

ABSTRACT

Title of Thesis: THE EFFECTS OF CO₂ AND TEMPERATURE ON THE SOIL MICROBIAL CARBON AND NITROGEN OF URBAN AND RURAL FORESTS

Elizabeth Kulka, Master of Science, 2015

Thesis Directed By: Professor Marla S. McIntosh
Department of Plant Science

This study investigated and compared the effects of elevated temperature and elevated CO₂ on the microbial biomass carbon (MBC) and nitrogen (MBN) of urban and rural forest soils. Soils analyzed from Baltimore Long-Term Ecological Research forests in June and October, 2014 had greater MBC and MBN quantities in rural than urban forests. A controlled environmental chamber study was conducted where June-collected soils were planted with hybrid poplars and exposed to ambient and elevated temperature and CO₂ levels. After exposure for 49 days, MBC and MBN quantities were again greater in rural than urban soils. Soil MBC was greater under elevated than ambient CO₂, while soil MBN was greater under elevated than ambient CO₂ and temperature. Results suggest that if temperature and CO₂ levels increase in the Baltimore area as predicted, microbial C and N pools in the studied forests will increase, and will remain greater in rural than urban soils.

THE EFFECTS OF CO₂ AND TEMPERATURE ON THE SOIL MICROBIAL
CARBON AND NITROGEN OF URBAN AND RURAL FORESTS

by

Elizabeth Kulka

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Master of Science
2015

Advisory Committee:
Professor Marla McIntosh
Dr. Richard Pouyat
Professor Joseph Sullivan
Assistant Professor Stephanie Yarwood

Acknowledgements

Firstly, I would like to sincerely thank my advisor, Dr. Marla McIntosh, for all of her patience and guidance throughout my graduate career. I would also like to thank my committee members Dr. Pouyat, Dr. Yarwood, and Dr. Sullivan, for their wonderful advice and support throughout my research. Finally I need to thank my parents, my ‘extended family’ in the Yeast Culture Club, and the brilliant students of the ‘Yarwin’ lab. Without their kindness and moral support, this thesis would not be possible.

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Rationale for Study

In a time of changing climate, the future of our global forests is a major concern. Forest community responses to changes in CO₂ and temperature are complex. Just as forest communities are altered by climate, local climate and nutrient cycles are altered by forests. Soil fungal and bacterial communities play an important role in the carbon (C) storage and nitrogen (N) dynamics of soils within forest ecosystems (van der Heijden et al. 2008, Cox et al. 2010). During the metabolism of both plant and microbially-derived compounds in the soil, microbes such as fungi and bacteria store soil C and N as biomass. Compounds from decayed microbial biomass (necromass) contribute a great deal to the recalcitrant soil organic C (SOC), which remains long-term in the soil (Schmidt et al. 2011, Schimel and Schaeffer 2012). By sequestering C, the recalcitrant SOC pool mitigates atmospheric CO₂ increases and the effects of climate change. Microbial biomass also constitutes a significant pool of soil N which, due to its high turnover rate, plays an important role in the nutrition of the plant and microbial community. As climate conditions change, the accumulation of C and N by the soil community will change in response, due to the effects of elevated temperature and CO₂.

Due to their significant role in long-term C storage and soil N pools, it is important to understand the interactions between forest soil microbes and climate change. Gradients of temperature and CO₂ that positively correlate with urbanization have been observed in cities such as Baltimore and New York (Ziska et al. 2004, Savva et al. 2010). Due to the elevated CO₂ and temperature levels in urban relative to rural forests, urban forests can be used as analogues for climate change (Carreiro and Tripler 2005). However, little is known about how climate change affects the MBC and MBN of forest

soils. This research investigated microbial biomass C (MBC) and microbial biomass N (MBN) differences between urban and rural forest soils, and the effects of elevated temperature and CO₂ on soil MBC and MBN, using urban forest soils as analogues for changes in temperature and CO₂. As soil N limitation may reduce long-term C storage, this research also investigated the effects of elevated temperature and CO₂ on soil ammonium and nitrate quantities in urban and rural forest soils. Through this research, we may be better able to predict how climate change will alter forest microbial N pools, as well as MBC production and storage in forest soils.

Background

Urban Forests, Urban Ecosystems, and Climate Change

Urban ecosystems are interacting networks of social, biological, and physical components, and are distinct from neighboring rural environments in many aspects (McKinney 2003). These distinctions extend to urban forests, whose trees and soils are critical to the storage of atmospheric carbon (C) and the cycling of terrestrial nitrogen (N). In the United States, estimates of the total amount of C sequestered by forested areas range from 0.149 to 0.33 Pg C yr⁻¹. Urban trees alone, whose canopies cover 27% of the 27.6 million square miles of urban land in the United States, are estimated to sequester 0.0228 Pg C yr⁻¹ (Nowak and Crane 2002, Woodbury et al. 2007, U.S. Department of Commerce 2010). The quantity of N retained in forest soils over time, however, is generally much smaller than the C sequestration rates. For instance, the total soil N retention for the forested rural watershed of Pond Branch (Cockeysville, MD) was estimated to be 0.0107 Mt N ha⁻¹ yr⁻¹, while the total soil N accumulation for the

suburban Glyndon watershed (Baltimore, MD) was estimated to be $0.0191 \text{ Mt N ha}^{-1} \text{ yr}^{-1}$ (Groffman et al. 2004).

The urbanization process is associated with environmental changes such as elevated temperatures, elevated CO_2 concentrations, and altered precipitation patterns. In most large cities, a combination of impervious heat-absorbing surfaces and heat trapping greenhouse gases creates an “urban heat island” (Carreiro and Tripler 2005). The temperature difference between urban and non-urban areas is often greater at night, as concrete surfaces slowly absorb heat during the day and slowly radiate heat at night (Aitkenhead-Peterson and Volder 2010). Large urban heat islands in the United States have been identified by satellite in Atlanta and Houston, and have been reported in New York and Baltimore (Bornstein and Lin 2000, Streutker 2003, Ziska et al. 2004, Carreiro and Tripler 2005, George et al. 2007). Urban forests within urban heat islands are also exposed to higher air and soil temperatures than nearby rural forests (Brazel et al. 2000, Savva et al. 2010). For the Baltimore area in particular, Savva et al. (2010) found that from 2000-2007, forest soils within Baltimore had an average annual temperature of $12.6 \text{ }^\circ\text{C}$ at 10 cm depth, while rural forest soils had an average annual temperature of $12.2 \text{ }^\circ\text{C}$. The presence of a higher average temperature in urban relative to rural areas has been linked to phenological changes in urban trees, notably earlier bloom times and longer growing seasons relative to rural trees (Carreiro and Tripler 2005). Gradients of CO_2 have also been documented in urban environments such as Baltimore, MD and New York City, NY (Gregg et al. 2003, Ziska et al. 2004, George et al. 2007). In particular, Ziska et al. (2004) found that in 2002 the average annual CO_2 level at the Baltimore Science Center (urban) was 466 ppm, while the average annual CO_2 level at an organic

farm in Buckeystown, MD (rural) was 385 ppm. Elevated CO₂ levels in urban areas are the result of a high concentration of fossil fuel consumers, including cars and factories. In fact, 75% of the world's fossil fuel consumption occurs in cities (Newman and Jennings 2008). It is no surprise then, that urban environments tend to have higher concentrations of atmospheric CO₂ than their rural counterparts (Jacobson 2010).

Environmental factors like temperature and CO₂ may affect the soil community, such that urban and rural soil microbial communities become structurally or functionally different over time (Allison and Martiny 2008). As microbial community members can vary in their life strategies, C and N requirements, and metabolic rates, distinct soil microbial communities may develop differing soil and microbial C and N quantities. The Intergovernmental Panel on Climate Change (IPCC) has predicted that the heightened temperatures and CO₂ amounts that cities currently experience will occur in non-urban areas in time due to climate change (IPCC 2007). Thus, urban environments can serve as proxies for climate change by constituting 'space-for-time' experiments, in which a forest's response to long-term elevated temperature and CO₂ is predicted to occur in the future in the forests of surrounding comparable rural areas (Carreiro and Tripler 2005). Therefore when examining soil microbial C and N storage, urban areas can serve as analogues for the effects of climate change (as elevated temperature and CO₂) on these variables. This type of study, however, can and should be further supported through controlled laboratory studies where cause and effect can be more clearly distinguished.

Average global CO₂ concentrations are currently around 400 ppm. By 2090, they are expected to rise to 600 ppm. Concurrently, temperatures in the Baltimore area are predicted to rise 2.5 °C (IPCC 2007, Earth System Research Laboratory: Global

Monitoring Division 2012). Few studies, however, have examined urbanization effects on forest soil microbial C and N (Pouyat and Carreiro 2003, Kaye et al. 2005, Pavao-Zuckerman and Coleman 2007, Pouyat et al. 2008, Groffman et al. 2009). Additional research is justified considering the significance of the microbial community to the long-term storage of C and soil N availability, and the impacts that future climate may have on these functions.

Soil Carbon Storage

Understanding how CO₂ cycles between terrestrial and atmospheric C pools is of growing importance, as atmospheric CO₂ is a key greenhouse gas that drives climate change. Each year around 11 Pg of C are released into the atmosphere as CO₂ from the burning of fossil fuels and vegetation (Woods Hole Research Center 2014). Deep ocean sediment and terrestrial soil are the two major carbon pools that store C and mitigate CO₂ accumulation in the atmosphere. The terrestrial soil system accumulates around 2.8 Pg of C yr⁻¹; more than half the amount accumulated per year in the atmosphere (Woods Hole Research Center 2014). The accumulation and transformation rates of C in terrestrial C cycles are expected to be sensitive to changes in climate, particularly to temperature and CO₂ (Cramer et al. 2001, Heimann and Reichstein 2008).

Plants, microbes, and animals all play a significant role in the breakdown, storage, and output of C from the soil. Earthworms, for instance, help drive the C cycle of forest soils by breaking down leaf litter and providing high nitrogen casts that stimulate microbial activity (Szlavecz et al. 2006). Fungi, on the other hand, actively break down complex soil organic matter (SOM), which facilitates the storage and respiration of C (Alberton et al. 2005). Bacteria facilitate C cycling in the soil subsurface and are themselves important sources of C to the soil community (van der Heijden et al. 2008).

SOM is composed of decaying biological matter from plants, microbes, and animals, and can be found in two different pools in the soil: labile (short-term storage) and recalcitrant (long-term storage) pools. This research will specifically investigate soil organic carbon (SOC), which contributes to the SOM pool. Labile C pools consist of compounds that are easily degraded by enzymes, either due to their structure or physical availability in the soil matrix. Living microbial biomass is an example of labile SOC that is metabolized during microbial turnover, although it represents <4% of SOC (Liang et al. 2010, Prescott 2010). Microbial necromass, however, forms during the degradation of living microbial biomass and represents a much greater portion of the total SOC and the labile dissolved organic carbon (DOC) pool (Liang et al. 2010). Recent studies have identified microbial C containing compounds, such as fatty acids, alkanes, alkenes, and amino sugars, within the recalcitrant SOC pool (Kindler et al. 2006, Simpson et al. 2007, Liang et al. 2010). These studies determined that, in many soils, a significant portion of these compounds are derived from microbial necromass. This refutes the historical view that recalcitrant SOC was primarily plant-derived (Jiao et al. 2010, Miltner et al. 2011). Recalcitrant SOC compounds can have half-lives of hundreds to thousands of years in the soil, which was thought to be due solely to their structural complexity (Kiem and Kogel-Knabner 2003). Now the prevailing view is that their recalcitrance is due primarily to their interaction with the soil matrix (inaccessible within aggregates and bound tightly to minerals) rather than their structural complexity (Schmidt et al. 2011, Schimel and Schaeffer 2012). In particular, the clay content of the soil seems to have a significant positive effect on the formation rate of recalcitrant soil C (Sulman et al. 2014).

Studies that follow the fate of isotopically labeled microbial biomass have provided insight into the contribution of microbial biomass to recalcitrant SOC pools (Miltner et al. 2009). A significant study done by Kindler et al. (2006) followed the decomposition of *E. Coli* in the soil and found that 40% of the original MBC remained in the soil as necromass after 224 days. In addition, 75% of amino acids and 25% of fatty acids remained in the soil as necromass after 224 days. While these studies identify the contribution of only *E. Coli*, they indicate that these microbial compounds contribute a great deal to the recalcitrant SOC pool (Miltner et al. 2011).

Soil Nitrogen Dynamics

Our atmosphere is 78% nitrogen gas and the largest global pool of N. In forests, soil N is derived primarily from atmospheric N through the processes of N fixation and N deposition. Nitrogen is a soil macronutrient critical to the growth and survival of the plant and microbial community. While many mature temperate forests are N limited to some degree, forest in urban or near agricultural areas can experience an abundance of N due to chronic N deposition. Elevated temperature and CO₂ levels are expected to occur in the future, and may exacerbate soil N limitation in forests that do not experience chronic N deposition (Luo et al. 2004). The N and C cycles of the soil are intertwined, such that N amounts in the soil may significantly affect the production of recalcitrant SOC (Wang et al. 2010, Zaehle et al. 2010). It is therefore important to understand the dynamics of N in these soils, and how this impacts the long-term storage of C, under future climate conditions.

A large pool of N can be found in SOM, of which microbial biomass is a major constituent (Simpson et al. 2007). N can occur in SOM as microbially-derived amino sugars and proteins (Kindler et al. 2006). The C:N of SOM is a major factor determining its decomposition rate and whether its N will be immobilized or mineralized by the soil microbial community (Lambers et al. 2008). Immobilization occurs at a C:N greater than 20:1, where N limits the decomposer community. At a C:N less than 20:1, mineralization occurs and N accumulates in the soil as ammonia or ammonium. Ammonium is the preferred form of labile inorganic N assimilated by a majority of the plant and microbial community (Lambers et al. 2008). Assimilated ammonium contributes to the production of important plant and microbial cell components such as amino acids, nucleic acids, and adenosine triphosphate (ATP). Ammonium is also used by the soil microbial community during nitrification, where ammonium is converted to nitrite by ammonia oxidizing bacteria and archaea in the first step, and nitrite is converted to nitrate by the genera *Nitrobacter* and *Nitrospira* in the second step. Nitrification occurs in aerobic forest soils, and is an important part of the soil nitrogen cycle. Nitrate is the secondary source of labile inorganic N to the plant community. Compared to ammonium, nitrate occurs in smaller quantities in the soil, is more mobile in the soil matrix, and requires more energy for plants to assimilate (Lambers et al. 2008). In the microbial community, nitrate is primarily used by denitrifying bacteria as a source of energy during denitrification,

during which nitrogen gas is produced. Denitrification, however, requires anaerobic conditions and is not a predominant process in temperate forest soils.

Ammonium, nitrate, and MBN are all N sources that contribute significantly to the N cycle of forest soils. The soil N cycle, however, is intertwined with the soil C cycle, such that a change in N accessibility can affect the long-term storage of C in the soil.

Prolonged elevations in temperature and/or CO₂ have been suggested to cause progressive nitrogen limitation (PNL), which reduces long-term soil C storage (Luo et al. 2004). The theory of PNL suggests that sustained elevated temperature and CO₂ levels will stimulate the growth of the plant community, such that over time available soil N will be assimilated by plants at rates greater than can be replenished by microbial mineralization, N fixation, or N deposition. Elevated temperature also stimulates nitrification, which converts ammonium to nitrite and nitrate. Nitrite and nitrate are highly mobile in the soil, and easily removed from the soil environment through runoff. If N becomes limiting in the soil, the C:N ratio of organic matter will increase, slowing its decomposition by the microbial community, and reducing the rate at which inorganic N is made available to the soil community. According to PNL, the growth of both plants and microorganisms will be reduced over time by the limited soil N availability, and the rate of microbial growth and storage of C and N will decrease (Luo et al. 2004).

However, some forests ecosystems may not experience N limitation over time due

balanced inputs from N deposition. Urban forests in particular have increased N deposition rates (as throughfall) relative to their rural counterparts (Bettez and Groffman 2013). This is due primarily to fossil fuel combustion which produces oxidized N compounds. Once released to the atmosphere, these compounds can return to the soil as dry/particulate deposition. Ammonium and ammonia are also important components of wet and dry N deposition, but are more commonly associated with agricultural areas, as their predominant sources are the volatilization of urea from fertilizers and the gaseous byproducts of industrial fertilizer production. In Baltimore in particular, Bettez and Groffman (2013) found that throughfall N deposition rates were $13.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ at Ronel Heights, an urban site, and $9.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ at Pond Branch, a rural site, and $13.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, an agricultural site. In order to fully understand how climate change will impact forest soil carbon storage as MBC, it is therefore also important to investigate how soil nitrate, ammonium, and the forest soil microbial N pool are affected by elevated temperature and CO_2 .

Effects of Climate Change on the Soil Microbial Community

CO_2 and the Microbial Community

The rhizosphere microbial community obtains its C from the external environment, where increasing atmospheric CO_2 promotes the growth of above and belowground plant biomass by some species, and the release of exudates and photosynthates by plants to the soil (Treseder et al. 2005, Phillips et al. 2011, Wang et al.

2012). Root exudates and photosynthates are important C sources for the rhizosphere microbial community, and positively affect MBC quantities (Ceulemans et al. 1999, White et al. 2002, Barron-Gafford et al. 2005, Lipson et al. 2005, Drigo et al. 2007, Phillips et al. 2011, Manna et al. 2013). For instance, Carrillo et al. (2014) found that soil MBC increased and the microbial respiration rate declined when a grass (*Bouteloua gracilis*) was exposed to increased CO₂ over a ten week period. This caused an increase in C use efficiency, which is defined as $(MBC / (MBC + \text{respired CO}_2))$ (Steinweg et al. 2008). Similarly, Lipson et al. (2005) found that long-term CO₂ treatments increased soil MBC at chaparral sites containing more than 0.03 g organic matter per gram of soil. In forested ecosystems, while MBC generally increases with CO₂, this can depend on the N levels in the soil (Hu et al. 2006, Dieleman et al. 2010, Phillips et al. 2011, Chen et al. 2012). Exudates, produced in greater quantities under elevated CO₂, are high in C and low in N. Increased exudate production has been shown to initially stimulate organic matter mineralization by the microbial community, as microbes search for available N (Phillips et al. 2011). This ‘priming effect’ can lead to large declines in the total SOC in the short-term, as CO₂ is respired and lost from the soil system during decomposition. The priming effect is expected to be particularly strong in forests with recalcitrant leaf litter (e.g. pine needles), and is expected to increase microbial biomass and the recalcitrant C that is eventually formed from it (Sulman et al. 2014). In the short-term, MBC and MBN are also expected to increase with CO₂ as long as accessible soil N is not limited. Under long-term elevated CO₂ conditions, however, increased C inputs and labile N assimilation by the growing plant community are expected to limit soil N for the microbial community. This in turn may reduce organic matter mineralization, N-fixation,

and N-deposition (Hu et al. 2001, Finzi et al. 2006). A meta-analysis by Dieleman et al. (2010) found that long-term (≥ 1 year) elevated CO₂ treatments had no overall effect on forest soil MBC, but a significant negative effect on N mineralization. They attributed this outcome to microbial N limitation, caused by N competition between the plant and microbial community.

A majority of the literature regarding the effects of elevated CO₂ on microbial biomass has focused on grasslands and agricultural sites, while none to my knowledge have examined urban forests. Grasslands, agricultural sites, and forest ecosystems can differ greatly in terms of their soil N and the N requirements of their vegetation. It is no wonder then that there are conflicting reports in the literature on the effect of elevated CO₂ on microbial C and N, with some claiming a positive effect while others have found no significant effect (Hu et al. 2006). The chosen duration of the study further complicates the issue, as long-term studies or mature forests are more likely to experience N limitation, which in turn affects microbial C and N storage. It is therefore important to keep in mind that the effect of CO₂ on soil MBC and MBN, which is mediated by vegetation, will depend on the type of vegetation and the N availability in the soil over the duration of the study.

Temperature and the Microbial Community

Temperature stimulates contrary processes in plants and the soil, which indirectly and directly influence MBC and MBN accumulation. By promoting the growth of vegetation, elevated temperatures increase the plant root biomass and exudate C sources available to the microbial community (Pregitzer et al. 2000, Uselman et al. 2000, Bardgett et al. 2013). The growth rates of microbes are also directly affected by temperature, increasing to the average optima at 30 °C (Singh et al. 2010, Gray et al.

2011). Therefore, the decay of organic compounds in the soil is also stimulated by elevated temperatures, with higher microbial turnover, decomposition of more complex compounds, soil N availability, and greater microbial respiration rates observed under these conditions (Davidson and Janssens 2006, Hagerty et al. 2014). As organic compounds in the soil decay, more N is made available to the soil community, stimulating plant and microbial growth and storage of C and N (Belay-Tedla et al. 2009, Melillo et al. 2011). This C and N is then returned to the soil as plant and microbial necromass.

The growth of any organism necessitates that some portion of their assimilated C or N is stored in biomass, some is released as a metabolic byproduct, and some is returned to the soil during decay. One or more of these processes may dominate such that the effects of temperature on MBC or MBN under natural or experimental conditions may not be significant. In many studies, temperature alone does not seem to significantly affect soil MBC amounts specifically (Zhang et al. 2005, Steinweg et al. 2008, Hagerty et al. 2014). In a study by Steinweg et al. (2008), this outcome was attributed to decreased C use efficiency, where respiration increases were much greater than MBC increases under elevated temperature (Steinweg et al. 2008). Additionally, Hagerty et al. (2014) found that elevated temperature greatly increased microbial respiration and turnover rates. In this case, while elevated temperature caused a significant increase in MBC production rate, the overall MBC concentration of the soil remained the same due to a microbial turnover rate that increased with temperature. While the effect of temperature on MBN has not been investigated by many studies, it has been found that elevated temperatures increase the availability of labile N in the soil through both increased mineralization and

plant root turnover (Melillo et al. 2002). This N can then be incorporated into the biomass of both microbes and plants. As long as the competition between plants and the microbial community is not so strong that N is limiting to microbial growth, MBN should increase in response to temperature. Belay-Tedla et al. (2009) found that labile C and N, along with MBC and MBN, both increased with temperature over a period of 2.5 years in prairie soils. They attributed this increase to increased root turnover and exudate production rather than organic matter decomposition, as their observed soil respiration rates were not significantly affected by temperature.

In summary, climate change has the potential to alter the amount of C and N stored by the soil community, both in the short-term as microbial biomass and in the long-term as microbially-derived recalcitrant SOM. The proposed study will investigate how the soil MBC and MBN of urban and rural forests is altered with elevated temperature and/or CO₂. Knowing this, we can better predict how soil C storage and the availability of microbial N pools may change in the future under elevated temperature and CO₂ levels.

Baltimore Field Study

Goals and Objectives

As cities continue to grow in size and number, it is important to understand how urbanization can affect pools of microbial N as MBN and C storage as MBC in forest soils. This study examined and compared soil MBC and MBN quantities in urban and rural forests in the Baltimore metropolitan area. These forests and their soils were similar except for their historical differences in temperature and CO₂ (Brazel et al. 2000, Ziska et al. 2003, Ziska et al. 2004). The study objectives were to investigate and compare: 1) the

storage of C as MBC in urban and rural forest sites during June and October, 2) an important pool of soil N as MBN in urban and rural forest soils during June and October, 3) additional labile soil C and N pools as dissolved organic C (DOC) and total dissolved N (TDN) in urban and rural forest soils during June and October, and 4) soil characteristics of urban and rural forested sites.

Materials and Methods

Site Description and Sampling Procedures

Six forested sites previously studied as Baltimore Long Term Ecological Research sites (BLTER), three urban and three rural, were selected for this research (Figure 1). The urban forest sites were: Leakin Park (LP), Cylburn Arboretum (CA), and Druid Hill (DH). The rural forest sites were Gunpowder Falls at Jerusalem Mills (GF), Loch Raven Reservoir (LR), and Oregon Ridge Park (OR). These BLTER sites were categorized as either urban or rural based on their distance from the city center (urban <9km and rural >19km), length of roads and highways, percent of urban land use, population density, and traffic volume on nearby major roads by Pouyat et al. (2008). In addition, Pouyat et al. (2008) determined that the urban and rural sites had similar forest composition, age, and minimal human disturbance. The soils within these sites were classified as *Ultic hapludalf* soils (United States Department of Agriculture 2013).

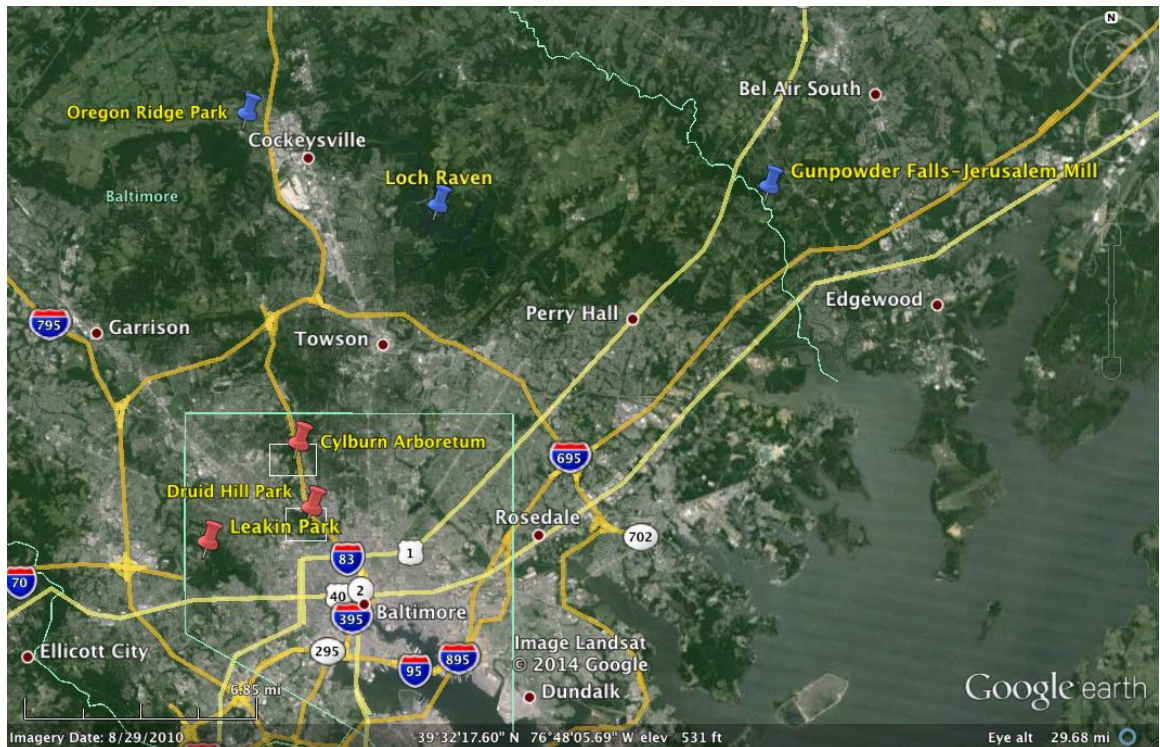


Figure 1. Six LTER sites in the Baltimore metropolitan area. The blue and red markers indicate rural and urban forests respectively.

Soil Sampling

Soil Chemical Analysis

Soil samples for the chemical analysis were collected on October 9 and 18 of 2013. At each site, soils were sampled around six selected tulip poplar trees (*Liriodendron tulipifera*), with three trees in the forest interior and three trees at the forest edge. Sampling locations were easily accessible, located on minimal slope, and located near other tulip poplar trees. Soil samples were taken near trees of the same species to minimize soil microbial community variation between sites due to differences in tree species association. Tulip poplar was chosen because it is the most common tree species in Baltimore area forests and was present at all sites. Tulip poplar trees grow in association with red maple (*Acer rubrum*), dogwood (*Cornus sp.*), black gum (*Nyssa sylvatica*), white oak (*Quercus alba*), sassafras (*Sassafras albidum*), black cherry (*Prunus serotina*), white hickory (*Carya tomentosa*), arrowwood viburnum (*Viburnum dentatum*),

and honeysuckle (*Lonicera sp.*) (Brush et al. 1997). Each tree around which the soil was sampled was marked with a small flag and geolocated. Soils were sampled on days without rain, so that there was no water-induced stimulation of microbial activity. Soil sampling methods followed Lilleskov et al. (2001). Around each selected tree, four soil cores (2 cm in diameter) were taken to 10 cm soil depth (excluding the litter layer) at a distance of 1.5 m from four sides of the tree. Soil samples taken within the interior or edge locations were separately composited, then homogenized, and refrigerated until analysis. Thus 12 composite soil samples, one interior and one edge sample at each site, were analyzed for soil properties.

Microbial Biomass, DOC, and TDN Analyses

Soil samples for the microbial biomass, DOC, and TDN analyses were collected on June 8 and 10 and October 11 of 2014. At each site, soils were sampled around the same three interior tulip poplar trees as the soil chemical analysis samples, using the same collection protocol, and frozen at -20 °C until analysis. Thus, 18 soil samples, three interior trees for each site, were analyzed in the June and October for MBC, MBN, DOC, and TDN.

Soil Chemical Analysis

Soils were analyzed for organic matter content (%OM), total N, total C, pH, cation exchange capacity (CEC), P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, S, Al, and Pb at the University of Delaware Soil Testing Laboratory using an Intrepid II XSP Duo View inductively coupled plasma spectrometer (Thermo Fisher Scientific, Waltham, MA).

Laboratory Analysis

Chloroform Fumigation Extraction

MBC and MBN were quantified before and after chloroform fumigation and extraction based on the methods of Howarth and Paul (1994). Two sets (fumigated and non-fumigated) of 10 g dry weight equivalent subsamples were taken from the 18 composite soil samples and placed in 25 mL beakers. The wet weights of the 10 g dry weight equivalent subsamples were determined by drying 10 g of each soil sample at 65 °C for two days and calculated as g of wet weight soil/g of dry weight soil. The average 10 g dry weight equivalents were 12.8 g and 12.5 g for soils collected in June and October, respectively. The non-fumigated set of subsamples were incubated at room temperature in the dark for five days. The other set was fumigated with chloroform before incubating in the dark for five days. The fumigated soils were placed in a vacuum desiccator containing a beaker with 50 mL of ethanol-free chloroform, which was evacuated until the chloroform boiled. After the fourth evacuation, the desiccator was sealed without venting. After the five day incubation period, the vacuum was released, the beaker of chloroform removed, and the desiccator evacuated and vented five times to remove excess chloroform in the soil. The microbial biomass of fumigated and non-fumigated soil samples was extracted with 50 mL of .5 M K₂SO₄, filtered through Whatman GF-C filter paper (1.2 µm pores), and frozen at -20 °C. Extracts were sent to the University of Delaware Soil Laboratory for analysis of total organic and inorganic C, and total bound N, using simultaneous electrochemical sensing and high temperature catalytic oxidation on a Vario TOC cube (elementar Americas Inc., NJ). Total dissolved organic C (DOC) and total dissolved N (TDN) were quantified from the extracts of unfumigated soils and excluded particles, such as living microbial biomass, that were

to large to pass through the 1.2 μm filter during extraction.

Statistical Analysis

The data were analyzed using JMP Pro, Version 11 (SAS Institute Inc., Cary, NC). Analysis of variance was used to determine the significance of the effects of site for MBC, DOC, TDN, MBN, and all soil characteristics. Means were compared using an LSD procedure at the 0.05 level of significance and were done in each case regardless of ANOVA results (Saville 2013). Planned comparisons were used to test for significant differences between rural and urban sites.

Results

Soil Characteristics

Forest soil characteristics impact the growth and activity of the plant and microbial community. When investigating and comparing MBC and MBN in urban and rural soils, it is important to take into account soil characteristics that may influence site differences in MBC and MBN. The soils of urban and rural forested sites were characterized by measuring soil nutrients (C, N, P, K, S, Ca, B, Mg), metals (Pb, Mg, Mn, Cu, Al, Fe, Zn), and properties (Total C, total N, C:N, %OM, pH, CEC).

Copper was the only soil characteristic to differ significantly among sites, while planned comparisons showed that Cu, Mg, Pb, Fe, and C:N were significantly greater in urban than rural forest soils (Table 2). The means of other soil characteristics ranged widely but not significantly between sites or urban/rural locations. Organic matter, an important source of C and N to the microbial community, ranged from 6.5 ± 0.3 to $9.0\pm 0.6\%$, and was highest at Oregon Ridge (Rural (R)). Soil pH, which affects the activity and structure of the microbial community, ranged from 5.2 to 6.2 ± 0.1 and was

least acidic at Gunpowder Falls (R) (Table 1). Sulphur, Mn, and Al did not differ between urban and rural locations (Table 2).

Table 1. Means of major forest soil characteristics by site and location. Urban and rural comparisons are included.

Type	Site	Total C (%)	Total N (%)	C:N	OM (%)	pH	P (kg/ha)	K (kg/ha)
Rural	Gunpowder Falls	4.1	0.32	12.9^B	8.6 ^{AB}	6.2	24.7	334
Rural	Loch Raven	4.0	0.30	13.2^B	6.5^B	5.8	51.6	363
Rural	Oregon Ridge	4.3	0.33	13.1^B	9.0^A	5.8	35.9	334
Urban	Cylburn Arboretum	6.2 [‡]	0.31	15.6^A	7.2 ^{AB}	5.2	42.6	236
Urban	Druid Hill	4.7	0.33	14.4 ^{AB}	6.8 ^{AB}	5.6	53.8	267
Urban	Leakin Park	4.8	0.35	14.0 ^{AB}	7.9 ^{AB}	5.6	63.9	328
	LSD	1.7	0.12	1.7	2.3	1.2	67.0	268
Rural	Mean	4.2	0.32	13.1[*]	8.0	5.9	37.3	344
Urban	Mean	4.8	0.33	14.4	7.3	5.5	55.6	285
	LSD	1.0	0.07	1.0	1.4	0.73	40.4	161

Means with the same letter are not significantly different. Means without letters indicate no significant difference between sites.

[‡] Cylburn Arboretum has no replication due to the exclusion of an outlier sample.

^{*} Indicates significance at $\alpha=0.05$ probability level.

Table 2. Means of minor forest soil characteristics by site and location. Urban and rural comparisons are included.

Type	Site	Ca (kg/ha)	Mg (kg/ha)	Pb (mg/kg)	Mn (kg/ha)	Cu (kg/ha)	Al (kg/ha)	Fe (kg/ha)	Zn (kg/ha)	S (kg/ha)	B (kg/ha)	CEC
Rural	Gunpowder Falls	4349	667^A	38^B	253 ^{AB}	6.3 ^{BC}	1279 ^{AB}	337 ^{AB}	20	30^B	2.8	14
Rural	Loch Raven	2959	417^B	49^B	217 ^{AB}	6.4 ^{BC}	1643^A	298^B	16	46^A	2.9	10
Rural	Oregon Ridge	3183	499 ^{AB}	53^B	229 ^{AB}	5.0^C	1022^B	317 ^{AB}	22	35 ^{AB}	2.6	10
Urban	Cylburn Arboretum	2576 [‡]	725^A	65 ^{AB}	236 ^{AB}	11.3 ^{AB}	1267 ^{AB}	376 ^{AB}	22	41 ^{AB}	1.68	12
Urban	Druid Hill	4035	669^A	98^A	92^B	14.9^A	1512 ^{AB}	402 ^{AB}	41	38 ^{AB}	2.1	14
Urban	Leakin Park	3632	673^A	62 ^{AB}	267^A	11.3^A	1496 ^{AB}	421^A	34	39 ^{AB}	2.5	13
	LSD	2567	248	40	173	4.8*	585	114	40	12	2.2	5.3
Rural	Mean	3487	526*	47*	233	5.9**	1314	318*	19	37	2.7	12
Urban	Mean	3575	681	77	191	12.7	1456	405	34	39	2.2	13
	LSD	1548	150	24	104	2.9	353	69	24	7.1	1.3	3.2

Means with the same letter are not significantly different. Means without letters indicate no significant difference between sites.

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level.

‡ Cylburn Arboretum has no replication due to the exclusion of an outlier sample.

Microbial Biomass Carbon and Dissolved Organic Carbon

The MBC of the soil sampled in June did not differ significantly among sites or between urban and rural sites (Table 3). In June, soil MBC site means ranged from 337 ± 15 to 495 ± 54 $\mu\text{g C/g}$ dry soil, with MBC significantly greater at Gunpowder Falls (R) than Cylburn Arboretum (Urban (U)) (Table 4). In contrast, MBC from soil collected in October differed among sites and between urban/rural locations (Table 3, Table 4). In October, soil MBC means ranged from 320 ± 14 to 476 ± 42 $\mu\text{g C/g}$ dry soil, similar to the June range, with all rural sites and Cylburn Arboretum (U) having significantly higher MBC than Druid Hill (U) (Table 4). The error variance (variation within a site), however, was much smaller in October than June (Table 3), resulting in statistically different significances.

The DOC of soil sampled in June did not differ significantly by site or between urban and rural locations (Table 3). In June, DOC ranged from 15.2 ± 2.7 to 22.2 ± 4.1 mg/L, with the greatest DOC levels at Leakin Park (U) and the lowest levels at Gunpowder Falls (R). Soil DOC collected in October was significantly greater in urban than rural locations (Table 3, Table 5). Soil DOC means ranged from 14.1 ± 0.28 to 21.4 ± 5.5 mg/L, similar to the June range.

Table 3. ANOVA for the effect of site on soil microbial biomass carbon (MBC) and nitrogen (MBN), dissolved organic carbon (DOC), and total dissolved nitrogen (TDN), for June and October. An urban and rural contrast is included.

Source of Variation	df	F-value and significance level of fixed effects MS of random error terms							
		MBC		DOC		MBN		TDN	
		June	October	June	October	June	October	June	October
Site	5	2.0	3.5*	1.24	1.13	3.2*	3.3*	0.43	2.54
Error	11 [†]	5731	2538	20.1	19.3	82.2	33.1	0.27	6.33
Contrast									
Urban vs Rural	1	1.9	6.2*	2.68	5.12*	8.3*	7.2*	0.78	6.35*

* Indicates significance at $\alpha=0.05$ probability level.

[†] One sample was lost from Loch Raven in June and one Cylburn Arboretum sample from October was removed as an outlier.

Table 4. Microbial biomass carbon (MBC) ($\mu\text{g C/g}$ dry soil) and nitrogen (MBN) ($\mu\text{g N/g}$ dry soil) means for June and October by site and location. Urban and rural comparisons are included.

		MBC		MBN	
Type	Site	June	October	June	October
Rural	Gunpowder Falls	495 ^A	435 ^A	66.0 ^A	38.9 ^A
Rural	Loch Raven	348 ^{AB}	431 ^A	48.4 ^{AB}	34.6 ^{AB}
Rural	Oregon Ridge	463 ^{AB}	476 ^A	54.7 ^{AB}	37.4 ^{AB}
Urban	Cylburn Arboretum	337 ^B	446 ^A	39.5 ^B	27.0 ^{BC}
Urban	Druid Hill	385 ^{AB}	320 ^B	42.6 ^B	23.8 ^C
Urban	Leakin Park	430 ^{AB}	392 ^{AB}	48.4 ^B	37.4 ^{AB}
	LSD	141	94*	16.8*	10.6*
Rural	Mean	446	447*	57.3*	37.0*
Urban	Mean	384	378	43.5	29.7
	LSD	81	54	9.7	6.1

In each column, means with the same letter are not significantly different.

* Indicates significance at $\alpha=0.05$ probability level.

Table 5. Dissolved organic carbon (DOC) (mg/L) and total dissolved nitrogen (TDN) (mg/L) means for June and October by site and location. Urban and rural comparisons are included.

Type	Site	DOC		TDN	
		June	October	June	October
Rural	Gunpowder Falls	15.2	14.1	2.67	9.81^A
Rural	Loch Raven	19.0	15.1	2.88	10.3^A
Rural	Oregon Ridge	15.5	16.2	2.42	8.02 ^{AB}
Urban	Cylburn Arboretum	17.3	21.4	2.94	8.81 ^{AB}
Urban	Druid Hill	21.0	20.1	2.93	5.11^B
Urban	Leakin Park	22.2	18.6	2.78	4.88^B
	LSD	8.3	8.1	0.96	2.07
Rural	Mean	16.2	15.1*	2.63	9.38*
Urban	Mean	20.2	19.9	2.88	5.95
	LSD	4.8	4.7	0.56	1.20

In each column, means with the same letter are not significantly different. Means without letters indicate no significant difference between sites.

* Indicates significance at $\alpha=0.05$ probability level.

Microbial Biomass Nitrogen and Total Dissolved Nitrogen

The MBN of the soils sampled in June and October differed significantly among sites and between urban and rural locations (Table 3). MBN means ranged from 39.5 ± 5.0 to 66.0 ± 5.8 $\mu\text{g N/g}$ dry soil in June, and from 23.8 ± 2.1 to 38.9 ± 0.3 $\mu\text{g N/g}$ dry soil in October (Table 4). While the MBN ranges in June and October are similar, the error variance was much smaller in October than in June (Table 3). In June, Gunpowder Falls (R) had a mean MBN of 66.0 ± 5.8 $\mu\text{g N/g}$ dry soil that was significantly higher than all urban sites. In October, Druid Hill (U) had a mean MBN of 23.8 ± 2.1 $\mu\text{g N/g}$ dry soil that was significantly lower than all rural sites.

The TDN of soils sampled in June did not significantly differ between sites or urban and rural locations (Table 3). In June, TDN means had a small range from 2.42 ± 0.33 to 2.94 ± 0.51 mg/L (Table 5). The TDN of soils sampled in October was significantly greater in rural than urban locations (Table 3, Table 5). Site TDN means

ranged from 4.88 ± 1.89 to 10.3 ± 1.70 mg/L, with Loch Raven (R) and Gunpowder Falls (R) having significantly greater soil TDN than Druid Hill (U) and Leakin Park (U) (Table 5).

Discussion

Soil Characteristics

Of the soil characteristics measured, pH, C:N, %OM, and the soil metals are arguably the most relevant to plant and/or soil microbial function. A soil pH of 7 is considered optimal for the growth of most microbes (Rosso et al. 1995). However, specific pH preferences of members within a community can result in large community shifts with changes in pH, with higher diversity at neutral pH levels (Fierer and Jackson 2006). The differences in the mean pH of individual soils, or mean pH of urban and rural soils, were not statistically significant. However, Gunpowder Falls (R) soil had the most neutral pH overall and among the highest quantities of MBC and MBN in both June and October. Thus, the more neutral pH of the soil possibly contributed to enhanced growth and activity of the microbial community at Gunpowder Falls, and the increased MBC and MBN at this site relative to other sites with lower average pH soils. Soil C:N is an important indicator of soil fertility. A low C:N suggests that more N is available in the soil for the growth of the plant and microbial community. The higher mean C:N of the urban soils relative to the rural soils may have reduced microbial growth and biomass accumulation, as plants compete more strongly with soil microbes for available soil N. In addition, microbial C:N was greater in urban (9.0 ± 0.37) than rural soils (7.8 ± 0.28) in June ($F_{1,11} = 6.1$, $p = 0.03$). Both microbial and soil C:N could have been affected by earthworm quantities. In particular, earthworms break down recalcitrant leaf litter and release high N castes in the soil, thereby increasing the N available to the microbial

community. Also, their castes can be favorable to bacterial growth by providing greater organic matter, moisture, and extractable inorganic N than the bulk soil, and detrimental to fungal growth by physically disrupting their structure (Zhu and Carreiro 1999). In a study of forest soils along an urban to rural gradient in New York City, Steinberg et al. (1997) found that the presence of earthworms increased microbial N mineralization and nitrification rates. Therefore, the low soil and microbial C:N of rural forest soils in this study may have been influenced by high earthworm quantities. Earthworm quantities were not measured, but should be considered in future research. Pouyat et al. (2008) also found higher metal concentrations in urban forest soils, where Cu and Pb correlated significantly and positively with urbanization along an urban-rural gradient in Baltimore, MD. In the present study, the soil metals Cu, Pb, and Fe were significantly greater in the urban than rural forest soils. Metals such as Cu, Pb, and Fe inhibit the activity of the soil microbial community, and may have played a part in reducing the microbial biomass quantities observed in urban relative to rural sites in this study (Kao et al. 2006, Khan et al. 2010).

Microbial Biomass Carbon and Dissolved Organic Carbon

The rural forest soils in this study had greater mean MBC quantities than the urban forest soils. However, due to higher error variance in June, the difference between the means of urban and rural sites was only statistically significant in October. This implies that the long-term soil C stored in microbial biomass is greater in the rural than the urban forest soils. This was unexpected, as previous studies have indicated that elevated CO₂ stimulated the metabolism and storage of C as MBC by the microbial community through increased production of plant root exudates (Lipson et al. 2005, Phillips et al. 2011). If CO₂ were the main factor influencing the MBC levels of these

forest soils, then urban forest soils, which have experienced higher levels of CO₂ than rural forests, should have had a greater mean MBC than rural forest soils. In this study, although the MBC was greater in rural soils, the soil DOC was greater in urban soils, suggesting that increased CO₂ increased labile C from plant root exudates in urban environments (Table 5) but factors other than temperature and CO₂ influence soil MBC.

A similar study by Groffman et al. (1995), investigating urban to rural forest gradients in and around New York City, found that the MBC of rural forest soil was significantly greater than urban forest soil. They attributed this difference to earthworm activity and leaf litter lignin content, both of which increased with urbanization. While neither of these factors were measured in the present study, MBC was found to be significantly negatively correlated with Cu ($R=-0.85$, $p=0.03$) and Pb ($R=-0.87$, $p=0.02$), both of which were significantly greater in urban than rural forest soils (Table 2).

Previous studies by Kao et al. (2006) and Khan et al. (2010) have found that the application of Cu and Pb to the soil negatively affect the respiration, enzymatic activity, and (to a lesser extent) the MBC of the soil microbial community. In a study where biosolids spiked with either Cu, Pb, or Zn were mixed with *Typic Udorthent* soil (16 mg/kg Cu; 18 mg/kg Pb) from Taiwan, Kao et al. (2006) found that elevated Cu and Pb reduced microbial respiration rates and MBC quantities relative to the untreated biosolid application. Microbial respiration rates in metal amended biosolid applications were significantly decreased from the untreated biosolid application at soil metal concentrations of 100 mg/kg Cu, 400 mg/kg Cu, 250 mg/kg Pb, and 1000 mg/kg Pb. Soil MBC also declined, although not significantly, with increasing metal concentration. Similarly, in a 12 week study of agricultural soil (1.79 mg/kg Pb) amended with varying

concentrations of Pb and Cd, Khan et al. (2010) found that MBC and enzyme activity declined with increasing Pb concentrations. For soil MBC, this general decline was not significant, while enzyme activity (acid phosphatase and urease) was significantly lower than the untreated soil at 150, 300, and 500 mg/kg Pb. These studies suggest that while MBC does decline with increasing soil Cu and Pb concentration, this decline is not significantly great for concentrations up to 400 mg/kg Cu and 1000 mg/kg Pb over a 12 week period. In the present study, average soil Pb amounts were 47 ± 7.4 mg/kg (Rural) and 77 ± 8.1 mg/kg (Urban), while average Cu amounts were 5.9 ± 0.8 mg/kg (Rural) and 12.7 ± 0.9 mg/kg (Urban). Therefore, while it is likely that Cu and Pb had some negative impact on the MBC of urban relative to rural soils, it is not likely that they were the only/primary environmental factors driving these differences.

Microbial Biomass Nitrogen and Total Dissolved Nitrogen

The rural forest soils in this study had greater mean MBN quantities than the urban forest soils in both June and October, and greater TDN quantities in October. The greater soil MBN in the urban compared to the rural sites could be related to the higher average C:N in urban (C:N 14.4 ± 0.4) versus rural (13.1 ± 0.1) sites. Also, the lower average October TDN of rural (9.38 ± 0.48 mg/L) compared to urban (TDN 5.95 ± 1.22 mg/L) sites may have also affected the MBN in October. Greater C:N and lower labile TDN in urban soil could have led to greater competition between plant roots and microbes for available N. High C:N, in turn, may have been driven by either the urban climate (elevated temperature and CO₂) or local tree species composition (Pouyat and Carreiro 2003). While tree species composition at each site was not evaluated in detail, the presence of high C:N tree species, such as oak, in the leaf litter may have reduced the MBN in urban forest soil samples relative to rural soils. Earthworms were another

unmeasured soil factor that could have affected C:N and TDN between urban and rural soils. Earthworm activity strongly affects the N cycle of the soil as earthworms break down leaf litter and increase available soil N more quickly than either fungi or bacteria (Pouyat and Carreiro 2003, Bohlen et al. 2004). Earthworms also disrupt soil fungal structures as they move through the soil profile, which reduces bacteria:fungi in the soil. This can cause subsequent increases in the microbial C:N, as bacterial biomass typically has a lower C:N than fungal biomass. In a similar study of deciduous forests along an urban to rural gradient in and around New York City, Zhu and Carreiro (2004) found that soil MBN was greater in rural than urban sites. They attributed this difference to %OM, which positively correlated with MBN and was 1.7 times greater on average in rural than urban forest soils. In the present study, %OM was not significantly different between urban and rural forest soils. More likely causes of reduced MBN in urban relative to rural soils in this study are metal concentration and C:N. Soil MBN was significantly negatively correlated with Pb ($R=-0.89$, $p=0.02$), Cu ($R=-0.85$, $p=0.03$), and C:N ($R=-0.81$, $p=0.049$), all of which were significantly greater in urban than rural site soils (Table 1, Table 2). A study by Kao et al. (2006) found that over twelve weeks, biosolids treated with both Pb and Cu and applied to soils had reduced soil MBN relative to the untreated biosolid applications. This decline in soil MBN was significant for Cu, but not significant for Pb. Soil application amounts ranged from 100-400 mg/kg (Cu) and 250-1000 mg/kg Pb, and the effects of Cu on MBN were significant at 100 mg/kg. In the present study, mean Pb concentrations at the urban and rural sites were 47 ± 7.4 and 12.7 ± 0.9 mg/kg, respectively. Mean Cu concentrations at the urban and rural sites were 77 ± 8.1 mg/kg and 5.9 ± 0.8 mg/kg, respectively. As with MBC, it is not likely that Pb and

Cu were the only environmental factors driving MBN differences between urban and rural soils. Although unmeasured environmental factors, such as leaf litter lignin content, soil texture, and earthworm activity may have contributed to the site variation in MBC, MBN, and TDN, sites were specifically chosen to have similar tree species composition and soil type and are not expected to be strongly impacted by these factors.

Finally, the residual error for both MBC and MBN in June was approximately twice as great as in October. The difference in residual error between June and October precluded the analysis of month effects on the combined dataset. High between- and within-site variation is typical for studies of forest soils. In order to minimize the residual error and type-II errors, future studies of this kind should have a greater within-site sample size and encompass a greater sample area.

Controlled Environmental Chamber Study

Goals and Objectives

In the Baltimore area, urban forests have historically experienced higher levels of temperature and CO₂ than rural forests (Brazel et al. 2000, Ziska et al. 2003, Ziska et al. 2004). The soil microbial communities of urban and rural forests may have adapted to these temperature and CO₂ differences, as the structure and function of soil microbial communities can be sensitive to CO₂ and temperature (Allison and Martiny 2008). Combined gradients of temperature, CO₂, and urbanization have been investigated in ‘space-for-time’ experiments, in which current urban ecosystem functions represent the future function of rural ecosystems along the gradient (Carreiro and Tripler 2005). However, few studies have examined the effects of elevated temperature and CO₂ on soil

MBC or MBN in both urban and rural forest soils (Groffman et al. 1995, Zhu and Carreiro 2004).

The study objectives were to investigate and compare the effects of elevated temperature and/or CO₂ on the soil microbial C storage and soil N pools of urban and rural forests. A controlled environmental chamber study was conducted to determine the effects of short-term exposure to elevated levels of CO₂ and temperature on C storage and N pools of urban and rural forest soils by quantifying C stored in soil MBC and soil N pools primarily as MBN, soil ammonium, and soil nitrate. The effects of elevated CO₂ and temperature on soil dissolved organic carbon (DOC), microbial C:N, above/belowground biomass of hybrid poplar trees (OP-367 (*Populus x canadensis*)) and tulip poplar (*Liriodendron tulipifera*) trees were also measured.

Environmental chambers were used to expose soils and trees to a 2x2 factorial combination of ambient and elevated levels of CO₂ and air temperature. Soils collected from three urban and three rural forest sites were placed in the chambers for seven weeks. During the experiment, hybrid poplars were grown in pots containing field soils to mimic field conditions and serve as an intermediate between CO₂ and the soil microbial community.

Materials and Methods

Controlled Environmental Chamber Treatments

Soils collected at the six sites were put in pots and placed in one of four controlled environments from July 8 To August 26. Four controlled environmental chambers (two growth chambers at the University of Maryland and two climate-controlled greenhouses at the USDA/BARC in Beltsville, MD) were randomly assigned a temperature/CO₂ treatment, which consisted of a factorial combination of ambient and elevated levels of

temperature and CO₂. The treatments were: ACAT = ambient CO₂ and ambient temperature, ACET = ambient CO₂ and elevated temperature, ECAT= elevated CO₂ and ambient temperature, and ECET= elevated CO₂ and elevated temperature. The ambient air temperature was set to 23 °C, the average of the minimum and maximum daily air temperatures in June 2010 at two urban and four rural weather stations in the Baltimore area based on weather records obtained from the National Oceanic and Atmospheric Administration (Appendix A1) (National Oceanic and Atmospheric Administration 2014). The elevated air temperature was set to 25.5 °C, which is a 2.5 °C increase from the ambient temperature as predicted for 2090 (IPCC 2007). Ambient CO₂ levels were approximately 450 ppm in the University of Maryland growth chambers and approximately 405 ppm in the USDA greenhouses. In both locations, the ambient CO₂ treatment was not regulated, but was continuously measured and recorded using an infrared gas analyzer and datalogger equipment. The elevated CO₂ level was set to 600 ppm, which is the 2013 global average atmospheric CO₂ concentration and the amount predicted for 2090 according to emission scenario A1B (IPCC 2007, Earth System Research Laboratory: Global Monitoring Division 2012).

The growth chambers were 3.7 m² and were illuminated with 16 400-watt T-15 clear (Metal Halide) lamps and 16 100-watt MB frosted (Tungsten Halogen) lamps from 6:00-20:00 UT each day. Both chambers were programmed for the ambient temperature treatments at a constant temperature of 23 °C. One chamber was maintained at ambient CO₂ levels (ACAT treatment), which ranged from 400 to 500 ppm over the 7-week experiment. The other chamber received supplemental CO₂ to maintain an elevated level of 600 ppm CO₂ (ECAT treatment).

The climate-controlled greenhouses received only natural sunlight, and the average within-greenhouse irradiance was 196 ± 8.1 W/m² per day over the course of the study. At the start of the experiment (July 8), daylight was from 5:48-20:35 UT. At the end of the experiment (August 26), daylight was from 6:30-19:46 UT (Time and Date AS 2015). Both greenhouses were programmed to receive elevated temperature treatments at a constant temperature of 25.5 °C, and maintained an average temperature of 25.3 °C throughout the study. One greenhouse was maintained at ambient CO₂ levels (ACET treatment), which averaged 416 ppm over the 7-week experiment. The other greenhouse received supplemental CO₂ to maintain a programmed elevated level of 600 ppm CO₂ (ECET treatment). Average CO₂ levels for the ECET treatment were 596 ppm over the course of the study. Although temperatures decrease at night in the field, this was not replicated in the controlled chambers/greenhouses due to technical difficulties.

Due to the limited availability of chamber/greenhouses able to provide supplemental CO₂ and time to repeat the experiment, the treatments were not replicated. Thus, the effects of temperature on the soils and plants were confounded with the differences between the growth chambers and the greenhouses, most notably the light intensity. Since the light intensity was higher in the greenhouses than the growth chambers, the increases between ambient and elevated temperatures were most likely caused by the combination of both increased temperature and light.

Soils

Soils used in this study were collected on June 28 and 30, 2014 at the same urban and rural forested sites analyzed in the field study. At each site, two and a half gallons of soil were collected to a depth of 10 cm (excluding the litter layer) near the same three

interior tulip poplar trees as in the field study. Soils at each site were homogenized and refrigerated for one week prior to their addition to pots.

Eighteen pots were filled with soil from each field site, with three pots per site were randomized in each controlled environment. A hybrid poplar cutting was planted in two of the three replicate pots in each chamber. The third replicate pot was left as an unplanted control to test whether or not the effects of elevated temperature and elevated CO₂ on soil MBC were significant without the hybrid poplar intermediate. Thus, there were 12 pots (6 sites x 2 reps) with a plant and six pots (6 sites x 1 rep) without a plant exposed to each CO₂/temperature treatment. Each controlled environment also contained two (USDA Beltsville) or three (University of Maryland) tulip poplar seedlings planted in potting soil. All pots were watered to soil saturation once every two days.

Trees

Hybrid Poplar

Trees were planted in the soils to mediate the effects of CO₂ on the soil community, as would occur at the forest sites. Hybrid poplar cuttings (genotype OP-367) (hybridpoplars.com, Glenmoore, PA) were used because they were genetically identical, of uniform size (approximately 7.6 cm), and lacked an initial root system. Prior to the start of the experiment, cuttings were grown in potting soil and misted every 15 minutes in the University of Maryland greenhouse. After three weeks, the rooted cuttings were rinsed and planted in 500 cm³ pots containing soils collected from the forest sites. Initially, the roots were approximately 3.8 cm long. After seven weeks, the hybrid poplar roots grew to have contact with a majority of the soil but were not observed to be root-bound.

Tulip Poplar

Tulip poplar seedlings (Cold Stream Farm LLC, Free Soil, MI) were planted in each controlled environment to help reduce the CO₂ build-up that may have occurred in the chambers due to the respiration of the soil microbial community. Each controlled environment contained two (USDA Beltsville) or three (University of Maryland) tulip poplar seedlings growing in potting soil. Tulip poplar seedlings were 76 cm tall and dormant when planted in potting soil, and were placed in controlled environments on July 8, 2014.

Laboratory Analyses

Woody Biomass

After seven weeks, the hybrid poplars and tulip poplars were harvested and separated into above and belowground woody biomass. Above and belowground

components were placed in separate paper bags and dried in drying ovens at the University of Maryland greenhouse at 40 °C for one week, and then weighed.

Soils

The protocol for MBC and MBN extraction and quantification was the same as detailed in the laboratory analysis section of the field study. DOC represents the total organic C quantified from the extracts of unfumigated soils. DOC excludes all C containing particles, such as living MBC, unable to pass through the 1.2 µm glass fiber filter.

Ammonium and nitrate were extracted from planted soils based on the methods of Baxter et al. (2002). The 10 g dry weight equivalent (13.5 g) was extracted with 30 mL of 2M KCl, then filtered through Whatman GF-C filter paper. Extracts were stored at -20 °C until analysis by Kreshnik Bejleri of the Environmental Science and Technology Department at the University of Maryland. Ammonium and nitrate were quantified from the soil extracts using a QuikChem® 8500 Autoanalyzer (Hach Company, Loveland, CO).

Statistical Analysis

The data were analyzed using JMP Pro, Version 11 (SAS Institute Inc., Cary, NC). Analysis of variance was used to determine the significance of the effects of site, temperature, CO₂, and their interactions on MBC, DOC, MBN, microbial C:N, nitrate, and ammonium quantities for the soils in pots with hybrid poplar cuttings. Analysis of variance was also conducted to determine the significance of these effects on hybrid poplar above/belowground woody biomass. For tulip poplar above/belowground woody biomass and the MBC of unplanted soils, ANOVAs were conducted to determine the

significance of the effects of site, temperature, CO₂, and all interactions except for site x temperature x CO₂ (Appendix A3). Means were compared using an LSD procedure at the 0.05 level of significance. Planned comparisons were used to test for significant differences between rural and urban sites.

Results

Microbial Biomass Carbon and Dissolved Organic Carbon

Soil MBC quantities differed significantly due to the main effects of CO₂ and site, but not temperature or the interactions of the main effects (Table 6). The mean MBC quantities of the main effects of temperature and CO₂ are given in Table 7. Overall, the mean MBC was 10% greater under elevated than ambient CO₂ levels (Table 7). However, at Oregon Ridge (rural (R)) and Cylburn Arboretum (urban (U)), the mean MBC of the elevated CO₂ treatments was not greater than the mean of the ambient CO₂ treatments (Table 7). Table 8 gives the MBC means for each treatment at each site. Overall, the mean MBC of rural sites (415±14 µg C/g dry soil) was significantly greater than urban sites (303±14 µg C/g dry soil) (Table 8). The difference between rural and urban mean MBC was also significant for ambient and elevated CO₂ treatments. Overall, Oregon Ridge (R) had the highest, and Cylburn Arboretum (U) and Druid Hill (U) had the lowest, mean MBC (Table 8).

Soil DOC quantities did not differ significantly due to the main effects of site, temperature, CO₂, or their interaction, and did not differ significantly between urban and rural locations (Table 6). The mean DOC quantities of the main effects of temperature and CO₂ are given in Table 7. Table 8 gives the DOC means for each treatment at each site.

Table 6. ANOVA for the effects of site, temperature, CO₂, and their interactions on the microbial biomass carbon (MBC), dissolved organic carbon (DOC), microbial biomass nitrogen (MBN), microbial C:N, ammonium, and nitrate of planted soils. Contrasts between urban and rural groups are included.

Source of Variation	df	F-value and significance level					
		MBC	DOC	MBN	Microbial C:N	Ammonium	Nitrate
Site	5	18.7***	0.58	3.7*	0.85	18.4***	3.3*
CO ₂	1	5.8*	0.01	7.4*	3.1	0.03	6.1*
Temperature	1	2.5	0.47	4.8*	5.2*	0.92	19.1***
Temperature x CO ₂	1	0.02	0.10	1.1	2.8	2.6	0.44
Site x CO ₂	5	1.6	2.0	1.0	0.72	0.82	0.66
Site x Temperature	5	0.54	0.24	0.20	0.77	0.29	0.48
Site x CO ₂ x Temperature	5	2.3	0.61	0.86	0.96	2.6	1.1
Error	24	--	--	--	--	--	--
Contrast							
Urban vs Rural	1	61.7***	0.55	8.3**	0.03	15.8***	2.8

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level ***Indicates significance at $\alpha=0.001$ probability level.

Table 7. Mean soil microbial biomass carbon (MBC) ($\mu\text{g C/g dry soil}$) and dissolved organic carbon (DOC) (mg/L) for temperature and CO_2 levels at each site. Soils were planted with hybrid poplar. A contrast between urban and rural groups is included. For all treatment level comparisons, the LSD was 72 for MBC and 5.9 for DOC. For all urban and rural contrasts, the LSD was 42 for MBC and 3.4 for DOC.

MBC									
Temperature Level	GF [†]	LR	OR	CA	DH	LP	Rural Average	Urban Average	Overall Average
Ambient	422	395	463	267	272	401	427***	313	370
Elevated	374	383	452	263	273	340	403***	292	348
CO ₂ Level									
Ambient	341**	375	456	272	260	346	390***	293	342
Elevated	455	403	460	259	284	395	439***	313	376
DOC									
Temperature Level	GF	LR	OR	CA	DH	LP	Rural Average	Urban Average	Overall Average
Ambient	17.8	17.1	16.1	18.2	15.4	17.8	17.0	17.1	17.1
Elevated	19.4	16.7	15.6	18.2	18.4	19.1	17.2	18.6	17.9
CO ₂ Level									
Ambient	15.6*	17.8	16.0	21.1*	15.6	18.3	16.5	18.4	17.4
Elevated	21.6	16.1	15.7	15.3	18.2	18.5	17.8	17.3	17.6

[†] GF= Gunpowder Falls, LR= Loch Raven, OR= Oregon Ridge, CA= Cylburn Arboretum, DH= Druid Hill, and LP= Leakin Park.

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level, *** Indicates significance at $\alpha=0.001$ probability level.

Table 8. Treatment means of soil microbial biomass carbon (MBC) ($\mu\text{g C/g}$ dry soil) and dissolved organic carbon (DOC) (mg/L) by site and location. Urban and rural comparisons are included.

Type	Site	MBC				Site Mean	DOC				Site Mean
		ACAT [†]	ACET	ECAT	ECET		ACAT [†]	ACET	ECAT	ECET	
Rural	Gunpowder Falls	344	338	500	410	398^B	16.5	14.7	19.1	24.1	18.6 ^A
Rural	Loch Raven	391	359	399	406	389^B	18.0	17.6	16.3	15.9	16.9 ^A
Rural	Oregon Ridge	492	419	434	485	458^A	17.1	15.0	15.1	16.3	15.9 ^A
Urban	Cylburn Arboretum	298	245	236	282	265^C	20.0	22.3	16.3	14.2	18.2 ^A
Urban	Druid Hill	259	262	285	283	272^C	14.8	16.4	15.9	20.4	16.9 ^A
Urban	Leakin Park	339	354	464	327	371^B	16.7	20.0	18.9	18.1	18.4 ^A
	LSD	102	102	102	102	51	8.3	8.3	8.3	8.3	4.1
Rural	Mean	409^{**}	372^{**}	445[*]	434^{***}	415^{***}	17.2	15.7	16.8	18.7	17.1
Urban	Mean	299	287	328	297	303	17.2	19.6	17.1	17.6	17.8
	LSD	59	59	59	59	29	4.8	4.8	4.8	4.8	2.4

Means with the same letter are not significantly different according to an LSD at 0.05 probability.

[†]ACAT = ambient CO₂ and temperature, ACET = ambient CO₂ and elevated temperature, ECAT=elevated CO₂ and ambient temperature, and ECET= elevated CO₂ and temperature.

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level, *** Indicates significance at $\alpha=0.001$ probability level.

Microbial Biomass Nitrogen and the Microbial C:N

Soil MBN differed significantly due to the main effects of temperature, CO₂, and site, but not their interactions (Table 6). The MBN temperature and CO₂ means for each site are given in Table 9. Mean MBN was 28% greater under elevated than ambient temperature levels and was 36% greater under elevated than ambient CO₂ levels. For all sites, MBN was consistently greater under elevated than ambient temperature levels. For all sites except for Cylburn Arboretum (U), MBN was consistently greater under elevated than ambient CO₂ levels (Table 9). Table 10 gives the MBN means for each treatment at each site. Overall, the mean MBN of rural soils (38.3±3.3) was significantly greater than urban soils (27.9±2.3) (Table 10). The difference between rural and urban mean MBN was also significant for the elevated CO₂ treatment (Table 9). There was also a significant location x CO₂ interaction ($F_{1,24} = 4.8, p=0.04$), in which the MBN of rural sites was significantly greater under elevated than ambient CO₂ levels ($F_{1,22}=9.7, p=0.01$), while the MBN of urban sites did not significantly differ between ambient and elevated CO₂ levels ($F_{1,22}=0.17, p=0.68$). Overall, Oregon Ridge (R) had a mean MBN that was significantly greater than those of Cylburn Arboretum (U), Druid Hill (U), and Loch Raven (R) soils (Table 10).

Soil microbial C:N differed significantly due to temperature, but not CO₂, site, or the interactions of the main effects (Table 6). The microbial C:N temperature and CO₂ means for each site are given in Table 11. Mean microbial C:N was 45.1% lower under elevated than ambient temperatures. For all sites, microbial C:N was lower under elevated than ambient temperatures. Table 12 gives the microbial C:N means for each

treatment at each site. Overall, the mean microbial C:N of urban and rural soils were not significantly different.

Table 9. Mean soil microbial biomass nitrogen (MBN) ($\mu\text{g N/g}$ dry soil) for temperature and CO_2 levels at each site. Soils were planted with hybrid poplar. A contrast between urban and rural groups is included. For treatment level comparisons, the LSD was 18.2. For all urban and rural contrasts, the LSD was 10.5.

Temperature Level	GF[†]	LR	OR	CA	DH	LP	Rural Average	Urban Average	Overall Average
Ambient	34.2	27.1	43.7	16.4	22.0	31.5	35.0*	23.3	29.1
Elevated	35.5	38.9	50.4	27.8	31.7	37.9	41.6	32.5	37.1
CO₂ Level									
Ambient	24.0*	24.6	39.6	22.1	25.5	33.1	29.4	26.9	28.2
Elevated	45.7	41.4	54.4	22.1	28.2	36.4	47.2**	28.9	38.0

[†] GF= Gunpowder Falls, LR= Loch Raven, OR= Oregon Ridge, CA= Cylburn Arboretum, DH= Druid Hill, and LP= Leakin Park.

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level.

Table 10. Treatment means of soil microbial biomass nitrogen (MBN) ($\mu\text{g N/g dry soil}$) by site and location. Urban and rural comparisons are included.

Type	Site	ACAT [†]	ACET	ECAT	ECET	Site Mean
Rural	Gunpowder Falls	19.3	28.7	49.0	42.4	34.8 ^{AB}
Rural	Loch Raven	12.4	36.8	41.7	41.1	33.0^B
Rural	Oregon Ridge	41.8	37.5	45.6	63.2	47.0^A
Urban	Cylburn Arboretum	12.5	31.8	20.4	23.9	22.1^B
Urban	Druid Hill	20.3	30.8	23.7	32.6	26.9^B
Urban	Leakin Park	28.0	38.2	35.1	37.7	34.7 ^{AB}
	LSD	25.7	25.7	25.7	25.7	12.9
Rural	Mean	24.5	34.4	45.4^{**}	48.9[*]	38.3^{**}
Urban	Mean	20.3	33.6	26.4	31.4	27.9
	LSD	14.9	14.9	14.9	14.9	7.4

Means with the same letter are not significantly different according to an LSD at 0.05 probability.

[†] ACAT = ambient CO₂ and temperature, ACET = ambient CO₂ and elevated temperature, ECAT=elevated CO₂ and ambient temperature, and ECET= elevated CO₂ and temperature.

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level.

Table 11. Mean soil microbial C:N for temperature and CO₂ levels at each site. Soils were planted with hybrid poplar. A contrast between urban and rural groups is included. For treatment level comparisons, the LSD was 18.2. For all urban and rural contrasts, the LSD was 10.5.

Temperature Level	GF[†]	LR	OR	CA	DH	LP	Rural Average	Urban Average	Overall Average
Ambient	14.9	25.7	11.2	30.6*	13.1	13.9	17.3	19.2	18.2
Elevated	11.0	9.90	10.2	10.5	8.72	9.95	10.4	9.73	10.0
CO₂ Level									
Ambient	15.3	25.4	12.4	28.6	10.7	11.6	17.7	16.9	17.3
Elevated	10.6	10.2	9.03	12.6	11.2	12.3	9.93	12.0	11.0

[†] GF= Gunpowder Falls, LR= Loch Raven, OR= Oregon Ridge, CA= Cylburn Arboretum, DH= Druid Hill, and LP= Leakin Park.

* Indicates significance at $\alpha=0.05$ probability level.

Table 12. Treatment means of soil microbial C:N by site and location. Urban and rural comparisons are included.

Type	Site	ACAT [†]	ACET	ECAT	ECET	Site Mean
Rural	Gunpowder Falls	19.0	11.7	10.9	10.2	13.0 ^A
Rural	Loch Raven	41.0	9.82	10.4	9.98	17.8 ^A
Rural	Oregon Ridge	12.5	12.3	9.90	8.16	10.7 ^A
Urban	Cylburn Arboretum	48.0	9.20	13.3	11.8	20.6 ^A
Urban	Druid Hill	12.7	8.61	13.5	8.83	10.9 ^A
Urban	Leakin Park	13.7	9.50	14.1	10.4	11.9 ^A
	LSD	25.7	25.7	25.7	25.7	12.9
Rural	Mean	24.2	11.3	10.4	9.46	13.8
Urban	Mean	24.8	9.10	13.6	10.4	14.5
	LSD	14.9	14.9	14.9	14.9	7.43

Means with the same letter are not significantly different according to an LSD at 0.05 probability.

[†] ACAT = ambient CO₂ and temperature, ACET = ambient CO₂ and elevated temperature, ECAT=elevated CO₂ and ambient temperature, and ECET= elevated CO₂ and temperature.

Ammonium and Nitrate

Soil ammonium and nitrate are important sources of N for plant and microbial communities. Quantifying their concentration in the soil after temperature and CO₂ treatments provided insight into the nutrient status of the soil, the use of ammonium and nitrate by the plant and microbial communities, and their relationship to MBC, MBN, and hybrid poplar biomass.

Soil ammonium concentration differed significantly among sites, but did not differ due to temperature, CO₂, or the interactions of the main effects (Table 6). The ammonium temperature and CO₂ mean concentrations for each site are given in Table 13. Table 14 gives the mean ammonium concentrations for each treatment at each site. The ammonium concentrations of the rural soils (11.1 ± 1.1 mg/kg) were significantly greater than those of the urban soils (7.4 ± 1.1 mg/kg) (Table 14). Of the six sites, the mean soil ammonium concentration of Gunpowder Falls (R) was largest, while the mean ammonium level of Druid Hill (U) was smallest (Table 14).

Soil nitrate concentration differed significantly due to the main effects of temperature, CO₂, and site, but not their interactions (Table 6). The nitrate temperature and CO₂ mean concentrations for each site are given in Table 13. The mean soil nitrate concentration was 46% lower under elevated than ambient temperatures and 29% lower under elevated than ambient CO₂ levels. For all sites, mean soil nitrate was lower under elevated than ambient temperature and CO₂ levels (Table 13). Table 14 gives the mean nitrate concentrations for each treatment at each site. The difference in mean soil nitrate

concentration between urban and rural soils was not significant. Overall, the mean soil nitrate concentration at Druid Hill (U) was lower than at all other sites (Table 14).

Table 13. Mean soil ammonium and nitrate (mg/kg dry soil) for temperature and CO₂ levels at each site. Soils were planted with hybrid poplar. A contrast between urban and rural groups is included. For treatment level comparisons, the LSD was 4.7 for ammonium and 7.6 for nitrate. For all urban and rural contrasts, the LSD was 2.7 for ammonium and 4.4 for nitrate.

Ammonium									
Temperature Level	GF[†]	LR	OR	CA	DH	LP	Rural Average	Urban Average	Overall Average
Ambient	17.4	7.7	8.2	4.1	3.8	11.6	11.1**	6.5	8.8
Elevated	17.0	8.6	7.8	6.3	4.6	14.0	11.1*	8.3	9.7
CO₂ Level									
Ambient	15.7	8.0	7.7	6.6	4.3	13.8	10.5	8.2	9.3
Elevated	18.7	8.3	8.3	3.7	4.2	11.8	11.8****	6.7	9.2
Nitrate									
Temperature Level	GF[†]	LR	OR	CA	DH	LP	Rural Average	Urban Average	Overall Average
Ambient	11.9	18.5**	16.9	15.7*	6.3	16.2	15.8	12.7	14.2
Elevated	7.3	8.3	10.4	7.4	3.3	9.3	8.7	6.6	7.7
CO₂ Level									
Ambient	9.8	17.2*	16.0	14.8	5.7	13.4	14.3	11.3	12.8
Elevated	9.4	9.5	11.3	8.3	3.9	12.1	10.1	8.1	9.1

[†] GF= Gunpowder Falls, LR= Loch Raven, OR= Oregon Ridge, CA= Cylburn Arboretum, DH= Druid Hill, and LP= Leakin Park.

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level, *** Indicates significance at $\alpha=0.001$ probability level.

Table 14. Treatment means of soil ammonium and nitrate (mg/kg dry soil) by site and location. Urban and rural comparisons are included.

		Ammonium					Nitrate				
Type	Site	ACAT[†]	ACET	ECAT	ECET	Site Mean	ACAT	ACET	ECAT	ECET	Site Mean
Rural	Gunpowder Falls	15.1	16.4	19.8	17.6	17.2^A	11.0	8.7	12.8	6.0	9.6 ^{AB}
Rural	Loch Raven	8.7	7.2	6.7	10.0	8.2^C	24.2	10.2	12.8	6.32	13.4^A
Rural	Oregon Ridge	8.2	7.3	8.3	8.3	8.0^C	16.8	15.2	17.0	5.6	13.6^A
Urban	Cyburn Arboretum	4.6	8.7	3.6	3.90	5.2 ^{CD}	20.2	9.5	11.2	5.3	11.5^A
Urban	Druid Hill	3.9	4.6	3.75	4.6	4.2^D	7.9	3.5	4.72	3.1	4.8^B
Urban	Leakin Park	8.4	19.2	14.8	8.9	12.8^B	19.7	7.1	12.8	11.5	12.7 ^A
	LSD	6.7	6.7	6.7	6.7	3.3	10.8	10.8	10.8	10.8	5.4
Rural	Mean	10.7^{**}	10.3	11.6[*]	12.0 ^{**}	11.1^{***}	17.3	11.4	14.2	6.0	12.2
Urban	Mean	5.6	10.8	7.4	5.8	7.4	15.9	6.7	9.6	6.6	9.7
	LSD	3.9	3.9	3.9	3.9	1.9	6.2	6.2	6.2	6.2	3.1

Means with the same letter are not significantly different according to an LSD at 0.05 probability.

[†]ACAT = ambient CO₂ and temperature, ACET = ambient CO₂ and elevated temperature, ECAT=elevated CO₂ and ambient temperature, and ECET= elevated CO₂ and temperature.

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level, *** Indicates significance at $\alpha=0.001$ probability level.

Woody Biomass

Hybrid Poplar

CO₂ indirectly affects the soil microbial community through its effects on the plant production of root exudates and photosynthates, which are metabolized by the rhizosphere community (Treseder et al. 2005). In the present study, soils were planted with hybrid poplars to simulate natural forest conditions in which trees mediate the interaction of CO₂ with the microbial community. The role of trees as mediators between CO₂ and the soil microbial community is an important component of the soil C and N cycles. The above and belowground biomass of hybrid poplar was measured to gain insight into relationships between tree biomass and soil MBC, DOC, MBN, microbial C:N, ammonium, and nitrate, as affected by elevated temperature and CO₂.

The hybrid poplar aboveground woody biomass differed significantly due to the main effects of temperature, site, and the site x temperature interaction (Table 15). The mean aboveground woody biomass for the main effects of temperature and CO₂ are given in Table 16. Overall, the mean aboveground woody biomass was 49% greater under elevated than ambient temperature levels. For all sites, aboveground woody biomass was consistently greater under elevated than ambient temperature levels, although this difference was only significant for Gunpowder Falls (R), Cylburn Arboretum (U), and Druid Hill (U) (Table 16, Figure 2). Table 17 gives the aboveground biomass means for each treatment at each site. Overall, hybrid poplars planted in urban soils had significantly greater mean aboveground woody biomass (1.87 ± 0.16 g) than hybrid poplars planted in rural soils (1.52 ± 0.11 g) (Table 17). The mean aboveground woody biomass of hybrid poplars planted in urban soils was significantly greater than those in rural soils only when exposed to elevated temperature treatments (Table 16). Overall,

aboveground woody biomass grown in Druid Hill (U) soil was significantly greater than all other sites regardless of temperature treatment. Conversely, Loch Raven (R) and Leakin Park (U) had the lowest aboveground woody biomass for ambient and elevated temperature levels, respectively (Table 16).

In contrast to aboveground biomass, hybrid poplar belowground woody biomass was not significantly affected by any of the main effects or their interactions, or between urban and rural soils (Table 15). The mean belowground woody biomass for the main effects of temperature and CO₂ are given in Table 16. Table 17 gives the belowground biomass means for each treatment at each site.

Table 15. ANOVA for the effects of site, CO₂, temperature and their interactions on the above and belowground biomass of hybrid poplar. Contrasts between urban and rural groups are included.

Source of Variation	df	F-value and significance level	
		Aboveground Woody Biomass	Belowground Woody Biomass
Site	5	11.4***	1.1
CO ₂	1	3.0	3.9
Temperature	1	35.3***	0.0004
Temperature x CO ₂	1	1.3	0.81
Site x CO ₂	5	0.88	0.57
Site x Temperature	5	2.9*	0.49
Site x CO ₂ x Temperature	5	0.51	0.18
Error	24 [†]	--	--
Contrast			
Urban vs Rural	1	11.1**	3.7

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level ***Indicates significance at $\alpha=0.001$ probability level.

[†] Df for aboveground woody biomass is 23 due to a lost sample from treatment ACET at Cylburn Arboretum.

Table 16. Mean hybrid poplar above- and belowground woody biomass (g) for temperature and CO₂ levels at each site. A contrast between urban and rural groups is included. For treatment level comparisons, the LSD was 0.57 for aboveground biomass and 2.2 for belowground biomass. For all urban and rural contrasts, the LSD was 0.33 for aboveground biomass and 1.3 for belowground biomass.

Aboveground Woody Biomass									
Temperature Level	GF[†]	LR	OR	CA	DH	LP	Rural Average	Urban Average	Overall Average
Ambient	1.50*	0.85**	1.40	1.33**	1.83***	1.25	1.25	1.47	1.36
Elevated	2.10	1.63	1.58	2.30	3.23	1.43	1.77**	2.31	2.03
CO₂ Level									
Ambient	1.85	1.28	1.20*	1.73	2.35	1.20	1.44	1.70	1.57
Elevated	1.75	1.20	1.78	1.90	2.70	1.48	1.58**	2.03	1.80
Belowground Woody Biomass									
Temperature Level	GF[†]	LR	OR	CA	DH	LP	Rural Average	Urban Average	Overall Average
Ambient	2.48	1.53	2.05	2.13	3.88	2.60	2.02	2.87	2.44
Elevated	2.35	1.78	1.95	3.15	2.60	2.78	2.03	2.84	2.43
CO₂ Level									
Ambient	2.00	1.58	1.05	1.95	2.65	2.83	1.54	2.48	2.01
Elevated	2.83	1.73	2.95	3.33	3.83	2.55	2.50	3.23	2.87

[†] GF= Gunpowder Falls, LR= Loch Raven, OR= Oregon Ridge, CA= Cylburn Arboretum, DH= Druid Hill, and LP= Leakin Park.

* Indicates significance at $\alpha=0.05$ probability level, ** Indicates significance at $\alpha=0.01$ probability level ***Indicates significance at $\alpha=0.001$ probability level.

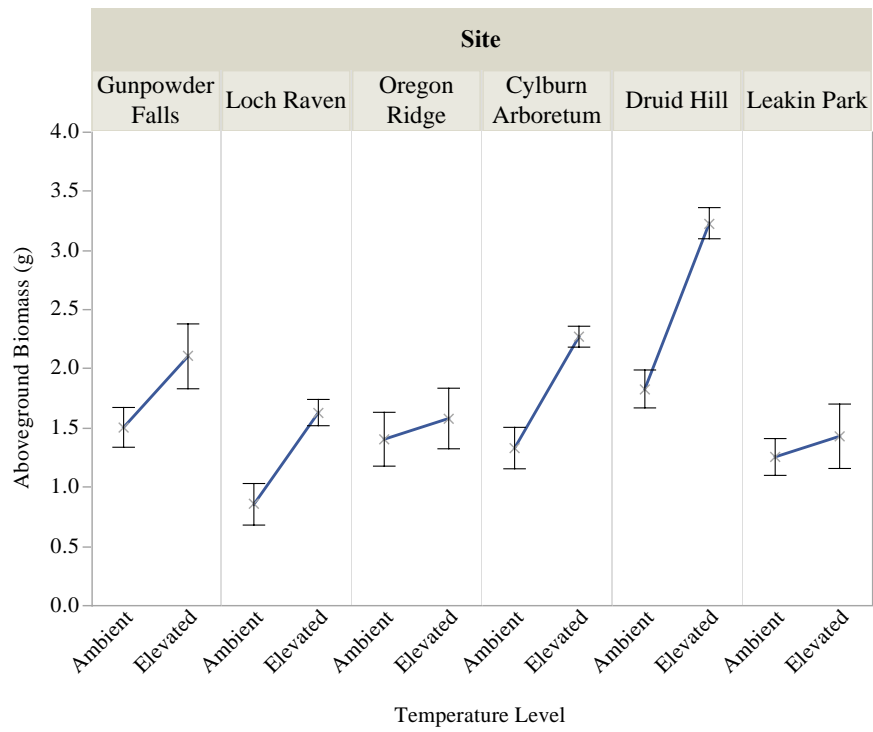


Figure 2. Site by temperature interaction shown for hybrid poplar aboveground woody biomass (g). Standard error bars are included.

Table 17. Treatment means of hybrid poplar above and belowground woody biomass (g) by site and location. Urban and rural comparisons are included.

Type	Site	Aboveground Woody Biomass				Site Mean	Belowground Woody Biomass				Site Mean
		ACAT [†]	ACET	ECAT	ECET		ACAT	ACET	ECAT	ECET	
Rural	Gunpowder Falls	1.4	2.35	1.65	1.85	1.80^B	1.45	2.55	3.5	2.15	2.41 ^{AB}
Rural	Loch Raven	0.85	1.70	0.85	1.55	1.24^C	1.35	1.80	1.7	1.75	1.65^B
Rural	Oregon Ridge	1.10	1.30	1.70	1.85	1.49 ^{BC}	1.15	0.95	3.0	2.95	2.00 ^{AB}
Urban	Cylburn Arboretum	1.05	2.40	1.60	2.20	1.81^B	1.20	2.70	3.1	3.60	2.64 ^{AB}
Urban	Druid Hill	1.65	3.05	2.00	3.40	2.53^A	3.20	2.10	4.6	3.10	3.24^A
Urban	Leakin Park	1.15	1.25	1.35	1.60	1.34^C	2.55	3.10	2.7	2.45	2.69 ^{AB}
	LSD	0.81	0.81	0.81	0.81	0.40	3.1	3.1	3.1	3.1	1.6
Rural	Mean	1.10	1.78	1.40	1.75^{**}	1.51^{**}	1.32	1.77	2.72	2.28	2.02
Urban	Mean	1.28	2.20	1.65	2.40	1.87	2.32	2.63	3.42	3.03	2.85
	LSD	0.47	0.47	0.47	0.47	0.23	1.8	1.8	1.8	1.8	0.90

Means with the same letter are not significantly different according to an LSD at 0.05 probability.

[†]ACAT = ambient CO₂ and temperature, ACET = ambient CO₂ and elevated temperature, ECAT=elevated CO₂ and ambient temperature, and ECET= elevated CO₂ and temperature.

** Indicates significance at $\alpha=0.01$ probability level.

Discussion

Microbial Biomass Carbon and Dissolved Organic Carbon

In this study, MBC quantities in planted soils were greater under elevated than ambient CO₂ levels, suggesting that as CO₂ levels increase in the Baltimore metropolitan area, the amount of C stored in the forest soil as MBC will also increase. As MBC increases, the long-term storage of C as microbial necromass will likely increase, which would prevent this C from returning to the pool of greenhouse gases. In unplanted soils, however, MBC quantities were not greater under elevated than ambient CO₂ levels (Table 18). This suggests that the effect of elevated CO₂ on the microbial community was mediated by the hybrid poplars. Some similar studies have suggested that either enhanced root biomass or exudate production were responsible for observed MBC quantities, which were greater under elevated than ambient CO₂. In a four-year study of soils from a subtropical forest in China, Chen et al. (2012) found that soils exposed to elevated CO₂ (700 ppm) in open-top chambers had significantly larger quantities of MBC than soils exposed to ambient CO₂ treatments. Chen et al. (2012) attributed this increase to an increase in new C inputs from plant root biomass. As part of the Duke Forest Free Air CO₂ Enrichment (FACE) experiment, Phillips et al. (2011) found that mature forest plots exposed to elevated CO₂ (585 ppm) had significantly greater soil MBC than forest plots exposed to ambient CO₂ treatments (385 ppm). They, however, attributed this increase in MBC to tree root exudate production, which also significantly increased in soils exposed to elevated CO₂. In the present study, soil DOC, which is primarily composed of small organic compounds from root exudates and plant/microbial necromass, was not significantly affected by temperature, CO₂, or their interaction. It is likely that the elevated CO₂ levels significantly increased MBC but not DOC because the increase in

these easily degraded C sources was balanced by increased C uptake by the living plant and microbial community.

In the literature overall, the effects of CO₂ on soil MBC in mature forest soils have been equally divided between positive and neutral responses, while mostly positive responses were observed in grassland ecosystems (Zak et al. 2000). A meta-analysis by Hu et al. (2006) found that 19 studies of mature forests observed positive effects, while 21 studies observed non-significant effects, of elevated CO₂ on soil MBC. The difference in response to CO₂ between mature forests and grasslands can be directly related to soil N availability, which is typically more limiting in mature forest than grassland ecosystems. When C additions (as exudates and root growth) increase soil C:N under elevated CO₂, N increasingly limits plant and microbial activity and growth to some degree. The present study involved a short-term (49 day) exposure of soils to elevated and ambient CO₂ and temperature levels. The observed effects of CO₂ on MBC and MBN may not hold with a longer exposure time, as N limitation becomes more prevalent.

The results of this study also suggest that rural forest soils in the Baltimore metropolitan area are larger microbial C stores on a per hectare basis than urban soils, and that this difference may persist in the future under elevated CO₂ and elevated temperature. The persistence of rural and urban MBC differences regardless of temperature and CO₂ exposure levels indicates that either 1) the soil microbial communities have adapted structurally to environmental differences between urban and rural forests such that, in the short-term, elevated temperature and CO₂ did not cause the MBC quantities from these communities to converge, or 2) soil factors unaffected by

short-term temperature and CO₂ treatments persisted from the field study to drive urban and rural soil MBC differences throughout the controlled chamber study.

The structure of microbial communities can be sensitive to changes in temperature and CO₂. A meta-analysis by Allison and Martiny (2008) found that microbial community structure was significantly altered by elevated CO₂ in 60%, and elevated temperature in 82%, of studies. Differences in average temperature and CO₂ levels between urban and rural forests can therefore result in divergent soil microbial communities. Microbial phyla vary in their metabolic requirements and changes in the structure of these communities (the fungi:bacteria for instance) in the present study could have caused the differences in microbial C storage (MBC) between urban and rural sites. In the present study, the microbial C:N was lower under elevated than ambient temperature treatments, and may have been influenced by a more predominant bacterial (lower biomass C:N than fungi) community under the elevated temperature treatment. Soil factors unaffected by short-term temperature and CO₂ treatments, but which affect soil MBC, could also have differentiated the MBC of urban and rural forest soils in this study, and include soil texture and metal content. While soil sampling locations in this study were all broadly designated as containing *Ultic hapludalf* soils through the USDA soil survey, soil texture may have varied across microsites in the forest soil. Soil texture was not measured in this study, but soils high in clay may have lower MBC due to the increased binding of, and aggregate formation around, organic matter used as C and N sources by the microbial community (Sulman et al. 2014). Soil metal content can also negatively affect soil MBC. In the field study, Cu and Pb were greater in urban than rural forest soils and were negatively correlated with MBC (Cu (R=-0.85, p=0.03) and Pb (R=-

0.87, $p=0.02$). Some previous studies have also found that urban soils had greater Pb and Cu concentrations than rural soils, and that MBC was negatively correlated with these metals (Kao et al. 2006, Khan et al. 2010, Zhao et al. 2013). The levels of Cu and Pb in the soils of the present study (Cu 8.9 ± 1.2 mg/kg; Pb 60.3 ± 7.0 mg/kg), however, were much lower than soil concentrations in the aforementioned studies. Therefore, while Cu and Pb may have inhibited microbial activity in the urban soils to some degree, it is not likely that they were the only/primary environmental factors driving the rural-urban differences in MBC.

Microbial Biomass Nitrogen and Microbial C:N

Microbial biomass has a short turnover time, and is an important source of organic N (such as amino acids) to the plant and living microbial community. The present study found that MBN was greater under elevated than ambient temperatures. However due to the design of the controlled environmental chamber study, the temperature effect may have become intertwined with the light effect, as irradiance was not likely identical between environment types (growth chambers versus greenhouses). The greater MBN observed in elevated than ambient temperature levels in the present study was consistent with previous studies showing that decomposition by the microbial community, and the general availability of N in the soil, increased with temperature (Melillo et al. 2002). However, observed MBN differences between ambient and elevated temperature treatments may be influenced by differences in irradiance. For instance, photosynthesis increases with irradiance until the carboxylation rate step becomes limiting (Lambers et al. 2008). As photosynthesis increases, more C is allocated to areas such as root growth and exudate production, two important sources of C for the microbial community. Root exudates, in particular, can stimulate the mineralization rates

of organic matter, and the assimilation of N, by the microbial community through priming effects. This in turn can increase the N taken up by the microbial community and stored as MBN. Therefore, while temperature has direct effects on the metabolic rates of the microbial community, light can have indirect effects through the stimulation of photosynthesis, and subsequent plant growth and exudate production.

In the present study, MBN was also greater under elevated than ambient CO₂ levels suggesting that as CO₂ levels increase in the Baltimore metropolitan area, available N as MBN in forest soils will increase as well. The positive effect of elevated CO₂ on MBN observed in the present study, however, conflicts with many studies of mature forest soils, which found no significant effect of CO₂ on soil MBN (Zak et al. 2003, Finzi et al. 2006). This contradiction could be due in part to competition for N between the plant and microbial community, which can be relatively strong in mature forest ecosystems that do not receive significant N input from deposition. As part of the Duke FACE experiment, Finzi et al. (2006) found that over six years, MBN did not differ due to elevated CO₂. There was, however, an interaction between CO₂ and time for the N content of tree woody biomass, such that N content was greater under elevated than ambient CO₂ treatments over time. This suggests that, in a mature forest setting, N uptake over time is much greater for trees than for the microbial community when exposed to elevated CO₂. The present study was conducted on hybrid poplar rooted cuttings over a seven week period. Over this time period, N competition between the small trees and the soil microbial community may not have become as severe as occurs in some mature forests, resulting in greater microbial N under elevated than ambient CO₂ in the present study. In a meta-analysis of studies investigating the C and N pools of forest

trees and soil over time, Yang et al. (2011) also found that while all studies observed positive increases in plant woody biomass N and 50% of studies observed increases in total litter and forest floor N, the magnitude of these N increases declined with forest age. This suggests that progressive N limitation may occur as some forests age and emphasizes the importance of tree age on the N dynamics of forest soils. In some forests, however, the N limitation that would occur with time in the soil is counterbalanced by N inputs from atmospheric deposition. In particular, chronic N deposition has been observed in forest near and within urban and agricultural areas, where large amounts of fossil fuel combustion and fertilizer production occurs (Bettez and Groffman 2013).

The soil microbial C:N was also smaller under elevated than ambient temperature levels. This smaller C:N was mainly due to significantly greater microbial N quantities, rather than smaller microbial C quantities, under elevated than ambient temperatures. As with the soil MBN, however, lower microbial C:N in elevated than ambient temperature treatments could have been influenced by either the effects of temperature or light. A smaller total microbial C:N under elevated compared to ambient temperatures may suggest that there was a greater bacterial:fungal in the soil, as soil bacteria typically have lower biomass C:N than fungi.

The results of this study also suggest that rural forest soils in the Baltimore metropolitan area have greater microbial N pools than urban soils, and that these differences will persist under elevated temperature and elevated CO₂ levels. As with MBC, observed urban and rural differences regardless of treatment may be explained either by the persistence of a microbial community structurally adapted to urban or rural soils, or the persistence of urban- or rural-defining soil factors unaffected by short-term

temperature and CO₂ treatments. In terms of soil factors, organic matter and soil metals affect MBN and have been found to differ between urban and rural forest soils. In a similar study by Zhu and Carreiro (2004), forest soil MBN along an urban-to-rural gradient in and around New York City decreased with urbanization. They attributed the elevated MBN in rural forest soils to increased organic matter, which is an important source of N for the microbial community. In the present study, there was no difference in organic matter between urban and rural forest soils before the experimental treatments began. Soil metal content can negatively affect soil MBN by negatively affecting microbial functions, such as N mineralization. In the present study, Cu and Pb were greater in urban than rural forest soils, and were also negatively correlated with MBN, in soils analyzed in the field study. As study by Kao et al. (2006) found that over twelve weeks, biosolids treated with either 250 mg/kg Pb or 100 mg/kg Cu and applied to soils had reduced N mineralization rates compared to untreated biosolid applications. As with MBC, the Cu and Pb concentrations at which significant effects on MBN are found in the literature, are much greater than found in the soil of the present study (Kao et al. 2006, Khan et al. 2010). Therefore it is not likely that Pb and Cu were the only environmental factors driving the observed rural-urban differences in MBN.

Ammonium and Nitrate

Soil ammonium is an important N source for the plant and microbial community. While soil ammonium was not affected by elevated temperature or elevated CO₂ during this study, the greater MBN quantities seen under elevated than ambient temperature and CO₂ levels indicates that there was microbial immobilization of soil N. In aerobic forest soils, the microbial community primarily uses ammonium as their source of inorganic N. A non-significant decrease in ammonium in response to microbial uptake may be due in

part to a counterbalance of ammonium input from increased mineralization. Greater mineralization rates occur with elevated temperature as the activity rate of microbial enzymes increases. In a long-term forest warming study by Melillo et al. (2011), a deciduous forest in Massachusetts was subjected to soil warming at levels consistently 5 °C above the ambient control. Over seven years they found that warmed soils maintained significantly higher net N mineralization rates than the ambient control. Studies have also found that mineralization rates increase under elevated CO₂ levels in the short-term due to priming, during which labile C inputs from plant roots stimulate microbial mineralization of organic matter (Treseder et al. 2005). However in the long-term, mineralization rates declined when N became more limiting in the soil due to plant uptake (Treseder et al. 2005, Dieleman et al. 2010). In the present controlled chamber study, a balance between mineralization and N uptake by the microbial and plant community may have resulted in no detected change of soil ammonium levels under elevated CO₂ treatments. While this is only speculative, the results imply that soil ammonium was not lower under elevated than ambient CO₂ and temperature treatments and was not limited in these soils.

Ammonium was greater in rural than urban forest soils overall. This difference likely persisted from the field study, where C:N was lower and June MBN was higher in rural than urban forest soils. Soil ammonium uptake by plants, however, is highly dependent on soil temperature and varies between seasons. In their study of beech and spruce dominated forests in Germany, Gessler et al. (1998) found that ammonium uptake for both spruce and beech dominated forests were highest in mid-summer, and correlated strongly with soil temperature. Forest soil temperatures increase with urbanization in the

Baltimore area (George et al. 2007, Savva et al. 2010). In the present study, therefore, the ammonium uptake by urban trees may have been greater than rural trees, causing urban soils to display lower MBN quantities in the field study and lower ammonium concentrations in the controlled chamber study.

Soil nitrate is an important source of inorganic N to the plant community, and is highly mobile in forest soils. In the present study, soil nitrate was lower under elevated than ambient temperature and CO₂. The microbial community of forest soils primarily uses ammonium as their inorganic nitrogen source, and smaller nitrate amounts in elevated relative to the ambient temperature and CO₂ treatments were not likely due greater microbial activity. However reduced activity by nitrifying bacteria, which convert ammonium to nitrite and nitrite to nitrate, may be partly responsible. The PNL theory suggests that under elevated temperature and CO₂, plant and microbial communities may compete more strongly for available N, as their N requirements increase with their growth and activity (Luo et al. 2004). If the plant community is able to outcompete the microbial community, nitrifying bacteria may reduce their activity, and the production of nitrate, as ammonium becomes more limiting. Soil N limitation, however, may not be as critical in forests near urban or agricultural areas, which typically experience greater N inputs into the soil through atmospheric deposition (Bettez and Groffman 2013). In addition, soil nitrate losses were not likely due to leaching. As all soils were given roughly the same amount of water during the study, soil nitrate losses from leaching would have occurred for all soils regardless of temperature or CO₂ treatment. These results imply that under a future climate of elevated temperature and CO₂, less nitrate may be mobilized from forest soils to pollute waterways, as plant nitrate

uptake increases. As previously stated, however, temperature and light effects were potentially intertwined, such that nitrate uptake due to plant growth could have been influenced by both elevated temperature and light.

While soil ammonium and nitrate are both important components of the forest N cycle, they differ critically in regards to: uptake preference by the plant community, soil temperature optima for plant uptake, and mobility in the soil. Nitrate is more mobile in the soil matrix than ammonium due primarily to its charge. Soil particles are generally negatively charged and bind to cations such as ammonium. Nitrate on the other hand has a negative charge and can be transported easily by water throughout the soil matrix. Due to the ease of its diffusion through the soil, nitrate can be more highly concentrated in the rhizosphere, relative to ammonium. Despite this, ammonium is more easily assimilated by plant roots than nitrate, as ammonium assimilation requires fewer steps (and thus less energy) to be converted to organic N in plant roots or leaves than nitrate assimilation. During assimilation, nitrate must first be reduced to ammonium before it can be converted to an amino acid (Lambers et al. 2008). The temperature optima for plant assimilation also differs between ammonium and nitrate. Several studies have shown that as soil temperatures increase from 10 °C, the ammonium:nitrate uptake of plant roots slowly decreases (Bassirirad 2000). While the mechanisms underpinning this change are not well understood, it has been suggested that greater uptake of nitrate under elevated temperature is related to increased N demand from aboveground plant growth, and the mobility of nitrate in the soil matrix. In the present study, greater hybrid poplar aboveground biomass, and corresponding lower quantities of soil nitrate, under elevated

than ambient CO₂ and temperature treatments may support this claim (Table 13, Table 16).

Above and Belowground Woody Biomass

Hybrid poplar aboveground woody biomass was greater under elevated than ambient temperatures, while belowground biomass did not differ between temperature levels. Hybrid poplar roots, however, were observed to grow throughout the study, filling the 500 cm³ pot after seven weeks. In the present study, less soil nitrate occurred under elevated than ambient temperature and CO₂ treatments, and were likely caused by hybrid poplar uptake during aboveground biomass growth. While plants in general provide C and N sources as above and belowground biomass for microbial growth, they are also in constant competition with the microbial community for soil nutrients. This competition, especially for N, increases as plants mature and their N demand becomes greater. Under elevated CO₂ and temperature levels, plants of all ages compete more strongly with the microbial community for soil N. Under these same conditions, however, more N may be accessible to plants and microbes due to increased microbial mineralization and turnover. Therefore, while most mature forests are N limited, plants and microbes may avoid the negative effects of PNL under elevated CO₂ and temperature levels through the replenishment of available soil N by the soil microbial community. The growth of the plant community is therefore necessarily intertwined with the microbial community, and the knowledge of one is necessary for the understanding of the other.

In addition, as with MBN, microbial C:N, and nitrate, aboveground woody biomass differences between temperature treatments could have been influenced by light differences between environmental chamber types. As irradiance positively affects plant

growth, up to the point where carboxylation rates are limiting, greater irradiance in the environments exposed to elevated temperature could have caused greater aboveground woody biomass growth there relative to the ambient temperature environments.

Table 18. ANOVA for the main effects of site, temperature, CO₂, and their interactions on the microbial biomass carbon of unplanted soils. A contrast between urban and rural groups is included.

Source of Variation	Df	F-value and significance level
Site	5	10.5**
CO₂	1	0.21
Temperature	1	0.10
Temperature x CO₂	1	1.8
Site x CO₂	5	1.6
Site x Temperature	5	0.73
Error	5	--
Contrast		
Urban vs Rural	1	46.5***

** Indicates significance at $\alpha=0.01$ probability level ***Indicates significance at $\alpha=0.001$ probability level.

Conclusion

The results of this research are preliminary, and replications of the controlled environmental chamber study need to be conducted to support these findings. Results from this study suggest that if CO₂ levels continue to increase, the soil C storage and microbial N availability of forests in the Baltimore metropolitan area will increase, while soil nitrate levels will decrease. Results also suggest that if temperature levels continue to increase, the microbial N availability of these forests will increase, while the microbial C:N and soil nitrate availability will decrease. However, observed effects of elevated temperature on MBN, microbial C:N, and nitrate in the controlled environmental chamber study may have been enhanced with light effects. Thus irradiance may be equally important in driving MBN, microbial C:N, and soil nitrate differences between ambient and elevated temperature treatments. This research also suggests that for the sites studied, rural forest soils have larger microbial C and N stores than urban forest soils, which may persist under elevated temperatures and CO₂ levels.

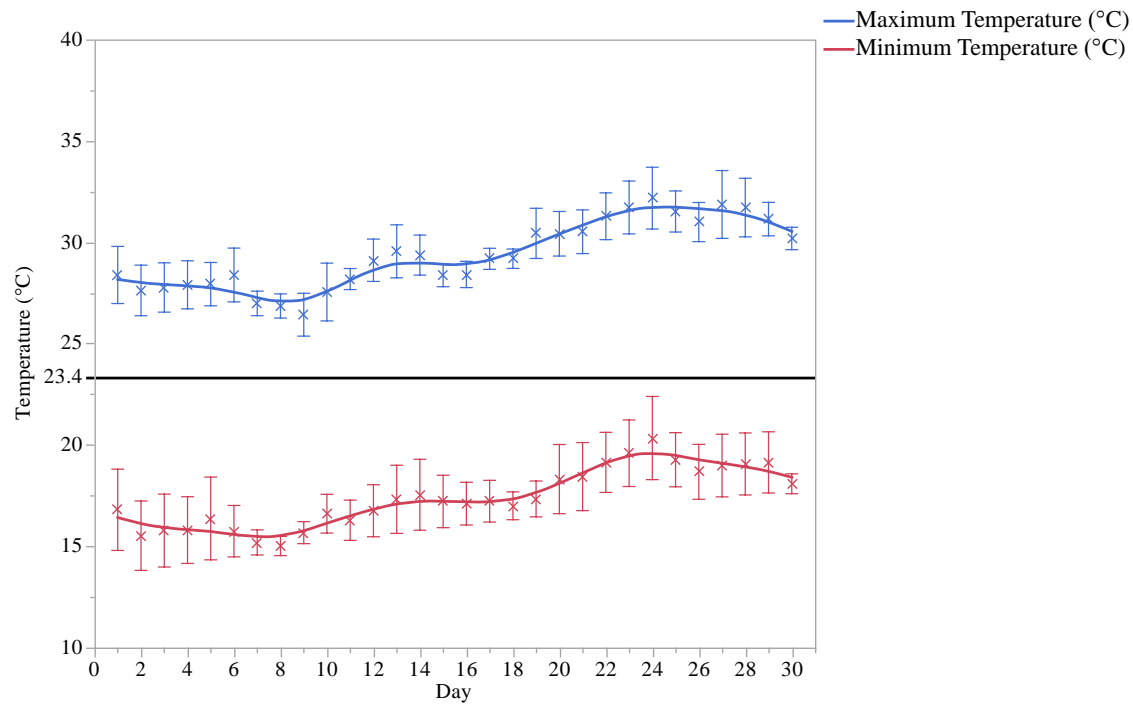
Some previous studies have found that MBC and MBN increase with elevated temperature and/or CO₂ levels (Lipson et al. 2005, Belay-Tedla et al. 2009, Melillo et al. 2011, Carrillo et al. 2014). As temperature and CO₂ levels have been shown to increase with urbanization in the Baltimore metropolitan area, it was thought that the studied urban forest soils would have had greater MBC and MBN quantities than rural soils. However, during the field study the opposite was found to occur. In the environmental chamber study, where CO₂ and temperature were carefully controlled, MBC was greater under elevated than ambient CO₂ levels and MBN was greater under elevated than ambient temperature/light and CO₂ levels. This could imply that there were unmeasured

environmental factors in the studied urban and rural forests that interacted with temperature and CO₂. This interaction may have effected microbial biomass C and N in a way that was contrary to expectations. In the present study, while Pb and Cu were greater in urban than rural soils, and negatively correlated with MBC and MBN, they were not likely primary factors in differentiating urban and rural forest soils in this study, as their levels are relatively low (Banu et al. 2004, Khan et al. 2010). Other factors that potentially influenced urban and rural microbial biomass differences, but were not measured in this study, include earthworm activity and leaf litter lignin content, as suggested by Groffman et al. (1995) and Pouyat and Carreiro (2003), and soil clay content, as suggested by Sulman et al. (2014). In addition, the controlled environmental chamber study was a short-term (49-day) experiment. If temperature and CO₂ treatments had been applied to soils for a longer period of time, their effects on MBC and MBN may have been non-significant, as is seen in many long-term forest studies (Zak et al. 2000, Hu et al. 2006).

Overall, this project illustrates the importance of temperature, CO₂, and urban versus rural forest location in determining the potential C storage and microbial N pools of forest soils. In recent years, soil C models have taken significant steps towards incorporating microbial processes and urban ecosystems (Pataki et al. 2006, Allison et al. 2010, Wieder et al. 2013). The present study suggests that there is clearly an environmental difference between urban and rural forests that influences soil microbial C and N quantities, and that is causing these differences to be maintained under elevated temperature and CO₂ conditions. The next step towards improving the accuracy of these models will involve distinguishing the C and N cycles of urban and rural forests, and the

environmental factors that differentiate them. Before this can occur, however, further research on the microbial components of these cycles under present and future climate conditions is critical.

Appendix



A1. The mean minimum and maximum daily temperatures of six locations in Maryland (four rural and two urban) in June 2010. Locations included Bel Air, Clarksville, Westminster, and Woodstock (rural), Cylburn Arboretum and the Maryland Science Center (urban). Averaged across sites, the daily temperature for June 2010 was 23.4°C. This was rounded to 23.0°C for the ambient temperature treatment of the environmental chamber study.

Tulip Poplar

Tulip poplars were planted and placed in environmental chambers to regulate any CO₂ build-up that may have occurred during the study due to the lack of CO₂ scrubbing equipment. The above and belowground woody biomass was measured to examine the effects of elevated temperature and CO₂ on this native tree species.

After 49 days of exposure to temperature and CO₂ treatments, tulip poplar aboveground woody biomass was not significantly affected by temperature, CO₂, or their interaction (Appendix A3). This likely occurred because the tulip poplar seedlings were around 76 cm and had a substantial root system when exposed to the treatments. Their growth under most conditions would likely be slower than the small and young hybrid poplar cuttings.

Tulip poplar belowground woody biomass differed significantly due to temperature, but was not significantly affected by CO₂, or the temperature x CO₂ interaction (Appendix A3). Tulip poplar belowground woody biomass was 46% lower under elevated than ambient temperature, which was likely a result of water stress (Appendix A4). The watering frequency under elevated temperature treatments was likely insubstantial for the tulip poplars, due to their large root system and well drained potting soil medium.

A2. ANOVA for the effects of CO₂, temperature, and their interaction on the above and belowground biomass of tulip poplar.

Source of Variation	Df	F-value and significance level	
		Aboveground Woody Biomass	Belowground Woody Biomass
CO ₂	1	0.61	0.08
Temperature	1	1.8	6.4*
Temperature x CO ₂	1	0.78	0.14
Error	6	--	--

* Indicates significance at $\alpha=0.05$ probability level.

A3. Tulip poplar above and belowground woody biomass (g) means for temperature and CO₂ levels.

Temperature Level	Aboveground Biomass	Belowground Biomass
Ambient	9.1	8.7
Elevated	7.4	4.7
LSD	3.2	3.8*
CO₂ Level		
Ambient	8.8	6.5
Elevated	7.7	6.9
LSD	3.2	3.8
Mean	8.4	7.1

* Indicates significance at $\alpha=0.05$ probability level.

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