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Carbon and nitrogen dynamics from slow pools of soil organic matter in a temperate forest: pyrogenic organic matter and and root litter

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OF SOIL ORGANIC MATTER IN A TEMPERATE FOREST:
PYROGENIC ORGANIC MATTER AND ROOT LITTER

by

FERNANDA SANTOS

A dissertation submitted to the Graduate Faculty in Earth and Environmental Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York
2014
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This manuscript has been read and accepted for the Graduate Faculty in Earth and Environmental Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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THE CITY UNIVERSITY OF NEW YORK
Abstract

CARBON AND NITROGEN DYNAMICS FROM SLOW POOLS OF SOIL ORGANIC MATTER IN A TEMPERATE FOREST: PYROGENIC ORGANIC MATTER AND ROOT LITTER

by

FERNANDA SANTOS

Advisor: Dr. Jeffrey A. Bird

Soil organic matter (SOM) is the dominant reservoir of organic carbon (OC) in terrestrial ecosystems, storing approximately three times the size of the C pool in the atmosphere. In temperate forests, a major fraction of the SOM consists of slowly decaying soil organic C (SOC) pools. While slowly cycling C pools constitute a large reservoir of stable C in soils, the dominant environmental factors controlling this C pool remain unresolved. This research investigates two significant, but poorly characterized slowly decaying C pools: fine root litter (< 2mm) and thermally altered plant biomass (pyrogenic organic matter, PyOM). Specifically, I used compound-specific stable isotope analysis ($^{13}$C and $^{15}$N) as my main methodological approach to examine the (1) decomposition of root litter and PyOM in temperate forest soils, and (2) the factors (soil type, nitrogen addition, and SOM) that affected the stability of these two SOC pools. This was accomplished by integrating the results of: a 180-d incubation study on PyOM...
decomposition, a study on the molecular composition and physicochemical structure of PyOM, δ^{13}C measurements of PyOM molecular markers (^{13}C-benzene polycarboxylic acids) in soils, measurements of atmospheric PyOM-C deposition, and a 2-yr field study on root litter decay. PyOM-C at 450°C had a centennial mean residence time (MRT) in temperate forest soils, and the mineralization of PyOM-N was affected by reactive mineral surfaces. Future tracing experiment studies will greatly benefit from the use of ^{13}C-BPCA approach to quantify PyOM turnover rates in soils. In northern Michigan, PyOM-C deposition fluxes from the atmosphere to soils were low, but provided background data relevant for future assessments of atmospheric PyOM-C concentration. Lastly, maple roots decomposed faster than those reported by previous studies in temperate ecosystems, suggesting that root litter C is not a stable SOM pool in northern temperate forest soils. To improve long-term predictions of the impact of climate change on SOC fluxes, ecosystem scale C models should consider root detritus as a fast-cycling C pool in northern forest soils, incorporate the effect of soil mineral assemblage on the stability of SOM, and no longer assume that PyOM has a millennial MRT in soils.
Dedication

I dedicate this work to my parents, Maria H. A. dos Santos and Antonio J. B. dos Santos (in memoriam), and my sister Ariana. I thank my parents for having given me the freedom to pursue my dreams; for their unconditional love, hard-work, dedication, and support.
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I thank the faculty, staff, and the students of University of Michigan Biological Station for providing a vibrant and supportive research community. Karen Slavik facilitated my research experience at the station; Mike Grant guided me through the GC-IRMS, giving me the confidence to carry out analyses independently; Tony and Rich were always in a good mood, and ready to assist me with logistics. I thank Jim LeMoine for sharing his knowledge on forest and soil ecology, for the valuable help in the lab and field, for the popcorn, recipe ideas, and rides; and Chris Vogel assisting me with the LICOR. And… a big thank you to the kitchen staff who worked hard to feed us all.

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Chapter 1: Introduction and background

Soils are important components of the global C cycle. Globally, soils store approximately 2344 Gt of organic carbon (OC) in the surface 3 m (1Gt = $10^{12}$ kg, Jobbágy and Jackson 2000). The C pool stored in soils constitutes approximately three and four times the size of the C pool in the atmosphere (800 Gt C, Schimel 1995, Batjes 1996, Lal 2004) and vegetation (550 Gt C, Houghton 2005), respectively. Soil C stocks in temperate forests account for 13% of the total soil C stocks in forest ecosystems (IPCC 2001). Given the large size of the C reservoir in soils, a small change in this pool triggered by a warming climate could stimulate heterotrophic soil respiration and result in larger emissions of CO$_2$ (and CH$_4$) into the atmosphere (Kirschbaum 1995, Kirschbaum 2000, Fang et al. 2005). Results from a 7-y soil warming study showed that increasing soil temperature leads to a cumulative net loss of soil C (Mellilo et al. 2011); supporting the hypothesis that soils can quickly become a C source as a response to warmer temperatures (Trumbore et al. 1996). However, there is still a considerable uncertainty associated with the response of SOC pools to different climate change scenarios because of limited knowledge of the interactions among climate variables and feedbacks between terrestrial ecosystem C fluxes and climate change (Davidson and Janssens 2006, Heimann and Reichstein 2008, DeLuca and Boisvenue 2012, Dungait et al. 2012). This knowledge gap is particularly significant for SOC pools thought to persist in soils from decades to millennia (Rumpel and Kögel-Knabner 2011, Schmidt et al. 2011, Dungait et al. 2012).

In an effort to better understand the stabilization mechanisms of SOC, as well as predict terrestrial ecosystem C fluxes and climate change, ecosystem C models, such as Rothamsted and CENTURY,
have been developed to simulate plant litter decomposition processes (Vitousek et al. 1994, Paul and Clark 1996, Falloon and Smith 2000). Rothamsted model simulates SOC turnover from days to centuries using five conceptually discrete compartments representing SOC pools that have distinct turnover rates: (1) decomposable (DPM) and (2) resistant plant materials (RPM), (3) microbial biomass (BIO), (4) humified organic matter (HUM), and (5) ‘inert’ organic matter (IOM), which is assumed to be a small pool, uncoupled from other compartments, and resistant to biological degradation (Jenkinson and Rayner 1977, Jenkinson et al. 1990). In the Rothamsted model, a decomposition rate constant ($k$, $y^{-1}$) is set for each of the ‘active’ compartments as follows: 10 $y^{-1}$ for DPM, 0.3 $y^{-1}$ for RPM, 0.66 $y^{-1}$ for BIO, and 0.02 $y^{-1}$ for HUM, whereas IOM is assumed to be 50,000 y old (Coleman and Jenkinson 1999). CENTURY model simulates the dynamics of C, N, P, and S in ecosystems, and uses three discrete SOC pools of different decomposition rates: active, slow, and passive (Parton et al. 1987). The active pool in the CENTURY model has a turnover time of months to years, and is represented by soil microbes and their products; the slow pool represents plant materials with a turnover time of 20 to 50 y; and the passive pool represents physically and chemically stabilized SOC with a turnover time of 400 to 2000 y (Parton et al. 1987).

Ecosystem-scale C models are important quantitative and predictive research tools. These models compartmentalize SOC into conceptually simple pools, and have been successfully validated against observations in systems for which they were developed (Dungait et al. 2012). Soil organic matter (SOM), however, is not a homogeneous class of organic compounds with a single turnover time (Trumbore 2000, Six and Jastrow 2002), but is a spectrum of above and belowground plant materials and microbial products with different turnover times. This range of compounds includes small molecular weight molecules, such as acetate and amino acids (van Hees et al. 2005) to recognizable
plant litters and microbial necromass. In soils, this assemblage of SOC compounds forms a continuum that ranges from fresh residues to gradually more complex and stabilized compounds (Marschner et al. 2008), each with distinct turnover times. Thus, the compartmentalization of SOM into three or five operationally defined C pools of different turnover times, as those used by Rothamsted and CENTURY models, could result in inaccurate interpretations of SOM dynamics (Six and Jastrow 2002). This is particularly critical when simulating the dynamics of the dominant SOC pools, the ‘slow’ and ‘passive’, as their stabilization mechanisms remain largely unclear, limiting an accurate prediction of these pools’ responses to climate change (Lutzow et al. 2006, Trumbore 2006, Schmidt et al. 2011, Dungait et al. 2012). Pyrogenic organic matter (PyOM) and roots are thought to represent the ‘passive’ and ‘slow’ SOC pools, respectively.

**PyOM**

PyOM is a product of the incomplete combustion of plant biomass and fossil fuels, and comprises a significant portion (up to 45%) of SOM in temperate regions (Goldberg 1985; Schmidt et al. 1999). PyOM is found in the environment as a heterogeneous group of thermally altered residues, ranging from slightly heated plant biomass to residues produced at progressively higher temperatures (e.g. 100 to 800°C). There is an increasing interest in understanding and quantifying the stability and dynamics of PyOM-C in soils given: (1) the assumption that inputs of PyOM to soils will increase as wildfire occurrences become more frequent and intense under future climate scenarios (Fried et al. 2004, Westerling et al. 2006, Flannigan et al. 2009); (2) the growing biochar energy industry, which promotes the use of PyOM as a soil amendment to improve soil fertility and sequester C (Lehmann 2007) and to assist with adsorbing soil contaminants (Nguyen et al. 2007, Wang and Xing 2007). However, it is clear from studies on PyOM-C decomposition in soils (Hammes et al. 2008, Singh et
al. 2012), PyOM-C export to marine systems (Jaffê et al. 2013), and on the impacts of biochar on N$_2$O and CH$_4$ fluxes (Gurwick et al. 2013), that the degradation, stability and mobility of pyrogenic C in the environment remains poorly understood. Thus, knowledge of the net contribution of PyOM-C to soil C will be crucial to predict the long-term effects of forest fires and biochar amendments on terrestrial C and N fluxes.

In the Rothamsted and CENTURY models, PyOM is considered an ‘inert’ C pool, and thought to have a turnover time of millennia (Jenkinson and Rayner 1977, Parton et al. 1987). The assumption that the microbial degradation of PyOM was negligible was initially supported by data from laboratory studies that showed PyOM was highly resistant to thermal (Cachier et al. 1989) and chemical oxidations procedures (Bird and Grocke 1997). Bird and Grocke (1997) showed that PyOM resisted to oxidations by acid dichromate, and a mixture of potassium hydroxide and hydrogen peroxide. In addition, data supporting the long-term PyOM stability included reported long mean residence times (2,400-13,900 years) in samples located in deep sea oceans (Masiello and Druffel 1998), their presence in rocks as fossils (Cope and Chaloner 1980), and the high contents of PyOM measured in tropical Anthrosols, which are reported to be 500-2500 years old (Glaser et al. 2001, Neves et al. 2003). However, almost three decades ago, Goldberg (1985) estimated that if PyOM were indeed resistant to degradation on this scale, it would take less than 100,000 years for the global surface C to be converted into PyOM-C. Nonetheless, for many decades PyOM was thought to be resistant to degradation and to have long mean residence time (MRT) in soils and sediments (Schmidt and Noack 2000, Forbes et al. 2006).
Over the last two decades, a new view on PyOM dynamics in soils has emerged from several lines of evidences supporting the hypothesis that PyOM-C turnover rates are significantly faster than previously predicted (Schmidt 2004, Ohlson et al. 2009, Knicket 2011, Singh et al. 2012b). For example, the MRT of PyOM has been reported to range from decades to centuries in savanna soils (Bird et al. 1999). Similarly, a centennial MRT was estimated for PyOM in a steppe soil in Russia (Hammes et al. 2008) and a temperate forest soil in Switzerland (Maestrini et al. 2014a). Despite numerous studies on PyOM in the environment, we still lack field-based turnover data to support the assumption of the long-term (i.e., >1000 years) stability of PyOM in soils (Gurwick et al. 2013). In addition, very limited data exist on the mechanisms by which PyOM-derived C and N is either mineralized or stabilized in soils (Schmidt et al. 2011). Chapter 2 reports and discusses the MRT of pine-derived PyOM- and its precursor wood- C in two contrasting soils. This was accomplished by following the fate of $^{13}$C/$^{15}$N-labeled Pinus ponderosa (ponderosa pine) PyOM and wood into soil-respired $^{13}$CO$_2$ during 180-day incubation study to answer the following specific question: what is the MRT of PyOM-C produced at 450 °C in temperate soils? The pine wood was included in this study for comparative purposes. The findings of this study were published in Soil Biology and Biochemistry (Santos et al. 2012).

Root litter C

While a significant fraction of the stable SOC is thought to be derived from root litter (Nierop 1998, Rumpel et al. 2002, Rasse et al. 2005, Nierop et al. 2006, Crow et al. 2009), the influence of root litter inputs on belowground SOM maintenance is much less well understood than that of aboveground litter (Melillo 1982, Ono et al. 2013). Results from field studies have shown that the turnover rates of root litter C can be up to nine times slower than those of aboveground litter C (e.g.,
Abiven et al. 2005, Rasse et al. 2005, Bird and Torn 2006, Xiong et al. 2013), suggesting that root litter C is an important source of slowly-degrading plant-derived SOC. For example, studying decomposition and stabilization of *Pinus ponderosa* -derived fine root (< 2 mm diameter) and needle in Sierra Nevada, Bird and Torn (2006) found that the decay rate of the resistant C pool of root litter was six times slower than that of needles. This apparent stability of root litter in soils has been attributed to the relatively high lignin concentrations in root tissues (Abiven et al. 2005). Lignin is the second most abundant biopolymer in nature after cellulose (Kogel-Knabner 2002), and white-rot fungi are presumably the only known microorganisms that can completely degrade it (ten Have and Teunissen 2001). Thus, higher levels of lignin in roots relative to other plant litters could retard the mineralization rates of root C in soils. Despite advances in quantifying the mineralization rates of root litter C and N (Bird and Torn 2006, Casals et al. 2010, Garcia-Pausas et al. 2012, Xiong et al. 2013), the long-term contribution of root litter to belowground C and N fluxes remains poorly understood. **Chapter 6 addresses this research gap and focuses on fine root decomposition and stabilization dynamics in soil by measuring the C and N mineralization dynamics of fine roots added to a northern temperate forest soil.** My approach was achieved by tracing dual-labeled \(^{13}C/^{15}N\) *Acer rubrum* (red maple) fine roots in soils within a long-term (i.e., ongoing, established in 2004) litter manipulation field study located at University of Michigan Biological Station (UMBS) to answer the following general questions: what is the MRT of fine root litter C in temperate forest soils? How much root C is lost as CO\(_2\) and DOC after 2-y *in situ*? How much root N is retained in soils after 2-y *in situ*? The specific questions addressed in Chapter 6 will be presented in the next sections of this chapter. The results of this study are planned for submission for publication during 2014 (Biogeochemistry, Santos et al. *in preparation*).
PyOM turnover in soils

While abiotic degradation (e.g., photochemical oxidation) has been shown to occur on PyOM surfaces (Cheng et al. 2006, Zimmerman 2010, Jones et al. 2011), several studies support that the degradation of PyOM in soils is primarily biotic. Results showing CO$_2$ evolution from PyOM in sterile and non-sterile matrices suggested that the biological degradation is the dominant mechanisms of PyOM-C losses (Potter et al. 1908, Shneour 1966, Zimmerman 2010). In an experiment carried out a century ago, bacteria were shown to be capable of degrading PyOM (Potter et al. 1908). Later observations demonstrated that microorganisms are able to access the interior of charcoals (Bird et al. 2008), providing evidence that BC surfaces can serve as a habitat for several microorganisms, such as saprophytic fungi (Pietikainen et al. 2000, Hockaday et al. 2006). More recently, Zimmerman (2010) observed higher C losses from PyOM as CO$_2$ when the media contained microbial inoculates. In a study using $^{14}$C-labeled PyOM, Kuzyakov et al. (2009, 2014) showed that PyOM-C was incorporated soil microbial biomass after 8 years of incubation. Utilization of PyOM-C by microorganisms has also been document by other recent studies (Steinbeiss et al. 2009, Farrell et al. 2013). While these findings support the hypothesis that the degradation of PyOM is primarily mediated by microorganisms, documented evidences supporting the biological degradation of PyOM remain scarce. Chapter 2 presents the microbial mineralization of PyOM C and N during a 180-d incubation study. Soil-respired C from sterilized soils with and without Pinus ponderosa PyOM was measured to quantify the relative importance of abiotic C mineralization, and answer the following specific question: is the C mineralization of PyOM predominantly biologically mediated? These findings were published in Soil Biology and Biochemistry (Santos et al. 2012).
Another missing element in PyOM research concerns the identification of the specific groups of soil microorganisms that utilize C from PyOM in temperate forest soils. Early laboratory experiments showed that bacteria were capable of degrading charcoal (Potter et al. 1908). Studies on tropical soils rich in PyOM, known as Terra Preta (or Amazonian Dark Earths), reported that these soils had higher and more diverse bacterial population, as well as higher fungi community when compared to adjacent PyOM-poor soils (Grossman et al. 2007; Kim et al. 2007, O’Neill et al. 2009). In a laboratory study, fungi and gram-negative bacteria were the groups that incorporated the most C from PyOM (Steinbeiss et al. 2009). Fungi were hypothesized to preferentially uptake PyOM-C, given that they produce the needed exoenzymes capable of degrading aromatic organic compounds, including lignin (Horwarth 2007). For example, white-rot fungi have been reported to degrade polycyclic aromatic hydrocarbons (Hammel et al. 1995), while saprophytic fungi colonies have been observed on charcoal particles (Hockaday et al. 2007). The identification of microbial communities utilizing PyOM-C can be accomplished by tracing a $^{13}$C-labeled substrate in phospholipid fatty acids (PLFA), a class of lipids that is common in membrane components of all living microbial cells (Zelles 1999). Fatty acids in microbial lipids are easily degraded upon cell death, making PLFA an excellent molecular biomarker for groups of microbial communities (Glaser 2005 and references therein). Chapter 2 summarizes and discusses the microbial groups that utilized PyOM-C during a 180-day incubation study. This was achieved by tracing the fate of $^{13}$C-labeled *Pinus ponderosa* PyOM and wood into microbial PLFAs to answer the question: which microbial community groups mostly utilize PyOM-C? These findings were published in Soil Biology and Biochemistry (Santos et al. 2012).
Elucidating the chemical structure of PyOM from forest fire events

In soils, PyOM is a heterogeneous class of thermally altered compounds that have different turnover rates. The stability of PyOM in soils has been shown to be influenced by its macromolecular structure, which is determined by the combustion or pyrolysis conditions, especially the temperature (Baldock and Smernik, 2002). In the last decade, significant progress has been made towards understanding the relationship between physicochemical properties of PyOM and its decomposition rates in soils (Singh et al. 2012b, Bergeron et al. 2013). Several recent studies have demonstrated the changes in the elemental composition and physical structures of the initial biomass (e.g. plant wood) with increasing charring temperatures. In these studies, an increase in temperature typically leads to larger proportions of highly condensed, and presumably less degradable, carbonaceous compounds (Hammes et al. 2006; Knicker et al. 2008, McBeath and Smernik 2009, Keiluweit et al. 2010, Schneider et al. 2010, McBeath et al. 2011). For example, an increase in temperature of oak and pine woods (350, 450, and 550°C) has been shown to result in greater proportion of aromatic compounds and higher degree of aromatic condensation in PyOM (McBeath et al. 2014). These changes further support previously observed decreases in H and O, and increases in C with a corresponding increase in PyOM formation temperature (Hammes et al. 2006, Bergeron et al. 2013).

Chapter 3 presents and discusses the physicochemical changes of Pinus ponderosa wood before and after pyrolysis at 450°C. This was accomplished by using a combination of isotopic and elemental composition (C, H, O, and N), solid state nuclear magnetic resonance, electron paramagnetic resonance, and diffuse reflectance Fourier transform infrared spectroscopy techniques. Chapter 3 answers the question: what is the chemical structure and composition of
pine PyOM produced at 450°C? These findings were published in Organic Geochemistry (Chatterjee, Santos et al. 2012).

The observations mentioned previously suggest that PyOM formed at lower temperature (e.g. 300°C) are likely to degrade faster than those formed at temperatures above 800°C (Hammes et al., 2006, Turney et al., 2006). Results from an incubation study shown that PyOM produced at 450°C decomposed faster than that produced at 550°C (Keith et al. 2011). The recent observation that mineralization rates for PyOM-C produced at 400°C were faster than those measured for PyOM-C produced at 550°C (Singh et al. 2012b) further support the hypothesis that different temperatures result in different PyOM degradation rates in soil.

Compound-specific isotope analyses are promising tools for shedding light to the stability and mobility of PyOM in soils and sediments. For example, compound-specific 13C analysis can be applied to track the origin and fate of 13C-PyOM compounds into different soil C pools (Glaser 2005). The amounts of PyOM can be quantified in soils and sediments by measuring benzene polycarboxylic acids (BPCAs, Glaser et al. 1998, Brodowski 2005). These molecular markers are the oxidation products of condensed aromatic structures, which are the main building blocks of most PyOM-C. The BPCA method was developed to overcome the methodological challenge of a prior lack of a direct analytical approach that could identify and measure PyOM-C in the environment (Glaser et al 1998; Brodowski 2005). In addition to quantifying PyOM, this method also provides qualitative information on the degree of condensation of the PyOM-C present in soils and sediments (Brodowski et al. 2007), providing a clearer picture of its degradability and mobility in the environment (Hammes et al. 2008). Another application of the BPCA method is the estimation of
PyOM-C turnover times in temperate forest soils with a known fire history (Hammes et al. 2008). The use of BPCA as a compound-specific method in combination with δ¹³C measurements can be used to differentiate between pyrogenic and non-pyrogenic sources of BPCAs (Glaser and Knorr 2008), and to shed light in the PyOM-C origin, movement, loss and degradation in situ. **Chapter 4 describes and discusses the applications of a new technique developed to measure δ¹³C-BPCAs and trace PyOM-derived C in soils. This chapter answers the question: is Ion Exchange Chromatography-Isotope Ratio Mass Spectrometry a suitable method for δ¹³C-BPCAs measurements?** The findings presented in Chapter 4 were published in Rapid Communication in Mass Spectrometry (Yarnes, Santos et al. 2012).

The stabilization of SOC has also been attributed to its sorption to reactive mineral surfaces (Guggenberger and Kaiser 2003, Lilienfein et al. 2004, Mikutta et al. 2007, Kalbitz and Kaiser 2008, Schneider et al. 2010, Saidy et al. 2012). For example, soils dominated by non-crystalline Al and Fe oxyhydroxides typically are able to store more SOC than those with lower contents of these minerals (Torn et al. 1997, Percival 2000, Masiello et al. 2004, Rasmussen et al. 2005). In an incubation study using PyOM-rich anthrosols, higher proportions of C were found in the more stable organo-mineral fraction of SOM, suggesting the soil minerals influenced the stability of PyOM (Liang et al. 2008).

**Chapter 2 assesses the influence of short range order (SRO) clay minerals on the MRT of PyOM- and wood-C during a 180-d laboratory study.** This was accomplished by quantifying the C mineralization rates of PyOM and its precursor wood in two temperate soils with contrasting SRO clay minerals. Given that the mineralization of C and nitrogen (N) in soils are closely coupled (Bird and Torn 2006, Manzoni et al. 2008), Chapter 2 also evaluates the effects of SRO clay minerals on mineralization of N from PyOM and wood. The specific question
addressed by Chapter 2 is: do SRO clay minerals affect the C and N mineralization rates of PyOM? These findings were published in Soil Biology and Biochemistry (Santos et al. 2012).

**Atmospheric deposition as an input pathway for PyOM**

Estimations of atmospheric PyOM deposition are important for future predictions of the long-term effects of forest fires on terrestrial C fluxes. However, PyOM deposition fluxes from the atmosphere remains a poorly quantified component of net PyOM additions to temperate terrestrial ecosystems. In addition, we lack sufficient data to elucidating the impacts of PyOM on regional and global climate (Bond et al., 2004, Bond et al. 2013). Chapter 5 summarizes and discusses the amounts of atmospheric OC and PyOM deposited in a temperate forest at the UMBS. This was accomplished by quantifying OC and PyOM-C in fine (< 2.5 µm) particulates (PM 2.5) using a Thermal/Optical method to answer the question: how much atmospheric PyOM-C is deposited in soils? These findings were submitted for publication in January 2014 (Atmospheric Environment, Santos et al. in review).

Once atmospheric PyOM is deposited in soils, its macromolecular structure and hence its degradation rate will be influenced by its formation temperature. For example, atmospheric PyOM derived from forest fires is typically produced at temperatures < 600 °C, while soot formation generally occurs at temperatures > 800 °C (Scott 2000, Turney et al. 2006, Hammes et al. 2006. Thus, PyOM particles formed under lower combustion temperature (e.g. biomass burning) are expected to degrade faster than those formed under higher temperatures (e.g. fossil fuel combustion). However, the relative contribution of these sources to the total atmospheric PyOM deposited in temperate forest soils remains unclear, yet important to understand the dynamics of atmospheric
Chapter 5 presents the dominant emission sources of fine (< 2.5 µm) particulates (PM 2.5) in a northern temperate forest located that at UMBS. This was achieved by using source-specific molecular markers to distinguish biogenic (e.g. biomass burning) versus anthropogenic sources (e.g. diesel fuel) to answer the following question: what are the predominant emission sources of atmospheric PyOM-C in a temperate forest soil?

The effects of PyOM on the turnover rate of native SOM

The addition of PyOM in mineral soils is thought to affect the degradation rates of native SOC through a biologically-driven phenomenon known as the ‘priming effect’ (Kuzyakov et al. 2000, Fontaine et al. 2003, Kuzyakov et al. 2010). A positive ‘priming effect’ of PyOM results from the observed stimulation of the SOC mineralization rates, whereas negative priming implies a suppression of the SOC mineralization rates following PyOM additions to soil.

Studies on priming have shown contrasting evidences for the effects of PyOM additions to soils on SOM decomposition. After 10 y in situ, litter bags containing a mixture of PyOM and humus lost twice as much C as what was expected based on litter bags containing only PyOM and only humus (Wardle et al. 2008). Losses of C from the PyOM+humus mixture were attributed to an increase in microbial activity, which potentially accelerated C losses from the mixture as respired C and/or through leaching (Wardle et al. 2008). Similarly, results from an incubation study showed that PyOM (possibly its labile fraction) stimulated the losses of native SOC (Keith et al. 2011). In a 10-month field study, PyOM was reported to induce positive priming on the native SOC pool associated to the free particulate (light) fraction in a temperate forest soil in Switzerland (Singh et al. 2014). In this same study, additions of PyOM to soils decreased native SOC concentration by 13% when
compared with control treatments, and was thought to be a result of greater microbial activity in PyOM microsites, which could have favored microbial growth (Singh et al. 2014). In contrast to the positive priming observed by the studies mentioned above, the negative priming effect of PyOM has been supported by several studies (Liang et al. 2010, Jones et al. 2011, Zimmerman et al. 2011). In a 532-day incubation study, the total C mineralized from PyOM-rich Anthrosols was 3 times lower than that from adjacent, PyOM-poor soils (Liang et al. 2010). During a 21-day incubation study, the addition of PyOM decreased the SOC mineralization net rate by 21% (Jones et al. 2011). In a 158-day incubation study, Maestrini et al. (2014b) found that PyOM induced a positive priming effect during the first 18 days of the study, whereas negative priming was observed from day 18 until the end of the incubation. These results were similar to those presented by Zimmerman et al. (2001), in which the addition of PyOM resulted in a positive priming effect at the early stages of the incubation period due to increased losses of labile PyOM-C, whereas a negative priming effect was observed at later phases due to the lower mineralization of native SOC. In other studies, however, SOC mineralization rates has been reported to be unaffected by the addition of PyOM to soils (Kuzyakov et al. 2009, Cross and Sohi, 2011, Maestrini et al. 2014a). Taken together, these contrasting results on priming clearly indicate that further research is needed to adequately elucidate the mechanisms regulating the direction (e.g. positive or negative) and magnitude of the PyOM priming effects in soils. It is possible that distinct soil horizons (e.g. organic versus mineral), as well as PyOM materials (e.g. grassland versus hardwood), formation temperature (e.g. 300°C versus 500°C) and decomposition stage, are among the factors driving these mechanisms (Zimmerman et al. 2011). In Chapter 2, the responses of SOC mineralization to PyOM amendments are presented. This was accomplished by measuring the mineralization rates of native (unlabeled) SOC in treatments with added $^{13}$C-labeled PyOM during a 180-day incubation study to answer the following
question: do PyOM amendments to soils affect the mineralization rates of SOC? These findings were published in Soil Biology and Biochemistry (Santos et al. 2012).

The influences of plant litter inputs on fine root decomposition in soils

In priming studies, the continuous supply of fresh and labile C (e.g. plant litters, root exudates) to surface soils has been shown to be a key regulator of the slow-cycling, non-pyrogenic SOM pools (e.g., root litter C). This process is thought to occur via co-metabolism, in which energy-limited soil microorganisms utilize fresh C as a source of energy to decompose older and less easily degradable OM (Fontaine and Barot 2005). The positive priming effect of labile C on slow-cycling SOM has been documented by laboratory incubation studies showing that the additions of plant-derived C (e.g., cellulose, root exudates) to soils stimulate (positive priming) the decomposition of native SOM (Cheng et al. 2003, Fontaine et al. 2004, Fontaine et al. 2007, Fontaine et al. 2011, Paterson et al. 2011). In a 161-day incubation study, a positive priming effect was observed following the addition of cellulose (Fontaine et al. 2007). In this study, soils that received cellulose additions released nearly twice as much old and less easily degraded SOC as control soils. In a field study in Panama, treatments that received litter additions lost 1.5 times more SOC (as CO$_2$) than control treatments (Sayer et al. 2011). Similarly, results from a litter manipulation study showed an increase in soil CO$_2$ effluxes following needle additions, although the priming of SOC was attributed to the influence of the rhizosphere and mycorrhizal fungi that live in symbiosis with plant roots (Crow et al. 2009b). In contrast, decreasing or removing plant C additions to temperate soils for more than 50 y, thus reducing the supply of energy-rich litter to microorganisms, has been reported to decrease the concentration of microbial biomass and OM contents in soils (Paterson et al. 2011). While a number of studies have addressed the priming effects of plant litter inputs on SOM decomposition, little is
known about the extent at which aboveground and belowground litter inputs affect the decomposition of roots. The use of litter manipulation experiments, when combined with stable isotopes techniques, can provide insights on the mechanisms associated with the effects of fresh litter inputs on C and N mineralization rates of SOM. Chapter 6 summarizes and discusses the results of a 2-year study that examined the influence of long-term litter inputs on root C and N dynamics in a temperate forest soil. This study was conducted in the Detritus Input and Removal Treatments (DIRT) located at the UMBS, and was accomplished by following the fate of $^{13}$C/$^{15}$N dual labeled *Acer rubrum* (red maple) fine-root litter in soil mesocosms within DIRT treatments that had (1) no belowground litter inputs, (2) no inputs, or (3) normal litter inputs (control). Chapter 6 answers the following specific questions: does the long-term removal of aboveground and belowground litter affect the retention and stabilization of fine root C and N in soils, the mineralization of fine root C as CO$_2$, and the vertical transport of fine root C as dissolved organic C?

**Nitrogen deposition effects on the degradation of slow and passive SOM fractions**

As mentioned previously, the continuous supply of fresh and labile C can alter the mineralization rate of the native SOC. This interaction between labile C and SOC is thought to be mediated by heterotrophic microorganisms mining for N (Craine et al. 2007). Thus, a greater supply of inorganic N to soil microorganisms could potentially retard the C losses from native SOM. In a recent study, Hobbie et al. (2012) concluded that N inputs have the potential to suppress the C losses from the slowly cycling (e.g. decadal and centennial MRT) SOM pool. While the influence of inorganic N deposition (from fossil fuels or fertilization) on SOC turnover rates has been extensively investigated (Pregitzer et al. 2008, Zak et al. 2008, Knorr et al. 2005, Janssens et al. 2010), little is known about
the responses of the slowly decaying SOM pools (e.g. decadal and centennial MRT) to N inputs to soils.

Greater amounts of inorganic N in soils have been reported to stimulate the decomposition of litter with low lignin contents, and to inhibit the decay of lignin-rich litter (Hobbie 2000, Knorr et al. 2005, Janssens et al. 2010). Similarly, additions of inorganic N to soils have been shown to promote the activity of polysaccharide-degrading enzymes, and suppress the losses of lignin after one year of litter decomposition (Talbot and Kathleen 2012). Given that lignin has been documented to be more abundant in root litter than in aboveground litters (Bird and Torn 2006, Xiong et al. 2013), long-term atmospheric N deposition is likely to increase the MRT of C from PyOM and root litter in N-limited soils. Indeed, cumulative PyOM mineralization was reported to decrease by 43% following the additions of N to soils in Switzerland (Maestrini et al. 2014a). Conversely, other studies have shown that PyOM mineralization is unaffected by N additions (Singh et al. in press, Maestrini et al. 2014b).

Chapter 2 presents the influence of N additions on the MRT of PyOM- and wood-C during a 180-day incubation study. This was accomplished by tracing the $^{13}$C-labeled PyOM and wood as soil-respired $^{13}$CO$_2$ in soils with and without added N (as NH$_4$NO$_3$) to answer the following questions: do inorganic N additions affect (1) C and N mineralization rates of PyOM, and (2) any PyOM priming effects on SOM?

Little data exist on the effects of N additions on fine root degradation. In a field experiment conducted in a Hawaiian forest, Ostertag and Hobbie (1999) found that at a 4.1-Myear old site, N additions accelerated the decomposition of fine root, whereas root decomposition was unaffected by the addition of N and P at a younger (300-year old) site. Studying root degradation in a Norway
spruce stand, Majdi (2007) observed an increase in root mass loss from soils that had received N and S additions. Clearly, our understanding of the interactions between N additions and availability and belowground litter decomposition is limited, and further investigations are needed to assess the impact of N deposition on slow-cycling SOM pools, and particularly, root litter. **Chapter 6 summarizes and discusses the influence of N additions on the dynamics of C and N from root litter during a 2-year field study in a northern temperate forest soil.** This was accomplished by tracing $^{13}$C/$^{15}$N-labeled *Acer rubrum* fine root litter in soils with and without additions of N (as NH$_4$Cl) to answer the following specific questions: do the long-term N additions to soils affect the (1) retention and stabilization of root C and N; (2) the mineralization of fine root C as CO$_2$; and (3) the vertical transport of fine root C as dissolved organic C? The findings of this study will be submitted for publication (Biogeochemistry, Santos et al. *in preparation*).

**Overview of dissertation research**

The overall goal of this dissertation research was to reevaluate the role of two important, but overlooked slowly decaying SOC pools – PyOM and root litter – in the global C cycle. The specific goal of this research was to examine the dynamics of C and N of PyOM and root litter and the factors that affect the long-term stability of these two SOC pools in temperate forest soils. Understanding the factors affecting the persistence of PyOM and fine roots in soils is relevant because while they represent a significant portion of the stable SOC, their stabilization mechanisms are not well understood (Schmidt et al. 2011). Another goal of this research was to assess the responses of PyOM and root litter decomposition rates to the additions of and interactions between inorganic N and SOM. Advancing our mechanistic understanding of these interactions could improve predictions of PyOM and root decomposition responses to future increases in anthropogenic
(e.g. fossil fuel and fertilization) N deposition to soils (Galloway et al. 2008). A clear picture of the dominant factors controlling the C pools of PyOM and root litter in temperate forests is fundamentally important for long-term predictions of belowground C responses to climate change.

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Chapter 2: Biological degradation of pyrogenic organic matter in temperate forest soils


Abstract

Pyrogenic organic matter (PyOM), derived from the incomplete combustion of plant biomass and fossil fuels, has been considered one of the most stable pools of soil organic matter (SOM) and a potentially important terrestrial sink for atmospheric CO₂. Recent evidence suggests that PyOM may degrade faster in soil than previously thought, and can affect native SOM turnover rates. We conducted a six-month laboratory incubation study to better understand the processes controlling the degradation of PyOM in soils using dual-enriched (¹³C/¹⁵N) PyOM and its precursor wood (Pinus ponderosa). We examined the effects of soil type and inorganic N addition on PyOM and wood C and N mineralization rates, microbial C utilization patterns, and native SOM turnover rates. PyOM charred at 450 °C or its precursor pine wood was incubated in two temperate forest subsoils with contrasting short range order (SRO) clay mineralogy (granite versus andesite parent material). Duplicates of experimental treatments with and without PyOM added were sterilized and abiotic C mineralization was quantified. In a second incubation, PyOM or wood was incubated in granitic soils with and without added NH₄NO₃ (20 kg N ha⁻¹). The fate of ¹³C/¹⁵N-enriched PyOM and wood was followed as soil-respired ¹³CO₂ and total extractable inorganic ¹⁵N. The uptake of ¹³C from PyOM and wood by soil microbial community groups was quantified using ¹³C-phospholipids fatty
acids (PLFA). We found that (1) The mean residence time (MRT) of PyOM-C was on a centennial time scale (390-600 yr) in both soil types; (2) PyOM-C mineralization was mainly biologically mediated; (3) Fungi more actively utilized wood-C than PyOM-C, which was utilized by all bacteria groups, especially gram (+) bacteria in the AN soil; (4) PyOM-N mineralization was 2 times greater in granite (GR) than in AN soils; (5) PyOM additions did not affect native soil C or N mineralization rates, microbial biomass, or PLFA-defined microbial community composition in either soil; (6) The addition of N to GR soil had no effect on the MRT of C from PyOM, wood, or native SOM. The centennial scale MRT for PyOM-C was 32 times slower than that for the precursor pine wood-C or native soil C, which is faster than the MRT used in ecosystem models. Our results show that PyOM-C is readily utilized by all heterotrophic microbial groups, and PyOM-C and -N may be more dynamic in soils than previously thought.
1. Introduction

Pyrogenic organic matter (PyOM) is a product of the incomplete combustion of plant biomass and fossil fuels, and comprises a significant portion (5-45%) of soil organic matter (SOM) in temperate regions (Goldberg, 1985; Schmidt et al., 1999). PyOM is a heterogeneous class of thermally altered residues that form a combustion continuum, ranging from slightly heated plant biomass to residues produced at progressively higher temperatures (e.g. 100-800°C). Despite recent advances in quantifying PyOM stocks in complex matrices such as soils (Hammes et al., 2008; Ohlson et al., 2009), limited data exist for PyOM C and N mineralization rates, and the main mechanisms responsible for PyOM degradation in soil remain unclear (Kuzyakov et al., 2009; Zimmerman 2010). Moreover, little is known about how edaphic factors such as the soil mineral assemblage and N additions may affect PyOM degradation rates, and the effects of PyOM additions on native soil C and N cycling.

While PyOM has been shown to undergo both microbial and abiotic (e.g. photochemical oxidation) degradation (Cheng et al., 2006; Zimmerman 2010; Jones et al., 2011), recent studies suggest that the biologically mediated degradation of PyOM may be the dominant pathway for PyOM degrading within soils and sediments (Kuzyakov et al., 2009; Steinbeiss et al., 2009). The microbial degradation of PyOM has been shown to occur via co-metabolism, because PyOM-C mineralization has also been stimulated by glucose additions in incubation studies (Hamer et al., 2004; Kuzyakov et al., 2009). While PyOM-C has been shown to be incorporated by microbial biomass, there are limited data on the utilization of PyOM-C within soil microbial communities (Steinbeiss et al., 2009).
Recent field studies have reported PyOM-C turnover in soils on decadal (< 100 years) or centennial (180- 540 years) timescales (Bird et al., 1999; Hammes et al., 2008). These data indicate a significantly faster turnover rate than the millennial scale turnover times estimated from radiocarbon analysis of PyOM recovered in soil profiles (Forbes et al., 2006; Preston & Schmidt 2006). Ecosystem-scale C and N models that include PyOM as a SOM pool, such as Rothamsted C model (Jenkinson et al., 1990), consider PyOM as inert or turning over at rates much slower than recent field studies suggest. Pyrolized organic inputs to soils have been predicted to increase in the future due to climate-change related increases in forest fire frequency (Harrison et al., 2010) and greater amounts of biochar available from low-temperature pyrogenic energy generation (Lehmann 2007). Consequently, a clearer picture of PyOM dynamics in soils is needed to better quantify PyOM dynamics for ecosystem-scale C and N cycling.

The soil mineral assemblage provides physical and chemical protection to SOM through mineral-associated interactions (e.g. Torn et al., 1997). For example, stable SOM has been found to interact with ferrihydrite and polymetric Fe and Al hydroxides (Mikutta et al., 2006). In other studies, soils dominated by non-crystalline and short range order (SRO) minerals (e.g. Al and Fe oxyhydroxides) have been shown to store substantially more C than soils with lower levels of these minerals (Torn et al., 1997; Masiello et al., 2004; Rasmussen et al., 2005; Parfitt, 2009). It is unknown, however, how soil mineral composition may influence mineralization rates of PyOM or wood.

The availability of N to heterotrophic microbial degraders may also affect the decomposition rates of C-rich PyOM. Long-term atmospheric deposition of reactive N species (Galloway et al., 2008) has been shown to affect microbial activity (Janssens et al., 2010) and increase C storage rates in
temperate forest soils (Pregitzer et al., 2008; Zak et al., 2008). N additions to soils via atmospheric deposition can inhibit the production and activity of oxidative enzymes (Waldrop & Zak 2006); thus, an expected increase in atmospheric N deposition may slow the decomposition of the highly aromatic PyOM in soil. In addition, N additions may likewise modify the direction and/or magnitude of soil C priming events caused by PyOM additions to soils.

The addition of PyOM to mineral soils has been reported to inhibit (Major et al., 2009; Jones et al., 2011; Zimmerman et al., 2011) or have no effect on native SOM mineralization rates (Kuzyakov et al., 2009; Hilscher et al., 2009; Cross & Sohi 2011). PyOM added to soils has also accelerated or “primed” native SOM turnover rates in organic horizons of a boreal forest (Wardle et al., 2008) and in mineral soils (Zimmerman et al., 2011). Positive priming effects of PyOM may result from enhanced soil nutrient retention and/or greater microbial biomass on PyOM surfaces (Kuzyakov et al., 2009), while reported inhibitory effects may be due to the physical trapping of SOM on highly reactive surfaces of PyOM particles or within PyOM pores (Major et al., 2009). The contrasting results mentioned above emphasize the need to better understand the mechanisms that control the magnitude and direction of SOM priming events from PyOM additions to soils.

We conducted a laboratory incubation experiment by adding $^{13}\text{C}/^{15}\text{N}$-labeled Pinus ponderosa-derived PyOM or its precursor wood to two soils that differed in their parent material to test the following hypotheses: (1) PyOM-C mineralization in soil will be predominantly biologically mediated; (2) PyOM-C produced at 450°C and incubated in soil will turnover on a centennial scale MRT; (3) PyOM C and N mineralization will be faster in granite-derived soil than in andesite-derived soil; (4) Soil C mineralization rates will be unaffected by PyOM amendments to soils (no
negative or positive priming). In addition, a companion incubation experiment was conducted with and without added inorganic N to investigate how added N might impact PyOM C and N mineralization rates and any SOM priming effects caused by the addition of PyOM or its precursor wood. We followed the fate of $^{13}$C and $^{15}$N from PyOM and its precursor wood as respired CO$_2$, inorganic N, and microbial C (phospholipids fatty acid-PLFA C) during 180 days.

2. Materials and methods

2.1. Soils
Soils for this laboratory investigation were collected from two mixed conifer stands within the University of California (UC) Blodgett Experimental Forest (38°53′83″N and 120°38′15″W) located in the western slope of the Sierra Nevada in El Dorado County, CA, USA. The climate is Mediterranean with dry, warm summers and wet, cool winters (Bird & Torn 2006). In November 2006, we excavated soils from two adjacent forest stands, which formed on two distinct parent materials, granitic (GR soil) and andesitic (AN soil). The AN soils were mixtures of andesite parent material with inclusions of fine-grained granodiorite (Rasmussen et al., 2005). Other climatic and edaphic characteristics of these sites were similar, including vegetative composition (ponderosa pine dominated), aspect, and slope. The forest stands were 90 (GR) and 60 (AN) years old. These well-drained soils, classified as Haploxeralfs (Soil Survey Staff 2010), were sampled from the same general locations (i.e. within ~25 m) as those investigated by Rasmussen et al. (2005) and are described therein. For this investigation, we used soil excavated from the Bt horizon of 4 soil pedons per forest site (GR, AN). The four separate pedon samples for each soil type served as experimental replicates for laboratory experiments.
2.1.1. Soil chemical and physical properties

Total soil organic C and N were measured from homogenized subsamples using a CHN gas analyzer (Costech Model 4010, Valencia, CA). Inorganic C was not detected in these soils. Natural abundance $\delta^{13}$C and $\delta^{15}$N in soils were analyzed using a PDZ Europa 20-20 Isotope Ratio Mass Spectrometer (IRMS, Sercon Ltd., Cheshire, UK). The natural abundance bulk soil $\delta^{13}$C values (GR soils, $-24.1 \pm 0.2\%o$; AN soils, $-24.0 \pm 0.1\%o$) and $\delta^{15}$N (GR soils, $6.4 \pm 0.2\%o$; AN soils, $7.2 \pm 0.4\%o$) values were expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and air for C and N, respectively. To identify differences in crystalline mineral species between GR and AN soils, X-ray diffraction (XRD) analysis was performed on one experimental replicate of each soil type using an X-ray diffractometer (Rigaku Geigerflex, Cu Kα radiation, Tokyo, JP) after the removal of organic matter from bulk soils, and the subsequent separation of soils into clay size fractions (Castanha et al., 2008). No large differences in clay mineral structures were observed between GR and AN soils (data not shown). The dominant clay species shown by XRD were gibbsite and disordered kaolinite or halloysite. Vermiculite was detected in GR soil only (data not shown), as peaks at 14Å shifted to 10Å after heating at 400°C. Selective dissolution and isolation of Al and Fe oxides were performed using an ammonium oxalate and sodium pyrophosphate extraction method (Dahlgren 1994). Extraction by ammonium oxalate predominantly removes Al ($Al_o$) and Fe ($Fe_o$) from organic complexes and short range order (SRO) Fe oxy-hydroxides and aluminosilicates. Sodium pyrophosphate predominantly extracts Al ($Al_p$) bound in organometal complexes (Soil Survey Staff 2004). Sample extracts were measured for Al and Fe using an inductive coupled plasma-atomic emission spectrometer (Optima 5300 DV, PerkinElmer, Waltham, MA, USA). In addition, soil samples were analyzed for nitric acid-extractable elemental Al and Fe (Hossner, 1996),
saturated paste pH (Thomas, 1996), ammonium chloride-extractable cation exchange capacity (Peech et al., 1947), and particle size distribution (Kilmer & Alexander, 1949). Soil physical and chemical data are summarized in Table 1. AN soils had significantly higher amounts of Al_0, Al_p, and inorganic aluminum (Al_0-Al_p), SOC, and silt-size fraction than did GR soils (n=4, P<0.05; Table 1). In contrast, GR soils had higher amounts of sand-size fraction than did AN soils.

### 2.2 13C/15N-enriched PyOM and ponderosa pine precursor wood

Ponderosa pine (*P. ponderosa*) saplings were grown and labeled with 13C as CO_2 and 15N as KNO_3 at UC Davis, as described by Bird & Torn (2006). Ponderosa pine was chosen for this study because it was the dominant vegetation type at the soil sampling sites. The wood was derived from the aboveground stems of 13C/15N-labeled ponderosa pine saplings (i.e. woody branches were not included). Pine stems were dried to a constant weight at 25°C, cut into 1 - 2 cm long pieces, and pyrolyzed at 450°C under N_2 for 5 h (Hammes et al., 2006) to produce 13C/15N-labeled PyOM (Table 2). This method approximates the oxygen-free conditions in the interior of naturally forming PyOM materials (Hammes et al., 2006). Both wood and PyOM were ground (< 2mm) prior to addition to soil. PyOM and wood subsamples were further homogenized by ball milling for elemental and stable isotope analysis. Carbon and N elemental and isotopic enrichment was measured on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 IRMS (Sercon Ltd., Cheshire, UK). Benzene carboxylic acids (BPCAs), which are used as molecular biomarkers for PyOM materials in soils and sediments (Glaser et al., 1998), were extracted from the enriched PyOM used in this experiment (Yarnes et al., 2011). The 13C enrichment of BPCAs averaged 2.03 atom% (Yarnes et al., 2011), similar to the 13C enrichment values of the bulk PyOM and its precursor wood (Table 2), suggesting that the isotopic label in the enriched wood and PyOM was uniform. Oxygen
elemental concentration and isotopic enrichment was determined using a PYROcube (ELEMENTAR Analysensysteme, Hanau, DE) interfaced to a Sercon 20-20 Isotope Ratio Mass Spectrometer (Sercon Ltd., Cheshire, UK), and H elemental concentration was determined using a high temperature elemental analyzer (Hekatech, Wegberg, DE). Provisional delta values, expressed with respect to Vienna Standard Mean Ocean Water, were corrected based on laboratory standards and international references analyzed with the samples. The summary of the isotopic and elemental composition of the PyOM and wood is shown in Table 2 as means of three analytical replicates.

2.3 Experiment I: soil mineral assemblage
Each soil (AN, GR) was incubated with and without $^{13}\text{C}/^{15}\text{N}$-labeled materials (wood and PyOM) to quantify C and N mineralization from the substrates and their response to soil mineral composition (n=4). Each soil was incubated in a 1L mason jar fit with a septa in the lid to allow for headspace CO$_2$ sampling. Soils without substrates served as unamended controls. Each of the four pedon samples served as a replicate for the study. Air-dried soils (<2 mm) were brought to 55% of soil water holding capacity (WHC) with de-ionized (DI) water. Ground (<2 mm) PyOM or wood was applied to soils at a rate of 7.5% of soil C (i.e. on a g substrate C per g soil C basis). The incubation was carried out in the dark at a constant temperature of 25°C for 180 days. The incubation period (180 days) was based on the pattern of C mineralization rates for all treatments. To test whether the mineralization of PyOM-derived C was biotic, an additional set of mason jars with either GR or AN soil, with or without added PyOM, was autoclaved three times at 121°C for 30 min before the incubation started. In addition, four non-sterilized experimental controls (i.e. vessels without soil or added PyOM) were monitored for evolved CO$_2$ throughout the incubation. Due to the imposed light restriction, photochemical degradation was not considered in these experiments.
2.4 Experiment II: nitrogen addition

To examine the effect of added N on C and N mineralization from PyOM, its precursor wood, and soil priming, a second parallel experiment was performed using the GR soil. GR soil with added PyOM, wood, or unamended controls received either 20 kg N ha\(^{-1}\) (as NH\(_4\)NO\(_3\)) or no added N. The inorganic N was added prior to the start of the incubation in DI water and homogenized with soil. Control soil that had no N added received the same amount of DI water and mixing as those receiving added N. Soils were incubated, sampled, and analyzed as described in Experiment I.

2.5 Soil and headspace C and N analyses

2.5.1 PyOM, wood and soil CO\(_2\) respiration

Soil-respired CO\(_2\) was measured as evolved CO\(_2\) into the vessel headspace on 10 sampling dates during the 180-d incubation. The accumulated headspace CO\(_2\) concentration was measured with an EGM-4 infra-red CO\(_2\) analyzer (PP Systems, Amesbury, MA). All jars were flushed with CO\(_2\)-free air after each sampling date, and 1 - 2 ml of DI water was maintained inside the vessels to maintain moisture content. \(^{13}\)C-CO\(_2\) gas samples were analyzed for \(^{13}\)C isotope enrichment using a ThermoScientific PreCon-GasBench system interfaced to a ThermoScientific Delta V Plus IRMS (ThermoScientific, Bremen, DE), and reported on a per mil (‰) basis using the standard convention relative to PDB standard. Carbon dioxide evolution was expressed on a soil mass and soil C basis, as described by Rasmussen et al., (2006). Mineralization rates of native soil C in soils incubated with and without PyOM or wood additions were measured to investigate priming effects on native SOM.
Isotopic signatures of measured soil-respired CO$_2$ were used to calculate the fraction ($f$) of PyOM- or wood-C in the resired CO$_2$ ($^{13}$C excess CO$_2$), as in Equation (1),

$$f = \delta^{13}\text{CO}_2\text{sample} - \delta^{13}\text{CO}_2\text{control} / \delta^{13}\text{C}_\text{S} - \delta^{13}\text{C}_\text{soil} \quad (1)$$

where $\delta^{13}\text{CO}_2\text{ sample}$ and $\delta^{13}\text{CO}_2\text{ control}$ are the isotopic composition of CO$_2$ evolved from sample treatment and unamended soil, respectively; $\delta^{13}\text{C}_\text{S}$ and $\delta^{13}\text{C}_\text{soil}$ are the C isotopic composition of added PyOM or wood, and soil C from unamended soil, respectively. CO$_2$ mineralization rates of enriched PyOM and wood were calculated by multiplying $f$ from Equation (1) by the rate of C evolved as CO$_2$ (mg C day$^{-1}$). Mineralization rates of CO$_2$ from unlabeled soil C was calculated as in Equation (2),

Unlabeled soil C = TCO$_2$ (1 - $f$) \quad (2)

where TCO$_2$ is the total CO$_2$ evolved and $f$ is as noted in Equation (1). The mineralization rates of CO$_2$ from added PyOM, wood, and native soil C were fit to a two-compartment first-order decay model to calculate the amount of C lost respired as CO$_2$ (Hess & Schmidt 1995) and the amount of C retained in soil from added PyOM, wood, and from initial unlabeled SOM. The model partitioned the mineralization rates of C into two conceptual C pools: an active, fast, or labile ($L$), and a slow or resistant ($R$), where:

$$-dC/dt = C_L e^{-k_L t} + C_R e^{-k_R t} \quad (3)$$

d$C$/d$t$ is (mg C day$^{-1}$), $C_L$ and $C_R$ are the size of the labile ($C_L$) and resistant ($C_R$) pools (mg C) and $k_L$ and $k_R$ represent decomposition rate constants (day$^{-1}$) for each pool. We assumed that decay resulted in CO$_2$ production, and there was no exchange of C between $C_L$ and $C_R$. Total pool sizes of the potentially mineralizable labile and resistant C pools were estimated by integrating the first-order equation to each pool from Equation (4):

$$C_{Ti} = C_i / -k_i (e^{k_i t} - 1) \quad (4)$$
where $C_{T,i}$ is the total size of pool $i$ (L or R) (mg C), and $C_i$ and $k_i$ are as noted in Equation (3). To calculate the MRT of the conceptual C pools, the proportion (%) of C remaining in soils from added PyOM and wood, calculated at time $t$, was fit to a double-exponential decay model [Equation (5)],

$$\text{Proportion of remaining C} = R_L e^{-k_L t} + R_R e^{-k_R t} \quad (5)$$

where $R_L$ is the easily decomposable fraction, $R_R$ is the resistant fraction, $k_L$ and $k_R$ are C decomposition constants, and $t$ is the time in days. Data of C remaining in soil from initial native SOM were also fit to Equation (5). The MRT of the labile and resistant pools was calculated as in Equation (6),

$$\text{MRT}_i = 1/k_i \quad (6)$$

where MRT$i$ is the mean residence time of pool $i$ (L or R), and $k_i$ is as noted in Equation (5). Curve fitting was performed using SigmaPlot for Windows (v. 10).

### 2.5.2 Microbial biomass and community composition

The quantity and $^{13}$C enrichment of PLFA biomarkers in all soils in Experiment I (item 2.3) were measured on three dates: (i) prior to incubation, (ii) day 42 of incubation, and (iii) 180 d of incubation using the procedure of Bird et al., (2011) based on White & Ringelberg (1998) to assess microbial community composition, biomass, and the utilization of C from native SOM, PyOM and wood during initial and latter stages of decomposition. Recovery of PLFAs from soil was determined using di-19:0 PC (1, 2-Dinonadecanoyl-sn-Glycero-3-Phosphocholine, Avanti Polar Lipids, Alabaster, AL, USA) added prior to extraction and 12:0 FAME (methyl dodecanoate, Matreya, Pleasant Gap, PA, USA) added prior to PLFA identification and quantification. FAMEs were analyzed using a Trace GC Ultra (Thermo Fisher Sci., Bremen, DE) with a 30 m x 0.25 mm x 0.25 mm VF-5ms column (Varian, Lake Forest, CA) connected via a Finnigan GC Combustion III
interfaced to a Delta V Advantage IRMS (Thermo Fisher Sci., Bremen, DE). The GC analyzed extracts in splitless injection, with an initial temperature of 110°C for 0.1 min., ramped to 220°C at 4°C min⁻¹, ramped to 290°C at 10°C min⁻¹ and held for 7 min. Peak identification was based on relative retention of known standards from Matreya (Pleasant Gap, PA).

We calculated ¹³C atom % excess in PLFA markers by subtraction of unlabeled (T₀) PLFA markers. We use the term unlabeled PLFA C to refer to the natural abundance ¹³C-PLFA of native PLFA C in soil. Twenty-six PLFA markers had an abundance threshold of greater than 0.4 mol% and were included in community analysis. Fifteen microbial PLFA biomarkers were assigned to 6 microbial categories: Gram (+) bacteria (15:0i, 15:0a, 16:0i, 17:0i, 17:0a), Gram (-) bacteria (16:1ω7, 18:1ω7), fungi (18:2ω6,9, 18:1ω9), actinobacteria (16:010Me, 17:010Me, 18:010Me), protozoa (20:4ω6,9), and cyclopropyl bacterial (17:0cyc, 19:0cyc), according to Zelles (1999) and Zogg et al., (1997). Unassigned PLFAs (14:0, 15:0, 16:1ω5, 16:1ω9, 16:0, 16:012ME, 17:0, 18:0, 20:1ω9) were included in total PLFA yields and all community analyses procedures. PLFA-defined fungal:bacteria ratios were calculated as fungal group PLFA yield divided by the sum of Gram (+) bacteria, Gram (-) bacteria, and cyclopropyl bacterial PLFA groups (Zelles 1999). PLFA nomenclature used consists of the total number of C atoms, the number of double bonds, followed by the position of double bonds and branching (iso or ante) as described by Ratledge & Wilkinson (1988). All unsaturated fatty acids are in the cis conformation.

2.5.3 Soil inorganic N
Total inorganic N (NH₄, NO₃) was determined on soil subsampled prior to incubation, and 42 and 180 d after the start of the lab incubation. Soil subsamples were extracted with 0.1M K₂SO₄ using a
5:1 extractant: dry soil ratio. Inorganic N (NH₄ + NO₃) was quantified colorimetrically on a GENESYS 10 UV spectrophotometer (Thermo Scientific, Madison, WI). The concentration of N in samples was measured using the Berthelot reaction for NH₄⁺ (Forster 1995), and the vanadium (III) chloride reduction method for NO₃⁻ (Doane & Horwath 2003). Inorganic N in soil extracts were diffused onto GF/A filters (Stark and Hart, 1996), and ¹⁵N enrichment was determined using PDZ Europa 20-20 IRMS (Sercon Ltd., Cheshire, UK).

2.6. Data analysis

To improve homogeneity of variance and normality, most isotope data required transformation using log₁₀-transformed data except PLFA C mole%, which was performed on data transformed by arcsine square root. Community analysis of PLFA data was performed by Non-metric Multidimensional Scaling (NMS) using PC-ORD v.5.31 (MjM Software, Glendenen Beach, OR, USA). The distance measure for NMS was Sorensen (Bray - Curtis). Comparisons between treatments for evolved ¹³CO₂, inorganic ¹⁵N, and individual and total PLFA yields were performed using repeated measures and individual ANOVA procedures for specific sampling dates using Systat v.10 (Systat Software, Inc., Chicago, IL, USA). We used a P < 0.05 as the a priori error for significance between means. Individual comparisons among means were performed using Tukey’s pairwise comparison test.

3. Results

3.1. C mineralization from PyOM and wood

After the 180-day incubation, total loss of PyOM as ¹³CO₂ excess (hereafter referred to as PyOM-C) was 68 times lower (0.39±0.02% of applied) than for its precursor wood-C (26.4±1.8% of applied) averaged across soils (Table 3). After 180 days, an average of 99.6 and 73.3% of the added PyOM-C
and wood-C, respectively, remained in the two soils. While the total loss of PyOM-C was unaffected by soil type, loss of wood-C was affected by soil type, with 7.3% greater loss of wood-C in GR soils than in AN soils after 180 days ($P<0.05$; Table 3). PyOM and wood C mineralization rates during the 180-d incubation are shown in the Supplementary Data section (Fig. S1).

For GR soils, sterile treatments (i.e. soil with or without PyOM) produced significantly more CO$_2$ than experimental controls (i.e. without soil or PyOM) on 4 of the 10 sampling dates ($P < 0.05$). AN soils, both with and without added PyOM, produced significantly more CO$_2$ than experimental controls on 6 of the 10 sampling dates ($P < 0.05$). However, the CO$_2$ evolved from all sterilized GR and AN treatments (i.e. soil with and without added PyOM) averaged less than 1.9% of their respective non-sterile GR and AN treatments during the incubation. Consequently, we were unable to reliably measure $^{13}$CO$_2$ isotope enrichment on these very low CO$_2$ concentrations to determine if this C included any PyOM-C. Addition of PyOM in sterile treatments did not affect the amount of CO$_2$ evolved (data not shown).

PyOM and wood mineralization rates were modeled with two kinetically-defined C pools: labile and resistant. As expected, the modeled labile ($C_{TL}$) and resistant ($C_{TR}$) pools of PyOM-C were smaller than those of wood-C by one and two orders of magnitude, respectively (Table 3). While the labile pool ($C_{TL}$) of wood-C was larger in GR than in AN soils ($P < 0.05$), the resistant pool ($C_{TR}$) was not affected by soil type. On average, the resistant C pool of PyOM and wood had a centennial and decadal scale MRT [$MRT_R$, Eqn. (6)], respectively (Table 3).

### 3.2. N mineralization from PyOM, wood, and soil
Net mineralization of PyOM-\(^{15}\)N excess (hereafter referred to as PyOM-N) measured on day 180 was approximately 20 times slower \((P < 0.05, \text{Fig. 1})\) than from wood-\(^{15}\)N excess (hereafter referred to as wood-N). Mineralization of PyOM- and wood-N in GR soils was two and four times larger than in AN soils, respectively \((P < 0.05)\). Native soil N mineralized approximately twice as fast in GR soils than in AN soils \((P < 0.05; \text{Fig. 1, Table 4})\).

### 3.3. C mineralization from native soil

Native soil C mineralized approximately twice as fast in GR soils than in AN soils \((P < 0.05; \text{Table 4})\). The labile \((C_{TL})\) and resistant \((C_{TR})\) native soil C pools had an estimated MRT of days and decades, respectively (Table 4). The MRT of the resistant soil C pool in AN soils was twice as large as in GR soils \((P < 0.05)\). The addition of wood or PyOM did not stimulate mineralization of native C from either GR or AN soils. CO\(_2\) mineralization rates from native SOM for each soil, with and without added wood or PyOM, are shown for the 180-d study in the Supplementary Data section (Fig. S2).

### 3.4. C and N mineralization responses to N additions

The addition of N fertilizer to GR soils (Experiment II) did not affect the mineralization rates of C and N from pine materials or native soil (data not shown).

### 3.5. Microbial biomass and community composition

Soil microbial community compositions as defined by soil PLFA C biomarkers were generally similar throughout the incubation for both AN and GR soils (Fig. 2). Unlabeled PLFA C (expressed
as mol%) followed the order gram (+) bacteria > gram (-) bacteria > fungi > actinobacteria in both soils throughout the incubation. On day 42, control (unamended) GR soils had a higher proportion of gram (-) bacteria PLFAs (15.3±0.6 mol%) than did control AN soils (12.6±0.2 mol%; n=4, P < 0.01). Also on day 42, control GR soils had a lower proportion of actinobacteria PLFAs (5.6±0.6 mol%) than did control AN soils (6.5±0.5 mol%; n=4, P<0.05).

Microbial community structure shifted between day 42 and the end (180 days) of the incubation in similar ways for AN and GR soils (Fig. 2). Specifically, cyclopropyl PLFAs increased from 8.5±0.6 to 12.0±0.9 mol%, while fungal PLFAs declined from 8.9±0.3 to 6.4±0.3 mol% averaged across soil type (n=8, P < 0.05). In addition, gram (-) bacterial PLFAs declined from 13.9±0.6 mol% on 42 d to 10.7±0.5 mol% after 180 days (averaged across control soils, n=8, P<0.01). Addition of PyOM had no effect on community composition during the incubation period in either soil (Fig. 2), whereas after 180 days, soils with added wood had greater fungal PLFA C (11.0±0.5 mol%) than for added PyOM (9.2±0.3 mol%) or for control soils (9.2±0.3 mol%) averaged across soil type (n=8; P < 0.05).

Total mass of unlabeled PLFA C was similar in AN and GR soils and averaged 4.0±0.4 µg PLFA C g⁻¹ soil after 42 days and 3.3±0.4 µg PLFA C g⁻¹ soil after 180 days of incubation. Total mass of unlabeled PLFA C was unaffected by PyOM or wood addition in either soil (Fig. 3). PLFA C was also unaffected by sampling time during the incubation period for AN soils with or without PyOM or wood amendment. Total PLFA C in control GR soils declined from 42 to 180 days when expressed on a soil C basis (Fig. 3; P < 0.001), while biomass in GR soils with PyOM or wood added was unaffected by sampling time.
3.6. Microbial utilization of PyOM and precursor wood

PyOM- and wood-C (as $^{13}$C- excess) was recovered as PLFA C after 42 and 180 days, indicating the uptake of C from these plant inputs by bacteria and fungal microbial groups (Fig. 4). In GR soils, total PyOM-C in PLFA biomarkers was significantly different from zero only at day 180. Wood-C in PLFA biomarkers did not vary with soil type or sample date (Fig.4).

Uptake ($^{13}$C distribution to PLFAs) and turnover dynamics of $^{13}$C-excess from enriched PyOM and wood varied significantly among PLFA biomarker groups and had a different pattern than the unlabeled PLFA C after 180 days of incubation (Fig. 5). PyOM-C was present in all PLFAs after 180 days, but was low to absent after only 42 days, in both soils (data not shown). In contrast, wood-C was recovered in all 24 PLFAs at day 42 and 180. In AN soil after 180 days, fungal PLFA C (as mol%) from wood additions exceeded that from PyOM and for native PLFA C in control soils (Fig 5; $P < 0.05$). Also at day 42, uptake of wood-derived C into PLFA was higher (16.2±0.7 mol%) than that of native C (8.9±0.3 mol%) averaged across soil type (n=8, $P<0.01$). In AN soil, movement of C into actinobacterial PLFAs was lower for PyOM and wood compared with unamended controls after 180 days (Fig. 5; $P < 0.05$). In GR soil after 180 days, gram (+) bacteria PLFA-$^{13}$C excess mol% from PyOM was greater than for wood and unamended control soils ($P < 0.05$). Consequently, after 180 days the PLFA C fungal - to bacterial - ratio for $^{13}$C excess from wood C (0.161±0.0004) exceeded that from PyOM C (0.141±0.003) averaged across soil type (n=8, $P < 0.01$). Distribution of wood-derived C in PLFAs increased significantly (<5 mol%) from 42 to 180 days for actinobacteria and cyclopropyl biomarkers and decreased for fungal biomarkers, and 16:0 in both soils (data not shown).
4. Discussion

4.1 PyOM-C turnover

The centennial scale MRTs calculated in this study for PyOM-C are consistent with recently reported results for an in situ study by Hammes et al., (2008) using a PyOM biomarker approach (i.e. BPCAs) and in the laboratory using $^{14}$C tracer by Kuzyakov et al., (2009). While centennial scale turnover of PyOM-C is faster than many models have assumed, it was also 32 times slower than we observed for the precursor wood in soil. Our mineralization rate constants for pine wood C are consistent with those estimated for woody debris via a mass loss approach (Weedon et al., 2009; Yoon et al., 2011).

Our results showed PyOM-N mineralization rates that were markedly slower compared with its precursor wood-N, illustrating the important role that chemical alterations during charring play in controlling PyOM-N availability. Most N in our PyOM was identified as pyrrole/amide-type N according to $^{15}$N solid state NMR analyses (data not shown), which is consistent with other recent results (Knicker 2010; Hilscher & Knicker 2011b). In a recent incubation study using $^{13}$C and $^{15}$N-enriched PyOM, Hilscher & Knicker (2011a) hypothesized that the observed N loss from PyOM was attributed to mineralization. We extend these findings by providing direct evidence for significant PyOM-N mineralization and the influence of soil type. PyOM additions did not affect the amount of unlabeled extractable inorganic N in either soil, while wood additions resulted in a 139 day-long period of net N immobilization in both soils (data not shown). The characterization of PyOM N functional groups, together with our reported N mineralization rates from PyOM-N and its precursor wood-N, suggest that the thermal alteration of pine wood increased the proportion of decomposition-resistant PyOM-N, and consequently suppressed N mineralization rates of PyOM compared with its wood precursor.
The slow mineralization rates of PyOM-C relative to its precursor wood-C are in agreement with laboratory studies that showed a sharp decrease in the mineralization rates for PyOM made under similar charring conditions (Baldock & Smernik 2002; Zimmerman 2010). We attribute the differences in C mineralization rates between PyOM and wood to the physicochemical and structural effects of charring, which resulted in a decrease in H:C and O:C elemental ratios, indicating the formation of more condensed structures in PyOM (Baldock & Smernik 2002; Hammes et al., 2006; Schneider et al., 2010). This is consistent with the dominance of the aromatic/alkene-C structures observed for the PyOM investigated here using $^{13}$C solid state nuclear magnetic resonance NMR analyses (data not shown). The physico-chemical and structural characteristics of the PyOM investigated here suggest that it is consistent with the ‘amorphous char’ category proposed for PyOM materials produced from temperatures in the range of 300 - 500°C (Keiluweit et al., 2010). As higher heating temperatures have been shown to reduce PyOM degradation rates (Baldock & Smernik 2002), the broad structural categories proposed by Keiluweit et al., (2010) may be a useful conceptual model for predicting typical PyOM MRTs in soils. We found centennial scale MRT for PyOM in the amorphous char category.

### 4.2 Microbial utilization of PyOM-C

Fungi utilized significantly less PyOM-C than its precursor pine wood-C or unlabeled soil C. Fungi have been shown to be the primary microbial group utilizing C from wood during decomposition in soil (Cornwell et al., 2009). Only a few studies have examined the microbial utilization of PyOM in soil. Fungi dominated the uptake of C from PyOM in a study of arable and forest soils (Steinbess et al., 2009). However, the PyOM materials examined by Steinbess et al., (2009) were yeast- and glucose-derived materials heated to 850°C. Thus, microbial utilization may have been affected by
both the starting material and its combustion temperature. Most PyOM produced in forest fires is considered to be made at charring temperatures under 500°C and under relatively low O₂ conditions (Turney et al., 2006; Preston & Schmidt 2006).

Gram (+) bacteria, the largest PLFA-defined microbial group present in both soils, utilized both PyOM-C and wood-C. In the AN soil, gram (+) bacteria utilized PyOM-C preferentially to wood-C and native SOM. Gram (+) bacteria are known to be significant consumers of aromatic and slowly decomposing soil C (Fierer et al., 2003; Kramer & Gleixner 2008; Bird et al., 2011), such as lignin and aromatic/alkenes-C that are found in high concentrations in wood and PyOM (Chapman & Koch 2007). Gram (+) bacteria have been shown to increase in proportional abundance with increasing soil depth (Fierer et al., 2003), where refractory SOM is common (Rumpel & Kögel-Knabner 2011). Based on our findings, Gram (+) bacteria found in subsoil horizons appear to be well suited to utilize PyOM-C.

Unlike Gram (+) bacterial PLFA biomarkers, actinobacteria showed low PyOM- and wood-C uptake, suggesting that actinobacteria might have a low preference for these soil inputs. Indeed, actinobacteria have been shown to have low preference for fresh plant C inputs, increasing their C utilization during the latter stages of decomposition in soil (Bird et al., 2011).

The microbial utilization of PyOM-C, along with the results from our sterile controls, suggests that the turnover of PyOM in these soils was primarily biologically mediated. Our laboratory conditions were performed in the dark and thereby did not assess photochemical oxidation (Austin & Vivanco 2006). Sterile treatments consistently produced a small amount of mineralized C, which was too
small to determine the possible contribution of PyOM-C. It is likely the sterile treatments were recolonized during the 180-d study during gas samplings. If there were abiotic decomposition processes occurring for the PyOM, the products may have included volatile compounds, as suggested by Zimmerman (2010).

In both AN and GR soil, the microbial community structure shifted by the end of the experiment, with an increase in cyclopropyl PLFA biomarkers and a decrease in Gram (-) bacterial biomarkers. These results suggest that the soil microorganisms may have been energy-limited during the latter part of the incubation. As PyOM turnover has been stimulated by glucose additions (Hamer et al., 2004; Kuzyakov et al., 2009), this energy limitation may have led to lower decomposition rates than if fresh plant inputs had been added to PyOM throughout the incubation period.

4.3 Soil type effects on PyOM, wood and native SOM mineralization

Carbon and N mineralization rates from wood and SOM were significantly reduced in the AN soil compared with the GR soil. The effect of soil type on mineralization rates was observed throughout the incubation period for wood, PyOM, and native SOM. Although total PyOM-C mineralization did not differ between soils, AN soil retained significantly more PyOM-N than GR soil. The soils investigated here were formed in the same forest site with similar soil forming factors except for parent material. Both GR and AN soils had similar microbial community structures and biomass on a per gram of dry soil basis. However, GR soil had much greater biomass per unit soil C, suggesting that a good portion of soil C in AN soils was inaccessible to microbes. These soils were also previously studied by Rasmussen et al., (2005), who reported higher Al-humus complexes and Fe-oxyhydroxide contents in AN compared with GR soils. In our study, AN soils had significantly
more Al-humus complexes, SRO Al-oxyhydroxide minerals, and silt than GR soils. The protective effects of noncrystalline and SRO minerals on soil C and N mineralization have been extensively reported in recent years (Torn et al., 1997; Rasmussen et al., 2008; Parfitt, 2009; Mikutta et al., 2010). Soil C mineralization rates have been shown to be negatively correlated with Fe-oxyhydroxides, Al-oxyhydroxides, and Al-humus complex content, suggesting mineral control of C mineralization (Rasmussen et al., 2006). We posit that the higher levels of SRO minerals and greater silt content in AN soils may have contributed to the enhanced stabilization of PyOM, wood, and SOM compared with GR soils by protecting C and N from microbial degradation throughout the 180-d study.

4.4. Priming effect from PyOM

Soil C turnover rates were unaffected by the addition of PyOM or wood to both soils. The isotope technique employed in our study allowed us to evaluate SOM priming by directly quantifying C mineralized (as CO₂) from the highly enriched ¹³C-labeled PyOM and wood, and unlabeled SOM separately. These findings are in accordance with several recent studies examining a range of PyOM materials in soils (Kuzyakov et al., 2009; Hilscher et al., 2009; Abiven & Andreoli 2010; Cross & Sohi 2011). In contrast, our results are not in agreement with some of the results presented by Zimmerman (2011) and Wardle et al., (2008), who both reported positive priming of SOM from added PyOM. The study by Wardle et al (2008) may have differed as they investigated priming effect from PyOM in a surface organic layer, whereas we used mineral soil from a subsurface horizon.
4.5. N addition effects

N addition had no effect on the MRT of PyOM-C or wood-C in GR soils. Numerous studies, however, have found that N additions suppress the decomposition of lignin-rich plant litter, such as pine wood (Knorr et al., 2005; Janssens et al., 2010). Given that the MRT of native soil C was also unaffected by N additions, we hypothesize that the microbial groups investigated in this study were either limited by an element other than N, such as phosphorous (Kunito et al., 2012), or the amount of N addition may have been insufficient to cause a measurable change in C mineralization. In addition, the presence of vermiculite in the GR soil may have immobilized some of the added ammonium N.

5. Conclusions

We demonstrated that pine-derived PyOM-C, charred at 450°C, had a centennial scale MRT in soils, and found that PyOM-N mineralization rates were significant and affected by soil type. These findings suggest that PyOM in soils is not inert as described in most ecosystem-scale soil C models. Moreover, it is evident from our study that PyOM-C mineralization was primarily biologically mediated, and that PyOM-C is accessible to all major groups of heterotrophic microorganisms. We found that Gram (+) bacteria – not soil fungi – were the primary utilizers of PyOM materials produced at temperatures between 400°C and 500°C in these Bt horizon soils. Soil derived from andesite parent material retained more N from PyOM, and more C and N from wood and native SOM, than did soil derived from granite. Taken together, our results provide supporting evidence that C and N pathways from PyOM are biologically mediated and may be more dynamic in soil than previously thought.
Acknowledgments

We thank K. Hammes and M. W. Schmidt for charring the pine wood. We appreciate the contributions by C. Castanha, R. Porras, E. Bisbee, and A. Liu to this research. This research was funded through a PSC-CUNY Research Award and from funds provided by The Office of Science, Office of Biological and Environmental Research, Climate and Environmental Science Division, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231 to Lawrence Berkeley National Laboratory. We appreciate the assistance of the research staff at UC Blodgett Forest Research Station and J. Matthews and C. Yarns at the UC Davis Stable Isotope Facility.
Table 1. Physical and chemical properties of granitic (GR) or andesitic (AN) derived Bt horizon soil used in the incubation.
Means and standard errors are shown (n=4).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Depth</th>
<th>pH</th>
<th>CEC</th>
<th>Al^4⁺</th>
<th>Fe^4⁺</th>
<th>Al₅⁻</th>
<th>Al₆⁺</th>
<th>Fe₅⁺</th>
<th>Al₆⁻ - Al₅⁺</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>C⁺</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR</td>
<td>26-50 cm</td>
<td>5.8 (0.1)</td>
<td>22.6 (5.2)</td>
<td>47.3 (1.3)</td>
<td>28.0 (1.2)</td>
<td>4.2 (1.2)</td>
<td>1.1 (0.2)</td>
<td>2.0 (0.7)</td>
<td>3.0 (1.2)</td>
<td>598 (27)</td>
<td>258 (8)</td>
<td>144 (23)</td>
<td>7.3 (0.7)</td>
<td>19.9 (0.7)</td>
</tr>
<tr>
<td>AN</td>
<td>33-51 cm</td>
<td>5.6 (0.1)</td>
<td>16.4 (4.9)</td>
<td>95.9 (8.0)</td>
<td>51.1 (2.8)</td>
<td>13.4 (1.8)</td>
<td>2.6 (0.4)</td>
<td>5.6 (1.5)</td>
<td>10.8 (1.6)</td>
<td>329 (14)</td>
<td>482 (34)</td>
<td>189 (33)</td>
<td>15.8 (2.4)</td>
<td>20.2 (0.4)</td>
</tr>
</tbody>
</table>

1 Average Bt horizon depth; 2 Saturated paste; 3 Ammonium chloride extraction; 4 Nitric acid digestion- extractable Al and Fe; 5 Ammonium oxalate- extractable Al and Fe; 6 Sodium pyrophosphate-extractable Al and Fe; 7 Inorganic Al. * Denotes properties that are significantly different (P<0.05) between the two soil types (GR, AN).
Table 1. 2 Isotopic and elemental composition of ponderosa pine-derived PyOM and its precursor wood.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>N</th>
<th>H</th>
<th>O</th>
<th>Mass ratio</th>
<th>Atomic ratio</th>
<th>13C</th>
<th>15N</th>
<th>18O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td>C/N</td>
<td>H/C</td>
<td>O/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PyOM</td>
<td>779</td>
<td>7.1</td>
<td>34.3</td>
<td>144</td>
<td>110</td>
<td>0.5</td>
<td>0.1</td>
<td>2.03</td>
<td>4.2</td>
</tr>
<tr>
<td>Wood</td>
<td>499</td>
<td>4.3</td>
<td>66.2</td>
<td>411</td>
<td>115</td>
<td>1.6</td>
<td>0.6</td>
<td>2.05</td>
<td>4.3</td>
</tr>
</tbody>
</table>

¹VSMOW, Vienna Standard Mean Ocean Water
Table 1. Total \(^{13}\)C excess respired as \(^{13}\)C-CO\(_2\) and \(^{13}\)C excess mineralization rate model parameters for PyOM and wood incubated for 180 days in AN and GR soils. Rate mineralization parameters were derived using two pool (labile and resistant) first-order decay equation (Eqn.3). Means and standard errors are shown (n=4).

<table>
<thead>
<tr>
<th>Litter</th>
<th>Soil</th>
<th>Total (^{13})C excess respired</th>
<th>(C_{TL})</th>
<th>(C_{TR})</th>
<th>(C_{TL}+C_{TR})</th>
<th>(k_L)</th>
<th>(k_R)</th>
<th>MRT(_L)</th>
<th>MRT(_R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PyOM</td>
<td>AN</td>
<td>0.37 (0.03)</td>
<td>1.1</td>
<td>3.1</td>
<td>4.3</td>
<td>3.9x10(^{-3})</td>
<td>9.2x10(^{-3})</td>
<td>29 (5)</td>
<td>605 (93)</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>0.41 (0.03)</td>
<td>1.5</td>
<td>4.2</td>
<td>5.7</td>
<td>5.6x10(^{-2})</td>
<td>2.1x10(^{-5})</td>
<td>21 (5)</td>
<td>389 (130)</td>
</tr>
<tr>
<td>Wood</td>
<td>AN</td>
<td>22.8 (1.7)</td>
<td>39.8</td>
<td>235</td>
<td>275</td>
<td>2.2x10(^{-3})</td>
<td>1.2x10(^{-4})</td>
<td>46 (2)</td>
<td>11 (4)</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>30.1 (1.8)</td>
<td>62.5</td>
<td>232</td>
<td>295</td>
<td>3.3x10(^{-3})</td>
<td>1.4x10(^{-5})</td>
<td>31 (2)</td>
<td>16 (1)</td>
</tr>
</tbody>
</table>

\(C_{TL}\) and \(C_{TR}\): size of labile and resistant C pools (Eqn.4), respectively; \(k_L\) and \(k_R\): decomposition rate constants for \(^{13}\)C remaining in labile and resistant pools (Eqn.5), respectively; MRT\(_L\) and MRT\(_R\): mean residence time for labile and resistant C pools (Eqn.6), respectively.
Table 1. 4 Unlabeled soil C mineralization rate model parameters for each soil (GR, AN) and woody debris amendment (i.e., non-amended, and amended with PyOM or wood), and total native soil C and N mineralized after 180 days of incubation. Rate mineralization parameters were derived using two pool (labile and resistant) first-order decay equation (Eqn.1). Means and standard errors are shown (n=4).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Amendment</th>
<th>$C_{TL}$</th>
<th>$C_{TR}$</th>
<th>$C_{TL} + C_{TR}$</th>
<th>$k_L$</th>
<th>$k_R$</th>
<th>MRT$_L$</th>
<th>MRT$_R$</th>
<th>Total soil C mineralized</th>
<th>Net soil N mineralized</th>
<th>% of initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>Non-amended</td>
<td>7.8(2.0)</td>
<td>59.3(9.2)</td>
<td>67.1(10.2)</td>
<td>2.5x10^{-2}(4.3x10^{-5})</td>
<td>1.1x10^{-4}(3.4x10^{-5})</td>
<td>40 (7)</td>
<td>27 (8)</td>
<td>4.0(0.5)</td>
<td>2.3(0.4)</td>
<td></td>
</tr>
<tr>
<td>GR</td>
<td></td>
<td>17.3(2.4)</td>
<td>106.1(7.4)</td>
<td>123.4 (9.2)</td>
<td>3.7x10^{-2}(1.1x10^{-5})</td>
<td>2.0x10^{-4}(2.3x10^{-5})</td>
<td>29(10)</td>
<td>14 (1)</td>
<td>6.6(0.3)</td>
<td>5.3(0.5)</td>
<td></td>
</tr>
<tr>
<td>AN</td>
<td>PyOM-$^{13}$C excess</td>
<td>4.9(0.7)</td>
<td>43.9(4.0)</td>
<td>48.8 (3.6)</td>
<td>2.1x10^{-5}(5.4x10^{-5})</td>
<td>5.7x10^{-5}(1.6x10^{-5})</td>
<td>49(12)</td>
<td>51(14)</td>
<td>3.6(0.5)</td>
<td>1.9(0.4)</td>
<td></td>
</tr>
<tr>
<td>GR</td>
<td>PyOM-$^{13}$C excess</td>
<td>13.2(1.0)</td>
<td>98.5(12.5)</td>
<td>111.7(12.7)</td>
<td>2.9x10^{-2}(9.4x10^{-5})</td>
<td>1.9x10^{-4}(3.5x10^{-5})</td>
<td>37(11)</td>
<td>15 (3)</td>
<td>6.6(0.3)</td>
<td>5.7(0.9)</td>
<td></td>
</tr>
<tr>
<td>AN</td>
<td>Wood-$^{13}$C excess</td>
<td>6.4(1.2)</td>
<td>57.9(3.3)</td>
<td>64.3(3.9)</td>
<td>2.3x10^{-2}(5.3x10^{-5})</td>
<td>9.3x10^{-5}(2.3x10^{-5})</td>
<td>45(10)</td>
<td>31 (7)</td>
<td>4.2(0.4)</td>
<td>0.8(0.3)</td>
<td></td>
</tr>
<tr>
<td>GR</td>
<td>Wood-$^{13}$C excess</td>
<td>9.1(0.7)</td>
<td>82.5(9.4)</td>
<td>91.6(9.7)</td>
<td>2.0x10^{-2}(1.8x10^{-5})</td>
<td>1.1x10^{-4}(4.8x10^{-5})</td>
<td>52 (5)</td>
<td>30(16)</td>
<td>7.0(0.4)</td>
<td>3.4(0.5)</td>
<td></td>
</tr>
</tbody>
</table>

$C_{TL}$ and $C_{TR}$: size of labile and resistant C pools (Eqn.4), respectively; $k_L$ and $k_R$: decomposition rate constants for % C remaining in labile and resistant pools (Eqn.5), respectively; MRT$_L$ and MRT$_R$: mean residence time for labile and resistant C pools (Eqn.6), respectively.
Figure 1. Proportion of total net $^{15}$N mineralized (as NH$_4$NO$_3$) from PyOM- and wood-$^{15}$N excess, and unlabelled soil nitrogen in AN and GR soils during 180 days of incubation. Net $^{15}$N mineralized data is expressed as the percent recovered of added for PyOM and wood, and percent of initial for native SOM. Significant differences ($P < 0.05$) between GR and AN are indicated with “*” for each pine material, and for native soil. Error bars shown are standard errors (n=4).
Figure 1. The non-metric multidimensional analysis of PLFA-defined microbial community structure changes based on unlabeled PLFA C (mole%) in AN (triangles) and GR soils (circles), with added PyOM (black symbols), wood (grey symbols) or unamended controls (white symbols). The first and second axes using non-metric multidimensional scaling (NMS) are shown. Soils were sampled on incubation day 42 (solid symbols) and day 180 (”+” inside symbol). Twenty-four PLFAs, represented as unlabeled, native PLFA C (mole%), were used in this analysis. Values shown are means ± standard errors (n=4).
Figure 1.3 Unlabeled soil microbial PLFA C during pre-incubation conditions, day 42 and day 180 of the incubation, for PyOM additions (black symbols), wood additions (grey symbols), and no addition control soils (white symbols), and for AN (triangles) and GR (circles) soils. Values shown are means ± standard errors (n=4).
Figure 1. Total soil microbial PLFA-\textsuperscript{13}C excess per mg of added \textsuperscript{13}C recovered from PyOM additions (black bars) and wood additions (grey bars) in AN (shaded) and GR (unshaded) soils. Values shown are means ± standard errors (n=4).
Figure 1. $^{13}$C excess mole% of select PLFA biomarkers for AN (top) and GR soils (top), with added PyOM (black bars), wood (grey bars) or unamended controls (white bars) after 180 d of incubation. Values shown are means ± standard errors (n=4). Significant differences between planted and unplanted treatments are indicated with “*” for each microbial group ($P<0.05$).
Supplementary Data

Figure S 1. Mineralization rates of $^{13}$C excess evolved (as CO$_2$) from PyOM (top) and wood (bottom) added to GR (circles) and AN (triangles) during the 180 incubation. Values shown are means ± standard errors (n=4).
Figure S 2. Mineralization rates of unlabelled native soil C evolved (as CO$_2$) from GR (circles) and AN (triangles) amended with PyOM (black), wood (gray) or unamended (white) during the 180-d incubation. Values shown are means ± standard errors (n=4).
6. References


Appendices

Appendix A 1 Pearson correlation coefficients (r) of the relationships among SRO minerals and soil C and N pools and fluxes during the 180 days of incubation with added PyOM, wood or no addition (controls). Coefficient values shown in bold indicate $P < 0.05$ (n=8).

<table>
<thead>
<tr>
<th></th>
<th>$\text{Al}_p$</th>
<th>$\text{Al}_o$</th>
<th>$\text{Al}_o-\text{Al}_p$</th>
<th>$\text{Fe}_o$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total PyOM-C mineralized</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PyOM-C mineralization rates:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 d</td>
<td>-0.31</td>
<td>-0.52</td>
<td>-0.54</td>
<td>-0.53</td>
</tr>
<tr>
<td>34 d</td>
<td>0.74</td>
<td>0.49</td>
<td>0.42</td>
<td>0.18</td>
</tr>
<tr>
<td>160 d</td>
<td>-0.34</td>
<td>-0.39</td>
<td>-0.39</td>
<td>-0.40</td>
</tr>
<tr>
<td><strong>Total Wood-C mineralized</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Wood-C mineralization rates:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 d</td>
<td>-0.33</td>
<td>-0.61</td>
<td>-0.64</td>
<td>-0.76</td>
</tr>
<tr>
<td>34 d</td>
<td>-0.76</td>
<td>-0.81</td>
<td>-0.79</td>
<td>-0.60</td>
</tr>
<tr>
<td>160 d</td>
<td>-0.32</td>
<td>-0.57</td>
<td>-0.60</td>
<td>-0.80</td>
</tr>
<tr>
<td><strong>Total unamended C (control) mineralized</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Soil-C mineralization rates:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 d</td>
<td>-0.60</td>
<td>-0.78</td>
<td>-0.79</td>
<td>-0.75</td>
</tr>
<tr>
<td>34 d</td>
<td>-0.57</td>
<td>-0.73</td>
<td>-0.74</td>
<td>-0.70</td>
</tr>
<tr>
<td>160 d</td>
<td>-0.56</td>
<td>-0.77</td>
<td>-0.79</td>
<td>-0.73</td>
</tr>
<tr>
<td><strong>Net N mineralized:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PyOM-N</td>
<td>-0.57</td>
<td>-0.68</td>
<td>-0.68</td>
<td>-0.72</td>
</tr>
<tr>
<td>Wood-N</td>
<td>-0.69</td>
<td>-0.75</td>
<td>-0.73</td>
<td>-0.66</td>
</tr>
<tr>
<td>Soil-N</td>
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<td>-0.73</td>
<td>-0.64</td>
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<tr>
<td><strong>Initial content in soil:</strong></td>
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<tr>
<td>C</td>
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<td><strong>0.84</strong></td>
<td><strong>0.78</strong></td>
<td>0.26</td>
</tr>
<tr>
<td>N</td>
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<td><strong>0.72</strong></td>
<td>0.65</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Microbial biomass C (μg PLFA C mg(^{-1}) soil C)</strong></td>
<td>-0.78</td>
<td>-0.63</td>
<td>-0.71</td>
<td>-0.78</td>
</tr>
</tbody>
</table>

$\text{Al}_o$ and $\text{Fe}_o$: ammonium oxalate-extractable Al and Fe; $\text{Al}_p$: sodium pyrophosphate-extractable Al; Inorganic Al: $\text{Al}_o-\text{Al}_p$.

Total soil C, N and microbial biomass (PLFA, mole%) are from prior incubation. C and N mineralization rates are from 3 sampling dates during incubation (12, 34 and 160).
Chapter 3: Elucidating the chemical structure of pyrogenic organic matter by combining magnetic resonance, mid-infrared spectroscopy and mass spectrometry


Abstract

Fire-derived organic matter (pyrogenic organic matter, or PyOM), despite its apparent long-term stability in the environment, has recently been reported to degrade faster than previously thought. Current studies have suggested that the composition and structure of PyOM can provide new insights on the mechanisms by which C and N from pyrolyzed biomaterials are stabilized in soils. To better understand the chemical structure of PyOM produced under typical fire conditions in temperate forests, samples of dual-enriched (13C/15N) Pinus ponderosa wood and the charred material produced at 450 °C were analyzed by solid-state nuclear magnetic resonance (ssNMR), electron paramagnetic resonance (EPR), diffuse reflectance Fourier transform infrared (DRIFT) spectroscopy, and both isotopic and elemental composition (C, H, O, and N). Notably, the use of high magnetic field strengths in combination with isotopic enrichment augmented the NMR detection sensitivity, and thus improved the quality of molecular information as compared with previously reported studies of pyrogenic materials. The key molecular groups of pine wood and the corresponding PyOM materials were determined using magic-angle spinning (MAS) 13C, 15N, and 1H NMR. Together with DRIFT and EPR measurements, ssNMR revealed the formation of a free radical-containing
disordered blend of nitrogenous aromatics and heat-resistant aliphatics in the PyOM due to incomplete combustion of the precursor wood. $^{13}$C ssNMR and DRIFT analyses showed the removal of oxygenated aliphatics due to pyrolysis of the precursor wood and the dominant contribution of multiply-bonded and oxygenated aromatic structures in the resulting PyOM. However, the $^{18}$O isotopic analyses indicated selective retention of ligneous moieties during charring at 450°C. $^{15}$N ssNMR studies implied that the nitrogenous species in PyOM corresponded to thermally altered rather than heat-resistant domains of the pine wood precursor. Our molecular characterization suggests that biomaterials pyrolyzed near 450 °C may degrade in soils faster than those pyrolyzed at higher temperatures and may not represent a stable C sink in terrigenous ecosystems.
1. Introduction

Pyrogenic organic matter (PyOM) consists of a heterogeneous class of thermally-altered plant materials and fossil fuel residues, ranging from mildly heated plant biomass to residues produced at progressively higher temperatures of up to 1000 °C (Goldberg, 1985). PyOM comprises a significant fraction (5 to 45%) of the soil organic carbon (C) pool, and represents an important sink for atmospheric CO₂ (Preston and Schmidt, 2006; Lehmann, 2007). However, recent studies have reported that PyOM may degrade at a centennial, not millennial time scale in soils (Hammes et al., 2008; Santos et al., 2012). Recent interest in better understanding the chemical structure, stocks and turnover rate of PyOM has come from the potential of PyOM to improve soil fertility in highly weathered soils (Glaser et al., 2002; Lehmann et al., 2006), and reduce the mobility of pollutants (e.g., heavy metals) in the environment (Koelmans et al., 2006; Nguyen et al., 2007). Furthermore, the amounts of PyOM produced are expected to increase from more frequent wildfires in a future warmer climate (Fried et al., 2004; Running, 2006; Bowman et al., 2009) and the production of biochar from the energy industry as a byproduct of low-temperature biomass pyrolysis (Lehmann, 2007; Brewer et al., 2009).

The dynamics of PyOM in soils are influenced by its physical and chemical structure. In an incubation study, Baldock and Smernik (2002) observed decreasing PyOM degradation rates with an increase in aromatic C and oxygenated aromatic ring structures. Additionally, the role of PyOM as a sorbent for organic contaminants (Oen et al., 2006) has been attributed to its porosity and aromatic structure (Wang and Xing, 2007; Keiluweit and Kleber, 2009). These findings suggest that a better understanding of PyOM molecular structure is essential to predict its turnover rate and sorption capacity in the environment.
The composition and structure of PyOM in the environment can vary widely depending on pyrolysis conditions and initial biomass. For instance, an increase in pyrolysis temperature has been reported to result first in a rapid decline in carbohydrates, then a decrease in lignin and fatty acids accompanied by an increase in aromatic C structures, followed by a condensation of these aromatic moieties (Nishimiya et al., 1998; Baldock and Smernik, 2002; McBeath and Smernik, 2009; Keiluweit et al., 2010). Supported by evidence from elemental analysis and $^{13}$C nuclear magnetic resonance (NMR), Knicker et al. (2008) proposed a model in which PyOM consists of a highly heterogeneous structure with N, O, and S substituents. In addition, PyOM produced under oxic conditions at 350 °C or 450 °C was claimed to consist of small polyaromatic clusters (Knicker, 2010), a finding compatible with the accepted hypothesis that aromatic C in PyOM contains a wide range of structures, including small cross-linked aromatic clusters that are susceptible to further decomposition (Schmidt and Noack, 2000; Preston and Schmidt, 2006). Recently, Keiluweit et al. (2010) proposed four categories for PyOM, each having a unique mixture of physical properties and chemical structures, and possibly, mean residence time (MRT) in soils. Moreover, there has been an increased interest in understanding how PyOM structure and stability in soils are influenced by the presence of thermally altered nitrogen (N), an important fraction of this material (Knicker, 2010; Hilscher and Knicker, 2011b).

The use of an isotope ($^{13}$C and $^{15}$N) enrichment approach, when integrated with high-field solid state NMR (ssNMR) data acquisition, can augment the detection of $^{13}$C and $^{15}$N signals from PyOM functional groups, improving the certainty of associated insights into their molecular structures. In particular, the use of isotopic enrichment provides an NMR spectrum in less time, helps to improve the spectral signal-to-noise ratio and to detect minor chemical species, and avoids excessive line
broadening during data processing that could compromise spectral resolution of the various chemical moieties. The use of higher magnetic field strengths for NMR may also allow better discrimination among chemically similar moieties via enhanced chemical shift dispersion. In addition, spectral editing and multinuclear data collection allow more confident peak assignments to particular structural groupings. Fast magic-angle spinning (MAS) techniques facilitate to directly observe $^1$H NMR in solid state. By augmenting cross-polarization magic-angle spinning (CPMAS) experiments with direct-polarization (DPMAS) measurements, it is possible to estimate the proportions of the molecular constituents quantitatively (Cusack et al., 2012). Finally, electron paramagnetic resonance (EPR) can detect the presence of unpaired electrons that may account in turn for technical challenges in the detection of $^{15}$N NMR spectra signals of the pyrogenic products.

The objective of the current study was to investigate the chemical structure of dual-enriched ($^{13}$C/$^{15}$N) PyOM produced under typical fire conditions ($450 \, ^\circ\text{C}$) using a combination of data from solid state NMR, EPR, diffuse reflectance Fourier transform infrared spectroscopy (DRIFT), and stable isotopic and elemental composition. A companion paper describes findings on the biological degradation of the $^{13}$C- and $^{15}$N- enriched PyOM in soils during a 180-day laboratory experiment (Santos et al., 2012).

2. Materials and methods

2.1 Wood and PyOM samples

Pine saplings were enriched using $^{13}$CO$_2$ and $^{15}$N fertilizer under controlled conditions during their third growth year (Bird and Torn, 2006). PyOM was produced using air-dried enriched pine stem wood cut into 1-2 cm long pieces and heated at 450 °C for 5 hrs under N$_2$ (Hammes et al., 2006).
For comparisons of the mid-infrared properties of the 450 °C *Pinus ponderosa* PyOM with related samples, chestnut (*Castanea sativa*) wood was treated following the same procedure described above (Hammes et al., 2006), but heated at 400 and 500 °C, respectively. Further details about these latter samples are described by Schneider et al. (2010). Cellulose was extracted from three pine wood subsamples using a Jayme–Wise oxidation method described by Leavitt and Danzer (1993) and Panyushkina et al. (2008).

Total organic C and N were measured using a CHN gas analyzer (Costech Model 4010, Valencia, CA, USA). Stable isotope contents (\(^{13}\)C, \(^{15}\)N) were determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope-ratio mass spectrometer (IRMS) (Sercon Ltd., Cheshire, UK). Oxygen elemental concentration and isotopic enrichment were determined using a PYROcube (ELEMENTAR Analysensysteme, Hanau, DE) interfaced to a Sercon 20-20 Isotope Ratio Mass Spectrometer (Sercon Ltd., Cheshire, UK), and H elemental concentration was determined using a high temperature elemental analyzer (Hekatech, Wegberg, DE). Provisional delta values, expressed with respect to Vienna Standard Mean Ocean Water, were corrected based on laboratory standards and international references analyzed with the samples. Elemental and isotopic compositions for pine wood and PyOM, showing high levels of \(^{13}\)C and \(^{15}\)N enrichment, are summarized in Table 1. The \(^{13}\)C enrichment of benzene carboxylic acids extracted from the enriched PyOM averaged 844 δ (Yarnes et al., 2011), similar to the bulk PyOM and its precursor wood, and suggestive of a uniformly distributed isotopic label in both samples.

2.2 Solid state \(^{13}\)C NMR spectroscopic analyses
The compositional comparisons of pine wood with subsequently produced PyOM and quantitative estimates of the observable $^{13}$C nuclear spins were made using ssNMR. Reproducibility of the spectroscopic measurements and spinning sideband identification were verified by obtaining $^{13}$C spectra on each sample at different rotor-spinning frequencies (10.000±0.015, 15.000±0.015 kHz, and 30.000 ±0.010 kHz). $^{13}$C CPMAS experiments were performed to identify the carbon chemical moieties, using cross-polarization times of 1.5-2 ms and a 3-sec recycle time. $^{13}$C DPMAS measurements were carried out for two replicate samples to estimate the relative numbers of each carbon type, using delays between spectral acquisitions of 160 s and 50 s for pine wood and PyOM, respectively (Table 2). The ramped-amplitude $^{13}$C CPMAS measurements were conducted with a $^{13}$C field strength corresponding to 79.6 kHz and a $^1$H field strength that was varied linearly by 10-20% to maintain the Hartmann-Hahn matching condition at a specific spinning frequency such as 30 kHz. High power heteronuclear $^1$H decoupling of 170-185 kHz was achieved by the SPINAL pulse sequence (Fung et al., 2000). A 4-channel Varian DirectDrive spectrometer operating at a $^1$H frequency of 600 MHz ($^{13}$C at 150 MHz) and equipped with a 1.6-mm fastMAS probe optimized for high-sensitivity data acquisition (4-6 mg samples; Agilent Technologies, Santa Clara, CA, USA) was used to perform both CPMAS and DPMAS experiments on solid samples of the pine wood and associated charred materials.

Typical experimental parameters for $^{13}$C MAS measurements were as follows: $^1$H (90°) pulse duration, 1.25 μs; $^{13}$C (90°) pulse duration, 1.25 μs; $^1$H-$^{13}$C cross polarization time, 1.5-2 ms; sweep width, 46 kHz; acquisition time, 25 ms; 1157 data points; number of transients, 20,000-24000 (CP), 500-3000 (DP). All spectra were zero filled to 8192 data points and processed with 50-100 Hz line
broadening; chemical shifts were referenced externally to the methylene (-CH₂-) group of adamantane (Sigma-Aldrich, St. Louis, MO, USA) at 38.4 ppm (Morcombe and Zilm, 2003).

CPMAS $^{13}$C measurements with a 10-kHz spinning rate and interrupted proton decoupling for periods of 10-40 µs prior to signal acquisition were carried out at 150 MHz to suppress signals from carbons relaxed efficiently by attached hydrogens and/or nearby unpaired electrons, while retaining nonprotonated and mobile carbon moieties (Opella and Frey, 1979). The proportion of NMR-observable carbon moieties in the wood samples was estimated using DPMAS measurements and the methodology of Smernik and Oades (2000a, b). For these $^{13}$C spin-counting measurements, the DPMAS $^{13}$C spectra at both 15 kHz and 30 kHz spinning rates were obtained by averaging 450-500 transients and referencing the intensity to an external alanine standard (Sigma-Aldrich #05129, St. Louis, MO) set to 100% spectroscopically observable carbon spins ($C_{\text{obs}}$). The total signal intensity for each sample was determined by integrating the DPMAS spectral region between -10 and +220 ppm, including any spinning sidebands (if any).

2.3 Solid state $^{15}$N and $^1$H NMR spectroscopic analyses

A 4 channel Bruker Avance II spectrometer operating at a $^1$H frequency of 750 MHz ($^{13}$C at 189 MHz) and equipped with a 4 mm HXY probe (~25 mg samples; Bruker BioSpin, Karlsruhe, Germany) was used for conducting ramped-amplitude CPMAS $^{15}$N experiments at 75 MHz to establish nitrogen chemical moieties present in the pine wood and the corresponding charred samples. The $^{15}$N CPMAS measurements were carried out with a cross-polarization of 0.300 ms, a 15-kHz spinning speed, a 1-sec recycle delay after acquisition, and a 20-50% linear ramp of the $^1$H field strength during cross polarization; heteronuclear $^1$H decoupling was achieved by the TPPM
(Bennett et al., 1995) method. In addition, $^{15}$N CPMAS measurements of the pine wood were carried out with 1- and 3-ms cross-polarization times. Typical experimental parameters on the Bruker spectrometer were as follows: $^1$H (90°) pulse duration, 3 µs; $^{15}$N (90°) pulse duration, 6 µs; $^1$H-$^{15}$N cross polarization time, 0.3-3.0 ms; sweep width, 100 kHz; acquisition time, 10ms; 2048 data points; number of transients, 85,000-170,000. Reproducibility of the spectroscopic measurements was assessed by obtaining replicate $^{15}$N spectra on each sample at 60 MHz on the Varian spectrometer (data not shown). Prior to Fourier transformation, the time-domain NMR signals were apodized using an exponential line broadening between 250 and 500 Hz; chemical shifts were referenced indirectly, using a calculation from gyromagnetic ratios of $^{15}$N and $^{13}$C according to International Union of Pure and Applied Chemistry (IUPAC) recommendations for solid samples (Harris et al., 2001).

Solid state $^1$H MAS spectra were acquired on the 600 MHz Varian spectrometer using rotor spinning speeds between 10 and 35 kHz with a 6-sec recycle delay and referenced indirectly following the same IUPAC guidelines (Harris et al., 2001). Typical experimental parameters for the solid-state $^1$H MAS measurements on the Varian spectrometer were as follows: $^1$H (90°) pulse duration, 1.25 µs; sweep width, 40 kHz; acquisition time, 100 ms; data points, 4096; number of transients, 32.

### 2.4 EPR spectroscopy

To examine the stable population of free electrons in PyOM, EPR spectroscopic measurements were carried out using a Bruker Elexsys e500 spectrometer (Billerica, MA, USA) operating with the following parameters: modulation frequency, 100 kHz; modulation amplitude, 1 G; microwave frequency, 9.469 GHz; microwave power, 1 mW and 0.1 mW. A suspension of PyOM (~2 mg/ml)
was prepared by mixing the solid sample with a pH 7.2 potassium phosphate buffer solution and vortexing the mixture vigorously for 20 min. The resulting suspension was frozen using liquid nitrogen while performing the EPR study at 77 K; the spectroscopic measurements were then repeated at room temperature. The compound 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, St. Louis, MO) was utilized as a standard set at a g value of 2.0031.

2.5 DRIFT spectroscopy

Mid-infrared spectra were acquired by diffuse reflectance infrared Fourier transform spectroscopy (DRIFT). Spectra were recorded using a Bruker TENSOR 27 spectrophotometer (Fällanden, Switzerland) from 4000 to 400 cm\(^{-1}\) (average of 16 scans per sample at 4 cm\(^{-1}\) resolution) on a powder containing 6 mg of ground sample and 194 mg of KBr. The samples were homogenized using a fine zirconium - ball mill for 2 min at a frequency of 25 min\(^{-1}\). Prior to measurement, the samples were dried in an oven at 70 °C. Assignments of the infrared absorption bands were based on a literature compilation (Table 3). The DRIFT spectra were measured and compared for three samples: the highly \(^{13}\)C/\(^{15}\)N-enriched PyOM and the two PyOM standards pyrolyzed at 400 °C and 500 °C, respectively, as described above.

3. Results

3.1 Solid state \(^{13}\)C and \(^1\)H NMR, and \(^{18}\)O isotopic signature of enriched PyOM from pine wood

The CPMAS \(^{13}\)C NMR spectra displayed resonances that are diagnostic for a range of molecular groups in the pine wood and corresponding PyOM (Fig. 1). The four major chemical shift regions represent alkyl (0-45 ppm), oxygenated alkyl (45-110 ppm), aryl and alkene (110-160 ppm), and
carbonyl (160-220 ppm) carbon moieties. As compared with previous NMR studies of chestnut, red
gum, and pine wood (Baldock and Smernik, 2002; Smernik et al., 2002; Hammes et al., 2006;
Solum, 2007; McBeath and Smernik, 2009) and noted above, $^{13}$C enrichment yielded high-quality
CPMAS $^{13}$C spectra efficiently and permitted data conditioning that could preserve subtle
differences among molecular structures. The spectral dispersion of chemically similar functional
groups (e.g., CH$_3$O-, two types of CH$_2$O-, two types of CHO) was also improved in this instance by
NMR spectroscopy conducted at high magnetic field strengths. The unaltered and charred pine
wood spectra exhibited significant differences in all chemical shift regions; the relative proportions
of carbonaceous chemical species in each sample were estimated from quantitatively reliable
DPMAS $^{13}$C spectra (Fig. 1) and presented in Table 2. Spin counting measurements of overall $^{13}$C
signal intensity also showed a noticeable reduction in the DP “NMR spectral observability” for the
PyOM compared to the precursor pine wood (Table 2), consistent with previously reported studies of
plant biomass (Baldock and Smernik, 2002; Hammes et al., 2006).

For the pine wood samples examined before charring, oxygenated aliphatics (45-95 ppm) made a
major contribution to both DPMAS and CPMAS $^{13}$C NMR spectra, implicating polysaccharides
(cellulose, hemicelluloses) as the (expected) principal structural components of the initial wood
materials (Kolodziejski et al., 1982; Czimczik et al., 2002; Maunu, 2002). Along with a relatively
weak carboxyl resonance (170-172 ppm), long-chain aliphatics and alkyl groups such as CH$_3$ and
CH$_3$-CH$_2$- resonating at 20-33 ppm and a methoxyl carbon signal at 56 ppm were also present in
both $^{13}$C MAS spectra. Finally, $^{13}$C spectra of the pine wood displayed several broad peaks in the
region 110-160 ppm, but overall a fairly low-intensity aromatic/multiply-bonded region, which is
consistent with the presence of lignin in the pine wood (Czimczik et al., 2002; Solum, 2007).
By contrast, a broad arene/alkene spectral envelope (110-160 ppm, centered at 127 ppm) was the dominant feature in the CPMAS and DPMAS $^{13}$C spectra of the PyOM, indicating that the charring process at 450 °C is associated with preferential removal of the polysaccharides as well as retention and/or formation of multiply bonded moieties. In addition, the PyOM exhibited fairly prominent alkyl signals (15-40 ppm) that could be differentiated from spinning sidebands by running the CPMAS experiments at two different rotor spinning frequencies (15 and 30 kHz). The breadth of the aryl peak could arise from overlapping signals of similar structural moieties and/or proximity to unpaired electrons. These possible explanations are discussed further below.

Unlike the precursor pine wood, the $^{13}$C spectra of the charred product displayed no carboxyl resonances (170-172 ppm). The presence of oxygenated aryl components in the charred product was supported by the observation of a $^{13}$C resonance at 150-160 ppm and confirmed by the invariance of its signal intensity in a series of interrupted decoupling (dipolar dephasing) experiments (Fig. 2). Conversely, a substantial (~30%) decrease in the overall intensity of multiply-bonded and/or aromatic resonances (110-150 ppm) was found in the PyOM dipolar dephasing spectra, attributable to spin relaxation mediated by attached protons and/or unpaired electrons (see below). The alkyl signals between 0 and 45 ppm dephased slowly in these spectral editing experiments, suggesting that they arise from highly mobile long-chain methylene and methyl groups (Sullivan and Maciel, 1982; Wilson et al., 1984; Kögel-Knabner and Hatcher, 1989; Czimczik et al., 2002).

Additional information about the aryl and/or alkene moieties was obtained for the first time from direct 35-kHz MAS observation of the PyOM solid-state $^1$H NMR spectrum, which exhibited two broad resonances at 3.4 ppm and 7.2 ppm (Fig. 3). This intriguing result demonstrates a diminution
of homonuclear dipolar couplings, suggestive of motional averaging in the solid state for protons that are covalently bound to aliphatic and aryl carbons, respectively. For the aryl protons, the narrowed spectral envelope may also indicate low proton density typical of fused aromatic units in the PyOM material.

The $\delta^{13}$C and $^{15}$N enrichments of PyOM were similar to the precursor pine wood (Table 1). In contrast, $\delta^{18}$O (13.2‰) was depleted compared with the precursor pine wood (26.0‰, Table 1) and cellulose extracted from pine wood (29.4 ± 0.2‰, n=3).

3.2 Solid state $^{15}$N NMR of enriched PyOM from pine wood

As with the $^{13}$C studies presented above, $^{15}$N enrichment and high magnetic field strengths facilitated the acquisition of high-quality CPMAS NMR spectra. For the pine wood sample before charring, Fig. 4 shows a $^{15}$N spectrum with a major peak at 120 ppm (attributed to amide or pyrrole moieties; (Gärdenäs et al., 2011) and a possible additional peak at 74 ppm (attributed to secondary amine groups). Whereas spectra for the wood precursor could be obtained with $^1$H-$^{15}$N contact times of 0.3 – 3.0 ms, $^{15}$N NMR signals were observed for the charred material only when a short 0.3-ms cross polarization time was used (Fig. 4). These PyOM data acquisition characteristics suggest rapid $^1$H spin relaxation during cross polarization, possibly due to unpaired electrons near the aromatic constituents of the charred material (Solum et al., 1995). As compared with the pine wood, the PyOM product displayed a broader nitrogen signal that was downfield shifted from 120 ppm, indicating the presence of more electronegative and chemically heterogeneous nitrogen-containing aromatic structures.
3.3 X-band EPR of PyOM from pine wood

The possibility of unpaired electrons in the pyrogenic product (Solum, 2007) was corroborated by the 77 K EPR spectrum shown in Fig. 5, which exhibited a strong symmetric signal with no hyperfine splitting. Observation of a symmetric EPR resonance with a g factor of 2.0023 and peak-to-peak width of ~6.1 G indicated a single chemical environment (or conceivably several averaged states) surrounding the unpaired free electrons within the charred wood (Solum et al., 1995; Solum, 2007).

3.4 Diffuse reflectance infrared fourier transform spectroscopy (DRIFT) of PyOM

DRIFT spectra of the labeled PyOM (Table 3 and Fig. 6, bands 1-10) provided independent structural comparisons of the initial biomass and charred products from different wood samples. Aromatic absorption bands were identified at 3050-3020 (1), 1730-1680 (4), 1430-1380 (7) and 880/805/745 cm⁻¹ (10). Bands 1 and 10 were observed to be less intense for the chestnut PyOM treated at 400 °C compared with 500 °C, whereas intermediate intensities displayed by the labeled pine PyOM indicated charring conditions in between these two temperatures and confirmed the usefulness of IR spectral analysis to verify charring temperature (Baldock and Smernik, 2002).

The bands corresponding to vegetal macromolecules (bands 6 at 1510-1500 cm⁻¹ for lignin, 8 at 1260-1210 cm⁻¹ for cellulose, 2 at 3000-2800 and 9 at 1060-1020 cm⁻¹ for aliphatic compounds) were diminished for PyOM samples as compared with the initial biomass. Whereas the lignin peak disappeared in the pine PyOM, it was still present for the 400 and 500 °C chestnut char. Even though plant structural constituents are largely replaced by aromatic structures during pyrolysis
(Baldock and Smernik, 2002), these IR spectra indicated that some of the initial compounds may still be present at 400-500 °C.

The residual structures are illustrated by aliphatic bands 2 and 3. Interestingly, features observed in both initial wood samples (one broad peak for the pine wood but a series of three peaks for the chestnut wood) were retained in the corresponding PyOM materials. As proposed previously by Wood (1988), such bands could be used to identify the initial wood type (hardwood vs. softwood) in PyOM.

4. Discussion

The strategy employed in this study was to utilize isotopically enriched wood and char materials with an interlocking set of spectroscopic methods to obtain more definitive structural and mechanistic information about the thermal transformation of P. ponderosa wood to PyOM at 450 °C. The discussion below addresses molecular characterization of the initial wood substrate, the materials that are consumed and formed during pyrogenic treatment, and the supramolecular organization of the resulting PyOM.

4.1 A molecular view of PyOM formed from pine wood

As noted above, the cellulose and hemicellulose polysaccharides reported previously in soft wood (Czimczik et al., 2002; Maunu, 2002; Solum, 2007) are evidenced by the predominant CHnO resonances in 13C NMR spectra of the pine wood precursor. Additional significant constituents such as lignin are represented by CH3O, C=O, and diverse aryl groups as reported previously (Kolodziejski et al., 1982; Hatcher, 1987; Hatfield et al., 1987; Baldock and Smernik, 2002;
Czimczik et al., 2002; Maunu, 2002; Hammes et al., 2006; Preston and Schmidt, 2006; Solum, 2007); a hemicellulose signature (CH$_3$ in an acetyl group) is also evident from the resonance at 21.5 ppm (Kolodziejski et al., 1982; Czimczik et al., 2002; Maunu, 2008; Popescu et al., 2010). The DRIFT stretching bands near 3000, 1500, 1235, and 1040 cm$^{-1}$ provide confirmatory evidence for both cellulose and lignin. Reference to the $^{15}$N NMR spectrum strengthens the proposal that some C=O carbons are situated in amide groups and some aryl carbons are found in pyrroles – but their modest contributions to the $^{13}$C spectrum indicate that such structures are chemically dilute in the wood materials. The alkyl resonances of pine wood samples could be attributed to CH$_2$ groups of hydrolytic lignin (Solum, 2007), long-chain aliphatics of waxes (Solum, 2007) and/or the CH$_3$ moiety in a hemicellulose acetyl group (Kolodziejski et al., 1982; Hatfield et al., 1987; Baldock and Smernik, 2002; Solum, 2007; Popescu et al., 2010).

Heat treatments of wood at relatively low temperatures (230-400°C) have been suggested to result in rapid depolymerization of polysaccharides, namely cellulose and hemicelluloses (Keiluweit et al., 2010). This hypothesis is supported by our thermal charring experiments on enriched pine wood at 450°C: the oxygenated aliphatic and carboxyl signals attributed to polysaccharides are diminished significantly in the solid state $^{13}$C NMR and corresponding DRIFT spectra. In addition, the present work provides evidence for chemical modification of lignins during pyrolysis, including diminution of the 1510-1500 cm$^{-1}$ DRIFT band and disappearance of CH$_3$O groups in the $^{13}$C NMR spectra. The latter observation is in accord with a recent charring study that used ssNMR to deduce considerable loss of aliphatics and removal of both methoxyl and phenolic carbons for an authentic lignin standard at 450°C (Knicker, 2010). However, the complete removal of ligneous aromatics cannot be verified in pinewood at 450°C due to the broadness of the aromatic resonance in the
PyOM product; lignin may require a higher temperature to undergo complete thermal conversion (Yang et al., 2007).

A proposed mechanism for this type of pyrogenic transformation, based on charring experiments conducted at progressively increasing temperatures (Baldock and Smernik, 2002; Keiluweit et al., 2010; McBeath et al., 2011), entails water loss under mild conditions but removal of various oxygenated aliphatic species and development of oxygenated aryl and condensed (layered) aromatic structures at the elevated temperatures we used to generate PyOM. For instance, dehydration and subsequent combustion during thermal treatment of wood, rice, straw, and grass (Baldock and Smernik, 2002; Hammes et al., 2006; Knicker, 2010) have been proposed to convert oxygenated aliphatic carbon moieties to multiply-bonded and oxygenated aromatic structures such as furans (Baldock and Smernik, 2002), pyranones, and anhydrosugars (Knicker et al., 2008).

These hypotheses regarding the structure of pyrogenic chars gain a measure of support from our spectroscopic characterization of PyOM: (1) aryl C-H, aryl C=C, and aryl carbonyl/carboxyl C=O stretching bands grow in the DRIFT spectra; (2) aromatic (and/or alkene) moieties are indicated by $^{13}\text{C}$, $^{15}\text{N}$, and $^1\text{H}$ NMR chemical shifts; (3) oxygenated aryl groups are implicated by $^{13}\text{C}$ chemical shifts and dipolar dephasing experiments; (4) fused or stacked aromatic rings (Knicker et al., 2008; Keiluweit et al., 2010) are indicated as plausible architectures for these molecular constituents by the broadened $^{15}\text{N}$ and $^{13}\text{C}$ aromatic resonances. Furthermore, the downfield shift of the $^{15}\text{N}$ NMR signal upon heat treatment shows that the nitrogenous species correspond to chemically altered rather than heat-resistant domains of the original pine wood precursor. As noted above, the broadness of the aromatic PyOM resonance precludes determination of whether the ligneous components of the
wood precursor have been removed completely or converted into new aromatics in the charred product. Since the incomplete combustion is also associated with 64% mass loss, it is not possible to validate the complete conversion of oxygenated aliphatics of the pinewood into newly generated aromatics of the PyOM product by solid-state $^{13}$C NMR or DRIFT analyses. However, our data do reveal significant heat-resistant and/or regenerated aliphatic domains within PyOM produced at 450 °C: (1) CH stretching bands appear in the DRIFT spectra; (2) a population of mobile alkyl groups is present in the $^{13}$C and $^1$H MAS NMR spectra.

Ecologists and paleobiologists have used changes in the $\delta^{18}$O of cellulose, lignin and whole wood from tree ring cores, along with $\delta^{13}$C, as a proxy for interpreting past climates (e.g., Borella et al., 1998). These studies have shown that because of metabolic fractionation, the $\delta^{18}$O of cellulose is often enriched by 4-5‰ compared with whole wood, while lignin is depleted in $\delta^{18}$O compared with whole wood and cellulose (Gray and Thompson, 1977). Whereas the pine wood studied herein was enriched artificially in $^{13}$C and $^{15}$N, the sample was unlabeled with respect to oxygen isotopes and thus displayed a typical signature for plant biomass (Table 1; (Dawson et al., 2002). We found a charring-associated shift towards the $\delta^{18}$O signature of lignin (Gray and Thompson, 1977), consistent with the preferential loss of cellulose in PyOM. $^{13}$C NMR also revealed the preferential removal of oxygenated aliphatics associated with the partial combustion of the pine wood at 450 °C.

4.2 Supramolecular organization of PyOM from pine wood
As noted above, amorphous structures comprised of small oxygenated aryl units have been proposed recently based on X-ray absorption spectroscopy and X-ray diffraction in biomass-derived charred materials formed above 400 °C (Keiluweit et al., 2010). In addition to their diagnostic value for
particular molecular groupings, our spectroscopic data on PyOM help to evaluate possible condensed polyaromatic architectures for such disordered aryl structures and can demonstrate their capability to host unpaired electrons.

For instance, the broader $^{15}$N and $^{13}$C NMR features observed for our PyOM as compared with the original pine wood could be attributed to paramagnetic constituents (Vassallo et al., 1987) or to the formation of disordered, heterogeneous nitrogen-containing aromatic moieties in the charred product. Support for the first possibility comes from EPR measurements at cryogenic temperatures, which show a strong signal from unpaired electrons in the structural framework of charred wood. Moreover, the relatively short cross-polarization time required for detection of $^{15}$N signals in cross-polarization NMR experiments suggests that these paramagnetic moieties are located close to the nitrogenous PyOM functional groups. Given the paucity of N-containing functional groups deduced above for the wood substrate (Table 1) and the modest size of the $^{15}$N signal obtained from a 3-day CPMAS NMR measurement on the corresponding PyOM, it can be deduced that nitrogenous moieties are also sparsely represented among the heterogeneous aromatics of this charred material.

It is also plausible that overlapped NMR signals result from slightly dissimilar aromatic groups in heterogeneous superstructures, such as heterocyclic aryl-nitrogen moieties formed from plant biomass via a proposed thermolytic degradation mechanism for incomplete combustion (Buena et al., 2010; Gärdenäs et al., 2011; Hilscher and Knicker, 2011b, a). For PyOM derived from pine wood at 450 °C, the broadened $^{15}$N and $^{13}$C resonances support the possibility that such structurally heterogeneous and disordered nitrogen-containing aryl structures develop during charring.
If the multiply-bonded moieties that develop during the charring process are present within highly condensed fused polycyclic aromatic structures (Czimczik et al., 2002; Brewer et al., 2009), then nonprotonated aromatic carbons would be expected to become prominent constituents. On the contrary, the uniform decrease in aromatic peak intensity observed in a series of interrupted heteronuclear decoupling $^{13}$C CPMAS experiments (Fig. 2) suggests the presence of protonated aromatic structural units as opposed to significantly proton-depleted condensed layers of aromatics in the PyOM from pine wood. An increase in the IR absorption band at the C-H aromatic stretching frequency (Fig. 6) qualitatively supports this contention. Thus although disordered heterogeneous aromatic moieties are evident in PyOM derived from the pyrolysis of the pine wood, the spectroscopic and isotopic measurements are consistent with the survival of some lignin-based aromatic residues upon charring at 450 °C.

In this study, the molecular characteristics of 450 °C pine PyOM were found to be consistent with the recently proposed amorphous char consisting of a heterogeneous mixture of thermally transformed biopolymers (Knicker et al., 2008; Keiluweit et al., 2010). Amorphous PyOM is thought to degrade faster than materials with turbostratic crystallite phases formed at temperatures above 500 °C (Keiluweit et al., 2010). In a recent study (Santos et al., 2012), the pine PyOM investigated herein was incubated in soils for 180d and reported to have a centennial scale MRT, which is shorter than that estimated (millennial) from radiocarbon analysis of pyrolyzed materials recovered in soil profiles (Forbes et al., 2006; Preston and Schmidt, 2006). Observed differences in turnover times for a variety of pyrolyzed materials have been reported elsewhere (Preston and Schmidt, 2006; Hammes et al., 2008). In our view, further assessments of the molecular composition and structure of PyOM produced at different temperatures, together with measurements of C and N mineralization from
these pyrolyzed materials, are needed to elucidate the apparent linkage between recently proposed PyOM categories (Keiluweit et al., 2010) and their degradation rates in soils.

5. Conclusions

Taken together, our studies of \(^{13}\)C and \(^{15}\)N-enriched pine wood support a pyrogenic product with chemically heterogeneous aromatic structures that can stabilize free electrons. Use of high-field ssNMR and fast magic-angle spinning, combined with DRIFT and \(\delta^{18}\)O analyses, permits a more confident assessment of the molecular moieties, the supramolecular architecture, and the transformation possibilities in these PyOM materials. Spectral analyses using \(^{13}\)C and \(^{1}\)H ssNMR complemented by infrared spectroscopy demonstrate the presence of both newly formed aromatics and heat-resistant aliphatics in the PyOM, indicating that the combustion of the precursor wood at 450 °C does not degrade all C-containing species into highly condensed aryl structures, but rather maintains a disordered blend of thermally altered biomaterials. Furthermore, \(^{15}\)N ssNMR supports the development of heterogeneous, free-electron-associated nitrogenous aryl constituents upon pyrogenic transformation of pine wood. Whereas the removal of oxygenated aliphatic carbon structures to form disordered multiply-bonded and oxygenated aromatic structures is indicated by \(^{13}\)C ssNMR, the possibility of selective retention of lignin moieties during 450 °C charring is nevertheless supported by \(^{18}\)O isotopic analyses. Correspondingly, the nitrogenous PyOM species are shown by \(^{15}\)N ssNMR to derive from thermally altered rather than heat-resistant domains of the pine wood precursor.

The pyrolysis of pine wood at 450 °C results in molecular structures that are likely to be more susceptible to degradation than structures formed at higher temperatures. Thus, consistent with prior
proposals (Schmidt and Noack, 2000; Preston and Schmidt, 2006), not all pyrolyzed materials should be considered inert by soil C models. We believe that insights from this and future work on the chemistry of PyOM will greatly benefit our efforts to understand the mechanisms involved in the stability and reactivity of pyrolyzed materials in the environment as well as the role of PyOM-nitrogen in soil fertility and C sequestration.

**Acknowledgements**

We thank K. Hammes and M.W.I. Schmidt from the Univ. of Zurich for preparing the PyOM from pine wood, C. Yarnes and J. Matthews at the University of California Davis Stable Isotope Facility for isotope analysis, and W. Horwath (Univ. of California, Davis) for growing the enriched pine. We thank S. Leavitt (Univ. of Arizona) for cellulose extractions. Dr. H. Wang and Mr. X. Guan (City College of NY) are acknowledged for valuable discussions about the NMR measurements. We thank Prof. R. Magliozzo and Mr. A. Khajo for assistance with EPR measurements performed at Brooklyn College. The 600 MHz NMR facilities used in this work are operated by The City College and the CUNY Institute for Macromolecular Assemblies, with additional infrastructural support provided by NIH 2G12RR03060 from the National Center for Research Resources and 8G12MD007603 from the National Institute on Minority Health and Health Disparities. The 750 MHz NMR spectrometer is operated by the New York Structural Biology Center, a STAR center supported by the New York State Office of Science, Technology, and Academic Research.
Table 2. Elemental and stable isotope composition of ponderosa pine wood and PyOM charred at 450 °C.

<table>
<thead>
<tr>
<th>Litter</th>
<th>C (g kg(^{-1}))</th>
<th>N (mass ratio)</th>
<th>H (atomic ratio)</th>
<th>O/C</th>
<th>C/N</th>
<th>H/C</th>
<th>O/C</th>
<th>(^{13})C (atom %)</th>
<th>(^{15})N (atom %)</th>
<th>(^{18})O (‰ (VSMOW))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine wood</td>
<td>499</td>
<td>4.3</td>
<td>66</td>
<td>411</td>
<td>115</td>
<td>1.60</td>
<td>0.62</td>
<td>2.05</td>
<td>4.3</td>
<td>26.0</td>
</tr>
<tr>
<td>PyOM</td>
<td>779</td>
<td>7.1</td>
<td>34</td>
<td>144</td>
<td>110</td>
<td>0.53</td>
<td>0.14</td>
<td>2.03</td>
<td>4.2</td>
<td>13.2</td>
</tr>
</tbody>
</table>

\(^{1}\)Vienna Standard Mean Ocean Water (VSMOW)
Table 2. Direct polarization (DP) $^{13}$C NMR signal intensities of pine wood and PyOM

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Total acquired NMR signal (ppm)$^a$</th>
<th>Spin counting $^{13}$C$_{\text{obs}}$-DP (%)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carboxyl</td>
<td>Aryl C/Alkenes</td>
</tr>
<tr>
<td>Pine wood</td>
<td>185-160 ppm</td>
<td>160-110 ppm</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>PyOM</td>
<td>-</td>
<td>162-100 ppm</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>93</td>
</tr>
</tbody>
</table>

$^a$ The total signal intensity estimates for PyOM are based on the DPMAS $^{13}$C spectrum at 30 kHz spinning rate. Estimated precision of 2-4% for PyOM or 10-15% for pinewood is derived from duplicate measurements of integrated signal intensity on a single sample obtained at two spinning speeds and independent measurements on replicate samples that were nominally identical.

$^b$ Percentage of potentially observable carbon ($^{13}$C) spins estimated by spin counting methodology, using alanine as an external standard with 100% observable carbons ($^{13}$C$_{\text{obs}}$) and DPMAS spectral acquisition techniques (Smernik and Oades, 2000a,b). Estimated precision of 15-18% is derived from nominally identical batches of PyOM.
Table 2. Major IR absorption bands and assignments (Wood, 1988; Guo and Bustin, 1998; Moore and Owen, 2001; Baldock and Smernik, 2002; Weiland and Guyonnet, 2003; Nuopponen et al., 2006)

<table>
<thead>
<tr>
<th>Band Number</th>
<th>Wavelength (cm(^{-1}))</th>
<th>Description (trend during charring)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3050-3020</td>
<td>C-H aromatic stretch (increase)</td>
</tr>
<tr>
<td>2</td>
<td>3000-2800</td>
<td>C-H aliphatic stretch (decrease)</td>
</tr>
<tr>
<td>3</td>
<td>2850-2820</td>
<td>Aliphatic C – H (difference between samples)</td>
</tr>
<tr>
<td>4</td>
<td>1730-1680</td>
<td>Aromatic carbonyl / carboxyl C=O stretch (increase)</td>
</tr>
<tr>
<td>5</td>
<td>1610-1570</td>
<td>C=C stretch (increase)</td>
</tr>
<tr>
<td>6</td>
<td>1510-1500</td>
<td>Lignin, Aromatic C=C stretch (decrease)</td>
</tr>
<tr>
<td>7</td>
<td>1430-1380</td>
<td>Aromatic C=C stretch (increase)</td>
</tr>
<tr>
<td>8</td>
<td>1260-1210</td>
<td>Cellulose (decrease)</td>
</tr>
<tr>
<td>9</td>
<td>1060-1020</td>
<td>Aliphatic C-O- and alcohol C-O stretch (decrease)</td>
</tr>
<tr>
<td>10</td>
<td>880, 805, 745</td>
<td>C-H aromatic bending deformation (increase)</td>
</tr>
</tbody>
</table>
Figure 2. 150 MHz CPMAS $^{13}$C NMR spectra of solid pine wood and the corresponding PyOM after charring at 450 °C. The spectra were acquired with a 30 kHz spinning frequency to remove spinning sidebands; chemical shifts were referenced externally to the methylene (-CH$_2$-) group of adamantane at 38.4 ppm. 150 MHz DPMAS $^{13}$C data (shown as an inset for PyOM) exhibit essentially the same spectral features.
Figure 2. Solid-state CPMAS $^{13}$C spectra of PyOM from pine wood, acquired at 150 MHz and with a 10-kHz spinning frequency. A series of time delays (10-40 µs) was inserted before the start of proton decoupling during spectral acquisition, attenuating the signals from rigid carbons with attached hydrogens or nearby electrons. Spinning sidebands are designated by stars (*); chemical shifts were referenced externally to the methylene (–CH$_2$–) group of adamantane at 38.4 ppm.
Figure 2. 3 600 MHz MAS $^1$H spectrum of PyOM from pine wood acquired with a 35-kHz spinning frequency, showing two broad spectral features. The $^1$H chemical shifts were referenced indirectly, using a calculation from the gyromagnetic ratios of $^1$H and $^{13}$C following IUPAC recommendations (Harris et al., 2001).
Figure 2. 4 75 MHz CPMAS $^{15}$N NMR spectra obtained with a 0.3 ms spin-lock time for solid pine wood and the corresponding PyOM after charring at 450 °C. The data were acquired with a 15-kHz spinning frequency. The $^{15}$N chemical shifts were referenced indirectly, using a calculation from gyromagnetic ratios of $^{15}$N and $^{13}$C, according to IUPAC recommendations (Harris et al., 2001).
Figure 2. 5 X-band EPR spectrum of PyOM at 77K (microwave power = 0.1 mW), demonstrating the presence of unpaired electrons in the charred sample.
Figure 2. 6 Diffuse reflectance infrared Fourier transform (DRIFT) spectra of powdered wood and PyOM samples. Numbered bands are discussed in the text.
6. References


Chapter 4: Stable isotopic analysis of pyrogenic organic matter in soils by liquid-chromatography isotope-ratio mass spectrometry of benzene polycarboxylic acids


Abstract

Pyrogenic organic matter (PyOM), the incomplete combustion product of organic materials, is considered stable in soils and represents a potentially important terrestrial sink for atmospheric carbon dioxide. One well-established method to measure PyOM in the environment is as benzene polycarboxylic acids (BPCA), a compound-specific method, which allows both qualitative and quantitative estimation of PyOM. Until now, stable isotope measurement of PyOM carbon involved measurement of the trimethylsilyl (TMS) or methyl (Me) polycarboxylic acids derivatives by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). However, BPCA derivatives can contain as much as 150% derivative carbon, necessitating post-analysis correction for accurate measure of $\delta^{13}$C isotope values, leading to increased measurement error. Here, we describe a method for $\delta^{13}$C isotope ratio measurement and quantification of BPCAs from soil-derived PyOM, based on ion-exchange chromatography (IEC-IRMS). The reproducibility of the $\delta^{13}$C measurement of individual BPCAs by IEC-IRMS was better than 0.35‰ (1σ). The $\delta^{13}$C-BPCA analysis of PyOM
in soils, including at natural and artificially enriched $^{13}$C-abundance, produced accurate and precise $\delta^{13}$C measurements. Analysis of samples that differed in $\delta^{13}$C by as much as 900‰ revealed carryover of <1‰ between samples. The weighted sum of individual $\delta^{13}$C-BPCA measurements was correlated with previous isotopic measurements of whole PyOM, providing complementary information for bulk isotopic measurements. We discuss potential applications of $\delta^{13}$C-BPCA, including the study of turnover rates of PyOM in soils and the partitioning of PyOM sources based on photosynthetic pathways.
1. Introduction

Fire is a major controller of carbon (C) cycling in terrestrial ecosystems, by converting plant biomass into atmospheric CO₂ and by contributing incompletely combusted biomass or pyrogenic organic matter (PyOM) to soils [1,2]. PyOM is ubiquitous in the environment and can be a sizable fraction of the stable portion of soil C [3,4,5]. Recent interest in better understanding the chemical properties, stocks, and turnover rates of PyOM has been driven by the potential role of PyOM as a stable C sink for atmospheric CO₂ [6,7], as a sorbent for pollutants [8,9], and to improve plant fertility in highly weathered soils [10,11]. Furthermore, the amount of PyOM added to the environment is expected to increase because of predicted increases in wildfire frequency and intensity with a warmer future climate [12,13] and significant contributions of biochar produced by the energy industry as a byproduct of low-temperature biomass pyrolysis [14,15].

One of the challenges to better understand the roles of PyOM in the environment is its methodological assessment, especially in complex, C-rich matrices like soil. PyOM represents a continuum of materials, whose structures are affected by the heat treatment temperature and conditions of combustion [16,17,18,19]. Recent approaches to the characterization of PyOM in environmental samples are diverse, and include spectroscopy [20,21,22], analytical pyrolysis [23,24], and molecular markers like levoglucosan [25] and benzene polycarboxylic acids [26,27]. A large, multi-laboratory study by Hammes et al. [28] compared seven methods of PyOM analysis on various environmental samples and observed that PyOM produced by wildfire conditions were well-estimated by the benzene polycarboxylic acids
(BPCA) molecular biomarker method. The BPCA approach was the only method that could reliably quantify PyOM and simultaneously characterize the chemical structure of PyOM materials in soil matrices. Hammes et al. [28] cautioned that the BPCA approach might underestimate the highly condensed soot part of the PyOM continuum and also include BPCA contributions from coal and shale. Recent advances in the BPCA extraction method [29], especially the addition of systematic standardization procedures [30], have addressed the inter-laboratory variability observed for BPCA [28].

During the last decade, the BPCA approach has been developed into a robust method to quantify the amount of PyOM materials in soils [31,32,33,34,35]. BPCAs are produced through the oxidation of substituted aromatic organic C and are, thus, a measure of the main building blocks of most PyOM materials. Consequently, BPCA can provide qualitative information on the degree of aromatic condensation of the PyOM present [30,35]. As the turnover rates of PyOM in the environment has been related to the heat treatment temperature and resulting degree of aromatic condensation [16,35], the BPCA approach can provide a clearer picture of PyOM degradability and mobility in the environment. When the BPCA approach is combined with stable and radiogenic C isotope tracers (i.e. $^{13}$C and $^{14}$C), the movement, loss and transformation of PyOM can be directly measured in situ [36].

Initial $\delta^{13}$C measurements of PyOM molecular markers have relied on gas chromatography-combustion-isotope-ratio mass spectrometry (GC-C-IRMS) of trimethylsilyl and methyl derivatives of BPCAs [37]. Unfortunately, GC-C-IRMS exhibits a number of shortcomings when applied to the measurement of stable isotopic composition of non-volatile BPCAs,
primarily due to the need for derivatization. For example, the TMS and methyl derivatives of mellitic acid (benzene hexacarboxylic acid; B6CA) add 18 or 6 C atoms, respectively, to the twelve C atoms present in B6CA. Because all of the C present in the derivatized molecule is ultimately oxidized to CO₂, and therefore contributes to the final δ¹³C-CO₂ measurement, significant correction factors must be applied to generate reasonable estimates of δ¹³C-BPCA. Moreover, BPCA derivative interactions[30], isotope dilution, kinetic isotope effects, and incomplete derivatization associated with GC-C-IRMS result in increased in δ¹³C measurement error[38].

High-performance liquid chromatography isotope-ratio mass spectrometry (HPLC-IRMS) may be a suitable alternative for the measurement of δ¹³C-BPCA. LC-IRMS would not require the derivatization of BPCAs prior to analysis, potentially providing better overall accuracy and precision without the requirement for correction factors. While two suitable methods for BPCA analysis by HPLC have been demonstrated [29,35], neither method is suitable for transfer to the current generation of LC-IRMS instrumentation, as separation methodologies for LC-IRMS cannot utilize organic solvents in the mobile phase [39]. In contrast, anion-exchange chromatography is particularly well suited to the separation of water-soluble ionic compounds without the use of organic solvents. Several applications of LC-IRMS have adopted ion-exchange chromatography (IEC-IRMS) for the separation of ionic organic compounds including, carbohydrates [40], amino acids [41], and amino sugars [42]. IEC-IRMS is suitable for application to a wide-range of other organic acids [43,44], including aromatic carboxylic acids [45].
In this study, we present an IEC-IRMS method for the measurement of $\delta^{13}$C-BPCA following hydroxide gradient separation of individual BPCAs by anion-exchange chromatography, taking advantage of the ionic properties of benzene polycarboxylic acids. The accuracy and precision of this method were evaluated using a series of plant-derived PyOM and soil samples, representing a range of sample matrices and isotopic composition. The sample included (1) PyOM from both C$_3$- and C$_4$-plants, (2) PyOM with and without a $^{13}$C-tracer (natural abundance and artificially enriched), (3) Mollisol soil from C$_3$-dominated ecosystem, (4) artificial mixtures of C$_4$-PyOM and C$_3$-Mollisol soils, and (5) Spodosol soil from C$_3$-dominated ecosystem. We briefly discuss the suitability of IEC-IRMS to potential applications of $\delta^{13}$C-BPCA measurements.

2. Experimental

2.1 Chemicals

Eight benzene polycarboxylic acids are used as biomarkers of PyOM (Fig. 1). Pure benzene polycarboxylic acids, including benzenetricarboxylic acid (B3CA), namely 1,2,3-B3CA (hemimellitic acid), 1,2,4-B3CA (trimellitic acid), 1,3,5-B3CA (trimesic acid); benzenetetracarboxylic acid (B4CA),1,2,4,5-B4CA (pyromellitic acid); and benzenehexacarboxylic acid (B6CA; mellitic acid) were purchased from Sigma-Aldrich (St. Louis, MO USA), except benzenepentacarboxylic acid (B5CA), which was supplied by Alfa Aesar (Heysham, UK). Two of the benzenetetracarboxylic acids (B4CA),1,2,3,5-B4CA (mellanophanic acid) and 1,2,3,4-B4CA (prehnitic acid), were not commercially available. A sodium hydroxide stock solution (50% w/w) was supplied by Acros Organics (Geel,

2.2 Sample descriptions

An overview of the samples used in this study is given in Table 1. The pyrolysis treatment for all PyOM materials examined in this study (i.e., chestnut, pine and maize) was performed at a constant temperature of 450°C for 6 hours under N2 \cite{21}. The standard PyOM material analyzed here was produced by pyrolysing chestnut (\textit{Castanea sativa}) wood. The $^{13}$C/$^{15}$N-labeled pine PyOM was produced by pyrolysing 2-3 year-old Ponderosa pine (\textit{Pinus ponderosa}) saplings which were grown in a stable isotope labeling chamber at the University of California, Davis in 2001 \cite{46}. The maize (\textit{Zea mays}) PyOM was sampled at maturity in an experimental field in Rotthalmünster, Germany \cite{47}. The maize stem was separated from the leaves and roots and chopped into 1-3 cm particles prior to charring.

Northern temperate forest soil samples (A horizon, 0-5 cm depth) were collected in 2009 from an on-going fire manipulation experiment located at the University of Michigan Biological Station (UMBS), Pellston, MI (45°35’N, 84°43’W). The 1 ha forested plot sampled at UMBS was last burned in 1911, and is located on an outwash plain derived from glacial drift. The UMBS soils are well-drained, sandy, mixed frigid Entic Haplothsods (Rubicon series), and consisting of 92 g kg$^{-1}$ sand and 1 g kg$^{-1}$ clay \cite{48}. Two composite soil samples (A and B) were included in this analysis, each from the same forest plot. Soil samples were collected at three sampling points equally spaced along a 120 m transect in
the plot. Soil samples were formed by combining 5 soil cores taken a meter radius around at each sampling point. In this study, Spodosol soil A is a composite sample from one sampling point in a transect, and contains 15.89 ± 0.06 g kg\(^{-1}\) C. Spodosol soil B was formed by combining soil composites from all sampling points in one single transect, and contains 11.92 ± 0.15 g kg\(^{-1}\) C. Soils were air-dried and sieved (< 2mm) before analysis.

Mollisol soils (Chernozem) were sampled at 20–60 cm depth in the Hildesheim-Braunschweig region, Harsum, Germany\(^{[49]}\) and used as a reference material in pyrogenic organic matter studies\(^{[28,30,34]}\). These soils are characterized as light sandy clay with 19 wt% clay content and 20.1 g C/kg.

In order to assess the ability of the method to detect \(^{13}\)C-BPCA differences for natural samples, we mixed C\(_4\)-maize-PyOM to C\(_3\)-Mollisol at 1:1 C\%. Ground samples were gently mixed, rewetted to field capacity and incubated for one day. The mixed samples were dried and milled again prior to analysis. Measured values are compared to expected values, calculated as the weighted average of \(^{13}\)C-BPCA from maize-PyOM and Mollisol soil.

### 2.3 BPCA extraction

We used the revised BPCA method as described by Schneider et al.\(^{[30]}\), which was first described by Glaser et al.\(^{[26]}\) and later modified by Brodowski et al.\(^{[27]}\). Briefly, the revised method replaces hydrochloric acid in the digestion procedure with trifluoroacetic acid (TFA) prior to the oxidation of samples with H\(_2\)O\(_3\) and phthalic acid as the internal standard.
Soil and PyOM samples were homogenized prior to extraction and completed in triplicate following Brodowski et al. [27].

2.4 Chromatography

Individual BPCAs were separated at 30°C on a Dionex IonPac® AS11 column (2 x 250mm) with AG11 guard column (Dionex Corp., Sunnyvale, California, USA.) using a sodium hydroxide gradient (NaOH) delivered by a Thermo Scientific Surveyor HPLC pump (Thermo Scientific, Madison, Wisconsin, USA). Solvents were (A) deionized water and (B) 100mM NaOH. The linear gradient profile was (B in A): 0-1 min, 30%; 1-10 min, 30-35%; 10-20 min, 35-40%; 20-45 min, 40-80%; 45-60 min, 80-100%; 60-70 min, 100%. Flow rate was 350µL min⁻¹. Sample injection was automated and performed with a 5 or 10µL injection loop. Targeted sample amount was 150-350 ng C for B5CA and B6CA.

All solvents were degassed by sonication under reduced pressure for 1 hr. In the case of the NaOH solvent, the carbonate-free NaOH stock solution (50% w/w) was not added until the water had first been degassed (1 hr); the prepared solution was then degassed for an additional 15 minutes. During use, solvents were bubbled with a continuous stream of helium (99.9999% purity, Praxair, Inc., Sacramento, California, U.S.A.) to prevent regassing. This procedure helped to reduce CO₂ contamination of the NaOH solvent and maintain a relatively low background of CO₂ in the IRMS.

The system was re-equilibrated for 15 minutes at initial conditions prior to each analysis. After every ten samples, the column was flushed with 200mM NaOH for 1h and allowed to
equilibrate for 30 min prior to subsequent analysis. After extensive use for the analysis of soil samples, the quality of separation became reduced and retention time shortened, probably due to the accumulation of metals and hydrophilic anions on the column. The column was then sequentially washed with (1) 2M HCl, (2) water, and (3) 2M NaOH, for 1h each, and re-equilibrated prior to subsequent use. Column cleanup was performed if average retention times had shifted more than 10 seconds, and expected retention was not reestablished by the hydroxide wash.

For six of the eight BPCAs, compounds were identified based upon retention times through the use of a prepared mixture of pure compounds. For the two commercially unavailable B4CA (1,2,3,4-B4CA and 1,2,3,5-B4CA), retention times and elution order were predicted from their published pK_a values [50], and comparison with GC data from soil samples and standards with variable individual B4CA content.

2.5 Combustion-oxidation and isotope-ratio mass spectrometry

After separation, the column eluent enters an oxidation interface, LC Isolink (Thermo Scientific, Bremen, Germany), where it is mixed with sodium peroxidisulfate (0.8 M) and phosphoric acid (1.5 M), separately pumped at 60 µL min⁻¹ and 90 µL min⁻¹ respectively. Individual BPCA peaks are then quantitatively oxidized to CO₂ as the mobile phase flows through an oxidation reactor heated to 99.9°C. Each CO₂ peak is transferred from aqueous phase to He carrier gas (2 mL min⁻¹) through a separation membrane. Following water removal using a two-stage Nafion™ dryer, the individual CO₂ peaks are transferred to an isotope-ratio mass spectrometer (Finnigan DeltaPlus Advantage, Thermo Electron, Bremen,
Germany) through an open split.

During analysis, three pulses of CO₂ (99.995% purity, Praxair, Inc., Sacramento, California, U.S.A.) of known δ¹³C, each 20 s in duration, were introduced into the IRMS instrument and used to calculate the provisional δ¹³C values of sample peaks (expressed in ‰, referenced vs. VPDB). Post-analysis, measurements were standardized with a prepared mixture of five BPCAs (BPCA-MIX) of varying concentration (50-500 ng C/µL each). BPCA-MIX was analyzed every five samples to allow for correction for instrument drift and used to evaluate linearity. Peak area integration parameters were set to a slope (mV/s) of 0.1 with a dynamic background algorithm with a step-width of 75 points⁵¹ to account for any changes in the background (Thermo Scientific, ISODAT v.2.5, Bremen, Germany).

For each BPCA, a final shift to δ¹³C values obtained through elemental analyzer-IRMS (EA-IRMS) was used to compensate for any potential offset produced by stationary phase-interactions during IEC-IRMS measurement⁵² (see Table 2). Briefly, 350 µg C of each pure compound was weighed into tin capsules, combusted and oxidized to CO₂ in an elemental analyzer, and purified and analyzed using an isotope-ratio mass spectrometer (20-20, Sercon, Crewe, United Kingdom). During analysis, samples were interspersed with several replicates of two different laboratory standards (nylon, δ¹³C = -27.41, and glutamic acid, δ¹³C = +43.74) that had been previously calibrated against NIST Standard Reference Materials (USGS-40 and USGS-41, National Institute of Standards and Technology, Gaithersburg, MD U.S.A.). The long-term standard deviation is ≤0.2‰ for δ¹³C. δ¹³C values obtained by EA-IRMS were assigned to each component of the BPCA-MIX.
3. Results and Discussion

3.1 Chromatography

Sufficient separation of all B3CA through B6CA, except 1,2,4-B3CA, was possible across a 30-100 mM sodium hydroxide gradient (mean resolution, \( R > 1.5 \); Fig. 2). The co-elution of the B3CAs with other di- and tricarboxylic organic acids presents the greatest obstacle to complete BPCA separation by IEC. Despite testing a range of chromatographic conditions, we were unable to completely separate 1,2,4-B3CA from a co-eluting non-BPCA compound (mean resolution, \( R = 1.1 \)). We found the non-BPCA compound to be more abundant in the PyOM standards than the soils, where the compound produces a small shoulder on the 1,2,4-B3CA peak (Fig. 2). The presence of this shoulder will increase the measurement error associated with 1,2,4-B3CA, as the \( \delta^{13} \)C measurement of overlapping regions will represent a mixture of the two compounds. The magnitude of this effect will depend upon on the relative concentration of the two compounds. Separation of the remaining BPCA did not differ significantly between the extracts of PyOM and soils (Fig. 2). The concentrations of non-BPCA organic acids concentrations were more abundant in BPCA extracts of woody PyOM (\( C. \) sativa, \( P. \) ponderosa) than those of non-wood-PyOM (\( Zea \) mays) and soils (Fig. 2B).

Further developments in sample purification, especially the removal of non-BPCA carbon will be critical to simplifying the separation of all B3CAs. We noted that the quality of separation was greatly affected by the presence of other organic anions and changes in column condition; this has also been shown to be problematic for more traditional HPLC-BPCA methods\(^{[29]} \). In addition, care must be exercised in the removal of polyvalent cations from soil samples during sample preparation \([e.g. 42]\). High concentrations of these ions in
the sample matrix may also greatly affect column retention through local electrostatic effects.

Some researchers have previously expressed concern over the use of hydroxide-based separations in IEC-IRMS, due to the loss of precision with potentially high CO₂ backgrounds. However, the careful preparation of solvents (as described above) and use of a relatively high acid flow rate (90 µL min⁻¹) resulted in a low background across a strong OH⁻ gradient (m/z 44 background: 400-900 mV; recommended maximum background[53]: <1V). Furthermore, significant changes in the precision of δ¹³C measurements with increasing hydroxide concentrations were not observed (Table 2).

### 3.2 Quantification

The relative abundance of Mollisol soil BPCAs measured by IEC-IRMS compared favorably with GC-FID measurements (Fig. 3). Measured proportions of B5CA and B6CA from this soil by both methods differed by less than 1%, while the relative abundance of B3CA (excluding 1,3,5-B3CA) was found to be marginally higher (~+2.8%) and B4CA slightly lower (~-1.7%) when measured by IEC-IRMS than by GC-FID. The concentrations of the individual BPCAs measured by IEC-IRMS were ≥ 1:1 of those measured by GC-FID (Fig. 4). Notably, the concentrations of BPCAs of Spodosol soils were higher when quantified by IEC-IRMS. This may be due to sample-specific differences in the completeness of BPCA derivatization in preparation for GC-FID analysis. However, the observed differences in quantification between soil types suggest the use of a native concentration standard until the underlying reasons for the differences are determined. 1,3,5-B3CA was not identified in the GC-FID analysis of the soils, yet was quantifiable by IEC-IRMS (range: 0.58-1.29 µg C mL⁻¹).
We did not observe a strong linear effect of injection amount on the \( \delta^{13}C \) measurements. A series of 15 measurements of 1,2,3-B3CA, ranging from 75 to 525 ng C per injection produced a standard deviation of <0.26‰ (1 SD, \( \delta^{13}C \) range: -22.17, -23.15; \( R^2=0.02 \)) with no relationship between \( \delta^{13}C \) value and 1,2,3-B3CA concentration (linear slope = -0.0002). Flow-injection analysis (direct injection) of the other BCPA-MIX constituents also did not reveal a significant linear relationship from 100 to 500 ng C per injection (\( \alpha = 0.05 \)). However, the use of concentration-variable reference mixture would be wise to account for potential changes in the combustion interface, as well as providing an efficient basis for validating the quantification of BPCA concentration.

### 3.3 Accuracy and precision

Overall, precision estimates of \( \delta^{13}C \)-BPCA values by IEC-IRMS compare favorably to GC-C-IRMS. The precision (1\( \sigma \), \( n=3 \)) of \( \delta^{13}C \)-BPCA values from pure compounds (BPCA-MIX) by IEC-IRMS was \( \leq 0.74\% \) (Table 2), while that of sample duplicates was \( \leq 0.2\% \) (Table 3). Precision estimates (mean standard error) reported by Glaser and Knorr [37] were \( \leq 2.4\% \) and \( \leq 1\% \) for TMS and Me derivatives, respectively. The precision of \( \delta^{13}C \)-BPCA from duplicate samples (1\( \sigma \), \( n=2 \)) of natural-abundance PyOM and Mollisol soils was \( \leq 0.36\% \), and \( \leq 8.8\% \) for the \( ^{13}C \)-labeled \( P. \) ponderosa PyOM.

Raw \( \delta^{13}C \) measurements of B4CA-B6CA obtained by IEC-IRMS matched well those obtained by EA-IRMS, while IEC-IRMS measures of B3CA were generally ~1% less than
those by EA-IRMS (Table 1). In addition, IEC-IRMS of BPCAs from C4-PyOM (Zea mays), C3-Mollisol soils, and artificial mixtures produced an expected range of δ\(^{13}\)C values (Table 3). Notably, the weighted sum of IEC-IRMS measurement of individual BPCAs approximated the bulk EA-IRMS measurements of the standard reference PyOM materials (within 5‰), both at natural abundance and artificially enriched in \(^{13}\)C (Table 3). We did not expect these measurements to exactly match the measurement of the bulk PyOM or soils, as these materials also contain non-BPCA carbon; however, this outcome was encouraging. The accuracy of δ\(^{13}\)C-BPCA values measured by GC-C-IRMS reflects the contribution of non-analyte carbon to the δ\(^{13}\)C measurement (as high as 11‰\[^{37}\]); derivatization procedures for δ\(^{13}\)C-BPCA measurement result in products containing up to 1.5 times as much non-analyte carbon and require appropriate correction procedures (TMS and Me derivatives\[^{37}\]). The measurement error of δ\(^{13}\)C values by GC-C-IRMS is propagated as the final estimate of the δ\(^{13}\)C value relies on measurements of both the derivatization agent and the analyte. Further, kinetic isotope effects associated with derivatization of BPCAs may also contribute significant error, as reported for other GC-C-IRMS applications\[^{38,52}\].

Limiting carryover, or the incomplete removal of analytes between samples, is essential for stable isotope analysis, especially when measurements include artificially enriched samples. Carryover between samples of differing δ\(^{13}\)C values was not significant for natural abundance and \(^{13}\)C-enriched PyOM. The difference between individual δ\(^{13}\)C-BPCA from C. sativa PyOM measured after \(^{13}\)C-labeled P. ponderosa PyOM ranged from -0.73‰ (1,3,5-B3CA) to 0.40‰ (1,2,4,5-B4CA), which is well within the measurement error for the \(^{13}\)C-enriched PyOM. This feature will be critical for partitioning the relative contribution of multifarious PyOM sources to sample δ\(^{13}\)C-BPCA.
3.4 Applications

While estimates of PyOM contents and transformations using BPCA provide an important measure of PyOM dynamics, researchers have shown that some BPCAs may derive from non-PyOM sources [37,54]. Consequently, the improved efficacy of combining BPCAs with stable isotope tracers (e.g. $^{13}$C-PyOM) allows researchers to directly follow PyOM-derived BPCAs in soils, sediments and leachates without concern for non-PyOM-derived BPCAs. The IEC-IRMS approach significantly improves both the reliability and accuracy of isotopic BPCA determinations by eliminating the complications related to derivatization using the conventional GC-C-IRMS approach.

The use of BPCA in association with highly-enriched $^{13}$C-labeled PyOM provides for a robust quantitative and qualitative measure of the decomposition and transport dynamics of PyOM in terrestrial and marine ecosystems. Due to the relatively long, century-scale turnover rates of PyOM, the utility of tracer-level studies would be most evident with short to decade-long studies. However, differences in the natural abundance $^{13}$C signature of PyOM (i.e., C3/C4 photosynthetic pathways) may provide an opportunity to examine longer time periods of PyOM dynamics.

Researchers have utilized $^{13}$C natural abundance signatures for several specific compounds, including lignin [55],[56], polysaccharides [57] or $n$-alkanes [58] to evaluate their decay rate and their related stabilization mechanisms in soil. Specifically for lignin, several researchers have observed patterns in lignin monomers, and associated these relative changes to corresponding decomposition patterns [59]. Like changes in lignin monomers, the BPCA
approach can provide a portrait of PyOM composition by measuring changes in BPCAs. The $^{13}$C-BPCA approach will facilitate the investigation of sites with significant wildfire histories, including sequences of vegetation changes between C4 grassland and C3 forest. In our study, IEC-IRMS was able to distinguish PyOM of C3- and C4-plants, as well as artificial mixtures of C4-PyOM and C3-Mollisol soils (Table 3). The measured isotopic values were very close to the expected values (i.e. less than 6% difference to the expected value of the sum), considering the BPCA variability in the PyOM and soils.

The utility of $^{13}$C-BPCA using IEC-IRMS should not be restricted to soils or terrestrial sediments, but would be useful for a range of environmental samples. For oceanographic studies, BPCA has been applied to examine the contribution of PyOM to marine dissolved organic matter [29], [60]. Furthermore, this approach may also provide utility for the analysis of sediments, since the proportions of BPCAs have been shown to depend on their origin, including terrestrial or oceanic sources [61],[62].

4. Conclusions

Suitable methods exist for the analysis of BPCA as PyOM molecular markers, both by GC-FID and HPLC-DAD. While the isotopic measurement of $\delta^{13}$C-BPCA has been restricted to GC-C-IRMS, IEC-IRMS appears to be a suitable alternative for the measurement of $\delta^{13}$C-BPCA. IEC-IRMS of $\delta^{13}$C-BPCA values does not require significant post-analysis correction for derivatization carbon, nor does it pose some of the other analytical issues often associated with GC-C-IRMS of derivatization products. IEC-IRMS is suitable for a range of sample types from PyOM to soils, at both natural and artificial $^{13}$C-abundance and should be appropriate for aqueous environmental samples. Potential improvements are primarily to be
made with sample preparation, specifically improvements in the removal of non-BPCA organic acids.

**Acknowledgements**

The authors would like to thank Joy Matthews and the staff at the University of California, Davis Stable Isotope Facility for assistance with EA-IRMS measurements. We acknowledge the University of Michigan Biological Station for facilities support. F.S. was funded by a NSF-IGERT Biosphere-Atmosphere Research and Training Fellowship. Swiss National Foundation funded S.A. and N.S. for this study.
Table 3. 1 PyOM and soils used for the evaluation of $\delta^{13}$C-BPCA analysis by IEC-IRMS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
<th>Organic C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PyOM$^1$</td>
<td></td>
<td>g kg$^{-1}$</td>
</tr>
<tr>
<td>Castanea sativa PyOM$^2$</td>
<td>Chestnut wood, Ticino, Switzerland</td>
<td>774</td>
</tr>
<tr>
<td>Zea mays (C$_4$) PyOM</td>
<td>Corn Stem, Rotthalmünster, Germany</td>
<td>755</td>
</tr>
<tr>
<td>Pinus ponderosa PyOM</td>
<td>Pine wood, California, United States of America</td>
<td>779</td>
</tr>
<tr>
<td>Soils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mollisol$^3$</td>
<td>Hildesheim, DE, 20-60 cm depth</td>
<td>19.3</td>
</tr>
<tr>
<td>Spodosol</td>
<td>Pellston, MI, US, 0-5 cm depth</td>
<td>15.9 (“A”),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.9 (“B”)</td>
</tr>
</tbody>
</table>

1 All PyOM materials were charred at 450°C under N$_2$ environment (Hammes et al. 2006$^{[21]}$)

2 Hammes et al. 2006$^{[21]}$, 2007$^{[28]}$

3 Schmidt et al. 1999$^{[48]}$, Hammes et al. 2007$^{[28]}$
Table 3. 2 Comparison of δ\(^{13}\)C values (‰, ‘per mil’) obtained by EA-IRMS and IEC-IRMS analysis of individual BPCAs (σ, n = 3). B5CA was not measured by EA-IRMS due to limited availability. 1,2,3-BPCA and 1,2,4-BPCA were measured under full and partial separation by IEC-IRMS.

<table>
<thead>
<tr>
<th></th>
<th>1,2,3-BPCA</th>
<th>1,2,4-BPCA</th>
<th>1,3,5-BPCA</th>
<th>1,2,4,5-BPCA</th>
<th>B5CA</th>
<th>B6CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>±</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.11</td>
<td>±0.02</td>
<td></td>
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</tr>
<tr>
<td>IEC-IRMS</td>
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<td>-29.63</td>
<td>-27.04</td>
<td>-25.97</td>
<td>-29.67</td>
<td>-26.28</td>
</tr>
<tr>
<td>±</td>
<td>±0.16</td>
<td>±0.44</td>
<td>±0.60</td>
<td>±0.70</td>
<td>±0.74</td>
<td>±0.22</td>
</tr>
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</table>
Table 3. δ\(^{13}\)C measurement of duplicate preparations of PyOM and mineral soils (‰, ‘per mil’). Sum total represents the weighted sum of the individual BPCA measurements. Reproducibility (σ) of sample duplicates was ±0.36‰ for natural abundance samples, and ±8.8‰ for the *P. ponderosa* PyOM.

<table>
<thead>
<tr>
<th>BPCA</th>
<th>B3CA</th>
<th>B4CA</th>
<th>B5CA</th>
<th>B6CA</th>
<th>Sum</th>
<th>EA-IRMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Castanea sativa</em> PyOM</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-29.72</td>
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<td>-28.71</td>
<td>-28.24</td>
<td>-29.05</td>
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<tr>
<td><em>Pinus ponderosa</em> PyOM</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+804.25</td>
<td>+843.03</td>
<td>+888.17</td>
<td>+821.9</td>
<td>+839.91</td>
<td>+844.79</td>
</tr>
<tr>
<td><em>Zea mays</em> (C(_4)) PyOM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C(_3) Mollisol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-27.42</td>
<td>-26.12</td>
<td>-25.31</td>
<td>-25.70</td>
<td>-25.27</td>
<td>n.m.</td>
</tr>
<tr>
<td>C(_3) Mollisol + <em>Zea mays</em> PyOM (expected)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-24.41</td>
<td>-20.73</td>
<td>-20.02</td>
<td>-21.72</td>
<td>-21.72</td>
<td>n.m.</td>
</tr>
<tr>
<td>C(_3) Mollisol + <em>Zea mays</em> (C(_4)) PyOM (measured)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-23.08</td>
<td>-20.03</td>
<td>-19.27</td>
<td>-19.31</td>
<td>-20.42</td>
<td>n.m.</td>
</tr>
</tbody>
</table>
Figure 3. Structure and nomenclature of benzene polycarboxylic acid biomarkers of PyOM.
Figure 3. 2 Chromatographs of BPCAs from (A) *Zea mays* PyOM, (B) *Pinus ponderosa* PyOM, (C) Spodosol soil (Soil A), and (D) Mollisol soil. 1: 1,2,3-B3CA, 2: 1,2,4-B3CA, 3: 1,3,5-B3CA, 4: 1,2,4,5-B4CA, 5: 1,2,3,5-B4CA, 6: 1,2,3,4 B4CA, 7:B5CA, 8: B6CA.
Figure 3. 3 Relative abundance of each class of BPCAs of Mollisol soil (n = 2, with two replicates) as measured by IEC-IRMS and GC-FID.
Figure 3. Quantification of BPCAs (µg C/g soil) by IEC-IRMS relative to GC-FID of Mollisol (n=2, with two replicates, squares; 0.9726x+0.429) and Spodosol soils (A & B; n=2, with two replicates, diamonds; 1.4763x+1.3504). Each point represents the mean value of individual BPCA.
6. References


Appendices

Appendix A 2 Relative abundance of each class of BPCAs of Spodosol soil A (composite sample from one sampling point in a transect, 0-5 cm depth) and Spodosol soil B (formed by combining soil composites from all sampling points in one single transect, 0-5 cm depth) as measured by GC-FID. Samples were collected in Pellston, MI, USA.
Chapter 5: Atmospheric black carbon deposition in a northern temperate forest

Santos, F., Fraser, M. P., Bird, J. A. 2014 Atmospheric black carbon deposition and characterization of biomass burning tracers in a northern temperate forest in MI, USA. Atmospheric Environment 95, 383-390. DOI: 10.1016/j.atmosenv.2014.06.038

Abstract

Aerosol black carbon (BC) is considered the second largest contributor to global warming after CO₂, and is known to increase the atmosphere’s temperature, decrease the albedo in snow/ice, and influence the properties and distribution of clouds. BC is thought to have a long mean residence time in soils, and its apparent stability may represent a significant stable sink for atmospheric CO₂. Despite recent efforts to quantify BC in the environment, the quantification of BC deposition rates from the atmosphere to terrestrial ecosystems remains scarce. To better understand the contribution of atmospheric BC inputs to soils via dry deposition and its dominant emission sources, atmospheric fine particle (PM₂.₅) were collected at the University of Michigan Biological Station from July to September in 2010 and 2011. PM2.5 samples were analyzed for organic C, BC, and molecular markers including particulate sugars, carboxylic acids, n-alkanes, polycyclic aromatic hydrocarbons, and cholestane. Average atmospheric BC concentrations in northern Michigan were 0.048 ± 0.06 µg m⁻³ in summer 2010, and 0.049 ± 0.064 µg m⁻³ in summer 2011. Based on atmospheric concentrations, particulate deposition calculations, and documented soil BC, we conclude that atmospheric deposition is unlikely to comprise a significant input pathway for BC in northern forest ecosystem. The major organic tracers identified in fine particulates (e.g. levoglucosan
and docosanoic acid) suggest that ambient PM$_{2.5}$ concentrations were mainly influenced by biomass burning and epicuticular plant waxes. These results provide baseline data needed for future assessments of atmospheric BC in rural temperate forests.
1. Introduction

Black carbon (BC), formed from the incomplete combustion of plant biomass and fossil fuels (Goldberg, 1985), is released to the atmosphere at a global rate of ca. 8 Tg (1 Tg = 1 x 10^3 Gt yr⁻¹) (Bond et al., 2004). BC affects climate by directly absorbing sunlight and increasing the temperature of the atmosphere, reducing the reflectivity of snow and ice surfaces, and either warming or cooling the atmosphere when interacting with clouds (Hadley and Kirchstetter, 2012, Bond et al., 2013, and references therein). Airborne BC particles are predominantly between 0.05 and 1 µm (Venkataraman and Friedlander, 1994), with a lifetime thought to vary from 2 to 11 days (Cooke and Wilson, 1996; Schulz et al., 2006). Given the current uncertainties in atmospheric BC concentrations and emission inventories (Bond et al., 2004, Bond et al. 2013), ground-based measurements of BC aerosol are critical to elucidating the impacts of BC on regional and global climate. In soils, BC is reported to comprise a significant fraction of the stable carbon (C) pool (Schmidt et al., 1999), and may represent a sizable stable sink for C (Schmidt et al., 2004, Lehmann 2007). Rates of BC addition to soils have been predicted to increase globally because of expected increases in wildfire frequency and intensity due to climate change (Westerling et al., 2006, Flannigan et al., 2013). Knowledge of the net contribution of BC to soil C will be needed to predict the long-term effects of forest fires on terrestrial C fluxes. However, BC deposition fluxes from the atmosphere remains a poorly quantified component of net BC additions to terrestrial ecosystems in temperate regions. Moreover, estimations of this total deposition of BC originating from biogenic aerosol sources (i.e. biomass burning) versus anthropogenic aerosol sources (i.e. fossil fuel combustion) are still scarce.
The stability of charred residues in soils can be influenced by the combustion temperature from which BC is generated (Ballock and Smernik, 2002). As charring temperature increases, the initial biomass undergoes physicochemical and structural changes, resulting in BC materials with larger proportions of highly condensed and presumably less degradable carbonaceous compounds (Hammes et al., 2006; Knicker et al., 2008, Keiluweit et al., 2010, Schneider et al., 2010). Thus, BC particles formed under lower combustion temperature (e.g. biomass burning) are expected to degrade faster than those formed under higher temperatures (e.g. fossil fuel combustion; Hammes et al., 2006, Turney et al., 2006). However, whether biomass- and fossil fuel-derived BC emission sources result in distinct degradation dynamics of atmospheric BC in soils remains unclear.

Biogenic (e.g. biomass burning) and anthropogenic sources (e.g. diesel fuel) of BC can be distinguished using source-specific molecular tracers (Simoneit 1999). Among specific tracers for biogenic emission sources is levoglucosan, an anhydrous saccharide formed from the thermal degradation of cellulose (Simoneit et al., 1999, Fraser and Lakshmanan 2000, Sullivan and Ball 2012). Levoglucosan has been successfully used as a tracer for biomass burning worldwide (Zdráhal et al., 2003, Claeys et al., 2004, Jordan et al., 2006, Medeiros et al., 2006, Jia et al., 2010, Giannoni et al., 2012, Rada et al., 2013). Among the organic compounds that have been used to characterize fossil fuel emissions are $n$-alkanes that lack odd-C isomers enrichment found in contemporary biomass, petroleum biomarkers (e.g. cholestane), and polycyclic aromatic hydrocarbons (Simoneit 1986, Fraser et al., 2002a, Fraser et al. 2002b, Simoneit 2002, Katianova et al., 2008, Wang et al., 2009). Bond et al., (2004) reported that fossil fuel/biofuel and open burning in North America may account for 5% and 1.5% of the global BC emissions, respectively. However, the relative contribution of
plant biomass and fossil fuels to the total amounts of atmospheric BC deposition to soils remains poorly documented.

In this study we measured atmospheric BC concentration in fine particulates at a rural forested site in northern Michigan to estimate atmospheric BC deposition inputs to forest soils during summer 2010 and 2011. We calculated BC deposition based on measured BC to estimate the potential flux of BC to the local ecosystem. Finally, we quantified particle bound molecular marker compounds to identify the dominant emission sources of measured BC aerosol.

2. Materials and methods

2.1 Ambient aerosol sampling

Particulate matter with an aerodynamic diameter smaller than 2.5 µm (PM2.5) was collected in summer 2010 and 2011 at the United States Department of Agriculture (USDA) UVB measurement site (45.56 N and 84.68 W) located at the University of Michigan Biological Station (UMBS) in Pellston, Michigan. The UMBS is located approximately 350 km and 400 km north from Detroit (MI) and Chicago (IL), respectively. The established sampling site is an open field (1 ha) surrounded by a mixed hardwood forest that represents the interface of northern temperate and boreal forest. Soil BC was quantified in a previous study using a benzene polycarboxylic extraction method, and averaged 3.3% of soil C (Yarnes et al., 2011).

In 2010, air samples were collected for 54 days between July 8 and September 15, while in 2011 samples were collected for 41 consecutive days between July 22 and September 1. Samples of PM2.5 were collected on ashed (550°C, 8 hr) and pre-weighed quartz-fiber filters
(Whatman, 8 x 10 in) by a high volume (1132 L min\(^{-1}\)) air sampler (Thermo Fisher Scientific, Franklin, MA, USA) with an air inlet located at 3 m above the ground (230 m). The air sampler was equipped with a size-selective inlet (Model 340 HVVI, MSP Corp, Shoreview, MN, USA) to only collect PM2.5. In 2010, collection time per sample varied from 24 to 216 hour periods, while in 2011 collection time varied from 24 to 72 hour periods. After collection, samples were weighed and stored at -20°C until extraction. Average mass concentration of PM2.5 (\(\mu\)g m\(^{-3}\)) was calculated dividing the mass of PM2.5 (\(\mu\)g) by the total volume of air sampled (m\(^{-3}\)).

2.2 Organic carbon and black carbon

Organic carbon (OC) and elemental carbon (EC; a method-specific measurement related to the quantity of black carbon in the sample) were measured on a Thermal-Optical Carbon Transmittance analyzer (Sunset laboratory Inc., Tigard, OR) at Arizona State University (ASU) following the method described by Birch and Cary (1996). Briefly, a small portion (1.5 cm\(^2\)) of the filter sample was subsampled and heated stepwise in a He atmosphere (i.e. 310°C for 60 sec, 475°C for 60 sec, 615°C for 60 sec, and 870°C for 200 sec), and then separately heated under an oxidizing atmosphere (10% O\(_2\), and He) at 550 °C (45 sec), 625 °C (45 sec), 700 °C (45 sec), 775 °C (45 sec), 850 °C (45 sec), and 870 °C (120 sec). At each temperature step, C was oxidized to carbon dioxide and then reduced to methane for quantification using a flame ionization detector. The precision of the method has been reported by Clements et al. (2014). The operational definition of OC includes C volatilized during heating in an inert atmosphere, which includes C pyrolysed to EC; while EC (hereafter referred to as BC) is operationally defined as C evolved during heating in an oxidizing
atmosphere and corrected for pyrolysed OC. Average mass concentration of OC and BC (µg m⁻³) was calculated as described in section 2.1.

2.2.1 Atmospheric black carbon deposition
Dry deposition fluxes of atmospheric BC were estimated by multiplying BC concentration in filter sample and dry deposition velocity:

\[ F = C \times V_d \]  

(1)

Where \( F \) is the dry deposition flux (µg m⁻² s⁻¹), \( C \) is BC concentration (µg m⁻³), and \( V_d \) is the dry deposition velocity (m s⁻¹), which is the inverse sum of the atmospheric aerodynamic, quasi-laminar sublayer, and surface or canopy resistances. We selected a lower- and an upper-limit \( V_d \) values of \( 7 \times 10^{-4} \) and \( 1.5 \times 10^{-2} \) m s⁻¹, respectively, to represent a typical range of \( V_d \) for forest and grasslands (Pryor et al., 2008).

2.3 Organic molecular markers
2.3.1 Sample extraction
Analysis of organic molecular markers was performed by extraction of one fourth of each quartz filter. Prior to analysis, isotopically labeled standards (D-Glucose-1,2,3,4,5,6,6-d7; decanoic acid-d19 and tetradecanoic acid-d27; n-tetracosane-d50 and n-triacontane-d62 and n-hexatriacontane-d74; chrysene-d12 and dibenz[ah]anthracene-d14) were spiked to each sample prior to monitoring extraction recovery and loss. Each sample was ultrasonically agitated in the presence of three 30 ml aliquots of dichloromethane:methanol (2:1, v/v) for a period of 10 min and solvent extract combined, filtered, and concentrated using a rotary evaporator (Medeiros and Simoneit 2007). The extract was further concentrated using a stream of high-purity N₂ gas to a volume of approximately 200 µl. Polar compounds,
including carboxylic acids, alcohols, and sugars were blown down to complete dryness using high-purity N\textsubscript{2}, and derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane and pyridine for 3 h at 70 °C. Non-polar and polar compounds converted to their trimethylsilyl derivatives were analyzed by gas chromatography-mass spectrometry (GC-MS) within 24 h.

2.3.2 Sample analysis

A 2 μl aliquot of the derivatized and non-derivatized extracts were analyzed by a HP model 6890 GC coupled to a HP model 5973 mass selective detector. Separation was achieved using a 30 m HP-5MS capillary column. The GC operating conditions were as follows: temperature hold at 65 °C for 10 min, increase from 65 to 300 °C at a rate of 10 °C/min with a final isothermal hold at 300 °C for 5 min. The sample was injected splitless with the injector temperature at 300 °C. The MS was operated in electron impact mode at 70 eV and scanned from 50 to 500 Dalton targeting analysis of organic molecular markers including sugars, carboxylic acids, \textit{n}-alkanes, polycyclic aromatic hydrocarbons (PAHs), and cholestane. Individual molecular markers were identified based on authentic standards and comparison of mass spectra to literature and library data. Quantification of marker compounds was performed by spanning the sample concentration range with authentic standards and monitoring instrument performance using a co-injection standard. The uncertainty and reproducibility of the method has been reported by Yue and Fraser (2004).

2.4 Backward trajectory analysis
Interpretation of aerosol sources was complimented by backward trajectory analysis conducted using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model developed at the US National Oceanic and Atmospheric Agency (Draxler and Rolph, 2013; Rolph 2013). While not intended to be definitive analysis of origin, the goal was to determine whether mass transport to our measurement site during the sampling period was consistent with that originated from urban and industrial regions or remote forested regions.

2.5 Data analysis
Spearman rank correlation analyses were performed on PM2.5, OC, BC, and molecular markers using Systat v.10 (Systat Software, Inc. Chicago, IL, USA).

3. Results and discussion:
3.1 Atmospheric black carbon concentration and deposition
Average atmospheric concentrations of total C (TC, sum of OC and BC), OC, and BC measured at the UMBS in summer 2010 were similar to those measured in summer 2011 (Table 1 and 2). The average TC concentrations were 2.28 ± 0.93 µg m⁻³ in summer 2010, and 1.77 ± 0.54 µg m⁻³ in summer 2011. The average atmospheric levels of OC were 2.23 ± 0.93 µg m⁻³ and 1.72 ± 0.54 µg m⁻³ in summer 2010 and 2011, respectively. BC levels averaged 0.048 ± 0.06 µg m⁻³ in summer 2010, and 0.049 ± 0.064 µg m⁻³ in summer 2011. BC had a high day-to-day variability (Figures 1A and 1B), and comprised up to 11 and 13% of TC in summer 2010 and 2011, respectively.

The average BC concentrations reported in our study are comparable to those reported for a forest site in central Amazonia in July 2001 (0.047 µg m⁻³ and 0.066 µg m⁻³, Graham et al.,
In contrast, BC concentration levels in our measurement site were nearly twice as low as those reported for sites in Central Asia (Miller-Schulze et al., 2011), and lower than those reported for our measurement site (0.6 µg m\(^{-3}\)) during winter (Cadle and Dasch, 1988), rural and remote locations (0.74 and 0.66 µg m\(^{-3}\), respectively) in Tennessee (Tanner et al., 2004), downtown Toronto (1.19 µg m\(^{-3}\), Knox et al., 2009), suburban site in Italy (0.19 to 0.65 µg m\(^{-3}\), Giannoni et al., 2012), Detroit and Cleveland (0.3 to 0.52 µg m\(^{-3}\), Snyder et al., 2010), and the average BC concentration across 187 U.S. counties during summertime (0.54 µg m\(^{-3}\), Bell et al., 2007). These results suggest that BC aerosol levels at the UMBS are at the lower end of the atmospheric BC range reported for sites worldwide (Koch et al., 2009).

Typical meteorological conditions resulted in transport of air masses from the north; on occasion, backward trajectory analysis indicated transport of air masses from the west, southwest, south and southeast of the measurement site (Figure 2). Air masses transported from the north originate over Canada, and during these periods, significantly lower levels of pollutants were observed at the UMBS (Cooper et al., 2001). In contrast, the highest pollutants concentrations have been attributed to air masses originated from the south, southwest, and southeast, where the large metropolitan centers are located (Carroll et al., 2001, Cooper et al., 2001). Interpretation of the backward trajectory analysis and measured aerosol OC and BC levels are consistent with this general rule, and suggest that the UMBS is influenced by long-range transport of pollutants.

BC dry deposition fluxes over the 54- and 41-day sampling in 2010 and 2011, respectively, were calculated based on BC concentration measurements and deposition flux calculations.
Dry deposition values over 54-day sampling in 2010 ranged between 133 and 2850 µg BC m\(^{-2}\), while BC deposition values over 41-day sampling in 2011 ranged between 128 µg BC m\(^{-2}\) and 2745 µg BC m\(^{-2}\). We used V\(d\) values previously reported for forest and grasslands (Pryor et al., 2008), which encompass the maximum V\(d\) value (2 x 10\(^{-3}\) m s\(^{-1}\)) documented for BC at our measurement site in the wintertime (Cadle and Dasch, 1988). If extrapolated for the entire year of 2010 or 2011, the maximum annual BC deposition flux (19.3 and 24.4 mg BC m\(^{-2}\) yr\(^{-1}\) estimated for 2010 and 2011, respectively) would represent approximately 0.1% of the soil BC stock estimated for the UMBS (Yarnes et al., 2011). However, the dry deposition measurements in this study were limited to six and ten weeks during summertime of 2010 and 2011, respectively, and did not include measurements of wet deposition.

3.2 Molecular markers in PM2.5

Analysis and quantification of organic target compounds are consistent with the primary emission sources of atmospheric carbon particles in northern Michigan being predominantly biogenic. In our study, sugars and alkanoic acids dominated the composition of target compounds during summer 2010 and 2011 (Figure 3). The concentration of total sugars ranged from 15 and 125 ng m\(^{-3}\) (47 ± 34 ng m\(^{-3}\)) in 2010, and from 11 to 40 ng m\(^{-3}\) (22 ± 7.4 ng m\(^{-3}\)) in 2011, with the predominance of levoglucosan (34%, 22.2 ± 30.5 ng m\(^{-3}\)) and mannitol (25%, 8.8 ± 5.7 ng m\(^{-3}\)) in 2010, and glucose (30%, 6.3 ± 1.5 ng m\(^{-3}\)) and levoglucosan (27%, 6.2 ± 4.4 ng m\(^{-3}\)) in 2011 (Figure 4). Total alkanoic acids average concentrations ranged from 5 to 130 ng m\(^{-3}\) (42 ± 40 ng m\(^{-3}\)) in summer 2010, and from 7 to 42 ng m\(^{-3}\) (23 ± 9.5 ng m\(^{-3}\)) in summer 2011. The distribution of alkanoic acids (Figure 5) was dominated by docosanoic acids in 2010 (47%, 20 ± 22 ng m\(^{-3}\)) and 2011 (41%, 9.4 ± 6.4 ng m\(^{-3}\)), while the \(n\)-alkane group (Figure 6) was dominated by \(n\)-heptacosane (47%) and \(n\)-
nonacosane (28%). PAHs was the least abundant and frequent tracer group, occurring in only two sampling periods in July 2010, and with Indeno(1,2,3-cd) pyrene (44.5%) and benzo(g,h,i)perylene (35%) as the dominant species (Figure 7), possibly from local sources. Cholestanone was not detected in PM$_{2.5}$ collected in either 2010 or 2011, indicating that the influence of fossil fuel emissions on PM$_{2.5}$ in our measurement site was negligible.

The contribution of biomass burning to fine particle emissions in northern MI was tracked by the detection of levoglucosan in all samples except August 25-26 in 2011 (Figure 4). The atmospheric concentration of levoglucosan was comparable to that of a suburban background site (6.2 ng m$^{-3}$, Giannoni et al., 2012). Levoglucosan levels in our study were lower than those reported for rural sites in Texas (Jia et al., 2010) and for a rainforest site (38 ng m$^{-3}$, Claeys et al., 2004). In contrast, levoglucosan concentration in our study was higher than that reported for a rainforest site (4.4 ng m$^{-3}$, Zdrahal et al., 2002), a maritime background site (2 ng m$^{-3}$, Puxbaum et al., 2007), a northern harwood conifer forest (1.0 - 5.3 ng m$^{-3}$, Medeiros et al., 2006), and a Canadian Arctic site (up to 1.08 ng m$^{-3}$, Fu et al., 2009).

The strong positive correlation between levoglucosan and OC observed in summer 2011 (Table 4) reveals the importance of biomass burning as a source of OC in northern MI. The correlation between levoglucosan and OC has also been observed during winter in a suburban background site in Italy (Giannoni et al., 2012) and an urban site in Spain (Reche et al., 2012). In addition to OC, levoglucosan was strongly associated with eicosanoic and docosanoic fatty acids in summer 2010 and 2011 (Tables 3 and 4). These fatty acid compounds, together with palmitic acid, have been detected in air particles emitted from conifer and grass combustion (Oros and Simoneit 2001a, Oros et al., 2006). There was no correlation between
concentrations of levoglucosan and BC (Tables 3 and 4), suggesting that they originated from different sources. However, in summer 2011 concentrations of levoglucosan and BC peaked in August 6-8, which was the end of a particular period (August 2-8, 2011) marked by a strong plant wax signature as revealed by the high concentrations of docosanoic acid in PM$_{2.5}$ (Figure 5B).

Plant waxes are composed by long-chain fatty acid and $n$-alkane compounds, and thought to be emitted into the atmosphere by mechanical entrainment of biogenic material (Hadley and Smith 1989, Conte and Weber, 2002). The contribution of vascular plant waxes to PM$_{2.5}$ emissions throughout the sampling period is supported by (a) the predominance of docosanoic and palmitic acids, both found in basic units of plant fats, oils, and phospholipids (Figure 5), and (b) the predominance of long-chain (C$_{25}$-C$_{33}$) $n$-alkanes homologs with odd C number (Figure 6), particularly of $n$-nonacosane, which is known contribute significantly to aliphatic lipids in plant waxes (Hankin and Kolattukudy, 1968). The positive correlation between concentrations of docosanoic and levoglucosan suggests that plant waxes emissions co-occur with biomass burning events in our measurement site. The relationship between docosanoic and levoglucosan in our study is in agreement with a previous study that detected a substantial increase in $> C20$ $n$-alkanoic acids and levoglucosan levels during a wildfire plume in Maine (Medeiros et al., 2006). Evidence for the presence of plant wax compounds in smoke particles detected in this study is also supported by the dominance of $n$-heptacosane (C$_{27}$), $n$-nonacosane (C$_{29}$), and $n$-hentriacontane (C$_{31}$) in the $n$-alkanes group in our study (Figure 6). A similar trend has been reported for grass burning emissions (Oros et al., 2006). Grass smoke has also been shown to contain significant amounts of palmitic and docosanoic acids (Oros et al., 2006). The association between palmitic acid and $n$-nonacosane in 2010, as well as
palmitic acid and n-heptacosane in 2011 (Tables 3 and 4) further supports the hypothesis that plant waxes emissions also contribute to PM$_{2.5}$ in our measurement site.

4. Conclusions

This study reported the ambient concentration of BC, OC, and molecular markers in ambient PM$_{2.5}$ samples collected at the UMBS during two consecutive summers (2010 and 2011). We demonstrated that atmospheric BC concentrations in northern Michigan are lower than those reported for urban centers, and that PM$_{2.5}$ is influenced by two main biogenic emission sources: biomass burning and epicuticular plant waxes. Based on aerosol measurements and deposition flux calculations, we conclude that atmospheric BC deposition is unlikely to contribute significantly to BC stocks in northern Michigan forest soils. Nonetheless, BC concentrations reported in this study can facilitate future assessments of changes in BC levels in northern Michigan ecosystems. Long-term and continuous in situ measurements that include dry and wet BC deposition are needed to better understand BC fluxes from the atmosphere to terrestrial and aquatic ecosystems.

Acknowledgements

We thank D. Bryck for assistance with OC/EC analysis, and A. Clements for assistance with extractions and GC-MS analysis. We acknowledge the University of Michigan Biological Station for logistics and research facilities support. F.S. was funded by a NSF-IGERT Biosphere-Atmosphere Research Training program, UMBS.
Table 4.1 Sampling schedule, PM2.5 concentration, OC and BC contribution to total PM2.5, and estimated BC dry deposition in summer 2010.

<table>
<thead>
<tr>
<th>Month</th>
<th>Days (1)</th>
<th>PM2.5</th>
<th>OC</th>
<th>BC</th>
<th>BC dry deposition (2)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>µg m$^{-3}$</td>
<td>%</td>
<td>%</td>
<td>µg m$^{-2}$</td>
</tr>
<tr>
<td>July</td>
<td>8-11</td>
<td>5.9</td>
<td>26.6</td>
<td>0.61</td>
<td>6.6 - 141</td>
</tr>
<tr>
<td></td>
<td>11-13</td>
<td>2.5</td>
<td>36.1</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>13-14</td>
<td>11.7</td>
<td>21.0</td>
<td>0.01</td>
<td>0.06 - 1.2</td>
</tr>
<tr>
<td></td>
<td>14-15</td>
<td>8.5</td>
<td>19.9</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>15-16</td>
<td>9.8</td>
<td>40.9</td>
<td>0.41</td>
<td>1.8 - 39.2</td>
</tr>
<tr>
<td></td>
<td>16-17</td>
<td>9.5</td>
<td>28.1</td>
<td>0.21</td>
<td>1.4 - 29.8</td>
</tr>
<tr>
<td></td>
<td>17-18</td>
<td>7.5</td>
<td>19.4</td>
<td>0.01</td>
<td>0.06 - 1.2</td>
</tr>
<tr>
<td></td>
<td>18-19</td>
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<td>87.8</td>
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<td>0.0</td>
</tr>
<tr>
<td></td>
<td>19-20</td>
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<td>31.1</td>
<td>0.18</td>
<td>0.66 - 14.1</td>
</tr>
<tr>
<td></td>
<td>21-27</td>
<td>6.2</td>
<td>26.9</td>
<td>0.13</td>
<td>2.9 - 63.2</td>
</tr>
<tr>
<td></td>
<td>27-28</td>
<td>5.9</td>
<td>30.9</td>
<td>1.47</td>
<td>5.2 - 112</td>
</tr>
<tr>
<td></td>
<td>28-29</td>
<td>9.7</td>
<td>40.6</td>
<td>0.02</td>
<td>0.14 - 3.0</td>
</tr>
<tr>
<td></td>
<td>31-1</td>
<td>14.1</td>
<td>17.9</td>
<td>1.23</td>
<td>15.8 - 338</td>
</tr>
<tr>
<td>August</td>
<td>1-2</td>
<td>20.2</td>
<td>11.4</td>
<td>0.68</td>
<td>8.2 - 175</td>
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<tr>
<td></td>
<td>2-3</td>
<td>15.2</td>
<td>16.3</td>
<td>0.89</td>
<td>8.1 - 174</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>8.1</td>
<td>28.1</td>
<td>1.82</td>
<td>8.8 - 189</td>
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<tr>
<td></td>
<td>4-5</td>
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<td>38.0</td>
<td>0.00</td>
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<td></td>
<td>5-14</td>
<td>10.7</td>
<td>16.1</td>
<td>0.05</td>
<td>2.8 - 59.7</td>
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<tr>
<td></td>
<td>19-26</td>
<td>4.0</td>
<td>22.4</td>
<td>2.80</td>
<td>47 - 1007</td>
</tr>
<tr>
<td></td>
<td>26-30</td>
<td>74.2</td>
<td>2.6</td>
<td>0.07</td>
<td>12 - 257</td>
</tr>
<tr>
<td>September</td>
<td>1-3</td>
<td>12.1</td>
<td>24.8</td>
<td>0.64</td>
<td>9.4 - 201</td>
</tr>
<tr>
<td></td>
<td>8-15</td>
<td>5.0</td>
<td>15.2</td>
<td>0.10</td>
<td>2.1 - 45.9</td>
</tr>
</tbody>
</table>

(1) 24-hr sampling except on July 8-11 (72-hr), 11-13 (48-hr), 21-27 (144-hr); August 5-14 (216-hr), 19-26 (168-hr), 26-30 (96-hr); September 1-3 (48-hr), 8-15 (168-hr).

(2) Range values estimated using Equation 1 as described in the Methods section (2.2.1)
Table 4. 2 Sampling schedule, PM2.5 concentration, OC and BC contribution to total PM2.5, and estimated BC dry deposition in summer 2011.

<table>
<thead>
<tr>
<th>Month</th>
<th>Days(^{(1)})</th>
<th>PM2.5</th>
<th>OC</th>
<th>BC</th>
<th>BC dry deposition (^{(2)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\mu g \text{ m}^{-3})</td>
<td>%</td>
<td>%</td>
<td>(\mu g \text{ m}^2)</td>
</tr>
<tr>
<td>July</td>
<td>22-24</td>
<td>7.7</td>
<td>22.6</td>
<td>1.10</td>
<td>10.2 - 218</td>
</tr>
<tr>
<td></td>
<td>24-25</td>
<td>5.7</td>
<td>43.7</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>25-27</td>
<td>5.0</td>
<td>16.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>27-29</td>
<td>9.3</td>
<td>18.3</td>
<td>1.07</td>
<td>11.8 - 253</td>
</tr>
<tr>
<td></td>
<td>29-31</td>
<td>7.3</td>
<td>23.9</td>
<td>0.28</td>
<td>2.57 - 55.1</td>
</tr>
<tr>
<td>August</td>
<td>31-2</td>
<td>7.6</td>
<td>25.3</td>
<td>0.09</td>
<td>0.83 - 17.7</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
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<td>34.0</td>
<td>0.18</td>
<td>1.41 - 30.3</td>
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<tr>
<td></td>
<td>4-6</td>
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<td>0.42 - 8.99</td>
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<td>0.00 - 0.77</td>
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<td>0.00 - 0.00</td>
</tr>
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<td>21.9</td>
<td>3.26</td>
<td>32.7 - 701</td>
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<td>32.9</td>
<td>2.26</td>
<td>6.21 - 133</td>
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<td></td>
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<td>32.8</td>
<td>0.00</td>
<td>0.00 - 0.07</td>
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<tr>
<td></td>
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<td></td>
<td>30-1</td>
<td>19.9</td>
<td>10.3</td>
<td>0.55</td>
<td>13.2 - 283</td>
</tr>
</tbody>
</table>

\(^{(1)}\) 48-hr sampling except on July 24-25 (24-hr), August 22-25 (72-hr), and August 25-26 (24-hr)

\(^{(2)}\) Range values estimated using Eq. (1) as described in the Methods section (2.2.1)
Table 4. 3 Spearman correlation coefficients of the relationships among levoglucosan, palmitic and eicosanoic acids, PM2.5, OC, and BC at the UMBS between July and September 2010. Coefficient values in bold denote $P < 0.05$ (N = 22).

<table>
<thead>
<tr>
<th></th>
<th>Levoglucosan</th>
<th>Palmitic</th>
<th>Eicosanoic</th>
<th>PM2.5</th>
<th>OC</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.00</td>
<td>0.50</td>
<td>0.39</td>
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<td>OC</td>
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<td>0.66</td>
<td>0.50</td>
<td>1.00</td>
<td>0.06</td>
</tr>
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<td>BC</td>
<td>0.11</td>
<td>-0.15</td>
<td>0.11</td>
<td>0.03</td>
<td>0.06</td>
<td>1.00</td>
</tr>
<tr>
<td>Levoglucosan</td>
<td>1.00</td>
<td>-0.37</td>
<td>0.67</td>
<td>0.50</td>
<td>0.38</td>
<td>0.11</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-0.04</td>
<td>0.40</td>
<td>0.22</td>
<td>0.04</td>
<td>0.35</td>
<td>0.26</td>
</tr>
<tr>
<td>Arabinol</td>
<td>-0.04</td>
<td>0.34</td>
<td>-0.11</td>
<td>-0.03</td>
<td>0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.04</td>
<td>0.37</td>
<td>0.28</td>
<td>0.09</td>
<td>0.43</td>
<td>0.23</td>
</tr>
<tr>
<td>Glycerol</td>
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<td>0.29</td>
<td>0.63</td>
<td>0.13</td>
<td>0.39</td>
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<tr>
<td>Sorbitol</td>
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<td>0.46</td>
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<td>Octadecanoic</td>
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<td>0.55</td>
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<td>0.13</td>
<td>0.35</td>
<td>-0.15</td>
</tr>
<tr>
<td>Palmitic</td>
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<td>1.00</td>
<td>-0.19</td>
<td>-0.18</td>
<td>0.08</td>
<td>-0.15</td>
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<td>Eicosanoic</td>
<td>0.67</td>
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<td>0.31</td>
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<tr>
<td>Myristic</td>
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<td>0.58</td>
<td>0.26</td>
<td>0.05</td>
<td>0.48</td>
<td>-0.13</td>
</tr>
<tr>
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</tr>
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<td>-0.26</td>
<td>0.35</td>
<td>0.24</td>
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Table 4. Spearman correlation coefficients of the relationships among levoglucosan, palmitic and eicosanoic acids, PM2.5, OC, and BC at the UMBS between July and September 2011. Coefficient values in bold denote $P < 0.05$ (N = 21).

<table>
<thead>
<tr>
<th></th>
<th>Levoglucosan</th>
<th>Palmitic</th>
<th>Eicosanoic</th>
<th>PM2.5</th>
<th>OC</th>
<th>BC</th>
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<tr>
<td>PM2.5</td>
<td>0.40</td>
<td>-0.19</td>
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<td><strong>0.53</strong></td>
<td>0.23</td>
<td><strong>1.00</strong></td>
</tr>
<tr>
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<td>0.40</td>
<td><strong>0.69</strong></td>
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<tr>
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<td>0.42</td>
<td>-0.13</td>
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<tr>
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<td>0.19</td>
<td>0.05</td>
<td>0.11</td>
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<tr>
<td>Glycerol</td>
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<td>0.16</td>
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</tr>
<tr>
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<td>0.42</td>
<td>-0.06</td>
<td>0.04</td>
<td>-0.03</td>
</tr>
<tr>
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<td>-0.19</td>
<td>-0.20</td>
<td>-0.30</td>
</tr>
<tr>
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<tr>
<td>Myristic</td>
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<td><strong>0.50</strong></td>
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<td>Docosanoic</td>
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<tr>
<td>Decanoic</td>
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<td>-0.10</td>
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<td><strong>0.46</strong></td>
<td>0.37</td>
<td>0.13</td>
</tr>
<tr>
<td>Octanoic</td>
<td>0.33</td>
<td>0.01</td>
<td><strong>0.46</strong></td>
<td>0.00</td>
<td>0.10</td>
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<tr>
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<td>Pentacosane</td>
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<td>-0.15</td>
<td>0.31</td>
<td>-0.15</td>
<td>0.25</td>
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</tbody>
</table>
Figure 4. BC concentration (µg m$^{-3}$) measured at the UMBS (A) from July 8 to September 15 2010 and (B) from July 22 to September 1 2011.
Figure 4. Two-day backward trajectories in 2011 showing the influence of air masses from the north on July 25-27 (A) and August 10-12 (B), and from the south on August 18-20 (C) and August 20-22 (D).
Figure 4. 3 Relative abundance of each class of molecular marker compound in PM2.5 collected in (A) 2010 and (B) 2011 at the UMBS.
Figure 4. Relative abundance of each target sugar compound in PM2.5 collected in (A) 2010 and (B) 2011 at the UMBS.
Figure 4. 5 Relative abundance of each target fatty acid compound in PM2.5 collected in (A) 2010 and (B) 2011 at the UMBS.
Figure 4. 6 Relative abundance of each target $n$-alkane compound in PM2.5 collected in (A) 2010 and (B) 2011 at the UMBS.
Figure 4. 7 Relative abundance of each target polycyclic aromatic hydrocarbon (PAH) compound in PM2.5 collected in 2010 at the UMBS.
5. References


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Chapter 6: Environmental controls of fine-roots decomposition dynamics in a northern temperate forest soil

Santos, F., LeMoine, J., Nadelhoffer, K., Bird, J. A. Environmental controls of fine-roots decomposition dynamics in a northern temperate forest soil. Biogeochemistry, in preparation

Abstract

Fine-roots (<2 mm diameter) are considered important sources of soil carbon (C) and nitrogen (N) in forest ecosystems. While a significant fraction of the stable soil organic C is thought to be derived from root turnover as litter, the primary factors that control fine-root C and N mineralization rates in temperate forest soils are not well understood. In a two-year field study we examined the influence of long-term aboveground litter and inorganic N additions on fine-root decomposition and vertical transport of root-derived C and N. The fate of dual-labeled (\(^{13}\text{C}/^{15}\text{N}\)) red maple (Acer rubrum) fine roots was followed in soils within a long-term litter manipulation study (Detritus Input and Removal Treatments or DIRT), located in Pellston, MI, USA. In Fall 2010, labeled fine-roots were applied to soil mesocosm within DIRT treatments that (1) received high levels of inorganic N addition as fertilizer; (2) had no belowground litter inputs, (3) no aboveground and belowground litter inputs, or (4) no removal of litter or additional N added (control). Zero-tension lysimeters were installed underneath mesocosms to collect soil leachate. Mesocosms without added fine-roots served as experimental controls. After one and two years in situ, the recovery of \(^{13}\text{C}\) and \(^{15}\text{N}\) from fine-roots in soil particulate (> 2 mm) and bulk soil (< 2mm) frations was determined within two depths (0-10 and 10-20cm). In addition, we quantified fine-root-\(^{13}\text{C}\) in dissolved organic carbon (DOC) leached below 20-cm soil depth, and periodically measured root-derived C loss
as $^{13}$CO$_2$ during the 2-yr study. In the first 2 years of decomposition, maple fine-root litter lost approximately 80 and 64% of their initial C and N, respectively. The removal of above and belowground litter (NI) in the DIRT plots significantly reduced the retention of maple root C in bulk soil within 10-20 cm increment depth after 2 years *in situ*, but had no effect on the total retention of maple root C in the mesocosm. Conversely, the overall retention of maple root N increased in the NI treatment in the entire mesocosm and with 0-10cm depth during the 2-yr field incubation study. Additions of inorganic N no effect on the recovery of root C, but reduced the retention of maple root N. Litter manipulation and inorganic N addition treatments had no effects of on the losses of root C as either CO$_2$ or DOC. Our results suggest that root C and N cycle relatively fast in northern temperate forest soils when compared with root C and N reported by previous studies, and eight years of litter manipulation and N additions was likely too short to significantly affect C fluxes from SOM in this ecosystem. Decadal time scale field studies that focus on the factors and mechanisms controlling the C and N pools of root litter in temperate forests are needed if we want to improve our ability to predict the long-term responses of belowground C to climate change.
1. Introduction

Soils store approximately three times the size of the C pool in the atmosphere (800 Gt C, Schimel 1995, Batjes 1996, Lal 2004), and a significant portion of the soil organic C (SOC) is supplied by roots via exudates or as root litter (Kramer et al. 2010, Rumpel and Kogel-Knabner 2011, Tefs and Gleixner 2012, Clemmensen et al. 2013). Root litter may account for up to 41% of the net primary production in temperate forests (Gough et al. 2008) and 88% of total roots reported in boreal forest soils (Persson 2012), its stability in soils has been inkoved by several studies (Balesdent and Balabane 1996, Nierop 1998, Rumpel et al. 2002, Rumpel et al. 2004, Nierop et al. 2006, Crow et al. 2009). Evidence to support this apparent stability includes the observation that fine root litter remains in soils longer than aboveground litter, which has been attributed to the higher lignin levels in fine roots (< 2mm diameter) relative to needles or leaf (Rasse et al. 2005, Bird and Torn 2006, Xiong et al. 2013). However, few studies have investigated root decomposition dynamics and its influence on belowground C and N pools and fluxes. In addition, little data exists on the influence of environmental factors (e.g. labile C and inorganic N inputs) on the decomposition rates of root litter in temperate forest soils. Understanding the importance of these factors in regulating C and N dynamics of root litter can improve our ability to predict soil organic matter responses to climate change.

The continuous supply of fresh or labile C to soils is thought to stimulate (positive priming) the decomposition rates of older and less easily degradable soil organic matter (SOM). For example, additions of plant-derived C (e.g. root exudates, cellulose) have been reported to accelerate the decomposition rates of native SOM by several incubation studies via co-metabolism (Cheng et al. 2003, Fontaine et al. 2004, Fontaine et al. 2011, Paterson et al.
Soils that received cellulose additions released nearly twice as much old and slowly-decaying SOC as control soils in a 169-day incubation study (Fontaine et al. 2007). In a field study in Panama, soils lost 1.5 times more SOC in treatments with litter additions relative to control treatments (Sayer et al. 2011). In contrast, the exclusion of litter inputs to soils for more than 50 years has been shown to decrease the SOM contents (Paterson et al. 2011). Despite the existing knowledge on the priming effects of plant litter inputs to soils on SOM decomposition, the extent at which these priming effects influence the C and N dynamics of root litter remains unknown.

Root litter decomposition rates could be inhibited by increasing levels of inorganic N in soils. Contrasting effects of inorganic N addition on litter decay have been shown by meta-analysis studies, with additions of N leading to faster decomposition rates of litter with low lignin contents, while slowing down the decay rate of lignin-rich litter (Knorr et al. 2005, Janssens et al. 2010). Similarly, experimentally adding N to soils have been shown to promote the activity of polysaccharide-degrading enzymes, whereas it suppressed the losses of lignin after one year of litter decomposition (Talbot and Treseder 2012). Given that lignin has been reported to be more abundant in root litter than in needle and leaf litter (Bird and Torn 2006, Xiong et al. 2013), long-term atmospheric N deposition could potentially increase the mean residence time of root litter C. While the additions of ammonium sulphate to soils have been reported to increase root mass loss in a Norway spruce stand (Majdi 2007), and additions of N-NO$_3$ have been shown to increase DOC concentration in northern hardwood forest soils (Pregitzer et al. 2004), little is known about the response of root C and N to the additions of inorganic N to soils.
The objective of this study was to assess the effects of litter inputs on the decomposition of dual-labeled ($^{13}$C/$^{15}$N) red maple (*Acer rubrum*) fine roots (< 2 mm diameter) during 2-yr using a long-term litter and N manipulation experimental site (the DIRT, Detritus Inputs and Removal Treatment, plots) located at the University of Michigan Biological Station (UMSB) in Pellston (MI). We hypothesized that the limitation of fresh litter inputs to soils would increase the C mineralization rates from added roots, and root-C leaching to lower soil depths, consequently decreasing the retention of root C and N in soils. In a second experiment, we examined the effects of inorganic N additions on the retention of fine root C and N in soils. We hypothesized that the addition of inorganic N to soils would increase the retention of root C and N to soils by inhibiting the C mineralization rates of fine roots, and decreasing losses of root C to lower soil depths as soil leachate.

2. Materials and methods

2.1 Study site

The DIRT plots (45° 33.6' N, 84° 42.6' W) are located at the UMB, in Pellston, MI. This experimental site is part of a larger network of DIRT projects (Nadelhoffer et al. 2004) established in Harvard Forest (MA), Bousson Environmental Research Reserve (PA), H. J. Andrews Experimental Forest (OR), and in Sikfikut Forest (Hungary). The canopy surrounding the DIRT plots at the UMBS is dominated by *Populus grandidentata* (bigtooth aspen), *Quercus rubra* (northern red oak), *Betula papyrifera* (paper birch), and *Acer rubrum* (red maple) in a transition zone between mixed hardwood and boreal forests. The site is located at 235 - 238 m elevation, with a mean annual temperature and precipitation of 6.8 °C and 838 mm, respectively (average from 1983-2013, Vande Kopple 2014). The soils are well-drained, sandy (92.9% sand, 6.5% silt, 0.6% clay, Curtis et al. 2005) spodosols developed on outwash plains, and classified as mixed, frigid Entic Haplorthods (Soil Survey Staff, 2014).
Atmospheric inorganic N deposition reported in 2012 for this area was 4.1 kg ha\(^{-1}\) (National Atmospheric Deposition Program 2014). The DIRT plots in northern MI were established in 2004, and consists of replicated treatments (5m x 5m, n = 3) in which the above and/or below-ground litter is either excluded or added (Nadelhoffer et al. 2004). Additional DIRT plots at the UMBS include N fertilization treatments.

2.2 \(^{13}\)C/\(^{15}\)N-enriched red maple fine roots

*Acer rubrum* (red maple) fine roots were grown and labeled with \(^{13}\)C and \(^{15}\)N between May and September 2009 in a greenhouse facility at Queens College, NY. The labeling of red maple saplings was conducted in a temperature-controlled growth chamber modified from Bird at al. (2003). \(^{13}\)C labeling was accomplished by exposing red maple saplings to enriched \(^{13}\)CO\(_2\) (25 atom %) once a week for a total of 18 weeks. \(^{15}\)N labeling was accomplished by fertilizing the maple saplings with \(^{15}\)NH\(_4\)Cl and K\(^{15}\)NO\(_3\) at (19.3\% atom excess), once a week for a total of 21 weeks. After harvesting, red maples fine roots were clipped, air-dried, and subsampled for chemical composition, elemental and stable isotope analyses (TABLE 2). Homogenized subsamples of fine roots were analyzed in duplicates for total organic C and N on a CHN gas analyzer (Costech Model 4010, Valencia, CA). Carbon and N isotopic enrichment (Table 2) was measured in duplicates on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20e20 Isotope Ratio Mass Spectrometer (IRMS, Sercon Ltd., Cheshire, UK). The initial chemical composition of labeled fine roots was determined on homogenized subsamples (n=3, Table 2) using a forest products determination of proximate C fractions according to Ryan et al. (1990). This method yields non-polar extractable component (waxes, fats, and chlorophylls), water soluble (simple sugars, hydroxyl phenol groups, and amino acids), acid soluble extractables (plant polysaccharides, proteins, polypeptides, some amino acids, and nuclei acids), and acid insoluble residue (lignin).
Calcium (Ca), potassium (K), phosphorous (P), and magnesium (Mg) concentrations of labeled fine roots (n=3, Table 2) were determined by inductively coupled plasma atomic emission spectrometry following hot plate acid digestion (Kalra 1997). Briefly, 0.3g of ground was digested at 120°C for 1hr and 180°C for 10min in a fluorocarbon vessel containing 4mL of acid mixture (60% HNO₃ and 40% HClO₄), and 1mL of HClO₄.

2.3 Field incubation design

In July 2010 mesocosms (10cm diameter and 22cm long PVC cylinder) were inserted into the soil. The mesocosms had two clusters of 0.4 cm diameter holes that were drilled 0.5 - 1 cm apart to allow fungal hyphae, fine roots, and earthworms to access the core. They were allowed to equilibrate for approximately eight weeks prior to the application of ¹³C/¹⁵N-labeled red maple fine roots.

2.3.1 Experimental treatment I: litter inputs

To test the effects of litter additions on the decomposition of labeled maple fine roots, soil mesocosms were placed within DIRT plots (Table 1) that had no belowground roots (NR), and no below- and above-ground litter additions (NI). In the NR treatment, roots were excluded by trenching and placing an impermeable plastic barrier around the perimeter of the plot. In the NI treatment, the aboveground litter inputs are removed using a mesh screen to collect the falling litter, while the roots were excluded as described for NR plots. Treatments with normal above and below ground litter inputs served as controls (C).

2.3.2 Experimental treatment II: nitrogen additions
To investigate the impacts of N additions on the decomposition of labeled maple fine roots, mesocosms were placed within plots that received nitrogen additions (Table 1) at a rate of 30kg N ha\(^{-1}\) yr\(^{-1}\) (as NH\(_4\)Cl) in three annual applications (May, August, and November). This application rate was approximately three times above background inorganic N deposition reported for this site (National Atmospheric Deposition Program 2014). Treatments with no experimentally added N served as controls.

### 2.3.3 Addition of \(^{13}\)C/\(^{15}\)N-enriched red maple fine roots to soils

On September 14, 2010, fine roots (1.1g C and 0.025g N) were added to mesocosms in DIRT plots from experiment I and II by mixing the labeled roots with the soil between 1 and 4 cm placement depth interval. Each plot had mesocosms with or without added \(^{13}\)C/\(^{15}\)N-labeled root. Soil mesocosms that received no addition of labeled roots received the same disturbance as those that received labeled roots, and served as controls. All soil mesocosms were left understurbed until excavation.

### 2.4 Root C and N recovery from soil

Intact mesocosms with or without applied \(^{13}\)C/\(^{15}\)N dual-labeled fine roots were excavated from each DIRT plot on two dates: September 15, 2011 (after 1 year \textit{in situ}), and August 18, 2012 (after 2 years \textit{in situ}). Following the excavation, soil mesocosms were brought to the laboratory at UMB where they was stored at 4 °C until processing and analysis (<7 days). For each mesocosm, soil was separated and subsampled by depth (0-10 and 10-20 cm). For both depth intervals, soil was sieved (2 mm mesh size), and the two soil size fractions (> and < 2 mm) were subsampled. Soil subsamples were homogenized by ball milling and analyzed for total elemental and stable isotope analyses. Total soil organic C and N were measured using a
CHN gas analyzer (Costech Model 4010, Valencia, CA). Carbon and N isotopic enrichment was measured on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20e20 IRMS (Sercon Ltd., Cheshire, UK). Isotopic composition was expressed in parts per thousand (‰) by using the delta (δ) notation. The isotopic ratios were calculated relative to the Pee Dee Belemnite (PDB) standard for C and relative to atmospheric N$_2$ for N. The total recovery of applied fine root-$^{13}$C in soil was fit to a single exponential model as in Eqn. (1):

$$^{13}\text{C} (S_t) = S_0 e^{(-kt)}$$  \hspace{1cm} (1)

where $S_0$ is the proportion (%) of C remaining in soil from added fine root, $S_t$ is the proportion of C remaining in soil at time $t$, and $k$ is the decay rate constant (day$^{-1}$). Curve fitting was performed using SigmaPlot for Windows (v. 10). The MRT of root C was calculated as in Eqn. (2),

$$\text{MRT} = 1/k$$  \hspace{1cm} (2)

where MRT is the mean residence time (days) of root C, and $k$ is as noted in Eqn. (1). In this study MRT was reported in years.

2.5 Soil CO$_2$ respiration and $^{13}$CO$_2$ efflux

Soil CO$_2$ flux rates were measured periodically in all DIRT treatments in 22 September 2010, 20 May 2011, 29 June 2011, and 19 August 2011, and 18 August 2012, during the 2-year study using a LI-6400 portable infrared gas analyzer and a soil respiration chamber (LI-COR, Lincoln, NE) modified for headspace gas collection (Torn et al. 2003). Soil-respired CO$_2$ efflux was measured three times per plot. In addition to CO$_2$ fluxes measurements, CO$_2$ was sampled at five time points per plot for $^{13}$C-CO$_2$ analysis. The $\delta^{13}$C signature was calculated as the y-intercept of the linear regression of $\delta^{13}$C versus [CO2]$^{-1}$ for the five data points per plot. The $\delta^{13}$C value in CO$_2$ was measured on a Finnigan Delta$^{\text{plus}}$ Continuous –Flow IRMS.
(Thermo Scientific) interfaced with a GasBenchII. Stable isotope values are reported in standard notation and referenced to Vienna PeeDee Belemnite (VPDB).

### 2.6 Soil C leachate and $^{13}$C-Dissolved organic C

In August 2010, each DIRT plot from experiment I and II had one zero-tension lysimeter installed underneath (20 cm depth) a soil mesocosm with, and without added labeled root to collect soil leachate. Soil solution collected after the addition of labeled fine roots were discarded to allow the lysimeters time to equilibrate. Collection of soil solution occurred after a rain event on August 13 (August 2011) and September 3 (September 2011), approximately 11 and 12 months after the addition of labeled fine roots to mesocosms. Upon collection, all samples were immediately transferred to the laboratory at the UMBS, where soil solution volume was recorded. Samples were filtered using a pre-combusted ($450^\circ$C for 5hr) filters (Whatman GF/F, < 0.7 µm), and stored at -20$^\circ$C until chemical analysis. Dissolved organic C (DOC) concentration in soil leachate was measured on a total organic C (TOC) analyzer (Shimadzu, TOC-V CPH, Kyoto, Japan). The $^{13}$C enrichment of DOC was determined using an O.I. Analytical TOC Analyzer (Model 1030, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) utilizing a GD-100 Gas Trap Interface (Gradn Instruments). Analytical precision for $^{13}$C of DOC was 0.4‰. Samples were acidified and purged with helium off-line to remove all dissolved inorganic carbon prior to measurement. The $\delta^{13}$C values were corrected based on laboratory standards and international references analyzed with the samples.

### 2.7 Calculations
The contribution of C ($f_{\text{root } C}$) and N ($f_{\text{root } N}$) from labeled fine roots to soil C and N fluxes and pools was calculated from a mass balance of isotopic signatures, as in Eqn. (3) and (4),

$$f_{\text{root } C} = (\delta^{13}C_{\text{soil sample}} - \delta^{13}C_{\text{control soil}})/(\delta^{13}C_{\text{labeled root}} - \delta^{13}C_{\text{control soil}})$$  \hspace{1cm} (3)

$$f_{\text{root } N} = (\text{atom}\%\text{ }^{15}N \text{ excess}_{\text{soil sample}})/(\text{atom}\%\text{ }^{15}N \text{ excess}_{\text{root}})$$  \hspace{1cm} (4)

where in Eqn. (3) $\delta^{13}C_{\text{soil sample}}$ and $\delta^{13}C_{\text{control soil}}$ are the $\delta^{13}C$ value (‰) in soil, evolved CO$_2$ or DOC from sample treatment (added labeled fine roots) and unamended soil (no added labeled fine roots), respectively; $\delta^{13}C_{\text{labeled root}}$ is the $\delta^{13}C$ value (‰) of labeled fine roots. In Eqn. (4), atom % $^{15}N$ excess is defined as atom% $^{15}N$ minus background atom% $^{15}N$; atom% $^{15}N$ excess$_{\text{soil sample}}$ and $^{15}N$ excess$_{\text{root}}$ refer to the $^{15}N$ enrichment in bulk soil or soil fractions, and in labeled fine root, respectively. The mass of added fine root C or N recovered in soil and pools was calculated by multiplying $f_{\text{root } C}$ by total amount of C in bulk soil, soil fractions, evolved CO$_2$ or DOC, and by multiplying $f_{\text{root } N}$ by total N mass in bulk soil or soil fractions.

2.8 Statistical analyses

The effect of DIRT treatments on root C and N recovered in different soil fractions and depths, as well as on DOC was tested using repeated measures and individual one-way analysis of variance (ANOVA) on individual sampling dates. Comparisons between treatments for soil CO$_2$ effluxes were performed using repeated measures one-way ANOVA. We used a $P < 0.05$ as the a priori error for significance between means, but $P < 0.10$ is also reported. All analyses were conducted using Systat v.10 (Systat Software, Inc., Chicago, IL, USA) for Microsoft Windows.
3. Results

3.1 Retention of labeled fine root C and N in DIRT treatments

After 2 yr *in situ*, 19.5 ± 3.6% of applied root C and 33.7 ± 5.4% of applied root N were recovered from soil mesocosms (0-20cm) within DIRT treatments that had received normal litter inputs, and no experimental additions of N (Table 3). At the end of the 2-yr decomposition study, the greatest retention of root C and N in all DIRT treatments *in situ* occurred in bulk soil (< 2mm fraction) within 0-10 cm depth interval. This fraction retained an average of 73 and 81% of the root C and N remaining in soil mesocosms, respectively, (Table 3).

On average, the C-to-N (C:N) ratio of roots remaining in soil mesocosms decreased during the 2-yr decomposition study (Figure 1). The C:N ratio of fine roots, initially 44, declined to an average of 27 ± 0.9 at the end of year 1, and to an average of 25 ± 1.1 at the end of year 2. After 2-yr *in situ*, the C:N ratio of roots recovered from mesocosms in treatments with no above or belowground litter inputs (NI) was 14% lower relative to control treatments (*P* = 0.047, one-way ANOVA). This trend was also noticeable at 0-10cm depth in year 2, but not statistically significant (*P* = 0.053, one-way ANOVA). Despite the lack of significant difference among DIRT treatments at 0-10cm depth, the C:N ratios of the labeled roots recovered in bulk soil was 19% lower in NI than in control treatments in year 2 (*P* = 0.044). After 2-yr in situ, while the C:N ratios of labeled roots within 10-20cm depth tended to be lower in NI than in control treatments, differences among these treatments were not statistically significant (*P* = 0.062). In year 2, fine roots C:N ratios in treatments with no belowground inputs (NR) were not statistically significant different from those in control
treatments ($P = 0.087$, one-way ANOVA). Similarly, C:N ratios of fine roots in treatments that received additions of N were not significantly different from control treatments (data not shown).

At the end of the 2-yr study, between 0.6 and 0.3% of the added root C was recovered within 10-20cm depth interval for all DIRT treatments (Table 3). The total retention of root C within 10-20cm depth was 58% lower in NI than in control treatments ($P = 0.03$, one-way ANOVA). Similarly, the retention of root C in the bulk soil fraction within 10-20cm depth interval declined by 55% in NI treatments when compared with control treatments ($P = 0.036$, one-way ANOVA). Despite the negative effects of NI treatments on the retention of root C within 10-20cm depth interval, less than 0.7% of the added root C and N was recovered within that depth interval at the end of the study. During the 2-yr study, NI treatment increased the total retention of root N in soil mesocosms ($P = 0.036$, repeated measures one-way ANOVA), and within 0-10cm depth interval when compared with control treatments ($P = 0.037$, r. m. one-way ANOVA, Table 3). The retention of root N in soil mesocosms was not affected by the removal of belowground litter inputs (NR) during the 2-yr study.

While the total recovery of root C in soil mesocosms was unaffected by DIRT treatments at the end of the 2-yr study, changes in root N recovery from soils during the 2-yr study were affected by treatments that received N additions (Table 3). Soils that received additions of N had 33% less root N retained in soil mesocosms (0-20cm depth) compared to control treatments ($P = 0.035$, repeated measures one-way ANOVA). Despite the differences observed between treatments with and without N additions, the recovery of root N at different
depth intervals (0-10 and 10-20cm) and soil fractions (> and < 2mm) was unaffected by N addition treatments.

### 3.2 C mineralization and leaching from labeled fine roots in DIRT treatments

Losses of root C losses as respired CO$_2$ within litter manipulation (NR or NI) and N addition treatments were not significantly different from control treatments during the 2-yr field incubation study (Figure 2). The highest rates of C-CO$_2$ losses from roots occurred eight days (September 2010) after the root application to soil mesocosms, and likely reflect the fast mineralization rates of the labile C pools from root litter. After that first sampling date, the variation observed in root C losses data appears to have been influenced by season, with the fastest and slowest root C mineralization rates occurring in the summer and fall, respectively. Similarly to soil C-CO$_2$ efflux from roots, losses of root C as DOC in soil leachate collected after two single rain events in August and September 2011 were not affected by either NR or NI treatment (Table 3). While treatments with added N tended to decrease losses of root C as DOC by 6.2% relative to control treatments, differences among treatment groups were not statistically significant ($P = 0.076$). Nonetheless, losses of root C as DOC were up to three orders of magnitude lower than those as CO$_2$ efflux.

### 4. Discussion:

#### 4.1 Root decomposition

In the first 2 years of fine root decomposition, red maple root litter lost approximately 80 and 64% of their initial C and N, respectively. On average, the retention of root litter C and N in this study was lower than that reported for a temperate forest (Bird and Torn, 2006), grassland site in France (Sanaullah et al. 2011), and mountainous grasslands (Casals et al. 2010, Garcia-Pausas et al. 2012). Studying the decomposition of ponderosa pine fine roots in Sierra Nevada
Bird and Torn (2006) found that 70.5 and 88% of the root C and N, respectively, remained in soils after 1.5-yr in situ. In a mountain grassland site in Spain, 62-78% of C and 88-98% of N from wheat root litter was recovered in soils after 1-yr in situ (Garcia-Pausas et al. 2012). The faster decomposition rates of maple roots relative to those reported by previous studies could be partially attributed to the quality of the maple root litter. For example, the initial C:N and lignin:N ratio of maple roots was approximately 10% lower than that reported for pine roots (Bird and Torn 2006), which is consistent with studies that show a negative relationship between litter decomposition rates and initial ratios of lignin:N and C:N in litter (Silver and Miya 2001, Tong et al. 2012). Moreover, water-soluble C concentrations in maple roots were 36% higher than in pine roots (Bird and Torn 2006). Thus, we hypothesize that microbial growth may have been stimulated by the addition of readily available root N and C, which in turn increased root decomposition rates.

In addition to the root litter quality, the sandy texture of the soils studied here likely favored the rapid decomposition of maple fine roots. Our hypothesis is supported by a study conducted at five AmeriFlux sites (Garten 2011), which suggested that soil texture was an important factor affecting the labile soil C at the UMBS. In addition, the C mineralization rates of maple root where similar to those reported for wheat roots incubated in sandy soils for 1 yr (Casals et al. 2010). Coarse-textured soils, such as the ones at the UMBS, have lower capacity to protect and stabilize soil C than do fine-textured soils. Thus, our results suggest that the edaphic factors in the northern temperate forests do not favor the long-term stabilization of root litter-derived C and N. The lower capacity of the UMBS soils to stabilize fine root litter when compared to other sites is further supported by a study that examined soil organic matter dynamics in a northern Michigan site located near the DIRT plots studied here.
In that study, most soil C in the UMBS site was found in the free light fraction, whereas only 37% of soil C was in the dense fraction (McFarlane et al. 2012).

Taken together, the findings presented in this study support the hypothesis that the decomposition rates of root litter are affected by a combination of litter quality, climate, and soil edaphic factors (Silver and Miya 2001, Zhang et al. 2008). The results of a meta-analysis of litter decomposition studies showed that litter C:N, total nutrients, latitude, and MAT explained 87.5% of the variation observed for the decay rates (Zhang et al. 2008). Studying litter degradation in 18 Canadian forest sites, Moore et al. (1999) reported that 73% of the variation for litter decay rates was explained by the combination of litter lignin:N ratio, MAT, and MAP. In a litterbag study in grassland soils, precipitation and percent lignin explained most of the variation in root decomposition rates (Bontti et al. 2009). Interesting, the multiple regression equation that related global root decomposition rates with a combination of initial root Ca, C:N, as well as MAT and MAP (Silver and Miya 2001), was able to predict the decay constant of maple root within DIRT control treatments. Root decay has been reported to be positively associated with initial root Ca concentrations (Wang et al. 2010), whereas it has been negatively correlated with root C:N ratios (Tong et al. 2012). In contrast to the results published by Silver and Miya (2001), other studies have been unable to correlate root Ca and C:N ratio with decomposition rates. For example, Hobbie et al. (2010) found no relationship between initial root Ca and root decay rates in a temperate forest, while Xiong et al. (2013) found that C:N ratio was not related to decay constants of temperate and subtropical tree roots. These contrasting results that more studies are needed to determine the factors that control the decomposition rates of root litter in temperate forest soils.
4.2 Aboveground and belowground removal effects

Surprisingly, the removal of above and belowground litter for eight years had no effect on the total retention of experimentally added maple root C. We expected to find greater retention of maple root C in soils that lacked litter and roots inputs. Soils that received no C or nutrient inputs for eight years were presumably depleted in energy-rich substrates. In a field study, root exclusion treatments tended to have higher proportions of actinomycetes, and sometimes higher ratios of cyclopropyl biomarkers to their precursor PLFAs, relative to other DIRT treatments, indicating microbial stress (Brant et al. 2006). More recently, the exclusion of plant inputs for more than 50 years has been reported to reduce SOM contents and microbial biomass size (Paterson et al. 2011). Thus, the addition of root litter to soils that had received no litter or root inputs was expected to stimulate microbial growth and increase the microbial utilization of this newly added root C. In addition to total root C recovery, root C losses as either soil-respired CO₂ or DOC were also unaffected by the litter manipulation treatments. Despite the lack of effect of litter manipulation on total root C recovery in the entire mesocosm, NI treatments significantly reduced the retention of root C in bulk soil within 10-20 cm depth after 2 years of the field incubation study, suggesting that within 10-20 cm depth soil microorganisms in the NI treatments were more energy-limited than in treatments that received regular additions of above and belowground litter (control). In addition to reducing root C retention in 10-20 cm depth increment, NI treatments also decreased the C:N ratio of added roots remaining in soil mesocosms after 2 years in situ, suggesting greater root C mineralization and root N immobilization in NI treatments than in control treatments. Indeed, NI treatment increased the overall retention of root N added to soils.

4.3 Nitrogen addition effects
The findings from this study did not support our hypothesis that the addition of inorganic N to soils would inhibit the losses of root C, and suggest that inorganic N additions had no effect on root decomposition. The lack of treatment effect on root C losses as CO₂ and DOC further support our conclusion that the mineralization rates of root C were unaffected by the additions of inorganic N in the DIRT plots. These results are consistent with those from several studies that showed no responses of litter decomposition on N additions (Hobbie and Vitousek 2000, Johnson et al. 2000). In contrast to our results, N fertilization in the form of ammonium sulphate has been shown to increase the decomposition rates of a Norway spruce root and root lignin (Madji 2007). In an incubation study, the addition of inorganic N agroforestry soils also increased the decomposition rates of pine roots, but did not stimulate (and sometimes inhibited) the decomposition of poplar root (Mao et al. 2011).

The experimental supply of inorganic N for eight years in the DIRT plots appears to have affected the overall changes in root-derived N by decreasing the proportion of maple root N retained in soils during the first 2 years of decay. Our results contrast with those reported by Talbot and Treseder (2012), who found no effect of N fertilization on the proportion of litter N lost after 1 year of decay. Our results suggest that the addition of inorganic N to soils may have stimulated the uptake of root N by soil microorganisms. However, the extent at which experimentally added N to soils affected the metabolic activity of microorganisms deserves further investigation.

5. Conclusions

In this study we assessed the effects of litter manipulation and inorganic N additions on the retention of C and N from maple fine roots to soils during 2-yr. Our results suggest that root
litter C and N are rapidly cycled in northern temperate forest soils, presumably due to a combination of litter quality, climate, and soil edaphic factors. The fast decomposition of roots observed in this study suggests that root litter might not be a dominant source of stabilized organic matter in this ecosystem. We demonstrated that N additions to soils and the removal of above and belowground litter for eight years imposed little influence on root C dynamics. Taken together, our results highlight the need for long-term studies on C and N dynamics of root litter and the environmental factors that affect root decomposition in different ecosystems. Finally, further studies that focus on the biotic and abiotic mechanisms that regulate the long-term root-C responses to changes in aboveground and belowground litter inputs and N additions are needed if we want fully understand the impact of climate change on SOC fluxes.

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Table 5. Isotopic and elemental composition of soils from DIRT treatments sampled in September 2011 from mesocosms without added labeled roots: C, received normal inputs of above and belowground litter, and no experimental N additions; added N, received additions of N as fertilizer; NR, received no additions of belowground inputs (e.g. no roots); NI, received no additions of above or belowground inputs. Reported values are means of three replicate plots (one standard error).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Depth cm</th>
<th>Size fraction</th>
<th>Bulk density g cm⁻³</th>
<th>Total C g kg⁻¹</th>
<th>Total N g kg⁻¹</th>
<th>C:N %</th>
<th>13C ‰</th>
<th>15N atom %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0-10</td>
<td>&gt; 2mm</td>
<td>1.2 (0.1)</td>
<td>172 (33)</td>
<td>4.7 (0.8)</td>
<td>36.7 (3.7)</td>
<td>-27.0 (0.3)</td>
<td>0.3648 (0.0002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 2mm</td>
<td>1.0 (1.5)</td>
<td>10.1 (1.5)</td>
<td>0.41 (0.02)</td>
<td>24.2 (2.6)</td>
<td>-26.8 (0.3)</td>
<td>0.3664 (0.0001)</td>
</tr>
<tr>
<td>Added N</td>
<td></td>
<td>&gt; 2mm</td>
<td>1.4 (0.2)</td>
<td>309 (67)</td>
<td>4.7 (0.2)</td>
<td>41.2 (5.1)</td>
<td>-27.5 (0.3)</td>
<td>0.3654 (0.0005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 2mm</td>
<td>9.5 (0.7)</td>
<td>0.35 (0.03)</td>
<td>27.2 (3.4)</td>
<td>-26.9 (0.3)</td>
<td>0.3671 (0.0004)</td>
<td></td>
</tr>
<tr>
<td>NR</td>
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<td>&gt; 2mm</td>
<td>1.1 (0.0)</td>
<td>242 (55)</td>
<td>5.9 (0.8)</td>
<td>40.0 (5.3)</td>
<td>-27.9 (0.4)</td>
<td>0.3654 (0.0007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 2mm</td>
<td>8.0 (0.9)</td>
<td>0.34 (0.02)</td>
<td>23.7 (4.0)</td>
<td>-26.8 (0.2)</td>
<td>0.3672 (0.0006)</td>
<td></td>
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<tr>
<td>NI</td>
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<td>&gt; 2mm</td>
<td>1.3 (0.0)</td>
<td>197 (18)</td>
<td>4.8 (0.6)</td>
<td>42.4 (5.0)</td>
<td>-27.1 (0.1)</td>
<td>0.3663 (0.0004)</td>
</tr>
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<td></td>
<td></td>
<td>&lt; 2mm</td>
<td>8.0 (1.8)</td>
<td>0.32 (0.07)</td>
<td>25.1 (1.5)</td>
<td>-26.7 (0.1)</td>
<td>0.3680 (0.0006)</td>
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<tr>
<td>C</td>
<td>10-20</td>
<td>&gt; 2mm</td>
<td>1.4 (0.0)</td>
<td>331 (29)</td>
<td>4.0 (0.2)</td>
<td>84.5 (10.5)</td>
<td>-26.7 (0.2)</td>
<td>0.3660 (0.0002)</td>
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<tr>
<td></td>
<td></td>
<td>&lt; 2mm</td>
<td>3.5 (0.6)</td>
<td>0.18 (0.03)</td>
<td>19.7 (1.4)</td>
<td>-25.3 (0.8)</td>
<td>0.3732 (0.0038)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 2mm</td>
<td>2.8 (0.1)</td>
<td>0.11 (0.01)</td>
<td>26.7 (3.5)</td>
<td>-24.5 (0.6)</td>
<td>0.3709 (0.0020)</td>
<td></td>
</tr>
<tr>
<td>NR</td>
<td></td>
<td>&gt; 2mm</td>
<td>1.5 (0.3)</td>
<td>255 (67)</td>
<td>2.9 (0.4)</td>
<td>86.8 (10.8)</td>
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<td>0.3669 (0.0002)</td>
</tr>
<tr>
<td></td>
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<td>&lt; 2mm</td>
<td>4.6 (0.8)</td>
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<td>21.8 (1.1)</td>
<td>-26.3 (0.3)</td>
<td>0.3699 (0.0002)</td>
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<td>NI</td>
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<td>&gt; 2mm</td>
<td>1.3 (0.1)</td>
<td>171 (21)</td>
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<td>44.5 (4.1)</td>
<td>-27.6 (0.5)</td>
<td>0.3670 (0.0003)</td>
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<td></td>
<td>&lt; 2mm</td>
<td>2.9 (0.1)</td>
<td>0.13 (0.03)</td>
<td>24.6 (5.3)</td>
<td>-25.0 (0.8)</td>
<td>0.3687 (0.0002)</td>
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</table>
Table 5. 2 Isotopic and elemental composition of Acer rubrum fine roots (dry matter) added to soils.

<table>
<thead>
<tr>
<th></th>
<th>Proximate C fractions1</th>
<th>Mass ratios</th>
<th>Nutrients2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (g kg(^{-1}))</td>
<td>13C (atom %)</td>
<td>15N (g kg(^{-1}))</td>
</tr>
<tr>
<td>Fine roots</td>
<td>527</td>
<td>5.20</td>
<td>11.5</td>
</tr>
</tbody>
</table>

1 All proximate C values are expressed on an ash-free dry basis. Fractions: NPE, non-polar extractives; WS, water soluble extractives; WS phenol, water soluble phenol expressed as percent tannic acid equivalents; WS glucose, water-soluble polysaccharide expressed as percent glucose equivalent; AHF, acid hydrolysable fraction; AHF glucose, acid hydrolyzable polysaccharides expressed as percent glucose equivalents; ARF, acid resistant fraction. The sum of NPE, WS, AHF, and ARF fractions equals to total C content.

2 After nitric- and perchlorid- acid digestion.
Table 5. Recovery of Acer rubrum fine root C and N in soils at different depths (0-10cm, 10-20cm) and fractions (> and < 2 mm) after 1 and 2 years in situ within litter manipulation (Experiment I) and N addition (Experiment II) treatments. Also reported: MRT, mean residence time of root C in soils; DOC, root C lost as dissolved organic C. Reported values are means of three replicate plots (one standard error). Different letters (superscript) denote statistical differences (one-way ANOVA, P < 0.05) among treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Years</th>
<th>Mesocosm</th>
<th>0-10 cm</th>
<th>10-20 cm</th>
<th>MRT</th>
<th>DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% of applied ¹³C or ¹⁵N</td>
<td>% of applied ¹³C</td>
<td>% of applied ¹⁵N</td>
<td>% of applied ¹³C</td>
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<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>1</td>
<td></td>
<td>35.0 (2.4)</td>
<td>63.2 (4.0)</td>
<td>20.7 (1.6)</td>
<td>12.5 (5.0)</td>
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<tr>
<td>NR</td>
<td></td>
<td></td>
<td>29.0 (2.6)</td>
<td>44.1 (2.7)</td>
<td>16.4 (1.5)</td>
<td>12.5 (3.4)</td>
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<td>Ni</td>
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<td>48.1 (10.6)</td>
<td>73.0 (13.7)</td>
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<td>C</td>
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<td>19.5 (3.6)</td>
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<td>28.6 (10.2)</td>
<td>41.3 (14.9)</td>
<td>5.0 (3.1)</td>
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<td>Ni</td>
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<td>22.4 (4.7)</td>
<td>45.0 (8.3)</td>
<td>7.9 (1.3)</td>
<td>14.2 (3.5)</td>
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<tr>
<td>Experiment II</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td>35.0 (2.4)</td>
<td>63.2 (4.0)</td>
<td>20.7 (1.6)</td>
<td>12.5 (5.0)</td>
</tr>
<tr>
<td>N added</td>
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<td></td>
<td>32.8 (5.6)</td>
<td>50.7 (6.1)</td>
<td>21.4 (3.3)</td>
<td>11.7 (2.5)</td>
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<td>No N added</td>
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<td>19.5 (3.6)</td>
<td>33.7 (5.4)</td>
<td>5.1 (2.2)</td>
<td>13.7 (3.5)</td>
</tr>
<tr>
<td>N added</td>
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<td>12.3 (2.4)</td>
<td>23.1 (3.5)</td>
<td>1.7 (0.5)</td>
<td>10.2 (2.7)</td>
</tr>
</tbody>
</table>

¹Losses of root-derived DOC in soil leachate from a single rain event on August 13 2011, and on September 3 2011.
Figure 5. 1 C:N ratios of root litter-13C and (b) root litter-15N retained in soil mesocosms (0-20 cm depth) after 1 yr (366 d) and 2 yrs (704 d) in situ within DIRT treatments that received normal additions of above or belowground litter (C, white circle); no belowground inputs (NR, gray circle); and no above and belowground litter inputs (NI, black circle). Values show means of treatments, and bars show standard errors (n = 3).
Figure 5. $^{13}$CO$_2$ mineralization from applied fine root C during the 2-yr study in DIRT treatments that received normal additions of above or belowground litter (2a, white circle); no belowground litter inputs (2a, light grey circle), no above and belowground litter inputs (2a, black circle), and treatments with (2b, dark grey circle) or without (2b, white circle) nitrogen additions as fertilizer. Values show means of treatments, and bars show standard errors (n = 3).
6. References


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Chapter 7. Conclusion and integration

Key findings and overall implications

This research assessed the C and N dynamics of pyrogenic organic matter (PyOM) and fine root litter in forest soils. The stability of these two significant and poorly characterized soil organic matter (SOM) pools and their contribution to ecosystem-scale C cycling remain unresolved (Schmidt et al. 2011). PyOM may account for up to 45% of SOM in temperate regions, and are considered to be inert (millennial mean residence time, MRT) in soils by most ecosystem C models. Similarly, root litter C is thought to have a slow cycling C pool. Evidences showing that fine root litter C is more stable in soils than is aboveground litter C suggest that root litter is an important source of stable SOM. Chapter 2 showed that charring pine wood at 450°C extended its mean residence time (MRT) from decades to centuries in two contrasting forest soils, which is consistent with a recent meta-analysis that showed that PyOM-C has a centennial, and not millennial, MRT in soils (Singh et al. 2012a). Further insights on the molecular composition and physicochemical structure of the pine PyOM-C (Chapter 3) revealed the preservation of heat resistant and/or regenerated aliphatic species, which may contribute to the initial, relatively fast biological degradation rates of pine PyOM in soils (Chapter 2). Future studies on PyOM degradation can take advantage of a newly developed method that quantifies and characterizes PyOM using a combination of molecular markers and stable isotopes (Chapter 4). This method builds on a previous approach that uses Benzene Polycarboxylic Acids (BPCAs) as molecular markers for PyOM (Glaser et al. 1998). More specifically, the method described in Chapter 4 measures $^{13}$C-BPCAs in soils and sediments, and can be used in tracing experiments to follow
the fate of $^{13}$C-BPCAs (PyOM) into different soil pools. PyOM-derived C and N interacted with and were significantly affected by reactive mineral surfaces (Chapter 2), providing compelling evidences that soil mineral assemblage can stabilize SOM, and should be considered in soil ecosystem C models (Schmidt et al. 2011). Furthermore, atmospheric PyOM-C deposition was not a significant PyOM-C input pathway in northern temperate forest soils for 2 consecutive summers. Despite the low PyOM-C deposition fluxes from the atmosphere to soils, PyOM-C concentrations reported in this study provides a baseline for future assessments of PyOM-C levels in northern Michigan ecosystems (Chapter 5). Lastly, the fast cycling of C and N from red maple root (Chapter 6) relative to that of root C and N reported for other temperate soils (Bird and Torn 2006, Garcia-Pausas et al. 2012) further supports the hypothesis that litter quality is not the primary factor driving the stabilization and transformation processes of SOM in soils (Kleber et al. 2010). Taken together, the main results of this study support the hypothesis that biomass converted into PyOM during wildfires might degrade at centennial time scale, which is faster than that assumed by ecosystem C models. Similarly, the fast decomposition rates of fine root litter in northern temperate forest soils relative to those reported earlier for other ecosystems should be taken into account by future assessments of ecosystem-scale C models.

**Implications for PyOM formation and stabilization research**

The pyrolysis of pine wood changed its physico-chemical and structural characteristics (Chapter 3), resulting in slower decomposition rates for PyOM relative to wood (Chapter 2). Interestingly, the characteristics of the PyOM formed at 450 °C (Chapter 3) are in accord to the ‘amorphous’ fire-derived OM category recently proposed for PyOM (~ 400–500 °C) comprised of mixed, unorganized assemblages of heat-resistant aliphatic and heteroaromatic domains (Keiluweit et al.
Given the growing evidence showing that an increase in charring temperature results in slower PyOM-C mineralization rates (Baldock and Smernik 2002, Keith et al. 2011, Singh et al. 2012b), the results presented here informs future studies aiming at articulating the molecular structure of PyOM and linking them to the MRTs of charred biomass in soils. A clearer picture of this relationship could provide new insights on the dynamics of the diverse array of PyOM in soils.

**Implications for northern high-latitude ecosystems in a changing climate**

A warming climate is likely to increase the amounts of fire-derived biomass (PyOM) in forest ecosystems, as higher temperatures and droughts are expected to increase wildfire frequency, intensity, and season length. The findings presented in this dissertation research indicate that charred wood formed during natural fires (range: 120 - 621 °C, median: 287 °C; Wolf et al. 2013) in temperate forests will likely persist in soils for centuries. However, climate change will likely reduce the MRT of PyOM in temperate soils, as slowly-decomposing SOM is thought to respond to changes in temperature faster than labile and fast-cycling SOM (Knorr et al. 2005). Thus, an increase in the global mean surface temperatures projected for the end of the 21st century (IPCC 2013) will have the potential to accelerate the C and N mineralization rates of PyOM in northern high latitude soils.

Greater amounts of charred plant biomass in soils could potentially increase the proportion of PyOM transported from soils to oceans through leaching as DOM. The relative mobility of PyOM from soils to oceans is supported by recent studies that reported dissolved pyrogenic C (DPyC) in marine and fresh water systems (Dittmar et al. 2012). Results from a global study
showed that DPyC accounted for 10% of the total DOC in riverine systems (Jaffe et al. 2013). Thus, despite the stability of PyOM, an increase in PyOM production during wildfires could potentially increase the fluxes of PyOM from soils to lakes, rivers, and oceans.

Soils in northern high-latitudes store large amounts of labile SOM (Billings 1987, Tarnocai et al. 2009, Waldrop et al. 2010). The findings of this dissertation research suggest that belowground C and N fluxes in high latitude ecosystems may take over a decade to respond to anthropogenic disturbances (e.g. harvesting) or extreme climatic events (e.g. drought). In addition, the results presented here also suggest that C and N fluxes from SOM in these systems may take over a decade to respond to an increase in N deposition to soils, given that the net primary production of high latitude ecosystems is N-limited (Shaver et al. 1986, Vitousek and Howarth 1991). Finally, this study suggests that SOC fluxes in the form of CO$_2$ and leached dissolved organic C (DOC) will take more than a decade to respond to environmental changes in northern forest soils. Taken together, the findings presented in Chapter 6 underscore the fundamental importance of long-term (e.g. decadal time scale) studies for accurate assessments of SOM’s responses to environmental disturbances in the transition zone between deciduous and boreal forests (or the temperate-boreal ecotone).

Atmospheric CO$_2$ concentration has increased by 40% from 1750 to 2011 (390.5 ppm), and will continue to increase in the next decades (Ciais et al. 2013). Elevated CO$_2$ concentration can increase live root productivity (Norby et al. 2004, Pritchard et al. 2008) and exudation (Phillips et al. 2011), resulting in greater supply of belowground C. Given that inputs of labile C to soils through live roots have been reported to stimulate the decomposition of the SOM pool (positive
priming) in temperate forests (Bird et al. 2011, Phillips et al. 2012, Cheng et al. 2013), similar mechanisms are likely to operate for interactions between fresh litter inputs and SOM in the tundra biome under elevated atmosphere CO₂. Nonetheless, significant inputs of C from roots and exudates would likely result in a net increase in SOC stocks in northern ecosystems (Drake et al. 2011).

**Recommendations for ecosystem-scale soil C models**

The results of this research confirm that soil C and N from the so called ‘slow’ and ‘passive’ cycle SOM pools are more dynamic in soils than previously thought in temperate forest ecosystems. Therefore, these findings suggest that C turnover models should (1) no longer assume that fire-derived organic matter is an ‘inert’ or a ‘passive’ C pool; (2) consider that root detritus might represent a ‘fast’-cycling C pool in northern temperate forest soils; (3) include soil mineral assemblage as a key variable driving the cycling of C and N from SOM in subsurface horizons.

The suggestions described above are consistent with those proposed by a growing number of studies that have challenged the assumptions of computer soil C models on SOM stability (Kleber et al. 2010, Schmidt et al. 2011, Dungait et al. 2012). One of these assumptions is that plant litter can be partitioned into ‘fast’ and ‘slow’ C pool based on the initial litter C chemistry. Based on this assumption, decomposition rates of lignin-rich litter would be expected to be slower than low-lignin litter. The long-term selective preservation of lignin in soils, however, has been unsubstantiated by several studies (Rumpel et al. 2002, Rumpel et al. 2004, Prescott 2010, Thevenot et al. 2010), including those that use compound-specific isotope analysis (Lichtfouse et
The emerging view that lignin-derived phenols might degrade faster than previously thought, especially in the presence of bioavailable C (Klotzbucher et al. 2011), suggests that other factors also control SOC turnover rates. This is consistent with the results of this dissertation research, which suggested that the relatively fast cycling of C and N from fine root litter in temperate soils was likely due to a combination of environmental factors, such as litter chemistry, soil texture, water availability, and microbial size and composition. The incorporation of the size and composition of microorganisms in ecosystem-scale C models as control factors during SOM decomposition has already been addressed by a few studies (Todd-Brown et al. 2012). Taken together, these findings suggest that ecosystem-scale C models should abandon their long-standing assumptions, and recognize the need to incorporate the environmental factors shown to affect the decomposition of SOM. These changes would enable us to advance our mechanistic understanding of the SOC cycling.

**Future research needs**

The findings presented in Chapter 2 derived from measurements performed under controlled temperature and moisture conditions in laboratory. An ongoing field experiment in Switzerland has been monitoring the effects of N additions on PyOM decay *in situ* (Singh et al. 2014). Future work should extend this study, and measure the long-term degradation of a suite of charred materials *in situ* to account for the effects of a wide range of environmental factors (e.g. snow) that will likely affect the persistence, transformations, losses, and mobility of PyOM in soils. This work could include, for example, the application of δ^{13}C-BPCA technique discussed in
Chapter 4. Field-based data on the MRT of pyrogenic C remains insufficient, yet extremely needed in SOM research (Gurwick et al. 2013).

In addition to the decomposition of PyOM \textit{in situ}, the amounts of atmospheric PyOM-C deposited in soils remains a poorly quantified input mechanism in the PyOM-C budget. While Chapter 5 aimed at addressing this gap in research, ground-based measurements of PyOM-C were conducted in one single site in northern Michigan and did not account for wet deposition of atmospheric particles. The establishment of multiple long-term monitoring sites could be used to extend these results and provide a more comprehensive dataset on wet and dry deposition of atmospheric PyOM-C in northern forest ecosystems. Future work should consider the total deposition of atmospheric PyOM-C during wintertime, given the negative impact of PyOM-C on snow albedo (Hadley and Kirchstetter 2012). For example, higher PyOM-C deposition on snow surface could increase snow melting.

Finally, future studies should consider exploring PyOM C and N cycling in different ecosystems around the globe, and under different experimental conditions (e.g. warming, drought, fire etc.). Researchers working in the field and laboratory are also encouraged to work more closely with scientists interested in modeling soil C and N cycling. Additionally, long-term and interdisciplinary studies should provide a better understanding of the mechanisms driving the changes in belowground C and N fluxes in northern high-latitude ecosystems frequently affected by wildfires (boreal forests), and highly sensitive to climate change (Arctic tundra).
References


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