PLANT TAXA AND PYROLYSIS TEMPERATURE CONTROLS OF MICROBIAL AMINO SUGARS IN A NORTHERN FOREST SOIL

by

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To my friends and family, who have instilled in me a curiosity for the natural world

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LIST OF ABBREVIATIONS

C = carbon

N = nitrogen

OM = organic matter

PyOM = pyrogenic organic matter

JP = jack pine

RM = red maple

MRT = mean residence time

NSC = native soil carbon

SOC = soil organic carbon

GluN = glucosamine

MurA = muramic acid

GalN = galactosamine

ManN = mannosamine

ABSTRACT

In fire-prone forests, tree taxa and burn temperature are the major controllers of the chemical and physical properties of pyrogenic organic matter (PyOM), the aromatic carbon-rich product of the incomplete combustion of plant biomass, that accumulates in soil in such settings. These controls also dictate how soil microbes can degrade plant C once it enters into soil as previous studies demonstrate that increased fire temperature results in low PyOM degradability but also impacts the decomposition of the original soil. However, we know little about how taxa and temperature of C-inputs impact the production and accumulation of cellular residues from soil microbes, which can be the dominant source of stabilized soil organic matter in many ecosystems.

This work presents the results of the analysis of soil microbial amino sugars, as proxies for soil microbial necromass, from long-term soil incubation experiments, 180 and 600 days, that were amended with jack pine (JP) and red maple (RM) wood or their PyOM produced at 300°C or 450°C. Both wood taxa amendments resulted in an increase in microbial sugar residues compared to non-amended soils but RM, the taxa with the highest proportion of soluble sugars and low tannin content, exhibited the highest percentage increase. Soils amended with PyOM exhibited lower amino sugar content as compared to their wood but no difference compared to controls (non-amended soils). There was no difference in soil amino sugars observed between the PyOM derived from the two taxa nor between the temperature of pyrolysis, possibly due to only small amounts of bioavailable C and N in the PyOM. Total amino sugar concentrations varied significantly between PyOM and fresh wood treatments, with PyOM treatments yielding 659 – 730 μg/ g soil while wood treatments yielded 757 – 930 μg/ g soil early in incubations. While fungal-derived amino sugars were dominated in all treatments, longer soil incubation time, 600 days vs 180 days, resulted in a proportionately greater decrease bacterial-derived amino sugars. Overall, at 180 days, PyOM treatments exhibited 19-27% of soil N and 4-5% of soil C quantifiable as amino sugars while wood treatments exhibited 27-28% of total soil N and 6-7% of total soil C as amino sugars. This work shows, for the first time, that on a per C or per N basis, PyOM versus fresh wood addition to soils will result in a net depletion of microbial residues. The variable response in amino sugars between treatments and incubation time highlights the importance and dynamic nature of

the physicochemical characteristics of organic matter input to soil in controlling the contribution of soil microbial residues to that soil.

CHAPTER 1. INTRODUCTION

1.1 Significance of Soil Organic Matter

Terrestrial ecosystems hold the largest global pools of organic carbon (C) and nitrogen (N), with soils estimated to account for 1550 Pg of organic C and 140 Pg of total N (Batjes 1996; Janzen 2005; Stevenson 1982). At a particular location, the amount of C and N in soil may change over time based on the rate of input by plant and microbial material (Miltner et al., 2012; Janzen 2005) and the output controlled by microbial decay and erosion (Miltner et al., 2012; Doetterl et al., 2012; Papanicolaou et al., 2015). Long term C storage in soil can be attained through environmental factors such as cool overall temperatures or waterlogged soils that slow decay (Leiros et al., 1999), introduction of thermally alter pyrogenic organic matter (Lehmann et al., 2015), strong association of soil organic matter (SOM) with mineral surfaces (Sollins et al., 2009; Kleber et al., 2010), complexation by cations (Schwarzenbach et al., 2017), or inclusion within microaggregated structures that limit access to organic matter by degrading enzymes of microbes (Jastrow and Miller 1998; Dungait et al., 2012). These mechanisms controlling long term C storage may have large impacts on ecosystem services.

Organic matter is a fundamental determinant on the health of soil and how it can deliver ecosystem services, thereby allowing for agricultural productivity and C sequestration (Loveland and Webb 2003; Kumar et al., 2018). There is concern that if SOM concentrations in soils decrease too much then the productive capacity of soil will be weakened by declines in soil physical properties and by impairment of soil C and N cycles (Loveland and Webb 2003). Furthermore, the sequestration of CO_2 into soil C may significantly diminish gas concentrations from the atmosphere (Griscom et al., 2017). In this context, studies on the chemical form and reactivity of SOM are needed to understand how we can better manage soil for agriculture (Loveland and Webb 2003), protect soil from environmental and land use stress (Kumar et al., 2018), and maximize soil's ability to sequester atmospheric CO_2 in the form of soil organic C to mitigate climate change (Griscom et al., 2017).

1.2 Sources of Soil Organic Matter

Organic C and N inputs may come from a variety of sources, such as dead or decaying plant and animal matter, microbial residues (Cotrufo et al., 2015; Liang et al., 2019), and charred organic matter (Schmidt et al., 2011), however the input rate of plant tissue controls the overall C and N input to soil and primary cycling dynamics (Cotrufo et al., 2013). Plant production and input rate often correlate with C and N accumulation in soil; this has been seen in studies with soil collected from forests (Finzi et al., 1998; Campbell and Gower 2000) and grasslands (Six et al., 1998; Luo et al., 2017), with greater C and N content being associated with greater plant litter inputs (Miao et al., 2019). These studies have also shown that taxa, amount, and type, (i.e. root, stem, leaf) of plant litter input impacts C and N content in soil (Liao et al., 2006), with greater C and N content from fresh plant litter of different species correlating with greater C and N in soil. Additionally, chemical structure of plant litter varies by source, with more easily degradable (labile) structures being associated with leaf tissues and more stable (recalcitrant) structures being associated with wood tissues (Cotrufo et al., 2013).

Recent models have been developed, such as the microbial efficiency – matrix stabilization (MEMS) framework that incorporates microbial substrate use efficiency and soil matrix interactions as the two key processes that control the fate of litter inputs to soils (Cotrufo et al., 2013). The MEMS framework suggests that more labile litter is decomposed more easily by microorganisms and becomes incorporated into microbial biomass rather than being mineralized or respired, as indicated by high microbial substrate use efficiency (Figure 1.1). Substrate use efficiency is controlled by litter quality, with recalcitrant structural molecules in plant matter (i.e. lignin) having lower use efficiency compared to labile metabolic molecules (i.e. glucose). This could also be applied to fire-derived organic matter, where organic matter (OM) formed at high temperatures is more recalcitrant compared to OM formed at lower temperatures (Lehmann et al., 2011; Bruun et al., 2011; Lehmann et al., 2015). Upon decomposition molecular byproducts follow similar trends in lability and recalcitrance as precursor molecules as indicated by decomposition product quality. The MEMS framework further suggests that with greater substrate use efficiency and greater decomposition product quality, compounds from litter inputs become stabilized by the surrounding soil matrix or become incorporated into SOM to a higher degree, suggesting that plant litter quality dictates C and N accumulation in soil.

A separate process has been demonstrated using the soil continuum model (Lehmann and Kleber, 2015), whereby the progressive decomposition of organic residues yields compounds ranging in size from large biopolymers to smaller single molecules. These molecules then become associated with both aggregates and mineral surfaces based on their size, complexity, and chemical composition (Lehmann and Kleber, 2015). Organic matter inputs are thus continuously participating in complex interactions with biotic and abiotic aspects of the soil around them. Type and amount of plant inputs can have a large impact on soil C and N accumulation, however, to assess organic matter, its stabilization in soil must also be considered.

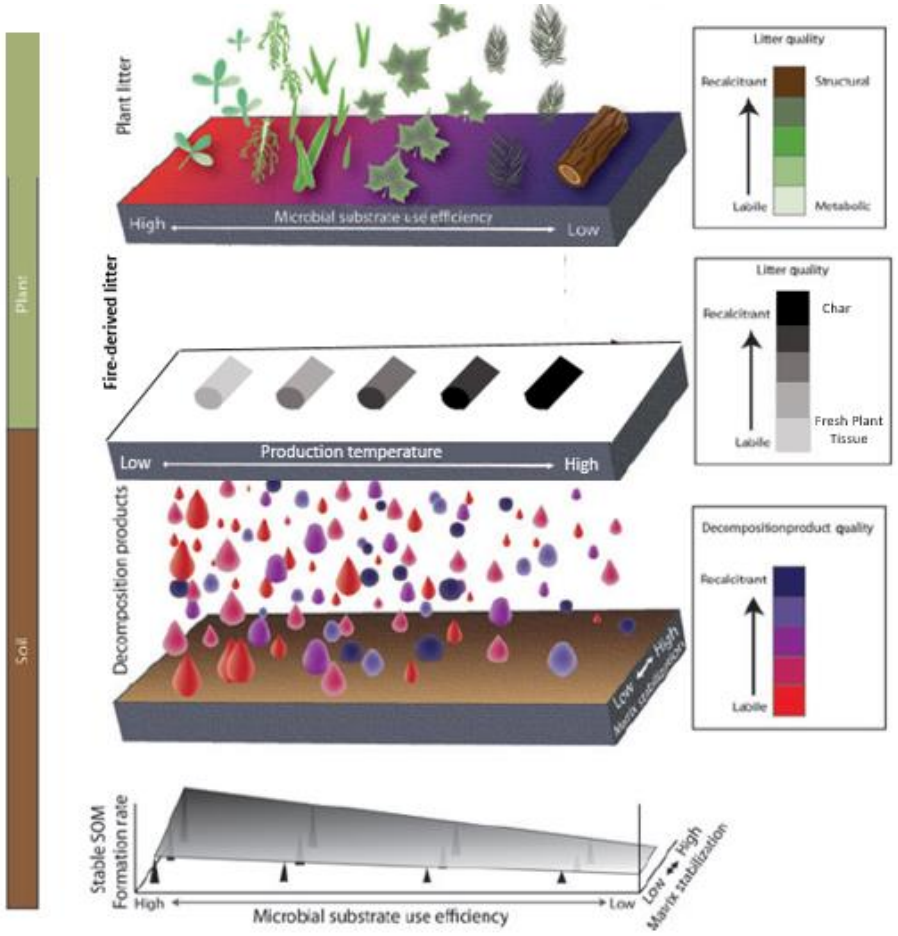


Figure 1.1. Microbial Efficiency-Matrix Stabilization framework showing the effects of plant and fire-derived litter quality on substrate use efficiency, matrix stabilization, and SOM formation (Modified from Cotrufo et al, 2013).

1.3 Protection of Soil Organic Matter

Components of SOM may be utilized as a nutrient source by microorganisms, but the ease of decomposition, once accessibility is overcome (Dungait et al., 2012), of SOM is largely dependent on physical and chemical protection mechanisms. Chemically, SOM can be resistant to decomposition based on its composition and molecular structure. Compounds with more double bonded, condensed, cyclical structures are more thermodynamically stable based on the energy of bond formation (LaRowe and Van Cappellen, 2011). Microorganisms that would normally break these bonds and utilize OM as an energy source may not be able to do so if compounds are energetically unfavorable, and thus OM is more resistant to biochemical decomposition by microorganisms (LaRowe and Van Cappellen, 2011; Schwarzenbach et al., 2017). Generally, more complex larger molecules have been thought to decompose at slower rates than smaller simple molecules due to their energetic constraints (LaRowe and Van Cappellen, 2011; Kleber, 2010). This type of protection, called biochemical recalcitrance, can be inherited from the original plant material, imparted to it based on diagenetic processes, or through temperature mediated alterations (Kleber, 2010; Paul, 2016). Organic matter may become so resistant to decomposition that it persists in soil for hundreds of years, such as with thermally altered pyrogenic organic matter (Lehmann et al., 2015). Though the concept of inherent recalcitrance has been thought to be a controlling factor for OM stability, the resistance of OM to decomposition has been shown to change based on physical environmental conditions as well.

An additional class of protection mechanisms relies on both physical and chemical characteristics of organic matter and mineral components of soil. Organic matter may become protected in soil by interactions with surrounding organic material, clay minerals, and metal oxides (Paul, 2016). Organic matter inputs may sorb to surrounding organic surfaces or interiors mediated by hydrophobic forces (Schwarzenbach et al., 2017; Paul 2016). Metal oxides and cations of clay minerals may interact with organic molecules through ionic bonding, creating a temporary induced charge, or through electron mediated Van der Waals interactions (Schwarzenbach et al., 2017; Paul 2016; Mikutta et al., 2006). This has been seen with aluminosillicates and iron hydroxide mineral surfaces where generally greater mineral content yields greater retention times of OM (Sollins et al., 2009; Mikutta et al., 2006). When on hydrophobic and charged clay surfaces microorganisms are unable to utilize the organic molecules that are too strongly associated with clay or surrounding OM to separate, resulting in greater OM persistence in soil.

Soil organic matter can also be physically protected by becoming spatially inaccessible to microbes (Six et al., 1998; Cotrufo et al., 2015). For example, SOM may become incorporated into soil aggregates, groups of soil particles that bind to each other more strongly than to other surrounding particles mediated by organic, mineral, and oxide interactions (Jastrow 1996; Paul 2016). In aggregates SOM can become physically protected by soil particles with greater physical protection resulting in lower microbial utilization and greater long term storage (Jastrow and Miller 1998; Filley et al., 2008; Cotrufo et al., 2015). Soil structure and the physical stabilization of SOM can be altered by factors such as rainfall, machinery use, and plant residue management. To improve the stabilization of SOM plant residues can be used through mechanisms such as, increasing soil aggregation, protecting soil aggregates from raindrop impact, and protecting soil from compaction (Turmel et al., 2015). Through these mechanisms SOM is protected to varying degrees, though the success of protection is also dependent on SOM decomposition by microorganisms.

As fundamental drivers of OM decomposition microorganisms play a large role in SOM formation and stabilization. To better understand how organic matter inputs impact SOM formation and stabilization more information is needed on how organic inputs influence microbial residues in soil. SOM is composed of both microbial and plant derived residues (Schmidt et al., 2011; Miltner et al., 2012; Liang and Balser, 2011). While fresh plant residues may persist in soil for years to decades, their thermally altered counterparts, fire derived residues, may persist in soil for hundreds of years (Figure 1.2). Both plant and fire derived organic matter sources can be utilized by microorganisms that largely control their presence in soil by means of biological decomposition. While the impact of select plant tissues on SOM and certain microbial residues are more known, less is known about the impact of alternative plant derived inputs, such as charred residues (Schmidt et al., 2011). In order to assess the impact of plant and fire derived organic inputs on SOM, microbial residues themselves must first be examined. In doing so more information can be obtained as to how OM inputs may impact SOM on a large scale.

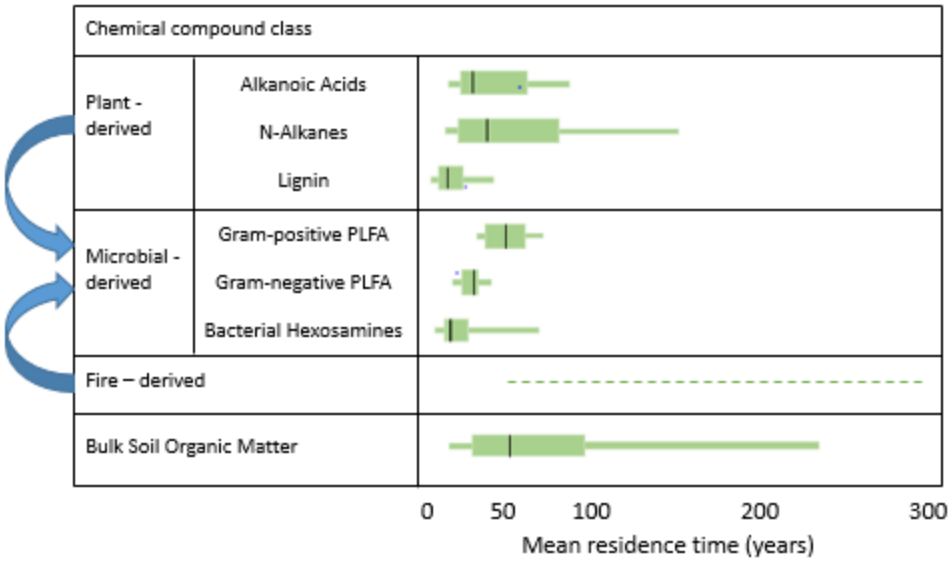


Figure 1.2. Persistence of compound classes and subsequent compounds found in soil organic matter highlighting the importance of plant and fire derived organic matter to microbial derived organic matter. (Modified from Schmidt et al., 2011).

1.4 Microbial Contribution to SOM

Microorganisms play key roles in the formation of C and N in soil, either by the stabilization of SOM or by directly contributing to SOM with cell residues. Liang and Balser (2011) have suggested a mechanism by which soil microbes create stable C, called the microbial carbon pump, where soil microbes degrade labile OM and leave recalcitrant byproducts. The carbon pump involves a series of processes through which CO_2 is fixed as OM in plants by photosynthesis and then transferred to soil by plant litter deposition. Once in soil microbial utilization of the more labile fraction of litter yields more stable partially decomposed plant material that can be stored for millennia. Liang and Balser (2011) further suggest that microbial growth, metabolism, and death have a role in the stabilization of SOM, where litter inputs become incorporated into microbial biomass and then enter labile or stable C pools upon cell death (Figure 1.3). Complimentary, recent studies have highlighted the importance of microorganism contributions to soil C and N through stabilization of their cell residues on mineral surfaces or in aggregates, though a process known as patchy fragment formation (Miltner et al., 2012). Positively charged mineral surfaces may attract patches of cell fragments by inducing a temporary charge or by ionic bonds, thus aiding in cell fragment retention in soil (Miltner et al., 2012; Sollins et al., 2009;

Schwarzenbach et al., 2017). Furthermore, cell residues may be incorporated into soil aggregates (Miltner et al., 2012; Jastrow and Miller 1998). This is based on the progressive decomposition of residues yielding compounds ranging in size from large biopolymers to smaller single molecules that become associated with both aggregates and mineral surfaces (Lehmann and Kleber, 2015). This is the case for microbial derived molecules, such as amino sugars - common structural units of large biopolymers, such as chitin and peptidoglycan, found in microbial cell walls (Zhang and Amelung 1996). Amino sugars are likely stabilized in soil on charged mineral surfaces or native soil organic matter, leading to potentially long term storage in soil (Glaser et al., 2004; Miltner et al., 2012). Regardless of mechanism, cell residues may become physically protected and may thus be less likely to degrade by biotic or abiotic means, thereby persisting in soil and contributing to soil C and N pools.

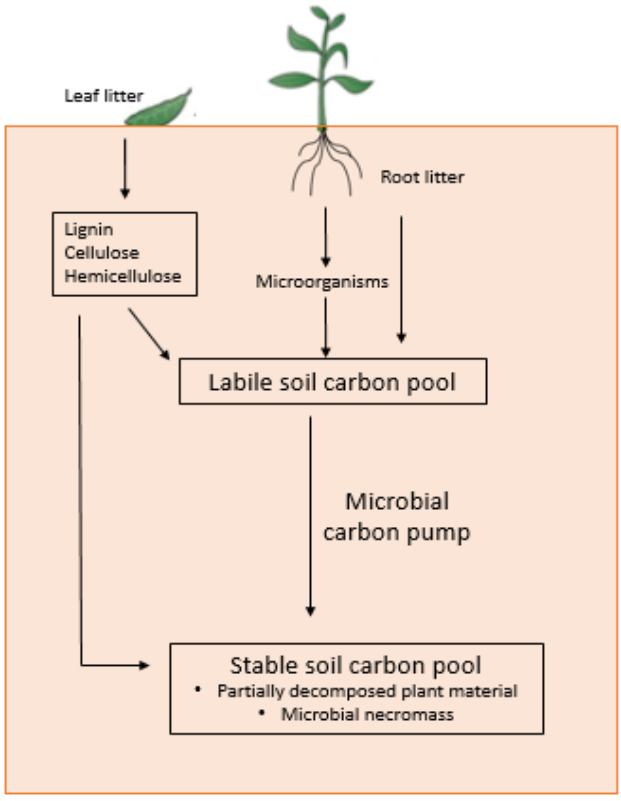


Figure 1.3. Conceptual model of microbial carbon pump (Modified from Liang and Balser, 2011).

Recent work has suggested the importance of microbial tissue contribution to soil C and N, with changes in the abundance of microbial residues leading to changes in soil C and N (Appuhn and Joergensen 2006; Joergensen 2018; Liang et al., 2019). The C and N content for known compounds can be calculated by obtaining concentrations of specific molecules and converting to concentration of C and N using known stoichiometry. When paired with soil C and N content, microbial tissues derived C and N contribution to soil can be determined. While only about 2% of total soil organic carbon (SOC) exists as living cell biomass, residues from dead and fragmented cells, also known as necromass, can account for substantially more (Liang and Balser, 2011; Miltner et al., 2012). Microbial necromass has been calculated to contribute about 60 – 80% of SOC in grasslands (Simpson et al., 2007; Liang et al., 2019), about 30-60 % in forests (Simpson et al., 2007; Liang et al., 2019), and 50 – 70% in agricultural soils (Srandnick et al., 2014; Liang et al., 2019). Global average model simulation estimates of soil microorganism suggest necromass may account for 47-80% of SOC (Fan and Liang, 2015). Furthermore, microbial necromass has been calculated to contribute about 80 – 90% of soil organic nitrogen (SON) in grasslands and about 80% in forests (Simpson et al., 2007). Model simulation estimates of soil microorganisms suggest necromass may also account for 40.5% - 100% of the total soil N (Liang et al., 2019), with a wide range of estimates due to certain parameters including the abundance of fungi and bacteria, lifecycle and degradation of microbial biomass and necromass, and microbial metabolic response to organic inputs. As microbial tissues may account for significant amounts of C and N, knowledge of soil microbial residue types, chemistry, and environmental factors that control their stability and abundance in soil are needed.

Microbial residues vary in their origin and chemistry. The chemical composition of soil microbial residues is based on the taxonomic origin of molecules within cells, with molecules coming predominantly from cell walls, membranes, and genetic material (Amelung et al., 2003). Cell wall tissues vary among taxa, such as fungal and bacterial cells. Chitin is a polymer found in fungal cell walls composed of chains of the amino sugar N-acetylglucosamine. Strong covalent bonds between glucosamine groups creates high chemical stability and yields long molecular chains (Figure 1.4). With variable length due to number of structural units, estimates of chitin molecular weights are difficult to assess but may range from 10^4 to 10^6 g/mol (Wieczorek et al., 2019; Funahashi et al., 2017). Peptidoglycan is a polymer consisting of sugars, amino-sugars, and amino acids found in bacterial cell walls. The amino sugar components of peptidoglycan are

primarily composed of chains of N-acetylglucoasmine and N-acteylmuramic acid. Like chitin, peptidoglycan can consist of long chains. The amount of peptidoglycan varies between bacterial cell types, but average chains range from 20 -40 structural units, with thinner layers of peptidoglycan sheets being present in gram negative bacteria compared to gram positive (Figure 1.5). With many possible combinations of amino acid chains associated with peptidoglycan average chemical compositions and subsequent molecular weights are difficult to determine, but may reach up to 10^9 g/mol (Vollmer et al., 2008; Vollmer and Holtje, 2004).

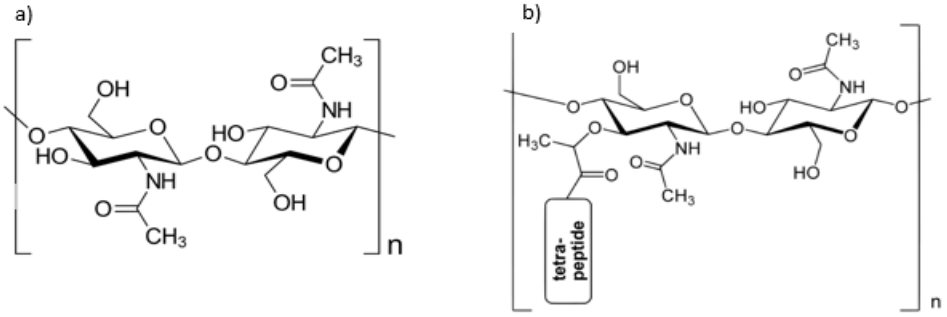


Figure 1.4. Structures of a) chitin and b) peptidoglycan

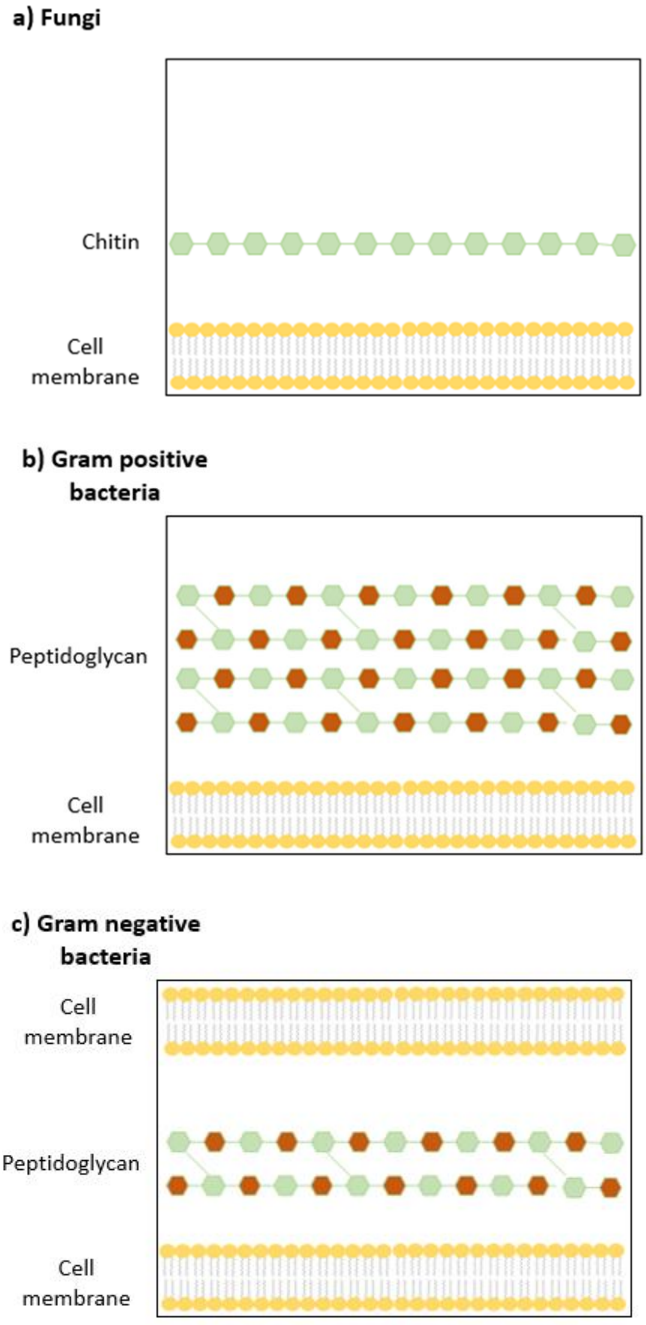


Figure 1.5. Cell wall and membrane structures for a) fungi b) gram positive bacteria and c) gram negative bacteria.

Similar to cell wall tissues, cell membrane and genetic material vary in chemical composition and differ among taxa. Fatty acids of cell membranes can differ by cell types such as in gram positive and gram negative bacteria, mycobacteria, and fungus, with specific fatty acids endings of lipids being found in select taxa (White et al., 1979; Kaur et al., 20005). Fatty acids vary in length and hydrogen saturation depending on origin, though are generally hydrocarbon chains of around 20 carbons or less (Kaur et al., 2005). Cell genetic material varies even more, with specific nucleic acids being found for individual organisms or genes. They are composed of nucleotides, which are units made of phosphate, N, and cyclical C (Verma and Eckstein 1998). With unique origin and composition, microbial cell residues such as these can be used to better understand changes in soil microbial type and abundance.

1.5 Assessing Microbial Soil Content Through Biomarker Quantification

To investigate the abundance or shifts in microbial residues in soil, several molecular cellular component can be used (Amelung et al., 2008). These molecules, known as biological markers or biomarkers, are the diagnostic chemical components of cells that can indicate the origin and amount of microbial residues. Common biomarkers used to study microbial residue type and abundance include fatty acids (Zelles, 1999; White et al., 1979), nucleic acids (Dyckmans et al., 2003), amino sugars (Zhang and Amelung, 1996; Joergensen, 2018; Liang et al., 2019), and amino acids (Cowie and Hedges, 1994; Amelung, 2003). A variety of methods have been developed using biomarkers as molecular proxies for microbial type to assess the accumulation of microbial residues in soils, along with their contribution to soil C and N. However, the degree to which molecules can be used for these metrics varies between biomarkers.

Fatty acids can be utilized to determine broad microbial abundance and composition fungal and bacterial groups (Kaur et al., 2005; White et al., 1979). Phospholipid derived fatty acids (PLFAs), a type of fatty acid, are present in the cell membranes of specific microorganisms, allowing for the quantification of a variety of bacterial and fungal groups. Recent studies have used PLFA extraction and quantification techniques to assess changes in microbial community structure with changes in soil land use and OM inputs. Fungal and bacterial PLFAs have been shown to increase with greater amounts of leaf matter input (Koyama et al., 2018). Meanwhile the exclusion of root litter has shown significantly lower fungal and bacterial PLFAs (Jing et al., 2019), suggesting that microbial PLFAs respond directly to plant inputs. Recent studies using PLFAs

have also shown that soil microorganism abundance and diversity are impacted by fire – derived OM addition to soil (Santos et al., 2012; Farrell et al., 2013), where total PLFA abundance has been shown to be greater for soils given a fire – derived wheat or eucalyptus amendment compared to a control after a short incubation period. However, it has been demonstrated that the addition of a fire – derived wood has no change on total PLFA abundance and reported no major changes to microbial community composition (Santos et al., 2012). It has also been reported that the composition of microbial communities changed over time, with gram positive bacteria dominating soil at first then subsiding to similar levels or other bacteria and fungi (Farrell et al., 2013). Elevated CO_2 and temperature of soil have shown limited changes in fungal or bacterial PLFAs over a 9 year period (Liang et al., 2015), suggesting that fatty acids may not respond over longer time periods. Fatty acids also have no N and may therefore be a less powerful estimators of microbial residue contribution to SOM compared to other biomarkers.

Nucleic acids have been used to gain insight into more specific microbial community compositions in soil. In particular, DNA is commonly used to investigate shifts in microbial communities (Xue et al., 2013). With the extraction of DNA and isolation of specific genes microbial composition and functionality have been determined in select soil systems. The relative abundance of fungal DNA and genes encoding enzymes involved in C and N cycling have shown to increase with crop litter input to soil (Xue et al., 2013; Lin et al., 2019). Litter inputs in forest soils have shown similar results and also suggest that increased diversity of litter inputs enhance the diversity of microbial DNA (Winsome et al., 2017). DNA has also been used to investigate the diversity of microbes following fire – derived manure (Dai et al., 2017) and wood amendments (Anderson et al., 2011). With unique chemical compositions and roles in C and N cycling, the abundance of certain microorganisms could then help explain the microbial contribution to soil C and N. However, nucleic acids are poor indicators of microbial abundance due to their fast decomposition and are therefore not commonly used to estimate microbial residue contribution to SOM (Miltner et al., 2009).

Unlike other microbial biomarker indicators, amino sugars can be used to both estimate fungal or bacterial abundance and estimate the microbial derived C and N contribution to overall soil C and N. Due to their persistence in soil, amino sugars are able to be used to estimate microbial residues, living or dead, in soils. Four amino sugars - glucosamine (GluN), muramic acid (MurA), galactosamine (GalN), and mannosamine (ManN) - have been commonly quantified in soil as

microbial indicators (Figure 1.6). MurA is common in bacteria while GluN is associated with fungi, though a smaller amount exists in bacteria as well (Joergensen, 2018). ManN and GalN are both of bacterial and fungal origin so they are not used as indicators of specific microbial origin, but have been used to estimate total amino sugar abundance (Liang et al., 2012). While MurA has a unique chemical composition GluN, GalN, and ManN exist as stereoisomers, molecules with the same composition and sequence of bonds but different three-dimensional arrangement. Together these four amino sugars can be used to estimate fungal, bacterial, and total microbial residues in soil (Joergensen, 2018; Liang et al., 2019). Furthermore, amino sugars may account for a large portion of soil C and N. As a portion of microbial biomass and necromass, amino sugars are estimated to account for 5-12% of total soil N (Stevenson, 1982) and 2-5% of SOC (Joergensen and Meyer, 1990) and as a result the study of amino sugars is of high importance for understanding soil C and N composition.

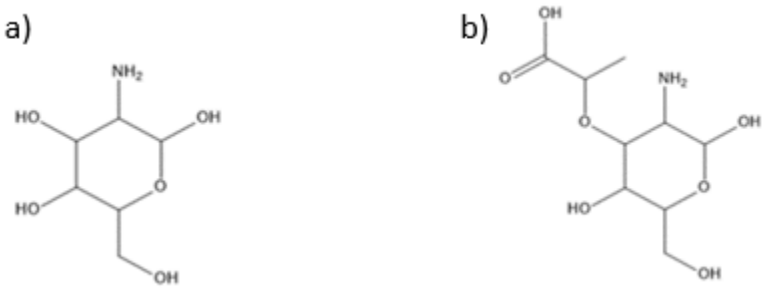


Figure 1.6. Structures of a) glucosamine and b) muramic acid

Liang et al (2019) outlined calculations to estimate microbial residue C and N from amino sugar content of soils. Amino sugar derived C and N can be estimated based on stoichiometry of C and N to amino sugar molecules. When compared to total C and N abundance in soil this suggests how much microbial derived C and N may account for total soil C and N. Calculations have been developed to further suggest the amount of microbial C and N in total microbial residues:

1. n µg MurA x 45 = µg bacterial residue C
2. n µg MurA x 6.67 = µg bacterial residue N
3. $(\frac{\text{m µg GluN}}{179.17})$ – 2 x $(\frac{\text{n µg MurA}}{251.23})$ x 179.17 x 9 = µg fungal residue C
4. $(\frac{\text{m µg GluN}}{179.17})$ – 2 x $(\frac{\text{n µg MurA}}{251.23})$ x 179.17 x 1.4 = µg fungal residue N

Where n is the mass of MurA, m is the mass of GluN, 251.23 is the molar mass of MurA, 179.17 is the molar mass of GluN, 45 and 6.67 are the conversion factors from C and N content respectively to MurA in bacterial cells, and 9 and 1.4 are the conversion factors from C and N content respectively to GluN in fungal cells (see Appendix A for sample calculations). Two times the mass of MurA is subtracted from equations 3 and 4 to account for GluN that may be of bacterial origin. Limitations for these equations exist in the use of conversion factors based on average microbial residue C, N, and amino sugar content which may change in soils based on microbial community composition. Nevertheless, in using these calculations greater insight could be achieved into the role microorganisms play in contribution of SOM.

Similar to the use of other biomarkers, recent studies have utilized amino sugars to evaluate the response of soil microbial residues to changes in climate and land use (Liang et al., 2015; Ding et al., 2011; Ding et al., 2017). Climate change impacts are reflected in amino sugar abundance in soil, where elevated CO_2 conditions and greater temperature decreased GluN and increased MurA, likely due to a shift from gram negative to gram positive bacteria which contain more MurA (Liang et al., 2015). Additionally, changes in land use for a variety of soils have been shown to alter abundance of amino sugars. The practices of tillage and continuous cropping have shown to yield lower amino sugar contents in soil, while rotation of crops or restoration to former dominant vegetation has shown to increase amino sugar abundance (Ding et al., 2011; Ding et al., 2017). With many types of changes that land can undergo, the quantification of amino sugars and their contribution to soil C and N is paramount. Similarly, studies have investigated the impact of fresh plant and fire-derived tissues on amino sugars in soil. However, to assess these impacts, the unique characteristics of plant and fire-derived tissues must first be considered.

1.6 Distinctions in Physicochemical Characteristics between PyOM and Fresh Plant Tissue

Fresh plant matter source can have a significant impact on SOM, where the input rate of plant tissue controls the overall C and N input to soil and primary cycling dynamics (Cotrufo et al., 2013). With chemical composition varying between plant sources, litter contribution to SOM can vary widely between ecosystems, but plant derived particulate organic matter may account for 10 - 20% of SOM on average (Ingham, 2019; USDA, 2014). To assess the degree to which plant matter can impact SOM formation and stabilization, physicochemical characteristics of fresh plant tissues must first be considered.

Plant litter stability in soil is determined primarily by differences in chemistry and structure of the plant material from which it is derived. Generally, plants are composed of carbohydrates, fats, proteins, and larger structural polymers such as lignin and cellulose (Berg and McClaugherty, 2008). These compounds differ in their size, structural complexity, and the type of chemical bonds, with larger more complex molecules, such as lignin and long chain aliphatics, taking longer to decay in soil due to difficulty of microbial degradation (Cotruo et al., 2015; Talbot et al., 2012). Lignin in particular decays more slowly as it is stabilized by the formation of complex polymers due to linkages with other molecules such as polysaccharides (Talbot et al., 2012). Large roots, bark, and woody plant tissues contain high amounts of lignin compared to conifer needles and leaf, grass stem, or fine root tissues (Berg and McClaugherty, 2008). In comparison, those tissues low in lignin are typically much higher in sugars, phenols, and short chain aliphatics (Cotrufo et al., 2015). Differences in plant tissue have been noted for various forest plant inputs with conifer needles having greater lignin and aliphatic content that degrades more slowly than leaves of deciduous trees (Soong et al., 2015; Klotzbucher et al., 2011; Crow et al., 2009). The quality of litter, based on its size, complexity, and composition then dictates its ability to persist in soil, however fresh plant litter may not be the only source of OM deposited to soil.

Another important contributor to long-lived SOC is pyrogenic organic matter (PyOM) - the carbon rich product of the incomplete combustion, or pyrolysis, of plant biomass produced during forest and grass fires (Santin et al., 2016; Lehmann et al., 2015). When exposed to extreme heat, but in the absence of oxygen, as found in a natural fire, plant matter will undergo severe physical and chemical changes controlled by the temperature of the fire, to become PyOM. The temperature of biomass burning during natural fires can vary depending on the specific environment, with forests ranging from 300 - 900 ˚C and grasslands ranging from 100 – 450 ˚C (Santin et al., 2016; Morgan, 1999). During fires plant tissue is altered on a molecular level, with a variety of compounds being released into the atmosphere in a gaseous state, but also with the production of high molecular weight aromatic, condensed PyOM. During a typical fire, most plant – C is released into the atmosphere as CO_2, but a significant fraction, 4-18 % in grasslands (Preston and Schmidt, 2006) and about 27 % in forests (Santin et al., 2016), can be converted to PyOM. Recent estimates suggest a global average of 13.7% of SOC is derived from PyOM (Reisser et al., 2016). Estimates vary between ecosystems with an average of 16% in agricultural soils, 12.1% in grasslands, and 15 – 17% being found in tropical and temperate forests (Reisser et al., 2016).

PyOM contribution to SOC can vary widely depending on specific locations with some estimates of up to 40% of grassland and forest SOC being derived from PyOM (Preston and Schmidt, 2006, Schmidt et al., 2011). With PyOM contributing potentially large amounts to SOC knowledge of its stability in soil is needed.

PyOM stability in soil is determined primarily by the temperature of pyrolysis and also by differences in chemistry and structure of the plant material from which it is derived (Lehmann et al., 2015). Changes in the physicochemical structure of plant matter occurs along a temperature gradient (Keiluweit et al., 2010). With increasing pyrolysis temperature plant tissues change in chemical composition from common structural molecules such as lignin, cellulose, and hemicellulose to condensed amorphous C and crystalline C (Lehmann et al., 2015; Keiluweit et al., 2010). Physically, plant tissues change as well when structural molecules from the fresh plant matter are volatilized at high temperatures yielding greater pore space and surface area until larger volume crystallinites are formed (Keiluweit et al., 2010). If not volatilized PyOM - C remains unchanged throughout pyrolysis such as with fixed C and ash content (Figure 1.7). Physical and chemical characteristics of PyOM may change depending on initial plant matter as well (Keiluweit et al., 2010). It has been shown for a variety of plant materials that with increased pyrolysis temperature there is a shift toward more stable, double bonded, cyclical molecular structures. Grass PyOM has shown to contain fewer amino and alkyl C groups, but more pyrrole and aromatic C groups compared to that of initial plant material (de la Rosa and Knicker 2011; Keiluweit et al., 2010). Similarly, wood has shown fewer long alkyl chains and o-alkly groups, but rather more condensed aromatic structures in PyOM compared to the source plant material (Hatton et al., 2016; Keiluweit et al., 2010; Bostick et al., 2018).

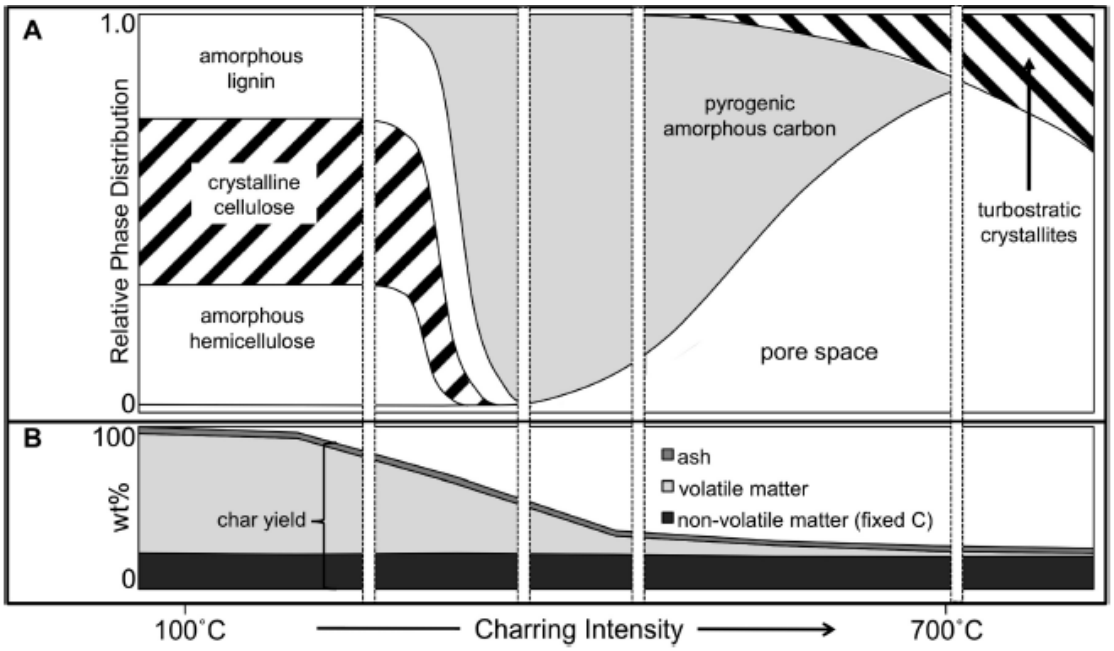


Figure 1.7. Trends in plant matter along a temperature gradient showing a) physical and chemical characteristics and b) char composition (From Keiluweit et al., 2010).

While PyOM may be more stable than the original plant material it is derived from due to its unique physicochemistry, all OM inputs to soil are susceptible to both biotic and abiotic decomposition processes. Plant tissues and PyOM have been shown to be decomposed by soil microorganisms, with microbes likely mineralizing more labile C fractions of OM inputs (Castellano et al., 2015; Santos et al., 2012; Wang et al., 2015) or taking them up into their biomass (Moorhead et al., 2014; Farrell et al., 2013; Sanstos et al., 2012). Abiotically, PyOM has been shown to degrade due to exposure to higher energy light and water-leaching in soils that lead to chemical changes, with photooxidation of PyOM increasing the aliphatic C content compared to aromatic C, water-leaching decreasing soluble O containing groups, and general abiotic oxidation leading to the presence of greater negative surface charges on PyOM (Wang et al., 2017; Gibson et al., 2016; Cheng et al., 2006). Concurrently, plant tissue has been shown to oxidize such as by photolysis or with naturally occurring chemical oxidants (Haruo 2012; Golanoski et al., 2012). Furthermore, partial abiotic oxidation of plant tissues and PyOM have been shown to stimulate microbial metabolisms and oxidative enzymes in soil (Wang et al., 2017). However, OM degradation varies based on initial plant taxa and temperature of pyrolysis.

Many studies have utilized field and laboratory incubations of soil with OM amendments where PyOM or unaltered plant biomass are added to soil to investigate how OM amendments degrade to CO_2 or persist in soil (Kogel-Knabner, 2002; Berg and McClaugherty, 2008; Soong et al., 2015). When given fresh OM, plant litter quality is the primary controller of litter decomposition in soil (Zhang et al., 2008; Cotrufo et al., 2013), with chemical differences in plant matter, such as lignin and cellulose content, leading to differences in decomposition rates of litter residues (Soong et al., 2015; Crow et al., 2009). Mean residence time (MRT) has been commonly used as a metric for OM persistence in soil, being the inverse of decomposition rates (Schmidt et al., 2011). Generally, low molecular weight, less complex compounds are degraded faster by microorganisms leading to shorter MRTs (Zhang et al., 2008; Schmidt et al., 2011). Plant inputs low in these labile compounds compared to larger complex compounds, such as lignin and long chain aliphatics, typically have longer MRTs (Klotzbucher et al., 2011; Berg and McClaugherty, 2008). Due to typically low lignin and aliphatic but high cellulose content leaf litter, fine roots, and grass stems persist in soil from < 1 - 5 years (Agostini et al., 2015; Zhang et al., 2010). Meanwhile wood, which is low in cellulose compared to lignin, persisting much longer from 30-100 years (Zhang et al., 2010; Profft et al., 2009). When converted to PyOM, however, tissues may persist in soil for much longer.

Exposure of plant matter to pyrolysis conditions has been shown to lead to longer MRTs in soil (Lehmann et al., 2015). This is likely due to lack of decomposition based on the volatilization of more labile components of PyOM, leaving more stable chemical structures (Keiluweit et al., 2010, Lehmann et al., 2015). Laboratory experiments using natural abundance or ^{13}C-enriched C have estimated the retention of PyOM in soil on the scale of decades to centuries (Lehmann et al., 2015; Schmidt et al., 2011; Singh et al., 2012). This has been seen with a variety of naturally occurring and agricultural plant sources. At lower pyrolysis temperature and with more labile plant sources estimates remain on the decadal scale, with temperatures of 200 – 350 ˚C straw, grass, and crop stalks has been reported to have a MRT as low as 6-62 years (Bruun et al., 2011; Lehmann et al., 2015; Cross and Sohi, 2011). Meanwhile the MRT for PyOM from woody plant inputs and those produced at higher pyrolysis temperatures are typically much greater, ranging from hundreds to even thousands of years (Lehmann et al., 2015). Wood based PyOM formed under thermal conditions ranging from 300 - 950 °C have exhibited average mean residence times (MRT) in soils from 200 - 600 years, which greatly exceeds that of initial plant materials (Santos et al., 2012;

Singh et al., 2012; Maestrini et al., 2014). Though it is clear the persistence PyOM and fresh plant litter may be in part due to inherent chemical and physical characteristics, and subsequent potential use by microbes, other interactions may be at play between OM inputs and soil microorganisms.

1.7 Changes in Soil Microbial Residues Based on Organic Matter Inputs

As such a large fraction of SOM, the factors that control microbial necromass and biomass abundance must be assessed to understand how soils respond to stressors that can alter soil C content (Liang et al., 2015). Recent studies have highlighted the significance of a variety of OM inputs on measured microbial residues in soil using both biomass and necromass estimates. Miltner et al. (2012) outlined a conceptual framework, known as patchy fragment formation, by which OM inputs, potentially from fresh plant and fire–derived residues, lead to changes in microbial biomass and necromass (Figure 1.8). Organic matter inputs may be used to support the growth and reproduction of microbes, though over time bioavailable OM becomes limited leading to microbial starvation and death. With eventual cell death and fragmentation, biomass in turn becomes necromass which may become more stable due to cell wall fragment sorption to mineral surfaces or inclusion in aggregates, suggesting that an increase in plant or PyOM inputs to soil could yield greater microbial necromass.

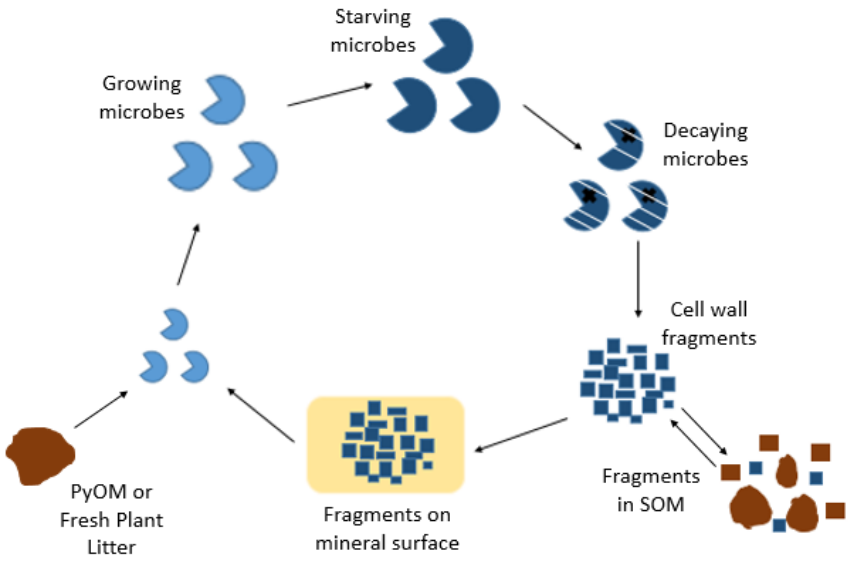


Figure 1.8. Conceptual model of microbial residue formation with organic matter input followed by residue fragmentation and stabilization in soil (Modified from Miltner et al., 2012).

Soil organic matter from fresh plant tissues has shown to impact microbial biomass (Moorhead et al., 2014; Zheng et al., 2018; Helfrich et al., 2015) and necromass (Ma et al., 2018; Wang et al., 2020; Ye et al., 2019). Strong positive correlations have been found between plant litter derived SOC and microbial residue abundance in grassland (Ma et al., 2018) forest (Wang et al., 2020), and agricultural soils (Ye et al., 2019). Changes in the abundance of OM inputs have also shown to alter microbial necromass in soil, with the exclusion of plant C input yielding a negative effect on microbial residues in cells as exemplified by lower concentrations of amino sugars (Jing et al., 2019). Besides abundance of plant litter residues, quality of plant derived OM may impact microbial residue abundance. Litter quality based on type (i.e leaf, root) has been shown to alter soil residue abundance, with lignin content and C to N ratio being the predominant controllers of litter decomposition and subsequent incorporation into biomass (Moorhead et al., 2014; Helfrich et al., 2015; Moore-Kucera and Dick, 2008), where lower lignin content and low C to N ratio yields greater microbial biomass. Plant litter quality also affects microbial community composition with greater fungal residue abundance generally associated with more recalcitrant wood and root tissues (Zheng et al., 2018; Moore-Kucera and Dick, 2008). While bacterial residues have been shown to be less abundant overall in most soils, they have been shown to increase early on with the decomposition of more labile leaf and needle litter (Moore-Kucera and Dick, 2008; Helfrich et al., 2015). While changes in microbial residues due to fresh plant matter are more apparent, less is known about how microbes respond to PyOM.

While fresh plant C inputs are shown to increase microbial residue production PyOM has been shown to impact the abundance of microbial residues in less predictable ways. Recent studies have utilized fatty acids to examine the impact of wood (Santos et al., 2012; Hardy et al., 2019, Singh et al., 2012) and crop (Farrell et al., 2013; Hardy et al., 2019) based PyOM on soil microbial biomass, which have shown that PyOM contains a small fraction of easily degradable C that may allow for the short term growth in microbial biomass, particularly gram positive bacteria. While few long term studies have examined the effects of PyOM on microbial residues it has been suggested that after depletion of the more labile fraction of PyOM microbial biomass could decrease (Maestrini et al., 2015). The labile fraction of C present in PyOM has been shown to change with pyrolysis temperature as well (Lehmann et al., 2011; Bruun et al., 2011; Lehamann et al., 2015) and has been reflected as such by microbial incorporation to biomass as seen with less incorporation of PyOM – C into biomass with greater production temperature (Dai et al., 2017;

Lui et al., 2016). Greater pyrolysis temperature has also shown a decrease in microbial diversity, resulting in PyOM – C use by specialized bacterial groups (Dai et al., 2017; Lui et al., 2016). While trends in microbial biomass due to PyOM additions may be clear for short term studies, it is apparent that less is known about how PyOM impacts microbial residues in the long term, particularly based on the use of amino sugars. To better assess how fire –derived or fresh plant OM impact soil, mechanisms other than direct microbial uptake must be examined.

1.8 Potential Impact of Organic Matter Inputs on Soil Microorganisms

Organic matter inputs may impact soil microorganisms through a variety of pathways, such as by providing a nutrient source, providing microhabitats on the OM surface, promoting or inhibiting microbial enzymatic activity, and physically protecting native SOM from microbial decomposition (Lehmann et al., 2015; Theis and Rillig 2009). Biological use of OM inputs as a nutrient source is one of the controlling factors of OM persistence in soil. Microorganisms may utilize the C and N in OM to provide energy for cellular functions or to increase cell growth and biomass (Hopkins et al., 2014). When OM is used as an energy source compounds containing C and N are mineralized, where organic molecules are decomposed to smaller inorganic molecules such as CO_2, NH_4^+, and NO_3^-. Many studies have relied on substrate use efficiency, the amount of substrate incorporated into biomass compared to the amount mineralized, as a metric to investigate how microorganisms may utilize OM inputs (Cotrufo et al., 2013). More labile compounds, such as sucrose, may have up to 75% of initial C mineralized, suggesting microorganisms convert 25% to biomass, though the actual amount incorporated into biomass versus the amount mineralized also depends on other pathways of OM loss or stabilization (Hopkins et al., 2014). Recent studies have stated assumptions that if not released as CO_2 then decomposed C may be incorporated into microbial biomass, become inaccessible such as by incorporation into aggregates, or may be lost from the system such as through the movement of dissolved organic carbon (DOC) (Hopkins et al., 2014, Gibson et al., 2018). To better assess the amount of OM input that is mineralized or incorporated into biomass OM type must also be considered.

While it is clear studies have examined OM uptake into microbial biomass (see chapter 1.7), recent studies have also examined mineralization of soil amended with fresh plant matter or PyOM. Soils have shown a positive correlation between amount of fresh plant biomass additions to soils and biomass mineralization (Zeng et al., 2010), where biomass mineralization changes between

fresh plant types with 35 - 60 % of grasses, fine roots, and leaf litter (Zeng et al., 2010; Mtambanengwe and Kirchman, 1995) and 25 - 50% (Mtambanengwe and Kirchman, 1995; Gibson et al., 2018) of wood amendment - C being released as CO_2. Microorganisms may also preferentially mineralize PyOM, with greater temperature of PyOM production yielding lower mineralization (Gibson et al., 2018; Zimmerman et al., 2011). Only small fractions of initial C have been shown to be mineralized for several plant sources, with 1- 0.05% of grass PyOM C being mineralized along a temperature scale of 200 – 650 ˚C (Zimmerman et al., 2011) and 30 – 0.6 % of wood PyOM C being mineralized from 200 – 600 ˚C (Gibson et al., 2018). Besides the degradation and use of OM as a nutrient source, other factors of OM inputs may affect microorganisms.

Organic matter amendments may also alter the decomposition of native SOM (Maestrini et al., 2014; Whitman et al., 2015; Zimmerman et al., 2011). This effect, known as priming, describes how PyOM or fresh plant biomass creates soil conditions that enhance or hinder the decomposition of SOM by microbes, with positive priming leading to increased decomposition and negative priming leading to decreased decomposition that would occur in non-amended soils, as measured by mineralized C and N (Figure 1.9). Priming of SOM by OM inputs may occur through several mechanisms. True priming effects occur due to acceleration or retardation of microbial mineralization of SOM, such as by increased microbial growth and reproduction and shifts towards microbial communities that mineralize SOM more quickly (Kuzyakov et al., 2000). Chemical characteristics of OM may impact real priming effects by changing soil pH, thereby creating more favorable or less favorable conditions for microbial metabolism, stimulating oxidative enzyme activity (Zimmerman et al., 2011), and allocating energy needed to break down the more metabolically expensive fractions of SOM (Moorhead et al., 2013). Simultaneously, physical characteristics of OM may impact real priming effects by creating suitable microbial habitats on PyOM or plant residue surfaces. Following greater pyrolysis temperature, plant material exhibits greater porosity and surface area (Hatton et al., 2016; Keiluweit et al., 2010), which could potentially yield habitats that may be beneficial for select microorganisms and yield greater microbial metabolism in soils. Organic matter inputs may also impact priming by protection of native soil C. The large hydrophobic chemical structure of PyOM and some plant residues increases long term sorption of SOM, resulting in less microbial utilization of SOM and ultimately lower metabolic activity (Whitman et al., 2015). Similarly, enzymes may become sorbed to PyOM

and plant residues inhibiting their ability to mineralize SOM (Whitman et al., 2015). Concurrently, apparent priming effects occur due to changes in observed mineralization rates, such as by the loss of amendment C or N to surrounding soil or by the uncontrolled loss of C and N through plant uptake (Kuzyakov et al., 2000). Some or all of these mechanisms may be at play when OM is added to soil depending on the physicochemical characteristics of the OM input and intrinsic properties of the native soil, resulting in various potential outcomes for the direction and magnitude of priming effects (Theis and Rillig, 2009).

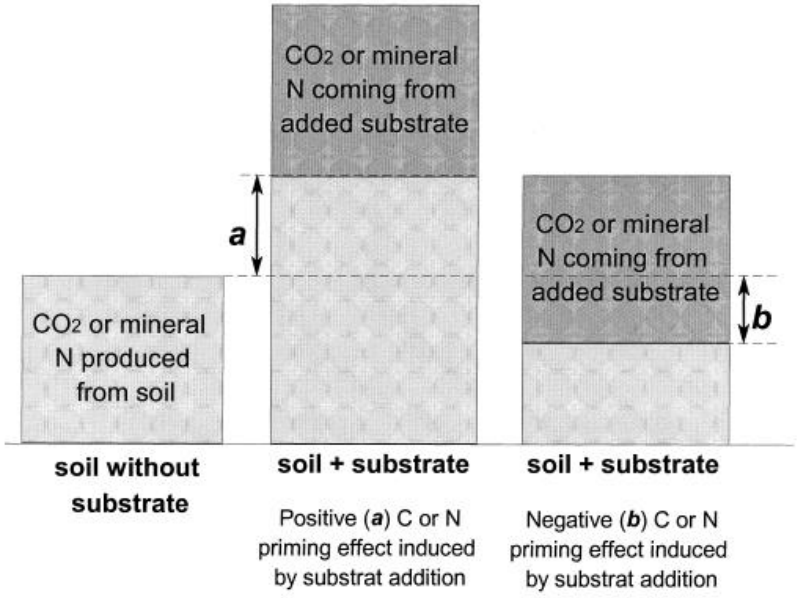


Figure 1.9. Conceptual model of priming effects due to substrate addition showing a) acceleration of SOM mineralization or b) retardation of SOM mineralization (From Kuzyakov et al., 2000).

Over time, priming effects of OM inputs on native SOM may change. Studies have reported that the addition of PyOM and fresh plant material generally creates a positive priming effect for the first 20 days after OM input likely due to initial mineralization of more labile components (Maestrini et al., 2015; Bastida et al., 2019). Later in incubations however, studies have reported decreased SOM mineralization due to physical protection of native SOM after being sorbed to OM inputs, especially PyOM (Maestrini et al., 2015). This has been seen particularly in grass and wood amendments in longer incubation studies, lasting 250-500 days (Zimmerman et al., 2011). After 10 months Gibson et al (2018) showed diminished native soil C mineralization with an

introduction of wood or PyOM, suggesting lower overall microbial activity. From these studies it is clear that mineralization of SOM declines after some point when no additional PyOM or other OM is placed into the system.

Meanwhile, additional organic inputs may induce priming effects on fresh plant litter or PyOM. To investigate the potential priming effects on OM inputs themselves, more labile C sources, such as sucrose, have also been used. Recent studies have shown variable responses of OM to sucrose, which may accelerate OM decay in some cases (Wang et al., 2016), and slow decay in others (Bastida et al., 2019). With many possible interactions between microorganisms, native SOM, and fresh or pyrolyzed OM inputs there is a need for studies to examine the impact of specific OM inputs to soil.

While there has been significant effort to investigate wood and PyOM induced native soil C priming effects, few studies have been able to quantify the true net impact of wood or PyOM on native soil C over long time periods (Maestrini et al., 2015; Lehmann et al.,2015). Due to variation in incubation conditions, land management practices, and the method by which priming is detected in studied soil, estimates of priming effects can vary widely (Lehmann et al., 2015). Variation in incubation conditions, such as moisture and temperature, have been shown to result in negative, positive and neutral priming effects (Wang et al., 2015; Maestrini et al., 2014). Land practices, such as tillage, can result in varying directions and magnitude of priming effects in agricultural and forested land (Whitman et al., 2015). To detect priming the introduction of isotopically labelled OM must occur, thereby indicating which mineralized material is from OM input and which is from native soil C. In incubation studies, by creating closed conditions that mimic natural conditions there are few risks of external variables influencing measured outcomes thereby allowing for more representative quantification the effects of plant tissue and PyOM on native soil C (Naga Raju et al., 2017). Thus, incubation studies are imperative to understanding soil priming and other possible interactions between select OM inputs and soil.

1.9 Recent Studies Using Jack Pine and Red Maple Wood and PyOM

Recent studies have examined differences in specific taxa and temperature controls of highly ^{13}C labeled jack pine (JP) and red maple (RM) wood and PyOM. This thesis leverages those studies to explore how wood and PyOM addition impact amino sugar based microbial residue abundance and contribution to SOM. These studies have examined physicochemical characteristics of wood

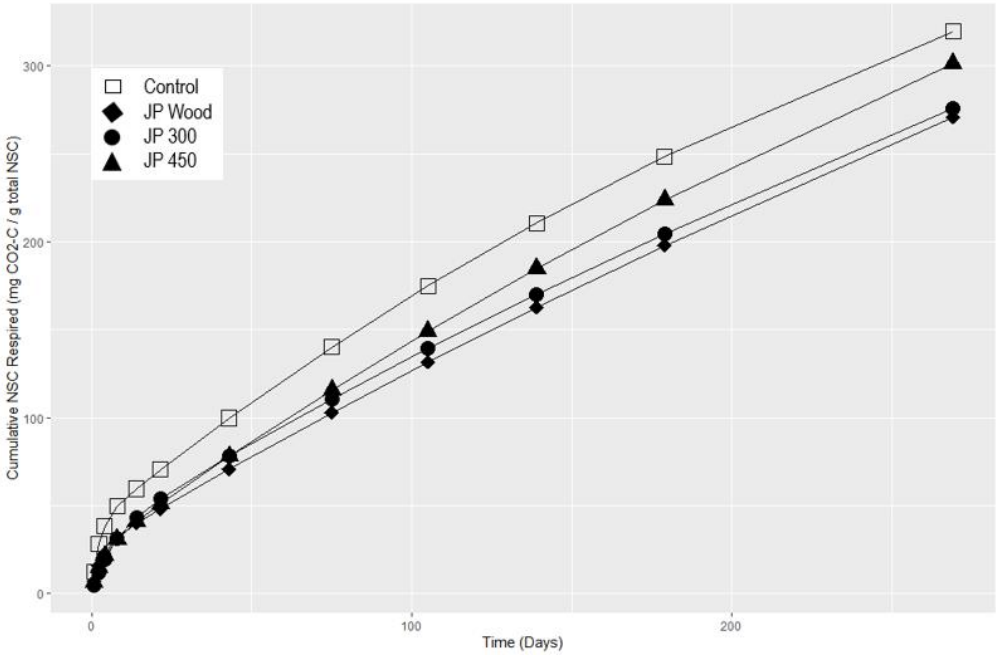
and PyOM from JP and RM (Hatton et al., 2016; Gibson et al., 2016; Gibson et al., 2018). RM wood has shown to contain more labile chemical structures, such as non-lignin phenols and carbohydrates, and a lower C to N ratio than JP wood (Gibson et al., 2018), which suggests that RM may be more easily incorporated into microbial biomass when in soil (Cotrufo et al., 2013). Additionally, JP wood has a higher concentration of condensed tannins and other extractives that might have microbial suppression qualities (Smith et al., 2012; Gibson et al., 2018; Saxena et al., 1995), suggesting that JP wood may yield lower microbial biomass than RM wood when in soil. Physicochemical differences for JP and RM were also compared along a temperature gradient of 200 – 600 ˚C (Hatton et al., 2016; Gibson et al., 2018), where greater production temperatures yielded less labile organic structures, suggesting potentially greater microbial utilization at lower production temperatures. Structural changes toward greater stability, with more double bonded, cyclical, and oxygen or nitrogen containing groups, occurred around 300 ˚C for JP and around 450 ˚C for RM, indicating that physicochemical changes at high temperatures of pyrolysis have a large degree of taxa-specific control (Hatton et al., 2016; Gibson et al., 2018).

The Gibson et al (2016, 2018) studies also investigated the impacts that JP and RM wood and PyOM have on soil. The MRT of wood and PyOM were investigated in soil, with greater MRTs being estimated with an increase in pyrolysis temperature (Gibson et al., 2018). Following similar trends in compositional changes at select temperatures, MRTs of PyOM were different between taxa at 300 ˚C and 450 ˚C. RM produced at 300 ˚C was calculated to remain in soil for 9 years but increased to 281 years when produced at 450 ˚C. Similarly, the MRT for JP increased from 40 years when produced at 300 ˚C to 292 years when produced at 450 ˚C. Altogether, the mean residence times of both tree taxa increased with pyrolysis temperature, but RM inputs had a greater difference between 300 ˚C and 450 ˚C, whereas this difference is not as significant for JP, suggesting that RM PyOM at 300 ˚C and 450 ˚C may have more distinct differences in microbial utilization.

The mineralization of wood and PyOM amendments in soil and amendment impacts on native SOM mineralization have also been examined. Greater proportions of wood amendments have been shown to be mineralized than subsequent PyOM, with lower mineralization for greater pyrolysis temperatures (Gibson et al., 2018). Differences between PyOM of both taxa have been observed, with RM - C mineralization being greater between 300 ˚C and 450 ˚C PyOM treatments than those of JP. When added to soil wood and PyOM from both taxa induced net negative priming

(Gibson et al., 2018). To investigate the impact of an additional labile C source, sucrose was added to several incubations. Gibson et al (2018) found no effect of sucrose on PyOM mineralization, while the addition of sucrose offset negative priming of native SOM for soils given JP PyOM (Figure 1.10). Altogether this information helps illuminate the potential mechanisms at play when investigating microbial responses to JP and RM wood and PyOM addition to soil.

a)



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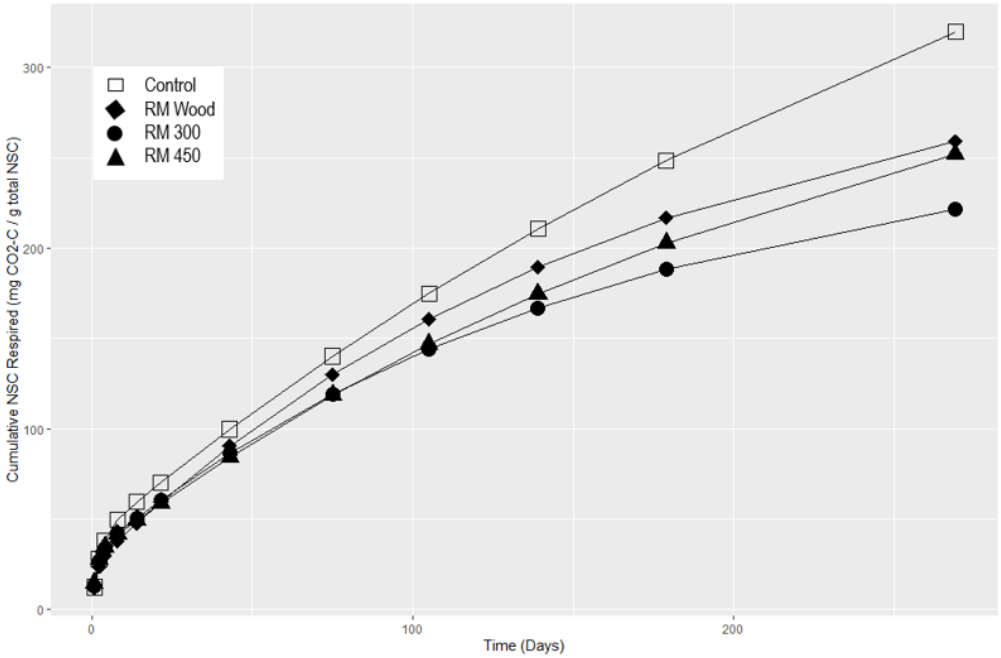


Figure 1.10. Cumulative native soil C respired for a) jack pine and b) red maple with a sucrose addition. (Modified from Gibson et al., 2018).

1.10 Goals and Hypotheses

The goal of this thesis work was to better understand the impact of biomass addition in the form of wood tissue and pyrogenic organic matter of various pyrolysis temperatures on the production and accumulation of microbial-derived amino sugars in soil, and overall SOM, in fire prone forest soil systems. Soils, from a previous study (Gibson et al., 2018), that were incubated with these plant sources were used herein. To accomplish this wood and PyOM produced at 300 ˚C and 450 ˚C from two tree taxa, JP and RM, were used as they represent major taxonomic groups in the northern temperate and boreal forests of North America. Amended soils we used were incubated for either 180 or 600 days to examine changes in amino sugars over time. This work differs from previous studies in its utilization of amino sugars as a biomarker for bacterial, fungal, and total microbial residue abundance in PyOM from woody plant tissues. In this study we sought to 1) gain information on the abundance of fungal and bacterial residues associated with select wood or PyOM amended soils 2) reveal how PyOM pyrolysis temperature and taxa affect such microbial residue abundance and 3) determine the impact this has on microbial contribution to soil organic matter.

Here, it is hypothesized that 1) pyrolysis temperature would be a large controlling factor for the abundance of amino sugars, with greater amino sugar abundance correlating with lower pyrolysis temperature for both taxa due to lower amendment quality; 2) there would be a more noticeable difference in abundance of amino sugars between 300 ˚C and 450 ˚C for RM than JP due to differences in quality of tissues produced at those temperatures; 3) there would be a difference in amino sugar content between both tree taxa and the control, with RM treatments having the greatest abundance of amino sugars; 4) there would be greater amino sugar abundance in soils incubated for 180 days compared to those incubated for 600; and 5) treatment effects for microbial C and N contribution to soil C and N would follow those of amino sugar content, where soils given RM and lower pyrolysis temperature treatments, and those incubated for shorter times would show greater microbial C and N contribution.

CHAPTER 2. MATERIALS AND METHODS

2.1 Wood and PyOM Production

Wood and PyOM were used as soil amendments in this study. Tree saplings are susceptible to charring during wildfires, with their remains being deposited onto the soil surface (Santin et al., 2016). In mixed hardwood forests a variety of sapling taxa contribute to charred residue deposits. Saplings of two tree species in mixed hardwood forests, jack pine (*Pinus banksania*) and red maple (*Acer rubrum*), were used in this study as they represent two major taxonomic groups, gymnosperms and angiosperms respectively. Furthermore, these taxa represent dominant taxa in boreal and northern temperate forests of North America, respectively, which have shown shifting taxonomic dominance in recent years (Gough et al., 2008). Jack pine (JP) and red maple (RM) were thus used as soil amendments to study the impact of tissue from different taxonomic origin on soils.

Saplings of JP and RM were previously generated to support a project to investigate wood and PyOM reactivity in soils. The methods of production of the PyOM and growing conditions of the seedlings are discussed in detail in Bird and Torn (2006). Pine and maple saplings were grown in a soil composed of fritted clay and washed sand. One year old JP was grown to 30-40 cm height (Itasca Greenhouse Inc., Cohasset, MN, USA) and 2 year old RM was grown to 30-60 cm height (Cold Stream Farms, Freesoil, MI, USA) before sampling. Stems of each species were dried at 25 ˚C and cut into 1-2 cm pieces before being exposed to heat at 300 ˚C or 450 ˚C under N_2 for 5 hours following a protocol by Hammes et al (2006), producing PyOM. Detailed information on physicochemical characteristics of this PyOM or wood has been published by Hatton et al (2016). PyOM samples were processed for subsequent use in a laboratory soil mesocosm experiment as described in Gibson et al (2018).

2.2 Soil Sampling and Laboratory Incubation

Soils used in this study were collected from a site within the University of Michigan Biological Station (UMBS). The site is located on a boreal to northern temperate forest boundary, where both taxa of interest are found, in Northern Michigan as shown in Figure 2.1 (45˚ 35.5’N, 84˚ 43’W), and has been previously used for fire chronosequence experiments. The soils at UMBS

have been described as excessively well-drained, medium, frigid Typic Haplorthods comprised of 92% sand, 7% silt and 1% clay (Gough et al., 2008). The mean annual temperature (1983–2013) was 6.8 ˚C and the mean annual precipitation was 838 mm (Santos et al., 2016). Soil organic C in the top 20 cm of the soil is largely in the form of particulate organic matter, with 50 – 55% of organic C coming from the free light fraction (MacFarlane et al., 2012). Soils were collected from four field blocks within the site in 2012 to a depth of 20 cm (Figure 2.2). As described in Gibson et al (2018), soils from cores within each of the four field blocks were sieved, dried, and homogenized by gently tumbling in preparation for laboratory use.

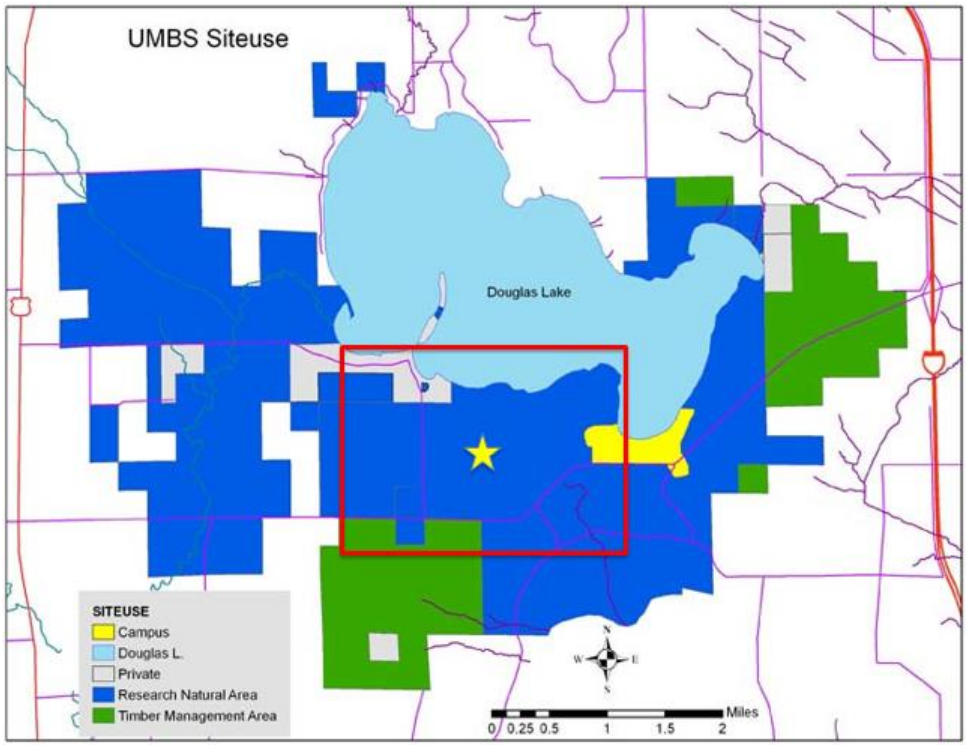


Figure 2.1. Location of University of Michigan Biological Station where soil samples were collected.

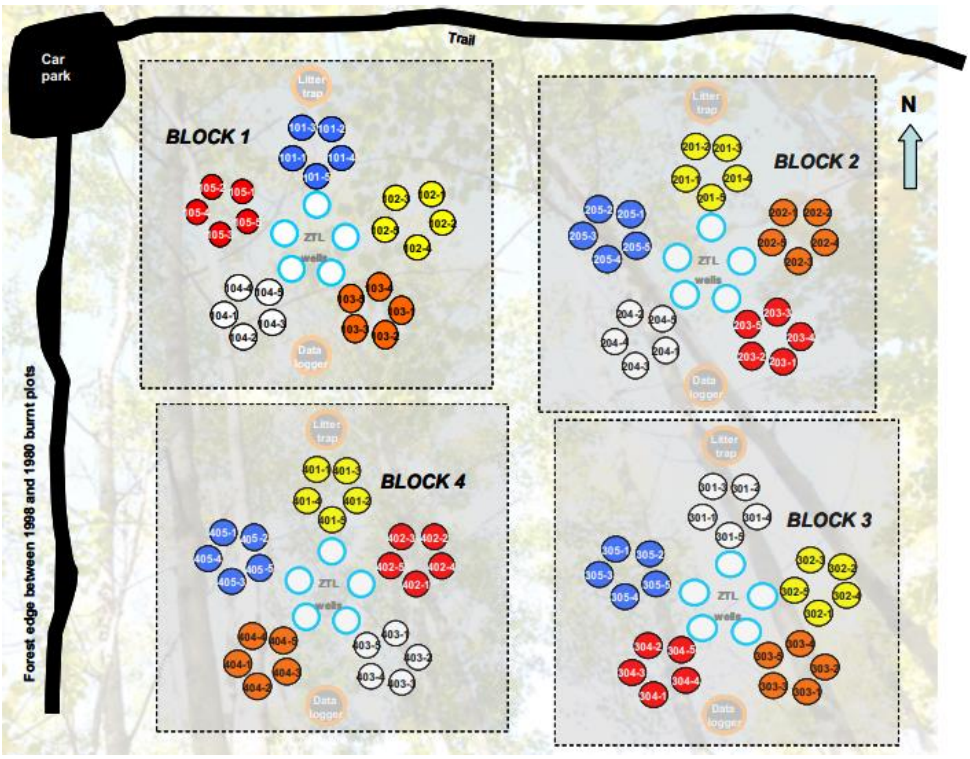


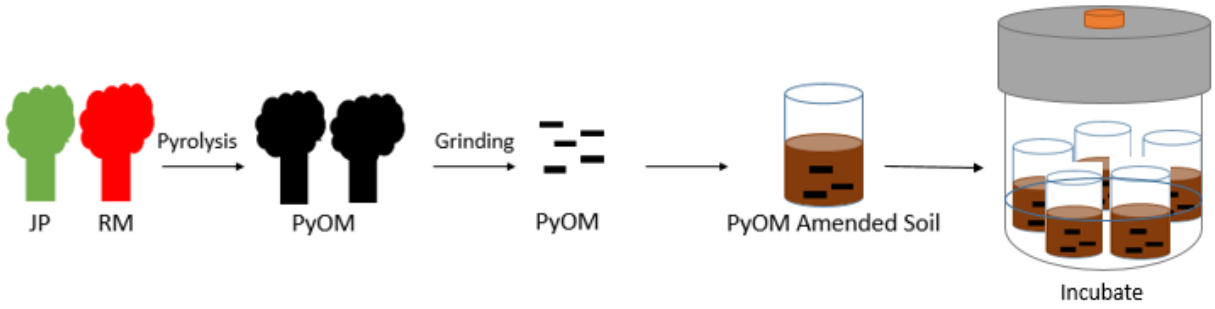
Figure 2.2. Schematic of field block design used for soil sampling at the University of Michigan Biological Station.

Collected soils were then incubated. Soils were placed into 20 ml scintillation vials and either PyOM or wood was added at 11% of soil C mass, about 20 mg of amendment per 20 g of soil per sample. PyOM or wood amendments were categorized by taxa and pyrolysis temperature; a matrix of sample amendments is given in Table 2.1. Autoclaved water was then added at 0.22 ml/g soil to achieve 60 % of water holding capacity. To examine possible effects of a more labile C source on microbial use of PyOM in soil, a beat sucrose solution was added to half of the samples at 0.15 mg/g soil (Appendix B). Five completed scintillation vials were then placed into a sealed 1 L jar with 20 ml of water. All samples were incubated as described for 180 or 600 days in the dark in a temperature-controlled room of 26 ˚C. Description of the full incubation were provided in Gibson et al (2018). Samples used in this study were soils given a temperature x taxa amendment for each field block. Soil with no amendment was used as a control and subjected to identical incubation conditions. A flowchart detailing sample preparation and incubation is shown in Figure 2.3.

Table 2.1. PyOM amendments by initial plant type and temperature of pyrolysis.

Taxa	Temperature	Taxa X Temperature
RM	0 (Wood)	RM Wood
RM	300	RM 300
RM	450	RM 450
JP	0 (Wood)	JP Wood
JP	300	JP 300
JP	450	JP 450

a)



b)

Figure 2.3. a) Flowchart of PyOM preparation and soil amendment and b) example vial containing soil from UMBS and PyOM amendment following incubation.

2.3 Sample Storage and Preparation for Analysis

Upon completion of the incubation experiments samples were stored in cold conditions and prepared for further analysis. After samples were removed from the incubation experiments and upon completion of the incubation after 180 and 600 days, the recovered soil was stored frozen

(-80 ˚C) for several years to halt microbial metabolism. For amino sugar extraction and elemental analysis the frozen incubation soils were freeze-dried, and then ground using a Retsch MM400 Ball Mill in preparation for analysis. Percent weight of C and N of samples were determined using an Elemental Analyzer-IRMS PDZ Europa linked to Sercon 2020 (Sercon Ltd., Crew, UK). Carbon and nitrogen content of amended soils following incubation are given in Table 2.2. The ground samples were then used for amino sugar extractions.

Table 2.2. Average carbon and nitrogen content of soil amended with wood or PyOM for JP or RM.

Incubation Time (Days)	Treatment	Weight % N	Weight % C
180	Control	0.022	0.51
	JP Wood	0.022	0.54
	JP 300	0.021	0.57
	JP 450	0.026	0.56
	RM Wood	0.027	0.55
	RM 300	0.029	0.64
	RM 450	0.027	0.60
600	Control	0.027	0.48
	JP Wood	0.024	0.40
	JP 300	0.022	0.45
	JP 450	0.018	0.38
	RM Wood	0.029	0.52
	RM 300	0.026	0.49
	RM 450	0.026	0.50

2.4 Amino Sugar Extraction

Amino sugars were extracted, purified, and chemically-derivatized for gas chromatographic analysis following the protocol outlined by Zhang and Amelung (1996). To extract amino sugars samples first underwent acid hydrolysis. Briefly, about 800 mg of soil was weighed into a 50 ml sealed hydrolysis flask with final mass determined by the intent of having a total of 0.3 mg N in the extraction vial. The vial was then given 10 mL of 6 M hydrochloric acid (HCl), sealed, and heated to 105 ˚C for 8 hours to hydrolyze the amino sugars.

Immediately after hydrolysis, purification steps began. The flasks were cooled to room temperature, spiked with 100 µl of a 1.0 mg/ml myo-inositol solution as a recovery standard, and

then hand mixed by swirling before subsequent extraction. Each sample was then gravity filtered through Whatman 9 cm filter paper and collected in a 250 ml pear shaped flask. The hydrolysis flasks were then rinsed with 5 ml of deionized (DI) water and then washed through the same respective filter as its sample. Filter paper was rinsed with an additional 5 ml of DI water. The filtrate was then dried by vacuum rotary evaporation at 45 ˚C, where upon the residue was transferred to 40 ml Teflon tubes with 10 ml of DI water, and pH adjusted to 6.6 – 6.8 using 1 M potassium hydroxide (KOH). The samples were then centrifuged at 3000 rpm for 10 minutes to remove any salt, mineral precipitates, or soil particles. The supernatants were then transferred to pear shaped flasks and rotary evaporated to dryness. The residues were then transferred to 15 ml Teflon tubes with 5 ml of dry methanol, and centrifuged again at 3000 rpm for 10 minutes. This second supernatant was then transferred to 5 ml glass conical reaction vials and dried under the gentle stream of dry N_2 gas. Next, 1 ml of DI water and 100 µl of 1.0 mg/ml n-methylglucamine, a second internal standard, were added to each reaction vial and then the resultant mixture was frozen at -20 ˚C and then freeze dried overnight.

The freeze-dried extract of amino sugars and internal standards then underwent several stages of chemical derivatization (Figure 2.4), as outlined by Zhang and Amelung (1996), to permit analysis by gas chromatography mass spectrometry (GC/MS). A derivatizing reagent was made using 4:1 pyridine – methanol, 32 mg/ml 4-dimethylaminopyridine, and 40 mg/ml hydroxylamine hydrochloride. Exactly 300 µl of the derivatizing reagent was added to each sample, mixed by vortexing for 30 seconds, and then placed in a hot water bath at 75 ˚C for 30 minutes with light vortexing every 15 minutes. The samples were then cooled to room temperature, where then 1 ml of acetic anhydride was added and the vials were vortexed again. Samples were placed back into a hot water bath for 60 minutes. The samples were then cooled to room temperature, before 1.5 ml of dichloromethane (DCM) was added followed by mixing by vortexing. One mL of 1M HCl was then added, to the solution, mixed, allowed to settle, and then the top aqueous phase was extracted and discarded from each sample. Three additional aqueous phase extractions using 1 ml of DI water were performed. The extracted organic solution was then placed under the gentle stream of N_2 gas until dry. Once dry, samples were dissolved with 300 µl of 1:1 ethyl acetate – hexane and transferred to 2 ml glass screw cap vials to be analyzed by GC/MS. A flowchart of amino sugar extraction, purification, and derivatization procedures are shown in Figure 2.5.

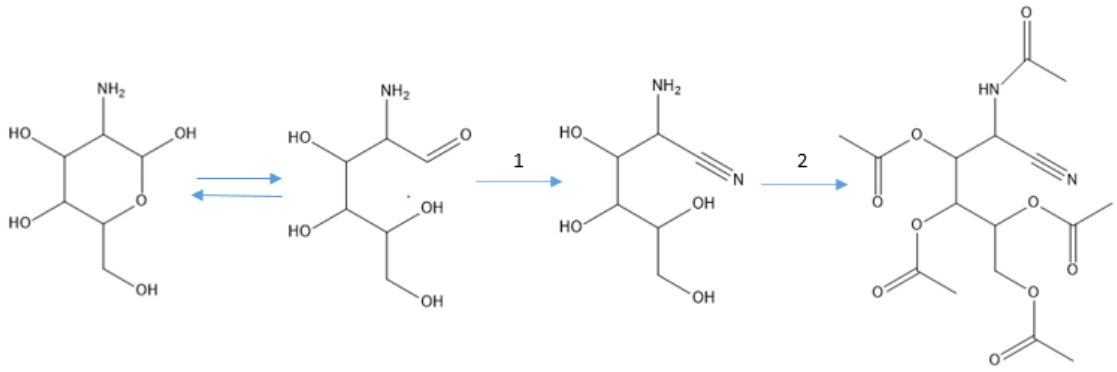


Figure 2.4. Derivatization steps for glucosamine. 1) shows the nitrile reaction and 2) shows the acetylation reaction.

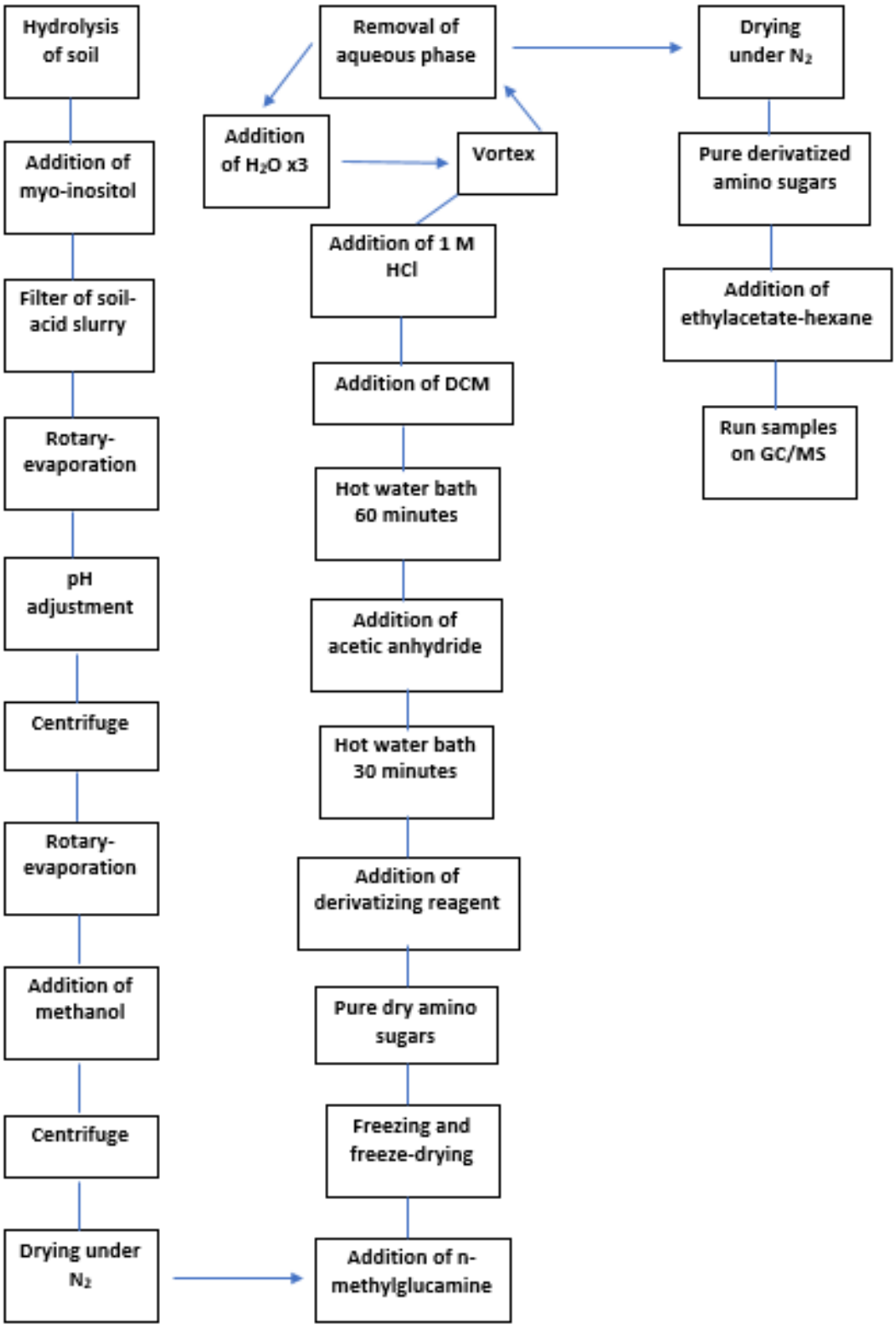


Figure 2.5. Flowchart of amino sugar extraction, purification, derivatization, and detection.

2.5 GC/MS Quantitation of Extracted Amino Sugars

The extracted amino sugars – glucosamine (GluN), mannosamine (ManN), galactosamine (GalN), and muramic acid (MurA) - were quantified using GC/MS analysis based on calibration curves of exact standards and derivatized in an identical way as described above. A Shimadzu GC 2010 system equipped with an AOC 20s auto-sampler, AOC 20i auto-injector, and Shimadzu QP2010 Plus mass spectrometer operated in electron ionization mode (Shimadzu Crop., Kyoto, Japan) was used for the separation and quantitation. The concentration of the calibration standards spanned the range 0.01 mg/ml to 2.0 mg/ml. ManN and MurA, compounds typically in low abundance in soils, were quantified using calibration standards of 0.01, 0.05, 0.1, and 0.25 mg/ml. GalN used calibration standards of 0.05, 0.25, 0.5, and 1.5 mg/ml. GluN, typically in greatest abundance, used calibration standards of 0.25, 0.5, 1.5, and 2.0 mg/ml. Peaks were identified as compounds of interest using comparison of retention time and specific mass fragments as compared to exact standards and then quantified using the total ion count (TIC) of their peak areas. Concentrations of each amino sugar were determined using the specific calibration curves described above. A calibration curve for each amino sugar referenced to myo-inositol TIC peak area was created per sample batch. Recovery adjustments were used for concentration calculations based on myo-inositol to n-methylglucamine peak area ratios. Chemical structures of each amino sugars and standards are shown in Figure 2.6.

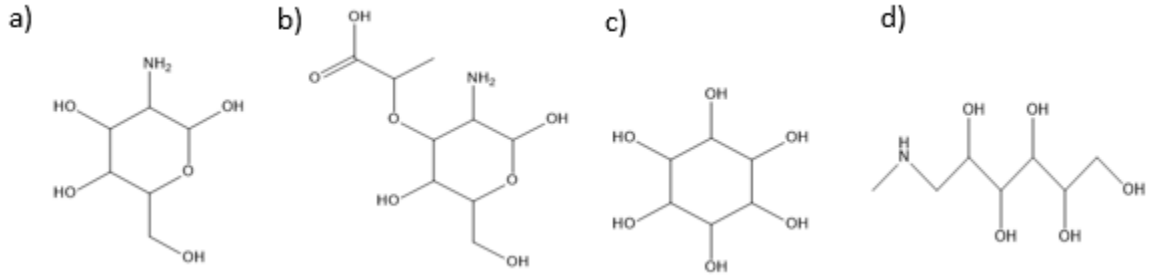


Figure 2.6. Structure of amino sugars and internal standards; a) glucosamine, mannosamine, and galactosamine b) muramic acid c) inositol d) n-methylglucamine.

Parameters for both GC and MS settings were modified from a protocol outlined in Liang et al (2012). The injection and oven parameters were as follows: 1 µL split injection (50:1) with the GC inlet set at 250 °C; initial oven temperature of 120 °C; hold 1 min; increase the oven temperature at 10 °C/min to 250 °C; hold 2.5 min; increase to 270 °C at 20 °C/min; hold 2 min; increase to 290 °C at 20 °C/min, hold 5 min. All quantification was done using a SPB-1 capillary column 30 m long, 0.25 mm internal diameter, and 0.25 um thickness. Amino sugars and internal standards were identified by known characteristic mass-charge ratios (Liang et al., 2012; Liang et al., 2011; He et al., 2006). Mass spectra and fragments for each target compound are shown in Figure 2.7.

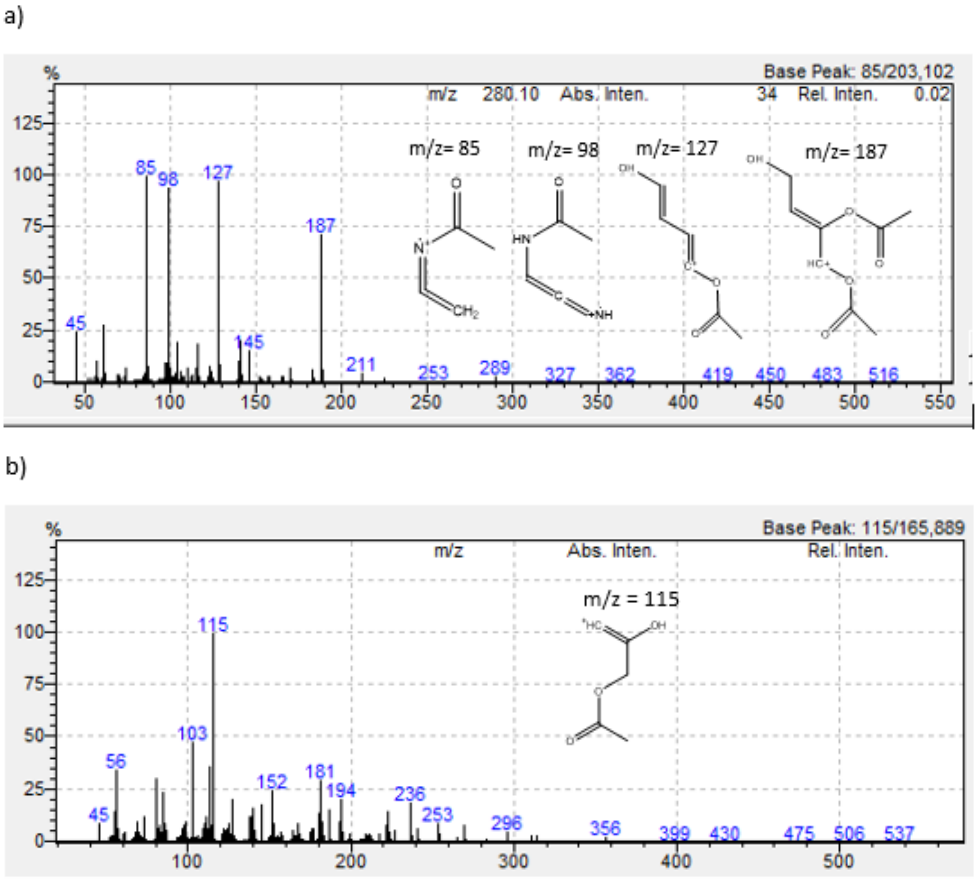
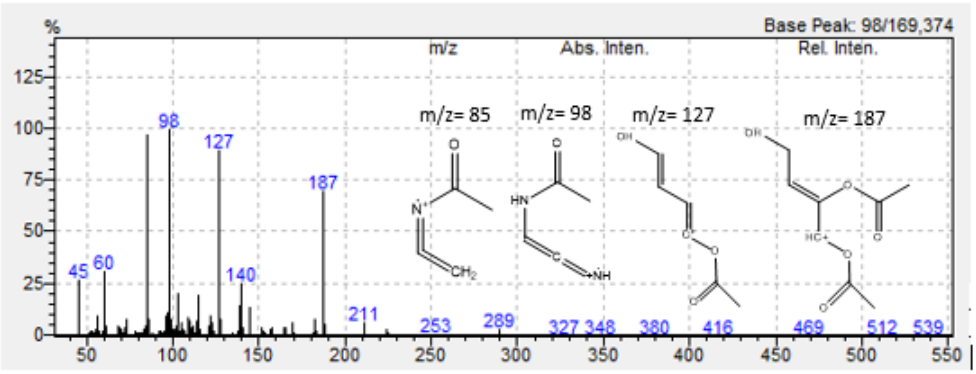


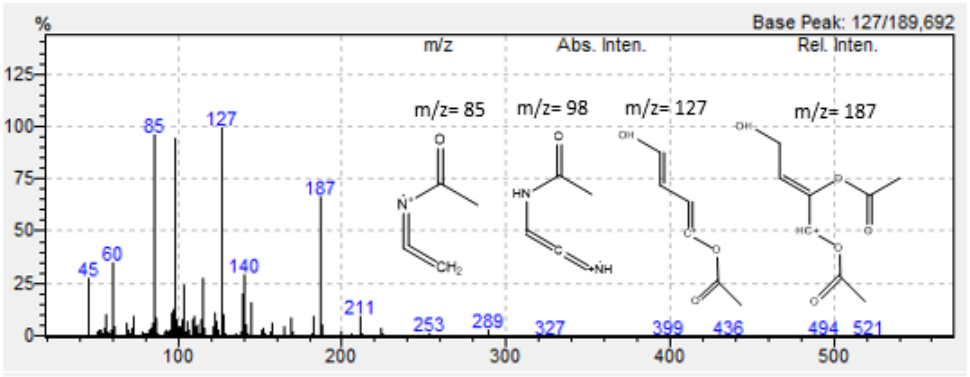
Figure 2.7. Mass spectra for a) glucosamine b) muramic acid c) mannosamine d) galactosamine e) inositol f) n-methylglucamine

Figure 2.7 Continued

c)



d)



e)

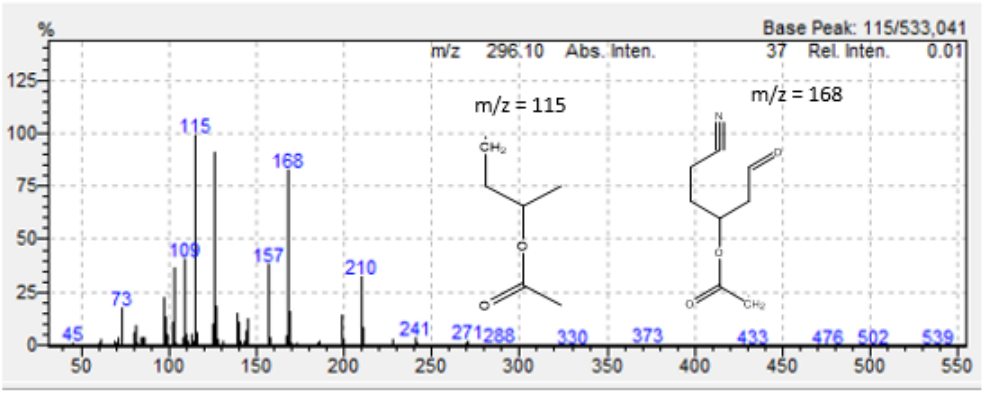
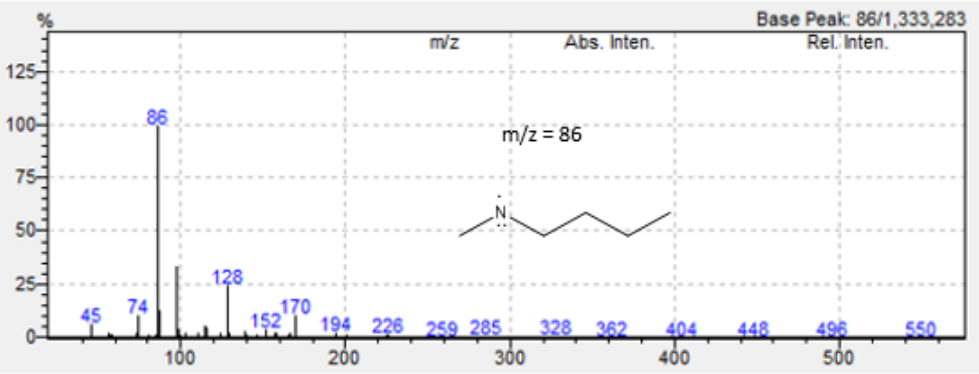


Figure 2.7 Continued

f)



2.6 Treatment of Data

Amino sugar concentrations were investigated by mass of soil, mass of C, and mass of N. First, amino sugars were investigated by mass of soil and then normalized by C and N mass of sample. From elemental analyzer data C and N samples were normalized using the following equations:

1. $$\frac{\mu g\ amino\ sugar}{g\ N} = \frac{\mu g\ amino\ sugar}{g\ soil} \text{ x } \frac{g\ soil}{g\ N}$$
2. $$\frac{\mu g\ amino\ sugar}{g\ C} = \frac{\mu g\ amino\ sugar}{g\ soil} \text{ x } \frac{g\ soil}{g\ C}$$

Following calculations outlined by Liang et al (2019) amino sugar concentrations were then converted to amino sugar derived C or N to assess microbial contribution to SOM. This was done with the following equations:

3. $$\frac{mg\ GluN{-}N}{g\ N} = \frac{mg\ GluN}{g\ N} \text{ x } \frac{14.01\frac{g}{mol}N}{179.17\frac{g}{mol}GluN}$$
4. $$\frac{mg\ GluN{-}C}{g\ C} = \frac{mg\ GluN}{g\ C} \text{ x } \frac{12.01\frac{g}{mol}C\ x\ 6}{179.17\frac{g}{mol}GluN}$$
5. $$\frac{mg\ MurA{-}N}{g\ N} = \frac{mg\ MurA}{g\ N} \text{ x } \frac{14.01\frac{g}{mol}N}{251.23\frac{g}{mol}MurA}$$
6. $$\frac{mg\ MurA{-}C}{g\ C} = \frac{mg\ MurA}{g\ C} \text{ x } \frac{12.01\frac{g}{mol}C\ x\ 9}{251.23\frac{g}{mol}MurA}$$

Equation 3 shows the conversion of glucosamine per sample N to glucosamine derived N per sample N using molar mass of glucosamine (179.17g/mol) and N (14.01g/mol). Equation 4 uses

glucosamine molar mass along with molar mass of the molecules 6 carbon atoms (12.01 g/mol x 6) to calculate glucosamine derived C per total sample C. Equations 5 and 6 follow similar calculations but for muramic acid (251.23 g/mol). Sample calculations are found in Appendix A.

Statistical analysis was completed using the program R Studio, with samples processed in duplicate within field blocks, though field blocks were ultimately pooled together (n=8 per treatment). The data collected showed a normal distribution thus analysis of variance (ANOVA) was performed. Data was then compared between each treatment using a post-hoc Tukey test for significance between groups.

CHAPTER 3. PHYSICOCHEMICAL DIFFERENCES IN WOOD TAXA AMENDED TO SOIL CONTROL ACCUMULATION PATTERNS OF MICROBIAL-DERIVED AMINO SUGARS DURING INCUBATION

3.1 Abstract

Recent literature has highlighted the importance of the conversion of plant litter to microbial tissue residues in soil as a fundamental process in the production of stable soil C and N pools. The rate and type of microbial decomposition of plant tissue is controlled by numerous factors including plant litter quality, soil physical characteristics, and the ability of plant litter to alter microbial interaction with native SOM. There is a knowledge gap, however, in how specific plant tissues derived from different taxa may differentially control the accumulation of microbial tissue residues in a soil. This work shows the results of the analysis of microbial-derived amino sugars from soils amended with jack pine (JP) and red maple (RM) wood following 180 and 600 days of incubation. Both taxa, amended at 11% of the total original SOC, resulted in an increase in microbial sugar residues compared to non-amended soils with RM showing the highest percentage increase. The difference in amino sugar content between taxa may be governed by chemical properties of the woods with labile components, present to a higher degree in RM, being incorporated into biomass and subsequent necromass. Amino sugar derived C and N contributions to total soil C and N partially followed patterns of amino sugar abundance, with soils given RM showing the greatest amino sugar C contribution to soil C. Amino sugar derived C accounted for 4-7% of soil C, while amino sugar derived N accounted for 14-28% of soil N. Longer incubation time resulted in lower amino sugar content, with bacterial residues decreasing more severely over time compared to fungal residues that dominated throughout incubation times. The variable response in amino sugars between plant taxa and time highlights the importance and dynamic nature of litter quality in controlling soil microbial responses to organic matter inputs.

3.2 Introduction

Fresh plant tissues control the overall C and N input and primary cycling dynamics of nutrients in soil (Cotrufo et al., 2013, Miltner et al., 2012; Janzen 2005). The sequestration of CO_2 from the atmosphere and deposition of C into soil as plant tissue is a prominent mechanism to mitigate the effects of global climate change while improving soil fertility (Griscom et al., 2017; Loveland and Webb 2003). However, shifts in vegetation type and amount may alter C cycling dynamics and change ecosystem ability to function as a C sink (Nave et al., 2010; Kumar et al., 2018). This is particularly important to consider in the context of continued climate change and land use change (Griscom et al., 2017; Kumar et al., 2018). As particulate organic matter from fresh plant tissues can make up a substantial portion of soil organic matter (SOM), with estimates ranging from 10 - 20% of SOM on average (Ingham, 2019; USDA, 2014), changes in plant inputs may have important implications on soil C and N dynamics and subsequent climate or land use change.

The persistence of plant tissues in soil varies based on physical and chemical protection mechanisms (Berg McClaugherty, 2008; Paul, 2016). Chemically, tissues may be more or less resistant to microbial or abiotic decay due to thermodynamic stability of chemical bonds, where double bonded, cyclical, large, and complex structures are more resistant to degradation (recalcitrant) while small simple structures are more easily degraded (labile) (LaRowe and Van Cappellen, 2011; Berg and McClaugherty, 2008). Large complex molecules, such as lignin and long chain aliphatics, are typically associated with woody plant tissues while leaf, needle, and fine root tissues are more abundant in smaller molecules, such as sugars, phenols, and short chain aliphatics (Berg and McClaugherty, 2008; Cotrufo et al., 2013). Chemical resistance to decomposition also changes between similar plant tissue types (i.e. wood, leaf, root) of different taxonomic origin, where tissues with greater recalcitrant molecule content are slower to decompose (Gibson et al., 2018; Agostini et al., 2015). Plant matter may become sorbed to surrounding organic surfaces or interiors mediated by hydrophobic forces (Schwarzenbach et al., 2017; Paul, 2016). Metal oxides and cations of clay minerals may interact with plant matter through charge mediated interactions (Schwarzenbach et al., 2017; Paul, 2016; Mikutta et al., 2006), such as with binding to aluminosillicates and iron hydroxide mineral surfaces (Sollins et al., 2009; Mikutta et al., 2006), where labile plant byproducts are more frequently mineral associated (Cordova et al., 2018; Cotrufo et al., 2015). Plant tissues can also be physically

protected by becoming spatially inaccessible to microbes or abiotic conditions such as by being incorporated into aggregates, which is characteristic of more recalcitrant particulate plant matter (Six et al., 1998; Cotrufo et al., 2015).

While the persistence of plant tissues in soil may be variable due to unique chemistry and stabilization mechanisms (Berg and McClaugherty, 2008; Paul, 2016; Schwarzenbach et al., 2017), they are also susceptible to decomposition by both abiotic and biotic means (Krishna and Mohan, 2017). Plant tissue decay can occur abiotically, such as by photolysis or with naturally occuring chemical oxidants (Haruo, 2012; Golanoski et al., 2012). Microbial degradation is the main biotic pathway for plant tissue decomposition in soil (Zeng et al., 2010; Zheng et al., 2018; Helfrich et al., 2015). It has been suggested that the ultimate control on the rate of plant tissue decay in soil is largely depedent on the relative proportion of slow-degrading, large complex molecules and labile molecules (Castellano et al., 2015). To assess microbial degradation on short time scales, from months to a few years, studies have investigated both mineralization and microbial uptake of plant tissues, and found that some tissue types are mineralized slower (Zeng et al., 2010; Mtambanengwe and Kirchman, 1995) and converted to microbial biomass to lower degrees (Moorhead et al., 2014; Zheng et al., 2018; Helfrich et al., 2015). Research on the competition between catabolic breakdown of molecules for energy production and the anabolic production of biomass, refered to as substrate use efficiency, has typically shown that a higher proportion of the mass contained in labile substrates is allocated toward microbial biomass compared to more recalcitrant substrates that have high energy costs of breakdown (Lekkerkerk et al., 1990; Cotrufo et al., 2013). While microorganisms may decompose plant matter they may also contribute to SOM through the deposition of their own residues.

Microbial cell residues play a large role in the production and stabilzation of SOM, and as such the microbial response to plant litter inputs is of high importance to the stability and production of SOM. While live microbial biomass may only account for about 2% of soil organic C (Liang and Balser, 2011; Miltner et al., 2012), dead cell tissue residues, known as necromass, may account for 47-80% soil C (Fan and Liang, 2015) and 40 – 100% of soil N (Liang et al., 2019). To investigate the abundance and diversity of microbial necromass, microbial cell wall chemical components, such as amino sugars, can be used a proxy indicators. Amino sugars, as a proxy for necromass, have been shown to vary in response to plant tissue inputs, with larger amounts of amino sugars being formed with greater plant tissue abundance in soil (Ma et al., 2018; Wang et

al., 2020; Ye et al., 2019) and with higher proportions of labile plant chemical input (Bai et al., 2013). Amino sugars can also be used to differentiate between microbial groups, with glucosamine (GluN) representing fungal origin and muramic acid (MurA) representing bacterial origin, while galactosamine (GalN) and mannosamine (ManN) are not origin specific (Joergensen, 2018). These amino sugars and biological molecules representing biomass, such as phospholipid fatty acids (PLFAs), have been used to investigate how plant litter quality affects microbial community composition with greater fungal residue abundance generally associated with more recalcitrant plant tissues (Bai et al., 2013; Zheng et al., 2018; Moore-Kucera and Dick, 2008). While bacterial residues have been shown to be less abundant overall in most soils, bacteria have been shown to play large roles in the decomposition of more labile plant litter (Bai et al., 2013; Moore-Kucera and Dick, 2008; Helfrich et al., 2015). In addition to being a proxy for microbial residue abundance and diversity, amino sugars have been used to assess the contribution of microbial C and N to overall SOM (Appuhn and Joergensen, 2006; Liang et al., 2019; see chapters 1.5 and 2.5), which may vary based on plant input type and abundance (Simpson et al., 2007; Ma et al., 2018; Ye et al., 2019). With microbial residues comprising potentially large fractions of soil C and N and plant input type and amount altering microbial residues, it is likely that changes in plant inputs alter microbial residue contribution to soil C and N.

Microbial residue abundance and composition also vary over time as recent studies have demonstrated that after initial plant tissue input microbial biomass and subsequent necromass increase (Miltner et al., 2012). During the course of plant litter decay the labile aspects of plant matter and SOM are preferentially consumed, leaving behind recalcitrant byproducts (Liang and Balser, 2011), which may then lead to microbial starvation and diminished biomass over time (Miltner et al., 2012). As microbial cell residues are composed of high N content sugars and amino acids they are also readily decomposable, leading to potential utilization of microbial residues as a nutrient source for living biomass. However, those residues may become stabilized on SOM particles or mineral surfaces and be protected from decay (Milter et al., 2012), resulting in variable residence times in soil (Schmidt et al., 2011). Additionally, specific microbial groups have been shown to change over time after plant tissue input. Bacteria have been shown to be early decomposers of plant tissues as seen by amino sugars and PLFAs though their abundance is typically smaller than that of fungi throughout the decomposition process (Ding et al., 2011; Bai

et al., 2013; Moore-Kucera and Dick, 2008). Besides the use of plant tissue as a nutrient source, other factors of plant inputs may affect microorganisms and their subsequent residues.

Organic matter amendments may also increase or decrease the mineralization of native SOM (Zimmerman et al., 2011; Kuzyakov et al., 2000). This effect, known as priming, describes how fresh plant tissue enhances or hinders the decomposition of native SOM, where positive priming leads to increased decomposition of native SOM and negative priming describes a decrease in decomposition rate of native SOM (Zimmerman et al., 2011; Kuzyakov et al., 2000). Soil amendment incubation experiments have shown that the positive priming effect generally occurs early, within the first month of addition to soil, in soil experiments (Zimmerman et al., 2011; Kuzyakov et al., 2000). Positive priming effects have been attributed to the stimulation of native SOM mineralization rates by the addition of labile components of plant tissues that are typically depleted in available N and thus inducing the microbes to "mine" soil organic matter N (Lyu et al., 2019). Negative priming effects have been shown to occur later in long term experiments and have many potential causes (Zimmerman et al., 2011). Observed negative priming effects could be caused due to plant-derived compounds that promote the use of substrates for microbial growth rather than respiration (Bastida et al., 2019), inhibit microbial or extracellular enzyme activity (Kuzyakov et al., 2000), or induce organo-mineral interactions and aggregation of SOM (Chen et al., 2019). With variable direction and magnitude of priming effects due to multiple mechanisms the investigation of how soil microbial respond to plant tissue inputs over time is needed.

Determining the impact of plant tissue deposition in forest systems that are under land use, invasive species, and fire stress are especially important as these ecosystems are undergoing impacts that could have important feedbacks to the soil C and N pools (Janzen 2005; Nave et al., 2010; Kumar et al., 2018). The University of Michigan Biological Station (UMBS) is located at the transition zone between Northern temperate and boreal forests (Gough et al., 2008), where older pine species and approaching deciduous species meet, and is projected to be susceptible to increased fire pressure under future climate projections (Reich et al., 2015; Santín et al., 2016). This site is a perfect location to test how changing deciduous and conifer dominance could influence soil microbial response. Plant tissues can have varying rates of decay dependent upon initial plant tissue chemistry, with conifers, being slower to decay than hardwood deciduous tissues in long term field studies (Shortle et al., 2012). Recent studies have investigated physicochemical differences of jack pine (JP) and red maple (RM) wood in particular, and their degradability in soil

from the UMBS locality (Gibson et al., 2018; Hatton et al., 2016, see chapter 1.9). RM wood contains more labile organic structures (carbohydrates and non-lignin phenols) compared to JP wood, which plays a role in the longer residence time and larger fast cycling C pool, measured by mineralization, for JP wood (Gibson et al., 2018). This may be the result of changes in substrate use efficiency, with more labile components of wood being incorporated into microbial biomass, while recalcitrant components are used for metabolic activity, as indicated by mineralization (Lekkerkerk et al., 1990; Cotrufo et al., 2013). In incubations with the UMBS soil, both taxa imparted a net negative priming effect during 300 days of incubation (Gibson et al., 2018). However, it is unknown how the tissues of these very different taxa may become incorporated into microbial tissues over time, and how these microbial tissues impact soil C and N.

The aim of this study was to better understand the impact of plant biomass amendments, in the form of wood tissue, on the production and accumulation of microbial-derived amino sugars in soil, and overall SOM, in forest soil systems. This work differs from previous studies in the use of amino sugars as microbial residue proxies for soil amended with two tree taxa that underwent varying incubation periods. Here it is hypothesized that a) there would be a difference in amino sugar content between tree taxas and the control, with RM treatments having the greatest abundance of amino sugars, b) there would be greater amino sugar abundance in soils incubated for shorter (180 days) compared to longer (600 days) times and c) treatment effects for microbial C and N contribution to soil C and N would follow those of amino sugar content, where soils given RM wood and incubated for shorter times would show greater microbial C and N contribution.

3.3 Materials and Methods

Saplings of JP and RM were previously generated to support a project to investigate wood reactivity in soils from UMBS (Hatton et al., 2016). The methods of growing conditions of the seedlings are discussed in detail in Bird and Torn (2006). Stems (two year old RM and one year old JP) were dried, cut to 2mm sections, and gently mixed into soil at 11% of total SOC and incubated in controlled laboratory conditions for 180 or 600 days (Gibson et al., 2018). After samples were removed from the incubation, the recovered soil was stored frozen (-80 ˚C) to halt microbial metabolism prior to additional chemical analysis.

Amino sugars were extracted, purified, and chemically-derivatized on duplicate soil samples following the protocol outlined by Zhang and Amelung (1996). Exact details of the extraction,

purification, gas chromatography/mass spectrometry (GC-MS) analysis, and normalization techniques are discussed in Chapter 2 of this thesis. Briefly, the extracted amino sugars were quantified using GC-MS analysis based on calibration curves of exact standards. Amino sugar concentrations were investigated by mass of soil, mass of C, and mass of N. Amino sugars were investigated by mass of soil and then normalized by C and N mass of sample. Percent weight of C and N of samples were determined on a flash combustion elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) using a Sercon ltd (Crew, UK) GSL EA interfaced to a Hydra IRMS. Amino sugar concentrations were then converted to amino sugar derived C or N to assess amino sugar based microbial contribution to SOM.

Statistical analysis was completed using the program R Studio, with samples replication at the block scale (n=4). Each individual block soil used in the incubation was a composite of eight cores (0-20 cm) from that block (Gibson et al., 2018). The data collected showed a normal distribution thus analysis of variance (ANOVA) was performed. Data was then compared between each treatment using a post-hoc Tukey test for significance between groups.

3.4 Results

3.4.1 Changes in amino sugar content per soil mass

Total concentrations of amino sugars in incubated soil (μg/ g soil) differed significantly between RM wood amendments and the control after both 180 and 600 days of incubation, while JP wood amendments only differed from the control at 180 days (Table 3.1). RM amended soils exhibited greater content in total amino sugars as compared to JP (Figure 3.1). These trends in total amino sugars were driven by the compounds in greatest abundance, GluN and GalN, while there was no difference between taxa observed for ManN or MurA content over the same incubation time period. On average, total amino sugar content increased by 12.5% for JP wood and 38.3% for RM wood after 180 days of incubation as compared to the control. Amino sugars increased 26.6% for RM wood above the incubation control after 600 days of incubation, while JP wood did not vary from the control. Additionally, the total amino sugar content was greater for each treatment, including control, at 180 days of incubation as compared to 600 days (Figure 3.1). The contribution of each individual amino sugar to total amino sugar concentration followed the order of GluN>GalN>MurA>ManN. Additionally, the GluN to MurA ratio varied significantly

between JP and RM wood when incubated for 180 days, while there was no difference between treatments when incubated for 600 days. Generally, 600 day treatments showed greater GluN to MurA ratios than 180 day treatments (Table 3.1).

Table 3.1. Concentration of total amino sugar, glucosamine (GluN), galactosamine (GalN), and muramic acid (MurA), mannosamine (ManN), as well as ratio of glucosamine to muramic acid per soil mass for wood treatments incubated for 180 and 600 days. Values are given as mean ± (standard deviation). Letters within each column represent a significant difference between groups. The same letter in a column represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

Treatment	Total (μg/g soil)	GluN (μg/g soil)	GalN (μg/g soil)	MurA (μg/g soil)	ManN (μg/g soil)	GluN/MurA
180 Day Control	673.0(41.9)c	492.0(30.3)bc	148.7(13.0)bc	24.2(2.9)b	8.1(0.6)b	20.5(2.4)bc
180 Day JP Wood	757.3(46.3)b	554.3(34.8)b	165.4(10.4)b	28.2(2.9)a	9.4(1.3)ab	19.8(1.6)c
180 Day RM Wood	931.0(85.4)a	695.1(70.0)a	198.2(15.9)a	27.6(4.1)ab	10.2(2.6)a	25.8(4.0)ab
600 Day Control	490.4(41.3)d	353.5(22.8)d	115.5(14.6)d	12.0(1.2)c	9.4(1.0)ab	29.8(4.2)a
600 Day JP Wood	474.4(36.5)d	340.7(29.8)d	111.2(6.8)d	12.4(1.5)c	10.1(0.6)a	27.6(2.7)a
600 Day RM Wood	614.2(60.7)c	454.8(43.2)c	134.9(15.1)c	14.4(2.1)c	10.1(1.1)a	31.9(3.1)a

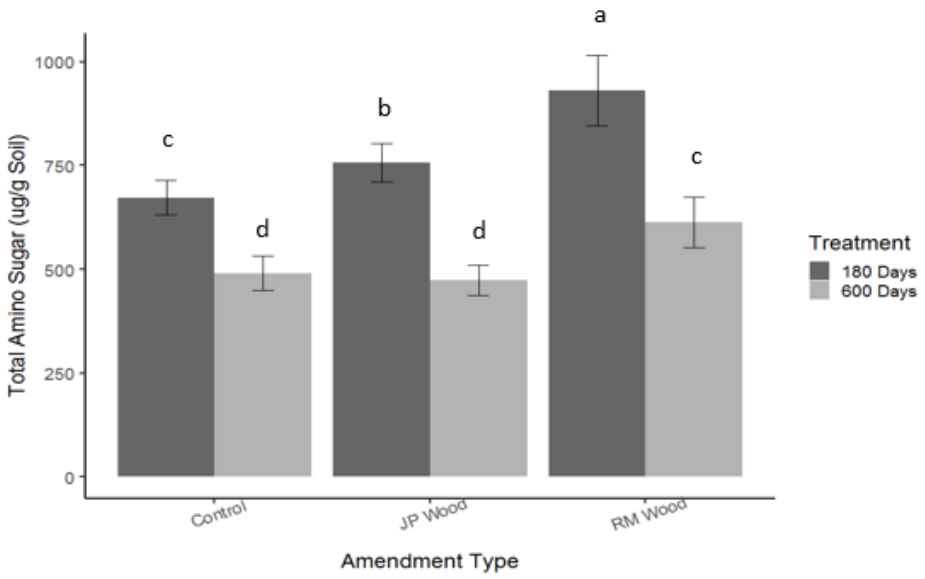


Figure 3.1. Total amino sugar concentrations per mass of soil for JP and RM wood treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

3.4.2 Change in amino sugar concentration per soil C and N

Total concentrations of amino sugars (mg/ g soil C) differed significantly between RM wood amendments and control after 180 days of incubation, while JP wood amendments did not differ from the control at either incubation time (Table 3.2). Additionally, a high degree of variability was exhibited among the field blocks. On average amino sugar concentration per mass soil C increased 31.8% for RM wood after 180 days of incubation as compared to the control, while a nascent trend for JP wood was shown with a 6.8% increase. Amino sugars did not significantly increase compared to the incubation control after 600 days of incubation. The total amino sugar concentration was generally greater for treatments after 180 days of incubation as compared to 600 days, though not for the control (Figure 3.2). Total concentrations of amino sugars (mg/ g soil N) did not differ significantly between wood amendments or the control after both 180 and 600 days of incubation (Table 3.2). The total amino sugar concentration (mg/ g soil N) was greater for each treatment after 180 days of incubation as compared to 600 days, including the control (Figure 3.3).

Table 3.2. Concentration of total amino sugars (AS) per mass of C and N, along with amino sugar derived C and N contribution to total soil carbon and nitrogen for wood treatments incubated for 180 and 600 days. Values are given as mean ± (standard deviation). Letters within each column represent a significant difference between groups. The same letter in a column represents no significant difference between treatments at P<0.05. n=8 for each treatment.

Treatment	AS (mg/g N)	AS (mg/g C)	AS N Contribution to Soil N (%)	AS C Contribution to Soil C (%)
180 Day Control	3057.2(120.7)ab	131.8(7.4)bc	23.7(0.9)ab	5.3(0.3)bc
180 Day JP Wood	3471.1(310.6)a	140.8(14.0)b	26.8(2.4)a	5.7(0.6)b
180 Day RM Wood	3580.4(605.5)a	174.1(31.3)a	27.8(4.7)a	7.0(1.3)a
600 Day Control	1830.0(219.8)c	105.2(14.9)c	14.2(1.7)c	4.2(0.6)c
600 Day JP Wood	2008.6(207.2)c	120.3(11.5)bc	15.6(1.6)c	4.8(0.5)bc
600 Day RM Wood	2441.2(856.3)bc	126.1(35.4)bc	19.0(6.6)bc	5.0(1.4)bc

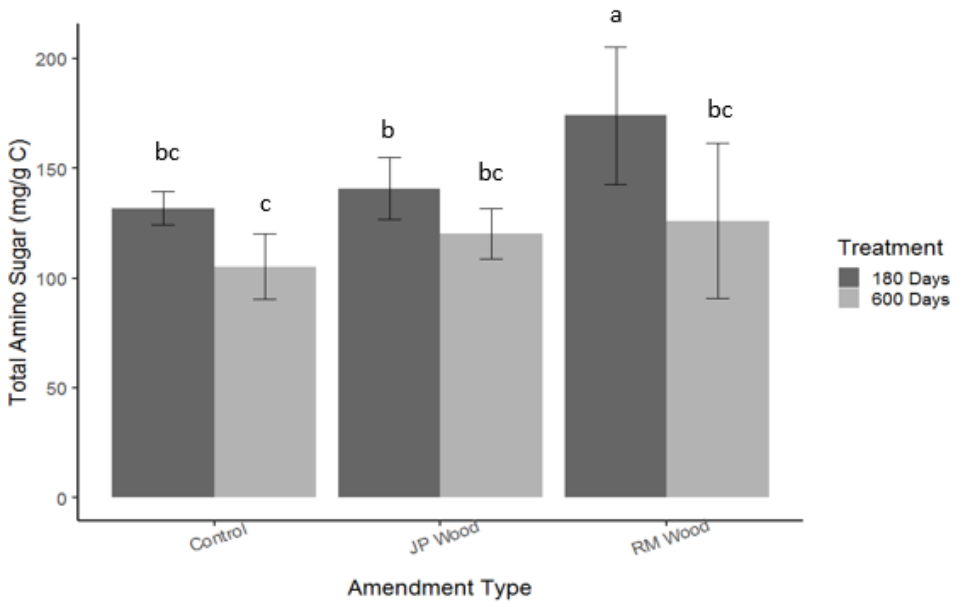


Figure 3.2. Total amino sugar concentration per mass soil C for JP and RM wood treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at P<0.05. n=8 for each treatment.

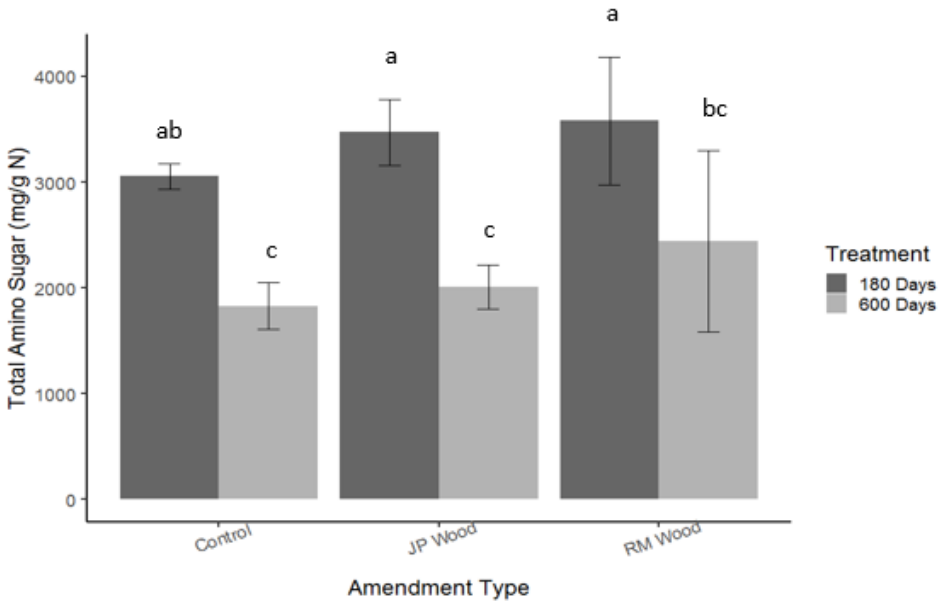


Figure 3.3. Total amino sugar concentration per mass soil N for JP and RM wood treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

3.4.3 Proportional contribution of amino sugar- C and N to total soil C and N

The contribution of amino sugar derived C to total soil C for RM wood amendments differed significantly from JP wood amendments and control after 180 days of incubation, while JP wood amendments did not differ from the control (Table 3.2). The proportion of amino sugar C to total C was generally greater for treatments after 180 days of incubation as compared to 600 days, and significantly so for RM wood (Figure 3.4). On average the control showed 5.3% microbial derived C contribution to soil C after 180 days of incubation, while RM wood amendments showed 7.0% (an increase of 32% from the control). There was no significant difference in microbial derived C in SOC between the control or either wood treatment after 600 days of incubation.

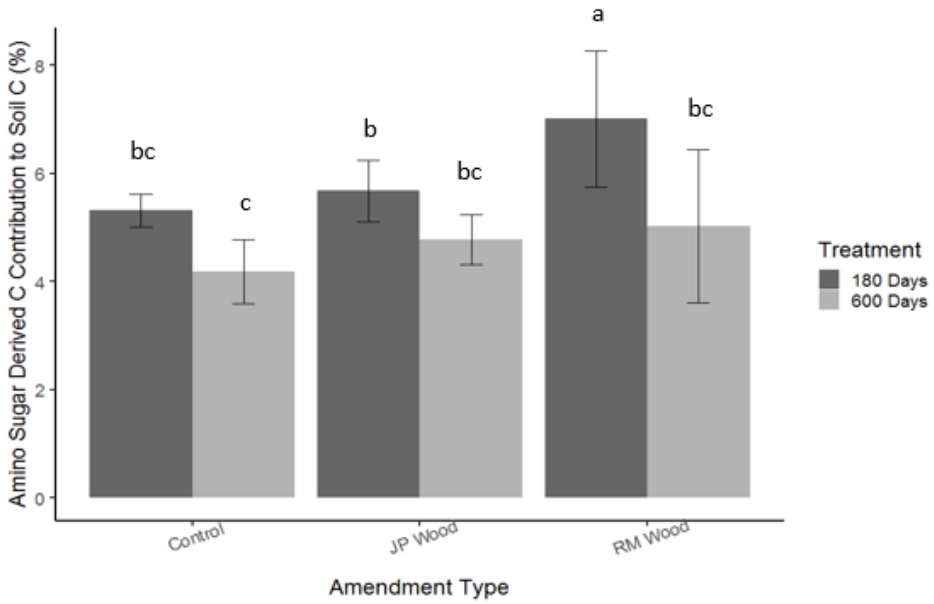


Figure 3.4. Total amino sugar derived C contribution to total soil C for JP and RM wood treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

The proportional contribution of amino sugar derived N to total soil N did not differ significantly between wood amendments or the control after both 180 and 600 days of incubation (Table 3.2). Total amino sugar content exhibited greater proportional enrichment for each treatment after 180 days of incubation as compared to 600 days (Figure 3.5).

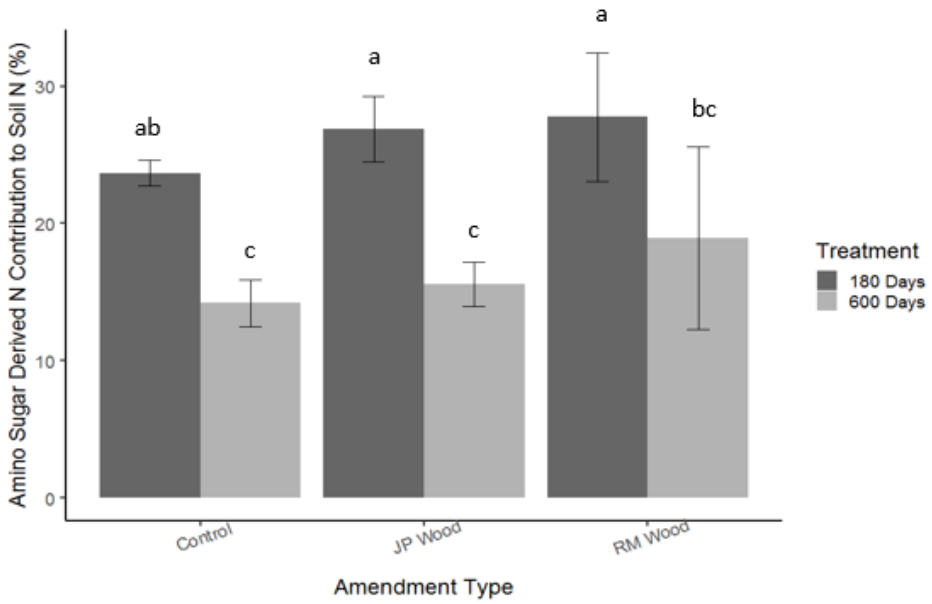


Figure 3.5. Total amino sugar derived N contribution to total soil N for JP and RM wood treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

3.4.4 Fate of amended wood during incubation: mineralization and microbial uptake

Based on work published on these same samples in Gibson et al (2018) it was determined that during the course of the 180 day incubation, of initial wood C amendment loaded at 11% by weight of the soil C mass that on average, about 59.7% (+/- 3.6%), was mineralized with no significant difference between JP or RM treatments. These values were calculated based on isotope modeling outlined in Gibson et al (2018) which indicated that up to 25% of native soil carbon was converted to CO_2 (Table 3.3). While it is unknown how much original wood amendment remained following the incubation, it can be estimated how much of the original amendment could have been converted to amino sugar carbon and microbial tissue. About 6% of initial amendment C was incorporated into amino sugars for JP wood and 19% for RM wood (Figure 3.6). Total microbial residue content was calculated from amino sugar content (Table 3.3., Equations 1-4, Chapter 1.5), suggesting that up to 100% (+/- 23%) of sample C for RM wood treatments was incorporated into necromass, along with 94% (+/- 10%) for JP wood, and 85% for the control (+/- 4%).

Table 3.3. Wood amendment, native soil carbon (NSC), and total sample (amendment and soil) mineralization (Gibson et al., 2018), estimates of incorporation into amino sugar C, and incorporation into overall microbial residues in soil samples incubated for 180 days. Values are given as mean ± (standard deviation). Letters within each column represent a significant difference between groups. The same letter in a column represents no significant difference between treatments at P<0.05. n=4 for each treatment.

Treatment	Initial Sample C Mineralized (%)	Initial NSC Mineralized (%)	Initial Amendment C Mineralized (%)	Initial Sample C Amino Sugar (%)	Initial Amendment C Amino Sugar (%)	Initial Amendment C Microbial Residues (%)
Control	24.8(1.5)a	24.8(1.5)a		5.7(1.0)a		
JP Wood	23.5(2.1)a	19.8(1.9)a	57.1(10.0)a	5.7(0.5)a	5.9(5.3)b	133(91)b
RM Wood	25.7(2.6)a	21.7(3.3)a	62.3(5.4)a	7.0(0.4)a	18.9(6.3)a	312(111)a

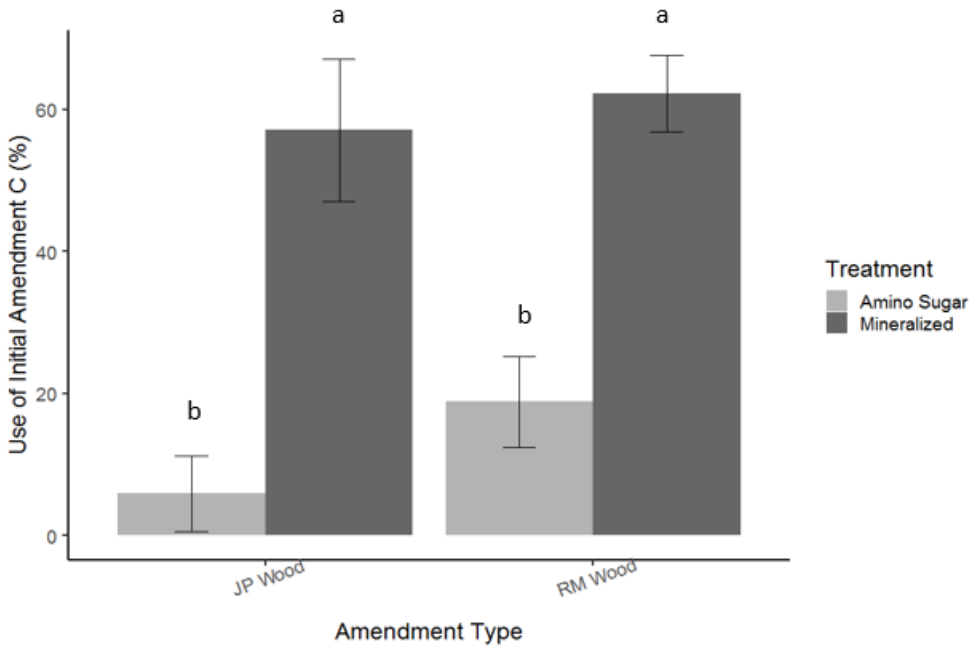


Figure 3.6. Graph showing mineralization of C from red maple and jack pine wood amendment (Gibson et al., 2018) and increase in total amino sugars (this study) for the same samples incubated for 180 days. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at P<0.05. n=4 for each treatment.

3.5 Discussion

3.5.1 Plant taxa physicochemical composition control microbial production of amino sugar

Total amino sugar concentrations calculated in this work were within range of previous studies that investigated North American forest soils (Liang et al., 2008), including boreal and temperate forest soils. It is reasonable that GluN dominated total amino sugar abundance while MurA was of lower abundance according to previous studies (Joergensen, 2018). Fungi have been shown to be the primary microbial group utilizing C from wood during decomposition in soil (Cornwell et al., 2009) and can be highly specialized (Boddy and Heilmann-Clausen, 2008) to break down most components of wood including sugars, lipids, hemicellulose, cellulose, and lignin. The role of bacteria in the decomposition of wood, on the other hand, is still less known (Cornwell et al., 2009).

This study suggests that microbial amino sugar abundance varies according to the type of fresh organic matter amended to soil. As expected, soils amended with RM wood typically showed greater concentrations of soil amino sugars compared to those given JP wood or the control. It is well established that OM quality, such as C to N ratio and presence of certain organic structures, is a fundamental control on microbial decomposition, with more labile structure and low C to N ratio sources being preferentially decomposed by microorganisms (Kleber, 2010, Cotrufo et al., 2013). The difference in taxa effects observed herein may be explained by chemical differences between the taxa as RM wood has a higher proportion of labile compounds, such as non-lignin phenols and carbohydrates compared to JP wood, while also having a lower C to N ratio (Gibson et al., 2018; Hatton et al., 2016). Additionally, JP has a higher concentration of condensed tannins and other extractives that might have microbial suppression qualities (Smith et al., 2012; Gibson et al., 2018; Saxena et al., 1995). These chemical differences may result in RM being more easily degraded by soil microorganisms and incorporated into microbial biomass – measured here as amino sugars (Cotrufo et al., 2013). Studies have also found a positive correlation with labile C content of plant matter inputs and microbial biomass (Jolivet et al., 2006) and necromass (Bai et al., 2013), which is consistent with the observed trends of greater RM labile C content corresponding to greater amino sugar abundance.

Recent incubation studies have also investigated differences in wood degradability based on mineralization (Santos et al., 2012; Gibson et al., 2018), where JP wood and RM wood have shown nearly equal levels of mineralization (Gibson et al., 2018). This may be a matter of substrate

use efficiency, with recalcitrant tissues found in both woods, being respired to a greater degree than labile tissues, present to a higher degree in RM wood, which are incorporated into microbial biomass (Cotrufo et al., 2013). It has been shown that substrate use efficiency decreases with recalcitrant compound content of plant litter due to high energy costs of molecule breakdown (Lekkerkerk et al., 1990). Meanwhile, long-term studies of wood tissues have shown varying rates of decay dependent upon plant tissue chemistry, with RM decaying more quickly than more recalcitrant conifers (Shortle et al., 2012). It is thus likely that the observed effect can be explained by the recalcitrant compound content of JP, which is less incorporated into microbial biomass, and higher labile content of RM which is highly incorporated into biomass and subsequent necromass. Previous studies have estimated that substrate use efficiency of more labile compounds, such as sucrose, with up to 60% of initial C mineralized, suggesting microorganisms may convert up to 40% of substrate C to biomass, though the actual amount incorporated into biomass versus the amount mineralized also depends on other pathways of OM loss or stabilization (Hopkins et al., 2014).

In contrast to the direct microbial mineralization or incorporation into microbial residues, wood additions may have induced priming of native soil C as found in previous mineralization studies (Gibson et al., 2018). Priming of native soil C by wood additions could cause a shift in substrate use away from soil C toward greater use of wood or vice versa, which could yield positive, negative, or no net priming (Maestrini et al., 2015; Whitman et al., 2015). While positive priming has been reported for some long-term incubations (Maestrini et al., 2015), the current samples exhibited net negative priming throughout this incubation (Gibson et al., 2018). With a suppression in native soil C mineralization it could be expected that incorporation of native soil C into microbial residues is also suppressed. However, the results from the current study suggest an increase in microbial residues with the addition of wood. As wood amendments have been shown to be highly mineralized and to be incorporated into microbial biomass, as indicated by fatty acids (Santos et al., 2012), it is likely that both JP and RM wood are directly incorporated into microbial amino sugars, though no direct mechanism is investigated here. Considering the measured negative priming effects induced by the wood amendment and the simultaneous mineralization of 57-62% of the added wood (Gibson et al., 2018), we surmise that increased amino sugar abundance for these same soils are primarily derived from the microbial utilization of wood.

3.5.2 Amino sugar content decreases with incubation time

We observed, as expected, that amino sugar content in soil decreased with incubation time. This could be explained by a number of factors including decreased availability of nutrients and labile C over time which caused microbes to mine microbial necromass. Additionally, microbial use of labile fractions of OM yields progressively more recalcitrant byproducts, as described by the microbial carbon pump (Liang and Balser, 2011). These OM fractions would then be less bioavailable and thus less able to be incorporated into microbial residues. Miltner et al. (2012) discussed a framework by which fresh plant residues lead to changes in microbial biomass and necromass, with plant inputs initially yielding greater biomass and necromass which then may become stabilized on mineral surfaces or incorporated into SOM. However, with progressively more recalcitrant OM sources, it could be expected that microbial residues, such as amino sugars are used as an energy source and mineralized thereby leaving the system, which is consistent with the current findings.

Specific amino sugars also changed over time as indicated by the GluN to MurA ratio, which expresses fungal-to-bacterial residues abundance. Fungi have been shown to favor decomposition of what are purportedly recalcitrant tissues, such as wood-lignin (Blanchette, 1991) with lower or constant fungal residues reported later in studies (Cornwell et al., 2009; Bai et al., 2013; Ding et al., 2011). Meanwhile, bacteria have been shown to play significant roles in plant litter decomposition within the first several weeks, but to a lesser degree than fungi and even less so later in incubation or field studies (Cornwell et al., 2009; Bai et al., 2013; Ding et al., 2011). This shift of weaker bacterial contribution to litter degradation over time is consistent with a greater GluN to MurA ratio at 600 days of incubation compared to 180 days (Table 3.1).

3.5.3 Plant taxa and incubation time control amino sugar abundance per C and N and amino sugar contribution to SOM

Current results suggest that a difference in fresh organic matter amendments and incubation time may cause a change in amino sugars per mass of soil C and N. As expected, soils amended with RM wood showed greater amino sugar abundance (mg/g soil C) compared to the control and JP wood, similar to trends in amino sugar abundance per sample mass. However, trends were also similar to total sample C content. Soils (0-20 cm) used in this study contained 0.3-0.9% total SOC with variability derived from natural heterogeneity of soil from different sampling blocks.

Similarly, total amino sugars (mg/g soil N) showed trends correlating to amino sugar abundance per sample mass and total sample N content. With soils containing only 0.02 – 0.05% total N and amino sugar content per mass of soil being typical for forest soils, the proportion of amino sugar derived-N may appear inflated due to low soil N content (Equation 1, Chapter 2.5). In low nutrient content soils, like those investigated herein, fresh OM inputs may provide an easily accessible C and N source for microorganisms (Jolivet et al., 2006), which may explain why only an 11% increase in C from wood inputs yielded greater amino sugar per mass soil C (31.8% RM and 6.8% JP). It has been shown that soils given RM wood exhibit smaller fast cycling C pools compared to JP wood (Gibson et al., 2018), suggesting fast microbial utilization of RM. More so, RM wood contains more extractable sugars compared to JP wood (Hatton et al., 2016), which are likely rapidly used as a nutrient source by microbes and reflected in amino sugar content presented here. While this taxa effect was present earlier in the incubation there was no difference in amino sugar C or N content later in the incubation, suggesting that time also impacts amino sugar content per mass of C and N.

Current results suggest that a difference in fresh organic matter amendments and incubation time may cause a change in microbial C and N contribution to SOM. Here, RM and JP additions yielded greater amino sugar C contributions to soil C than non-amended soil. In the present study, amino sugar C contributed 4-7% of soil C, which is within range of previous estimates (Ni et al., 2020; Joergensen and Meyer, 1990). However, amino sugar derived N contribution to soil N (14-28%) was higher than previous estimates of 5-12% (Stevenson, 1982), which may be explained by the presence of non-bioavailable inorganic N in other soils with high clay contents. Soils with high clay content have been suggested to make N less accessible than soils dominated by sand through organo-metallic complexation and electrostatic interactions (Rasmussen et al., 2006; Santos et al., 2012). If soil C and N are inaccessible through complexation, microbial communities are more reliant on wood amendments, and may increase their biomass and subsequent necromass, yielding higher percent contribution values than expected when introduced to an OM amendment. This would be especially true for bacterial amino sugars, which have shown to quickly incorporate NH_4^+ and NO_3^- in comparison to soil N (He et al., 2011). However, the soils used in this study were of low clay content so there are likely other controlling mechanisms. Another possible explanation for the observed trends in amino sugar C and N contribution is by the loss of gaseous C and N over time. The loss of CO_2 was monitored during the course of this incubation, with consistent losses

of native soil C and wood derived C accounted for up to 300 days (Gibson et al., 2018). With potentially high mass losses of C through mineralization there is room for variability as seen between treatments at 180 days. However, studies have reported minimal losses of gaseous N, such as N_2O, during plant tissue incubations (Abbasi et al., 2015; Santos et al., 2012), which may be due to microbial immobilization of N (Abbasi et al., 2015). With limited release of N from the soil system it could be expected that treatments do not vary.

3.5.4 Fate of amended wood during incubation: mineralization and microbial uptake

Gibson et al (2018), through tracking the isotopes labeled in the amended wood, found for these same samples that during the course of the 180 day incubation that, on average, 59.7% (±3.6%) of the amended wood was mineralized to CO_2 with no significant difference between JP or RM treatments. While it is unknown how much original wood amendment remained, mostly as cellulose and lignin, following the incubation it can be estimated how much of the original amendment could have been converted to amino sugar C and microbial tissue. About 6% of initial amendment C was incorporated into amino sugars for JP wood and 19% for RM wood (see Appendix A). The possible incorporation of wood-derived C to amino sugars C was lower compared to mineralized C (Gibson et al., 2018) at 180 days of incubation. However, it should be noted that amino sugars only represents a single compound class of microbial residues. When amino sugars were used to estimate the amount of initial wood C used by microorganisms (see chapter 1.5), up to 100% may have been incorporated into or have previously existed as microbial residues. This suggests that wood C could be utilized to a high degree for microbial growth and highlights the growing importance to understand microbial contributions to SOM.

3.6 Conclusion

The results of this study provide new information on the effects of different plant tissue source taxa on soil microbial residues, and microbial contribution to SOM, using amino sugars as a proxy for C and N contribution to soils. It has been determined that wood amendments of different physicochemical properties yield increases in microbial residues with more easily degradable taxa, like RM, showing proportionately higher microbial responses in residue accumulation than taxa with higher abundances in lignin and potentially anti-microbial extractives,

like JP. Early in incubations, bacteria may have a large role in OM decomposition, as indicated by the presence of bacterial residues, though their abundance decreases greatly over time. Fungal amino sugar residues dominate throughout incubation periods though diminish over time as well. Amino sugar derived C and N contributions to total soil C and N partially followed patterns of amino sugar abundance, with soils give RM showing the greatest amino sugar C contribution to soil C.

These findings confirms soil conceptual models (i.e., MEMS framework, microbial carbon pump, patchy fragment formation) that suggest microbial residue accumulation is controlled by plant litter quality, where more labile RM wood is incorporated into microbial tissues than its more recalcitrant counterpart, JP wood. While estimates for amino sugar contribution to soil C in this study are in agreement with previous findings across numerous ecosystems, the contribution of amino sugar N to total soil N are larger than expected, possibly due to differences in gaseous release of N from soil throughout the incubation or organo-metallic complexation. From these values it is possible that microbial residues could contribute larger amounts to SOM than traditionally thought. While further analysis is needed to provide a direct mechanism for the incorporation of wood tissue to amino sugars and total microbial residue estimates may need to be refined, this study has provided insight on the connection between plant source taxa and microbial amino sugars in soil.

CHAPTER 4. PHYSICOCHEMICAL DIFFERENCES IN PYOM AMENDED TO SOIL PARTIALLY CONTROLS ACCUMULATION PATTERNS OF MICROBIAL-DERIVED AMINO SUGARS DURING INCUBATION

4.1 Abstract

Recent literature has highlighted the importance of the conversion of plant litter and pyrogenic organic matter (PyOM) to microbial tissue residues in soil as a fundamental process in the production of stable soil C and N pools. The rate and type of microbial decomposition of PyOM is controlled by a combination of PyOM physicochemical characteristics, soil physical conditions, and microbial interactions with native soil organic matter (SOM). However, there is a knowledge gap in how the properties of PyOM, and it source plant tissue, act to control the accumulation of microbial residues in a soil. This work presents the results of the analysis of amino sugars extracted from soil incubation experiments that were amended with jack pine (JP) and red maple (RM)-sourced PyOM produced at 300°C or 450°C as well as the source JP and RM wood. Soils amended with PyOM exhibited lower amino sugar content as compared to their wood source but no difference compared to controls (non-amended soils). No differences in soil amino sugar content were observed between the PyOM derived from the two taxa nor between the temperature of pyrolysis, possibly due to only small amounts of bioavailable C and N in each PyOM amendment. As a result total amino sugar concentrations varied between pyrolyzed and fresh wood treatments, with PyOM treatments yielding 659 – 730 μg/ g soil while wood treatments yielded 757 – 930 μg/ g soil early in incubations. Longer soil incubation time, 600 days vs 180 days, resulted in a lower amino sugar content, with bacterial-derived sugars decreasing more over time compared to the fungal-associated ones that dominated throughout incubation times. While PyOM treatments exhibited 19-27% of soil N and 4-5% of soil C from amino sugars early in the incubation, wood treatments exhibited 27-28% of soil N and 6-7% of soil C derived from amino sugars. Meanwhile amino sugar contribution to soil C and N declined over time. Thus, this work shows that on a per C or per N basis, PyOM versus source wood addition to fire prone soils will result in a net depletion of microbial residues. The variable response in amino sugars between treatments and incubation time highlights the importance and dynamic nature of the physicochemical characteristics of organic matter input to soil in controlling the contribution of soil microbial residues to that soil.

4.2 Introduction

Fire is a significant facilitator of C and N cycling in forests, contributing to atmospheric CO_2 and NO_x, as well as soil C and N through the deposition of organic matter (Santin et al., 2016; Driscoll et al., 1999; Sprichtinger et al., 2001). The impacts to soil and many other atmospheric reponses come from the formation of fire-derived pyrogenic organic matter (PyOM), the product of the incomplete combustion of biomass in the absence of oxygen (Lehmann et al., 2015). Numerous studies have shown that PyOM addition may have a large role in the C and N content, stability, and cycling dynamics of nutrients in soil (Driscoll et al., 1999; Santin et al., 2016). Fire is a major ecosystem disturbance that shifts a forest from being a C sink to a large source of CO_2 to the atmosphere. However, deposition of PyOM to soil can shift a forest from being a large source of atmospheric C and N to a C and N sink or vice versa depending on the amount of organic matter (OM) stabilized in soil versus the amount of OM mineralized (Palviainen et al., 2018; Santin et al., 2016). Furthermore PyOM can alter the rate of mineralization or immobilization of C and N once in soil (Palviainen et al., 2018). Thus, for a clearer understanding of regional and global C and N budgets, the impact of PyOM production and addition to soils in fire prone settings must be clearly understood. Recent estimates suggest a global average of 13.7% of soil organic C (SOC) is derived from PyOM (Reisser et al., 2016). While estimates vary between ecosystems and locations, PyOM is present in most soils and can contribute up to 40% of forest SOC (Preston and Schmidt, 2006, Schmidt et al., 2011). This is particularly important to consider as fire frequency and intensity are expected to increase in many ecosystems with climate change and associated impacts of invasive species with a projected increased PyOM production (Reich et al., 2015; Santín et al., 2016; Palviainen et al., 2018). With PyOM composing potentially large amounts of soil organic matter (SOM), the study of PyOM persistence and its impact on SOC destabilization/stabilization processes are paramount.

PyOM stability in soil is determined primarily by the temperature of pyrolysis and by differences in chemistry and structure of the plant material from which it is derived (Lehmann et al., 2015; Gibson et al., 2018). In the production of PyOM, changes in the physicochemical structure of plant matter occur along, in a non-linear fashion, gradients in the production temperature (Keiluweit et al., 2010). With increasing pyrolysis temperature, plant tissues change in chemical composition from the dominant structural macromolecules such as lignin, cellulose, and hemicellulose to condensed amorphous C and crystalline C (Lehmann et al., 2015; Keiluweit

et al., 2010). Physically, plant tissue changes when structural molecules from the fresh plant matter are volatilized at high temperatures yielding greater pore space and surface area until larger volume crystallites are formed (Keiluweit et al., 2010). At low PyOM production temperatures (< 300 °C) initial plant chemistry may only be marginally altered (Hatton et al., 2016) and thus PyOM produced at low temperatures may have similar characteristics as the original plant matter. At high PyOM production temperatures (> 400°C) many of the physiochemical characteristics of initial biomass are diminished as pyrolysis temperatures cause chemistry, surface area, and internal pore space of PyOM to become highly altered (Kleber et al., 2015; Keiluweit et al., 2010; Hatton et al., 2016). Though the specific temperature of chemical shifts may change, PyOM generally shifts towards more recalcitrant, condensed, cyclical, double bounded structures at higher production temperatures, which has been shown to play a significant role in PyOM persistence in soil (de la Rosa and Knicker 2011; Keiluweit et al., 2010; Bostick et al., 2018; Hatton et al., 2016; Gibson et al., 2018).

PyOM persistence in soil is also dependent on the physical protection by SOM, mineral surfaces, and aggregates (Lehamann et al., 2015; Brodowski et al., 2006; Liang et al., 2008; Laird et al., 2008). Fragments of PyOM have been found within varrying sizes of soil aggregates, which may reduce its accessibility to microorganisms that would otherwise deccompose it (Brodowski et al., 2006; Liang et al., 2008). PyOM may also be inaccessible through interactions with clay mineral surfaces, where partial positive charges of aluminum (Al), silicon (Si), and iron (Fe) may interact with negatively charged PyOM surfaces mediated by ligand or anion exchange (Lehmann et al., 2015). Surrounding SOM may interact with PyOM through sorption, where high fractions of PyOM surface area are coated with SOM and fresh plant or microbial residues (Laird et al., 2008; Lehmann et al., 2015), thereby decreasing microbial accessibility and slowing decomposition.

While the persistence of PyOM in soil may be variable due to unique chemistry based on production temperature and initial plant source, as well as phyiscal protection by surrounding soil particles (Lehmann et al., 2015; Keiluweit et al., 2010), it is also susceptible to decomposition by both abiotic and biotic means (Wang et al., 2017; Gibson et al., 2016; Cheng et al., 2006). Many studies have estimated the persistence of PyOM in soil from decades to centuries compared to the much shorter persistance of plant parent material (Santos et al., 2012; Maestrini et al., 2014; Singh et al., 2012) based on biotic and abiotic decomposition. It has been shown that PyOM may be

degraded abiotically by photolysis (Gibson et al., 2016), ozone (Cheng et al., 2006), water (Bostick et al., 2018) and chemical oxidants (Liu et al., 2013). Partial abiotic decompostion of PyOM can lead to increased susceptibility to biotic decomposition (Wang et al., 2017) through increased porosity, surface area, and surface oxidation, which can improve microbial habitats and enhance the utilization of PyOM as an energy source (Whitman et al., 2015; Lehmann et al., 2015). Biotically OM, such as PyOM, can be degraded by microorganisms that typically utilize the C and N in OM to provide energy for cellular functions or to increase cell growth and biomass (Jiang et al., 2016; Cotrufo et al., 2013). However, the degree to which PyOM is utilized by microorganisms is dependent on its chemical stability, where more stable residues produced at higher production temperatures exhibit slower decomposition (Cross and Sohi, 2011; Santos et al., 2012; Singh et al., 2012; Maestrini et al., 2014).

To assess microbial degradation, studies have investigated both mineralization and microbial uptake of PyOM, where PyOM produced at higher temperatures, associated with more recalcitrant tissues, are generally less mineralized (Zimmerman et al., 2011; Gibson et al., 2018) and found in microbial biomass to lower degrees (Lui et al., 2016). The trade off between catabolic breakdown of molecules and the anabolic production of biomass, refered to as substrate use efficiency, has typically shown that a higher proportion of the mass contained in labile substrates is allocated toward microbial biomass compared to more recalcitrant substrates that have high energy costs of breakdown (Cotrufo et al., 2013). Previous studies have estimated that substrate use efficiency of more labile compounds, such as sucrose, with up to 60% of initial C mineralized, suggesting microorganisms may convert up to 40% of substrate C to biomass, though the actual amount incorporated into biomass versus the amount mineralized also depends on other pathways of OM loss or stabilization (Hopkins et al., 2014) and may vary based on OM chemistry (Cotrufo et al., 2013). With potentially large amounts of PyOM or other OM being incorporated into microbial biomass, the study of microbial residues is imperative.

Microbial residues play a large role in the production and stabilzation of SOM, and as such the microbial response to plant and PyOM inputs is of high importance. While live microbial biomass may only account for about 2% of soil organic C (Liang and Balser, 2011; Miltner et al., 2012), dead cell tissue, known as necromass, may account for 47-80% soil C (Fan and Liang, 2015) and 40 – 100% of soil N (Liang et al., 2019). To investigate the abundance and diversity of microbial necromass, microbial cell wall chemical components, such as amino sugars, can be used

a proxy indicators. Amino sugars, as a proxy for necromass, have been shown to vary in response to plant tissue inputs, with larger amounts of amino sugars being formed with greater plant tissue abundance in soil (Ma et al., 2018; Wang et al., 2020; Ye et al., 2019, Chapter 3) and with higher proportions of labile plant chemical input (Bai et al., 2013, Chapter 3). However, less is known about how microbial amino sugars respond to PyOM inputs to soil.

Amino sugars can also be used to differentiate, broadly, between fungal and bacterial origins of the cell residues in soil, with glucosamine (GluN) being of predominantly fungal origin, muramic acid (MurA) of bacterial origin, and galactosamine (GalN) and mannosamine (ManN) of mixed origin (Joergensen, 2018). These amino sugars and other biological molecules linked to biomass origin, such as phospholipid fatty acids (PLFAs), have been used to investigate how OM input quality affects the microbial community composition. In general, fungal residue abundance is associated with more recalcitrant plant litter input to soil (Bai et al., 2013; Zheng et al., 2018; Moore-Kucera and Dick 2008; Santos et al., 2012). While bacterial residues have been shown to be less abundant overall in most soils, they have been shown to play large roles in the decomposition of more labile plant litter and PyOM (Bai et al., 2013; Moore-Kucera and Dick, 2008; Helfrich et al., 2015; Dai et al., 2017, Santos et al., 2012). However, with limited information on microbial diversity in PyOM amended soils, less is known about the microbial contribution based on specific amino sugars.

In addition to being metrics for microbial residue abundance, amino sugars have been used to assess the contribution of microbial C and N to overall SOM (Appuhn and Joergensen, 2006; Liang et al., 2019; see chapters 1.5 and 2.5), which may vary based on type of plant input (Simpson et al., 2007). Amino sugars have been estimated to account for 5-12% of total soil N (Stevenson 1982) and 2-8% of SOC (Ni et al., 2020; Joergensen and Meyer, 1990). However, less is known about how amino sugar contributions to SOM may change for soils given PyOM. With microbial residues comprising potentially large fractions of soil C and N it is likely that changes in soil nutrients following OM input are largely controlled by microbial residue responses.

Microbial residue abundance and composition also vary over time as recent studies have demonstrated that after initial OM input microbial biomass and subsequent necromass increase (Miltner et al., 2012). As microbes initially utilize the more labile components of C amendments (e.g. plant litter, PyOM) they leave behind in the SOM progressively more recalcitrant byproducts, part of a process recently described as the microbial carbon pump (Liang and Balser, 2011), which

may then lead to microbial starvation and diminished biomass over time (Miltner et al., 2012). However, microbial residues may become stabilized within SOM aggregates, on PyOM, or on mineral surfaces and be protected from decay (Milter et al., 2012), resulting in variable residence times in soil (Schmidt et al., 2011). Additionally, specific microbial groups, which have different decay propensities and strategies, have been shown to change over time after plant and PyOM input. For example, bacteria have been shown to be early decomposers of plant tissue though their abundance is typically smaller than that of fungi throughout the decomposition process (Ding et al., 2011; Bai et al., 2013; Moore-Kucera and Dick, 2008). In the presence of PyOM both bacteria and fungi have been shown to be early decomposers (Santos et al., 2012; Farrell et al., 2013), but the relative contribution of fungal and bacterial to the overall decomposition process remains unknown and is probably highly system dependent (Lehmann et al., 2015). Besides the use of plant litter and PyOM as a nutrient source, other factors of OM inputs may affect microorganisms and their subsequent residues.

PyOM amendments may also increase or decrease the mineralization of native SOM (Zimmerman et al., 2011; Whitman et al., 2015; Lehmann et al., 2015). This effect, known as priming, describes how PyOM, or any organic matter amendment (Kuzyakov, 2010), enhances or hinders the decomposition of SOM, with positive priming leading to increased decomposition and negative priming leading to decreased decomposition that would occur in non- amended soils. The positive priming effect of PyOM additions generally occurs early, within the first several days to weeks, in soil experiments (Zimmerman et al., 2011; Lehmann et al., 2015). Positive priming effects have been attributed to the stimulation of native SOM mineralization rates by the addition of labile components of PyOM (Lehmann et al., 2015), and changes in substrate preferences (Farrell et al., 2013). Traditionally, negative priming effects occur later in long term experiments (Maestrini et al., 2015). Observed negative priming effects could be caused due to toxic PyOM-derived compounds that inhibit microbial extracellular enzyme activity (Whitman et al., 2015), PyOM surface sorption of microbial tissues, signaling factors, and extracellular enzymes (Whitman et al., 2015; Zimmerman et al., 2011), binding of labile native soil compounds and added low molecular weight compounds (Keith et al., 2011), or increased aggregation and organo-mineral interactions (Liang et al., 2010). With variable direction and magnitude of priming effects due to multiple mechanisms, the investigation of how soil microbes respond to PyOM inputs over time is needed.

Determining the impact of PyOM deposition in forest systems is especially important as these ecosystems are vulnerable to changing climate and fire regimes, which could have important feedbacks to the soil C and N pools (Sprichtinger et al., 2001; Palviainen et al., 2018). The University of Michigan Biological Station (UMBS) is located at the transition zone between Northern temperate and boreal forests (Gough et al., 2008), where older pine species and approaching deciduous species meet, and is projected to be susceptible to increased fire pressure under future climate projections (Reich et al., 2015; Santín et al., 2016). This site has been used in recent studies to test how changing PyOM deposition could influence soil microbial response (Gibson et al., 2018; Hatton et al., 2016, see chapter 1.9 and 2.2). Specifically, these studies explored how the physicochemical differences of jack pine (*Pinus baksiana*) and red maple (*Acer rubrum*) wood and associated PyOM produced at a range of temperatures, impacted the response of the local soil system and their degradability in those soils. For the plant tissue explored in those studies it was shown that there were interactions between taxa chemistry and structure and PyOM prodcution temperature to control the reultant PyOM physiochemical properties (Hatton et al., 2016). Specifically, early carbonization, loss of carbohydrates, and loss of non – lignin phenols, occurr at a lower pyrolysis temperature for jack pine (JP) than red maple (RM). Addtionally, the mean residence time (MRT) of the wood and PyOM amended in soil increased and mineralization decreased with the pyrolysis temperature (Gibson et al., 2018). While the slow cycling MRT of both tree taxa increased with pyrolysis temperature, RM exhibited greater difference between 300 ˚C and 450 ˚C, whereas this difference is not as significant for JP. Similarly, RM PyOM exhibited greater differences in mineralization between 300 ˚C and 450 ˚C compared to JP PyOM. When added to soil wood and PyOM from both taxa induced net negative priming (Gibson et al., 2018). However, it is unknown how the tissues of these different woods and PyOM may become incorporated into microbial tissues over time, and how these microbial tissues impact soil C and N.

The purpose of this study was to determine how the physiochemical properties of JP and RM derived PyOM influence microbial amino sugar contribution in a forest soil system following a laboratory incubation. Amino sugars responses for wood amendments are discussed in detail in Chapter 3 but are included in this chapter for reference to display the relative effect of heat treatment on microbial response. This work differs from previous studies in the use of amino sugars to investigate the impact of wood based PyOM on soil microorgansims, especially when

investigating a range of pyrolysis temperatures – non-pyrolyzed (wood), 300 ˚C, and 450 ˚C - using two tree taxa. We hypothesized that a) pyrolysis temperature would be a large controlling factor for the abundance of amino sugars, with greater amino sugar abundance correlating with lower pyrolysis temperature for both taxa; b) there would be a more noticeable difference in abundance of amino sugars between 300 ˚C and 450 ˚C for RM than JP based on previously measured chemical differences; c) there would be greater amino sugar abundance in soils incubated for shorter (180 days) compared to longer (600 days) times; and d) treatment effects for microbial C and N contribution to soil C and N would follow those of amino sugar content, where soils given lower pyrolysis temperature amendments and incubated for shorter times would show greater microbial C and N contribution.

4.3 Materials and Methods

Saplings of JP and RM were previously generated to support a project to investigate wood and PyOM reactivity in soils from UMBS (Hatton et al., 2016). The methods of growing conditions of the seedlings are discussed in detail in Bird and Torn (2006). Stems (two year old RM and one year old JP) were dried, cut to 2mm sections, pyrolyzed at 300 ˚C or 450 ˚C, and gently mixed into UMBS soil at 11% of total SOC and incubated in controlled laboratory conditions for 180 or 600 days (Gibson et al., 2018; see chapter 2.1 and 2.2). After samples were removed from the incubation, the recovered soil was stored frozen (-80 ˚C) to halt microbial metabolism prior to additional chemical analysis (see chapter 2.3).

Amino sugars were extracted, purified, and chemically-derivatized on duplicate soil samples following the protocol outlined by Zhang and Amelung (1996). Exact details of the extraction, purification, gas chromatography/mass spectrometry (GC-MS) analysis, and normalization techniques are discussed in Chapter 2 of this thesis. Briefly, the extracted amino sugars were quantified using GC-MS analysis based on calibration curves of exact standards. In the treatment of the data, amino sugar concentrations, as well as embodied C and N, were normalized with respect to mass of soil, mass of soil C, and mass of soil N. Amino sugars were investigated by mass of soil and then normalized by C and N mass of sample. Percent weight of C and N of samples were determined on a flash combustion elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) using a Sercon ltd (Crew, UK) GSL EA interfaced to a Hydra IRMS.

Statistical analysis was completed using the program R Studio, with samples replication at the block scale (n=4). Each individual block soil used in the incubation was a composite of eight cores (0-20 cm) from that block (Gibson et al., 2018). The data collected showed a normal distribution thus analysis of variance (ANOVA) was performed. Data was then compared between each treatment using a post-hoc Tukey test for significance between groups.

4.4 Results

4.4.1 Changes in amino sugar content per soil mass

Amino sugar concentrations (μg/ g soil) generally did not differ between pyrolyzed wood amendments and the control at 180 or 600 days of incubation (Table 4.1). JP wood amended soil showed a higher total amino sugar content compared to PyOM after 180 days of incubation, but no difference after 600 days of incubation. Meanwhile soils given RM wood were greater than all other treatments for both 180 and 600 day incubations. Similar trends were seen with the individual amino sugars GluN and GalN. There was no significant change in total amino sugar content between soils given 300 ˚C and 450 ˚C PyOM for either JP or RM. Similar trends were seen for GluN, GalN, and MurA. For all treatments, the contribution of each individual amino sugar to total amino sugar concentration followed the order of GluN>GalN>MurA>ManN. Average amino sugar content was greater for soils with PyOM amendments after 180 days of incubation compared to 600 days. Additionally, GluN to MurA ratio did not vary significantly using JP PyOM and RM PyOM amendments in soils incubated for 180 or 600 days. Generally, 600 day treatments showed greater GluN to MurA ratios than 180 day treatments (Figure 4.1).

Table 4.1. Concentration of total amino sugar, glucosamine (GluN), galactosamine (GalN), and muramic acid (MurA), mannosamine (ManN), as well as ratio of glucosamine to muramic acid per soil mass for PyOM and wood (data copied from Chapter 3) treatments incubated for 180 or 600 days. Values are given as mean ± (standard deviation). Letters within each column represent a significant difference between treatments. The same letter in a column represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

Treatment	Total (μg/g soil)	GluN (μg/g soil)	GalN (μg/g soil)	MurA (μg/g soil)	ManN (μg/g soil)	GluN/MurA
180 Day Control	673.0(41.9)bc	492.0(30.3)bc	148.7(13.0)bc	24.2(2.85)ab	8.09(0.61)ab	20.5(2.44)b
180 Day JP Wood	757.3(46.3)b	554.3(34.8)b	165.4(10.4)b	28.2(2.93)a	9.44(1.26)a	19.8(1.61)b
180 Day JP 300	730.9(17.0)b	533.5(13.9)b	162.7(6.44)b	26.7(1.89)ab	7.92(0.85)ab	20.0(1.55)b
180 Day JP 450	700.2(43.7)b	514.4(35.9)b	152.9(9.35)bc	25.5(1.54)ab	7.36(1.01)a	20.3(2.57)b
180 Day RM Wood	930.0(85.4)a	695.1(70.0)a	198.2(15.9)a	27.6(4.10)a	10.2(2.59)a	25.8(3.97)ab
180 Day RM 300	659.0(49.9)bc	485.4(39.7)bc	143.3(10.2)c	23.4(2.60)b	6.91(0.97)b	20.8(1.49)b
180 Day RM 450	672.6(39.1)bc	490.0(27.4)bc	150.2(13.5)bc	25.0(2.27)ab	7.43(1.04)ab	19.8(2.04)b
600 Day Control	490.4(41.3)d	353.5(22.8)d	115.5(14.6)d	12.0(1.21)c	9.43(0.98)a	29.8(4.23)a
600 Day JP Wood	474.4(36.5)d	340.7(29.8)d	111.2(6.77)d	12.4(1.46)c	10.1(0.64)a	27.6(2.65)a
600 Day JP 300	494.9(42.3)d	354.7(32.1)d	119.6(8.75)d	13.3(1.46)c	8.19(0.98)ab	26.6(0.99)ab
600 Day JP 450	454.0(20.2)d	322.1(14.8)d	110.6(9.94)d	13.1(0.96)c	8.16(0.85)ab	24.7(2.41)ab
600 Day RM Wood	614.2(60.7)c	454.8(43.2)c	134.9(15.1)c	14.4(2.12)c	10.1(1.13)a	31.9(3.09)a
600 Day RM 300	467.9(24.8)d	332.7(19.1)d	113.4(7.78)d	12.9(0.74)c	8.95(0.62)ab	25.8(1.78)ab
600 Day RM 450	473.3(19.8)d	338.2(20.4)d	113.8(5.02)d	12.8(0.86)c	8.43(1.33)ab	26.6(2.91)ab

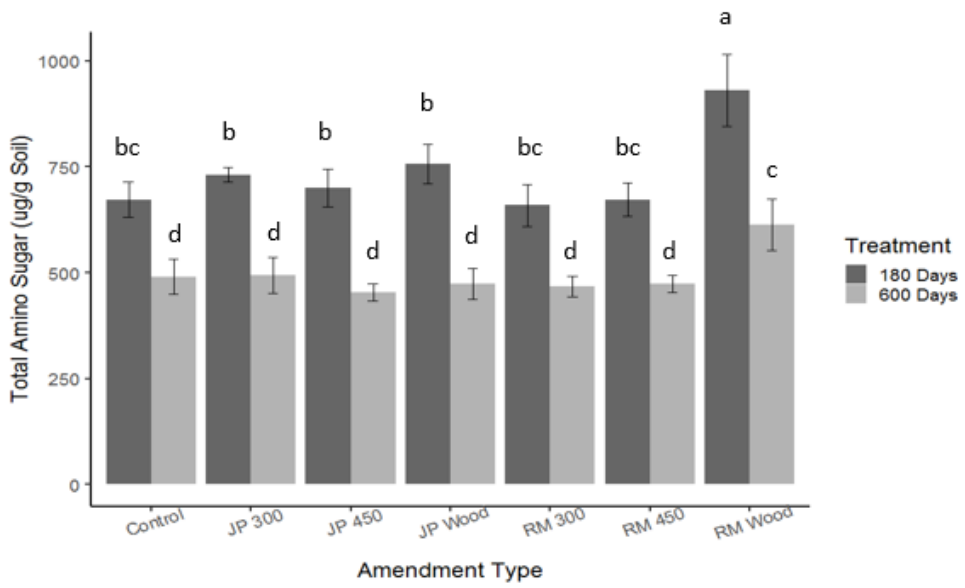


Figure 4.1. Total amino sugar concentrations per mass of soil PyOM and wood (data copied from Chapter 3) treatments at 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

4.4.2 Change in amino sugar content per soil C and N

Total concentrations of amino sugars (mg/ g soil C) from PyOM amendments exhibited no difference from control soils based either on taxa or temperature (Table 4.2). However, RM wood was statically higher than the PyOM amendments and control at 180 days of incubation, though JP wood was not. After 600 days of incubation there was no difference in amino sugar content between any treatments. There was no significant change in total amino sugars between 300 ˚C and 450 ˚C PyOM for either JP or RM. The total amino sugar content did not differ for treatments after 180 days of incubation as compared to 600 days, except for RM wood and the control (Figure 4.2).

Table 4.2. Concentration of total amino (AS) sugars per mass C and N, along with amino sugar derived C and N contribution to total soil C and N for PyOM and source wood (data from Chapter 3) treatments incubated for 180 and 600 days. Values are given as mean ± (standard deviation). Letters within each column represent a significant difference between groups. The same letter in a column represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

Treatment	AS (mg/g N)	AS (mg/g C)	AS N Contribution to Soil N (%)	AS C Contribution to Soil C (%)
180 Day Control	3057.2(120.7)ab	131.8(7.4)b	23.7(0.9)ab	5.3(0.3)b
180 Day JP Wood	3471.1(310.6)a	140.8(14.0)b	26.8(2.4)a	5.7(0.6)b
180 Day JP 300	3494.6(489.2)a	131.0(16.4)b	27.0(3.8)a	5.3(0.7)b
180 Day JP 450	2759.4(161.8)b	127.5(13.6)b	21.3(1.2)b	5.1(0.5)b
180 Day RM Wood	3580.4(605.5)a	174.1(31.3)a	27.8(4.7)a	7.0(1.3)a
180 Day RM 300	2441.3(559.7)bc	108.3(24.2)bc	18.9(4.3)bc	4.4(1.0)bc
180 Day RM 450	2517.4(177.3)b	111.5(9.9)bc	19.5(1.4)b	4.5(0.4)bc
600 Day Control	1830.0(219.8)c	105.2(14.9)c	14.2(1.7)c	4.2(0.6)c
600 Day JP Wood	2008.6(207.2)c	120.3(11.5)bc	15.6(1.6)c	4.8(0.5)bc
600 Day JP 300	2285.9(119.2)c	112.4(10.8)bc	17.7(0.9)c	4.5(0.4)bc
600 Day JP 450	2534.1(219.3)b	120.4(11.5)bc	19.6(1.7)b	4.8(0.4)bc
600 Day RM Wood	2441.2(856.3)bc	126.1(35.4)bc	19.0(6.6)bc	5.0(1.4)bc
600 Day RM 300	1839.2(242.2)c	100.3(18.8)c	14.3(1.9)c	4.0(0.7)c
600 Day RM 450	1870.4(249.5)c	96.2(14.2)c	14.5(1.9)c	3.8(0.6)c

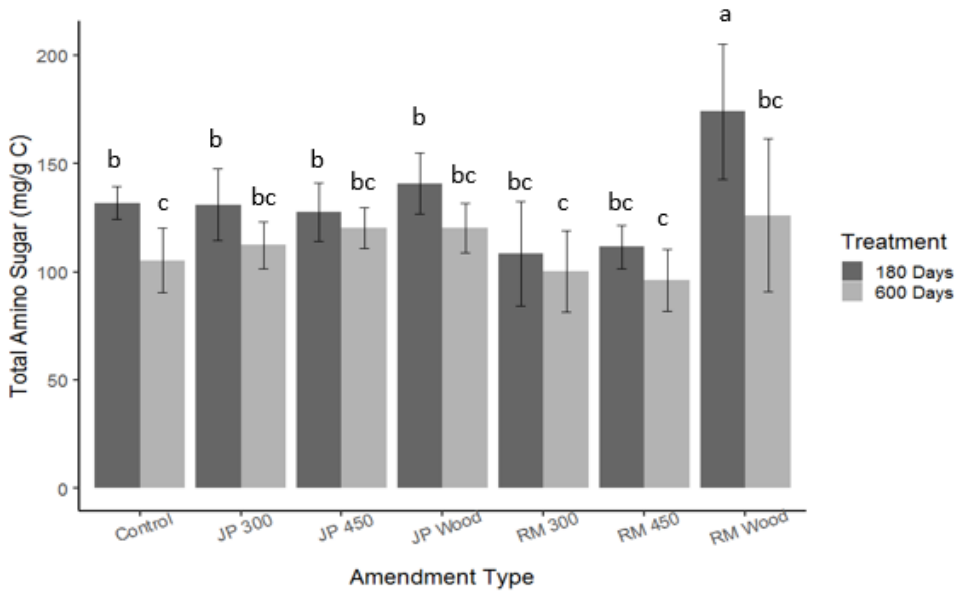


Figure 4.2. Total amino sugar concentration (mg/ g soil C) for PyOM and source wood (data from Chapter 3) treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

Total concentrations of amino sugars (mg/ g soil N) for both wood amendments were significantly greater than JP 450, RM 300, and RM 450 after 180 days of incubation (Table 4.2). Similarly JP 300 was greater than both JP 450 and RM 300 and RM 450. However, no treatment differed from the control at 180 days. After 600 days of incubation amino sugar content for JP 450 was greater than all other treatments except RM wood. The total amino sugar content was greater for treatments after 180 days of incubation compared to 600 days, except for RM 300 and JP 450 (Figure 4.3).

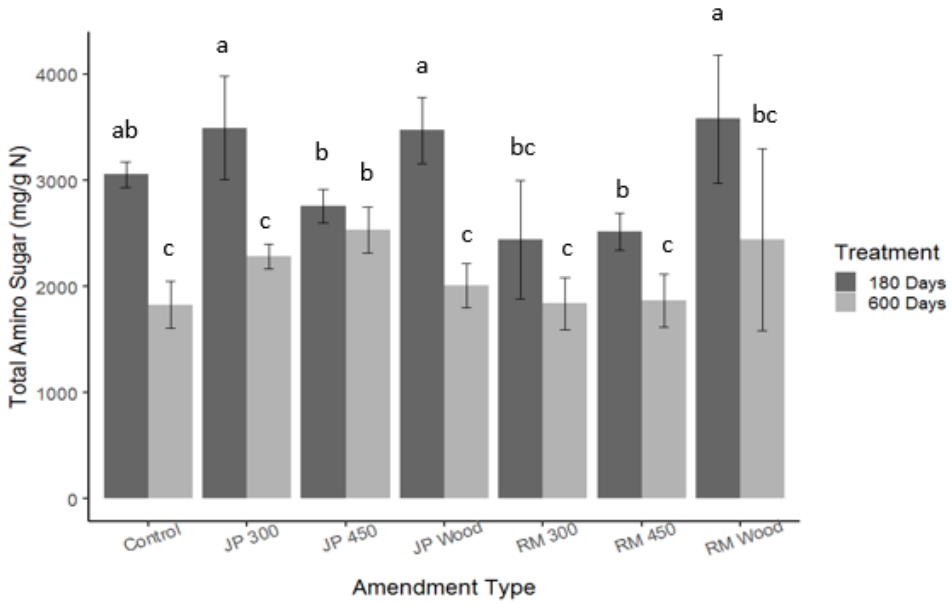


Figure 4.3. Total amino sugar concentration (mg/ g soil N) for PyOM and source wood (data from Chapter 3) treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

4.4.3 Proportional contribution of amino sugar- C and N to total soil C and N

The contribution of amino sugar derived C to total soil C (mg C/ g soil C) for PyOM amendments exhibited no statistical difference from control soils or from each other at each incubation period (Table 4.2). RM wood, however, differed from all other treatments after 180 days of incubation. On average, microbial sugar derived-C in the control soil was 5.3% of the total C at 180 days of incubation, while the RM wood amendment increased to 7.0% of total soil C (an increase of 32% from the control). After 600 days of incubation there was no difference in amino sugar content between any treatments and the control. Additionally, there was no significant change in total amino sugars between 300 ˚C and 450 ˚C PyOM for either JP or RM. The total amino sugar content did not differ for PyOM treatments at 180 days of incubation as compared to 600 days, differing from the RM wood and the control (see chapter 3), which showed a decrease over time (Figure 4.4).

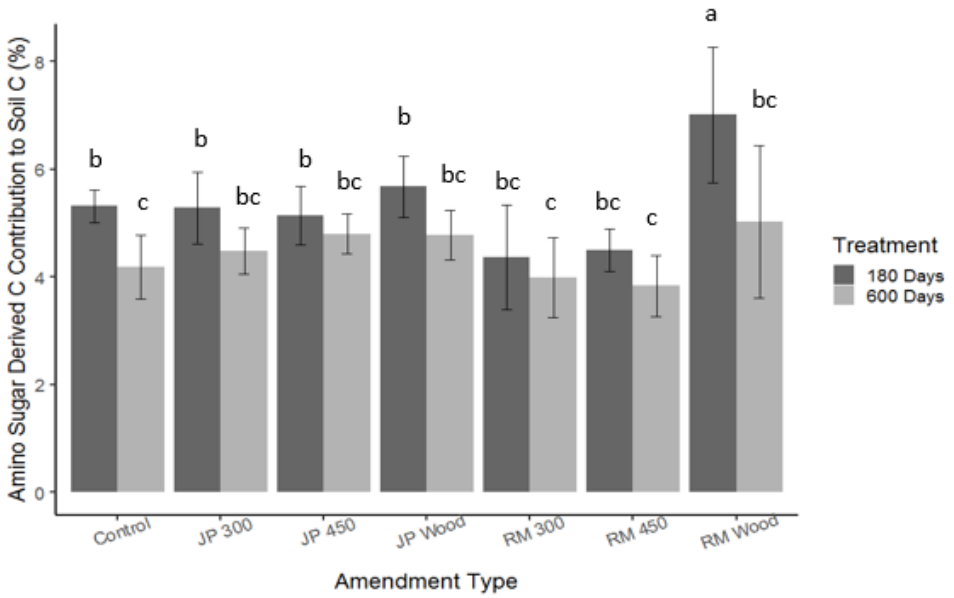


Figure 4.4. Total amino sugar derived C contribution to total soil C (mg C/ g soil C) PyOM and wood (data from Chapter 3) treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

Contribution of amino sugar derived N to total soil N (mg N/ g soil N) for both wood amendments were significantly greater than JP 450, RM 300, and RM 450 after 180 days of incubation (Table 4.2). Similarly JP 300 was greater than JP 450 and RM pyrolyzed amendments. However, no treatment differed from the control at 180 days. After 600 days of incubation amino sugar content for JP 450 was greater than all other treatments except RM wood. The total amino sugar content was greater for treatments after 180 days of incubation compared to 600 days, except for RM 300 and JP 450 (Figure 4.5).

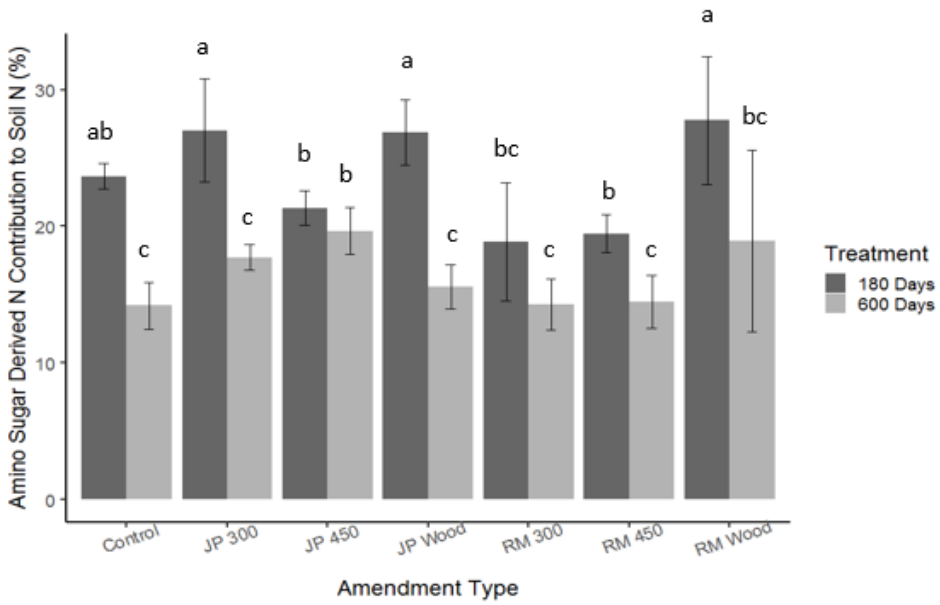


Figure 4.5. Total amino sugar derived N contribution to total soil N (mg N/ g soil N) for PyOM and wood (data from Chapter 3) treatments along with 180 day or 600 day incubation period. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

4.4.4 Fate of Amended PyOM during incubation: Mineralization and Microbial Uptake

Based on work published on these same samples (Gibson et al., 2018), it was determined that during the course of the 180 day incubation that 10(±1.9)% of RM 300 and 2.7(±0.3)% of JP 300 were mineralized. At 450 ˚C 0.6-0.7% of PyOM was mineralized with no difference between JP or RM treatments. Meanwhile, wood C was much more highly mineralized, with 57.1(±5.9)% of JP wood and 62.3(±18.8)% of RM wood being released from the soil at 180 days (Table 4.3). These values were calculated based on isotope modeling outlined in Gibson et al. (2018), which indicated that up to 25% of native soil carbon was also converted to CO_2 during the same time frame (Table 4.3). While it is unknown how much original wood or PyOM amendment remained following the incubation, it can be estimated how much of the original amendment could have been converted to amino sugar carbon (measured herein) and microbial tissue with additional calculation estimates (Liang et al., 2019). Based on calculations outlined in Appendix A, it is estimated that approximately 4.2% of initial PyOM C was incorporated into amino sugars for JP 300 and 1.6% for JP 450, while no RM PyOM was incorporated into amino sugars. Meanwhile, about 6% of initial amendment C was incorporated into amino sugars for JP wood and 19% for

RM wood (Figure 4.6). This may account for up to 100% of wood amendment degradation and incorporation into overall microbial tissues for both JP and RM, but substantially less for RM 300, RM 450, and JP 450 (Table 4.3).

Table 4.3. Wood and PyOM amendment, native soil carbon (NSC), and total sample (amendment and soil) mineralization (Gibson et al., 2018), estimates of incorporation into amino sugar C, and incorporation into overall microbial residues in soil samples incubated for 180 days. Values are given as mean ± (standard deviation). Letters within each column represent a significant difference between groups. The same letter in a column represents no significant difference between treatments at $P<0.05$. n=4 for each treatment.

Treatment	Initial Total Sample C Mineralized (%)	Initial NSC Mineralized (%)	Initial Amendment C Mineralized (%)	Initial Total Sample C Amino Sugar (%)	Initial Amendment C Amino Sugar (%)	Initial Amendment C Microbial Residues (%)
Control	24.8(1.5)a	24.8(1.5)a		5.7(1.0)a		
JP Wood	23.5(2.1)a	19.8(1.9)a	57.1(10.0)a	5.7(0.5)a	5.9(5.3)b	133.5(91.4)ab
JP 300	18.7(0.6)a	20.5(0.6)a	2.7(0.3)c	5.6(0.7)a	4.2(1.9)b	92.8(36.7)b
JP 450	20.3(2.7)a	22.4(2.9)a	0.7(0.1)c	5.3(0.5)a	1.6(5.3)b	52.7(61.0)bc
RM Wood	25.7(2.6)a	21.7(3.3)a	62.3(5.4)a	7.0(0.4)a	18.9(6.3)a	312.5(111.2)a
RM 300	18.0(2.4)a	18.8(2.6)a	10.0(1.9)b	5.0(0.9)a	0(4.2)b	21.8(33.8)c
RM 450	18.3(2.8)a	20.3(3.1)a	0.6(0.1)c	5.1(0.5)a	0(4.5)b	22.1(44.2)c

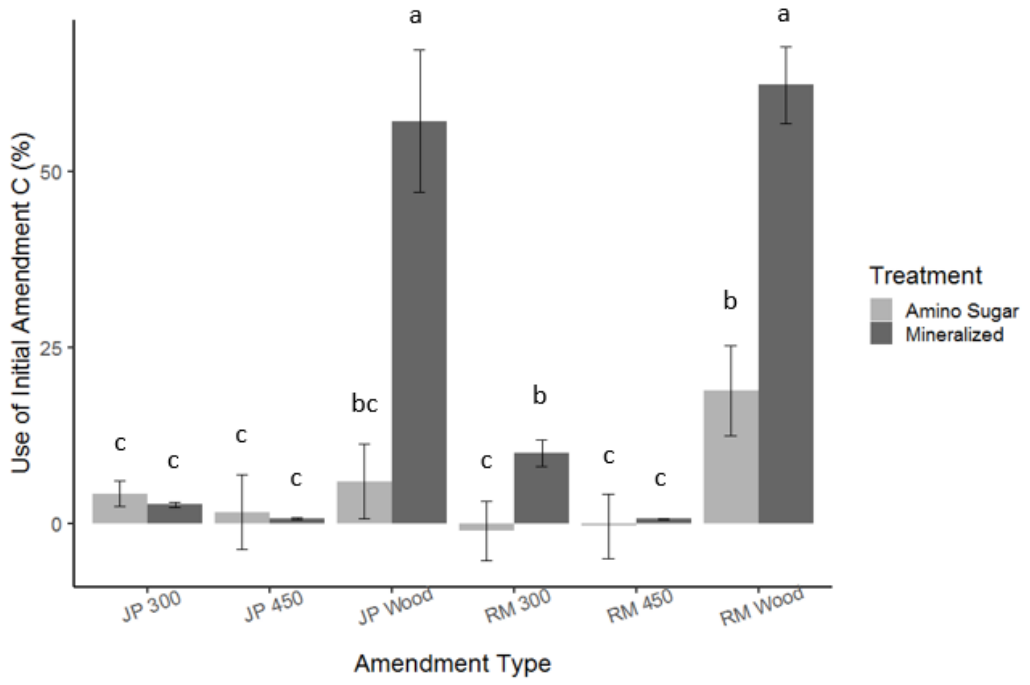


Figure 4.6. Graph showing mineralization of C from red maple and jack pine wood and PyOM amendments (Gibson et al., 2018) and increase in total amino sugars (this study) for the same samples incubated for 180 days. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=4 for each treatment.

4.5 Discussion

4.5.1 Greater pyrolysis temperature proportionally decreases amino sugar abundance in soil

Soils amended with wood (Chapter 3) and PyOM amendments showed amino sugar contents within range of North American boreal and temperate forests (Liang et al., 2008). Although few changes in specific amino sugars were found between PyOM treatments, there was consistently and significantly more GluN than MurA present in soil for all treatments. This is in line with previous findings that state forest soil systems have substantially more fungal residues than bacterial (Liang et al., 2019). Additionally, soils amended with PyOM exhibited less GluN and MurA than treatments without PyOM, indicating that both fungal and bacterial domains preferentially utilize wood over PyOM, which is supported by previous mineralization and fatty acid studies (Gibson et al., 2018, Santos et al., 2012, Farrell et al., 2013). Gram positive bacteria have been shown to incorporate wood based PyOM into PLFAs to a higher degree than precursor wood (Farrell et al., 2013, Santos et al., 2012), but with bacterial residues in lower abundance

compared to fungal residues and only 65% of bacteria on average being gram positive (Appuhn and Joergensen, 2006) there may be no discernable difference in bacterial incorporation into amino sugars. While results may be reasonable for North American forest soils, variability was still observed between select treatments.

This study suggests that pyrolysis temperature of OM amendments partially controlled amino sugar abundance in that non-pyrolyzed wood amendments increased amino sugar concentrations compared to soils given PyOM or no amendment. The effect could have been induced due to chemical changes in the wood and PyOM, with less lignin, cellulose, and carbohydrates but more stable aromatic structures being found for plant tissues at greater pyrolysis temperatures, which yields lower microbial utilization of OM inputs (Lehmann et al., 2015, Wang et al., 2016). This is in agreement with recent studies that have found trends toward greater recalcitrance with increased temperature, as seen by lower phenol and carbohydrate content but more C content per mass of OM with increasing pyrolysis temperature of both JP and RM (Gibson et al., 2018, Hatton et al., 2016). Wood has been shown to mineralize (Gibson et al., 2018) and become incorporated into microbial biomass for fungi and some bacteria (Santos et al., 2012) to a greater degree than PyOM, which is consistent with current findings.

The trend in microbial incorporation into amino sugars was less apparent for JP than RM possibly due to the recalcitrant nature of JP wood and its PyOM, in comparison to RM wood (Hatton et al., 2016). With less labile but more recalcitrant compounds, JP amendments may be less utilized by microorganisms altogether, and appear to be incorporated into amino sugars to a similar degree as PyOM amendments. Additionally, JP has a higher concentration of condensed tannins and other extractives that might have microbial suppression qualities (Smith et al., 2012; Gibson et al., 2018; Saxena et al., 1995). These chemical differences may result in RM having a lower barrier to degradation by soil microorganisms and incorporation into biomass – measured here as amino sugars (Cotrufo et al., 2013).Thus it is surmised that recalcitrant tissues of JP wood and its PyOM, and potentially suppressive properties of JP wood, may yield fewer changes in microbial utilization of JP wood and its PyOM treatments compared to RM.

Changes in amino sugar content between wood and PyOM treatments could also be due to physical differences. Typically, PyOM exhibits greater surface area and pore volume that may yield increased habitation by microorganisms (Lehmann et al., 2015). The hydrophobic nature of PyOM may also encourage the sorption of microbial residues (Whitman et al., 2015) thereby

allowing for greater amino sugar persistence in soil. However, such hydrophobic nature may also sorb extracellular enzymes and signaling factors from microorganisms (Whitman et al., 2015) thereby decreasing PyOM decomposition and utilization. This may be supported by current findings of diminished microbial residues in the presence of PyOM compared to at least RM wood, however additional factors, such as priming effects, may also play a role in observed trends.

The addition of wood and PyOM to soil may have also influenced the decomposition of native soil C. Priming of native soil C by wood or PyOM additions could cause a shift in substrate use away from soil C toward greater use of any OM amendment or vice versa, which could yield positive, negative, or no net priming (Maestini et al., 2015; Whitman et al., 2015). While positive priming effects have been reported for some long term incubations (Maestrini et al., 2015), the current samples have exhibited net negative priming during the course of this incubation (Gibson et al., 2018). With a suppression in native soil C mineralization it could be expected that incorporation of native soil C into microbial residues is also suppressed. However, the addition of sucrose to all the experiments, has been shown to offset negative priming for soils given JP PyOM (Gibson et al., 2018). With minimal mineralization of JP PyOM and no net priming effect, soils given JP PyOM exhibit no large changes in microbial biomass and necromass. Soils given RM PyOM have still shown negative priming and minimal mineralization compared to RM wood (Gibson et al., 2018). As wood and PyOM amendments have been shown to be mineralized and be incorporated into microbial fatty acids (Santos et al., 2012), it is likely that both JP and RM wood OM are directly incorporated into amino sugars, though the mechanism is beyond the scope of this work. Even with potential negative priming effects, with increased amino sugar abundance for soils given wood amendments compared to the control, it is possible that the microbial utilization of wood is greater than the negative priming effects they might have on surrounding SOM. Potential lower microbial utilization of PyOM may negate negative priming effects on SOM, yielding no observable difference in amino sugar content between PyOM treatments and the control, which is consistent with the current results. Considering the measured negative priming effects induced by the all OM amendments used here, the simultaneous mineralization of 57-62% of the added wood, and mineralization of 0.6 -10% of added PyOM (Gibson et al., 2018), we surmise that increased amino sugar abundance for these same soils are primarily derived from the microbial utilization of wood and PyOM.

4.5.2 PyOM addition to soil shows no impact on soil amino sugar concentration

Contrary to our hypothesis, there were no differences in amino sugar concentrations between PyOM treatments for either JP or RM. This may be the result of only small portions of bioavailable C in PyOM produced at both temperature. Several plant tissues, such as wood and crop shoots, have been reported to have 0.1 - 20% of PyOM – C incorporated into microbial biomass at low production temperatures (< 350 ˚C) (Farrell et al., 2013; Luo et al., 2013). Meanwhile, at high temperatures (> 400 ˚C) 0-2% of PyOM – C is incorporated into biomass (Farrell et al., 2013; Santos et al., 2012; Luo et al., 2013). Recent studies have shown that JP and RM PyOM are significantly less mineralized than precursor wood (Gibson et al., 2018), suggesting potentially negligible differences in microbial utilization. It is possible that the more recalcitrant PyOM could be incorporated into biomass and necromass to a lesser extent, resulting in little to no discernable difference between PyOM treatments in comparison to the more labile wood tissues. Additionally, the equal amino sugar content of PyOM amended soils may be mediated by more than PyOM chemical differences, such as by priming effects. Negative priming effects of PyOM to soil may also be in effect (Gibson et al., 2018) which may counteract the small fractions of PyOM used by microbes. With the amount of PyOM being mineralized or incorporated into microbial biomass and similar studies reporting no priming effects (Santos et al., 2012), it is possible that PyOM amendments have little net impact on microbial biomass and necromass accumulation in soil which is consistent with the current results.

4.5.3 Amino sugar content in soil decreases with incubation time

We observed, as expected, that amino sugar content in soil decreased with incubation time. This could be explained by a number of factors including decreased availability of nutrients and labile C over time which caused microbes to mine microbial necromass. Additionally, microbial use of labile fractions of OM yields progressively more recalcitrant byproducts, as described by the microbial carbon pump (Liang and Balser, 2011). These OM fractions would then be less bioavailable and thus less able to be incorporated into microbial residues. Miltner et al (2012) discussed a framework by which changes in fresh plant residues, which may be extended to PyOM as well, lead to changes in microbial biomass and necromass, with plant and PyOM inputs initially yielding greater biomass and necromass which then may become stabilized on mineral surfaces or

incorporated into SOM. However, with progressively more recalcitrant OM sources, it could be expected that microbial residues, such as amino sugars are used as an energy source and mineralized thereby leaving the system, which is consistent with the current findings.

Specific amino sugars also changed over time as indicated by the GluN to MurA ratio, which expresses fungal-to-bacterial residue abundance. Fungi have been shown to favor recalcitrant tissues overall in decomposition studies, with lower or constant fungal residues reported later in studies (Cornwell et al., 2009; Bai et al., 2013). Meanwhile, bacteria have been shown to play significant roles in PyOM decomposition within the first several weeks, but to a lesser degree later in incubation studies (Santos et al., 2012; Farrell et al., 2013). This shift of weaker microbial contribution to OM amendment degradation over time, especially with bacteria, is consistent with a greater GluN to MurA ratio at 600 days of incubation compared to 180 days.

4.5.4 Pyrolysis temperature and incubation time control amino sugar abundance per C and N and amino sugar contribution to SOM

Current results suggest that a difference in pyrolysis temperature of amendments and incubation time may cause a change in amino sugars per mass of soil C and N. As expected, soils amended with RM wood (Chapter 3) showed greater amino sugar abundance (mg/g soil C) than RM PyOM, however this was not true for JP wood and its PyOM, similar to trends in amino sugar abundance per sample mass. However, trends were also similar to total sample C content. Soils (0-20 cm) used in this study contained 0.3-0.9% total SOC with variability derived from natural heterogeneity of soil from different sampling blocks. Similarly, total amino sugars (mg/g soil N) showed trends correlating to amino sugar abundance per sample mass and total sample N content. Soils used in this contained small amounts of total N, only 0.02 – 0.05%, yet amino sugar content per mass of soil was typical for forest soils. This may have led to the proportion of amino sugar per soil N appearing inflated due to low soil N content (Equation 1, Chapter 2.5). In low nutrient content soils, like those investigated herein, fresh OM inputs may provide an easily accessible C and N source for microorganisms (Jolivet et al., 2006), which may explain why only an 11% increase in C from wood inputs yielded greater amino sugars per mass soil C for RM (31.8%). However, with more recalcitrant or microbially suppressive organic structures, JP wood and most PyOM treatments likely did not provide easily assessable C and N. While this temperature effect for RM treatments was present earlier in the incubation there was no difference in amino sugar C

or N content later in the incubation, suggesting that time also impacts amino sugar content per mass of C and N.

Current results suggest that a difference in wood or PyOM amendments and incubation time may cause a change in microbial C and N contribution to SOM. Here, RM wood additions yielded greater amino sugar C contributions to soil C than PyOM amended and non-amended soil. These results indicate that amino sugar C contributed between 4-7% of soil C, which is within range of previous estimates (Ni et al., 2020; Joergensen and Meyer, 1990). However, amino sugar derived N contributed 14-28% of soil N, which is higher than previous estimates (Stevenson, 1982). The high contribution to soil N may be explained by the presence of non-bioavailable inorganic N and altered N mineralization. Soils with high clay content have been suggested to make N less accessible than soils dominated by sand through organo-metallic complexation and electrostatic interactions (Rasmussen et al., 2006; Santos et al., 2012). If soil C and N are inaccessible through complexation, microbial communities are more reliant on OM amendments, and may increase their biomass and subsequent necromass, yielding higher percent contribution values than expected when introduced to an OM amendment. This would be especially true for bacterial amino sugars, which have shown to quickly incorporate substrate N, in the form of NH_4^+ and NO_3^-, in comparison to soil N (He et al., 2011). However, the soils used in this study have low clay content so soil C and N inaccessibility and subsequent reliance on OM inputs may only play a small role in the observed results. Another possible explanation for the observed trends in amino sugar C and N contribution is by the loss of gaseous C and N over time. Gibson et al (2018) monitored the loss of CO_2 during the course of this incubation, and reported consistent losses of native soil C and wood derived C accounted for up to 300 days. With potentially high mass losses of C through mineralization there is room for variability as seen between treatments at 180 days. However, studies have reported minimal losses of gaseous N, such as N_2O, during plant and PyOM incubation studies (Abbasi et al., 2015; Zhang et al., 2014), which may be due to microbial immobilization of N (Abbasi et al., 2015) or decreased bioavailable N at greater pyrolysis temperatures (Zhang et al., 2014). With limited bioavailable N at greater pyrolysis temperatures amino sugar N would remain low, such as those exhibited by RM 300, RM 450, and JP 450.

4.5.5 Fate of amended PyOM during incubation: mineralization and microbial uptake

Gibson et al (2018), through tracking the isotopes labeled in the amended wood and PyOM, found for these samples that during the course of the 180 day incubation that 57-62% of the amended wood and 0.6-10% of amended PyOM was mineralized to CO_2. While it is unknown how much original amendment remained in soil following the incubation, it can be estimated how much of the original amendment could have been converted to amino sugar C and microbial tissue. For the same incubation time 6-19% of wood C and 0-4% of PyOM C was converted into amino sugar C and, thus possible incorporation of amendment C to amino sugars C was lower compare to mineralized C (Gibson et al., 2018; see Appendix A). However, it should be noted that amino sugars only represents a single compound class of microbial residues. When amino sugars were used to estimate the amount of initial sample C utilized by microorganisms (see chapter 1.5), up to 100% may have been incorporated into or have previously existed as microbial residues. These values are above previous estimates of 47-80% soil C being derived from microbial residues (Fan and Liang, 2015). This may be due to the use of conversion factors used to calculate current results that are based on average microbial residue C, N, and amino sugar content which may change in soils based on microbial community composition (Liang et al., 2019). Nonetheless, current estimates suggest that native soil C could be utilized to a high degree for microbial growth and highlights the growing importance to understand microbial contributions to SOM.

4.6 Conclusion

This study provides new information on the effects of PyOM production temperature on microbial residues and microbial contribution to SOM, using amino sugars as a proxy for C and N contribution to soils, when added to North American forest soils. It has been determined that OM amendments of different physicochemical properties yield increases in microbial residues with more easily degradable OM, like non-pyrolyzed wood, showing proportionately higher microbial responses in residue accumulation than OM higher in recalcitrant tissues, such as PyOM. However, no differences in amino sugars were observed between PyOM of different production temperatures. Earlier in incubations, bacterial residues have a large role in OM decomposition though their abundance decreases greatly over time. Fungal residues dominate throughout incubation periods though diminish over time as well. Amino sugar derived C and N contributions to total soil C and

N partially followed patterns of amino sugar abundance, with soils give RM showing the greatest amino sugar C contribution to soil C. This work shows that on a per C or per N basis, PyOM versus source wood addition to fire prone soils will result in a net depletion of microbial residues.

These findings support soil and PyOM conceptual models that suggest microbial residue accumulation is controlled by OM quality, where more labile non-pyrolyzed wood is incorporated into microbial tissues than its more recalcitrant PyOM. With little bioavailable C and N in PyOM at either temperature, microbes were likely unaffected by physicochemical differences between PyOM production temperatures. While values for amino sugar contribution to soil C are in agreement with previous findings, values of contribution to soil N are larger than expected, possibly due to differences in gaseous release of C and N from soil or organo-metallic complexation. From these values it is possible that microbial residues could contribute larger amounts to SOM than traditionally thought. While further analysis is needed to provide a direct mechanism for the incorporation of wood and PyOM tissue to amino sugars and total microbial residue estimates may need to be refined, this study has provided insight on the connection between PyOM production temperature and microbial amino sugars in soil.

CHAPTER 5. CONCLUSIONS

5.1 Summary

Understanding the effects of wood and PyOM source taxa and pyrolysis temperature are essential for determining the impact of increased wood and PyOM deposition on microbial residues in ecosystems vulnerable to changing vegetation dominance and fire regimes. The use of amino sugars, as a proxy for microbial residues, in this study provided the unique opportunity to assess these potential impacts. This work has shown that the addition of non-pyrolyzed jack pine (JP) and red maple (RM) wood to soil resulted in an increase of amino sugars, especially for RM (Chapter 3). The difference in amino sugar content between taxa may be governed by chemical properties of the woods with labile components, present to a higher degree in RM, being incorporated into biomass and subsequent necromass. However, the addition of PyOM produced at either temperature and for both taxa resulted in no change in amino sugar content compared to control soils, likely due to limited bioavailable C and N in PyOM (Chapter 4). When amino sugar content was normalized to soil C and N, PyOM versus wood addition to soils resulted in a net depletion of microbial residues, particularly for RM treatments. Furthermore, longer incubation time resulted in lower amino sugar content, with bacterial residues decreasing more severely over time compared to fungal residues that dominated throughout incubation times. The variable response in amino sugars between plant taxa, pyrolysis of tissues, and incubation time highlights the importance and dynamic nature of organic matter physicochemical quality in controlling soil microbial responses to organic matter inputs.

5.2 Implications of wood and PyOM deposition to soil affected by fire and land change

Wildfires are of growing importance as their severity and frequency are expected to increase in the next century (Whitman et al., 2019; Santin et al., 2016). Fires in North American forests have been shown to impact soil through biotic and abiotic means including diminished soil microbial biomass (Dooley and Treseder 2012; Holden and Treseder 2013), increased soil temperature, and altered soil pH and moisture (Whitman et al., 2019). As fires remove large amounts of organic C due to volatilization of plant biomass and near surface SOM (Santin et al., 2016; van der Werf et al., 2010; Neff et al., 2005), there is less bioavailable organic C in soil

following fire events. Through volatilization large amounts of C can be released to the atmosphere thereby contributing to rising global C emissions and subsequent climate change (Santin et al., 2016). However, PyOM left behind may also serve as a sink for atmospheric C as it may persist in soil for significantly longer time periods than the source wood (Palviainen et al., 2018). With large amounts C being deposited to the soil surface during fires that is essentially inaccessible to microbial decay, PyOM may sequester large amounts of unreactive C into soil that persist for decades to centuries. Current findings suggest that if unused as direct microbial C sources, PyOM may still alter the use of surrounding OM. Due to the large contribution of PyOM derived C to forest soil systems further investigation into large scale estimates of C fluxes from soils affected by fire is needed.

Shifts in the use of land and dominant plant species are expected to change as human population and the effects of climate change increase (Liu et al., 2018; Kumar et al., 2018). As plants play a critical role in the sequestration of CO_2 from the atmosphere and deposition of C to soil as plant tissue (Griscom et al., 2017; Loveland and Webb 2003), the alteration of plant type and amount in an ecosystem are paramount to C cycling dynamics. Shifts in plant taxa have been shown to impact soil through altering microbial biomass, necromass, and microbial community structure through the utilization of plant tissue C and N (Bai et al., 2013; Moore-Kucera and Dick, 2008; Helfrich et al., 2015; Ma et al., 2018; Ye et al., 2019). As soils in North American forests, such as those found at UMBS, experience shifts in dominant taxa away from coniferous to deciduous trees, these soils can expect to see plant tissue mediated responses of microorganisms in the soil, with deciduous tissues such as RM contributing to increased microbial tissue storage as amino sugars. With plant tissues being the dominant nutrient source for microorganisms in forest soils more information is needed on how a greater variety of plant taxa may impact the storage of soil C in the form of microbial residues.

The carbon use efficiency of microorganisms is closely related to the capacity of soil microorganisms to regulate the carbon cycle through a balance of anabolic and catabolic processes (Dijkstra et al., 2011), such as the mineralization of organics and release of C into the atmosphere or the incorporation of organics into microbial residues and subsequent stabilization in soil. This topic is of growing concern in recent decades as global temperatures continue to rise, land use continues to change, and fires increase, thereby altering the dynamics of C and SOM stabilization. Understanding the cycle of C and N from plants, PyOM, microorganisms, soil, and surrounding

environment is the basis for understanding how microbes contribute to the long-term consequences of controlling CO_2 and nutrients in soil, and thus climate change and soil fertility as a whole (Liang et al., 2019).

5.3 Future work in microbial tissue response to OM inputs

Microbial residues account for a substantial amount of soil C and N as seen by amino sugars, a single class of microbial derived compound, which contribute approximately 4-7% of total soil C and 14-28% of total soil N in North American forest soils investigated in this study. Based on conversion factors from amino sugar mass to microbial residue C and N content (see chapter 2), values calculated in this work suggest that up to 100% of soil C and N could come from microbial residues. These values are larger than expected even using the lower end estimates for these conversion factors (Appuhn and Joergensen 2006; Joergensen 2018; Liang et al 2019). Limitations exist in the use of conversion factors based on average microbial residue C, N, and amino sugar content which may change in soils based on microbial community composition. Nevertheless, in using these calculations greater insight could be achieved into the role microorganisms play in contribution of SOM. Given the wide range of microbial residue contribution to soil C and N, future consideration should be taken into the application of specific soil conditions that may impact microbial residue contribution estimates. However, the present work is intended to highlight the important role of microbial necromass in soil C and N stabilization in soil.

The use of amino sugars in this work is limited by the precision and accuracy of each amino sugar. While laboratory procedures are robust improvements can be made in the recovery of amino sugars, particularly ManN and MurA which are typically low in abundance. Furthermore, the accuracy of amino sugar quantification can be improved through the use of well-established soil standards for various soil types. As amino sugar abundance varies substantially between soils based on organic matter content and soil texture, robust standards should be developed in order to more appropriately assess amino sugar content with high accuracy.

To better assess total microbial residue response to wood and PyOM addition to soil, other microbial biomarkers could be used, such as PLFAs. The use of PLFAs may not only better suggest the role of this specific biomarker, as a proxy for microbial biomass, but it may also illuminate the relative contribution of specific subgroups of bacteria and fungi. Conversion factors used to estimate bacterial and fungal C and N rely on assumptions of gram positive to gram negative

bacteria ratios, a change in which upon introduction of wood or PyOM, may substantially alter the estimated microbial contribution to soil C and N. Furthermore, PLFAs may suggest variable microbial residue retention times in soil, where fatty acid tissues typically respond more quickly and persist in soil for shorter times than amino sugars. With the consideration of multiple microbial residues a more complete understanding of how specific microorganisms respond to OM amendments and contribute to SOM may be obtained.

While this thesis has estimated the incorporation of wood and PyOM C into amino sugar C and overall microbial C, no direct mechanism for uptake was investigated. Here, calculations of microbial uptake of amendment C are based on differences from non-amended soils, suggesting that the introduction of amendment C contributed to observed change in amino sugars and microbial residues. To better assess the actual fate of amendment C in microbial residues the analysis of amino sugar ^{13}C could be implemented following addition of ^{13}C-labeled woody tissue and PyOM. As the amendments used in this study are isotopically enriched, the analysis of amino sugar C by EA-IRMS may directly suggest microbial uptake into amino sugars. Similarly, amendment N incorporation into microbial residues could be examined following similar procedures as OM amendments were highly labeled with ^{15}N. These considerations would provide a more complete picture of the interactions of fresh wood tissues, PyOM, and microorganisms within soil.

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APPENDIX A. EXAMPLE CALCULATIONS

Box A1. Amino sugar per mass soil, C, and N

The mass of amino sugars per mass of soil, C, and N were calculated based on total amino sugar mass (μg) and mass of soil, soil C content, or soil N content where:

$$\frac{\mu g\ amino\ sugar}{g\ soil} = \mu g\ amino\ sugar\ /\ g\ soil$$

$$\frac{\mu g\ amino\ sugar}{g\ N} = \frac{\mu g\ amino\ sugar}{g\ soil} \times \frac{g\ soil}{g\ N}$$

$$\frac{\mu g\ amino\ sugar}{g\ C} = \frac{\mu g\ amino\ sugar}{g\ soil} \times \frac{g\ soil}{g\ C}$$

So if there is 1000 μg total amino sugar/g soil and 0.005 g C/g soil then

$\frac{1000\ \mu g\ amino\ sugar}{1\ g\ soil} \times \frac{1\ g\ soil}{0.005\ g\ C}$ = 200,000 μg amino sugar/g C or 200 mg amino sugar/g C

If there is 0.0005 g N/g soil for the same soil then

$\frac{1000\ \mu g\ amino\ sugar}{g\ soil} \times \frac{g\ soil}{0.0005\ g\ N}$ = 2,000,000 μg amino sugar/g N or 2000 mg amino sugar/g N

Box A2. Amino sugar C and N contribution to soil C and N

The contribution of amino sugar C and N to soil C and N is determined by specific amino sugar mass per g C or N, molar mass of that specific amino sugar, molar mass of C or N, and the number of C or N atoms per amino sugar molecule. The following equations show GluN C and N contribution to soil C and N, which can be used similarly for GalN and ManN:

$$\frac{mg\ GluN{-}N}{g\ N} = \frac{mg\ GluN}{g\ N} \text{ x } \frac{14.01\frac{g}{mol}N}{179.17\frac{g}{mol}GluN}$$

$$\frac{mg\ GluN{-}C}{g\ C} = \frac{mg\ GluN}{g\ C} \text{ x } \frac{12.01\frac{g}{mol}C\ x\ 6}{179.17\frac{g}{mol}GluN}$$

Where 14.01 is the molar mass of N, 12.01 is the molar mass of C, 179.17 is the molar mass of GluN, GalN, or ManN, and 6 is the number of C atoms per amino sugar molecule. If a sample contained 100 mg GluN/g C and 1000 mg GluN/g N then:

$$\frac{mg\ GluN{-}N}{g\ N} = \frac{1000\ mg\ GluN}{g\ N} \text{ x } \frac{14.01\frac{g}{mol}N}{179.17\frac{g}{mol}GluN} = 78 \text{ mg GluN – N /g N or 7.8 \% of soil N}$$

$$\frac{mg\ GluN{-}C}{g\ C} = \frac{100\ mg\ GluN}{g\ C} \text{ x } \frac{12.01\frac{g}{mol}C\ x\ 6}{179.17\frac{g}{mol}GluN} = 40 \text{ mg GluN – C/g C or 4.0\% of soil C}$$

The following equations show MurA C and N contribution to soil C and N:

$$\frac{mg\ MurA{-}N}{g\ N} = \frac{mg\ MurA}{g\ N} \text{ x } \frac{14.01\frac{g}{mol}N}{251.23\frac{g}{mol}MurA}$$

$$\frac{mg\ MurA{-}C}{g\ C} = \frac{mg\ MurA}{g\ C} \text{ x } \frac{12.01\frac{g}{mol}C\ x\ 9}{251.23\frac{g}{mol}MurA}$$

Where 251.23 is the molar mass of MurA and 9 is the number of C atoms per molecule of MurA. If a sample contains 10 mg MurA/g C and 100 mg MurA/g N then:

$$\frac{mg\ MurA{-}N}{g\ N} = \frac{100\ mg\ MurA}{g\ N} \text{ x } \frac{14.01\frac{g}{mol}N}{251.23\frac{g}{mol}MurA} = 5 \text{ mg MurA – N/g N or 0.5\% of soil N}$$

$$\frac{mg\ MurA{-}C}{g\ C} = \frac{10\ mg\ MurA}{g\ C} \text{ x } \frac{12.01\frac{g}{mol}C\ x\ 9}{251.23\frac{g}{mol}MurA} = 4 \text{ mg MurA – C/g C or 0.4\% of soil C}$$

The sum of all four amino sugar C or N contributions to soil C or N yields total amino sugar C or N contribution as discussed in this thesis.

Box A3. Microbial residue C and N contribution to soil C and N

Overall bacterial and fungal residue C and N can be calculated based on equations outlined in Liang et al (2019). The following equations show mass of bacterial C and N based on mass of MurA:

n μg MurA x 45 = μg bacterial residue C

n μg MurA x 6.67 = μg bacterial residue N

Where n is the specific mass of MurA in the sample, 45 is the conversion factor from MurA to bacterial C, and 6.67 is the conversion factor from MurA to bacterial N. So in a sample with 50 μg of MurA:

50 μg MurA x 45 = 2250 μg bacterial residue C

50 μg MurA x 6.67 = 333 μg bacterial residue N

The following equations show mass of fungal C and N based on GluN and MurA:

$$\left(\frac{\text{m μg GluN}}{179.17}\right) - 2 \text{ x } \left(\frac{\text{n μg MurA}}{251.23}\right) \text{ x } 179.17 \text{ x } 9 = \text{μg fungal residue C}$$

$$\left(\frac{\text{m μg GluN}}{179.17}\right) - 2 \text{ x } \left(\frac{\text{n μg MurA}}{251.23}\right) \text{ x } 179.17 \text{ x } 1.4 = \text{μg fungal residue N}$$

Where m is the specific mass of GluN in the sample, n is the specific mass of MurA, 179.17 is the molar mass of GluN, 251.23 is the molar mass of MurA, 9 is the conversion factor from GluN to fungal C, and 1.4 is the conversion factor from GluN to fungal N. So in a sample with 500 μg GluN:

$$\left(\frac{500 \text{ μg GluN}}{179.17}\right) - 2 \text{ x } \left(\frac{50 \text{ μg MurA}}{251.23}\right) \text{ x } 179.17 \text{ x } 9 = 3860 \text{ μg fungal residue C}$$

$$\left(\frac{500 \text{ μg GluN}}{179.17}\right) - 2 \text{ x } \left(\frac{50 \text{ μg MurA}}{251.23}\right) \text{ x } 179.17 \text{ x } 1.4 = 600 \text{ μg fungal residue N}$$

The sum of fungal and bacterial residues may then be used to represent total microbial residue C and N content. Also note that conversion factors may change based on estimated C and N content of microbial residues and C/N ratios. Variability in C content in biomass may yield a wide range in conversion factors, 30-90 for bacteria and 8-11 for fungi. Meanwhile, variability in C/N content of biomass may additionally yield a wide range in N based conversion factors, 5.5-8.3 for bacteria and 1.1-1.7 for fungi (Joergensen, 2018; Appuhn and Joergensen, 2006).

Box A4. Amendment C incorporation into amino sugar C and overall microbial residue C

The percent of amendment C incorporated into amino sugar C (% amendment C amino sugar) is dependent on change in total amino sugar C content for a sample compared to the control and initial amendment C content where:

% amendment C amino sugar = $\frac{total\ amino\ sugar\ C\ change\ from\ control\ (mg)}{initial\ amendment\ C\ (mg)}$ x 100

So if there is an additional 2 mg of total amino sugar C for a sample compared to the control, and the amendment in that sample contained 10 mg C then,

% amendment C amino sugar = $\frac{2\ mg}{10\ mg}$ x 100 = 20%

The percent of amendment C incorporated into overall microbial C (% amendment C microbial residue) is dependent on change in total microbial residue C content for a sample compared to the control and initial amendment C content where:

% amendment C microbial residue = $\frac{total\ microbial\ residue\ C\ change\ from\ control\ (mg)}{initial\ amendment\ C\ (mg)}$ x 100

So if there is an additional 7 mg of total microbial residue C for a sample compared to the control, and the amendment in that sample contained 10 mg C then,

% amendment C microbial residue = $\frac{7\ mg}{10\ mg}$ x 100 = 70%

APPENDIX B. SUPPLEMENTAL MATERIAL: SUCROSE ADDITION SHOWS NO IMPACT ON AMINO SUGAR CONCENTRATIONS

Table B1. Concentration of total amino sugar, glucosamine (GluN), galactosamine (GluN), and muramic acid (MurA), mannosamine (ManN), as well as ratio of glucosamine to muramic acid per soil mass for treatment incubated for 600 days with and without a sucrose addition. Values are given as mean ± (standard deviation). Letters within each column represent a significant difference between groups. The same letter in a column represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

Treatment	Total (µg/g soil)	GluN (µg/g soil)	GalN (µg/g soil)	MurA (µg/g soil)	ManN (µg/g soil)	GluN/MurA
Control	508.5(42.2)c	366.4(32.6)c	118.7(10.6)b	13.8(1.5)b	9.6(0.7)ab	26.8(2.4)ab
JP Wood	591.8(51.1)ab	423.3(47.8)ab	141.5(8.5)a	16.0(1.6)a	11.1(2.2)a	26.9(5.2)ab
JP 300	531.3(28.8)bc	378.4(23.6)bc	127.7(9.5)b	14.9(1.1)ab	10.3(1.6)ab	25.5(2.2)b
JP 450	470.1(63.8)c	327.3(48.9)c	117.8(15.3)b	14.0(2.2)b	11.0(1.7)a	23.5(1.8)b
RM Wood	642.6(44.4)a	466.6(39.1)a	147.5(12.2)a	17.3(3.3)a	11.1(0.9)a	27.9(6.5)ab
RM 300	505.0(36.7)c	359.8(29.7)c	122.3(8.7)b	13.5(1.6)b	9.4(0.7)b	27.0(4.2)ab
RM 450	490.3(64.3)c	346.0(51.5)c	119.2(14.7)b	14.7(2.7)b	10.4(1.0)ab	24.1(5.0)b
Control + Sucrose	490.4(41.3)c	353.5(22.8)c	115.5(14.6)b	12.0(1.2)b	9.4(1.0)ab	29.8(4.2)a
JP Wood + Sucrose	474.4(36.5)c	340.7(29.8)c	111.2(6.8)b	12.4(1.5)b	10.1(0.6)ab	26.6(1.0)ab
JP 300 + Sucrose	494.9(42.3)c	354.7(32.1)c	118.6(8.8)b	13.3(1.5)b	8.2(1.0)b	24.7(2.4)b
JP 450 + Sucrose	454.0(20.2)c	322.1(14.8)c	110.6(9.9)b	13.1(1.0)b	8.2(0.9)b	27.6(2.7)ab
RM Wood + Sucrose	614.2(60.7)a	454.8(43.2)a	134.9(15.1)a	14.4(2.1)b	10.1(1.1)ab	25.8(1.8)ab
RM 300 + Sucrose	467.9(24.8)c	332.7(19.1)c	113.4(7.8)b	12.9(0.7)b	9.0(0.6)b	26.6(2.9)ab
RM 450 + Sucrose	473.3(19.8)c	338.2(20.4)c	113.8(5.0)b	12.8(0.9)b	8.4(1.3)b	31.0(3.1)a

Table B2. Concentration of total amino sugars (AS) per mass of C and N, along with amino sugar derived C and N contribution to total soil C and N for wood and PyOM treatments incubated for 600 days with or without a sucrose addition. Values are given as mean ± (standard deviation). Letters within each column represent a significant difference between groups. The same letter in a column represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

Treatment	AS (mg/g N)	AS (mg/g C)	AS N Contribution to Soil N (%)	AS C Contribution to Soil C (%)
Control	1921.0(386.0)c	110.5(24.4)b	14.9(3.0)c	4.5(1.0)b
JP Wood	2496.8(173.9)ab	149.4(7.30)a	19.4(1.4)ab	6.0(0.3)a
JP 300	2477.8(356.5)ab	122.8(26.2)ab	19.2(2.8)ab	5.0(1.1)ab
JP 450	2603.4(200.3)a	125.3(22.6)ab	20.2(1.6)a	5.1(0.9)ab
RM Wood	2520.3(800.0)ab	131.8(34.0)ab	19.5(6.2)ab	5.3(1.4)ab
RM 300	2003.2(377.5)bc	109.6(26.6)b	15.5(2.9)bc	4.4(1.1)b
RM 450	1937.3(359.6)c	99.8(19.4)b	15.0(2.7)c	4.0(0.8)b
Control + Sucrose	1830(219.8)c	105.2(14.9)b	14.2(1.7)c	4.2(0.6)b
JP Wood + Sucrose	2009(207.2)bc	120.3(11.5)ab	15.6(1.6)bc	4.8(0.5)ab
JP 300 + Sucrose	2286(119.2)ab	112.4(10.8)b	17.7(0.9)ab	4.5(0.4)b
JP 450 + Sucrose	2534(219.3)ab	120.4(11.5)ab	19.6(1.7)ab	4.8(0.4)ab
RM Wood + Sucrose	2441(856.3)ab	126.1(35.4)ab	19.0(6.6)ab	5.0(1.4)ab
RM 300 + Sucrose	1839(242.2)c	100.3(18.8)b	14.3(1.9)c	4.0(0.7)b
RM 450 + Sucrose	1870(249.5)c	96.2(14.2)b	14.5(1.9)c	3.8(0.6)b

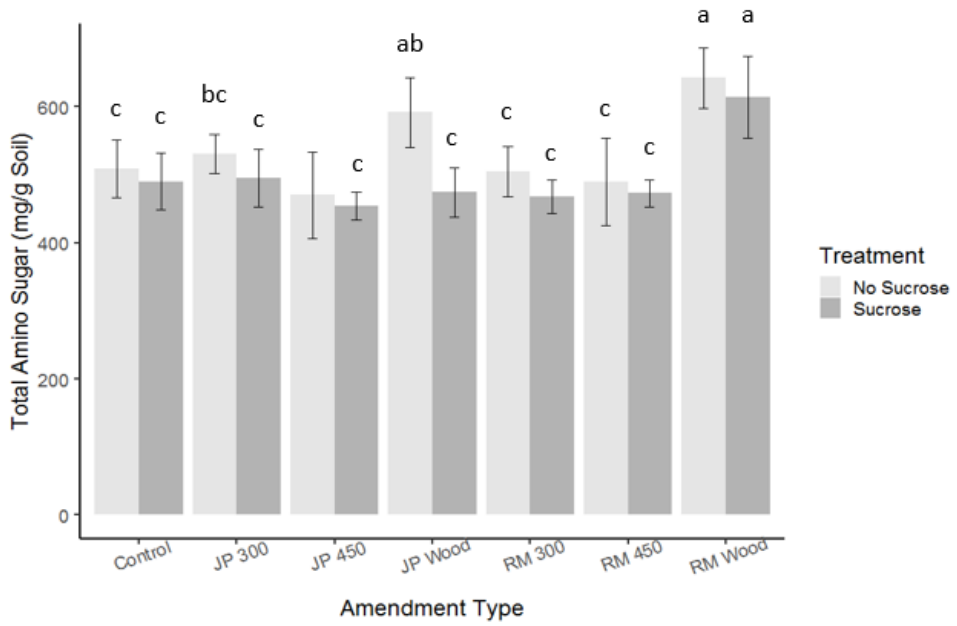

Figure B1. Total amino sugar concentrations per mass of soil for wood and PyOM treatments along with 600 day incubations with or without sucrose. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at P<0.05. n=8 for each treatment.

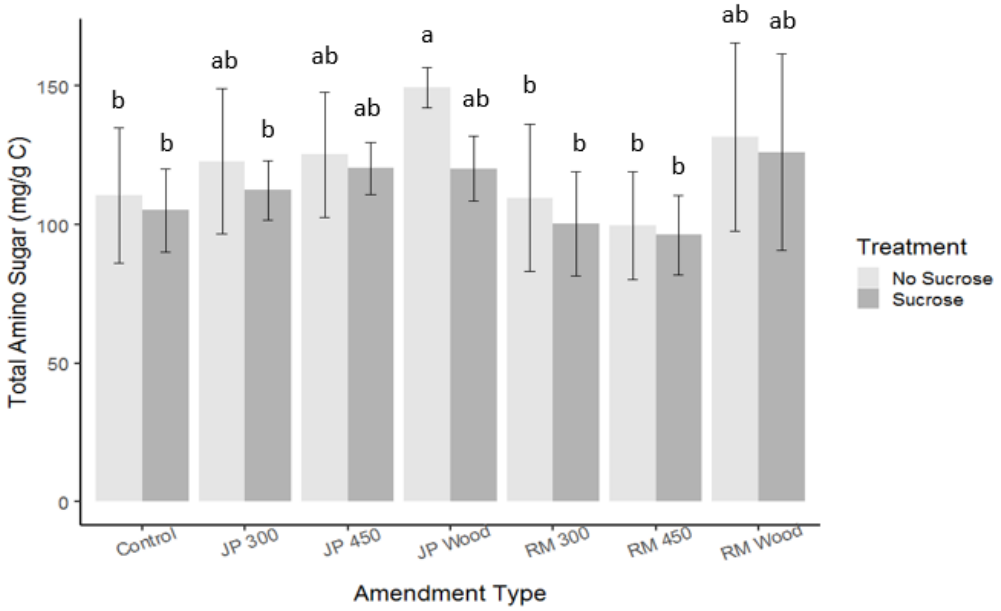

Figure B2. Total amino sugar concentration per mass soil C for wood and PyOM treatments incubated for 180 days with or without a sucrose addition. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at P<0.05. n=8 for each treatment.

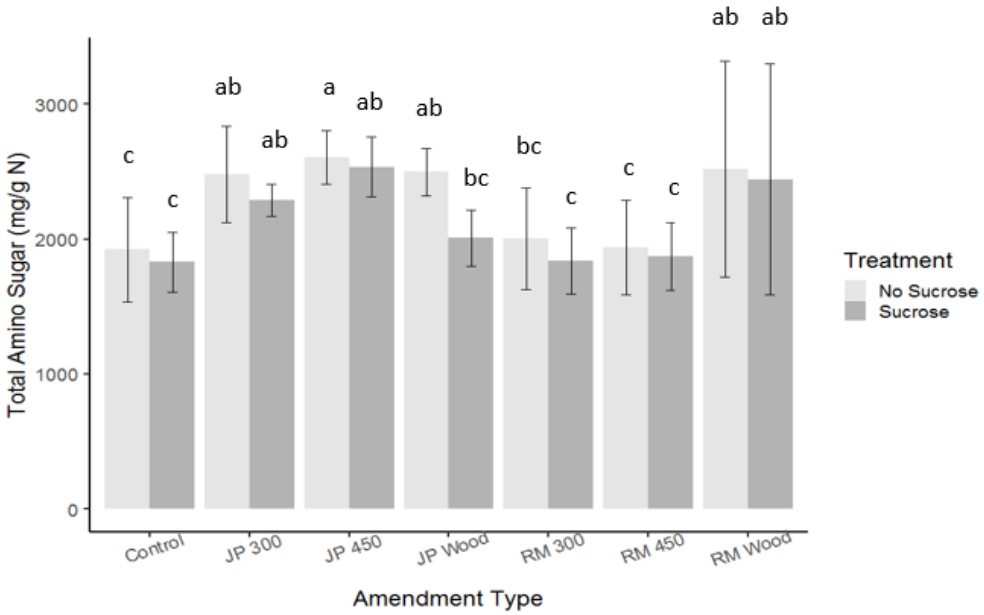

Figure B3. Total amino sugar concentration per mass soil N for wood and PyOM treatments incubated for 180 days with or without a sucrose addition. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at P<0.05. n=8 for each treatment.

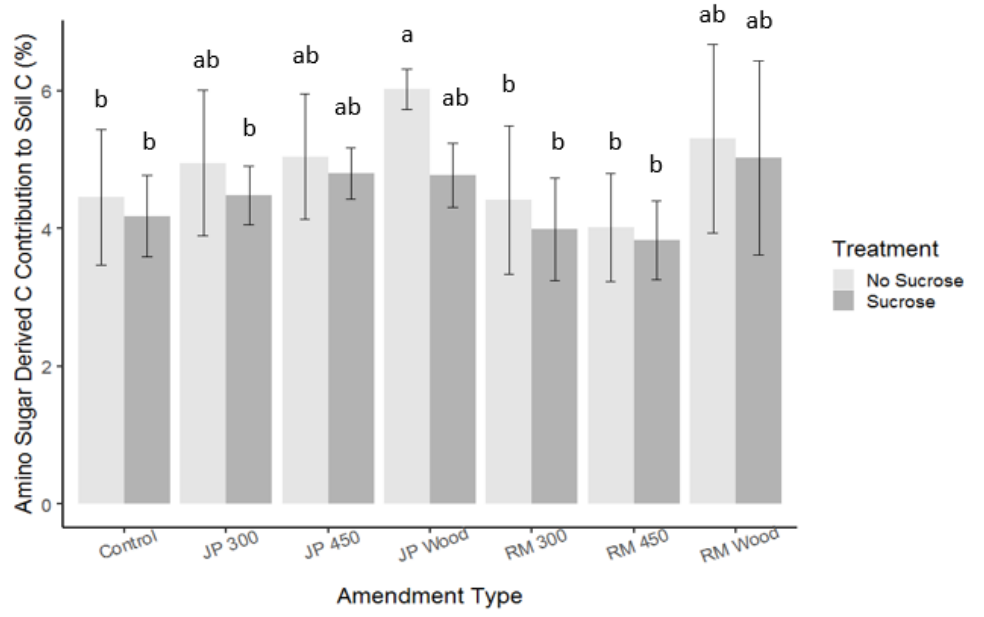

Figure B4. Total amino sugar derived C contribution to total soil C for wood and PyOM treatments incubated for 180 days with or without a sucrose addition. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at P<0.05. n=8 for each treatment.

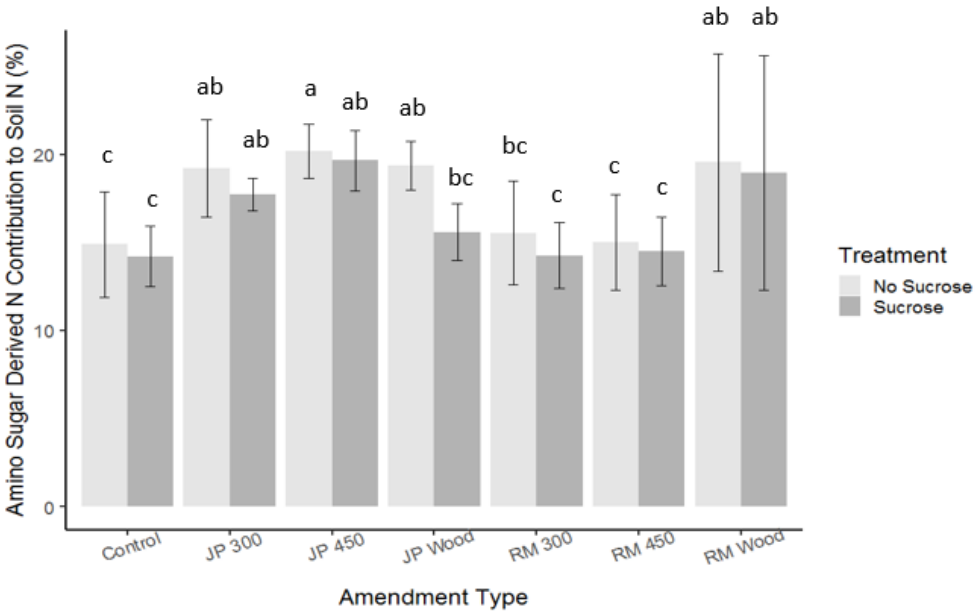

Figure B5. Total amino sugar derived N contribution to total soil N for wood and PyOM treatments incubated for 180 days with or without a sucrose addition. Bars shown are treatment means with error bars as standard deviation. Letters for treatments represent a significant difference between groups. The same letter represents no significant difference between treatments at $P<0.05$. n=8 for each treatment.

APPENDIX C. SUPPLEMENTAL MATERIAL: PHYSICAL AND CHEMICAL CHARACTERISTICS OF ORGANIC MATTER AMENDMENTS

Table C1. Elemental composition and isotopic composition of soil, jack pine, and red maple PyOM and source wood, porosity, surface area, and energy density for jack pine and red maple source wood, and PyOM produced at 300 and 450 °C (Hatton et al., 2016).

PyOM or soil properties	C ($g\ kg^{-1}$)	N ($g\ kg^{-1}$)	^{13}C (atom %)	^{15}N (atom %)	Porosity (%)	BET - N_2 surface area ($m^2\ g^{-1}$)	Energy density ($J\ mg^{-1}\ C$)
JP Wood	464.00	2.8	2.2	19.2	53	2.6	21.1
JP 300	669	5.4	2.37	18.8	75	4.3	26.4
JP 450	786	5.4	2.33	18.9	--	13	27.2
RM Wood	451	6.4	3.81	8.9	60	0.5	20.0
RM 300	574	9	3.74	8.9	72	2.3	23.4
RM 450	761	9.4	3.85	9.1	78	3.3	30.5
Soil	4.9	0.08	-28%	--	--	--	--