# ASSESSING THE AGRONOMIC AND ECOLOGICAL RELEVANCE OF MINERAL-ASSOCIATED ORGANIC MATTER

BY

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# ABSTRACT

ASSESSING THE AGRONOMIC AND ECOLOGICAL RELEVANCE OF MINERAL-ASSOCIATED ORGANIC MATTER

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As the largest terrestrial sink for carbon (C) and a critical source of nitrogen (N) for plants, soil organic matter (SOM) is a major driver of ecosystem function. It is critical to understand the mechanistic controls on SOM in order to improve models of global C cycling and to develop accurate measures of soil fertility. SOM consists of a wide spectrum of compounds, varying in chemical characteristics and function. The chemical and physical fractionation of SOM is a valuable tool for distilling this complexity into meaningful and distinct pools: detrital or particulate organic matter (POM), which contains mostly recent litter inputs at early stages of decomposition, and mineral-associated organic matter (MAOM), which is far more processed, consisting of small organic compounds bound to reactive mineral surfaces. For decades, MAOM has been studied primarily for its capacity to sequester soil C and N. In this dissertation, my research reveals the under-appreciated role of clay minerals in mediating the short-term accrual and turnover of SOM. I examine the mechanistic controls on MAOM and specifically, how agricultural management and plant-microbe interactions influence C and N within MAOM.

Agricultural practices can directly impact the capacity for soils to store MAOM. Approaches that minimize soil disturbance, such as conservation tillage, and those that increase crop residue input and diversity, such as cover cropping, can facilitate the rapid accrual of N within MAOM (Chapter 1). Through this research, I found that MAOM N may also be an

important, but overlooked, source of N for crops. This work led me to develop a conceptual framework in which I synthesized literature from the fields of geochemistry and soil biology to investigate the potential mechanisms that drive MAOM turnover. Although this conceptual work stands alone (Chapter 2), the hypotheses and ideas therein form the basis for my experimental work. My overarching hypothesis addresses the biochemical strategies that plants employ to disrupt mineral-organic interactions and release both C and N from MAOM. Specifically, I examine two mechanisms by which plant root inputs may stimulate the destabilization and turnover of both C and N within MAOM: belowground root C inputs, specifically in the form of sugars and organic acids, can stimulate MAOM decomposition through indirect and direct mechanisms, respectively.

Through a series of laboratory incubations, I demonstrate that simulated root exudates can stimulate the mobilization of both C and N from MAOM through microbial and non-microbial pathways (Chapter 3). Additions of a sugar substrate, glucose, were associated with the microbial-mediated mineralization of C and N from MAOM. The organic acid substrate, oxalic acid, was associated with the direct and concomitant mobilization of DON and metals into exchangeable and soluble pools. Most notably, both substrates stimulated the respiration of MAOM-C (i.e., positive priming), with total increases ranging from 35–89%. Our results provide evidence for pathways of MAOM destabilization, and more generally reveal that a pool of soil nutrients generally considered passive or inert has the potential to function as a significant source of C and N.

# INTRODUCTION

Changes in soil organic matter (SOM) stocks have immediate and long-term implications for environmental health and human well-being. On the short term, SOM can supply critical quantities of nutrients for crop growth. SOM is also the largest active terrestrial reservoir for carbon and so, on the longer-term, will play an important role in either the mitigation or exacerbation of atmospheric $CO_2$ concentrations (Adhikari and Hartemink, 2015). In order to promote the sustainable management of soils and improve predictive models for ecosystem C and N cycling, it is critical to understand the fundamental mechanisms and processes that contribute to the formation and preservation of SOM.

SOM is generated through the degradation and transformation of fresh organic inputs. Upon entry into soil, organic residues can proceed through many decomposition pathways. The majority of these transformations are microbially mediated, but a portion of organic inputs can resist decomposition, remaining minimally altered or undecomposed due to their chemical recalcitrance (Keil and Mayer, 2014). Within the traditional view of SOM cycling, it is believed that this residual and recalcitrant material are the main constituents of stable and persistent SOM (Stevenson, 1994). However, a burgeoning body of empirical evidence has revealed that stable SOM is microbially-derived compounds associated with mineral surfaces (Grandy and Neff, 2008). These findings have steered an emerging view on SOM formation and stabilization: the primary pathway of SOM formation is microbially mediated; further, the decomposition and stabilization of SOM is a continuum where litter inputs are successively processed, moving from detrital or particulate forms into mineral-associated forms of organic matter.

Organic compounds associated with mineral surfaces are considered the most stable and resistant to decay. Silt and clay particles can bind organic compounds via chemical associations due to a high availability of charged binding sites on their surfaces (Kleber et al., 2015). As such, mineral-associated organic matter (MAOM) is considered the most stable or persistent pool for soil nutrients. Unlike detrital or particulate organic matter (POM), which consists mostly of recent litter inputs at early stages of decomposition, MAOM is more processed, having been oxidized and decomposed both biotically and abiotically (Lützow et al., 2006). The stabilization of compounds on mineral surfaces is responsible for the long-term sequestration of soil C and N in agricultural systems, and the accumulation of MAOM is thus considered a metric of soil health and function (Lal, 2016). However, mineral surfaces are also dynamic zones of nutrient exchange and recent evidence suggests a portion of MAOM may be cycling on rapid timescales not easily captured by radiocarbon-based measures of turnover (Hall et al., 2015). MAOM is generally not considered in studies on short-term nutrient cycling despite the growing body of research pointing to the dynamic qualities of this pool.

My first chapter provides a glimpse into these potentially dynamic qualities of MAOM and questions this long-held perspective that MAOM is an inert or passive pool of C and N. In a field experiment replicated across three sites in the north central and mid-Atlantic United States, I found that conservation tillage and cover cropping can lead to rapid accrual of N across all fractions, particulate and mineral-associated. As expected, POM fractions were most sensitive to tillage, but the responses in MAOM fraction varied by site and management. In two low-SOM soils, both reduced tillage and cover cropping significantly increased N within MAOM. In a contrasting high-SOM soil, cover cropping increased MAOM N while tillage had no effect. I also found that both particulate and fine fractions were positively associated with select measures

of N availability and crop performance. Current conceptual models and analytical methods do not account for this potentially dynamic role of MAOM as N-supplier in soils. Moreover, many indices used to predict plant-available N often disregard the effect of growing roots and their localized effects over microbial and nutrient dynamics. This work led me to investigate at greater depth the potential contribution of MAOM as an N source, specifically in rhizosphere environments.

At the core of my research is a conceptual and experimental framework in which I demonstrate how MAOM could be an important and overlooked source of N for plants. Although the conceptual work stands alone as my second chapter and as a published manuscript (Jilling et al., 2018), hypotheses therein form the basis for my experimental work described in my third chapter. My overarching hypothesis addresses the biochemical strategies that plants employ to disrupt mineral-organic interactions and release N from MAOM. Within this framework, belowground plant C inputs, specifically in the form of root exudates, can stimulate MAOM decomposition rates indirectly, by way of the microbial community, or directly, via metal-destabilizing organic acids. I tested two pathways of MAOM destabilization in my third chapter through a series of laboratory incubations. We applied a sugar, glucose, and an organic acid, oxalic acid, to simulate potentially different pathways by which root exudates may mobilize C and N within MAOM.

We applied substrates to assembled mixtures of sand and MAOM to isolate the effects of simulated substrates on MAOM, specifically. Both substrates stimulated the release of C and N and the effects were a product of both biological and non-biological action upon MAOM—corresponding to the indirect and direct pathways of MAOM degradation, respectively. Glucose additions activated the microbial community to a greater extent, based on increases in enzyme

activities, MAOM-C and MAOM-N mineralization. The effect of organic acid additions was both microbial and non-microbial: oxalic acid caused a smaller but still significant increase in MAOM-C respiration; the substrate was also associated with an abiotic release of DON and metals into exchangeable and soluble pools. My findings provided proof of concept for different pathways of MAOM destabilization and led me to consider the broader implications of MAOM destabilization. The study described in the third chapter reveals the potential for MAOM turnover in assembled mixtures with MAOM as the only source of C and N. We considered this a first step in understanding the mechanisms that provide plants and microbes access to C and N within MAOM.

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# CHAPTER 1

## RAPID AND DISTINCT RESPONSES OF PARTICULATE AND MINERAL-ASSOCIATED ORGANIC NITROGEN TO CONSERVATION TILLAGE AND COVER CROPS

**Abstract**

Particulate organic matter (POM) is considered an "active" source of nitrogen (N) in cultivated soils, responding readily to management and being more physically accessible to decomposers than mineral-associated forms of organic matter. However, there is increasing evidence that mineral-associated organic matter (MAOM) can also exhibit short-term changes to management that may impact plant and microbial N dynamics. In this study, we investigated how N within soil organic matter fractions responded to three years of tillage and cover crop treatments. We collected soils from a row-crop (maize-soybean rotation) field experiment replicated across three sites in the north central and mid-Atlantic United States: a high-soil organic matter site in Illinois (IL) and two low-soil organic matter sites in Michigan (MI) and Pennsylvania (PA). Management treatments included two levels of tillage (chisel plow and ridge tillage) and two levels of cover crop (with or without rye cover crop). Using an optimized sonication method coupled with particle size separation, we isolated and analyzed for N content free POM, occluded POM, a coarse silt fraction, and MAOM. Using partial least squares regression, we explored broad cross-site relationships between soil organic matter (SOM) fractions, soil N availability, and crop performance.

Both particulate and fine fractions responded to tillage and cover crop treatments, but patterns varied by site and fraction. In the low-SOM MI and PA soils, ridge tillage and cover cropping both increased N within POM fractions. The response to ridge tillage was most pronounced, with a 76% and 24% increase in occluded POM N content in MI and PA, respectively. In contrast, at the IL site (high-SOM), the inclusion of cover crops led to higher N, specifically within the fine fractions (coarse silt and MAOM). Cover cropping increased MAOM N content in IL by 24%. When analyzing all sites together, variables associated with fine fractions were more closely associated with N mineralization and crop performance. MAOM can be responsive to short-term management practices and, along with POM, may also be potential sources of N for crops.

## Introduction

In managed systems, soil organic matter (SOM) plays a critical role as a reservoir and source of potentially bioavailable nitrogen (N) (Knicker, 2011). Up to 95% of soil N is organic in form (Bingham and Cotrufo, 2016), with the bulk of this N widely considered to be physically or chemically inaccessible to decomposers. The long-term storage of N within SOM provides many ancillary ecosystem services, such as sequestering N that may otherwise pollute waterways and contributing to a soil's physical structure. At the same time, the mineralization of SOM is necessary to support microbial and plant growth (Janzen, 2006). SOM can thus serve multiple, seemingly opposing functions, due to its physical and chemical heterogeneity (Grandy and Neff, 2008). SOM is a mixture of compounds that range in molecular-size, chemical composition, and

extent of oxidation—properties that in turn, influence its behavior, such as potential interactions with mineral surfaces and accessibility to microbes (Kleber et al., 2015). In order to sustainably manage our agricultural soils, it is important to understand how management practices alter the accrual and distribution of carbon (C) and N within SOM.

Fractionation is used to partition SOM and isolate components that are distinct in physical and chemical traits (Cambardella and Elliott, 1994). Measurable differences often translate to differences in function, such as potential N-supplying capacity (Sollins et al., 1984). Generally speaking, particulate (sand-sized) or light fractions (< ~1.7 g/cm$^3$) are considered the primary source of plant-available N. Particulate organic matter (POM) responds rapidly to changes in soil and crop management (Gartzia-Bengoetxea et al., 2009; Six et al., 2000) whereas mineral-associated (silt and clay-sized) fractions accumulate or lose N more slowly (Llorente et al., 2010). Mineral-associated organic matter (MAOM) is an important long-term sink for nutrients, in which compounds can persist for decades or centuries (Trumbore, 2009). As such, studies on short-term N cycling tend to focus on POM or light fractions while MAOM is studied primarily for its ability to store and sequester nutrients longer-term. However, in cultivated soils, the majority of N is held within MAOM fractions due to the depletion of POM by repeated disturbance and residue removal (Denef et al., 2013). Compared to POM, MAOM is also dominated by low molecular weight, N-rich compounds and so tends to have a lower C/N ratio. Plant root inputs may stimulate the turnover and release of N from MAOM (Jilling et al., 2018), which given its low C/N ratio, could be more easily mineralized into plant-available forms. The lack of total change often observed in MAOM may be due to decomposing POM flowing into this pool and offsetting any release of C or N from MAOM (Mitchell et al., 2018). Given this emerging awareness that MAOM may be dynamic on short timescales, and that these dynamics

may affect crop growth, more research is needed to understand the behavior of N across fractions, especially in agricultural systems, where N management is so central to productivity.

The majority of research thus far has focused on how management influences POM (Porter and Griffin, 2004; Spargo et al., 2011; Willson et al., 2001). Above- and below-ground crop residues contribute directly to particulate and light fractions of SOM and their contribution is essential to building SOM (Mitchell et al., 2018). However, POM is also an initial substrate in a sequence of decomposition that eventually leads to mineral-associated SOM (Grandy and Neff, 2008; Hatton et al., 2012). Agricultural practices that increase crop residue inputs, slow decomposition, or increase the efficiency of litter conversion to SOM may increase the N within POM and MAOM fractions and, in turn, enhance the N-supplying capacity of soils.

Cover cropping is one strategy often employed specifically for its role in increasing both POM and MAOM by counteracting the removal of cash crop residues. Indeed, cover cropping can be an effective strategy to increase soil SOM stocks (Poeplau and Don, 2015) and can also help offset the negative effect of tillage on SOM (Garcia-Franco et al., 2015). While greater crop diversity is generally associated with increased soil aggregation and SOM (Tiemann et al., 2015), there is an even greater benefit with the inclusion of cover crops (McDaniel et al., 2014). The positive effect of cover crops on SOM may derive from the accumulation of SOM specifically in MAOM, which may be promoted by the efficient conversion of belowground cover crop residues into these mineral-associated pools (Austin et al., 2017; Kallenbach et al., 2016). To date, research has largely focused on soil C responses to cover crops, but much less is known about the effect on SOM N, both in terms of total quantity and distribution across fractions including MAOM.

Tillage can also influence residue decomposition rates, and strongly influence SOM stabilization processes and the distribution of C and N across SOM fractions (Grandy and Robertson, 2007). Disturbance from tillage tends to disrupt physical aggregation processes that otherwise contribute to the stabilization of SOM in more physically protected pools (Jastrow, 1996; Six et al., 2004a, 1999; Wander and Bidart, 2000). Tillage practices vary widely in terms of the extent and nature of physical disturbance they cause—i.e., the depth, intensity and frequency of tillage influences how and when residues are incorporated (Reicosky, 2015). Reduced or no-till (i.e., conservation tillage) practices can enhance residue retention, minimize physical disturbance and, in turn, slow the decline or allow the accumulation of SOM compared with conventional tillage systems, such as chisel plow (Lal and Kimble, 1997).

As with cover crop effects, short-term responses to tillage are most pronounced in POM fractions (Chan et al., 2002; Miller et al., 2019; Zotarelli et al., 2007). However, MAOM can also respond to changes in soil disturbance. In experimental plots under continuous barley for 25 years, soils under no-till had 16% more organic C and 5% more organic N than soil under chisel tillage (Plaza et al., 2013). In this case, the mineral-associated pool accounted for 65% of the difference in total organic C content. In a separate study, the heavy or mineral-associated fractions accounted for the majority of gains in soil C after 12 years of no tillage (Grandy and Robertson, 2007), however in other experiments of similar duration, most increases in C occurred in particulate fractions following cessation of tillage (Bayer et al., 2001). The effect of tillage intensity on MAOM may depend largely on time—tillage effects over MAOM do not always emerge in short-term (<10 year) experiments (Salvo et al., 2010). Likewise, the effect of tillage on MAOM may depend on the specific form of tillage being assessed.

Ridge tillage, a form of conservation tillage, establishes permanent raised beds that are rebuilt annually when soil and residues are translocated from the inter-row space to the base of plants (a process known as re-ridging; Fig. 1). Unlike the homogenizing and disruptive effect of chisel plow, ridge tillage is more conservative, allowing time for more stable aggregate structures to develop (Zhang et al., 2012). While physical disturbance does occur during re-ridging, it is restricted to the surface soil and so overall, ridge tillage is less invasive physically than chisel plow. Due to the presence of limited soil disturbance, ridge tillage is considered intermediate between conventional and no-tillage, and is hypothesized to provide the benefits of no-tillage, namely SOM accrual, while also promoting nutrient turnover in the soil surrounding plant roots (Williams et al., 2016b).

Slower aggregate turnover facilitates the formation and protection of macro-aggregate structures that protect SOM from decomposition and provide opportunities for organo-mineral associations to develop (Six et al., 2004b). However, aggregate turnover can also lead to SOM formation and stabilization (Plante and McGill, 2002; Virto et al., 2010), suggesting that other mechanisms beyond aggregate protection also drive SOM formation. Microbial necromass and by-products are significant constituents of MAOM and SOM more broadly (Kallenbach et al., 2016; Miltner et al., 2012). Management that influences microbial activity and turnover, therefore, such as the quality of residue inputs or the increased presence of plant roots, may also influence the rate of MAOM formation.

The objective of this study was to investigate how soil and crop management affected the distribution of N across SOM fractions and, further, how these changes corresponded to potential N mineralization and crop performance. We examined how cover crops and tillage practices

influenced SOM dynamics within field experiments replicated across three growing sites located in north-Central and mid-Atlantic US.

## Materials and Methods

### *Site description and experimental design*

Field experiments were established in 2011 across three sites located in north-Central and mid-Atlantic United States (Table 1). The three sites are comparable in average precipitation and temperature, but differ more significantly in soil texture and C concentration. The IL and PA soils are dominated by silt-sized mineral particles and the MI soil by sand-sized particles. The C and N concentration of the IL soil is approximately two to three times that of MI and PA soils, respectively. We refer here to IL soil as high-SOM and MI and PA soil as low-SOM soils.

Field treatments were applied in a randomized complete block factorial design with four blocks. Each block contained four chisel plow plots and four ridge tillage plots. Within the four plots for each tillage system, two plots were planted with maize (*Zea Mays* L.) and two with soybean (*Glycine max* (L.) Merr.) then maintained in an annual rotation. Of the two plots planted with the same crop, one plot was planted with winter rye (*Secale cereale* L.) after crop harvest and another was left fallow. Cover crops were planted in the third week of October at all sites. Each plot was further split to create a fertilized and non-fertilized subplot. For more specific details on the tillage treatments, please refer to Williams et al. (2016a).

*Soil sampling*

We sampled in June of 2014 (3 years after experimental plots were established), about 10 days after re-ridging was completed in ridge till plots. We sampled from the tillage and cover crop plots that were unfertilized and in the corn phase of rotation. Corn plants were at or near growth stage V6, an exponential stage of growth and one of high crop N demand. We chose to sample in unfertilized subplots to isolate the effects of tillage and cover crops on the distribution among fractions of N derived from organic sources. In total, we collected four field replicates per treatment at each site.

We used 1.9-cm diameter cores to collect and composite 10 soil cores from each experimental plot. Soils were sampled to a 5-cm depth. We chose this sampling depth because our interest after only three years was not in profile N accumulation, *per se*, but shifts in surface soil N dynamics. These are relevant to soil health and agronomic performance, as well as highlight potential mechanisms that underlie longer-term SOM responses to management (Franzluebbers, 2002; Grandy and Robertson, 2007). Moreover, the depth of soil displacement through re-ridging is generally restricted to the top ~3-5 cm (Williams et al., 2016a) and previous work has shown the positive effects of ridge tillage on soil N are concentrated at the 0-5 cm depth (Kane et al., 2015). A subset of soils was processed immediately for potentially mineralizable nitrogen. Remaining soil was air-dried and stored until further analysis.

*CEC, texture, and N analyses*

Cation exchange capacity (CEC) was estimated through summation of the quantities of $Ca^{2+}$, $Mg^{2+}$, and $K^{+}$ extracted with a Mehlich 3 solution (Mehlich, 1984). Texture was determined by

the hydrometer method (Gee and Bauder, 1986). Soil fraction organic N concentrations were determined on an elemental analyzer (Costech ECS 4010).

*SOM fractionation*

To separate organic matter fractions, we used a particle size-based technique, combining sonication with wet-sieving and centrifugation (Fig. 2). No chemical dispersants or density agents were used with this method. Ultrasonication was used to disperse the soils in a step-wise fashion. Soils were first dispersed gently in water (10 ± 0.1 g in 50 mL $H_2O$) in order to release the free particulate organic matter (f-POM) (Cerli et al., 2012). Floating particulates were recovered with suction and cleaned on a 53 μm sieve. The remaining soil and water suspension was centrifuged at 10,000 x g for 35 minutes. The centrifuge speed and duration were determined according to Stoke's Law (Elliott and Cambardella, 1991). The supernatant was discarded and the pellet combined with the remaining soil sample. The soil and water suspension received an initial low energy sonication of 60 joules/mL to release occluded particulates. The soil and water suspension were then passed through a 53 μm sieve to recover the occluded-POM fraction (o-POM).

The suspension recovered below the 53 μm sieve was centrifuged, the supernatant discarded and the pellet resuspended for the subsequent sonication step. The second sonication step was optimized for each soil type as we expected soils to respond variably to sonication, depending on the specific nature of the clays and organics that bind aggregates, floccules and particles together (Kaiser and Berhe, 2014). Excessive dispersion can lead to fragmentation of organics or other transformations that may alter the chemistry of recovered fractions. In order to identify the sonication energy that would maximize dispersion of the fine fractions, we tested

increasing levels of sonication intensity and used the relative increase in MAOM recovered to determine the optimal and final sonication level. We selected the following sonication levels for each soil type based on optimization tests: 210 joules/mL for both IL and PA and no additional sonication for MI. Following this second sonication step, the suspension was passed through a 20 μm sieve; material recovered on the sieve we termed the coarse silt fraction (c-Silt). Material less than 20 μm included fine silt and clay-associated organic matter and we refer to here as MAOM. f-POM, oPOM and c-Silt fractions were dried at 60º C, and MAOM was dried at 105º C. All fractions were ground and stored until further analysis.

*Soil N availability and crop performance*

Soil inorganic N, potentially mineralizable nitrogen (PMN) and plant tissue N were analyzed as described in Kane et al., (2015). Potentially mineralizable nitrogen was assessed with a 7-day anaerobic incubation following the methods of Drinkwater et al. (1996) and Waring and Bremner (1964).

Leaf chlorophyll content was measured at growth stage V6 with a SPAD meter. Maize was harvested at full physiological grain maturity, designated by the development of a black abscission layer at the base of kernels. Within two, 3 m-long rows in each plot, all maize ears were hand harvested. Kernels were mechanically separated from cobs, and fresh grain mass determined. Grain was then dried to constant mass in a forced air oven, and dry mass determined. To determine yield, grain weights were measured on a 6 plant subsample then scaled up to a hectare basis. Maize yields were expressed in kg dry mass $ha^{-1}$ at 15.5% moisture content.

To determine plant N uptake and partitioning, six plants were randomly selected in each plot, harvested and separated into three fractions: grain, reproductive tissues (cob, silks, husk, tassel), and vegetative tissues (stem, leaves). Fractions from all six plants were then combined, processed, and weighed. Each sample was dried in a forced air oven at 60°C for 7 d. Once dried, samples were reweighed to quantify the biomass of each fraction and ground to pass through a 1-mm sieve. Tissue N concentrations were determined by dry combustion of the samples using a Costech ECS 4010. To calculate the average per-plant mass N within each plant fraction, dry biomass was multiplied by the N concentrations and divided by the number of plants in each.

*Statistical Analyses*

To assess the effect of management on SOM fraction properties, we used two-way linear mixed effects models (package *nlme* in R version 3.3.2) with tillage and cover coded as fixed effects. Initial three-way mixed effects models included location as a fixed effect. Results indicated significant location by management treatment interactions across the majority of response variables. As such, we ran separate mixed effects models within each location with block coded as a random factor. Within-group variances due to soil texture were modelled with a power or exponential variance function using varPower or varExp using the *nlme* package. Mixed effect models and ANOVA were performed using R.

We examined the relationships between SOM fraction variables, soil N availability, and crop performance measures using both univariate and multivariate approaches. As we were interested in the broad-scale patterns, irrespective of treatment or site effects, we analyzed all sites together. Partial least squares regression (PLSR) was selected for a more holistic analysis to

examine the potential correlations between soil properties, N availability and crop performance. PLSR is similar to multiple regression analysis, but can tolerate multicollinearity and violations of homogeneity (Carrascal et al., 2009). PLSR generates a model that maximally explains the variation in predictor and response variables. A full model was initially run, with SOM fraction variables (SOM fraction N content and distribution), CEC, pH, and texture as predictor variables and crop-N content, yield, potentially mineralizable N, KCl-extractable $NH_4^+$ and $NO_3^-$ as response variables (Appendix Table 1.2). Data were centered and scaled and the number of latent factors chosen based on the predictive residual sum of squares (PRESS). A K-fold cross-validation and nonlinear iterative partial least squares (NIPALS) method were applied. The model was reduced using only the variables with variable of importance for projection (VIP) threshold scores greater than 0.8. PLSR analysis was performed in JMP Pro Version 14.0.

**Results**

Total mass recovery was on average 93%, 97%, and 96% of the pre-fractionated sample mass for IL, MI, and PA, respectively. Due to the varied degree of mass recovery between soil types, all fraction properties were calculated with the total recovered mass or N, rather than with the mass of the original sample.

*SOM fraction mass proportion*

SOM fraction mass proportion at the IL and PA sites was dominated by MAOM (>60% of total recovered mass for both; Fig. 3a). In contrast, o-POM constituted around 65% of total recovered mass in MI. Although f-POM comprised less than 1% of total recovered soil mass, there was still

a detectable management effect. In MI and PA, there was a significant increase in f-POM mass proportion under ridge tillage, irrespective of cover crop treatments (Fig. 3b). In IL, ridge tillage increased f-POM proportion when coupled with the cover crop treatment. While there were no treatment effects on o-POM in MI, there was a significant management effect in PA, where cover cropping caused a 20% increase in o-POM mass proportion (Fig. 3c). In IL, there was a cover by tillage interaction on o-POM. Coarse silt was significantly altered by management only within IL, where cover crops decreased the mass proportion by 16.7% (Fig. 3d). There was also a significant effect of tillage on c-Silt in IL. Cover cropping caused a 5.5% decrease in MAOM mass proportion in PA (Fig. 3e).

*Nitrogen distribution and content in SOM fractions*

Tillage and cover cropping influenced the distribution of N across all SOM fractions, however responses were site-specific and varied when expressed as content (mg N $g_{soil}^{-1}$) or distribution (% of total soil N). Out of total recovered N, the majority of N was concentrated in MAOM fractions; when averaged across management treatments, 78.9, 62.6, and 74.7% of total recovered N was stored within MAOM in IL, MI, and PA soils, respectively (Fig. 4a). A smaller, but still significant portion of total N resided in o-POM fractions: 15, 25.7, and 18.9% in IL, MI, and PA, respectively.

Across all treatment and soil type combinations, the mean N content of POM fractions ranged from 0.02 – 0.06 mg N $g_{soil}^{-1}$ in f-POM and 0.15 – 0.47 mg N $g_{soil}^{-1}$ in o-POM. In the MI and PA soils, ridge tillage and cover cropping increased N within POM fractions. Specifically, ridge tillage increased fPOM-N content compared to chisel plough (Fig. 4g). For o-POM N content, there was a main effect of tillage and cover in MI and PA: cover cropping caused a

22.4% and 8.6 % increase and ridge tillage a 75.7% and 23.8% increase in MI and PA, respectively (Fig. 4h).

Mean N content ranged from 0.05 – 0.13 mg N $g_{soil}^{-1}$ in c-Silt and 0.52 – 2.19 mg N $g_{soil}^{-1}$ in MAOM fractions. The tillage effect was also significant in c-Silt-N and MAOM-N, with ridge tillage increasing c-Silt-N by 22.1% and 20.8% (Fig. 4i) and MAOM-N by 7.5% and 10.1% (Fig. 4j) in MI and PA, respectively. In contrast, in the high-OM soil at the IL site, POM and fine fractions were less responsive to tillage. Rather, the inclusion of cover crops led to increases in N content, especially within the fine fractions (c-Silt and MAOM). Including a cover crop increased c-Silt N by 16.8% and MAOM N by 23.7%.

*Relationships between soil properties and N availability/crop performance*

Management effects on soil N availability and crop performance are provided in Appendix Table 1.1. These measures were not the focus of this study, but rather were used as correlates in a PLSR analysis. An initial PLSR model included 14 soil variables as predictors (Appendix Table 1.2) and explained 87.2% of the variation in predictor variables and 49.3% of the variation in N availability and crop performance. A reduced model was fit using only the variables with a Variable Importance of Projection (VIP) > 0.8 and explained 92.4% and 48.8% of the variation in predictor and response variables, respectively. The correlation loading plot summarizes the relative direction and magnitude of the correlation between predictor and response variables along the first two factors (Fig. 5). Of the SOM fraction variables, oPOM-N, c-Silt-N, and MAOM-N were positively correlated with PMN along both factors 1 and 2 as indicated by their close proximity in the correlation loading plot. MAOM-N relativized to total N (MAOM-N as a percentage of total soil N) and silt content were positively correlated with yield while clay

content was associated most closely with SPAD measurements (chlorophyll content) along both factors. The majority of the variables associated with the particulate fraction, including sand content, were negatively associated with PMN, yield, and SPAD, but most positively associated with KCl-extractable $NH_4^+$, reproductive tissue-N and grain-N.

We observed similar patterns in the univariate correlations between SOM fraction N content and measures of soil N availability and crop performance (Appendix Fig. 1.1). Spearman correlation analyses conducted across sites indicate that soil texture was significantly associated with fraction properties. Coarse silt and MAOM fraction N content and distribution were positively associated with the respective soil texture variable: e.g., MAOM N content was positively correlated with clay content (Spearman's rho = 0.50; $P < 0.001$) and coarse silt N content with silt content (Spearman's rho = 0.43; $P < 0.01$). The same pattern in correlation was observed with MAOM and coarse silt N distribution. Unlike the fine fractions, fPOM and oPOM N content were not associated with sand content. However, we did observe positive correlations with respect to fPOM N (Spearman's rho = 0.45; $P < 0.01$) and oPOM N distribution (Spearman's rho = 0.64; $P < 0.001$). MAOM N content, when relativized to total N, was negatively associated with KCl-extractable $NH_4^+$ (Spearman's rho = -0.38; $P < 0.01$) and positively associated with PMN (Spearman's rho = 0.35; $P < 0.05$), yield (Spearman's rho = 0.55 ; $P < 0.001$), and SPAD measurements at the V6 stage of crop growth (Spearman's rho = 0.44; $P < 0.01$). In contrast, o-POMN relativized to total N was negatively correlated with PMN (Spearman's rho = -0.30 ; $P < 0.05$), yield (Spearman's rho = -0.47; $P < 0.001$) and SPAD measurements (Spearman's rho = -0.41; $P < 0.01$).

**Discussion**

*Mass and N distribution across SOM fractions*

The relative mass distribution of SOM fractions was largely driven by particle size distribution. The IL and PA sites are dominated by fine-grained minerals, with sand comprising less than 15% of total texture. These soils have high proportions of silt and clay particles that can form strong bonds with organic compounds, and so contained the majority of mass within the MAOM fraction. In contrast, the MI soil has a sandy loam texture with most of the recovered mass distributed in the occluded POM and c-Silt fractions.

The high proportion of sand in the MI soil likely influenced this soil's relatively low N content. Sandy soils are often limited in their capacity to build SOM (Plante et al., 2006) due to limited capacity to protect C long-term by various mechanisms such as aggregation, direct mineral surface interactions or incorporation into outer-sphere SOM complexes. However, given the large size of the o-POM fraction in the MI soil, when expressed as a proportion of total soil N or on a whole soil basis (N content), o-POM fraction N was comparable to the other soils. While the IL and PA soils were quite similar in their overall mass and N distribution of fractions, there were clear differences in how management influenced the distribution and content of N across SOM fractions. Soil texture does not uniformly limit how SOM fractions accrue N or respond to soil and crop management.

*Management effect on SOM fraction C and N*

Our results demonstrate the potential for both conservation tillage and cover cropping to increase N at 0-5 cm depth across all measured SOM fractions in a relatively short time-span—in this case, after three years of management. The POM and MAOM fractions responded distinctly to

management with divergent patterns emerging also based on site. In the two low-SOM sites (MI and PA) the increase in total organic N was due primarily to changes in POM-N; ridge tillage caused a pronounced increase in POM-N, which aligns with other studies on reduced or no-till in SOM-depleted soils (Franzluebbers, 2002; Motta et al., 2007; Wander et al., 1998). POM fractions are often early indicators of management changes (van Wesemael et al., 2019), especially those related to physical soil disturbance (Chan et al., 2002) or that impact residue input quality, quantity, and its decomposition rate (Chivenge et al., 2007). A previous study showed a single tillage event can have dramatic effects on aggregation and SOM decomposition in a sandy soil, similar in soil type and texture to the MI site studied here (Grandy and Robertson, 2007).

Compared to chisel plow, ridge tillage limits physical disturbance throughout and between the growing seasons. Ridge tillage establishes permanent raised beds, which are rebuilt annually when soil and residues are translocated from the inter-row space to the base of plants, causing a spatial zonation in the soil's physical structure (Williams et al., 2016b) and a concentration of nutrients near the plant roots (Kane et al., 2015; Williams et al., 2016b). In SOM-depleted soils, ridge tillage may provide opportunities to replenish POM pools, which although transient, can serve as important initial substrates in a decomposition sequence that eventually leads to aggregate-protected and/or mineral-associated organic matter (Hatton et al., 2012).

The high-OM soil in IL showed negligible response to either tillage or cover cropping in POM fractions. Instead, the management effect was strongest in fine fractions, indicating this pool, often considered resistant to short-term change, can accrue N in a relatively short time-span. Specifically, cover cropping caused a significant increase in N within c-Silt and MAOM

while tillage had no effect. The positive effect of cover cropping on fine fractions may be due in part to soil mineralogy; the high proportion of reactive silt and clay particles in IL was likely conducive to the formation of mineral-organic associations (Hemingway et al., 2019). Specifically, the presence of reactive mineral surfaces in combination with cover crop inputs may have contributed to the observed positive effect on MAOM N content.

It is also possible that in IL, the high-SOM soil, the accrual of stable SOM depends less on the quantity of litter inputs but on the quality, diversity, and/or extent of physical interaction between roots and soil. Cover crops increase the time period in which plant roots interact with the soil environment (Tiemann et al., 2015). Root inputs are known to contribute to stable SOM pools, often more-so than aboveground residues (Kong and Six, 2010; Lian et al., 2016). They deliver an additional source of root litter and exudates, providing greater diversity in belowground inputs (Austin et al., 2017). This enhanced interaction of root inputs may support microbial growth and turnover in rhizosphere hotspots, processes that can enhance the formation of stable and mineral-associated organic matter (Kallenbach et al., 2016). Thus, the additional inputs from cover crop roots may have facilitated the accrual of MAOM.

Overall, the effect of tillage on fine fraction N was most pronounced in the low-SOM soils in MI and PA. The soil series at these sites are characterized by more weathered and less reactive clays (Cremeens and Mokma, 2010; Jackson, 1959) in comparison to the dominant soil series in the high-SOM soil (Mohanram et al., 2010). The low-SOM soils may thus be limited in their ability to form macro-aggregates that would otherwise promote SOM decomposition, microbial turnover and the formation of more stable forms of SOM in fine fractions (Jastrow, 1996). In a previous study conducted on fertilized sub-plots within the same experiment, the low-SOM sites, MI and PA, sites had lower permanganate-oxidizable carbon (POX-C) within macro-

aggregates compared to the IL site (Williams et al., 2017). Additionally, chisel plow caused a significant decrease in macro-aggregate POX-C relative to ridge tillage (Williams et al., 2017). POX-C represents a more processed form of C and is associated with more stable fractions of POM (Culman et al., 2012; Hurisso et al., 2016). Ridge tillage may have provided enhanced opportunities for POM accrual (Cambardella and Elliott, 1994), thus allowing these more stable forms of C to accumulate in the low-SOM sites.

*Comment on soil sampling depth and fractionation technique*

The response to short-term management across all SOM fractions was likely accentuated due to the depth of sampling. The largest treatment differences are often seen at this shallow depth (Chan et al., 2002) and changes in SOM fractions, even MAOM, are expected to be pronounced at 0-5cm depths (Duval et al., 2016). We would expect that treatment effects would attenuate with depth. Here, we chose to sample to -5cm depth as this represents a critical zone of nutrient accumulation and turnover and is a zone of high microbial activity (Purnomo et al., 2000; Woods, 1989).

We believe our fractionation method successfully isolated functionally distinct components of SOM as evidenced by the distinct responses of fractions to management. However, in terms of the overall patterns and for conceptual purposes, the f-POM and o-POM fractions could be grouped into one unified POM fraction due to similarities in management effects. In our approach, f-POM is a minor constituent compared to the proportion typically isolated with other methods. It is likely that the o-POM fraction we recovered included what other methods would define as f-POM, especially those that include a density agent (Ludwig et al., 2015). In our case, the purpose of isolating f-POM prior to sonication was to minimize the

release of dissolved N from coarse litter fragments (Wagai et al., 2009). Using a density agent like sodium polytungstate to isolate f-POM is less effective than particle size-based methods in separating a meaningful active fraction with distinct turnover rates (Poeplau et al., 2018).

The c-Silt fraction was isolated as suggested by Kaiser and Berhe (2014) as this fraction can be dominated by weathering-resistant primary minerals (Bayer et al., 2006; O'Brien et al., 2013) that could dilute dynamics associated with the more reactive fine silt and clay particles. In the case of MI, isolating this fraction was beneficial as it clearly responded uniquely to management compared to MAOM fractions. In isolating c-Silt we also believe it is advisable to conduct preliminary optimization of sonication levels. Excessive sonication can increase the potential for POM destruction, MAOM mobilization and SOM redistribution, possibly altering the N and C dynamics within pools (Kaiser and Berhe, 2014). Thus, rather than using the same standard high-energy dispersion for all soils (e.g., 450 Joules/mL), we recommend that sonication be optimized to consistently separate different soils into fractions while minimizing SOM alterations and redistribution.

*Synthesis: Correlations between SOM fraction properties, soil N availability and crop performance*

Our exploration of associations between SOM fraction properties, soil N availability and crop performance aggregated all soil data and employed a statistical method well-suited for preliminary exploration of data. The results of the PLSR analysis are correlative and thus should be interpreted with caution. We found that fine fraction variables were associated with nutrient provisioning to plants while the coarse fractions and POM were not consistently associated with measures of N availability or crop performance. Along with oPOM-N, many of the variables

associated with the fine fractions, including silt, clay, c-Silt-N and MAOM-N, were positively associated with PMN and yield. In contrast, variables associated with the POM fractions—namely, fPOM-N, both f-POM N and oPOM-N relativized to total N, and sand content—were negatively associated with yield and SPAD.

The association between silt and clay fractions of SOM, plant available N, and crop performance may have been driven indirectly by clay and total N. That is, soils with a higher proportion of silt and clay will likely store more SOM and in turn, support greater crop productivity. However, consistent with findings from other agricultural soils, in the soils we studied MAOM contained the majority of total soil N. The divergent patterns in association between fraction N content, when relativized to total soil N, and PMN or yield suggest that some portion of the fine fraction could be an active source of bioavailable N. Several studies point to this dynamic role of MAOM as both an important pool for the long-term stabilization of N and C, but also a potential supplier of bioavailable nutrients (Jilling et al. 2018). Indeed, while POM is often most sensitive to management, it is not always a good predictor of crop N uptake (Schwenke et al., 2002). Osterholz et al. (2017) found that non-POM C content, which could be interpreted as mineral-associated C, was strongly correlated with gross N mineralization. Similarly, C and N content of dense fraction SOM, also analogous to MAOM, was positively associated with gross N mineralization in a grassland ecosystem (Fornara et al., 2011).

We understand the limitations of our correlative approach and a single yield measurement. Yet taken together with these and other studies, the relationships suggest that there is a need to better understand the SOM pools that, in many systems, can supply crops over half of their annual N uptake (Gardner and Drinkwater, 2016). These results also highlight a need to examine directly how soil and crop management interacts with soil type to influence the potential

behavior of SOM pools. Certain soil types may be well-suited for the specific management of MAOM, but it is important this is not achieved at the expense of SOM accumulation over the long-term.

*Conclusion*

This study demonstrates how tillage and cover cropping can lead to rapid (i.e., within 3 years) changes in N across all SOM fractions, particulate and mineral-associated. In particular, our results show that MAOM is a large and dynamic sink that can rapidly accumulate N. The specific effect of management on MAOM was similar in the two low-SOM soils, where both reduced tillage and cover cropping significantly increased N within MAOM. In the high-SOM soil, cover cropping increased MAOM N while tillage had no effect. We also found in exploratory analyses that particulate and fine fractions may be positively associated with select measures of N availability and crop performance. Building a more N-rich MAOM pool, such as by providing higher quality residues with cover cropping and supporting and protecting the microbial processing of SOM via conservative tillage, may potentially increase the N-supplying capacity and productivity of agricultural soils long-term. More research is needed to understand the potential contribution of MAOM as an N source as it has typically been studied for its role as long-term reservoir for nutrients.

**Table 1.1**. Site and soil characteristics. Mean Annual Precipitation (MAP) and Mean Annual Temperature (MAT) figures are 30-year growing season means (April–October in IL; May-October for MI and PA)

| **Site ID** | **Location** | **MAP** (cm) | **MAT** (°C) | **Soil Taxonomy** (Series; Classification) | **Texture** | | | **pH** | **CEC** (cmol $kg^{-1}$) | **C Conc.** (mgC $g^{-1}$soil) | **C/N** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Sand (%) | Silt (%) | Clay (%) | | | | |
| IL | Champaign, IL (40º 3', –88º 15') | 61.6 | 18.3 | Drummer; Typic Endoaquoll | 14.0 | 65.0 | 21.0 | 6.2 | 21.4 | 30.5 | 12.0 |
| MI | Mason, Michigan (42º 24', –85º 24') | 48.0 | 17.3 | Marlette; Oxyaquic Glossudalf | 59.4 | 33.2 | 7.4 | 5.8 | 8.54 | 10.1 | 11.1 |
| PA | Rock Spring, Pennsylvania (40º 47', –77º 51') | 55.0 | 17.9 | Hagerstown; Typic Hapludalf | 12.0 | 49.7 | 38.3 | 6.0 | 11.4 | 13.8 | 8.6 |

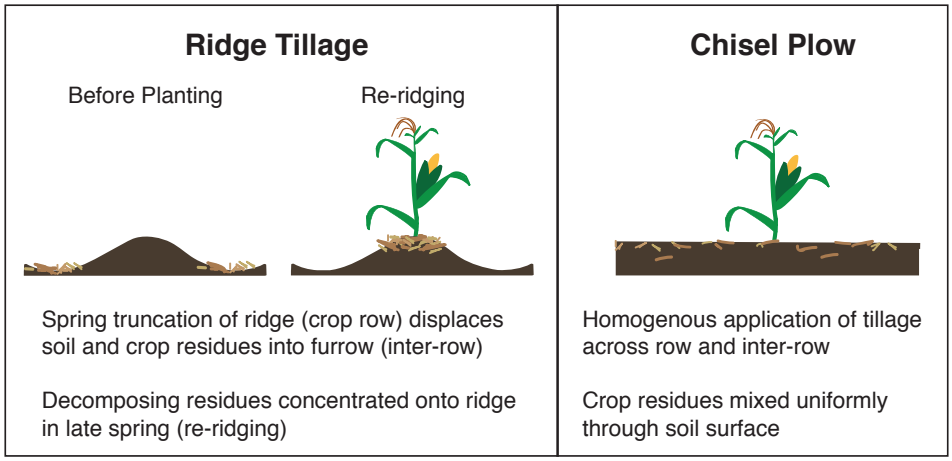


**Figure 1.1**. Illustration of key differences between ridge tillage and chisel plow (adapted from Williams et al., 2016)

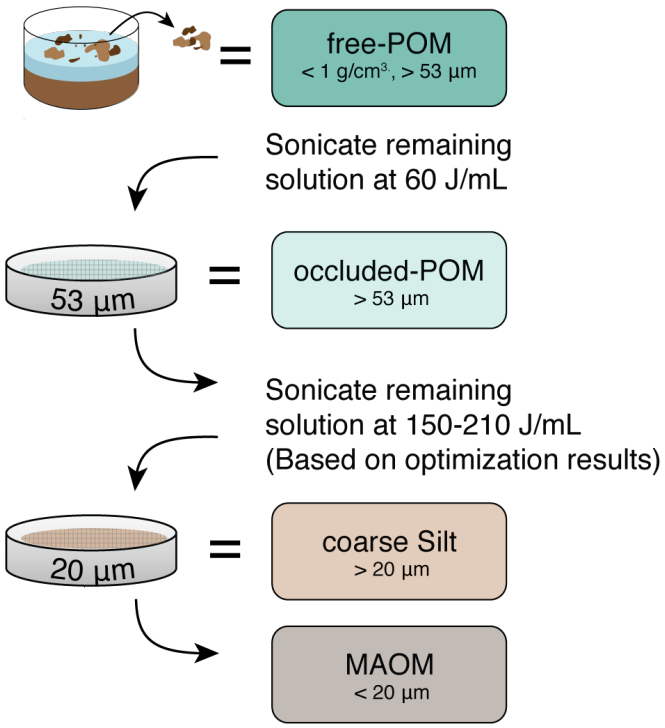


**Figure 1.2.** Fractionation scheme

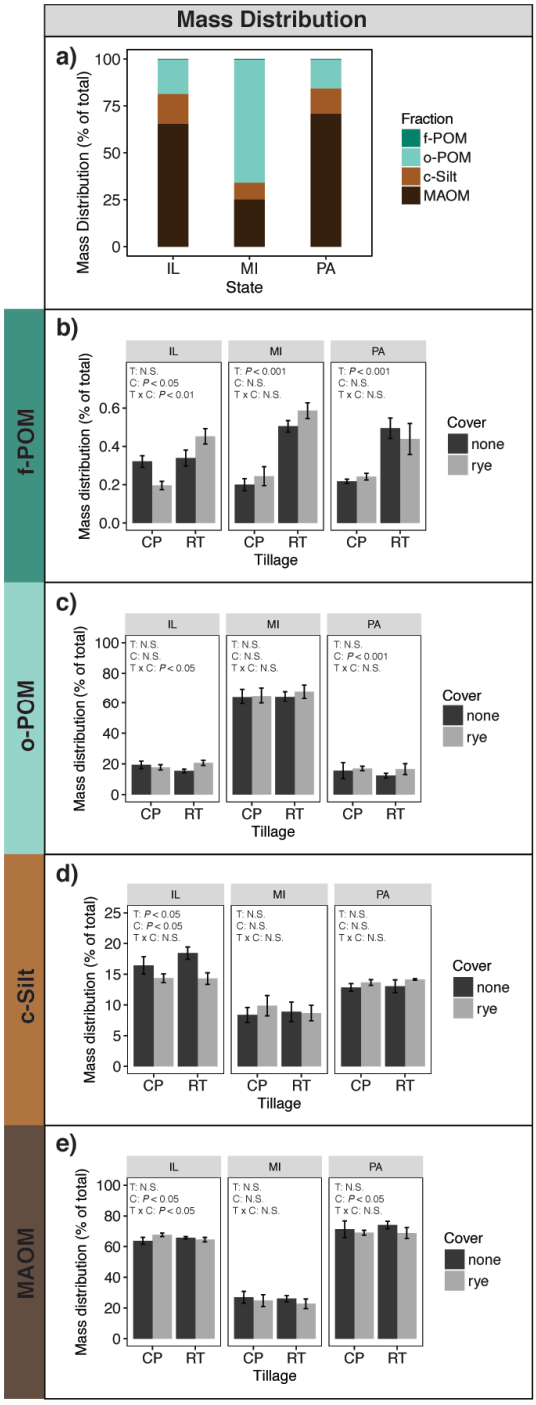


**Figure 1.3**. Mass distribution across SOM fractions. Top panel for distribution (a) shows averages within site. Specific treatment effects shown in the four color-coded panels. Error bars indicate standard error. ANOVA results for variables provided within the respective panel.

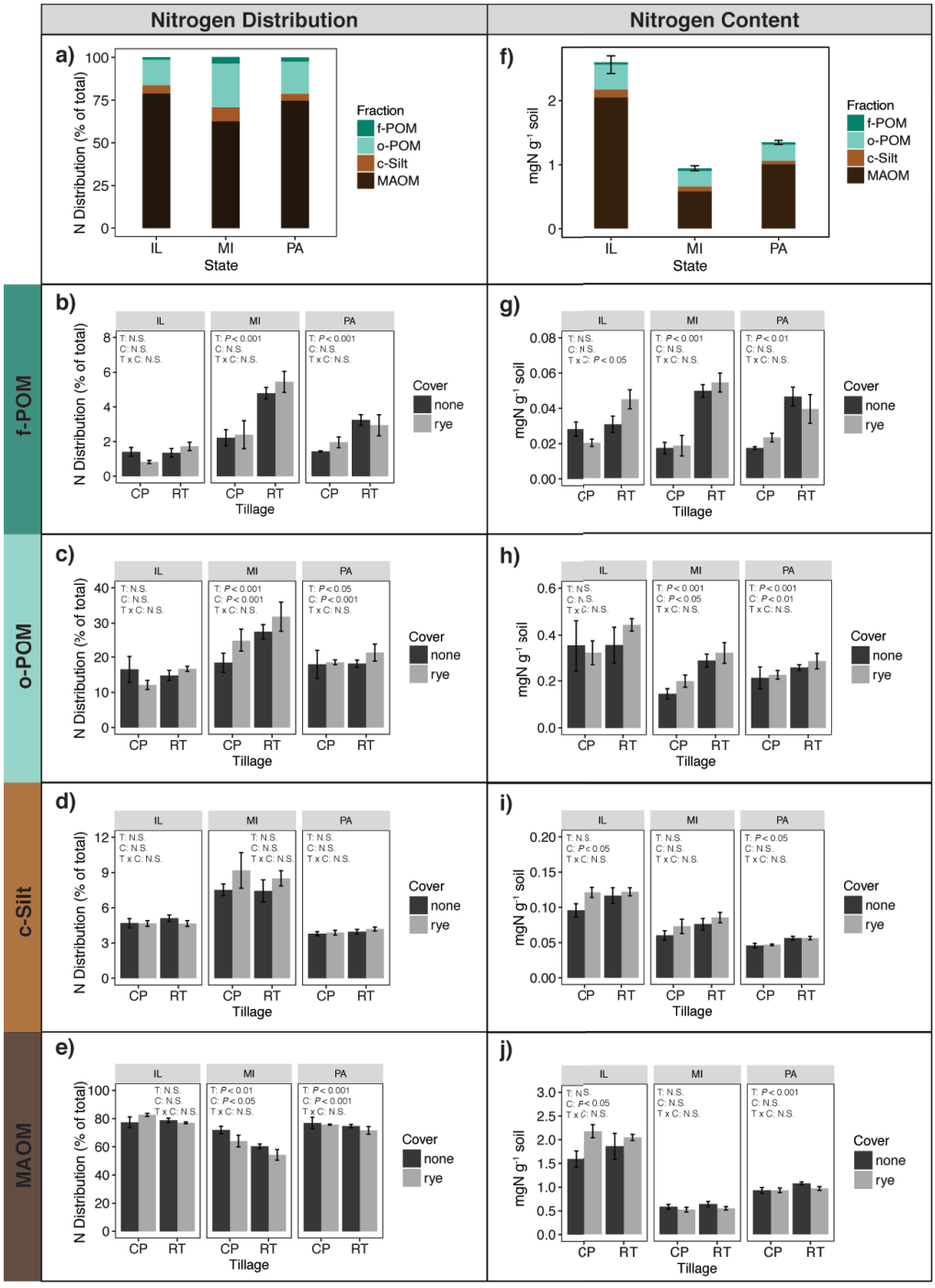


**Figure 1.4**. Nitrogen distribution (a–e) and content (f–j) across SOM fractions. Top panel for distribution (a) and content (f) shows averages within site. Specific treatment effects shown in the four color-coded panels. Error bars indicate standard error. In Fig. 4f, error bars indicate standard error for total N. ANOVA results for variables provided within the respective panel.

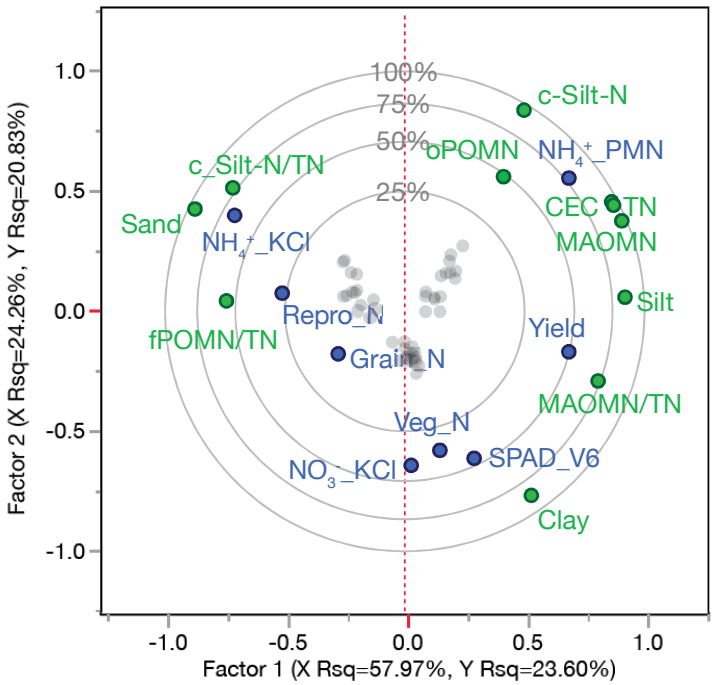


**Figure 1.5**. Partial least squares regression correlation loading plot for the final reduced model. Predictor variables (in green) include f-POM N content (fPOMN), o-POM N content (oPOMN), c-Silt N content (cSilt-N), MAOM N content (MAOMN), f-POM N content relativized to total N content (fPOMN/TN), o-POM N content relativized to total N content (oPOMN/TN), c-Silt N content relativized to total N content (cSilt-N/TN), MAOM N content relativized to total N content (MAOMN/TN), and texture variables (Sand, Silt, and Clay). Response variables (in blue) include potentially mineralizable N ($NH_4^+$_PMN), KCl-extractable ammonium ($NH_4^+$_KCl), KCl-extractable nitrate ($NO_3^-$_KCl), reproductive tissue N concentration (Repro_N), grain tissue N concentration (Grain_N), vegetative tissue N concentration (Veg_N), SPAD measurements at V6 growth stage (SPAD_V6) and Yield.

# CHAPTER 2

## MINERALS IN THE RHIZOSPHERE: OVERLOOKED MEDIATORS OF SOIL NITROGEN AVAILABILITY TO PLANTS AND MICROBES

**Abstract**

Despite decades of research progress, ecologists are still debating which pools and fluxes provide nitrogen (N) to plants and soil microbes across different ecosystems. Depolymerization of soil organic N is recognized as the rate-limiting step in the production of bioavailable N, and it is generally assumed that detrital N is the main source. However, in many mineral soils, detrital polymers constitute a minor fraction of total soil organic N. The majority of organic N is associated with clay-sized particles where physicochemical interactions may limit the accessibility of N-containing compounds. Although mineral-associated organic matter (MAOM) has historically been considered a critical, but relatively passive, reservoir of soil N, a growing body of research now points to the dynamic nature of mineral-organic associations and their potential for destabilization. Here we synthesize evidence from biogeoscience and soil ecology to demonstrate how MAOM is an important, yet overlooked, mediator of bioavailable N, especially in the rhizosphere. We highlight several biochemical strategies that enable plants and microbes to disrupt mineral-organic interactions and access MAOM. In particular, root-deposited low-molecular-weight exudates may enhance the mobilization and solubilization of MAOM, increasing its bioavailability. However, the competitive balance between the possible fates of N

monomers—bound to mineral surfaces vs. dissolved and available for assimilation—will depend on the specific interaction between mineral properties, soil solution, mineral-bound organic matter, and microbes. Building off our emerging understanding of MAOM as a source of bioavailable N, we propose a revision of the Schimel and Bennett (2004) model (which emphasizes N depolymerization), by incorporating MAOM as a potential proximal mediator of bioavailable N.

## Introduction

Nitrogen (N) bioavailability limits plant productivity in most ecosystems. However, it remains challenging to predict bioavailable N dynamics, particularly in mineral soils, because the specific origins and ultimate fate of N mobilized from soil organic matter (SOM) are unclear (Niu et al., 2016). Currently, the enzymatic depolymerization of SOM to monomers (e.g. from proteins to amino acids) is thought to be the key rate-limiting step in soil N mineralization and cycling (Schimel and Bennett, 2004). Both microbes and plants can use the N-containing monomers generated by depolymerization (Bardgett et al., 2003; Roberts and Jones, 2012), and the resulting competition for monomers can have cascading effects on N mineralization and bioavailability. Thus, recent research has focused on the mineralization path that N takes—from polymeric to monomeric and finally to inorganic N forms—and how these pools and processes influence N bioavailability or accessibility (Darrouzet-Nardi and Weintraub, 2014; Mooshammer et al., 2014a).

The focus on pathways of N mineralization has emphasized N release from plant litter; by contrast, there has been limited focus on other sources of bioavailable organic N that may also be important in mineral soils. Moreover, to understand N dynamics in mineral soils (the main

rooting zone in most systems) we must also consider live roots and their localized effects on microbial and nutrient dynamics. These plant-soil interactions are not captured by most laboratory measures of soil N availability, which usually rely on soils that have live roots cut off during sampling (Hart et al., 1994), and any remaining roots are often removed by sieving. This has led to uncertainties in our understanding of the variability in soil N pools, availability of N to organisms, and plant-microbe competition. For example, in many agricultural soils laboratory measures of SOM depolymerization and mineralization correlate only weakly with plant N uptake or yield (Luce et al., 2011). Field based measurements of net N mineralization also poorly predict plant N availability (Abril et al., 2001) and uptake (Monaco et al., 2010). Such results often lead to fertilizer overapplication in agricultural systems (Liu et al., 2010) and then to N losses from leaching and denitrification. While gross rates of mineralization are higher and may better correlate with plant N uptake, they are rarely measured at the temporal and spatial scales that are needed to resolve the complex processes that regulate bioavailable N (Osterholz et al., 2017a). In addition, many natural ecosystems exhibit a "missing sink" phenomenon wherein soils store more N than can be accounted for by N budgeting (Bernal et al., 2012; van Groenigen et al., 2015; Yanai et al., 2013). This suggests there are other pathways of N storage and production that are not adequately represented by standard laboratory measures of N availability. Such discrepancies can make it challenging to predict how SOM cycling will respond to environmental stressors such as climate change or N deposition.

Current conceptual models do not fully account for the pools and processes that supply bioavailable N. Given the emphasis on mineralization from plant litter and microbial detritus, conventional perspectives on SOM maintain that SOM pools that are not physically protected—defined operationally based on particle size or density—are the primary source of plant-available

N. Such pools consist of partially decomposed plant material and are referred to as "light fraction" or "particulate" organic matter pools (Gosling et al., 2013; Haynes, 2005; Wander, 2004). However, these particulate organic matter (POM) fractions, which are notoriously vulnerable to disturbance (e.g., tillage) (Grandy and Robertson, 2007), store only a small proportion of total organic N in some soils (Table 1). In contrast, mineral-associated organic matter (MAOM) fractions, which are defined based on particle size (< 53 μm) and/or density (>~1.7 g $cm^{-3}$), often hold an order of magnitude or more total N than POM fractions.

MAOM stores a lot of N, but has a longer turnover time than POM fractions; as a result, it has historically been considered inaccessible to microbes and plants (Denef et al., 2013; Fabrizzi et al., 2003; Paul, 2016). Yet, certain attributes of MAOM could make it a substantial N source. MAOM is enriched in low-molecular-weight plant compounds (Haddix et al., 2016) and microbial byproducts (Kopittke et al., 2018; Miltner et al., 2012; Schmidt et al., 2011). MAOM thus possesses a low C/N ratio (Sollins et al., 2006), which generally promotes N mineralization (Sollins et al., 1984; Whalen et al., 2000); this is in contrast to POM, which often has a relatively high C/N and thus, early in its decomposition, acts as a sink for N (Fornara et al., 2011; Luce et al., 2011; Whalen et al., 2000). Other recent discoveries also collectively point to the mineralization potential of MAOM (Table 2), which is not uniform; for example, it includes a range of N-rich molecules whose turnover times vary depending on their molecular structure and interaction with different minerals and microbial communities (see sections below). Finally, changes in pH or soil solution composition can cause organic matter to be mobilized off mineral surfaces, or alternatively to be sorbed onto them (Avena and Koopal, 1998; Rashad et al., 2010; Singh et al., 2016). Such variability points to MAOM's potential to serve as an active N source and sink.

Taking into account the pool size, composition, and mineralization potential of MAOM, we synthesize emerging research to show that the processes regulating MAOM mobilization may be critical, proximal mediators of dissolved organic N cycling and bioavailability in mineral soils. We discuss the mechanisms that facilitate the release of mineral-associated N, with a particular focus on the rhizosphere where root exudates and microbes enhance its bioavailability to plant roots. There is ample research on the physical and chemical mechanisms that mobilize other nutrients (e.g., phosphorous) from mineral surfaces (Hinsinger, 2001; Sharma et al., 2013) and increasing attention on factors controlling organic N stabilization (Bingham and Cotrufo, 2016). However, there is a need to highlight and synthesize evidence for the biological pathways and plant-microbe interactions that govern N turnover *from* MAOM. In the sections that follow we describe mechanisms that control the destabilization of MAOM and the role of clay minerals as mediators of bioavailable N supply, distinguishing between processes that proceed through chemical mechanisms (direct) and those that occur via microbial activities (indirect). Finally, we integrate these ideas into a conceptual framework that extends the Schimel and Bennett (2004) model by incorporating MAOM as a mediator of rhizosphere N transformations and N bioavailability (Fig. 1).

**Plant exudates enhance the destabilization, solubilization, and accessibility of MAOM**

Belowground plant C inputs, specifically in the form of root exudates, can suppress or stimulate C and N decomposition rates, through a suite of microbial processes collectively known as "priming" (Bingeman et al., 1953; Kuzyakov et al., 2000). Exudates can interact directly with the mineral-organic bonds and thus mobilize MAOM from clays (Keiluweit et al., 2015). Exudates

can indirectly influence MAOM mobilization by stimulating a concentrated zone of microbial activity (Kuzyakov, 2010) that may facilitate the enzymatic degradation and/or assimilation of MAOM. Both direct and indirect priming mechanisms originate with belowground plant rhizodeposits and could potentially enhance rhizosphere turnover of N from MAOM, providing a means for both plants and microbes to access N from this large pool.

*Direct destabilization pathways*

In our first pathway, plants increase MAOM bioavailability by producing root exudates that directly interact with mineral surfaces and MAOM. These exudates mobilize MAOM by modifying organo-mineral interactions and sorption dynamics through mechanisms that do not require microbial intermediaries. Low-molecular-weight organic acids, which are commonly released by plant roots (Bowsher et al., 2015; Oburger et al., 2013), mobilize formerly protected MAOM and thus expose new substrates to microbial degradation and assimilation (Clarholm et al., 2015) (Fig. 2). This destabilizing effect can release sorbed organic compounds into solution. Organic acids can dissolve minerals by decreasing pH at a local level (Zhang et al., 1985; Zinder et al., 1986). In this case, protonation of a mineral surface can weaken metal-oxygen bonds (Furrer and Stumm, 1986; Xu and Gao, 2008). Organic acids may also chelate with formerly organic-bound surface cations (Golubev et al., 2006; Kleber et al., 2015; Wang et al., 2014) or compete with binding sites and directly displace MAOM via ligand exchange (Keiluweit et al., 2015; Oburger et al., 2009). These processes can affect both metal oxides and silicate clays, although the potential for MAOM destabilization through this mechanism is generally greater among the former (Golubev et al., 2006).

Recent studies show the capacity of organic acids to destabilize MAOM-C and enhance its degradation. For instance, Keiluweit et al. (2015) used an artificial rhizosphere environment to demonstrate how the metal-chelating capacity of oxalic acid was directly responsible for increased microbial respiration and soil C loss. Using a natural soil, Wang et al. (2014) observed negligible release of organic C from mineral surfaces when soil particles were in water, but up to 228 mg C $L^{-1}$ $g^{-1}$ organic C was released with the addition of organic acids (citric and malic acid). This release was attributed to dissolution of metals like $Fe^{3+}$ and $Al^{3+}$, components of the mineral fraction that can stabilize organic compounds via strong ligand bonds. The evidence for organic acid-induced destabilization and solubilization of organics from mineral surfaces suggests this could be an important chemical pathway for mobilizing MAOM in the rhizosphere. Further, considering the low C/N ratio of MAOM, organic acids could destabilize even greater amounts of N relative to the C they mobilize. Although organic acid production is associated with enhanced N availability (Pan et al., 2016), no study has directly assessed how organo-mineral structures modulate the effect of organic acids on N cycling.

More broadly, exogenous dissolved organic matter (DOM) inputs such as root exudates can interact with endogenous organic compounds to mobilize MAOM (Toosi et al. 2012a) or cause dissolved materials to precipitate onto minerals (Halvorson et al., 2016). For example, new DOM inputs exchange rapidly with existing mineral-sorbed OM, and this exchange can mediate both the quantity and quality of OM in solution (Sanderman et al., 2008). Mineral-bound organic N is particularly vulnerable to exchange by incoming DOM (Scott and Rothstein, 2014) due to the propensity for hydrophobic compounds to displace N-rich and typically more hydrophilic compounds. This is frequently observed across soil profiles where DOC is preferentially retained in surface soils while N-rich and hydrophilic compounds dominate

subsurface and mineral horizons (Lajtha et al., 2005). Minerals also serve as hotspots for biogeochemical interactions between mineral surfaces, microbes, and OM (Kaiser and Kalbitz, 2012; Leinemann et al., 2018). Thus, the exchange of OM between free and mineral-bound pools may also be microbially mediated. In the rhizosphere, plants could potentially intercept this microbially transformed and N-rich DOM. It is important to account for the role of root exudates in facilitating these sorption-desorption processes and how it may be a means for plants to acquire N from MAOM.

*Indirect destabilization pathways*

Root exudates can also enhance SOM degradation indirectly by stimulating microbial activity and "priming" (Kuzyakov, 2010; Zhu et al., 2014). The leading priming hypothesis states that labile exudates enhance microbial activity by alleviating microbial C or energy limitations (Chen et al., 2014; DeAngelis et al., 2008; Zhu et al., 2014). This priming effect has been studied mainly for its role in altering whole soil C or N cycling and without regard for the specific origin of mobilized nutrients. However, given the quantity and chemistry of N within MAOM, and the varied nature of organo-mineral interactions, this pool may be vulnerable to priming and an important source of N in the rhizosphere.

The priming effect can elicit a strong N-mining response (Rousk et al., 2016) by stimulating N-degrading microbial communities (Fontaine et al., 2011), accelerating N-acquiring enzyme activity (Meier et al., 2017; Phillips et al., 2011) and enhancing gross N-mineralization rates (Dijkstra et al., 2009; Finzi et al., 2015; Herman et al., 2006; Zhu et al., 2014). Resulting increases in soil N cycling can also be tied to greater N uptake by trees (Drake et al., 2011). Along with the positive rhizosphere effect on N-acquiring enzyme activities (Ciadamidaro et al.,

2014; Koranda et al., 2011; Loeppmann et al., 2016), plants can also stimulate the microbial production of oxidative enzymes, like laccase, peroxidase and phenol oxidase (Carney et al. 2007; Phillips et al. 2011; Partavian et al., 2015).

As opposed to hydrolytic enzymes, which operate on a lock-and-key mechanism, oxidative enzymes do not exhibit substrate specificity (Allison, 2006). They can attack a variety of chemical bonds, such as those in aromatic compounds, and can also catalyze decarboxylation and demethylation reactions (Call and Mücke, 1997). Such versatility in catalytic capacity may allow oxidative enzymes to initiate the first step in the breakdown of complex organic matter or destabilization of mineral-bound compounds—thereafter exposing substrates to downstream attack by hydrolytic enzymes. Oxidative enzymes are generally associated with the release of bioavailable C, but may also be a means to access organic N in the rhizosphere (Kieloaho et al., 2016; Sinsabaugh, 2009). For example, Zhu et al. (2014) observed a positive relationship between oxidative enzyme activity and gross N mineralization rates in response to priming, while Kieloaho et al. (2016) found both oxidative and protease enzymes activities were needed to release N from both protected and bioavailable N pools.

The preferential synthesis of N over C-acquiring enzymes by rhizosphere microbes can slow C mineralization and accelerate N mineralization, decoupling the processes. For example, Rousk et al. (2016) found that glucose amendments increased gross N mineralization by 100-300%, but reduced C mineralization up to 60%. The authors argued that increased root exudation can stimulate a shift from C to N-acquiring enzymes—indicative of an N-mining response, or a specific shift toward N-rich compounds (Rousk et al., 2016). Similarly, Murphy et al. (2015) observed that simulated root exudates mobilized a pool of SOM with a relatively low C/N ratio when compared against unprimed soils. In other studies, C exudation and N mineralization rates

were positively correlated with increased N-acquiring enzyme activity (Brzostek et al., 2013), phenol oxidase activities, and subsequent N transformation rates (Yin et al., 2013).

It remains unclear whether MAOM or other fractions are targeted by N-mining microbes, but the silt- and clay-sized soil fraction consists of biologically active microenvironments with an intrinsic N-supplying capacity that could benefit rapidly growing and N-limited rhizosphere microbes. While conventional thinking is that enzymes are no longer active following sorption onto mineral surfaces (e.g. Quiquampoix et al. 1993), there are many reported cases of sorption either enhancing (Tietjen and Wetzel, 2003) or having no influence (Gianfreda and Bollag, 1994) over enzymatic activity. Further, mineral surfaces can protect enzyme activity from environmental changes in pH, temperature, and ionic strength (Kim et al. 2012; Schimel et al. 2017), and the highest enzyme activities are typically found in MAOM fractions (Grandy et al., 2008; Lagomarsino et al., 2012; Q. Zhang et al., 2016).

Land use and global change studies provide a platform for understanding the root-driven priming of MAOM and any potential ecosystem-level consequences. Under elevated $CO_2$ and ozone ($O_3$), enhanced rhizodeposition accelerates decomposition, microbial N uptake, and N mineralization (Hofmockel et al. 2011; Phillips et al. 2012). This acceleration of C and N cycles has been tied to a greater vulnerability of MAOM to priming (Carney et al., 2007). Likewise, elevated $CO_2$ can lead to significantly more loss of C and N from older or mineral-associated fractions (Dorodnikov et al., 2011; Hofmockel et al., 2011). In a study investigating long-term reforestation, Mobley et al. (2015) and Richter et al. (1999) observed significant losses in subsoil SOM, possibly as a result of priming by new root growth. Thus, rhizodeposition may accelerate the cycling of mineral-associated pools of SOM; this increased turnover of N from MAOM may either prevent or ameliorate plant N-limitation. As terrestrial ecosystems experience global

change effects, MAOM may become an increasingly important source of N for plants and microbes.

## Drivers of direct and indirect MAOM destabilization

While MAOM is N-rich and is a potentially accessible soil fraction, specific properties of the interacting clays, organic matter, and microbes will influence its bioavailability. Minerals have a wide range of chemical and physical properties; similarly, organic compounds vary in molecular structure, functional group chemistry, and charge characteristics. Collectively, these properties determine the strength of organo-mineral associations and in turn, their susceptibility to destabilization. Aspects of the microbial community, such as the physiological and compositional response to labile C inputs, may additionally mediate the extent of MAOM destabilization and degradation.

### *Strength of organo-mineral associations*

The bond strength between organic compounds and mineral surfaces influences the vulnerability of MAOM to destabilization. The strength and nature of surface interactions are controlled by properties of the organic compound (i.e., type, abundance, charge characteristics of surface functional groups) and of the mineral particle (i.e. size, shape and topography) (Kleber et al., 2015). Although chemical sorption can establish a bond that makes a molecule resistant to oxidation and degradation, many organo-mineral sorption interactions are also reversible. Sorbates interact via short- or long-range atomic interactions with varying bond strengths—e.g., polar covalent bonds have bond energies greater than 100 kJ $mol^{-1}$ while bond energies

associated with weaker van der Waals or hydrogen bonds range between 2 and 12 kJ $mol^{-1}$ (Kleber et al. 2015; Violante and Caporale 2015).

Mineral identity plays a major role in the nature of organo-mineral bonds (Singh et al., 2017) (Fig. 3) and potentially in MAOM bioavailability. For example, relatively low-charge clays such as kaolinite will bind organic N more loosely than smectites or other expandable clays with a negative surface charge (Mikutta et al., 2010; Yu et al., 2013). These less reactive phyllosilicates may be more likely to deliver N to plants. Clays with greater sorptivity, such as 2:1 phyllosilicates, may compete more strongly against microbes for organic N monomers or oligomers (Dippold et al., 2014). Iron and aluminum oxides have an even stronger capacity to bind organic N than most phyllosilicate clays (Kaiser and Zech, 2000; Kleber et al., 2005), but some microbial metabolites associated with these surfaces can still be readily available to microbes (Swenson et al., 2015). Certain minerals, such as manganese oxides can catalyze the fragmentation of proteins (Reardon et al., 2016). Thus, the relationship between clay identity and MAOM-N stability warrants further investigation. In a recent case, including mineralogical parameters significantly improved multivariate models describing N mineralization in agricultural soils (Wade et al., 2018). Similar work is needed across a wide range of ecosystems to understand how mineralogy mediates the turnover of soil N. While mineralogical composition can drive the extent of soil C priming by fresh litter inputs (Rasmussen et al., 2007), its role in priming soil N is poorly understood, despite the clear influence of mineral composition over SOM chemistry and binding strength.

Mineral surface chemistry further interacts with the specific chemistry of organic compounds to determine the binding mechanisms ultimately involved in stabilizing organic matter. While there is a range of compound types and binding mechanisms, here we focus on

amino acids, which are the most abundant form of N-containing monomer in many soils (Knicker, 2011) and likely a dominant by-product of degradation processes occurring at the organo-mineral interface. In addition, mineral surfaces can re-adsorb amino acids generated in this zone of dynamic exchange. Minerals "compete" with plants and microbes for liberated amino acids, thus making mobilization from mineral surfaces a potential rate-limiting step for amino acid bioavailability.

Amino acids bind to mineral surfaces readily, but the interaction depends on their overall charge and side group chemistry as well as solution pH (Dippold et al., 2014; Lambert, 2008; Theng, 1974). However, sorption does not necessarily reduce bioavailability. Microbes colonize mineral surfaces (Uroz et al., 2015), bringing substrates and their potential degraders into proximity. In some cases, permeases (membrane transport proteins) have a higher affinity for amino acids than do adjacent mineral surfaces (Dashman and Stotzky, 1986) allowing microbial uptake of amino acids to outcompete mineral sorption (Fischer et al., 2010). The rate of amino acid sorption can also depend on the chemistry of pre-existing MAOM, where some compounds, such as phenolic acids, can enhance the sorption of amino acids (Gao et al., 2017). The specific chemistry of binding sites may therefore matter more than the number of binding sites in determining overall sorption potential of minerals. In the rhizosphere, the high density of microbial populations coupled to changes in pH could stimulate the release of mineral-associated amino acids.

*Microbial community dynamics*

The availability of N to plants is largely regulated by root-associated microbes and their access to, and utilization of, soil nutrients (Reynolds et al., 2003; Richardson et al., 2009). As such, specific properties of rhizosphere microbes, such as their abundance, physiology, and community composition, may mediate the magnitude/direction of the priming effect and the amount of MAOM destabilized via priming (Zechmeister-Boltenstern et al., 2015). For example, the C and N use efficiency (CUE, NUE) of the microbial community, coupled with its cellular stoichiometry, will determine nutrient demand (Geyer et al., 2016; Sinsabaugh et al., 2013; Wieder et al., 2015). As microbial CUE increases, N demand and NUE may also increase in order to maintain cellular C/N ratios (Mooshammer et al., 2014a), resulting in an enhanced N-mining response to root exudation. That is, as root exudates provide a labile C source and microbial C needs are satisfied, microbes will invest more resources into enzyme production in order to acquire N from MAOM. In contrast to scenarios where CUE is high, microbes that use C inefficiently will require less N, and will consequently show a depressed N mining response to root exudation (Mooshammer et al. 2014). Further, microbial CUE and NUE also constrain microbial biomass, turnover, and activity, which will have feedbacks to the cycling and bioavailability of MAOM-N (Sinsabaugh et al., 2016).

Microbes also produce a diverse array of metabolites and enzymes that likely vary in their capacity to destabilize MAOM. For example, some microbes produce organic acids such as malate, citrate, and oxalate, which have a high affinity for trivalent metal cations that bind organics to mineral surface (Jones, 1998); by mobilizing the metals, it could release associated organic matter. In contrast, microbial taxa that produce organic acids with limited metal complexing capacities, such as lactate, formate, and acetate (Jones, 1998), may be less able to access MAOM. Microbes capable of oxidative enzyme production may have a disproportionate

impact on MAOM destabilization. Microbial synthesis of lignin-degrading and other oxidative enzymes serves to release N from organic matter (Craine et al., 2007; Rinkes et al., 2016; Shukla and Varma, 2011); more specifically, oxidative attack may be an important pathway for fungi to acquire N in macromolecular structures, like protein-polyphenol complexes (Bending and Read, 1996). Indeed, peroxidase enzymes have been characterized as a "proximate control for C and N mineralization" (Tian et al., 2010).

Important microbes that produce oxidative enzymes to access N include ectomycorrhizal fungi (EMF) and lignin-degrading saprotrophs (Sinsabaugh, 2009; Talbot et al., 2008). Certain groups of EMF have the capacity to liberate and assimilate mineral-bound forms of organic N; hydrophobic EMF may be better equipped in this regard due to their capacity to access more insoluble forms of N (Chen et al., 2016; Hobbie et al., 2012). Not all lineages of EMF are uniformly equipped to produce oxidative enzymes (Pellitier and Zak, 2018) and other fungi, such as saprotrophic or ericoid mycorrhizal fungi, have an overall greater capacity to produce such enzymes (Bending and Read, 1996; Wu, 2011). More studies are needed to identify the fungal taxa that can produce the enzymes needed to mobilize N from MAOM.

By extending the root surface area for plants, mycorrhizal fungi may increase the opportunity for a plant to acquire N from MAOM, especially MAOM contained within aggregates. Both EMF and arbuscular mycorrhizal fungi are known to enhance soil aggregate formation and stabilization (Rillig and Mummey, 2006; Zheng et al., 2014), but they also have the capacity to explore the interior of aggregates. Mycorrhizal hyphae can penetrate aggregates and so overcome the physical barrier a macro- or micro-aggregate might present that would protect MAOM. However, research thus far has not addressed if or how fungal, and specifically EMF, entry into a macro- or micro-aggregate mobilizes N-containing MAOM contained within.

Microbes can also weather minerals through both physical and biochemical mechanisms, which could ultimately solubilize compounds from MAOM. Mineral colonization by fungi can lead to biomechanical weathering—hyphal pressure can be strong enough to physically alter clay surfaces (Bonneville et al., 2009). Biochemical weathering can result from the action of bacterial and fungal chelators (Burford et al., 2006), protons or siderophores that can alter the chemical structure or behavior of clays (Courty et al., 2010; Gadd, 2010). Among the fungal groups, ectomycorrhizal fungi may play an outsized role in weathering (Van Breeman et al., 2000) although recent evidence suggests both arbuscular and ectomycorrhizal fungi may have comparable effects on mineral weathering rates (Koele et al., 2014; Remiszewski et al., 2016).

Generally, clay weathering is considered a slow pedogenic process, but it can occur over decadal or sub-annual time scales, especially within the rhizosphere. Processes occurring at the root-soil interface can accelerate pedogenesis such that mineral dissolution or alteration can occur within 20 years (Calvaruso et al., 2009; Mareschal et al., 2013) or even within a growing season (Paola et al., 2016). Through these microbial-driven weathering processes, it is possible that MAOM could become released into the surrounding soil solution. However, these studies are typically conducted in pure culture environments and with the mineral substrate being minimally weathered primary mineral or pure clay minerals. It is also important to consider the potential contribution of bedrock N—up to 17% of N in natural systems may be derived from modern-day rock N, upending the conventional assumption that the atmosphere is the original and primary source of N (Houlton et al., 2018). Thus, it is possible that in the rhizosphere, plant and microbial-induced weathering can mobilize N from both the minerals and the associated SOM.

**Synthesis: proposed conceptual framework**

Building on the mechanisms of destabilization described here, we propose to reconsider Schimel and Bennett's model of soil N bioavailability by incorporating MAOM as a mediator of rhizosphere N transformations and N bioavailability (Fig. 1). In our revised model, SOM is split into particulate and soluble pools. Particulate SOM consists of polymeric compounds—essentially plant litter—which must be enzymatically degraded by microbes in order to release soluble components that can be accessed. The soluble pool contains some polymers, such as protein oligomers, and monomers, such as amino acids (Warren, 2013). This retention of smaller soluble SOM compounds by mineral surfaces can restrict their accessibility to microbes and plants. Byproducts from microbial turnover can accumulate on surfaces and so contribute to further building MAOM (Grandy and Neff, 2008). However, plant root exudates can remobilize MAOM into the soil solution, thus making SOM "free" and accessible to microbes (and their enzymes) and to plants.

Our revised framework emphasizes spatially-explicit processes occurring in the rhizosphere, where high microbial activity and the influx of low molecular weight C compounds (i.e., exudation) intensifies sorption/desorption of organic N and plant-microbial competition for these compounds. Root exudation, by modifying the soil solution and microbial community, regulates the accessibility of N in MAOM pools. It is likely that the direct and indirect pathways of MAOM destabilization, as previously described, interact synergistically. That is, as organic acids target MAOM, adjacent enzymes can degrade newly freed polymers. At the same time, the chemical properties of mineral surfaces may mediate the accessibility of MOAM in the

rhizosphere. For example, N-containing compounds that are weakly associated with weathered silicate clays may be more vulnerable to destabilization.

In both Schimel and Bennett's model and our own, a critical source of bioavailable N is protein depolymerization. However, whereas Schimel and Bennett (2004) developed their model with a focus on plant material and how N moves from litter to microbes and plant roots; in mineral-dominated soils there's now ample evidence that minerals are a dynamic source and sink for bioavailable N that can strongly influence overall N-availability. By providing an additional and distinct N source, MAOM is also likely to have consequences for plant and microbial N acquisition. For example, plant-microbe competition for MAOM in the rhizosphere is likely distinct from competition occurring in bulk soil or within particulate organic matter patches. In bulk soil, where roots and microbes are disconnected, diffusion limitations drive competition for N between plants and microbes (Kuzyakov and Xu, 2013) (Fig. 4a); N recycles through the microbial community and may only reach the plant when microbial N needs are met (Mooshammer et al., 2014a). Alternatively, in POM-rich patches colonized by roots and their associated hyphae, plants and microbes are in more direct competition. Plants can more effectively intercept N when associated with mycorrhizal fungi (Cavagnaro et al., 2012) and they can also intercept N released from the turnover of N-limited microbes (Fig. 4b). Due to temporal niche differentiation, both plants and microbes can acquire N from these litter patches: while microbes take up N quickly, their populations turn over rapidly, cycling N back into the form that plants can compete for and store for a more extended time. Thus, over time, N moves from the microbial biomass into plants (Hodge et al., 2000; Kuzyakov and Xu, 2013).

These scenarios are likely distinct from those in which plants and microbes acquire N from MAOM in the rhizosphere. Root exudates stimulate microbial activity, driving microbial

mining and mineralization of N, some of which could be derived from MAOM. As this is driven by root exudate inputs, it occurs along the root plane where plants can access the released N. Plants deplete N from solution, which further accelerates the N-mining response and enhances desorption from mineral surfaces. This plant-microbial interaction serves to drive N off of mineral surfaces. Thus, a tight coupling of C and N cycling provides microbes C and plants N, leading to a cooperative dynamic distinct from the more competitive ones occurring in patches of labile POM or in the bulk soil (Fig. 4c).

**Future research opportunities**

Developing a more quantitative understanding of MAOM's N cycling capacity has been limited in part by the analytical scale of most SOM studies. Prevailing perspectives on SOM mineralization are based on analyses conducted on whole-soil samples, whereas critical zones of activity, such as those in the rhizosphere or on mineral surfaces, operate at a much smaller scale ($nm^3$–$mm^3$). Standard chemical extractions and fractionations may attenuate or overlook the micro- and nano-scale processes that govern MAOM transformations. Even isotope-based studies, which can disentangle chemical processes, are still usually applied to whole soil samples—although NanoSIMS can analyze N dynamics at the micro-scale (Herrmann et al., 2007).

Disentangling the functional role of MAOM and the ways in which plants and microbes access and interact with it will require fine-scale and minimally destructive techniques. Tracing and quantifying the N-supplying capacity of MAOM will also require experiments that include a live plant or that mimic the action of roots. The mechanisms described in this paper may be most

relevant where there is a constant drawdown of soluble N. This would maintain the N-mining activities of both roots and microbes, which may otherwise be suppressed in standard incubation experiments where soluble nutrients can accumulate (Craine et al., 2007; Fog, 1988). We also recommend that future research employ high resolution techniques such as stable-isotope probing, which can be used to trace the fate of C and N through living and non-living soil pools. Combining stable-isotope methods with emerging techniques for characterizing molecular and chemical composition will be valuable for examining the cycling of nutrients at the organo-mineral interface and the role of plants (e.g., see Pett-Ridge and Firestone 2017). By incorporating these ideas into existing and future models, we may also improve our theoretical understanding and practical management of the N cycle.

Our revised model emphasizing minerals as a source and sink for bioavailable N should encourage new avenues of research that foster collaborations among biogeochemistry, ecology, and soil science. For example, priming studies, thus far, have largely focused on specific mechanisms and short-term (days- to months-long) effects (Huo et al., 2017). To better understand how priming influences MAOM turnover and the potential consequences for ecosystem function, it will be important to link fine-scale geochemical processes with higher-level and longer-term ecosystem responses. By integrating across diverse spatial and temporal scales, we can explore MAOM's role through a more interdisciplinary lens.

To that end, we suggest the following potential avenues for future research, broadly categorized by spatial scale. This is intended to highlight emerging topics related to MAOM destabilization and the release of bioavailable N.

1) At the *meter to ecosystem scale*, the most basic questions remain: how much N do plants and microbes acquire from MAOM in different ecosystems, and what is the

turnover time of N compounds in the MAOM pool? We have provided evidence based on pool size, variable turnover rates, "missing" N sources and sinks, and our emerging understanding of priming, which all suggest that MAOM is a dynamic pool of N. Yet more direct measurements of plant and microbial uptake of MAOM-N are needed. Further, questions remain regarding how plant traits, such as root exudate profiles, influence the potential for MAOM degradation. Certain plant species may host rhizosphere microbial communities that are more effective at solubilizing MAOM. It is possible that such traits could confer plants with a competitive advantage, thus altering plant-community dynamics and succession over time.

2) At the *micro-meter scale*, patterns of enzyme induction and microbial investment in organic N acquisition will depend on microbial traits. We know how individual morphological and physiological traits may translate into different abilities to access MAOM-N, but we do not fully understand how this manifests within diverse microbial communities. In theory, CUE and NUE should be tightly coupled to MAOM-N acquisition. If this is true, changes in environmental conditions that alter microbial physiology could have strong effects on MAOM-N dynamics. We need to better understand the role that mycorrhizal fungi play in accessing MAOM. By extending root surface area, transporting N, and producing degradative enzymes, they may enhance MAOM-N bioavailability.
3) At the *nano-meter scale*, we need to better define and measure the "kinetic zone" at the organo-mineral interface (Kleber et al., 2007). It is not clear how or under what conditions enzymes overcome organo-mineral associations. Oxidative enzymes can generate free radicals that further decompose SOM (Dashtban et al., 2010). Can these

small, diffusible oxidizers access and decompose MAOM regardless of the strength of the mineral-organic bond? Alternatively, are enzymes only capable of attacking weakly held compounds, such as those associated with highly weathered minerals?

4) Finally, there is a need to investigate processes occurring at *pedon scales*: the role of soil physical properties, such as pore and aggregate dynamics, and how specific microbial community members may be more or less equipped physiologically to access MAOM. Recent research begins to address the interactions of pore size/connectivity and soil moisture in driving the turnover of SOM cycling (Bailey et al., 2017; Smith et al., 2017); this remains a key question in understanding the fate of MAOM and microbes' abilities to access the N in these protected pools.

The importance of MAOM as a source of N to plants and microbes will likely vary among ecosystems depending on management as well as climate, vegetation and edaphic soil properties. The proportion of N stored as MAOM relative to particulate sources may determine the potential rate of MAOM-N turnover within the rhizosphere. For example, in organic soils or N-limited environments, which served as the context for the model originally proposed by Schimel and Bennett (2004), depolymerization (Delgado-Baquerizo and Gallardo, 2011) or mycorrhizal N transfer (Högberg et al., 2017) may more strongly limit N availability than minerals. In contrast, MAOM may be a more significant source of N in agricultural soils, where proportionally more N is stored as MAOM than POM. However, the relative distribution of N across SOM fractions will not be the only determinant. Other interacting factors, such as MAOM chemical composition (e.g., the abundance of monomeric vs. polymeric N) and mineral assemblage (e.g., the abundance of highly reactive vs. low-charge clays) will influence if and how plants and

microbes can access N within MAOM. In certain ecosystems, mineral subsoils may be a more important source of N where plants with deeper rooting systems could access and extract N from MAOM. Moisture and temperature are additional controls, which may alter the rate of MAOM turnover and/or mediate whether mobilized N remains within a physically isolated microbial community or can translocate through the pore spaces. New research on the role of MAOM in N cycling across different ecosystems and spatio-temporal scales is needed to understand how these factors interact and to determine where MAOM-N cycling is most relevant. This will also allow us to move from the conceptual framework we have provided here to more quantitatively rigorous simulation models and experimental evidence.

**Table 2.1.** Examples from published literature that illustrate the average relative distribution of N across particle size-based or density fractions in agricultural and non-agricultural systems. The maximum and minimum values are provided in parentheses. If MAOM-N not explicitly provided, value was calculated as POM-N subtracted from total soil N. POM (particulate organic matter), LF (light fraction), NaI (sodium iodide), NaPT (sodium polytungstate). Note: Selected studies do not represent a comprehensive, unbiased literature review but instead are provided to show ranges across varied ecosystem and management contexts.

| System and location | Depth (cm) | Fractionation method and cutoff | %POM-N or LF-N out of total soil N | %MAOM-N out of total soil N | Reference |
|---|---|---|---|---|---|
| **Agricultural Systems** | | | | | |
| Tillage studies across southern USA | 5 | POM > 50µm | 26.0 (3.4 - 49.6) | 74.0 (50.4-96.6) | (Schomberg et al., 2009) |
| Tillage studies in Illinois, USA | 5 | POM >53µm | 22.5 (17.1 - 25.8) | 77.5 (74.2 - 82.9) | (Wander and Bidart, 2000)[a] |
| Cotton cropping systems in Texas, USA | 5 | POM > 53µm | 16.5 (13.2-18.9) | 83.5 (81.1-86.8) | (Bronson et al., 2004)[b] |
| Cropping sequence, tillage, and fertilization studies in North Dakota, USA | 7.5 | POM > 50–53 µm | 32.4 (12.9 - 43.5) | 67.6 (56.5 - 87.1) | (Liebig et al., 2004) |
| Crop rotation study in Saskatchewan, Canada | 7.5 | LF < 1.7 Mg $m^{-3}$ NaI | 5.8 (3.4 – 8.2) | 94.2 (91.8 – 96.6) | (Biederbeck et al., 1994) |
| Grass and cereal production sites in Alberta, Canada | 10 | LF < 1.7 Mg $m^{-3}$ NaI | 5.5 (4.1 – 6.9) | 94.5 (93.1 – 95.9) | (Arshad et al., 2004) |
| Grassland and plantation sites in south-western Australia | 10 | POM > 45µm | 65.3 (56.7–73.8) | 34.8 (26.2–43.3) | (Mendham et al., 2004) |
| Comparing cultivated soil and field edge in the UK | 30 | LF < 1.7 Mg $m^{-3}$ NaI | 13.6 (4.3 – 23) | 86.4 (77-95.8) | (Bending et al., 2002)[c] |
| **Non-Agricultural Systems** | | | | | |
| Temperate deciduous forests in northeastern USA | 5 | LF < 1.65 Mg $m^{-3}$ NaPT | 37.3 (29.9 – 44.7) | 62.7 (55.3 –70.1) | (McFarlane et al., 2013) |
| Primary and secondary forests in Puerto Rico | 10 | LF < 1.85 Mg $m^{-3}$ NaPT | 18.6 (17.8 – 19.6) | 81.4 (80.4 – 82.2) | (Marin-Spiotta et al., 2009) |
| Temperate shrubland/grassland in Denmark | 12.3 | LF < 1.6 Mg $m^{-3}$ NaPT | 18 (7-29) | 82 (71-93) | (Thaysen et al., 2017) |
| Grasslands in China | 15 | LF < 1.8 Mg $m^{-3}$ NaI | 19 | 74 | (Ming Shi et al., 2010)[d] |
| Alpine forests in Austrian Alps | 20 | LF < 2.0 Mg $m^{-3}$ NaPT | 40.4 (26.7-53.2) | 59.6 (46.8-73.3) | (Schnecker et al., 2016) |

[a]Values from "location" averages

[b]Values reported for cotton cropping systems only

[c]Proportion of total organic N

[d]Single average

**Table 2.2.** Evidence for the mineralization potential of mineral organic matter fractions: DF (density fraction/fractionation), PSF (Particle-size based fractionation), LF (light fraction), HF (heavy fraction). Note: Selected studies do not represent a comprehensive, unbiased literature review but instead are provided to show ranges across varied ecosystem and management contexts.

| SOM fraction | Ecosystem Type and Location | Methods | Results and Implications | Reference |
|---|---|---|---|---|
| DF | Agricultural and forest sites in northwestern USA | LF and HF fractions were added to whole soils. Rates of C and N mineralization compared against basal rates in whole soil samples | HF additions to soil maintained net N mineralization rates while LF additions led to N immobilization | (Whalen et al., 2000) |
| DF | Forest sites in northwestern USA and Costa Rica | Compared net N mineralization from whole soils and isolated LF and HF fractions. Soils incubated under anaerobic conditions. | Net N mineralization greater from HF than LF | (Sollins et al., 1984) |
| DF | Grassland site in Upper Midwest, USA | Assessed how the plant effects on SOM fraction C and N concentrations mediated changes in soil N mineralization | Plant belowground biomass positively related to C and N concentrations of SOM fractions. Total soil C and N of recalcitrant fraction positively associated with gross N mineralization. | (Fornara et al., 2011) |
| Acid hydrolysis | Agricultural site in northeast China | Evaluated the fate and seasonal dynamics of fertilizer N across different organic N fractions | The acid insoluble pool stabilized but not completely protected from degradation; can be made available depending on supply and demand. | (Lu et al., 2013) |
| PSF | Agricultural site in the North Island of New Zealand | Compared N mineralization from sand, silt, and clay fractions in 18-week incubation | Most N mineralization derived from silt and clay fraction | (Parfitt and Salt, 2001) |
| N/A | Grassland site in north-central Switzerland | Assessed how elevated $CO_2$ altered N cycling from the recalcitrant SOM pool and into plant and microbial pools. | Delivery of N from stable organic matter; N taken up by plants coming from non-extractable pools. | (de Graaff et al., 2009) |
| DF of particle size fractions | Grassland sites in western Belgium | Evaluated the relationship between C and N content of SOM fractions and gross N mineralization rates | N contents of <50 and 50-150µm fractions explain up to 97% of variability in gross N mineralization rates; Larger relative importance of <50µm fraction in the regression model | (Accoe et al., 2004) |
| PSF | Grassland site in England | Examined the contribution of macro-organic matter N to gross N mineralization | Macro-organic matter only contributes 2.3-3.4% to inorganic N pool; inferred that majority of N derived from mineral-associated pools | (Monaghan and Barraclough, 1997) |
| DF | Forested chronosequence in the South Island of New Zealand | Measured microbial N cycling and N functional gene abundance in incubations of HF fraction and bulk soil | N cycling rates and N-related N functional gene abundance similar or higher in HF compared to bulk soil | (Turner et al., 2017) |
| DF | Harvard Forest DIRT site in northeastern USA | Determined how inclusion/exclusion of above and belowground plant inputs influenced the accumulation of SOM pools | Root inputs did not increase MAOM pool compared to soils with all inputs excluded. | (Lajtha et al., 2014) |

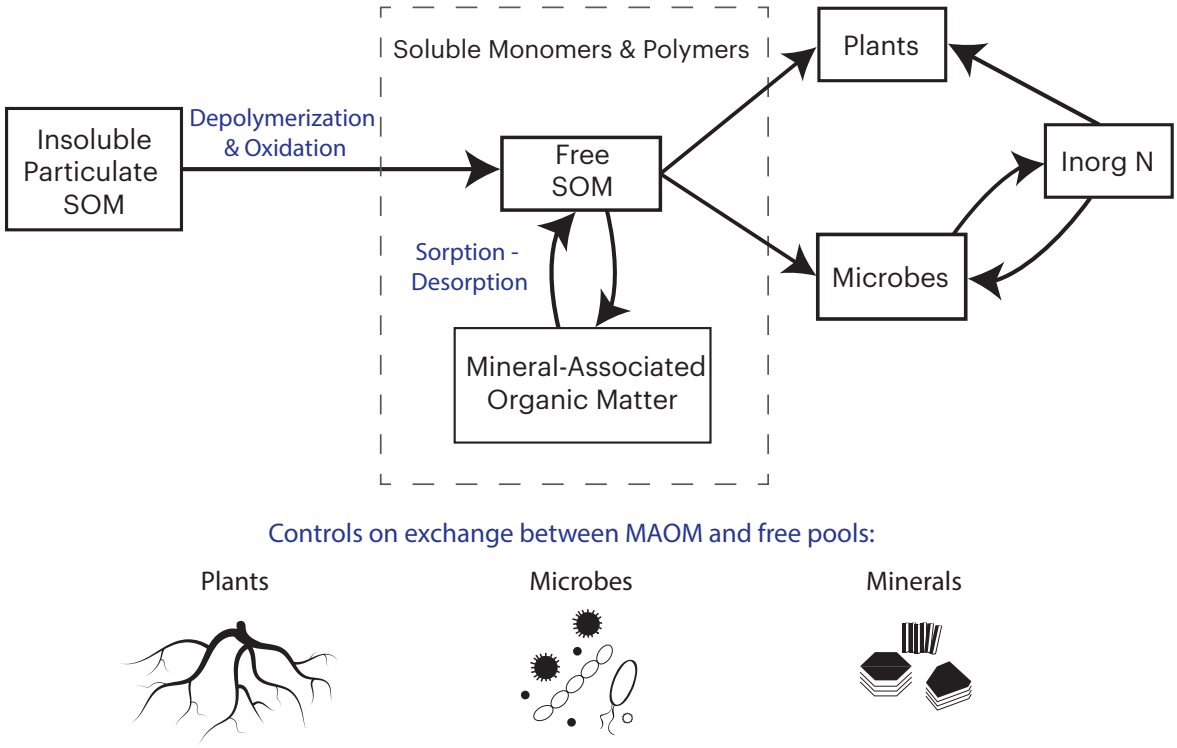


**Figure 2.1.** Conceptual model demonstrating the biological pathways for the production of plant-available N. Adapted from the model originally proposed by Schimel and Bennett (2004), our model highlights the role of mineral-associated organic matter (MAOM) and its continuous and dynamic exchange with the free and soluble soil organic matter (SOM) pool. Properties of the plants, microbes, and mineral particles will further mediate the exchange of N between mineral-associated and free pools. Note: The sizes of the boxes do not relate to pool size.

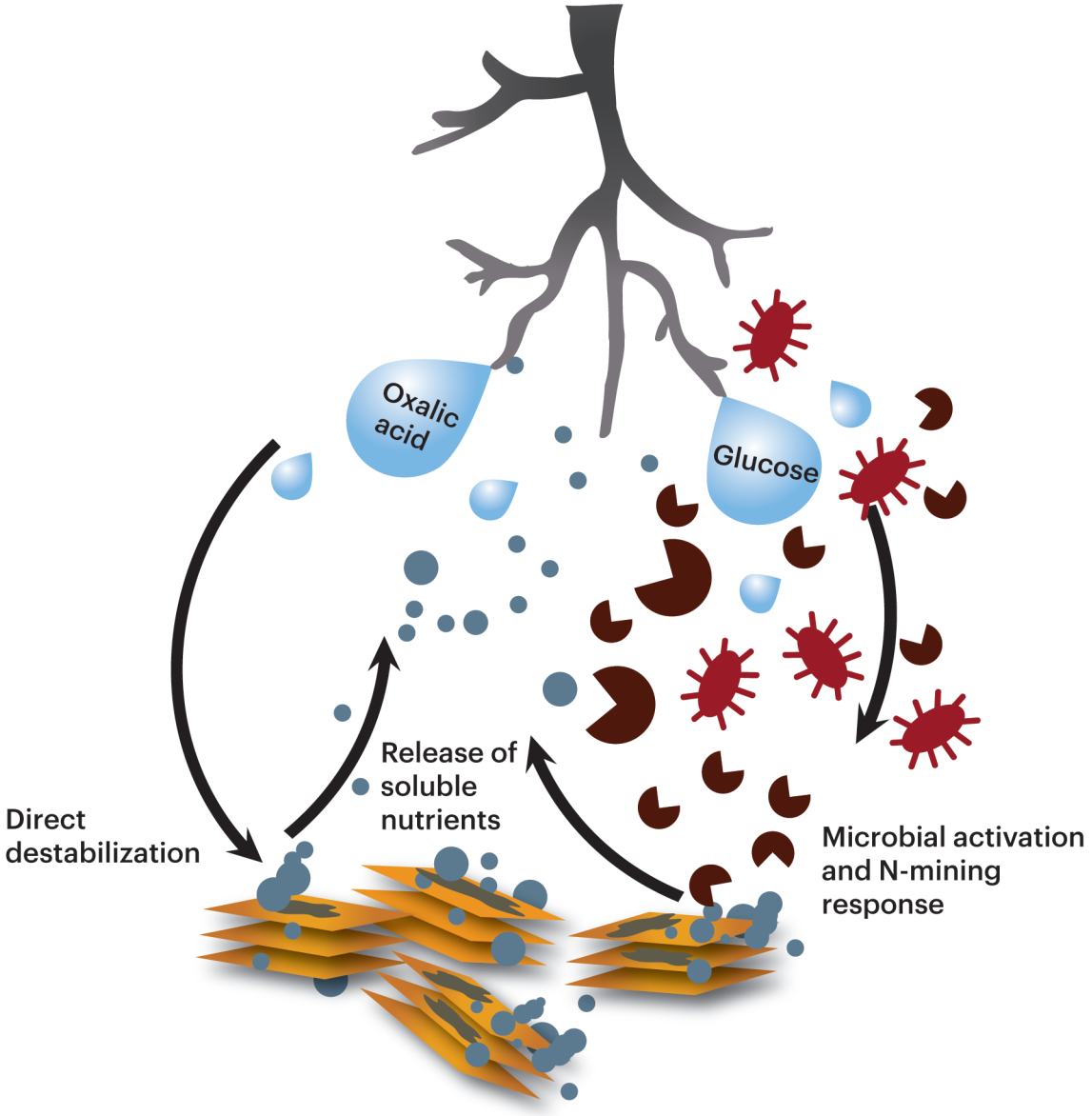


**Figure 2.2.** Illustration depicting the direct and indirect pathways to MAOM destabilization. On the left, oxalic acid can target the organo-mineral bond directly. This pathway bypasses the microbial community to stimulate the release of organic compounds from mineral surfaces. On the right, glucose stimulates the microbial community and activates a microbial-mediated N-mining response. These two pathways can work synergistically to further accelerate the degradation and turnover of MAOM within the rhizosphere

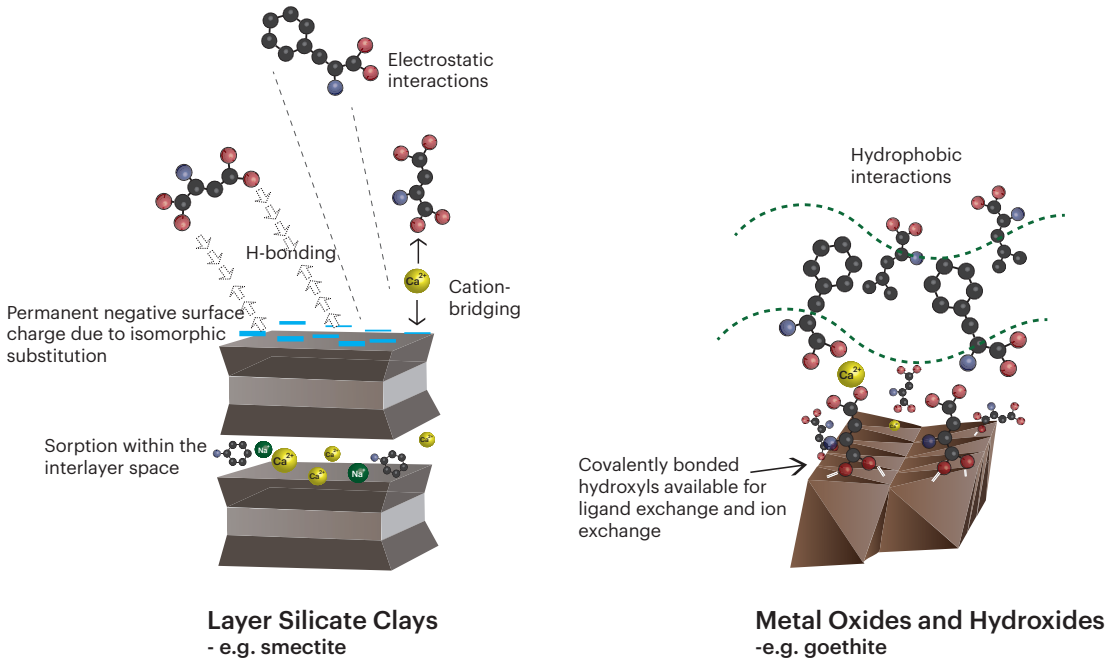


**Figure 2.3.** Illustration of two mineral types and examples of the variety of bonding mechanisms that can be associated with each. While the organo-mineral associations are not mutually exclusive, the predominant mechanisms of association differ across clay types leading to variation in bonding strength among minerals. Blue and red circles represent nitrogen and oxygen, respectively.

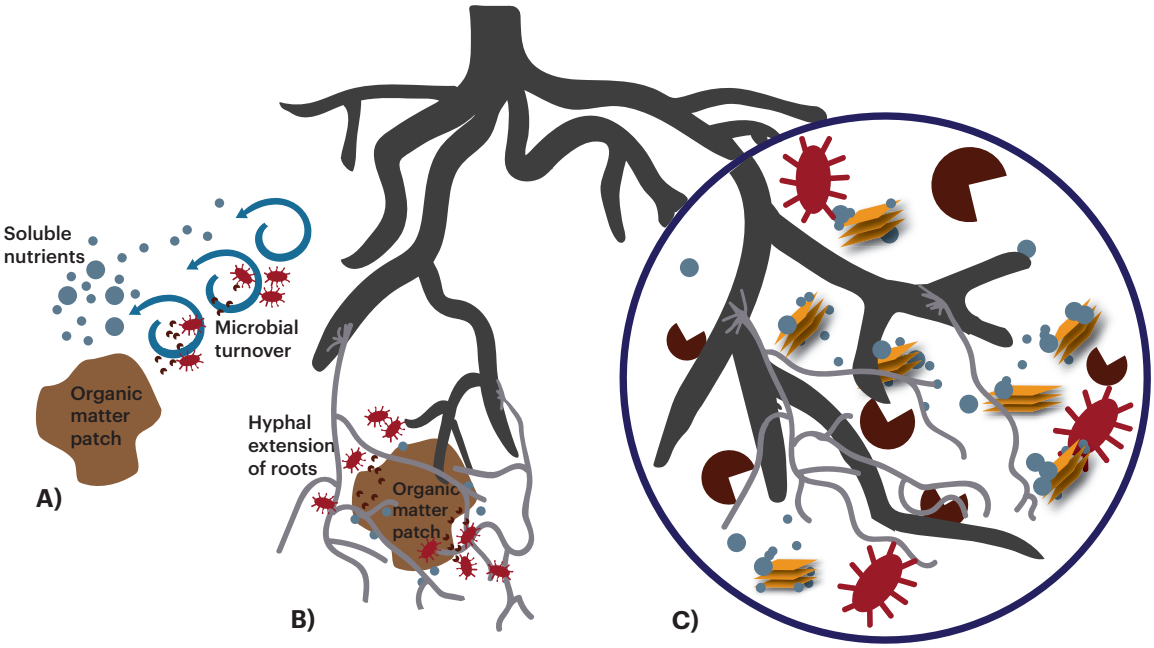


**Figure 2.4.** Illustration of three distinct scenarios of plant-microbial competition for N. In scenario A, diffusion limitations drive competition for N between plants and microbes. In scenario B, where roots and their associated hyphae colonize POM- or litter-rich patches, plants and microbes are in more direct competition. In scenario C, a tight coupling of C and N cycling provides microbes C and plants N, leading to a cooperative dynamic

CHAPTER 3

# PRIMING MECHANISMS PROVIDING PLANTS AND MICROBES ACCESS TO MINERAL-ASSOCIATED ORGANIC MATTER


## Abstract

Mineral-associated organic matter (MAOM) is a critical long-term reservoir for soil nutrients. However, recent experimental and theoretical advances suggest that plant root inputs may accelerate the turnover and bioavailability of this otherwise stable pool. In laboratory incubations of physically isolated MAOM, we tested potential pathways by which root C inputs destabilize this fraction. To assembled soil mixtures containing MAOM and sand, we added water (to serve as a control) or one of two treatments simulating common root exudates: $^{13}$C-labelled glucose, to stimulate microbial activity and potentially the production of extracellular enzymes capable of decomposing MAOM, or $^{13}$C-labelled oxalic acid, which has been demonstrated to disrupt metal-organic bonds. In a follow-up incubation, we added substrates to sterilized MAOM + sand mixtures to assess the non-microbial pathways of MAOM destabilization.

Over the course of the 12-day incubation we measured $CO_2$ respiration rates, $^{13}$C-$CO_2$ efflux, enzyme activities, inorganic N pools, gross N mineralization and microbial community composition via phospholipid fatty acid (PLFA) analysis. Both substrates stimulated the mineralization of MAOM-C (i.e., positive priming), with mineralization increasing by up to 428–553% and total increases ranging from 35–89%. Likewise, C treatments stimulated hydrolytic and oxidative enzyme activities; glucose additions enhanced the production of an exo-cellulase and a chitinase, while oxalic acid enhanced oxidative enzyme activities. Finally, gross

ammonium production was positively associated with MAOM-C priming and oxidative enzyme activities, but only in glucose treatments. In sterilized MAOM + sand mixtures, oxalic acid additions also stimulated the concomitant release of metals and dissolved organic nitrogen into exchangeable pools. Our results indicate that common root exudates, like glucose and oxalic acid, can significantly increase the turnover and potential release of C and N from MAOM through microbial (e.g., enzyme induction) and non-microbial (e.g., mobilization of metal oxides) mechanisms.

## Introduction

Mineral-associated organic matter (MAOM) is a critical reservoir for soil nutrients and often accounts for the majority of soil carbon and nitrogen (N) in terrestrial systems (Gentsch et al., 2015). As such, its turnover has significant implications for the cycling of reactive N and potential $CO_2$ emissions from soils. Surface charge properties confer clay minerals with high capacity to accumulate organic compounds (Kleber et al., 2015). Once associated with mineral surfaces, organic compounds can resist further decomposition, potentially persisting for up to centuries or millennia (Torn et al., 1997; Trumbore et al., 1995). However, recent work demonstrates a proportion of MAOM can cycle on short-term (i.e., sub-annual) time-scales (Gentsch et al., 2015; Torn et al., 2013), which challenges the representation of MAOM as an inert or passive pool. It is critical to identify the soil properties and processes that are conducive to MAOM turnover given the importance of this pool as a long-term reservoir for both C and N (Bingham and Cotrufo, 2016).

Organo-mineral bonds vary in strength (Newcomb et al., 2017; Schulten and Leinweber, 2000) and are sensitive to shifts in the biochemical environment. The rhizosphere, the zone of

soil surrounding roots, hosts hotspots of microbial and biochemical activity that may render mineral-associated compounds vulnerable to decomposition (Jilling et al., 2018). Root activity can cause significant shifts in pH (Kreuzeder et al., 2018), and alter soil solution chemistry with the drawdown of soluble nutrients (SOM), deposition of low molecular weight C compounds, and activation of microbial communities. These biochemical changes can stimulate or inhibit the degradation of SOM through a process referred to as the "priming effect" (Kuzyakov, 2002). The net effect on SOM degradation can be significant; in a meta-analysis of 31 priming studies, the rhizosphere itself caused an average 59% increase in soil C mineralization (Huo et al., 2017). To date, research on the priming effect has focused largely on overall changes in nutrient pools, while the specific origin of primed C and N and, in particular, the potential vulnerability of MAOM to priming has been largely overlooked.

MAOM typically stores the majority of soil N and is often dominated by low C:N compounds (Giannetta et al., 2018). The lower molecular weight and N-rich compounds in MAOM are ideal substrates for microbial use; fewer physiological steps and less energy are required to access the C or N within an amino acid or nucleotide compared to a long-chain aliphatic compound or a more complex molecule like tannin (Allison and Vitousek, 2005). N-rich compounds within MAOM may also be more physically accessible to microbes than other molecules because they are more prone to desorption (Coward et al., 2019; Scott and Rothstein, 2014). Given the strong N-mining response observed with priming (Murphy et al., 2015), and that N within MAOM is more vulnerable to turnover, MAOM may also be targeted in rhizosphere environments. Despite the role of root exudates in accelerating nutrient turnover, few studies have identified which soil pools are primed.

Microbial activation through priming by roots is an indirect pathway by which plants may facilitate the mobilization of N contained in MAOM. Root inputs can also stimulate a direct pathway (i.e. non-microbial) of MAOM destabilization that specifically involves organic acids (Clarholm et al., 2015; Jilling et al., 2018). Low-molecular-weight organic acids are commonly released by roots (Bowsher et al., 2015; Oburger et al., 2013). Organic acids, such as oxalic acid, can destabilize organo-mineral bonds and expose new substrates to microbial degradation and assimilation (Clarholm et al., 2015). Specifically, they can dissolve minerals through protonation of mineral surfaces and subsequent weakening of metal-oxygen bonds (Xu and Gao, 2008; Zinder et al., 1986). Organic acids can also chelate with formerly organic-bound surface cations (Golubev et al., 2006; Kleber et al., 2015; Wang et al., 2014). For example, oxalic acid in rhizosphere environments is believed to mobilize N in alkaline calcareous soils, such as those common to karst ecosystems (Pan et al., 2016). The mobilization of Ca within organo-metal associations may be an important mechanism by which plants acquire N (Tu et al., 2007). Finally, organic acids can compete with binding sites and directly displace MAOM via ligand exchange (Keiluweit et al., 2015; Oburger et al., 2009).

Our objective in this research was to examine if labile C substrates stimulate the degradation of MAOM through the aforementioned microbial and non-microbial mechanisms (Fig. 3.1). We assessed the potential for C and N release from MAOM by treating physically isolated silt and clay fractions with labile C substrates to simulate the action of a plant root.

## Materials and Methods

### *Soil sampling and fractionation*

Soil samples were collected in 2014 from agricultural sites located in Illinois (IL) and Michigan (MI) (Table 3.1). The IL soil is of the Drummer soil series and classified as a silt loam (Typic Edoaquoll). The MI soil is of the Marlette soil series and is classified as a sandy loam (Oxyaguic Glossudalf). X-ray diffraction analyses indicated the presence of smectite in IL and that both soils are characterized by low-reactivity minerals such as quartz, chlorite and feldspar.

Field experiments were established at the sites in 2011 and maintained annually. For more specific details regarding the field site and management histories, please refer to Williams et al. (2016a) and Kane et al. (2015). Samples were collected in May of 2014 from all subplots that did not receive any nitrogen fertilizer throughout the field experiment. We used 1.9-cm cores to collect and composite 10 soil cores to a 5-cm depth from each subplot. Samples were sieved to below 4 cm, air-dried, and combined to produce a single composite soil sample for each site. Air-dried soil samples were crushed gently in a mortar and pestle and passed through a 2-mm sieve.

MAOM was physically isolated using a particle size-based technique optimized for each soil as described in Chapter 1. Soils were fractionated and dispersed in water as we wanted to avoid any chemical residues that might influence microbial growth in subsequent incubations. Soils were first dispersed gently in water (10± 0.1 g soil in 50 mL $H_2O$) to release the free particulate organic matter. Floating particulates were recovered with suction and cleaned on a 53 μm sieve. The remaining soil and water suspension was centrifuged at 10,000 x g for 35 minutes. The supernatant was discarded and the pellet resuspended for ultrasonication. The soil and water suspension received an initial low energy sonication of 60 joules/mL to release occluded particulates. The soil and water suspension was then passed through a 53 μm sieve to recover the occluded-POM fraction (o-POM).

The suspension recovered below the 53 μm sieve was centrifuged, the supernatant discarded and the pellet resuspended for the subsequent sonication step. The second sonication step was optimized for each soil type as described in Chapter 1. Following this second sonication step, the suspension was passed through a 20 μm sieve. Material less than 20 μm included fine silt and clay-associated organic matter that we refer to here as MAOM. Coarse silt was excluded from MAOM as this fraction can be dominated by weathering-resistant primary minerals (Bayer et al., 2006; O'Brien et al., 2013) that could dilute dynamics associated with the more reactive fine silt and clay particles. f-POM, oPOM and c-Silt fractions were dried at 60º C and MAOM was dried at 105º C. All fractions were ground and stored until further analysis.

*MAOM priming incubation*

Mixtures of MAOM and acid-washed sand were incubated in 176 mL glass jars. Each microcosm contained 4g of MAOM and 6g of sand, thus creating a sandy clay texture. Soil mixtures were brought to 60% of water-holding capacity and then pre-incubated for 7 days. Three levels of substrate treatment were applied separately to the microcosms: glucose, oxalic acid and water (control). In priming studies, C addition rates are typically a percentage of microbial biomass C (Blagodatskaya and Kuzyakov, 2008). However, attempts to measure microbial biomass in our soils using chloroform fumigation extraction techniques were ineffective. This may have been due to low microbial biomass and/or re-sorption of compounds lysed during the fumigation process. The chloroform fumigation extraction technique is also known to have issues with extracting biomass associated with clay surfaces (Alessi et al., 2011) and in our artificial soils, clay surfaces may have been more physically accessible to microbes

compared to a natural soil. In addition, the microbes that survived the sonication and fractionation process were more likely those closely associated with and potentially protected by clays (Dandurand et al., 1994). Due to these limitations in measuring biomass, we applied C substrates at a rate of 40% of estimated microbial biomass. We estimated microbial biomass to be 2.5% of C in the MAOM + sand mixture. We applied each substrate pulse at a rate of 140.8 μg C $g^{-1}$ soil to IL and 89.4 μg C $g^{-1}$ soil to MI mixtures. $^{13}$C-enriched (99 atom percent excess) and unenriched glucose and oxalic acid were dissolved in water to obtain a target level of 25 atom percent excess for each substrate solution.

Over the course of the 12-day incubation, substrate solutions were applied to soil microcosms on day 0, 6, and 11 in three distinct pulses (Fig. 3.2). A subset of samples was processed two days after each substrate addition, making number of substrate additions and thus total C applied a separate treatment level. We chose to process soils two days after substrate addition as we expected shifts in enzyme activity and microbial processing to require at least 24-48 hours to manifest. While changes in respiration can occur immediately after substrate addition, other processes requiring more energy and metabolic steps, such as enzyme synthesis, likely require several more hours to manifest. We decided to apply multiple pulses as we suspected the effects on microbes, such as enzyme activities and priming of MAOM, to intensify with each successive pulse of substrate. The three pulse treatment levels (glucose, oxalic acid, and water) and three pulse additions (1, 2 and 3) were applied, in combination, to three replicate microcosms in both soil types (IL and MI). This resulted in a total of 36 individual microcosms.

*$CO_2$ analyses*

Headspace gas sampling was conducted on a 5-day schedule following the first two substrate additions: initially, 4.8, 12, 24, 36, and 48 hours after substrate addition and then daily until the subsequent substrate addition. Following the third and final substrate addition, gas sampling was also conducted at hours 4.8, 12, 24, 36, and 48, but then stopped for the final harvest. Prior to each gas sampling interval, jars were left open to equilibrate with atmospheric concentrations. Lids fitted with rubber septa were then used to seal the jars. At the end of each sampling interval, we removed 15 mL of headspace for $^{13}C$ isotopic analyses. Isotope samples were transferred to pre-evacuated and helium-flushed 12 mL exetainers (LabCo Limited) and then analyzed at the UC-Davis Stable Isotope Facility using gas bench/isotope ratio mass spectrometry. Gas samples from blank jars were analyzed to correct for background $CO_2$ concentration and $^{13}C/^{12}C$ isotope ratio.

We used an isotope mixing model to quantify the MAOM and substrate-derived $CO_2$ in gas samples after accounting for blank $CO_2$ and $\delta^{13}C$ values (Gearing et al., 1991):

$$[\mathrm{C}]_{\mathrm{CO_2}} \cdot r_{\mathrm{CO_2}} = [\mathrm{C}]_{\mathrm{BG}} \cdot r_{\mathrm{BG}} + [\mathrm{C}]_{\mathrm{appG}} \cdot r_{\mathrm{appG}}$$

$$[\mathrm{C}]_{\mathrm{CO_2}} = [\mathrm{C}]_{\mathrm{BG}} + [\mathrm{C}]_{\mathrm{appG}}$$

$$f_{\mathrm{MAOMC-CO_2}} = \frac{\delta^{13}C_{prime} - \delta^{13}C_{substrate}}{\delta^{13}C_{soil} - \delta^{13}C_{substrate}}$$

$$f_{\mathrm{SubstrateC-CO_2}} = 1 - f_{\mathrm{MAOMC-CO_2}}$$

$CO_2$ = sample, BG = background, appG = primed

*Enzyme analyses*

Potential enzyme activities were determined using fluorometric and colorimetric-based microplate assays (Saiya-Cork et al., 2002). For fluorometric assays of hydrolytic enzyme activities, we used the following 4-methylumbelliferyl labeled substrates: β-D-cellobioside for cellobiohydrolase (CBH), N-acetyl-β-D-glucosaminide for N-acetyl-glucosaminidase (NAG), and β-D-glucopyranoside for β-glucosidase (BG). We used 7-amino-4-methyle coumarin-labelled substrates for the following proteases: leucine (LAP) and tyrosine amino-peptidase (TAP). All hydrolytic enzymes were incubated at 140 min at room temperature in a sodium acetate buffer (pH 5.5) and measured fluorometrically at excitation 365 nm and emission 450 nm. Phenoloxidase (PHENOX) and peroxidase (POX) enzyme activities were determined colorimetrically using L-3,4-dihydroxyphenylalanine (DOPA) as a substrate.

*PLFA analyses*

Following the first substrate addition, 1.2 g subsamples were collected and freeze-dried for phospholipid fatty acid extraction. To each sample, we added a phosphate/chloroform/methanol buffer (0.8:1:2, v:v) in order to extract all microbial lipids from the soil matrix. The solution was vortexed for 30 seconds then sonicated in the dark for 15 minutes. Following centrifugation for 20 minutes at 400 x g, the supernatant was separated, saved, and the extraction repeated two more times. Phosphate buffer and chloroform was added to combined supernatants (0.8:1, v:v), shaken for 1 minute, then centrifuged for 5 minutes at 400 x g to allow the phases to separate. The organic phase was removed and evaporated under $N_2$ gas until just dry but still appearing

greasy. 150 µl of chloroform was added to each sample, then stored at –0º C until extracted further with silica column chromatography. Polar lipids were first extracted and purified with silica column chromatography and methanol washes as described in Bligh and Dyer (1959). Lipids were converted to fatty acid methyl esters (FAMES) following additions of methanolic potassium hydroxide and heat treatments. FAMES were dried under $N_2$ gas and stored until analysis on a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (Palo Alto, CA). We compared peaks against a standard library to determine the biomass and relative abundance of microbial groups.

Individual lipids were used as biomarkers for broad groups of microorganisms: 16:1ω5c for arbuscular mycorrhizal fungi (AMF), 18:2ω6,9c for saprotrophic fungi (fungi) (Balser et al., 2005), 16:1ω7c for Gram-negative bacteria, 15:0 iso for Gram-positive bacteria (Wilkinson et al., 2002), and 16:0 10 methyl as an indicator of actinomycetes Frostegård et al. (1993). Biomass of PLFA (nmol lipid $g^{-1}$ dry weight) was determined using an internal standard (19:0 nonadecanoic methyl ester). Relative abundance of individual PLFAs was expressed as mole percentage (mol%) of total PLFA.

*Inorganic N pools and transformation rates*

The C and N content of samples was measured using a Costech Elemental Analyzer. Soil pH was determined on fresh samples using a 1:5 soil/water ratio. Fresh soil samples were extracted with 2M KCl, filtered through ash-free cellulose paper, and analyzed for inorganic and organic N. Ammonium ($NH_4^+$) and nitrate ($NO_3^-$) concentrations in extracts were determined colorimetrically (Foster, 1995; Miranda et al., 2001). Gross rates of microbial $NH_4^+$ transformation were determined using $^{15}N$ pool dilution-based methods (Barrett and Burke,

2000). Briefly, 250 µL of $^{15}NH_4Cl$ (0.125 mM, 10 atom% excess) was applied uniformly to 2g of sample and shaken vigorously by hand. $NH_4^+$ added was under 20% of background concentrations. Samples were incubated for a period of 4 hours and 24 hours. Incubations were stopped by extracting samples with 7.5mL of 2M KCl and shaken horizontally for 30 minutes. Samples and blank solutions were filtered through ash-free paper and frozen until diffusion. The extracted ammonium was diffused to acid traps in a basic solution to concentrate the $^{15}N$ for analysis on the Elemental Analysis – Isotope Ratio Mass Spectrometry (EA-IRMS). One set of soils were extracted after 4 hours and a second set was extracted after a 24-hour incubation. The following equation was applied for the calculation of gross N mineralization.

$$M = \frac{([NH_4^+]_0 - [NH_4^+]_t)}{t} \cdot \frac{\log(APE_0/APE_t)}{\log([NH_4^+]_0/[NH_4^+]_t)}$$

M = Gross-mineralization in mg $kg^{-1}$ $d^{-1}$

$[NH_4^+]_0$, $[NH_4^+]_t$ = Concentration of ammonium at time 0 (=4h) and t (=24h) in mg $kg^{-1}$ $APE_0$, $APE_t$ = Atom percent-$^{15}N$ of $NH_4^+$-Pools at time 0 and t

Due to incomplete diffusion of ammonium, we applied a background correction to measured concentrations and isotopic signatures. We determined diffused concentrations and isotopic signatures for samples of known concentration and isotopic enrichment across a range of concentrations. The difference between diffused and non-diffused standard was used to calculate the blank correction (Hart et al., 1994; Stark and Hart, 1996).

*Follow-up experiment with sterile and non-sterile soils*

We conducted a follow-up experiment to assess the mobilizing effect of C substrates on DON and metals. We set up microcosms with identical mixtures of MAOM and acid-washed sand but created an additional set to be sterilized. We expected oxalic acid to act directly (non-microbially) on organo-mineral associations, thus we required a sterile "control" to determine the effect of oxalic acid in the absence of an active microbial community. After bringing microcosms to the target moisture levels (60% of water holding capacity), and following a 2-week pre-incubation, we sterilized a subset of samples using gamma irradiation. Samples were irradiated to 54 kGy and immediately returned to the lab to initiate the experiment. Sterility was confirmed by aseptically mixing soil and autoclaved water in a 1:100 and 1:1000 soil/water ratio. After vortexing the suspension for 5 seconds, aliquots of the supernatant were removed and spread onto lysogeny broth and yeast malt agar plates. Single colony isolates did not develop on the plates, which were monitored daily for 7 days, thus confirming sterility. Glucose, oxalic acid, and water were added in a single pulse to sterile and non-sterile microcosms using the same rate as in Experiment 1. Microcosms were incubated for two days. Fresh soil samples were extracted with 1M KCl, filtered through 0.2 µm syringe filters (Whatman GD/X) and analyzed for dissolved organic N (DON) concentrations.

Freeze-dried subsamples were extracted sequentially to isolate reactive mineral phases (Heckman et al., 2018; Keiluweit et al., 2015). Water was first used to isolate the freely available metals, followed by ammonium acetate (1M; pH 4.8) to isolate acid soluble and exchangeable metals, then finally ammonium oxalate (0.2M; pH 3.4), to isolate metals associated with short range order minerals (Dold, 2003). 1g of soil was mixed with 30 mL of extractant solution in a 50 mL polypropylene copolymer centrifuge tube. Samples were vortexed for 20 seconds, shaken

for 4 hours at 200 RPM in end-to-end orientation, then centrifuged for 30 minutes at 4,500 x g. After centrifugation, the supernatant was filtered through 0.2 µm nylon syringe filters (Whatman GD/X). Following filtration, samples were stored at -20 until analysis through inductively coupled plasma optical emission spectrometry (ICP-OES).

*Priming effect calculations and statistical analyses*

The C-substrate induced priming of measured response variables was calculated as follows:

$$\text{Priming Effect (\%)} = \frac{\text{Response}_{\text{treatment}} - \text{Response}_{\text{control}}}{\text{Response}_{\text{control}}} \times 100$$

Given our experimental design, we were unable to completely separate the effects of time and pulse number; as such, we also assessed the cumulative effect by summing values for select measurements from all pulse treatments. We used one-way ANOVA to analyze the effect of C substrate on cumulative $CO_2$ respired and enzyme activities. Potential enzyme activities were also analyzed with two-way ANOVA to assess the effect of substrate identity, number of substrate additions, and their interaction. For all measures, individual differences due to substrate identity were tested by Tukey test when ANOVA was significant. Data that violated assumptions of normality were normalized with square root transformations Due to limitations on replication, we chose a probability level of 0.10 in our statistical tests. All statistical analyses were carried out with R Studio (Version 1.0.136).

We performed a principal component analysis (PCA) that included $NH_4^+$ concentrations, gross N mineralization, DON concentrations, and MAOM-C respiration rates at the time point corresponding most closely to the time of soil processing. PLFA relative abundance and $^{13}C$-

incorporation was analyzed with non-metric multidimensional scaling (NMDS). The Gower distance measure was chosen as it is robust in the case of missing data (Marshall Brown et al., 2012). The relationship between extractable metals and DON was analyzed with Spearman correlation as the data were non-symmetric and thus not suitable for parametric analyses.

## Results

### *Experiment 1: $CO_2$ respiration*

Relative to the control (water only), glucose-treated soils respired 173% and 104% more C in IL and MI samples, respectively (Fig. 3.3). Oxalic acid-treated soils respired 129% and 68% more C in IL and MI, respectively. Overall rates of $CO_2$ production were comparable between oxalic acid treated and control soils following the first substrate addition. Rates began to increase significantly following the second oxalic acid addition and gradually increased over time and by the third addition, $CO_2$ respiration rates were, at their peak, 247-452% higher than control rates.

In both soil types we observed a net positive priming effect following glucose and oxalic acid additions—i.e., microbes were degrading and respiring C in MAOM at a faster rate than in control soils. The priming effect increased in IL up to 553% in glucose treated soils and up to 428% in oxalic acid-treated soils. In MI, the priming effect increased up to 342% and 211% in glucose and oxalic acid-treated soils, respectively. Both substrate types caused significant increases in cumulative MAOM-C respired ($P < 0.05$). Glucose additions increased total MAOM-C respired by 89.2% and 55% relative to the control in IL and MI, respectively. Over time, the patterns in MAOM-C degradation aligned with those of gross flux rates. Relative to the control, oxalic acid additions caused a 35% increase in total MAOM-C respired in MI and a 61%

increase in IL (Fig. 3.4; $P < 0.05$). Both IL and MI were characterized by an initial suppression of MAOM-C respiration by oxalic acid additions until the second pulse addition, after which positive priming of MAOM-C respiration was maintained.

*Experiment 1: Potential enzyme activities*

Pulse addition number had a significant effect on all enzyme activities where, in general, enzyme activities increased with each additional pulse and differences between treatments became most apparent by the final pulse addition (Appendix Table 3.1). Substrate identity also influenced the microbial production of hydrolytic and oxidative enzymes. Patterns between soil types were similar but between enzyme groups (i.e., C-acquiring, N-acquiring and oxidative), the priming effect varied in magnitude and direction. When summing enzyme activities across pulse additions, glucose additions enhanced the production of exo-cellulases, cellobiohydrolase and β-glucosidase, in both soil types (Fig. 3.5). Glucose additions enhanced the activities N-acquiring activities only in the IL soil, where N-acetyl-glucosaminidase increased by 39.3% and leucine aminopeptidase by 28%. Oxalic acid additions either caused no change or a decrease in all hydrolytic enzyme activities. Peroxidase was the only enzyme to respond positively and significantly to oxalic acid additions; we observed a 45.3% increase in IL and a 91.1% increase in MI. Glucose additions also stimulated peroxidase and phenoloxidase activities in MI alone and by 109.9% and 88.1%, respectively.

*Experiment 1: Soil physicochemical measures*

There were no significant differences in total soil C concentrations between substrate treatments (data not shown). Oxalic acid caused significant declines in pH in all soil types and pulse treatments ($P$ <0.05). In IL soils, pH dropped on average from 6.8 to 6.2 and in MI, from 6.8 to 5.8. Nitrate concentrations were consistently below detection limits. As such, for inorganic N pools, only ammonium data are presented (Table 3.2). Glucose and oxalic acid additions led to net decreases in ammonium and DON with concentrations declining further with each additional pulse. Gross N mineralization data were below detection limits for oxalic acid-treated soils receiving two pulses. Substrate identity did not affect rates of gross ammonification. However, in a PCA that included gross N mineralization, $NH_4^+$ concentrations, rates of MAOM degradation at time of soil processing and enzyme activities, there was a visible separation of points by substrate treatment (Fig. 3.6). The first two axes accounted for 69.5% of the variation. Separation between control and glucose treatments occurred primarily along PC axis 1, which explained 42.6% of the variation, and along which MAOM-C priming and gross N mineralization were most closely associated. Separation between control and oxalic acid treatments occurred primarily along PC axis 2, with oxalic acid treatments positively associated with DON concentrations.

*Experiment 1: Microbial community composition*

PLFA were not detectable for certain microbial groups leading to a large proportion of missing data in the microbial community dataset. We did not detect significant differences in overall microbial biomass or biomass within microbial groups (Appendix Fig. 3.1). Due to the sparse

nature of the data, we used an NMDS to examine community composition. Substrate treatments caused significant shifts in microbial community composition ($P$ <0.01) with separation significantly and positively associated with the relative abundances of fungi ($P$ <0.001) and Gram-positive bacteria ($P$ <0.05; Fig. 3.7).

*Experiment 2: Metal and DON concentrations in exchangeable pools*

In both sterile and non-sterile soils, oxalic acid additions were associated with increased metal concentrations in exchangeable pools. Between sterile and non-sterile treatments, overall treatment effects were similar. While the release of Fe and Al was not significant in MI, it was significant in IL: oxalic acid caused a 96-105% increase of Fe into water-extractable and an 89-173% increase into ammonium acetate-extractable in sterile and non-sterile soils. Changes in Al concentration were similar: 39-62% increase into water-extractable and 42-93% increase into ammonium acetate-extractable pools. Data presented in Fig. 3.8 are the total metal concentrations in exchangeable pools (water-extractable + ammonium acetate-extractable).

Sterilization with gamma irradiation caused substantial release of DON, as indicated by the near doubling of DON in the sterile relative to the non-sterile control samples. As such, we corrected measured values in sterile soils receiving glucose and oxalic acid to account for the net release of DON by sterilization. When aggregating data from both sterility treatments and soil types, total Fe concentrations in exchangeable pools correlated positively and significantly with DON (Appendix Fig. 3.2). After sub-setting data by treatment, the correlation between DON and exchangeable Fe was only significant in oxalic acid-treated soils (Appendix Fig. 3.3).

## Discussion

The root exudate-induced suppression or acceleration of SOM degradation can significantly alter the net balance of soil C and N (Meier et al., 2017; Zhu et al., 2014). The priming effect occurs across an array of ecosystems (Huo et al., 2017) and in response to a wide range of C compounds (Dungait et al., 2013; Renella et al., 2007). Priming is a ubiquitous process and yet, the specific origins of primed C or N within SOM remains a critical knowledge gap. The focus in priming studies on wholesale changes to C or N stocks overlook the chemical, physical and functional diversity of compounds within SOM.

Here, we present evidence that suggests priming accelerates turnover of C and N in MAOM, a pool considered far more stable, even inert or relatively passive, in prevailing theories on SOM cycling (Campbell and Paustian, 2015). Our study is the first to demonstrate the specific mechanisms by which simulated root exudates mobilize C and N, specifically from MAOM. Two compounds commonly released by plant roots, glucose and oxalic acid, stimulated MAOM turnover through microbial and non-microbial mechanisms. Both substrates increased the activity of microbial communities and triggered the mobilization of additional C and N from MAOM thus providing proof of concept for the hypothesized pathways of MAOM destabilization. We expected the two substrates to mobilize MAOM through distinct mechanisms—glucose, through an indirect and microbially mediated pathway (Mechanism 1; Fig. 3.1), and oxalic acid, through primarily the direct destabilization of organo-mineral bonds (Mechanism 2; Fig. 3.1). Our results point to the stimulation of these different mechanisms by different types of simulated exudates, and more generally reveal that a pool of soil nutrients generally considered passive or inert has the potential to function as a significant source of C and N.

*Higher priming of MAOM decomposition and microbial activity in glucose-treated soils*

$CO_2$ respiration rates increased significantly following substrate additions, with glucose causing distinctive spikes in respiration and returning to near basal rates generally within 36 to 48 hours. Oxalic acid treatments, on the other hand, were characterized by more gradual increases in respiration. Based on the $^{13}C$ signature of the $CO_2$ respired, we also determined that both substrates stimulated the increased degradation of MAOM-C (positive priming), with net increases in MAOM-C respiration occurring within hours of the first glucose addition in both soil types. The immediate and relatively short-lived (< 48-hour) spikes in respiration following glucose additions align with patterns observed in other priming studies (Hamer and Marschner, 2005; H. Zhang et al., 2016). Glucose is used readily by microbes and typically allocated quickly toward metabolic pathways (Chiellini et al., 2007).

*Evidence of N-mining in glucose-treated soils*

Increased MAOM-C degradation occurred in tandem with net immobilization of ammonium, likely due to N-limitations imposed by the labile C substrates (Landi et al., 2005). As microbial activity and MAOM-C use increased, N demand increased accordingly. Overall, gross N mineralization rates were at low rates typical of mineral soils (Wild et al., 2018). We did not detect significant substrate effects on gross N mineralization based on ANOVA, but we did observe different patterns in control and glucose treatments when assessing MAOM-C degradation and N data, collectively. In a multivariate analysis, priming of MAOM-C respiration

and gross N mineralization were associated with glucose-treated soils while DON and ammonium concentrations were associated with the control treatment. Microbes were degrading N from MAOM at a faster rate in glucose-treated soils, but the N released was immediately immobilized leading to a net decrease in ammonium pools. This suggests glucose may be stimulating the microbial mining of both C and N from MAOM, but that microbes are retaining extra N released by mineralization.

The extent to which labile C inputs stimulate the release of C or N from SOM is mediated partly by microbial demand for N. Labile C substrates subsidize energy demands of microbes and support the mining of N from SOM to achieve stoichiometric balance (Mooshammer et al., 2014b). The N-mining hypothesis is based on the principle that priming of SOM should decrease with increased N availability and applies specifically to environments of low N and high C availability (Chena et al., 2013). Labile C substrates released by roots stimulate microbial activity and proliferation, which in turn leads to microbial N-limitation. Given the low C:N of MAOM in our microcosm, it is probable that the C within MAOM was derived from N-rich and low molecular-weight organic compounds. The coupled C and N release from MAOM and the overall magnitude of MAOM-C priming may be in part due to the chemical composition of the MAOM itself. In a priming study using $^{13}C$ and $^{15}N$ based approaches to assess the relative release of C and N following C addition, the primed (i.e., glucose-treated) flux had a significantly lower C:N ratio than that of the control, leading the authors to conclude that priming is a "response mechanism to increase soil N supply" (Murphy et al., 2015).

*Oxalic acid additions trigger the concomitant release of DON and metals*

We observed significant mobilization of metals into exchangeable pools following oxalic acid additions. This release occurred in both sterile and non-sterile soils, which suggests microbial activity did not significantly alter the mechanism acting on metal-organic associations. Our results thus provide evidence for the direct destabilization of MAOM by oxalic acid—that is, without microbial intervention. The release of Fe and Al into exchangeable pools was also positively associated with DON, indicating the destabilizing effect of oxalic acid acts upon both metals and DON bound up in MAOM. The release of metals by oxalic acid was more pronounced in IL soils. This suggests that in this particular soil type, metal-organic bonds may play a more important role in the stabilization and destabilization patterns of MAOM. The IL soil has a higher CEC, and x-ray diffraction (XRD) analyses I conducted indicates this may be related to a higher proportion of reactive 2:1 clays smectite. MAOM fractions in this soil may support a higher proportion of metal-organic interactions.

*Simulated root exudates caused shifts in microbial physiology and community*

The initial attenuated response to oxalic acid additions may have been due to pH changes suppressing the microbial community (Fernández-Calviño and Bååth, 2010) or favoring the growth of a more specialized, potentially slower growing, microbial community (Favilli and Messini, 1990). In a multivariate analysis of PLFA relative abundance, oxalic acid-treated soils were associated with increases in fungal relative abundance. This was likely related to the near absence of detectable fungi in control and glucose-treated soils. Oxalic acid appears to have

activated the fungal community, but otherwise did not influence bacterial biomass. Both bacteria and fungi can use oxalic acid, however, bacteria may require the presence of fungi in order to metabolize the substrate (Martin et al., 2012).

It has been proposed that fungi serve as a highway by which bacteria access insoluble forms of oxalic acid, such as Ca-oxalate (Palmieri et al., 2019) and that oxalic acid may play an important role in fungal-bacterial interactions. Oxalic acid may promote the co-occurrence of bacteria and fungi in mineral environments that are often bacterial-dominated in non-rhizosphere environments (Smith et al., 2014). As we did not isolate PLFA after the second or third pulse, we are unable to speak to potential shifts in microbial community over the course of the experiment. Following substrate additions, Chena et al., (2013) reported shifts toward K-selected (i.e., slower-growing) microbial species, but the kinetics of microbial growth rate were measured 9 days after substrate addition. We suspect that in our microcosms, successional processes would have led to increased divergence in community composition over time.

We observed distinct responses in enzyme activities to C substrates, which provides further evidence that glucose and oxalic acid were supporting microbial physiological shifts. However, for most enzymes, differences were significant only by the third and final substrate addition. The activities of both C-acquiring hydrolytic enzymes and the chitinase increased in glucose treatments. Oxalic acid additions caused either no change or a decrease in hydrolytic enzyme activities. Glucose additions led to higher rates of enzyme production compared to oxalic acid, suggesting a higher degree of microbial activation overall. Oxalic acid additions stimulated the production of peroxidase, an oxidative enzyme, which may be related to shifts in microbial physiology, community composition (i.e., toward fungal dominance), or an abiotic effect of the oxalic acid. Oxalic acid can act as a proton donor, providing reactive oxygen species

that activate hydrolytic and oxidative enzymes (Palmieri et al., 2019). The observed increase in peroxidase enzymes may have been due to the activation of enzymes already present in the mineral environment. Minerals can bind enzyme molecules, rendering them inactive and inaccessible to potential decomposers (Quiquampoix et al., 1993). Organic acids may have thus provided an abiotic mechanism by which mineral-bound enzymes can be reactivated.

*Synthesis and future directions*

The overall patterns in priming of MAOM-C degradation and enzyme activities were similar between the two soil types. The primary differences between soil types were in the magnitude of MAOM-C priming and in the response to oxalic acid additions. As we only included two soil types, we cannot speak specifically to the properties or processes that drove these differences. Given that mineralogy can influence the strength of organo-mineral associations, as well as microbial community composition (Hutchens et al., 2010), succession (Ditterich et al., 2016), and physiology, we believe there is a need to assess the extent and nature of priming across a wide range of soil types.

The stimulation of enzyme activities and $CO_2$ respiration in primed soils is consistent with priming studies using similar trace-level concentrations. As the rate of exudate input is known to mediate the magnitude and direction of priming (de Graaff et al., 2010), we were careful to select substrate concentrations that would not trigger substrate switching, or negative priming, in which the microbial communities degrade mostly or exclusively the new labile C input (Kuzyakov, 2002). Both oxalic acid and glucose additions can cause negative priming, but this is generally at concentrations much higher that used in our experiment (Zhang et al., 2019). Our chosen exudate concentrations appeared to be able to stimulate a C-limitation that then

triggered the microbial degradation of C from MAOM. The additional release of C occurred without an apparent increase in microbial biomass, as indicated by PLFA analyses. Thus, the observed increases in overall respiration and MAOM degradation were not due simply to increased microbial biomass. Microbial communities were shifting in physiology, intensifying enzyme production and degrading MAOM-C, likely to meet internal metabolic needs.

In mining N within MAOM, microbes serve as a temporary reservoir for N as evidenced by the net immobilization of inorganic N. The N previously associated with mineral surfaces was thus transferred into a more active fraction, increasing the probability of that N becoming plant-available. If glucose and oxalic acid had been added in combination, then we may have observed a greater extent of N release into soluble pools. Indeed, our follow-up experiment with sterilized soils indicated that oxalic acid can facilitate the release of DON, likely through the destabilization of metal-organic bonds. If glucose and oxalic were added in combination, we may have observed higher rates of gross N mineralization and potentially net mineralization of inorganic N. N-containing compounds released by an organic acid would then become available to microbes that are activated but N-limited due to glucose consumption. We expect a synergism would occur wherein glucose and oxalic acid, together, would facilitate the net release of inorganic N that could be made available to plants.

Applying C substrates as distinct pulses led to clear spikes and crashes in C mineralization. Likely N mineralization and other microbial processes experienced similar fluctuations, which may have influenced the cumulative effect of simulated exudates on MAOM turnover. A continuous application of substrates would have sustained and potentially enhanced microbial activity, growth, and N-mining. When applied as pulses, substrate depletion leads to rapid declines in activity, which may have increased microbial stress and turnover. Without the

fluctuations in substrate availability and activity, we expect microbes would have become more consistently N-limited.

*Conclusion*

Our results demonstrate the potential for simulated root exudates to trigger both microbial and non-microbial pathways of MAOM destabilization. Although the soils in our microcosms lacked the full range of physiochemical and biological properties of a natural soil, our results provide proof of concept for the plant-mediated mechanisms that increase MAOM turnover. Both C and N were transferred from MAOM into bioavailable pools, with glucose working primarily via the microbial community and oxalic acid directly destabilizing metal-organic interactions. Our findings counter the prevailing perspective that MAOM is an inert or passive pool. Rather, MAOM is dynamic and can potentially behave as a source of C and N in rhizosphere environments.

**Table 3.1**. Site, soil, and MAOM characteristics. Mean Annual Precipitation (MAP) and Mean Annual Temperature (MAT) figures are 30-year growing season means (April–October in IL; May-October for MI)

| Site and Soil Properties | | | | | | | | MAOM Properties | |
|---|---|---|---|---|---|---|---|---|---|
| **Soil ID** | **Location** | **MAP** (cm) | **MAT** (ºC) | **Texture** | | | **CEC** (cmol $kg^{-1}$) | **C Conc.** (mg C $g^{-1}$ MAOM) | **C/N** |
| | | | | Sand (%) | Silt (%) | Clay (%) | | | |
| IL | Champaign, IL (40º 3', –88º 15') | 61.6 | 18.3 | 14.0 | 65.0 | 21.0 | 21.4 | 35.2 | 11.3 |
| MI | Mason, MI (42º 24', –85º 24') | 48.0 | 17.3 | 59.4 | 33.2 | 7.4 | 8.54 | 22.4 | 9.03 |

**Table 3.2.** Substrate treatment effects on dissolved N pools and transformations. Average values are presented with standard error in parentheses. Letters indicate significant differences as determined by Tukey tests.

| | | Illinois | | | Michigan | | |
|---|---|---|---|---|---|---|---|
| **Pulse** | **Substrate** | **DON** (ug N $g^{-1}$soil) | **$NH_4^+$** (ug N $g^{-1}$soil) | **Gross N Min.** (mg N-$NH_4^+$ $kg^{-1}$ $day^{-1}$) | **DON** (ug N $g^{-1}$soil) | **$NH_4^+$** (ug N $g^{-1}$soil) | **Gross N Min.** (mg N-$NH_4^+$ $kg^{-1}$ $day^{-1}$) |
| 1 | Co | 11.5 (0.6) a | 28.6 (1.6) b | 0.5 (0.1) | 23.2 (1.4) a | 25.8 (1.5) ab | 0.6 (0.1) |
| | Gu | 16.0 (3.3) a | 19.4 (2.2) a | 0.7 (0.3) | 18.4 (2.6) a | 23.8 (1.4) a | 0.7 (0.1) |
| | Ox | 26.6 (1.7) b | 26.7 (0.3) b | 0.6 (0.2) | 22.7 (1.8) a | 30.4 (1.9) b | 0.5 (0.2) |
| 2 | Co | 22.4 (0.9) a | 30.4 (0.6) b | 0.3 (0.1) | 26.3 (5.8) b | 30.4 (1.6) b | 0.2 (0.0) |
| | Gu | 19.2 (1.8) a | 13.0 (1.4) a | 0.5 (0.3) | 10.4 (3.1) a | 21.8 (2.5)a | 0.2 (0.1) |
| | Ox | 29.2 (1.8) b | 30.7 (0.6) b | 0.0 (0.0) | 27.7 (1.4) b | 32.2 (1.5) b | NA |
| 3 | Co | 19.5 (1.9) a | 33.2 (1.2) c | 0.4 (0.1) | 17.5 (1.2) b | 40.5 (1.6) c | 0.3 (0.1) |
| | Gu | 16.0 (0.8) a | 6.8 (0.5) a | 0.8 (0.3) | 4.2 (1.3)a | 18.6 (0.7) a | 0.3 (0.1) |
| | Ox | 25.6 (1.3) b | 25.5 (1.6) b | 0.3 (0.1) | 26.1 (1.0) c | 25.0 (0.6) b | 0.2 (0.0) |
| **ANOVA Results** | | | | | | | |
| | Substrate | *** | *** | NS | *** | *** | NS |
| | Pulse | ** | * | NS | * | NS | *** |
| | Sub. x Pulse | † | *** | NS | * | *** | NS |

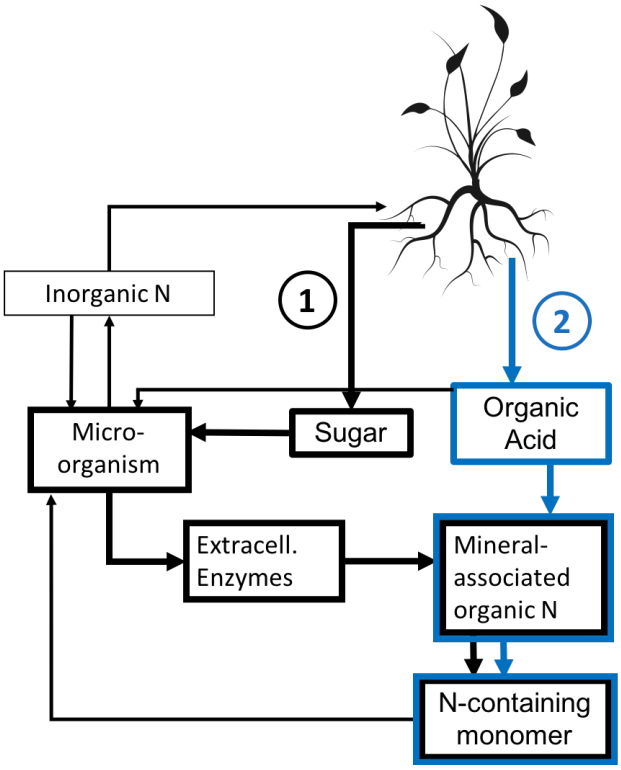


**Figure 3.1.** Conceptual diagram depicting the two pathways of MAOM destabilization. 1) The microbial (indirect) pathway of MAOM destabilization is indicated in bold and black. 2) The non-microbial (direct) pathways of destabilization is indicated in bold and blue.

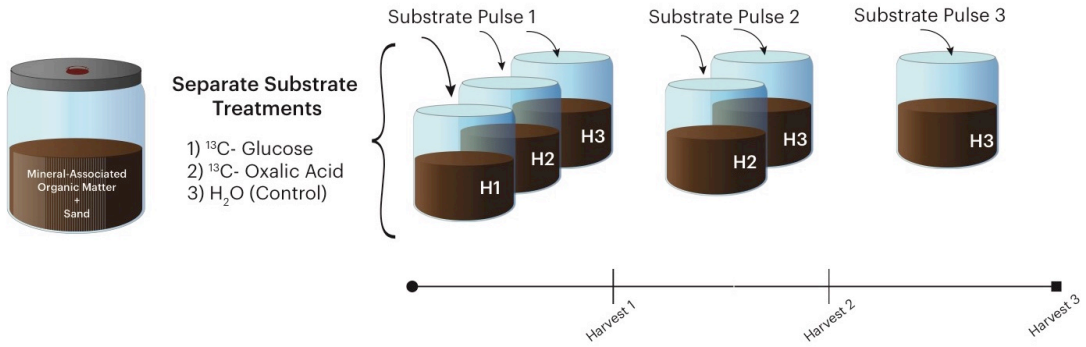


**Figure 3.2.** Schematic of experimental set-up

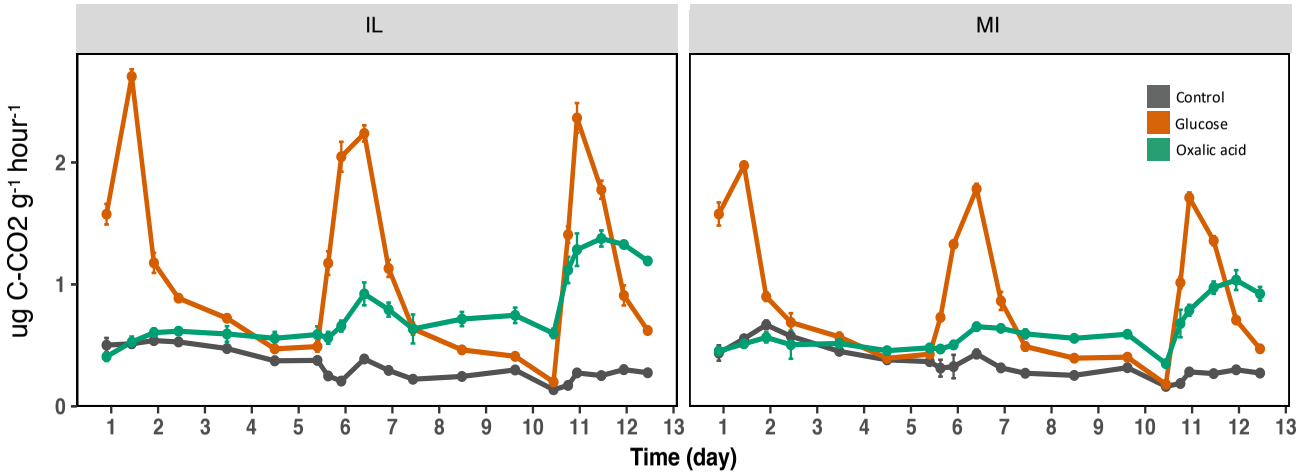


**Figure 3.3.** $CO_2$ respiration within each soil type

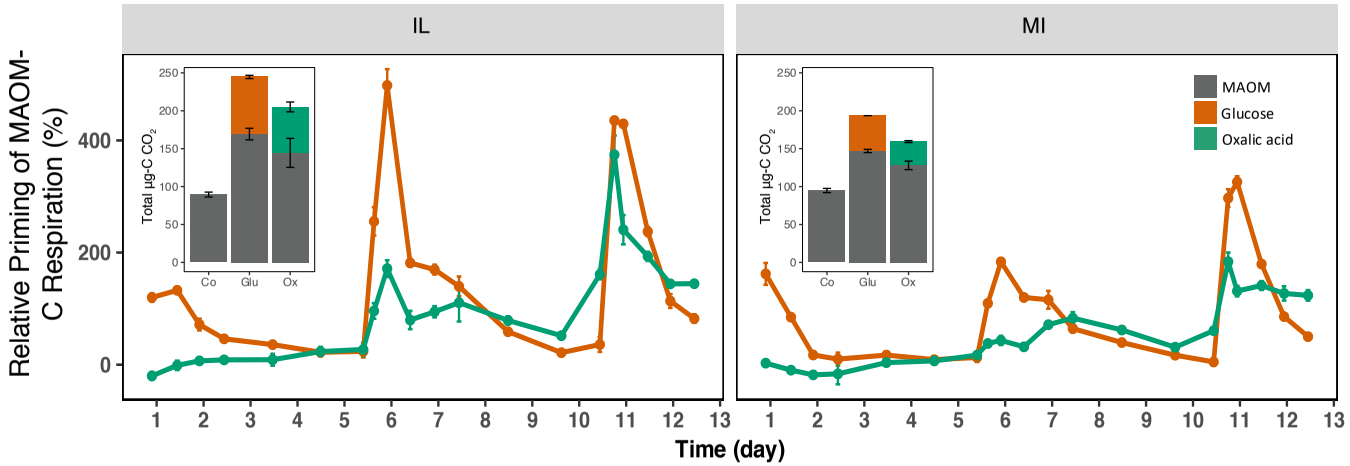


**Figure 3.4.** Relative priming of MAOM-C respiration in each soil type. Bar graph inlay shows the cumulative amount and origin of C respired (MAOM-C vs. substrate-C)

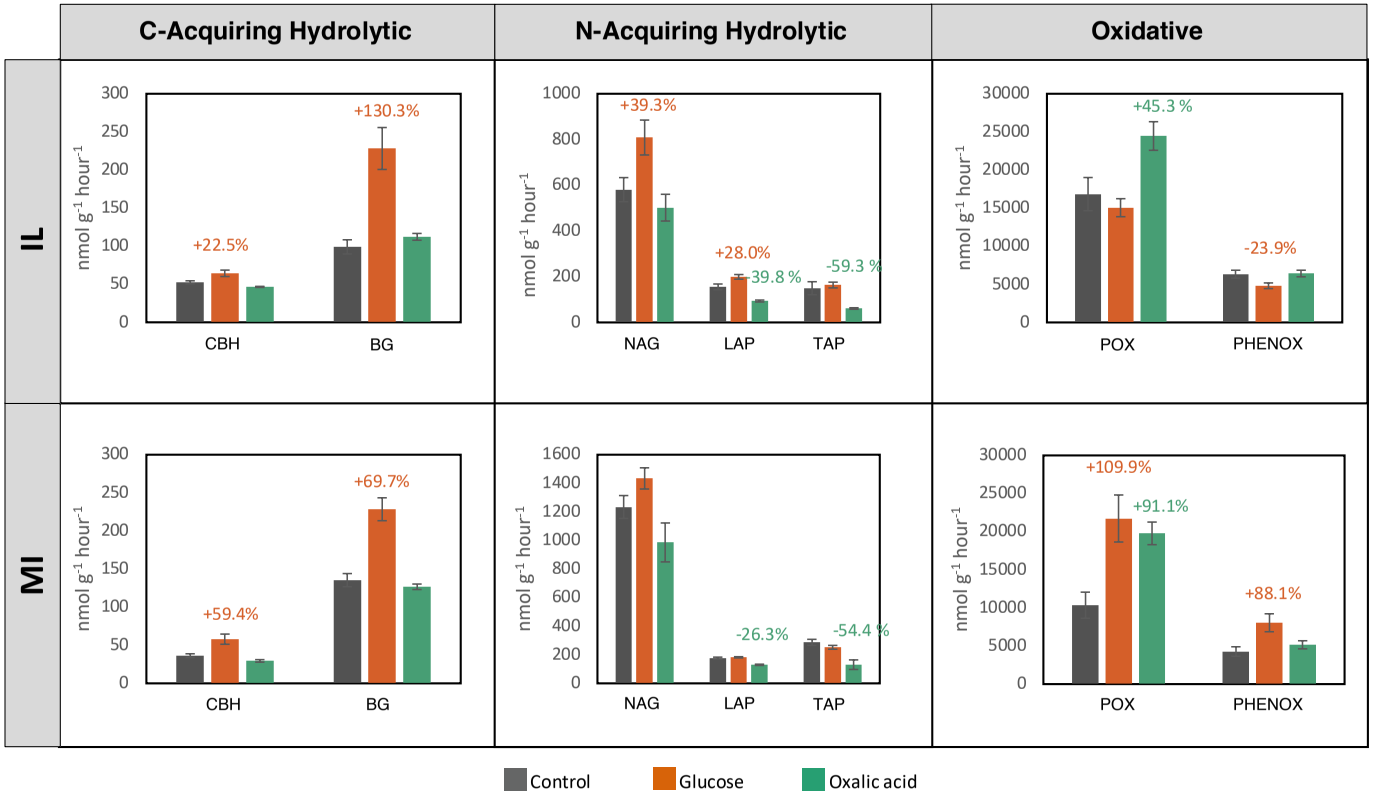


**Figure 3.5.** Substrate treatment effects on hydrolytic and oxidative enzyme activities. Average and standard error from each pulse number treatment were summed to produce the data presented. Values above orange (glucose) and green (oxalic acid) bars represent the relative priming of that enzyme's activity relative to the control. Bars lacking a percent priming values were not statistically significant from the control ($P > 0.10$). CBH = Cellobiohydrolase; BG = β-glucosidase; NAG = *N*-acetyl-glucosaminidase; LAP = leucine aminopeptidase; TAP = tyrosine aminopeptidase.

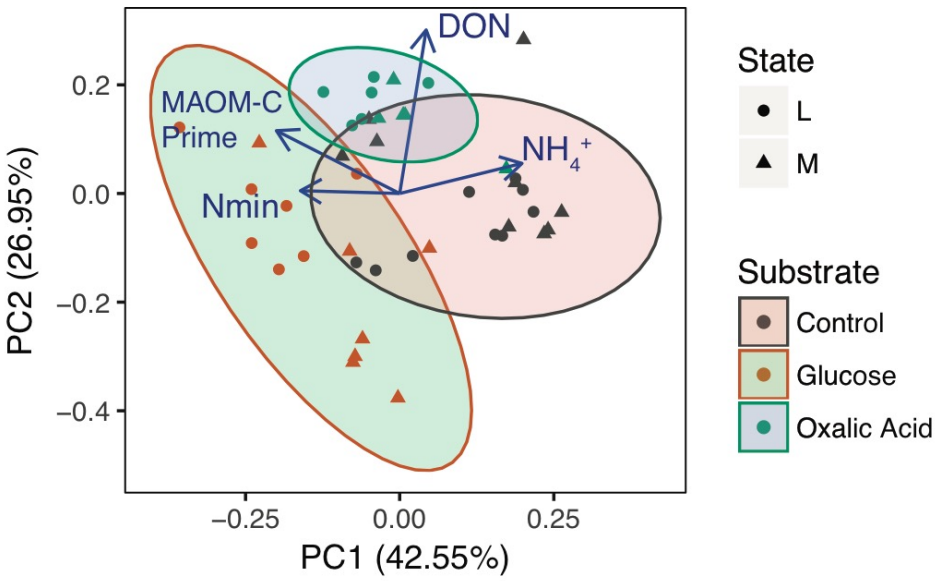


**Figure 3.6.** Principal component analysis (PCA) of DON, gross N mineralization, and MAOM-C respiration rate at time of soil processing. Eigenvectors represented by arrows on the plot.

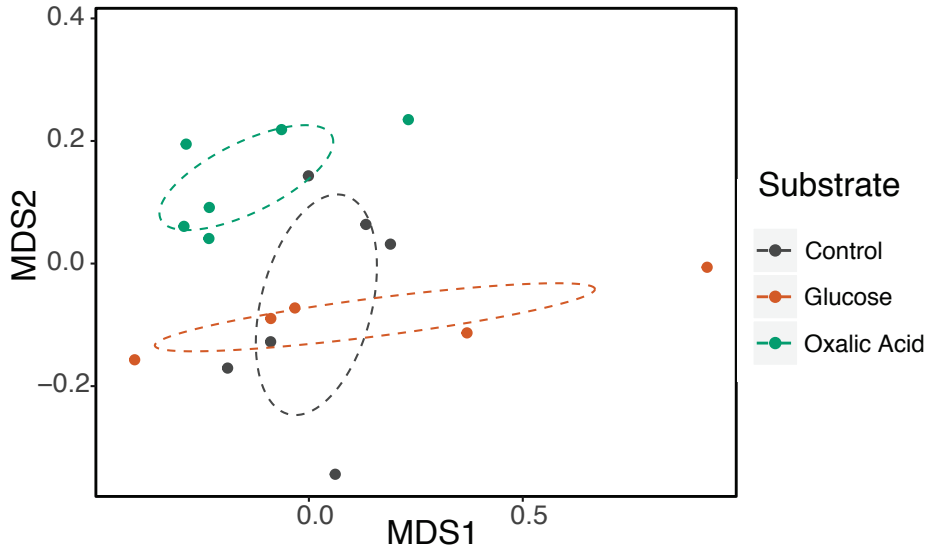


**Figure 3.7.** Dissimilarity of the PLFA relative abundance as determined by non-metric multidimensional scaling (NMDS). Microbial groups included: Gram-positive, Gram-negative, fungal, and actinomycete microbial groups. Stress value = 11.1.

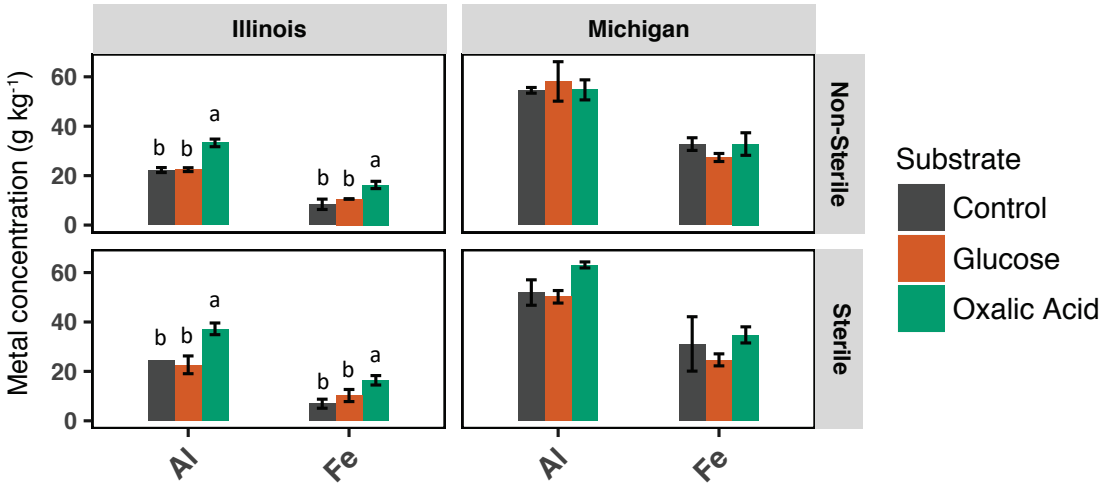


**Figure 3.8.** Fe and Al concentrations in exchangeable pools (water-extractable + ammonium acetate-extractable) separated by state and sterilization.

# CONCLUSION

The research described in this dissertation provides insight into the mechanisms that regulate MAOM turnover and overall, suggests a need to reconsider historical viewpoints on the function and ecological relevance of this pool. Research thus far has characterized MAOM primarily as a sink for soil nutrients. However, my research adds to a now growing body of evidence that demonstrates MAOM is a heterogenous pool and is sensitive to perturbations across varied spatial scales. As I present in Chapter 1, MAOM can respond rapidly to agricultural management, such as to changes in soil disturbance and residue inputs, and may serve as a potential source of N to crops. More research is needed to understand the N-supplying capacity of MAOM and to understand if and how management can be targeted for this function. MAOM accounts for the majority of C and N storage in agricultural soils and aggressive methods to facilitate its turnover could have long-term consequences for SOM stocks. Future research should pursue agricultural management that balances both the nutrient provisioning and stabilizing aspects of this fraction.

In chapters two and three, I examined the micro-scale perturbations that occur within rhizosphere environments. I found that MAOM can be vulnerable, specifically in the soil surrounding roots, where labile root C inputs destabilize MAOM through microbial and non-microbial mechanisms. Clay minerals have the potential to significantly alter the provisioning of soluble C and N. Future experimental work addressing SOM cycling processes will need to test for mineralogical properties more directly and possibly use features of MAOM as treatment variables themselves. Research in this regard could help resolve some of the remaining noise in our current methods for modeling or predicting SOM turnover. More broadly, there is a need to address the mechanisms that control microbial access to MAOM and to determine if these

processes can be scaled up to influence ecosystem-level properties. With the well-established perspective that SOM stabilization is microbially-mediated, future studies should focus on the specific activity and attributes of the microbial community associated with mineral surfaces. Finally, future avenues of research on SOM dynamics may also benefit from looking outside of the sequential framework of litter decomposition and SOM formation; the movement of litter from particulate to mineral-associated and physically protected pools still invokes a linear and one-way progression. This may overlook the feedbacks and nonlinearities of SOM transformations. Specifically, plant-microbial interactions, moisture fluctuations, and the feedback processes therein may impact stabilization and turnover dynamics unpredictably.

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# APPENDIX

**Appendix Table 1.** Predictor variables used in the final reduced PLSR model indicated in bold.

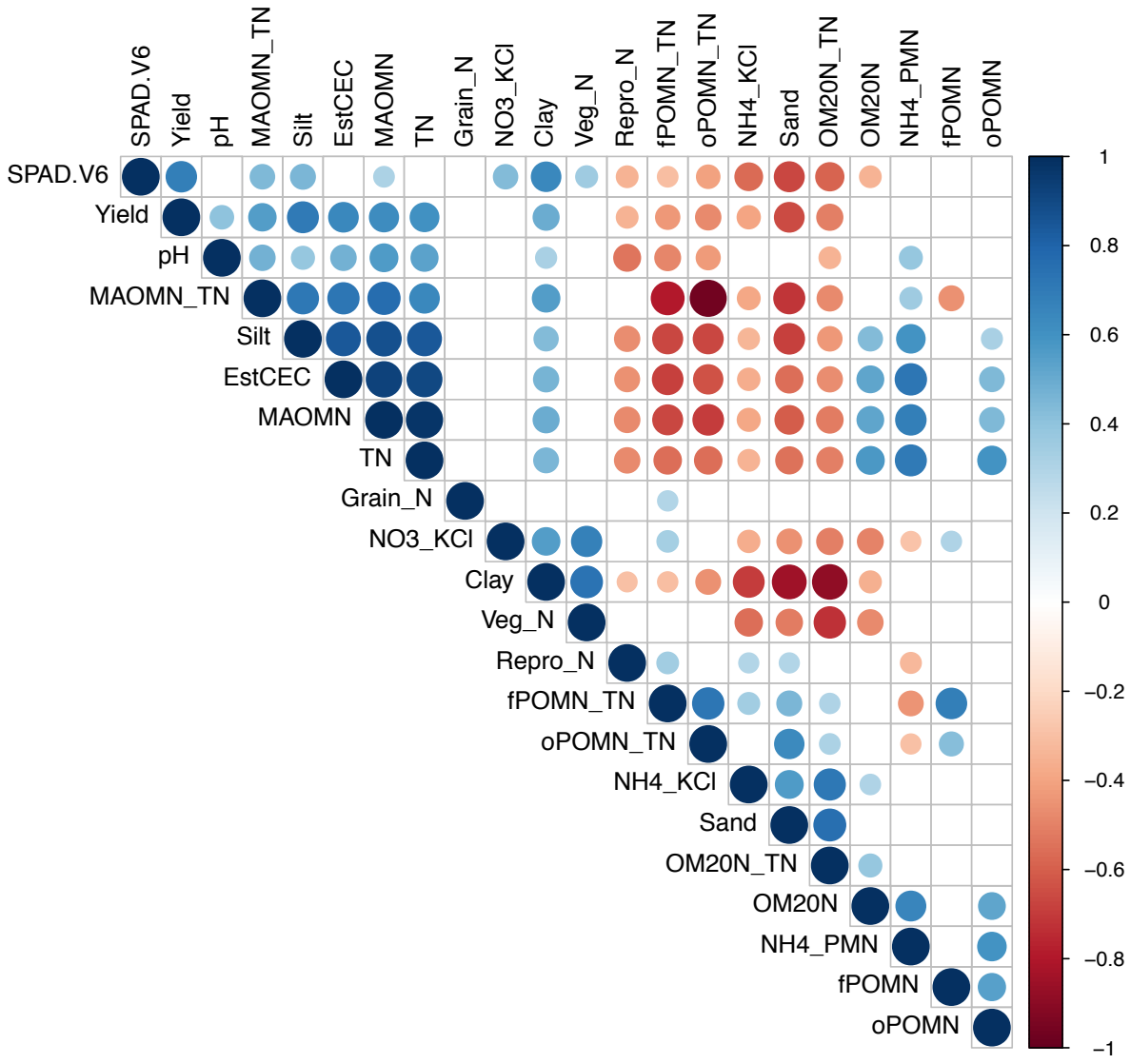
| Predictor | Response |
|---|---|
| pH | Grain - N |
| **CEC** | Reproductive Tissue – N |
| **Sand** | Vegetative Tissue – N |
| **Silt** | KCl-extractable $NH_4^+$ |
| **Clay** | KCl- extractable $NO_3^-$ |
| f-POM N content (fPOMN) | Potentially mineralizable $NH_4^+$ |
| **o-POM N content (oPOMN)** | SPAD at V6 growth stage |
| **c-Silt N content (cSiltN)** | Yield |
| **MAOM N content (MAOMN)** | |
| **f-POMN content relativized to total soil N (fPOMN_TN)** | |
| o-POM N content relativized to total soil N (oPOMN_TN) | |
| **c-Silt N content relativized to total soil N (cSiltN_TN)** | |
| **MAOM N content relativized to total soil N (MAOMN_TN)** | |
| **Total soil N (TN)** | |

**Appendix Table 1.2.** Average values and ANOVA results for measures of soil N availability and crop performance. Standard error in parentheses

| State | Tillage | Cover Crop | Grain_N | Repro_N | Veg_N | SPAD.V6 | Yield | $NH_4^+$ | $NO_3^+$ | PMN |
|---|---|---|---|---|---|---|---|---|---|---|
| IL | Chisel | None | 0.84 (0.07) | 0.43 (0.03) | 0.30 (0.01) | 52.8 (0.6) | 10,563.2 (420.1) | 4.2 (1.5) | 1.7 (0.7) | 5.7 (0.7) |
| | | Rye | 0.82 (0.11) | 0.42 (0.03) | 0.29 (0.02) | 51.0 (1.5) | 10,389.2 (1,377.3) | 3.9 (0.4) | 1.4 (0.5) | 6.3 (0.9) |
| | Ridge | None | 0.80 (0.05) | 0.42 (0.04) | 0.36 (0.04) | 48.3 (3.8) | 7,276.7 (983.6) | 5.9 (0.6) | 5.7 (1.9) | 7.9 (2.0) |
| | | Rye | 0.74 (0.07) | 0.47 (0.02) | 0.36 (0.04) | 50.3 (1.3) | 8,703.1 (560.9) | 5.1 (0.5) | 3.6 (0.7) | 9.8 (0.9) |
| MI | Chisel | None | 0.89 (0.01) | 0.57 (0.03) | 0.29 (0.02) | 48.9 (1.6) | 5,374.7 (304.6) | 19.3 (7.8) | 1.8 (0.4) | 0.5 (0.9) |
| | | Rye | 0.82 (0.01) | 0.48 (0.03) | 0.28 (0.02) | 46.8 (2.3) | 4,892.1 (276.9) | 13.7 (5.2) | 2.6 (1.0) | 1.1 (1.0) |
| | Ridge | None | 0.88 (0.02) | 0.51 (0.04) | 0.30 (0.02) | 47.0 (1.1) | 5,242.0 (334.1) | 33.9 (3.4) | 4.4 (0.5) | -0.4 (0.5) |
| | | Rye | 0.89 (0.02) | 0.67 (0.06) | 0.32 (0.05) | 44.9 (1.2) | 5,009.1 (258.1) | 35.2 (2.1) | 4.7 (1.2) | 0.7 (0.4) |
| PA | Chisel | None | 0.83 (0.05) | 0.44 (0.07) | 0.55 (0.04) | 54.5 (1.6) | 8,311.3 (659.2) | 3.1 (0.7) | 10.2 (1.7) | 1.4 (1.7) |
| | | Rye | 0.84 (0.05) | 0.52 (0.03) | 0.55 (0.03) | 55.1 (1.3) | 8,841.5 (435.6) | 1.7 (0.2) | 13.4 (3.2) | -0.1 (0.0) |
| | Ridge | None | 0.87 (0.04) | 0.43 (0.06) | 0.90 (0.26) | 55.9 (3.0) | 8,592.5 (876.8) | 1.5 (0.5) | 30.2 (4.6) | 0.7 (0.8) |
| | | Rye | 0.98 (0.06) | 0.42 (0.03) | 0.60 (0.03) | 52.0 (1.1) | 6,457.5 (1,038.0) | 1.8 (0.4) | 24.4 (2.5) | 2.0 (1.5) |

**ANOVA Results**

| State | Source | F-Statistic | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| IL | Tillage | 0.1 | 0.0 | 1.9 | 2.6 | 14.6** | 4.1 | 1.9 | 2.4 |
| | Cover Crop | 0.0 | 0.0 | 0.0 | 0.4 | 2.5 | 0.1 | 0.4 | 0.8 |
| | Tillage x Cover | 0.1 | 0.9 | 0.0 | 1.0 | 0.5 | 0.2 | 0.3 | 3.2 |
| MI | Tillage | 3.1 | 1.4 | 0.2 | 0.4 | 0.1 | 4.1 | 4.8 | 0.8 |
| | Cover Crop | 139.7*** | 2.5 | 0.1 | 0.1 | 1.4 | 0.6 | 0.5 | 0.4 |
| | Tillage x Cover | 21.2** | 10.2* | 0.4 | 1.9 | 0.1 | 0.5 | 0.1 | 0.1 |
| PA | Tillage | 6.4* | 0.0 | 0.8 | 4.1 | 0.1 | 5.7* | 16.1** | 0.2 |
| | Cover Crop | 0.0 | 1.5 | 0.7 | 3.7 | 0.3 | 4.5 | 0.4 | 0.8 |
| | Tillage x Cover | 0.8 | 0.9 | 0.4 | 0.2 | 3.4 | 3.5 | 1.8 | 1.5 |

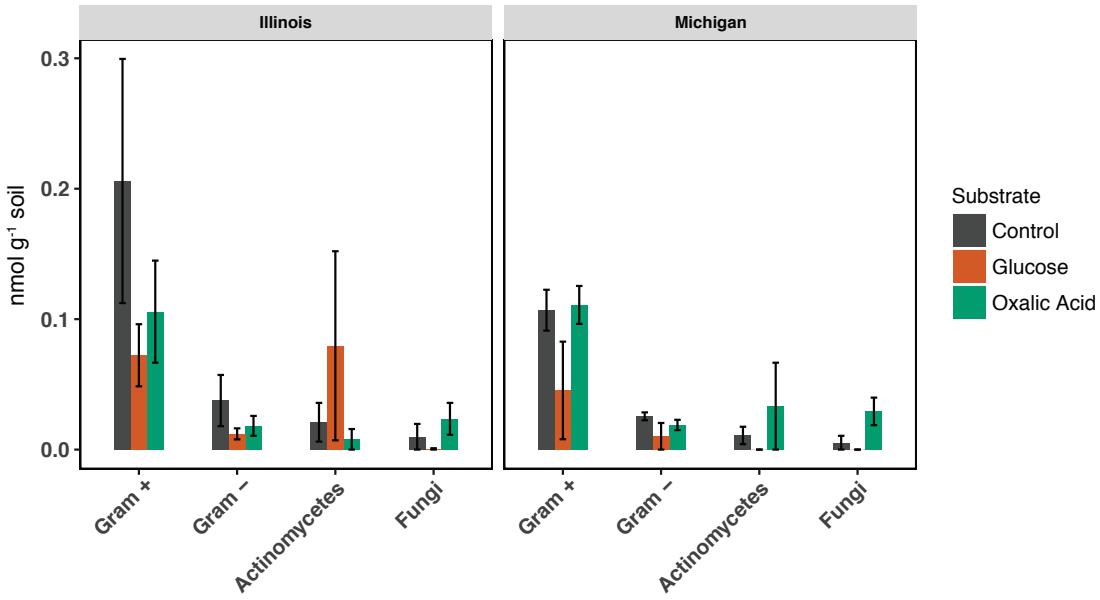



**Appendix Figure 1.1.** Spearman correlation matrix of soil and crop performance variables. Color and size of circle within cells indicate strength and direction of correlation. Blank cells indicate non-significant associations between variables. See Appendix Table 1.2 for information on variable names.

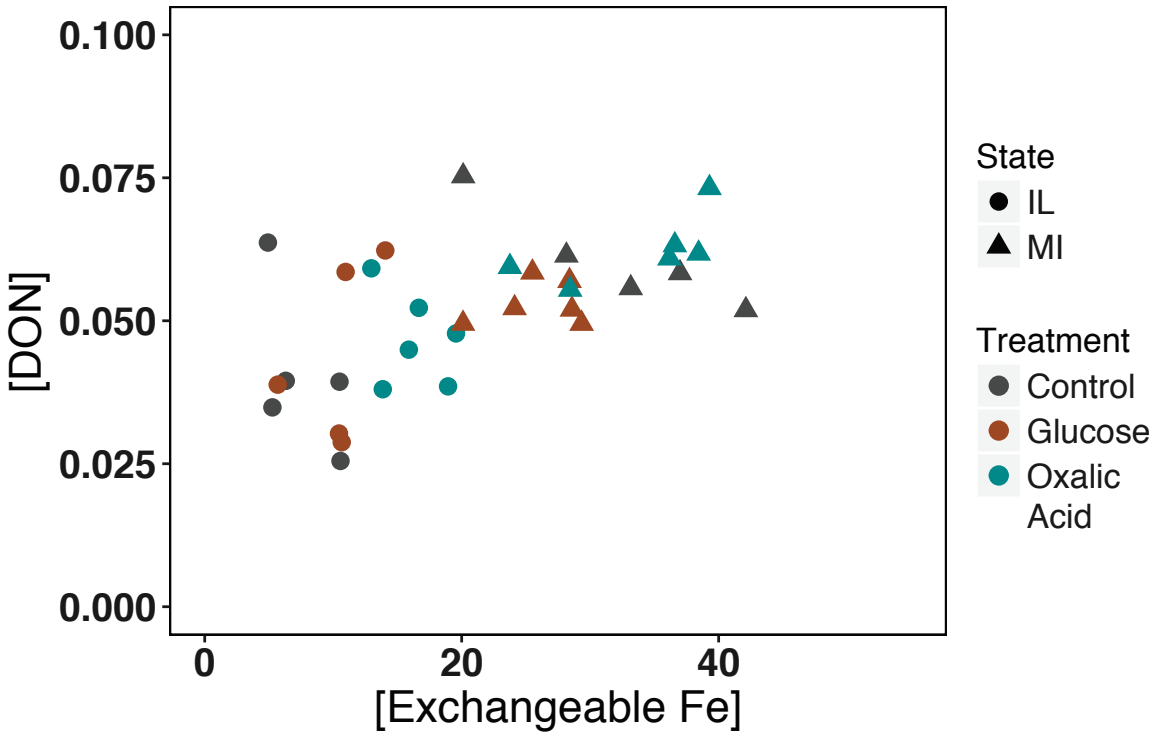
**Appendix Table 3.1.** Substrate treatment effects on hydrolytic and oxidative enzyme activities showing average values within each pulse treatment. CBH = Cellobiohydrolase; BG = β-glucosidase; NAG = *N*-acetyl-glucosaminidase; LAP = leucine aminopeptidase; TAP = tyrosine aminopeptidase. *** : P<0.001, **:  P<0.01, *: P<0.05,  †: P<0.10

| | | Illinois | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Pulse** | **Substrate** | **CBH** | **BG** | **NAH** | **LAP** | **TAP** | **POX** | **PHENOX** |
| 1 | Co | 13.5 (0.1) a | 19.1 (0.7) a | 11.1 (0.7) a | 55.9 (6.4) | 46.9 (7.3) | 7,198.2 (534.4) | 2,653.5 (204.7) |
| | Gu | 13.7 (0.3) a | 18.3 (2.6) a | 9.7 (2.2) a | 72.5 (2.6) | 61.0 (9.3) | 5,228.4 (1,087.4) | 1,716.5 (266.5) |
| | Ox | 12.1 (0.1) a | 13.2 (0.6) a | 6.7 (0.9) a | 34.4 (1.8) | 15.8 (1.1) | 9,312.7 (1,597.7) | 2,681.0 (381.8) |
| 2 | Co | 14.7 (1.2) a | 40.0 (0.6) ab | 326.3 (31.1) b | 36.0 (2.4) | 34.9 (5.7) | 1,958.1 (137.7) | 850.2 (23.3) |
| | Gu | 17.3 (0.3) a | 52.6 (2.0) b | 302.9 (30.4) ab | 48.9 (3.1) | 32.4 (5.1) | 2,639.8 (175.2) | 955.8 (31.6) |
| | Ox | 14.7 (0.4) a | 31.8 (1.7) a | 192.6 (16.4) a | 21.5 (2.0) | 15.7 (1.2) | 4,618.0 (220.2) | 1,170.8 (63.2) |
| 3 | Co | 24.3 (1.6) b | 39.9 (9.2) a | 243.3 (42.5) a | 63.8 (10.3) | 68.0 (26.9) | 7,674.1 (2,110.7) | 2,826.4 (478.5) |
| | Gu | 33.3 (4.2)c | 157.2 (27.3) c | 496.0 (70.0) b | 78.0 (9.3) | 70.2 (6.7) | 7,186.2 (428.8) | 2,142.0 (255.2) |
| | Ox | 19.7 (0.5) a | 67.3 (4.1) b | 302.7 (56.1) a | 37.9 (3.2) | 29.4 (2.9) | 10,527.6 (959.8) | 2,568.2 (193.7) |
| **ANOVA Results** | | | | | | | | |
| | Substrate | *** | *** | ** | *** | *** | ** | * |
| | Pulse | *** | *** | *** | *** | * | *** | *** |
| | Sub x Pulse | ** | *** | ** | NS | NS | NS | NS |
| | Substrate Effect | | | | Ox<Co<Glu | Ox<Co=Glu | Co=Glu<Ox | Glu<Co=Ox |

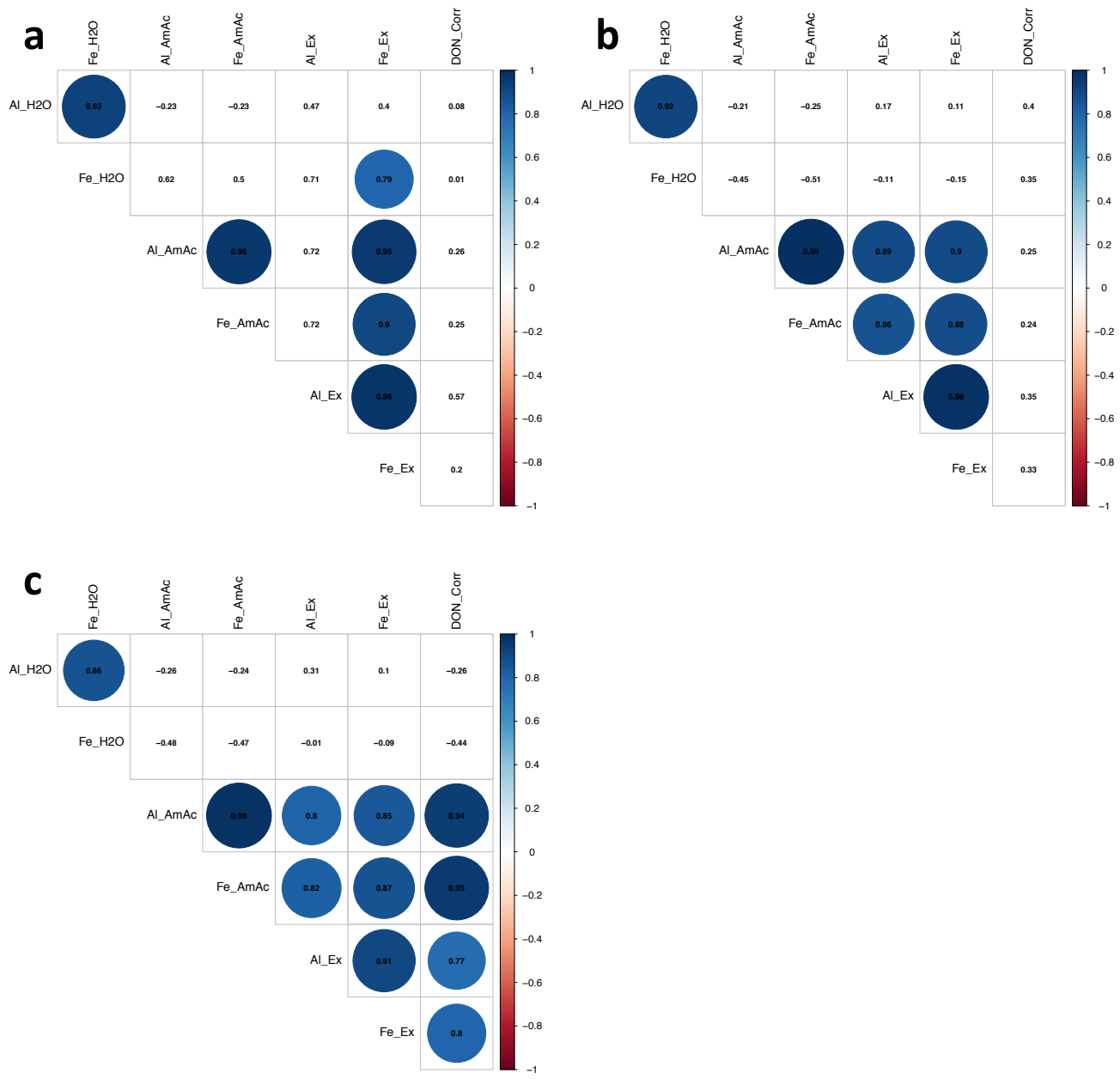
| | | Michigan | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Pulse** | **Substrate** | **CBH** | **BG** | **NAH** | **LAP** | **TAP** | **POX** | **PHENOX** |
| 1 | Co | 4.1 (0.3) a | 19.2 (0.8) a | 34.1 (7.5) a | 36.7 (2.8) a | 33.1 (6.5) a | 4,037.8 (1,504.7) a | 1,496.9 (402.9) |
| | Gu | 6.5 (0.8) a | 24.2 (0.4) a | 38.2 (1.2) a | 38.2 (1.9) a | 45.2 (4.5) a | 10,345.0 (3,079.3) b | 3,503.3 (952.9) |
| | Ox | 3.9 (0.7) a | 17.1 (0.7) a | 42.6 (4.0) a | 35.0 (2.7) a | 23.8 (0.5) a | 5,739.8 (719.5) a | 1,809.0 (247.3) |
| 2 | Co | 12.6 (0.6) a | 46.1 (3.7) a | 506.5 (21.5) a | 65.8 (5.1) b | 91.8 (6.3) b | 2,445.1 (262.9) a | 933.6 (37.4) |
| | Gu | 13.7 (0.5) a | 69.9 (1.2) b | 645.4 (47.7) b | 67.2 (1.3) b | 55.2 (5.1) ab | 2,736.1 (113.4) a | 1,659.9 (570.5) |
| | Ox | 9.5 (0.4) a | 35.5 (9.3) a | 393.7 (28.3) a | 48.0 (1.6) b | 30.4 (5.8) a | 5,915.5 (461.0) b | 1,385.6 (84.4) |
| 3 | Co | 19.7 (2.4) a | 70.1 (7.8) a | 693.4 (76.9) b | 73.7 (4.5) b | 162.2 (19.1) b | 3,867.2 (780.2) a | 1,848.5 (470.6) |
| | Gu | 38.4 (6.6) b | 133.4 (15.0) b | 792.7 (56.8) b | 78.3 (3.5) b | 147.5 (11.6) b | 8,743.9 (155.7) b | 2,488.7 (390.9) |
| | Ox | 16.1 (0.3) a | 74.2 (2.3) a | 549.9 (54.8) a | 46.9 (2.1) a | 76.7 (33.0) a | 8,120.6 (1,203.2) b | 1,964.2 (398.8) |
| **ANOVA results** | | | | | | | | |
| | Substrate | *** | *** | *** | *** | *** | ** | * |
| | Pulse | *** | *** | *** | *** | *** | ** | * |
| | Sub x Pulse | ** | * | * | ** | † | * | NS |
| | Substrate Effect | | | | | | | Co=Ox; Ox=Glu; Co<Glu |




**Appendix Figure 3.1.** Biomass of microbial functional groups isolated through PLFA analysis.




**Appendix Figure 3.2.** DON (mg $g^{-1}$) and Exchangeable Fe (g $kg^{-1}$) significantly correlated across all treatments in spearman correlation ($P$ <0.01).




**Appendix Figure 3.3.** Spearman correlation matrix of metal and DON concentrations in control (a), glucose (b) and oxalic acid (c) treatments. Color and size of circle within cells indicate strength and direction of correlation. Blank cells indicate non-significant associations between variables. Al_H2O and Fe_H2O refer to water-extractable Al and Fe, respectively. Al_AmAc and Fe_AmAc refer to ammonium acetate-extractable Al and Fe, respectively. Al_Ex and Fe_Ex

refer to total exchangeable Al and Fe, respectively (water-extractable + ammonium acetate-extractable). DON_Corr refers to the DON concentrations corrected to account for the effect of sterilization.