure, or substrate availability, and not from inherent differences in substrate E_a of decay.	– Intrinsic kinetics of decay can be quantified in controlled conditions and under varying environmental parameters such as pH and temperature. – The C : N flow ratio can be computed as environmental conditions change, reflecting how C and N availability can change even in the absence of microbial adaptation.

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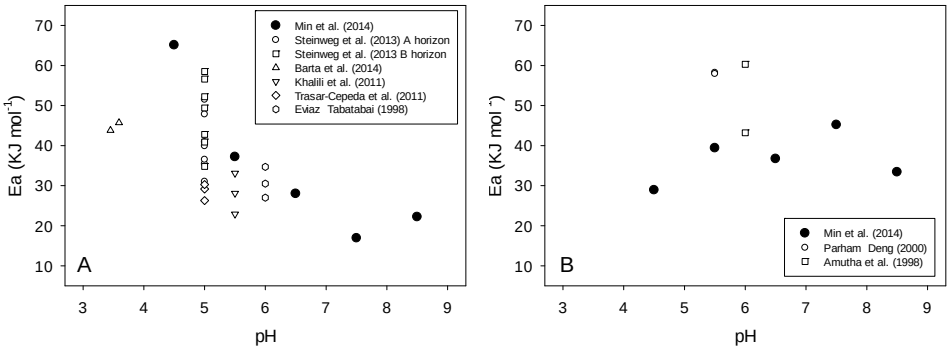

Figure 1. Estimates of intrinsic (closed symbols) and apparent (open symbols) E_a for the BGase/BG reaction **(a)** and the NAGase/NAG reaction **(b)**. The literature values for apparent E_a are shown at the pH the reaction was actually observed, and does not necessarily correspond to the pH of the soils the samples were taken. See Sect. 3 for interpretation.

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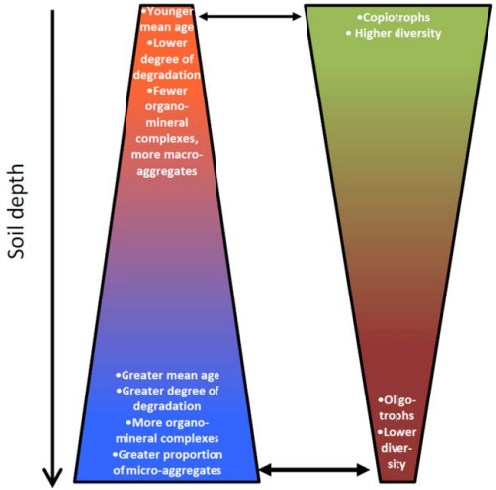

Figure 2. Depiction of parameters describing drivers of SOM decay and retention with depth. Salient physical and chemical features are described on the left, and microbial features on the right. Key features both resulting from and driving patterns of SOM decay are the mean age of SOM and its associated degree of degradation, and the degree to which it forms organo-mineral complexes and micro- vs. macro-aggregates. All of these typically are enhanced with depth. A greater mean residence time is often associated with a greater degree of microbial processing of that material, hence the greater degree of degradation. When coupled with the greater amount of organo-mineral complexes that form with depth, these features drive more energy intensive SOM decay at depth, increasing the activation energy (E_a) of decay and associated temperature sensitivity of decay. In turn, these physical and chemical changes with depth govern the diversity, physiology, and functional guild of microbial groups in shallow vs. deep soil horizons. Thicker arrow at depth represents likely greater interaction strength in deep soil horizons among energy availability in substrates, temperature sensitivity and microbial physiology, given the generally greater E_a and lower energy available at depth. Importantly, the microbial community can serve as both an agent of decay and of production of SOM compounds with apparently long residence times; this concept has only recently been explored in the soils literature.

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