Impacts of urbanization on carbon balance and human health risk: I. & II. Soil carbon dynamics under impervious surfaces and III. Water quality influences on a common West Nile virus vector.

by

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Abstract

A significant increase in urbanization will be observed during the next fifty years. Global environmental changes cannot be addressed without a better understanding of biogeochemical cycles in urban areas. Thus, more insight into the effects of urbanization on (i) soil biogeochemical properties, and (ii) water quality and its interactions with public health including increasing population of urban mosquitoes, are of critical importance. In this research, the effects of soil sealing by urban impervious surfaces such as building, roads, sidewalks, and parking lots on soil carbon and nitrogen dynamics investigated. In a second study, the effects of urbanization and expansion of urban lawns on survival rates of mosquitos related to infection of West Nile virus was examined.

In order to study the effects of urban impervious surfaces on soil carbon and nitrogen dynamics two separate approaches were required: 1) A field controlled study consisting of three different treatments (i) concrete slabs, (ii) mock-up of houses built on a crawl space and (iii) grassed (reference) plots. In this study, we found that the major cause of carbon loss beneath impervious surfaces was top soil removal . Soil carbon content decreased by 48.39 % (± 16.29 %) and 56.99 % (± 8.59 %) beneath the concrete and homes compared to reference plots at top 10 cm. The soil C beneath the concrete plots decreased over time while it fluctuated beneath the homes. The carbon loss to atmosphere beneath the homes at top 10 cm was the major mechanism of carbon loss following the initial disturbance. Moreover, nitrogen, microbial biomass, and net nitrogen mineralization rate were significantly lower beneath impervious surfaces compared to reference plots. However, accumulation of nitrate and phosphate beneath the impervious surfaces was evident. 2) A field chronosequence study was necessary to verify the result from the control study and shed light on true gain or loss of carbon beneath impervious surfaces over time. In this study, we quantified soil carbon (C), nitrogen (N), and influential parameters affecting them beneath homes built on a crawl space ranging from 11 to 114 years in age. The average soil C and N content in the top 10 cm were 61.86 % (± 4.42 %), and 65.77 % (± 5.65 %) respectively lower beneath the homes in comparison to urban lawns. The soil C and N loss beneath the homes revealed a quadratic correlation with age and maximum C loss occurring at approximately fifty years in age. Microbial biomass carbon (MBC), and nitrogen (MBN) were significantly lower beneath the homes compared to the urban lawns.

The third chapter was to determine the effects of urbanization and formation of urban lawns on survival rates of mosquitoes using a laboratory microcosm approach. Abscised leaves from three common overstory tree species in the southeastern United States and turfgrass clippings were used to simulate potential backyard breeding habitats for *Culex*. Urban lawns also alter water quality which may be affected by the surrounding vegetation which serves as a source of organic matter. Abscised leaf treatments had significantly lower survival rates than grass treatments with maximum pupae development of 3.93 ± 2.29 % in the pine treatment and 1.92 ± 1.72 % adult development in the sweetgum treatment. Grass treatments developed 28.50 ± 5.75 % and 22.50 ± 4.79 % pupae and adult mosquitoes respectively, suggesting that urbanization and formation of urban lawns create a suitable habitat for growth of mosquitoes when water ponding exists.

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Chapter1: Introduction

Effects of land use change on soil carbon dynamics have been an important aspect of global climate change research in recent years (Pouyat et al., 2009; West and Six, 2007). Anthropogenic sources emit approximately 8.7 Gt (1 gigaton = 1 billion tons) of carbon to the atmosphere per year (Menon et al., 2007). However, the increase in atmospheric carbon is approximately 3.8 Gt per year (Stockmann et al., 2013). This highlights the importance of biosphere carbon sinks. Soil is the second largest pool of carbon following the oceans (Stockmann et al., 2013) and its potential to be used as a potential solution to increasing atmospheric carbon concentrations have been widely discussed (Pouyat et al., 2009; Raciti et al., 2012a; Stockmann et al., 2013; Wang et al., 2013; Wei et al., 2014b). The soil carbon capacity is determined by the nature of vegetation (or land cover), precipitation and temperature (Lal, 2004), and thus can be altered through land use change. Land use change may alter the inputs and outputs of carbon (Lal, 2004) and, as a result, the soil may act as a source or sink of carbon. The top one meter of the soil stores approximately 1500 Gt of organic C with more than 40% of it stored in the top 20 cm (Jobbágy and Jackson, 2000; Stockmann et al., 2013). Consequently, it is of great importance to study the effects of land use and land cover changes on soil carbon storage. In particular, the effect of urbanization and conversion of forests and grassland to urban and exurban uses is critical (Pataki et al., 2006).

A significant increase in urbanization will be observed during the next fifty years (United Nations et al., 2014). Urbanization has been shown to impact soil physical, chemical, and biological properties including carbon (C) and nitrogen (N) storage and sequestration rates (Elvidge et al., 2007; Milesi et al., 2005). For instance, studies in Baltimore, MD (Raciti et al., 2008), Colorado (Golubiewski, 2006; Kaye et al., 2006), Oakland, CA and Chicago, IL (Pouyat et al., 2006a) concluded that urban land use may increase C storage. However, they have only measured soil carbon in urban grassed areas such as lawns and parks. Similar studies also have reported a decrease in carbon in areas with inherently high soil C storage such as Boston, MA (Pouyat et al., 2006b; Raciti et al., 2012b) and Syracuse, NY (Pouyat et al., 2006a). However, these estimates have not considered the effect of urban impervious surfaces on soil C and N storage.

Artificial soil sealing is a consequence of urbanization and infrastructure construction. The earth's total impervious surface (580,000 Km^2) is estimated to encompass an area bigger than the size of Spain (Elvidge et al., 2007; Raciti et al., 2012b). The United States has the largest amount of impervious surface per person (297 m^2/ per person (Elvidge et al., 2004) in world. Impervious surfaces such as buildings, pavements, and sidewalks may alter soil biogeochemistry in many ways including limiting the exchange of gasses, water, energy, and organic matter (Byrne, 2007; Scalenghe and Marsan, 2009a).Top soil removal which is a common practice in the installation of most of the impervious surfaces results in the elimination of highly active soil biota and organic matter which have a critical role in upper soil biogeochemistry (Lorenz and Lal, 2009). Installation of impervious surfaces may increase the soil bulk density which limits water and air penetration thereby affecting the biogeochemical cycling of C and N (Lehmann and Stahr, 2007). Moreover, soil sealing can have a great influence on heat exchange within the soil and between the soil and the atmosphere.

Impervious surfaces such as roadways, parking lots, and roofs have a relatively low albedo (Takebayashi and Moriyama, 2007), and would have a higher surface temperature during the day resulting in a lower temperature in the soil beneath them while the soil may have a higher temperature compared to open areas during the night. Temperature affects the chemical and microbial processes in the soil including organic molecules adsorption and desorption as well as microbial activities such nitrification (Sollins et al., 1996). The data suggest that a 1°C increase in temperature could ultimately lead to a loss of over 10% of soil organic C in regions of the world with an annual mean temperature of 5°C whereas the same temperature increase would lead to a loss of only 3% of soil organic C for a soil at 30°C (Kissel et al., 2009).. Sealing changes soil water regimes as well. The direct infiltration of water will be limited by impervious surfaces and the temperature regime described above will alter the moisture regime. Moreover, soil sealing increases runoff which will increase the risk of erosion, flooding and ponding (Shuster et al., 2005).

Despite the importance and magnitude of changes that may be caused by the impervious surfaces, there have been only a few studies where soil beneath impervious surfaces was sampled (Raciti et al., 2012b; Wei, 2014; Yan et al., 2015a). Soil C beneath impervious surfaces was 2.29 kg C m^{-2} [top 15 cm, New York, U.S. (Raciti et al., 2012b)]; 2.35 kg C m^{-2} [top 20 cm, Nanjing City, China (Z.-Q. Wei et al., 2014)]; 2.46 [top 20 cm, Yixing City China (Z. Wei et al., 2014a)]; and 3.55 [top 20, Urumqi City, China (Yan et al., 2015a)]. Normalizing these values to the top 100 cm (minimum value of 5.8 kg C m^{-2} in New York) reveals a significant underestimation of C in studies were sampling did not occur. In these studies. Assumptions ranged from zero (Tomlinson and Milne, 2006) to 1 kg m^{-2} (Cannell et al., 1999; Schaldach and Alcamo, 2007) to 3.3 kg m^{2} (Pouyat et al., 2006a) for the top 100 cm.

The few studies that have sampled beneath the impervious surfaces have included soil beneath road pavements, paved residential squares (Raciti et al., 2012b; Z. Wei et al., 2014b), and residential driveways (Edmondson et al., 2012; Yan et al., 2015b). However, various impervious surfaces such as home, road, sidewalks, and parking lots may have carry different effects on carbon storage due to variation in material, age, and size. Moreover, it is not clear if changes beneath impervious surfaces are only a result of top soil removal, and the initial disturbance or soil sealing. The true gain or loss of carbon beneath these surfaces following the installation of impervious surfaces have not been investigated. It is not clear whether the soil beneath impervious surfaces becomes biologically inactive, or may act as a sink or source of carbon.

To address these knowledge gaps, two separate studies were required. A field controlled study conducted consisting of three different treatments (i) concrete slabs, (ii) mock-up of houses built on a crawl space and (iii) grassed (reference) plots. This study was designed to monitor changes in soil properties and in particular soil carbon and nitrogen dynamics over the first year to understand if changes in soil properties are solely the result of top soil removal, or soil sealing. Moreover, this study also addressed the question regarding whether different types of impervious surfaces have different effects on soil carbon.

In the second chapter, we quantified soil carbon (C), nitrogen (N), and influential parameters affecting them beneath homes built on a crawl space. All homes were sampled in the Piedmont region of Alabama and Georgia, USA, and ranged from 11 to 114 years in age. Of the homes in the United States, 21.4 % are built on crawl spaces, while less than 24% were constructed on concrete slabs (U.S. Census Bureau, 2013). This chronosequence approach enabled us to study net gain or loss of carbon beneath urban impervious surfaces.

The goal of the third chapter was to determine the effects of urban vegetation on the survival rates of *Culex* mosquitoes, the primary vector of West Nile virus. The majority of urban open lands consist of decorative grassed lawns including parks, and green spaces attached to homes (Milesi et al., 2005, 2003). Urbanization may affect mosquito habitat through its influence on the quality of water that serves for larval development. This includes pollution and inputs of different types of urban vegetation which is necessary for mosquitoes as a carbon source. For instance, it has been shown that sweetgum leaves leach more dissolved organic carbon (DOC) than longleaf pine, bald cypress, and water tupelo leaves (Majidzadeh et al. 2015). The effect of vegetation on water quality may be magnified when native forests are replaced with lawns. The forest floor, naturally covered with dead and decomposed leaves, is replaced by highly managed lawns (i.e. fertilized, weed removal, clipped regularly). These changes in water quality can create habitat changes for urban mosquitoes including *Culex* which is the vector of West Nile virus.

Disease vectors and in particular urban mosquitoes have long been a global public health threat (Benelli et al., 2015; Kilpatrick and Randolph, 2012). Ongoing outbreaks of West Nile (WNV), Zika (ZIKV), and chikungunya (CHIKV) viruses are examples of urban mosquito-borne diseases (Attar, 2016; Ezenwa et al., 2006). West Nile virus (WNV), unlike the ZIKVand CHIKV, has caused epidemics every summer (Coffey and Reisen 2016) with over 1500 deaths since it was introduced to the United States in 1999 (Allan et al., 2009; Petersen LR et al., 2013). *Culex quinquefasciatus* (the southern house mosquito), inhabits regions with tropical and temperate climate and is considered a vector between species since it can infect birds, livestock, and humans (Allan et al., 2009). Currently, no vaccines are available for WNV. Therefore, strategies to minimize *Culex* populations are required to prevent the spread of the disease (Benelli et al., 2015; Benelli and Mehlhorn, 2016; Jeffries and Walker, 2016). Considering the life cycle of *Culex* and its critical dependence on water, deeper insight into the effect of water quality on the survival rate of the mosquitoes is required. To that end, a laboratory microcosm study was designed which included abscised leaves from three native overstory tree species in the southeastern United States and turfgrass clippings to simulate influences of lawns.

Research purpose and objectives

Global environmental changes cannot be addressed without a better understanding of biogeochemical cycles in urban areas (Rao et al. 2013). In order to influence urban development policies that minimize environmental degradation and enhance urban ecosystem services, more insight into the (i) biogeochemical consequences of urban soil disturbance, and (ii) effects of urbanization on public health are of critical importance. In this research, possible changes in soil biogeochemistry, and water quality which can affect the expansion of urban mosquitoes were studied through (i) field controlled study, (ii) field chronosequence study, and (iii) laboratory microcosm study. The primary objectives of this study are to gain insight into:

1. Changes in soil carbon and nitrogen storage and dynamics beneath impervious surfaces.
2. Changes in physical properties such as temperature, and moisture over time.
3. Effects on biological properties such as microbial biomass, nitrogen mineralization, and nitrification beneath impervious surfaces over time.
4. Influences on the survival rate of *Culex quinquefasciatus* in containers using turfgrass (*ryegrass*) and abscised leaves from three common tree species in the southeastern United States including sweetgum (*Liquidambar styraciflua* L.), water oak (*Quercus nigra* L.), and loblolly pine (*Pinus taeda* L.) as carbon sources.
5. Document how the initial nitrate and phosphate concentrations in water affect the survival rate of mosquitoes. Elevated levels of these nutrients are common pollutants associated with urban streams in the southeastern United States.

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Chapter2: Soil carbon and nitrogen dynamics beneath urban impervious surfaces: A field controlled study

Abstract

Soil sealing by impervious surfaces such as buildings, roads, sidewalks, and parking lots are some of the most extreme disturbances caused by urbanization. Previous studies have reported dramatic changes in soil properties, and in particular carbon and nitrogen content due to the installation of impervious surfaces. However, it is not clear if these changes are solely the result of initial disturbance and top soil removal, or if soil sealing alters soil properties over time by limiting the inputs of organic matter, water, and gas exchange. Moreover, the effects of different impervious surfaces (i.e. concrete slabs vs. homes) on soil biogeochemical properties have not been investigated. To address these knowledge gaps and to understand if the soil beneath impervious surfaces is acting as a sink or source of carbon and nitrogen, monitoring the changes in soil properties immediately after soil sealing is necessary. Here, a field controlled study was conducted consisting of three different treatments. Treatments consisted of concrete slabs, mock-up of houses built on a crawl space and grassed (reference) plots. Soil physical, chemical, and biological properties were monitored beneath these impervious surfaces for 15 months at depth 0-10 cm (layer one) and 10-20 cm (layer two).

Soil carbon content decreased by 48.39 % (± 16.29 %) and 56.99 % (± 8.59 %) beneath the concrete and homes respectively compared to reference plots at top 10 cm.

Temporally, soil carbon content decreased by 0.04 kg m^{-2} and 0.02 kg m^{-2} per month beneath the concrete plots at layer one and two respectively. Soil nitrogen, microbial biomass, and net nitrogen mineralization rates were significantly lower beneath impervious surfaces compared to reference plots. Other parameters that affecting soil carbon and nitrogen dynamic such as soil volumetric water content, temperature, pH, as well as nitrate, ammonium, Ca and K content were monitored over the experimental period.

Introduction

Urbanization alters soil physical and chemical properties including soil carbon storage and sequestration rate (Pouyat et al., 2006; Scharenbroch et al., 2005). To evaluate the effects of urbanization on the ecosystem, and the global carbon cycle, it is of great importance to quantify these changes in various urban land uses and land covers (Lal, 2004; Yan et al., 2015). Soil sealing by impervious surfaces such as buildings, roads, sidewalks, and parking lots is one of the most extreme disturbances caused by urbanization. It affects soil physical and chemical properties by limiting water and gas exchange and restricting the input of organic matter (Jazaei et al., 2014; Lorenz and Lal, 2009; Montague and Kjelgren, 2004). Moreover, the construction process usually involves compaction and removal of top soil which is rich in organic matter (Scalenghe and Marsan, 2009; Yan et al., 2015). Impervious surfaces cover more than one-third of urban areas in the United States exceeding 130,000 km^2 (Churkina et al., 2010; Elvidge et al., 2004; Wu et al., 2016). Despite the importance and magnitude of changes caused by the impervious surfaces, there have been only a few studies in which the soil beneath impervious surfaces were sampled (Raciti et al., 2012; Wei, 2014; Yan et al., 2015).These studies have reported 68 % to 75 % decrease in soil carbon underneath sidewalks, residential pavements, and residential driveways compared to open areas. A significant decline in soil nitrogen (Raciti et al., 2012; Z. Wei et al., 2014), and microbial activity (Z.-Q. Wei et al., 2014) also have been reported. However, it is not clear if these changes are a result of top soil removal and the initial disturbance or long-term soil sealing that alters soil properties over time. The true gain or loss of carbon beneath these surfaces over time following the disturbance has not been investigated. As yet, there are no reports stating whether soil beneath impervious surfaces becomes biologically inactive, or if the soil acts as a sink or source of carbon following disturbance and sealing. The soil beneath impervious surfaces has a very limited input of organic matter through leaching, roots, and insects (Lehmann and Stahr, 2007; Scalenghe and Marsan, 2009). The outputs of carbon are also limited in these systems because of a decrease in oxygen levels, microbial activity, and leaching (Wei et al., 2013). Moreover, various types and sizes of impervious surfaces may have different effects on soil properties. Yan et. al (2015) showed that the size of impervious surfaces would affect the soil properties and that soil carbon decreases horizontally from the edge of the pavement to the center. However, they did not demonstrate whether impervious surfaces type (i.e. homes vs. concrete slabs) would have the same effects on soil properties.

Homes and concrete slabs are two major types of impervious surfaces. Homes cover more than 20% of impervious surfaces in the United States (U.S. Census Bureau, 2013). Multi-unit homes and tall building construction involve intensive disturbance as well as major topsoil removal and is assumed to have zero carbon storage (Yan et al., 2015). However, only 23% of the homes in the United States are multi units. More than 53% of the houses in the United States are manufactured homes (6%), homes built on crawl space (16%) and home built with basement (31%) (U.S. Census Bureau, 2013).

To elucidate how soil sealing alters soil physical and biological properties, a field controlled study consisting of three different treatments were conducted. Treatments included concrete slabs (concrete plots), mock-up of houses built on a crawl space (home plots), and grassed (reference) plots. Soil physical, chemical, and biological properties were monitored beneath these surfaces for 15 months. The objectives of this study were to (i) monitor changes in physical properties such as temperature, and moisture over time; (ii) evaluate changes in soil carbon and nitrogen storage and dynamics beneath impervious surfaces; (iii) quantify biological properties such as microbial biomass, nitrogen mineralization, and nitrification beneath impervious surfaces over time.

Material and methods

The site was constructed near Auburn, AL (latitude 32.60° N longitude 85.48° W) which is located on the fall line between the Coastal Plain and the Piedmont Plateau with a mean annual air temperature of 17.4 ^{o}C and mean annual precipitation of 1337 mm. The research site was built on an unfertilized Piedmont meadow covered with grasses and shrubs. The soil on the site was classified as fine, kaolinitic, thermic Rhodic Kanhapludults. Treatments were built on 5 m × 5 m plots and replicated four times based on randomized complete block design (RCBD), totaling twelve plots (Figure 2.1). There was a four-meter buffer zone between treatments to prevent increased runoff from adjoining treatment having any effect. Vegetation and the top 10 cm of soil were removed for construction of concrete slab, and home plots. The soil was compacted by tamping prior to the installation of a concrete slab. There were 18 blocks in the middle of each concrete slab that could be opened for sampling (Figure 2.1b). The cracks between blocks were sealed after each sampling. All twelve plots were sampled (cores of 5 cm) before treatment installation at three depths: 0-10 cm, 10-20 cm, and 20-30 cm. Following treatment installation, samples were collected at 0, 1, 3, 5, 7, 9, 13, and 15 months. The reference plots were sampled at three depths (0-10 cm, 10-20 cm, and 20-30 cm) while the soil beneath impervious surfaces were sampled only at two depths since the first 10 cm was removed during the construction process. The first 10 cm beneath impervious surfaces was called as layer one and the second 10 cm was named layer two.

Soil temperature and volumetric water content were measured every 15 minutes at a depth 10 cm and 20 cm for all treatments using HOBO TidbiT temperature probes (Part UTBI-001, Onset Corp., Pocasset, MA) and EC-5 soil moisture probes (Onset Computer, Bourne, MA) respectively. An automated LabFit AS-3000 pH analyzer was used to measure pH of air-dried soil samples in a 0.01 molar solution of calcium chloride. This method provides more stable results between seasons compared to the 1:1 mixture of soil: water method (Kissel et al., 2009). The measured values were converted to 1:1 mixture of soil: water values by adding a conversion factor of 0.6. The Mehlich-1 method (Jones, 1998) was used to quantify concentrations of extractable P, Ca, and K using inductively coupled plasma spectrograph (ICAP61E, Madison, WI). Total C and N were measured using Perkin-Elmer 2400 Series II CHNS/O analyzer (PerkinElmer, Waltham, MA).

The chloroform-fumigation method was used for measuring soil microbial biomass carbon (Vance et al., 1987). Soil samples were homogenized by sieving through a 2 mm mesh sieve and dividing into two sets of 18.5 g. The first set was fumigated by exposing soil to alcohol-free chloroform for 24 hours. Fumigated and unfumigated samples were extracted with 100 mL 0.5 mol L-1 K_2SO_4 for 30 minutes. The extracts were filtered (Whatman no. 42 filters) and then frozen. After thawing, the samples were analyzed for organic C and total N using a Shimadzu TOC-V and total N combustion analyzer (Shimadzu Scientific Instruments, Columbia, MD). The differences between fumigated and unfumigated samples represent microbial C and N.

Net N mineralization was measured using the in situ bag method (Bottomley et al., 1994). The soil sample (from layer 1) were split into two approximately 500 mL polyethylene whirl pack bags (Nasco, Fort Atkinson, WI). Polyethylene bags permit the gas exchange while it prevents water entering the bags. One bag was immediately reburied and incubated for approximately 28 days, and the other bag brought back to the laboratory. Post-incubation samples and pre-incubation samples were processed for ammonium and nitrate. Processing consisted of passing the soil through a 2 mm sieve and then extracting 10 g of the < 2mm field moist soil in 100 mL of 2 mol L^{-1} KCl. The extracted KCl solutions were filtered and then frozen. Extracts were analyzed using standard colorimetric techniques, with the developed color analyzed using a microplate reader (Sims et al., 1995).

Statistical analysis

"R" software, version 3.1.2 was used for all statistical analysis in this study. A linear mixed-effects model (the nlme package of "R" software) with repeated measures of soil properties and block as the random effect was utilized to compare soil properties (i.e. soil C, N, microbial biomass C, nitrate, net nitrogen mineralization rates) between the treatments. The Tukey's honestly significant difference (HSD) following an analysis of variance (ANOVA) test was used to differentiate the means (Agricola package, $\alpha = 0.05$). There was an interaction between depths and treatments (lawns, homes, concrete), thus instead of a two-way ANOVA, soil properties beneath impervious surfaces were studied at two separate depths (0-10 cm, and 10-20 cm).

Results

Soil volumetric water and temperature

Soil volumetric water content in reference plot was significantly lower (*p*-value < 0.01) in the warm periods (May-October, average temperature = 21.54^{o}C ±1.66 ^{o}C) than in the colder periods (November-April, average temperature = 8.32^{o}C ± 4.54 ^{o}C). In cold periods, soil water at layer one followed the order of reference ≈ concrete > home treatments (Figure 2.2a). In the warm period, soil water beneath the concrete plots declined less than the reference and home plots probably due to limited evaporation. Thus, the soil water content was significantly higher beneath the concrete plots compared to reference and home plots at layer one in the warm period of the year (*p*-value <0.01, Figure 2.2a). The soil water at layer two was higher beneath the homes than two other treatments in both warm and cold periods (p-value < 0.001). There was no significant difference between concrete and reference plots during the cold period of the year in this layer. However, in the warm period, soil water content was higher beneath the concrete plots compared to reference plots in layer two (p-value < 0.001).

The soil temperature at depth 10 cm was on average 2 ^{o}C higher beneath the concrete plots compared to the reference and home plots during warm periods of the year, although this difference was not statistically different probably due to high variation in reference plots temperature (Table 1). In cold periods of the year, soil temperature was very similar for all treatments at both depths. Higher temperature beneath concrete plots is probably due to solar energy absorption of the concrete during the day. This heat will be stored within the concrete and during the night can be released to the soil and atmosphere (Maria et al., 2013).

Soil carbon and nitrogen

The mean soil carbon in layer one, over the experiment period was not significantly different beneath the concrete slab (2.31 ± 040 kg m^{-2}) and homes (1.75 ± 0.19 kg m^{-2}), which were 48.39 % (± 16.29 %) and 56.99 % (± 8.59 %) lower than reference plot at depth 0-10 cm, respectively. Considering the top soil removal, the soil carbon in layer one beneath these two impervious surfaces was not significantly different from that of at depth 10-20 cm in reference plots (Figure 2.3a). The construction process (month 0) which involved removing the top 10 cm of soil resulted in 44.00 % (± 16.16 %) and 51.52 % (± 10.71 %) decrease in carbon content beneath concrete and home plots, respectively. The initial disturbance and top soil removal were the primary cause of depletion in carbon content. However, the maximum carbon loss in layer one reached 56.06 % and 65.01 %, at the end of the experimental period (February 2016) beneath the concrete and homes respectively (Figure 2.3a). Soil carbon decreased with depth in all treatments. In layer two, the soil carbon beneath the homes (1.14 ± 0.21 kg m^{-2}) was higher than that beneath the concrete (0.93 ± 0.23 kg m^{-2}, *p*-value = 0.01) and both were significantly lower than soil carbon in reference plots at depth 10-20 cm (1.98 ± 0.42 kg m^{-2}, *p*-value < 0.001) but similar to that at depth 20-30 cm (Figure 2.3b).

Soil carbon beneath the concrete plots decreased 0.04 kg m^{-2} (± 0.01, *p*-value= 0.001) and 0.02 kg m^{-2} (± 0.005, *p*-value< 0.001) per month at layer one and two respectively. The soil carbon beneath the homes and in reference plots did not show a trend. The soil carbon beneath the homes peaked in November (both 2014 and 2015) at layers one and two, while the soil carbon in reference plots reached its maximum in February in the top 10 cm, and did not change over time at depth 10-20 cm. Soil carbon beneath the homes had a positive correlation with soil volumetric water content, and for each one percent increase in soil water content, a 0.23 kg m^{-2} (± 0.008 kg m^{-2}, *p*-value = 0.01) increase in soil carbon was observed. However, for reference and concrete plots no significant relation between soil water and carbon content was detected.

Soil total nitrogen content at layer one beneath the concrete and homes were on average 42.43 % (± 16.90 %) and 53 % (± 13.40 %) lower than that in the reference plots at depth 0-10 cm respectively (*p*-value < 0.001) (Figure 2.3c). The maximum decrease in nitrogen was observed in February with 71.18 % decrease beneath the homes and 77.4 % decrease beneath the concrete plots. At layer two, soil total nitrogen decreased by 60.8 % (±17.38

%) beneath the concrete and 50.4 % (±12.9 %) beneath homes compared to reference plots at depth 10-20 cm. There was no statistical difference in soil nitrogen content beneath the homes and concrete plots at both layers.

The construction process resulted in 46.24 % (± 11.89 %) and 58.41 % (± 3.82 %) decreases in soil N beneath concrete and homes, respectively. Following that, the soil N decreased beneath the concrete slabs over time in both layers. In layer one, the soil N decreased 4.53 g m^{-2} (± 1.71, *p*-value= 0.01), while in layer two, it decreased 2.63 g m^{-2} (± 0.99, *p*-value= 0.01) for each month of soil sealing. The soil N in reference plots and beneath the homes were fluctuating seasonally (Figure 2.3c, d).

The average soil C: N at layer one was not statistically different between treatments at layer one. However, at layer two it was higher beneath the homes (31.15 ± 5.63) compared to the reference (19.89 ± 3.98) and the concrete plots (18.25 ± 5.90, *p*-value = 0.03).

Soil Microbial Biomass

The soil microbial biomass (C_{mic}) at month 0 was surprisingly high beneath concrete plots which may be due to extra moisture provided with the wet concrete. However, after a month the C_{mic} significantly decreased due to the lack of carbon source and the C_{mic} did not significantly change over time beneath the concrete plots. The C_{mic} had seasonal variation in reference and home plots and did not show a significant relation with time although it was significantly higher in reference plots at both depths (Figure 2.4a, b). The C_{mic} decreased with depth for all treatments (*p*-value < 0.001) and had the same pattern as layer one. Microbial biomass carbon was significantly correlated with soil volumetric water content and increased 34.99 µg g^{-1} (±17.39 µg g^{-1}, *p*-value = 0.04), for each one percent increase in soil water at layer one.

The C_{mic}: C ratio is an indicator of readily metabolized carbon (Beyer et al., 1995). The mean C_{mic}: C ratio during the experiment was significantly higher beneath the homes (3.5 %) compared to reference (2.6 % ± 0.48 %, *p*-value = 0.06) and concrete plots (2.1 % ± 0.48 %, *p*-value = 0.004) at layer one. However, there was no significant difference in the C_{mic}: C ratio between different treatments at layer two.

Net nitrogen mineralization

The mean soil nitrogen mineralization rates during the experiment at layer one followed the order of reference (29.23 ± 10.03 mg N m^{-2} day^{-1}) ≈ concrete (14.86 ± 10.12 mg N m^{-2} day^{-1}) > home (2.33 ± 10.12 mg N m^{-2} day^{-1}, Figure 2.5a). The warm period of the year (March to July, average temperature = 21.69 ^{o}C) had significantly higher (*p*-value < 0.001) net nitrogen mineralization rates than the cold periods of the year (October to January, average temperature = 9.68 ^{o}C). Net ammonium production beneath the concrete slabs and homes were close to zero or negative during the experimental period with an average of -5.88 (± 5.42), and – 8.73 (± 5.33) mg N m^{-2}, respectively. Thus, the net N mineralization rates were primarily related to nitrification especially beneath impervious surfaces.

Soil nitrate contents at the top 10 cm differed significantly among all treatments and followed the order of concrete >> crawl space > reference treatments (Figure 2.5b).

Initial soil nitrate contents decreased with depth in reference and homes plots (*p*-value < 0.01), however, there was no correlation between depth and nitrate contents beneath the concrete plots. Moreover, the nitrate content increased 95.39 mg N m^{-2} (± 46.16. p-value = 0.05) and 93.97 mg N m^{-2} (± 18.62, *p*-value < 0.001) per month beneath the concrete and home plots respectively. However, the nitrate content in reference plots did not change significantly over time. In contrast to soil nitrate, soil ammonium content was higher beneath the homes compared to concrete slabs and decreased by 24.16 mg N m^{-2} (± 8.42. *p*-value = 0.008) beneath the homes and 24.11 mg N m^{-2} (± 9.01, *p*-value = 0.01) per month beneath the concrete plots (Figure 2.5c). There was no relation between time and initial ammonium content in reference plots.

Other soil physiochemical properties

Mean soil pH over the experimental period increased beneath the impervious surfaces and was followed the order of concrete (6.13 ± 0.40) ≈ homes (6.05 ± 0.35) > reference (5.68 ± 0.15) at layer one (*p*-value < 0.001). The soil pH increased 0.088 (± 0.016) per month beneath the concrete slabs and 0.028 (± 0.008) per month beneath the homes. However, the soil pH in reference plots did not change significantly over time. Soil pH decreased with depth in all treatments and had the similar pattern as layer one. Soil potassium (K) concentration was significantly higher beneath the concrete slabs compared to reference and homes treatments at layer one (*p*-value < 0.001). Moreover, for each month, soil K increased 23.10 mg kg^{-1} (±4.40, *p*-value< 0.001) beneath the concrete slabs while it had no significant change over time in the other treatments. Potassium accumulation beneath concrete slabs was only observed in layer one, and in layer two it was similar to that in reference plots. Soil calcium (Ca) concentrations in reference plots at top 10 cm was significantly higher than that beneath the impervious surfaces (*p*-value<0.001). The soil beneath the concrete slabs had higher Ca concentrations compared to the home treatment (*p*-value = 0.02). There was no significant difference in Ca concentrations at layer two. The soil phosphorous (P) content was not statistically different among the treatments. However, soil P concentrations increased beneath the homes by 0.12 mg kg^{-1} per month (± 0.06, *p*-value = 0.05), while there was no significant relation between time and soil P in other treatments.

Discussion

Top soil removal was the primary cause of the decrease in soil carbon and nitrogen content beneath impervious surfaces. Moreover, the decrease in carbon sequestration rates or decrease in carbon content over time can increase the difference between open soils and soil beneath impervious surfaces. Removal of the top 10 cm of soil resulted in 44.00 % (± 16.16 %) and 51.52 % (± 10.71 %) decrease in carbon content beneath concrete slabs and homes, respectively. Majidzadeh et al. (2016) studied the soil carbon beneath the homes built on crawl spaces that ranged from 11 to 114 years in age and reported 61.86 % (± 4.42 %) decrease in carbon beneath the homes compared to adjoining urban lawns. A study in New York reported a 66% decrease in carbon beneath pavements compared to nearby open soils in the top 15 cm (Raciti et al., 2012). A similar study in China reported a 68% carbon depletion in top 20 cm beneath the paved residential squares (Wei et al., 2013). The highest carbon loss beneath impervious surfaces is reported by (Yan et al., 2015), who noted a 75% decrease in carbon content in the top 10 cm. However, this study was in a dry region which limited the input of organic matter beneath impervious surfaces. Assuming that these impervious surfaces also included removal of vegetation and at least 10 cm of soil, top soil removal is the major cause of the decrease in carbon beneath impervious surfaces. Thus, strategies to use the removed topsoil in a way that protects the soil from atmospheric loss of carbon, would be of great importance.

Soil C beneath the concrete slabs decreased over time suggesting that it is decoupled from sequestration. Soil carbon sequestration in urban lawns can range from 0.026 kg m^{-2} (Huyler et al., 2014) to 0.355 kg m^{-2} (Selhorst and Lal, 2011) based on the irrigation, management, grass type, and region (Huyler et al., 2014; Pouyat et al., 2009; Qian and Follett, 2002). Soil carbon content beneath the homes and concrete slabs was not significantly different at layer one. However, home treatments had higher carbon at layer two, suggesting that the soil beneath the homes built on a crawl space may not be as sealed as the soil beneath the concrete slabs. This was further confirmed by higher volumetric water content beneath the homes at layer two. Similar carbon content at layer one is probably due to high carbon loss beneath the homes from oxidation since the top soil is not protected by vegetation or concrete. However, since loss through oxidation in layer two is not as pronounced, we observed higher carbon content beneath homes compared to concrete plots. This pattern further confirmed by higher C_{mic}: C ratio beneath the homes compared to the other treatments at layer one. This ratio has a positive relation with the proportion of metabolized carbon (Scharenbroch et al., 2005), thus the increase in C_{mic}: C suggests C loss over time (Beyer et al., 1995). Majidzadeh et al.

(2016) also found that gain and loss of C beneath the homes may reflect two different processes.

Soil N was similar beneath the concrete plots (0.13 ± 0.05 kg m^{-2}) and homes (0.11 ± 0.4 kg m^{-2}). Similar N content was expected since denitrification is a microbial process (Groffman et al., 2004) and there was no significant difference in microbial biomass beneath the home and concrete plots.

Soil microbial biomass was significantly lower beneath impervious surfaces compared to reference plots. Lower C_{mic} was expected beneath impervious surfaces considering the lack of bioavailable C and N (Wardle, 1992). Moreover, the gas and water exchange was limited by soil sealing which can reduce microbial activities (Zhao et al., 2012). However, while C_{mic} did not change over time beneath the concrete plots, it was fluctuating beneath the homes. This was probably due to limited inputs of organic carbon through infiltration which enables some microbial activity beneath the homes. This was further confirmed by a significant relation between soil volumetric water content and C_{mic}.

Soil net nitrogen mineralization rate was affected by both temperature and treatments. Although this increase was expected during the warm periods of the year, the increase beneath the concrete plots during this period was extraordinary high such that in May and July it was higher than that in reference plots. These high mineralization rates are probably due to higher soil volumetric water content beneath concrete plots during the warm periods of the year. Thus, the microbial community in warm periods of the year benefits from high temperature unless water stress occurs. Net ammonium production rates were negative, or zero beneath impervious surfaces since the input of litter and organic matter was limited. However, the soil initial ammonium content was enough to support the nitrification during the experimental period. This pattern may slow down through time since initial ammonium content would be limited. However, further studies are required. Z.-Q. Wei et al. (2014) reported that total soil mineral nitrogen (NO^{-}_{3} + NH^{+}_{4}) concentration was lower beneath pavements compared to open soils. However, they did not study nitrate and ammonium separately.

The soil pH increased beneath the impervious surfaces while it had no relation with time beneath the homes. P accumulation beneath the home may be a result of the increase in soil pH. The availability of phosphorous increases from pH 6 toward acidic pH since the adsorption of P onto clay mineral decreases (Devau et al., 2009). Majidzadeh et. al (2016) also reported higher P content beneath the homes compared to urban lawns and attributed it to lower P content in urban lawns due to plant uptake. P accumulation beneath the homes was not expected considering that phosphorous rarely leaches in aerobic conditions.

In this study, the biochemical process beneath two common types of impervious surfaces was investigated. We found that the major cause of carbon loss beneath impervious surfaces was top soil removal. However, the soil C beneath the concrete plots decreased over time while it fluctuated beneath the homes. The carbon loss to atmosphere beneath the homes at top 10 cm was the major mechanism of carbon loss following the disturbance. Thus, the application of vapor barriers beneath homes may have a significant role in preventing carbon loss. Considering these variations, assumptions regarding a constant value for soil carbon beneath impervious surfaces (Pouyat et al., 2006; Z.-Q. Wei et al., 2014) may be oversimplified. Soil microbial activities including C_{mic} was significantly decreased following the soil sealing. Thus, the net nitrogen mineralization rates decreased beneath impervious surfaces. However, accumulation of nitrate due to nitrification of soil initial ammonium was observed. As a result, soil ammonium content decreased over time beneath the impervious surfaces.

The results of this study reveal that the soil carbon and nitrogen dynamics beneath different impervious surfaces (homes built on crawl space vs. concrete in this study) are not the same, and parameters such as the type of structure, topsoil removal, depth, age, and climate would affect these dynamics.

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Tables and graphs

Table 2.1. Mean soil temperature (^{o}C) in warm and cold periods of the year beneath different treatments at depth 10 cm and 20 cm.

	Depth = 10 cm		Depth = 20 cm	
Treatments	May-Oct	Nov-Apr	May-Oct	Nov-Apr
Reference	25.82 ± 3.41	11.57 ± 5.56	25.64 ± 3.26	11.68 ± 5.25
Concrete	27.27 ± 3.83	12.00 ± 4.91	27.11 ± 3.67	12.19 ± 4.64
Home	24.61 ± 3.53	11.58 ± 4.05	24.54 ± 3.34	11.84 ± 3.79

Figure 2.1. 5m x 5m Reference (A), Concrete (B) and Home (C) plots. Each plot has a 4 meters buffer zone around it.

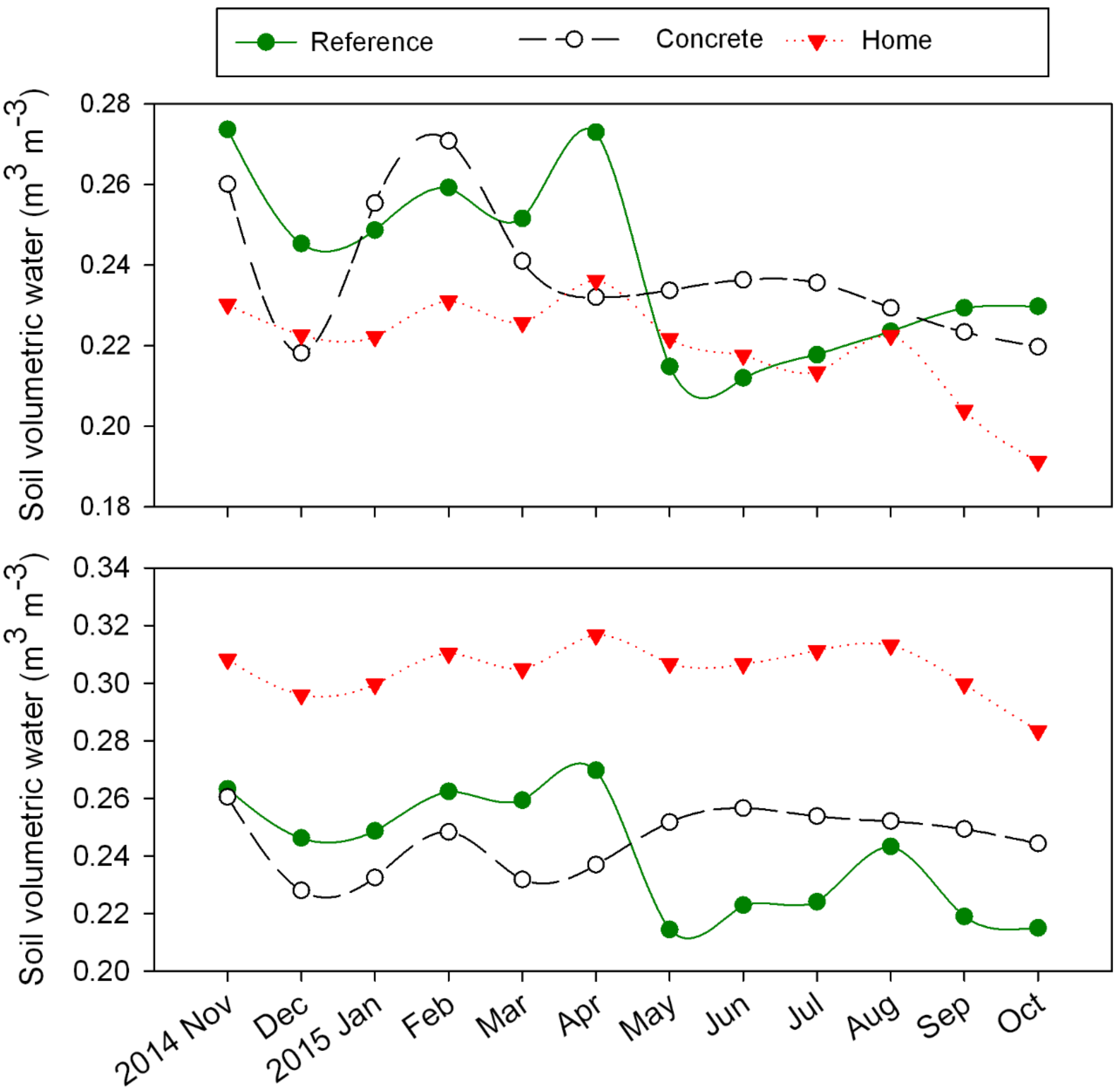

Figure 2.2. Soil volumetric water content pattern over a year at depth 10 cm (A) and 20 cm (B) at reference plots and beneath concrete and homes.

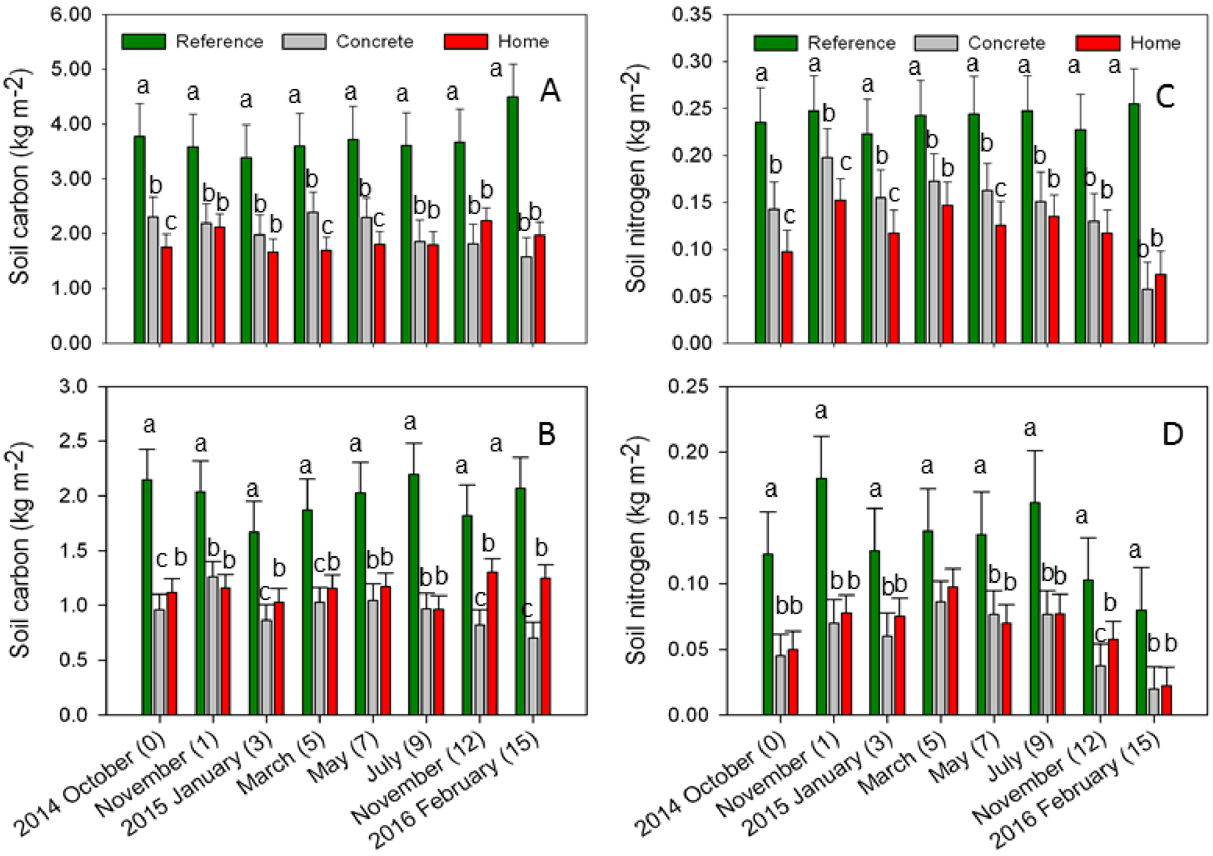

Figure 2.3. Monthly variation of mean soil carbon by treatment (n=4/ month) at layer one (A), and two (B). Monthly variation of mean nitrogen content by treatment at layer one (C) and layer two (D). Means are significantly different with different letters based on Tukey’s honest significant difference (HSD) tests at α = 0.05. The number in the parenthesis in the x-axis refers to the month following the start of the project.

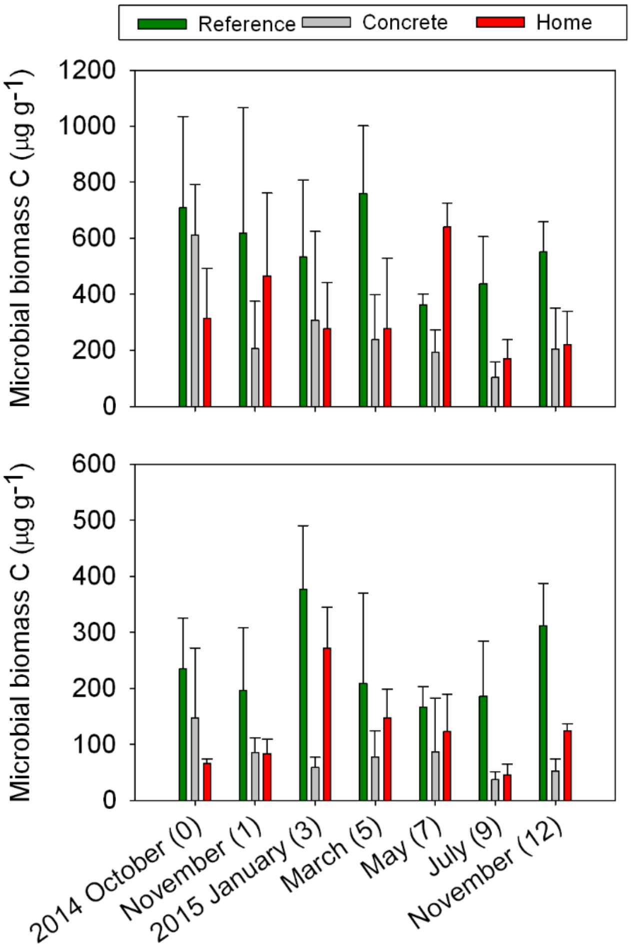

Figure 2.4. The monthly dynamic of soil microbial biomass carbon by treatment (n=4/ month) at layer one (A), and two (B). Means are significantly different with different letters based on Tukey's honest significant difference (HSD) tests at α = 0.05.

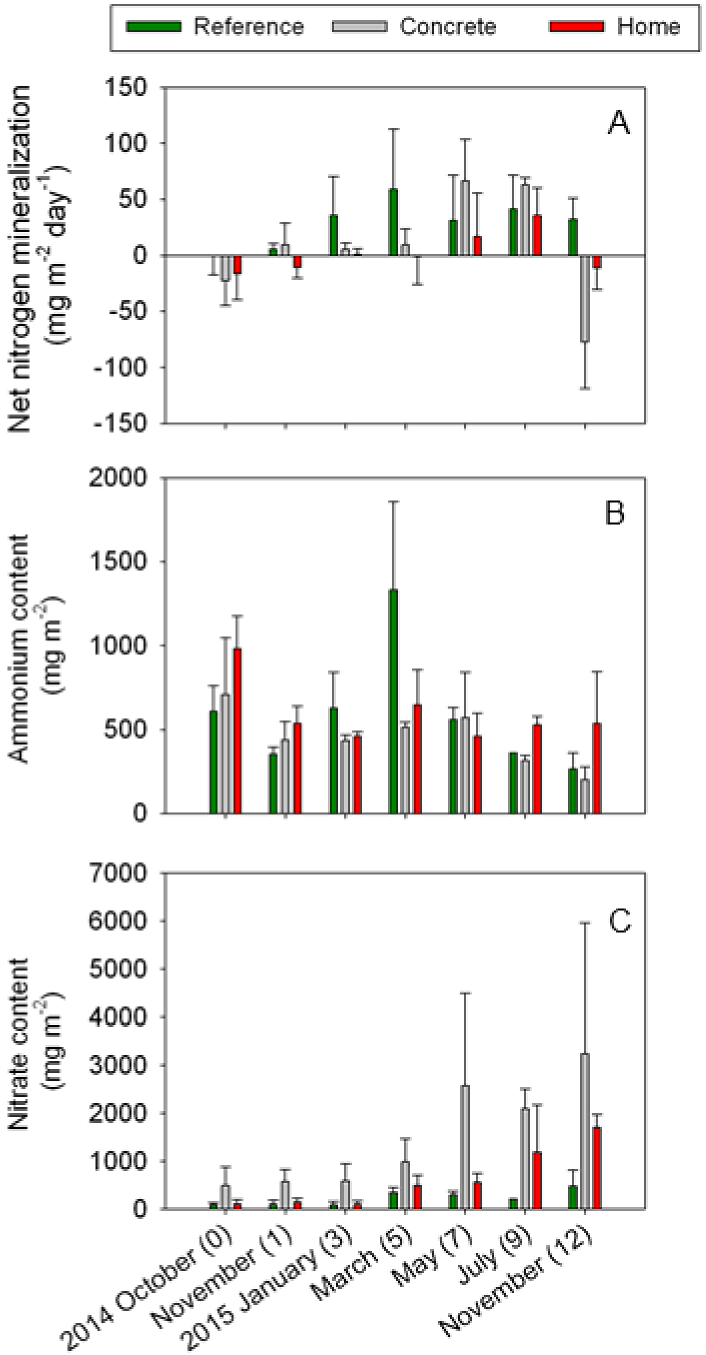

Figure 2.5. (A) Variation in soil nitrogen mineralization rate over time by treatment (n = 4/ month). (B) in soil ammonium content (n = 4/ month). (C) in soil nitrate content (n = 4/ month) over time.

Chapter 3: Effect of home construction on soil carbon storage in the Piedmont Region of the Southeastern United States

Abstract

Increased urbanization has resulted in rapid expansion of impervious surfaces. To access the ecosystem services provided by urban areas such as carbon sequestration, the estimation of carbon storage beneath the impervious surface is crucial. However, a knowledge gap exists due to the paucity of data. A systematic approach to measure soil carbon beneath impervious surfaces requires studies of various classes and sizes of impervious surfaces. Since homes cover more than 20% of impervious surfaces in the United States, we quantified soil carbon (C), nitrogen (N), microbial activity, bulk density, soil moisture, and macronutrients beneath homes built on a crawl space at two depths (0-10 cm, and 10-20 cm). All homes were sampled in the Piedmont region of Alabama and Georgia, USA, and ranged from 11 to 114 years in age. This enabled the use of a chronosequence approach to estimate carbon loss or gain beneath the homes sampled. The average soil C and N content in the top 10 cm were 61.86 % (± 4.42 %), and 65.77 % (± 5.65 %) respectively lower beneath the homes in comparison to urban lawns. The soil C and N loss beneath the homes revealed a quadratic relation with age and maximum C loss occurring at approximately fifty years in age. Microbial biomass carbon (MBC), and nitrogen (MBN) were significantly lower beneath the homes compared to the urban lawns, while bulk density and phosphorus were higher beneath the homes in the top 10 cm.

Introduction

The global urban population has increased from 746 million in 1950 to 3.9 billion in 2014 and is expected to surpass 6.4 billion by 2050 (United Nations et al., 2014). One important aspect of urban expansion is its effect on soil C storage and the global C cycle (Pataki et al., 2006; Pouyat et al., 2009; West and Six, 2007). Urban expansion has changed native land use and land cover, and substantially increased the area covered by urban lawns and impervious surfaces such as buildings, roads and parking lots (Jazaei et al., 2016; Lorenz and Lal, 2009; Milesi et al., 2003; Scalenghe and Marsan, 2009a; Shuster et al., 2005). While soil C storage in lawns has been widely studied in recent years, only a few field studies have investigated soil C storage beneath impervious surfaces. Most of these previous studies suggest that urban lawns have higher soil C storage compared to native ecosystems (Kaye et al., 2006; Milesi et al., 2005; Pouyat et al., 2009; Rao et al., 2013). For instance, turfgrass soils had twice as much C storage in the top 20 cm compared to rural forests in Baltimore, MD (Pouyat et al., 2009). However, in areas with very high native C content such as Boston, MA., urban lawns had similar levels of C storage to that of native soils (Pouyat et al., 2009).

Ecosystem services provided by urban areas cannot be understood without considering the effects that impervious surfaces have on soil biogeochemical processes. Impervious surfaces cover more than 580,000 km^2 globally (Elvidge et al., 2007). In the United States, the impervious surface area is greater than 83,337 km^2 which is larger than the total area of herbaceous wetlands (Elvidge et al., 2004). Installation of impervious surfaces may involve removal of the top soil which is rich in organic matter

(Lorenz and Lal, 2009; Yan et al., 2015b). It also limits the input of organic matter and will alter soil physical properties such as bulk density, temperature, and moisture (Scalenghe and Marsan, 2009b; Shuster et al., 2005). Nevertheless, the soil beneath these surfaces may still serve as habitat for microorganisms and insects, and provide space for rooting and leaching (Lehmann and Stahr, 2007). Soil C storage beneath impervious surfaces has been assumed to range from zero (Tomlinson and Milne, 2006) to 1 kg m^2 (Cannell et al., 1999; Schaldach and Alcamo, 2007) to 3.3 kg m^2 (Pouyat et al., 2006a) for the top 100 cm. However, studies that have sampled beneath the impervious surfaces do not confirm the previously mentioned assumptions. Soil C beneath impervious surfaces was 2.29 kg C m^{-2} [top 15 cm, New York, U.S. (Raciti et al., 2012b)]; 2.35 kg C m^{-2} [top 20 cm, Nanjing City, China (Z.-Q. Wei et al., 2014)]; 2.46 [top 20 cm, Yixing City China (Z. Wei et al., 2014a)]; and 3.55 [top 20, Urumqi City, China (Yan et al., 2015a)]. Normalizing these values to the top 100 cm (minimum value of 5.8 kg C m^{-2} in New York) reveals a significant underestimation of C in studies where sampling did not occur. In addition, Edmondson et al. (2012), sampled seventeen excavation sites in England and did not find any significant difference in C storage between urban lawns and beneath impervious surfaces. However, they excluded the topsoil removal from their calculations. These five sampling studies included road pavements, paved residential squares (Raciti et al., 2012b; Z. Wei et al., 2014b), and residential driveways (Edmondson et al., 2012; Yan et al., 2015b), but did not include homes. Yan et al. (2015), reported that the soil C storage decreased with distance from the edge of impervious surfaces, suggesting that soil C storage beneath narrow impervious surfaces (i.e.

sidewalks, residential pavements) may be higher than impervious surfaces with a larger footprint (i.e. buildings and parking lots).

Another component of soil C is microbial biomass, the non-plant portion of the soil organic matter. Microorganisms have a key role in C cycle feedbacks (Enloe et al., 2015; Fanin et al., 2014), and thus, microbial biomass C and N can be used as an early indicator of changes in soil quality (Ge et al., 2010; Groffman et al., 2004). Microbial activity and diversity may decrease by as much as 80% beneath the impervious surfaces due to lack of carbon, sunlight and temperature stresses ((Wei et al., 2013).

Specific objectives of this study included: 1) determination of C and macronutrient content within 0-10, and 10-20 cm depths beneath homes built on a crawl space and associated lawns; and 2) to quantify microbial biomass within the same soil samples.

Material and Methods

Study area

Samples were collected from Auburn, AL (latitude 32.60° N longitude 85.48° W), and Opelika, AL (latitude 32.64° N longitude 85.38° W), and Barnesville, GA (latitude 33.05° N longitude 84.15° W). Barnesville is situated in the Piedmont upland section of the Piedmont province of the Appalachian Highlands (Miller and Robinson, 1995). Auburn and Opelika are located on the fall line between the Coastal Plain and the Piedmont Plateau. All soil cores were collected from the Piedmont plateau. The taxonomic classes of the sampled soil included Fine, kaolinitic, thermic Typic Kanhapludults (Pacolet, and Cecil series) and Fine-loamy, kaolinitic, thermic Typic

Kanhapludults (Marvin series). The region has a mean annual air temperature of 17.4 °C and an average precipitation of 1337 mm. Trees common on urban properties included red maple (*Acer rubrum* L.), pecan (*Carya illinoensis*), sweetgum (*Liquidambar styraciflua* L.), oaks (*Quercus* spp.), loblolly pine (*Pinus taeda*), and shortleaf pine *(Pinus echinata).*Turf grasses were mostly Bahiagrass (*Paspalum notatum*), Bermudagrass (*Cynodon dactylon*), Zoysiagrass (*Zoysia*).

Twenty-four homes (n=17 in AL. and n=7 in GA), constructed on a crawl space, were sampled and ranged from 11 to 114 years in age. Age data obtained from the city of Auburn Planning Commission and Lee County Courthouse, Opelika, Al. Homes selected randomly when volunteers were available.

Soil properties

At each homestead, two soil cores (5 cm diameter) at two depths (0-10 cm and 10-20 cm) were collected for chemical analysis in the crawl space (n=4) and in the adjoining lawns (n=4), totaling 192 soil cores collected from the 24 homes. All soil cores removed from beneath the homes were at least two meters from the exterior wall. Bulk density samples were collected next to each chemical soil sample location. The bulk density samples were dried at 105 °C, and soil bulk density was calculated using the core method (Soil Survey Laboratory Staff, 2004). The air-dried soil samples were used to measure pH in a 0.01 molar solution of calcium chloride using an automated LabFit AS-3000 pH analyzer. The measured values were converted to 1:1 mixture of soil:water values by adding a conversion factor of 0.6. Using a diluted solution of salts to measure soil pH will result in more stable values between seasons and years (Kissel et al., 2009).

The Mehlich-1 method (Jones, 1998) was used to quantify concentrations of extractable P, Ca, K, Mg, Mn, and Zn using Inductively Coupled Plasma Spectrograph (ICAP61E, Madison, WI). Total C and N were measured using a PerkinElmer 2400 Series II CHNS/O analyzer (PerkinElmer, Waltham, MA). Soil gravimetric water (W_m) calculated by drying 10 g of soil in oven at least for 24 h at 80 ^{o}C and using the following equation:

$$W_m = \frac{Moist\ soil\ weight - dry\ soil\ weight}{dry\ soil\ weight}$$

Soil percent volumetric water (Wv) was quantified by multiplying W_m and soil bulk density.

Microbial biomass

The chloroform-fumigation method (Vance et al., 1987) was used for measuring soil microbial biomass C, and N. Soil samples were homogenized by sieving through a 2 mm mesh sieve and dividing into two sets of 18.5 g. The first set was fumigated by exposing soil to alcohol-free chloroform for 24 hours. Fumigated and unfumigated samples were extracted with 100 mL 0.5 mol L^{-1} K_2SO_4 for 30 minutes. Extracts were filtered (Whatman no. 42 filters) and then frozen. After thawing, samples were analyzed for organic C and total N using a Shimadzu TOC-V and total N combustion analyzer (Shimadzu Scientific Instruments, Columbia, MD). The differences between fumigated and unfumigated samples represent microbial C and N.

Statistical analysis

"R" software, version 3.1.2 was used for all statistical analysis in this study. A linear mixed-effects model using the nlme package of "R" software was utilized to compare soil properties beneath the houses and in urban lawns. The Tukey's honestly significant difference (HSD) following an analysis of variance (ANOVA) test was used to differentiate the means (Agricola package, $\alpha = 0.05$), for soil C, N, microbial biomass C and N, and bulk density between urban lawns and beneath the homes at two depths. There was an interaction between two depths and treatments (urban lawns, and beneath the houses), thus instead of a two-way ANOVA, soil properties were examined at two separate depths (0-10 cm, and 10-20 cm).

Results

Soil Carbon and Nitrogen

The soil C content in the top 10 cm and 10-20 cm were respectively, 61.86 % (± 4.42 %), and 56.82 % (± 3.67 %) lower beneath the homes compared to urban lawns (p-value <0.001, Fig. 3.1a). The soil C content decreased with depth and was 1.25 (± 0.18) kg C m^{-2} at 0-10 cm and 0.78 (± 0.12) kg C m^{-2} at 10-20 cm beneath the homes. In urban lawns, soil C was 3.38 (± 0.13) kg C m^{-2} at 0-10 cm and 1.88 (± 0.13) kg C/m^2 at 10-20 cm (p-value < 0.001). The difference in carbon content beneath the homes and adjoining grassed area was calculated using the following equation for each home:

$$\text{Difference (\%)} = \frac{\textit{Soil carbon in adjoining lawn} - \textit{Soil Carbon beneath the home}}{\textit{Soil carbon in adjoining lawn}} \times 100$$

The difference in C had a quadratic relation with home age (p-value = 0.004, $r^2 = 0.31$, Fig. 3.2a) which could be separated by two periods of time. For the first 53 years, the difference increased in the upper 10 cm (p-value =0.01, $r^2 = 0.49$, Fig. 3.2b). Following that, the difference in C content decreased between age 53 and 114 years (p-value =0.05, $r^2 = 0.19$, Fig. 3.2c).The soil N beneath the homes compared to urban lawns decreased by 65.77 % (± 5.65 %), and 55.11 % (± 5.31 %) in the top 10 cm and 10-20 cm respectively (*p*-value < 0.001, Fig. 3.1b). Similar to C, a quadratic relation between home age and the difference in N was observed (*p*-value = 0.01, $r^2 = 0.33$, Fig. 3.3).

In contrast to C and N content, the C:N ratio was significantly higher (*p*-value <0.001) beneath the homes (20.26 ± 1.48) in comparison to lawns (15.72 ± 1.34). A significant relation between C and N content was observed both beneath the houses ($r^2 = 0.76$, *p*-value <0.001) and in lawns ($r^2 = 0.74$, *p*-value <0.001, Fig. 3.4).

Microbial biomass carbon and nitrogen

A 66.90 % (± 3.84 %), and 67.80% (± 5.31 %) decline in microbial biomass carbon (MBC) was observed beneath the houses compared to urban lawns in the top 10 cm and 10-20 cm, respectively (*p*-value <0.001, Fig. 3.5a). The soil microbial biomass nitrogen (MBN) was 89.36% (± 2.9 %), and 81.49% (± 2.55 %) lower than that of in urban lawns in top 10 cm and 10-20 cm respectively (Fig. 3.5b). The MBC and MBN both showed a negative relation with depth in lawns (*p*-value <0.001). However, no significant relation between MBC, or MBN and depth was observed beneath the homes.

Other soil physical and chemical properties

Soil bulk density was significantly higher beneath the houses in comparison to that in urban lawns (*p*-value <0.001, table 1). Soil bulk density increased with depth in urban lawns (*p*-value <0.001) but displayed no significant increase beneath the homes. Soil C concentrations were negatively related with bulk density. In top 10 cm, soil C concentrations decreased 0.55 g kg^{-1} (± 0.15; $r^2 = 0.22$, *p*-value = 0.001) and 1.6 g kg^{-1} (± 0.4; $r^2 = 0.21$, *p*-value = 0.003) for each 1 Mg m^{-3} increase in soil bulk density in urban lawns, and beneath the houses, respectively (Fig. 3.6a, b). In depth 10-20 cm soil C concentration and bulk density were also negatively related in both urban lawns and beneath the homes (Fig. 3.6c, d).

Soil moisture beneath the houses was higher than urban lawns at both depths (*p*-value <0.01). The samplings were conducted in summer and higher evaporation rates in grassed areas were expected. Soil moisture and carbon content were positively related in lawns at both depths (Fig. 3.7a, b). However, the soil moisture beneath the homes had a negative relation with soil carbon content at the top 10 cm, and no significant relation observed at depth 10 -20 cm (Fig. 3.7c, d). Higher pH, extractable Ca, Mg, and phosphorus content was observed beneath homes compared to urban lawns in top 10 cm (table 1). Lower concentrations of P in urban lawns in comparison to underneath the houses may be due to plant uptake and removal of P by grass clippings (Soldat and Petrovic, 2008).

Discussion

Soil C content in urban lawns of this study (5.26 kg C m^{-2}) was higher than findings of (Huyler et al., 2013) who reported C content of 2.37 kg C m^{-2} in the top 15 cm of residential lawns in Auburn, AL. Moreover, normalizing our data to 1 meter by extrapolating the lower portion of the core data, (Pouyat et al., 2006a), the C storage in the top 1 meter of urban lawns (7.69 kg C m^{-2}), was similar to C storage in native forests of the southeast United States (7.02 kg C m^{-2}). This similar C storage is expected in temperate areas due to low C storage of native soils and fewer management practices which are required for turfgrass growth in comparison to arid regions (Pouyat et al., 2009).

Soil C content beneath the houses was 63.30 % lower than the adjoining urban lawns. The decrease in C beneath the homes was similar to that of beneath pavements in two other studies in China and the United States (Raciti et al., 2012b; Z. Wei et al., 2014b). Higher decreases in C content (75 %) also have been reported in a study in China (Yan et al., 2015a). The equilibrium value assumption for soil C beneath impervious surfaces suggested by Wei et al., 2014 may be oversimplified. In this assumption, a constant value would be considered for the soil C content beneath the impervious surfaces. If due to the lack of field data, any assumptions are to be made, equilibrium decrease in C (68.07 % ± 4.3, an average of four field studies) beneath the impervious surfaces in comparison to urban lawns would be a better estimation. In areas that the data is available, using native forests as the reference instead of urban lawns would provide even a better estimation since the C levels would not be affected by management practices.

Homes cover approximately 18,000 km^2 of the United States (U.S. Census Bureau, 2013), which would be approximately, 20% of the total impervious surface in the U.S. (Elvidge et al., 2007). Of these homes, 21.4 % are built on crawl spaces, while less than 24% were constructed on concrete slabs (U.S. Census Bureau, 2013). The soil C storage beneath homes built on a crawl space in the southeastern U.S. is approximately 2.96 million metric tons of C which translates to a loss of 8.06 million metric tons of C to the atmosphere. This estimation is based on assumptions that (a) removed top soil has not been protected or re-used (b) only 21% (national average) of the 4,888 km^2 homes in the southeastern U.S. are constructed on a crawl space, and (c) the average C loss beneath these homes is similar to the findings of this study.

The soil C: N ratios were significantly higher beneath the homes in comparison to urban lawns, suggesting that soil beneath the impervious surface has lower N.This was also confirmed by our microbial biomass data. Microbial biomass C dropped 65.14 %, while MBN dropped by 80.51 % beneath the homes. This decrease in microbial biomass is linked with lack of bioavailable C and N (Wardle, 1992). Moreover, increased soil pH (Table 1), lack of sunlight, lower temperatures, and oxygen limitations may also reduce microbial biomass (Zhao et al., 2012).

While the carbon content in lawns and beneath the homes did not show any relation with time, the difference in C content increased over time and reached its maximum (more than 90 %) at age 53 years. Following that, it decreased between ages 53 and 114 years. This pattern can be due to the accumulation of C in urban lawns and/or loss of C beneath the homes. In order to shed light on this process, MBC: C ratios were calculated for both land covers. The MBC: C ratio is an indicator of the proportion of metabolized carbon (Scharenbroch et al., 2005). The increase in this ratio over time is a sign of C loss, while a decrease in it would be indicative of C sequestration (Beyer et al., 1995). The MBC: C ratio in lawns showed no relation with age suggesting that soil carbon in the age range of studied lawns is almost stabilized. However, this ratio increased for the first 53 years ($r^2 = 0.27$, *p*-value = 0.03), and was decreasing between ages 53 and 114 ($r^2 = 0.15$, *p*-value = 0.05) beneath the homes (Fig.3.8). These trends suggest that C loss may be the prevalent process for the first 50 years beneath the homes while the C sequestration would be the dominant process afterward. We may speculate that C loss and gain have different mechanisms beneath the homes. The decomposition of C to the atmosphere as CO_2 may be the major driver of C loss which would be limited to the surface soil and all the decomposable carbon may have decomposed in the first fifty years. However, the major process of carbon gain may be dissolved organic carbon leaching from surroundings of homes which would not be linked to the age of the structure and become the source of carbon. Moreover, crawl spaces in the southeastern U.S. have lower temperatures during summer months which can reduce evaporative loss of C. The lower temperatures also provide an excellent shelter for insects such as ants and termites which can increase the soil C.

The U.S. has the largest impervious surface area per person (297 m^2/ per person) (Elvidge et al., 2007), and is expanding rapidly with approximately $480 billion per year investment in construction (Elvidge et al., 2004). Thus, a better understanding of C storage beneath these surfaces and strategies to minimize C loss there is of great importance. Top soil removal accounts for a major portion of C loss beneath the impervious surfaces. Protecting the top soil would minimize the contribution of home construction on atmospheric C. Further studies are required to compare soil C beneath the houses built on crawl spaces to those constructed on concrete slabs in order to add clarity regarding C storage beneath the homes.

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Tables and graphs

Table 3.1. Mean ± standard error of soil characteristics sampled at two depths (0-10 cm (n=2) and 10-20 cm (n=2)) beneath the homes constructed on a crawl space (n=24) and in urban lawns (n=24). Row means are significantly different with different letters based on Tukey's honest significant difference (HSD) tests at α = 0.05.

Soil properties	Beneath Houses	Urban Lawns	Beneath Houses	Urban Lawns
	(0-10 cm)	(0-10 cm)	(10-20 cm)	(10-20 cm)
Bulk Density (Mg/m^3)	1.47 ± 0.03 a	1.07 ± 0.03 c	1.48 ± 0.03 a	1.31 ± 0.04 b
pH	5.95 ± 0.12 a	5.60± 0.14 ab	5.61 ± 0.12 ab	5.44 ± 0.11 b
Soil water (m^3/ m^3)	1.58 ± 0.11 b	1.36 ± 0.16 c	1.82 ± 0.10 a	1.37 ± 0.11 c
Ca ($mg\ m^{-2}$)	93.98 ± 18.08 a	75.90 ± 12.21 ab	45.48 ± 11.36 b	57.89 ± 11.21 ab
K ($mg\ m^{-2}$)	6.54 ± 0.79 a	6.57 ± 0.72a	6.39 ± 0.77 a	4.93 ± 0.76 a
P ($mg\ m^{-2}$)	4.16 ± 0.49 a	3.59 ± 0.65 b	1.00 ± 0.50 c	3.31 ± 0.48 a
Mg ($mg\ m^{-2}$)	12.61 ± 1.01 a	10.79 ± 2.02 ab	8.41 ± 0.99 b	9.94 ± 0.98 b
Mn ($mg\ m^{-2}$)	1.89 ± 0.20 ab	2.38 ± 0.26 a	1.43 ± 0.19 b	2.06 ± 0.19 ab
Zn ($mg\ m^{-2}$)	2.73 ± 0.55 a	2.46 ± 0.66 a	1.76 ± 0.54 a	1.25 ± 0.53 a

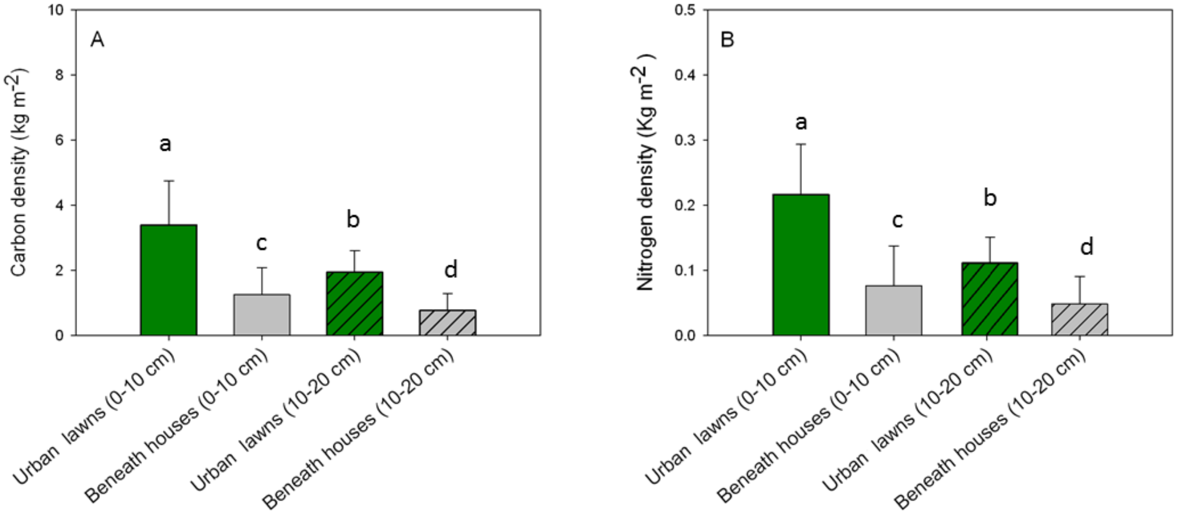

Figure 3.1. Mean ± standard error soil a) C and b) N content (kg m^{-2}) in urban lawns and beneath the homes built on a crawl space at two depths (0-10 cm and 10-20 cm). Means are significantly different with different letters based on Tukey's honest significant difference (HSD) tests at $\alpha = 0.05$.

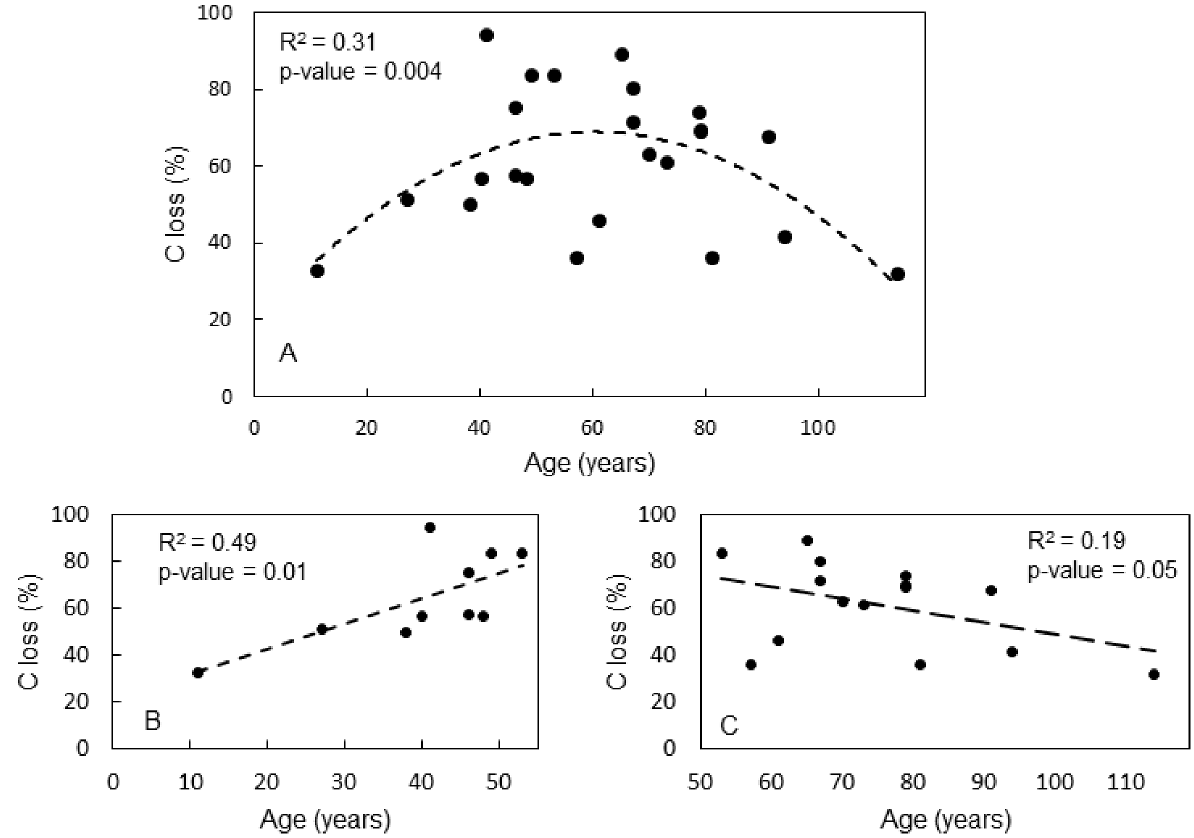

Figure 3.2. Carbon loss from the soil beneath the crawl space of homes a) 11-114, b) 11-53, and c) 53-114 years old. Second order polynomial (a) and linear trend line (b, c) used to illustrate the patterns.

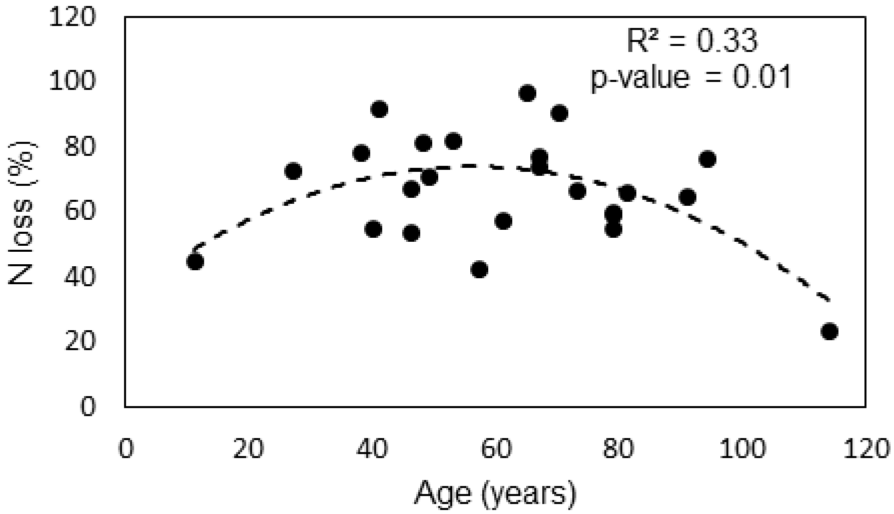

Figure 3.3. Nitrogen loss from the soil beneath the crawl space of homes age 11 to 114 years. Second order polynomial trend line used to illustrate the patterns. R-square reported for the polynomial trend line.

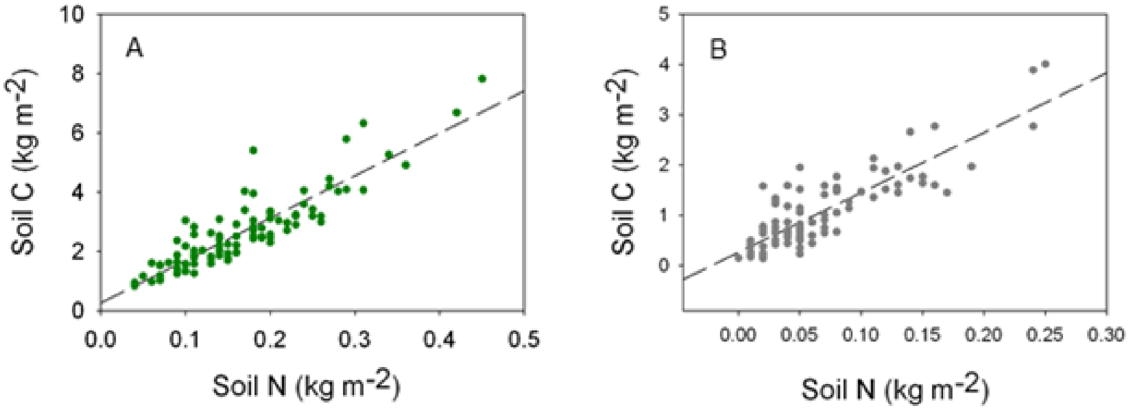

Figure 3.4. The relation between soil C and N in a) urban lawns and b) beneath the crawl space of homes.

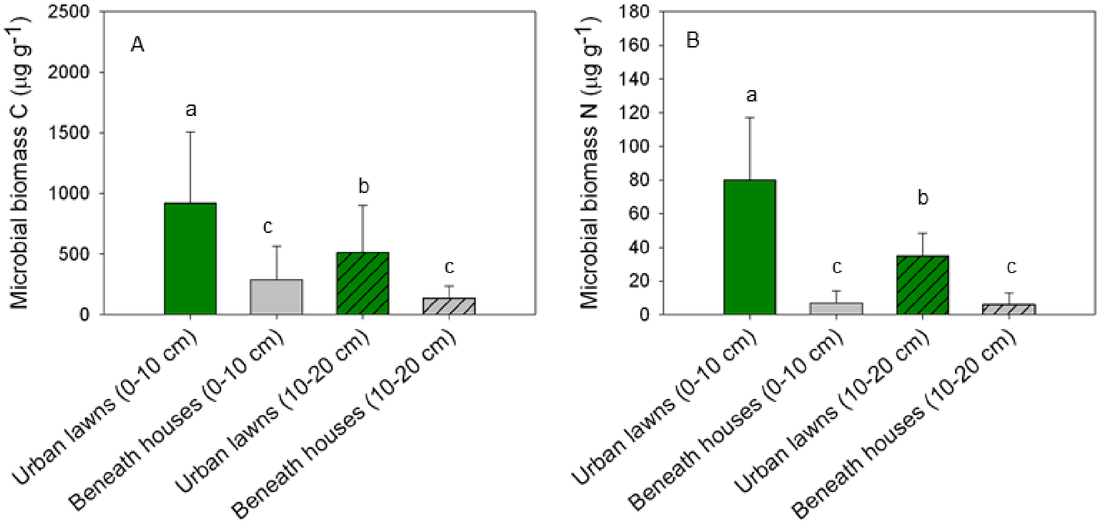

Figure 3.5. Soil a) microbial biomass C (and b) microbial biomass N (ug g^{-1}) in urban lawns and beneath the crawl space of homes at two depths (0-10 cm and 10-20 cm). Means are significantly different with different letters based on Tukey's honest significant difference (HSD) tests at α = 0.05.

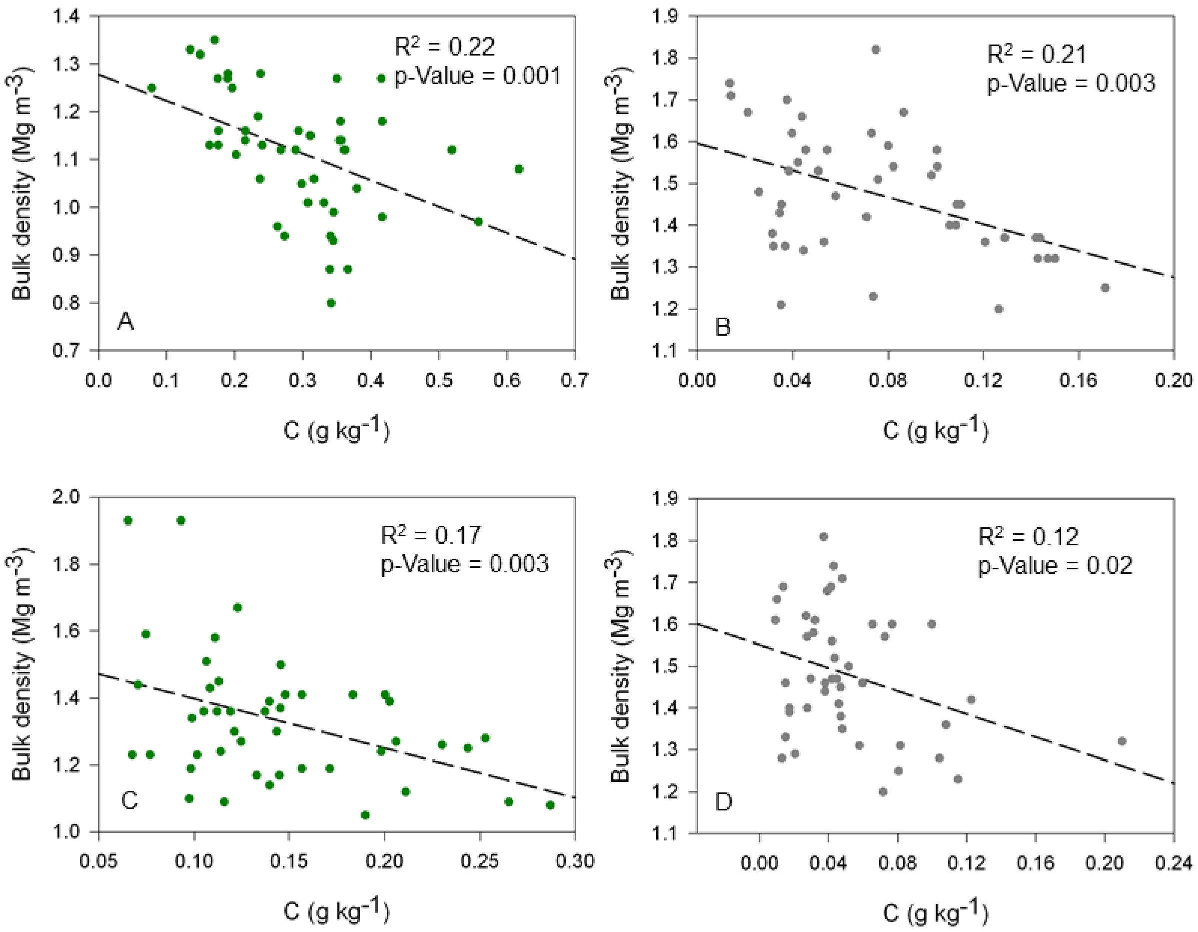

Figure 3.6. Relation between C concentrations (g kg^{-1}) and soil bulk density (Mg m^{-3}) in urban lawns at depth 0-10 cm (a) urban lawn at depth 10-20 cm (b) beneath the houses at depth 0-10 cm (c), and beneath the houses at depth 10-20 cm (d).

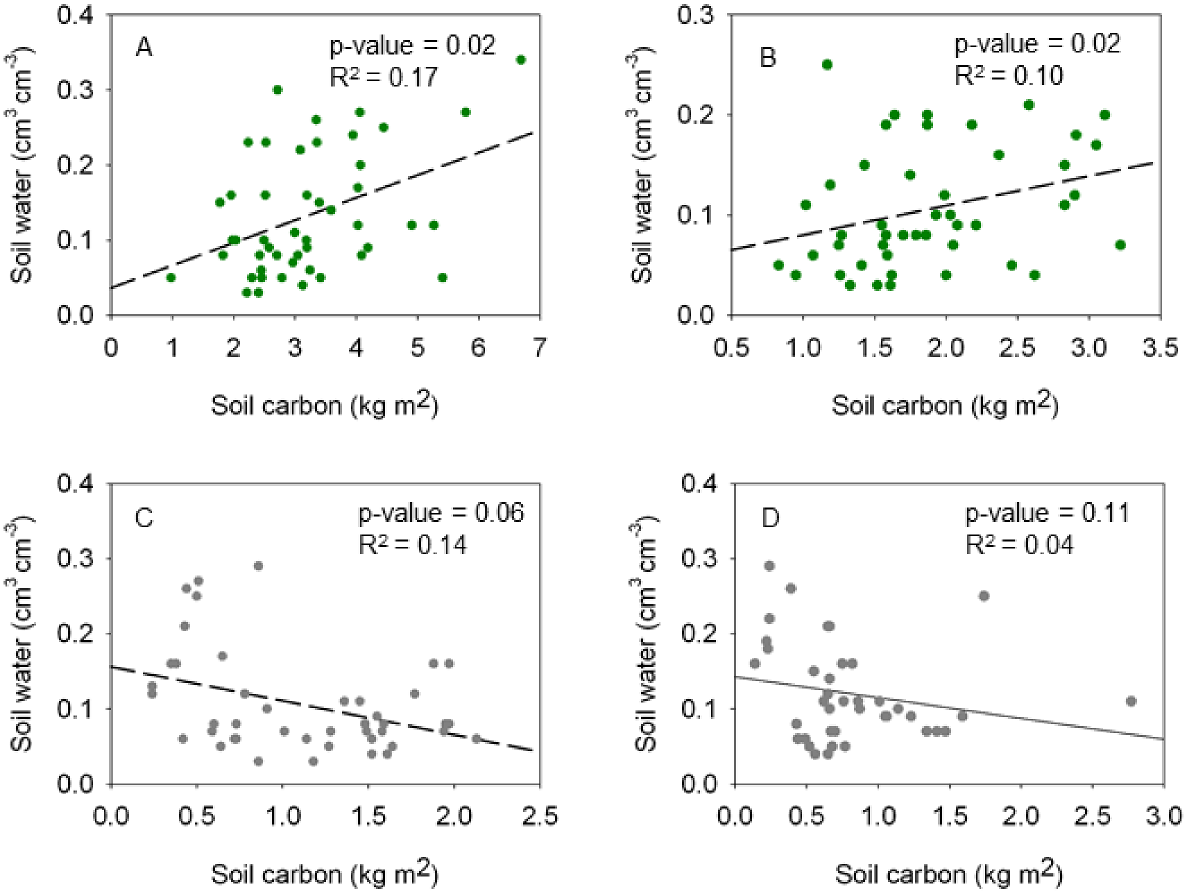

Figure 3.7. Relation between carbon content and soil water in A) urban lawns (0-10 cm), B) urban lawns (10-20 cm), C) beneath homes (0-10 cm), and D) beneath homes (10-20 cm).

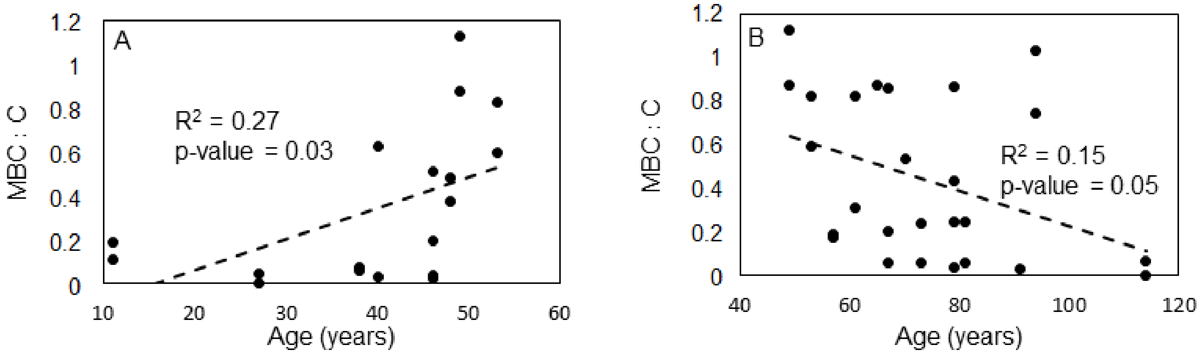

Figure 3.8. Relation between Age and the MBC: C ratio a) between age 11 and 53 and b) between age 53 and 114.

Chapter 4: Effects of Urbanization and Urban Lawns on Survival Rate of Mosquitoes

Abstract

Mosquito-borne diseases, especially in urban areas, are a global threat to public health. West Nile virus (WNV), transmitted by the southern house mosquito (*Culex quinquefasciatus* Say) has caused over 1500 deaths in the United States since 1999. Minimizing breeding habitat would be an effective, eco-friendly method to reduce urban mosquitoes. However, the determination of ideal breeding habitat must first be understood. To that end, a laboratory microcosm study was designed which included abscised leaves from three common overstory tree species in the southeastern United States and turfgrass clippings to simulate potential backyard breeding habitat for *Culex*. Pupae and adult male numbers were positively correlated with dissolved organic carbon (DOC) and negatively correlated with dissolved phosphate (DP). The number of adult females was similarly affected by DOC and DP while dissolved organic nitrogen (DON) was also a key factor for their development. Survival rates were significantly lower in abscised leaf treatments than those found in grass treatments with maximum pupae development of 3.93 ± 2.29 % in the pine treatment and 1.92 ± 1.72 % adult development in the sweetgum treatment. Grass treatments developed 28.50 ± 5.75 % and 22.50 ± 4.79 % pupae and adult mosquitoes respectively, suggesting that urbanization and formation of urban lawns create a suitable habitat for growth of mosquitoes when water ponding exists. This study highlights the importance of variation in carbon,

nitrogen, and phosphorous sources in the ponded water on survival rates of the *Culex* mosquitoes.

Introduction

Disease vectors and in particular urban mosquitoes have long been a global public health threat (Benelli et al., 2015; Kilpatrick and Randolph, 2012). Ongoing outbreaks of West Nile (WNV), Zika (ZIKV), and chikungunya (CHIKV) viruses are examples of urban mosquito-borne diseases (Attar, 2016; Ezenwa et al., 2006). West Nile virus (WNV), unlike the ZIKVand CHIKV, has caused epidemics every summer (Coffey and Reisen 2016) with over 1500 deaths since it was introduced to the United States in 1999 (Allan et al., 2009; Petersen LR et al., 2013). WNV, transmitted by *Culex quinquefasciatus* Say (the southern house mosquito), inhabits regions with tropical and temperate climate. *Culex* is considered a vector between species since it can infect birds, livestock, and humans (Allan et al., 2009). Currently, no vaccines are available for WNV. Therefore, strategies to minimize the mosquito population are required to prevent the spread of the disease (Benelli et al., 2015; Benelli and Mehlhorn, 2016; Jeffries and Walker, 2016).

The life cycle of the *Culex quinquefasciatus* has four major developmental stages: First, the adult female lays egg rafts on surface water or in small natural or man-made containers filled with flood water or rain (Kaufman et al. 2002). Second, within two days eggs hatch and form aquatic larvae (Jeffries and Walker, 2016). During this stage, larvae consume microorganisms (Barrera et al., 2006; Kaufman et al., 2002) and algae in the water to grow (Johnson and Borror, 2005). Third, larvae develop into aquatic pupae. The time for this to occur depends upon environmental conditions such as temperature and nutrient levels (Johnson and Borror, 2005; Norris, 2004). Fourth, within two to three days the pupa develop into adult mosquitoes (Johnson and Borror, 2005). Since only adult females transmit the viral infection, controlling their population is a priority (Petersen LR et al., 2013). Considering the life cycle of *Culex* and its critical dependence on water, deeper insight into the effect of water quality on the survival rate of the mosquitoes is important especially when considering the physical and monetary toll of the disease on the human population. Water quality may be affected by the surrounding vegetation that serves as a source of organic matter. For instance, it has been shown that sweetgum leaves leach more DOC than longleaf pine, bald cypress, and water tupelo leaves (Majidzadeh et al. 2015). The effect of vegetation on water quality may be magnified when native forests are replaced with lawns due to urbanization. The forest floor, naturally covered with dead and decomposed leaves, is replaced by highly managed lawns (i.e. fertilized, weed removal, clipped regularly). Urban lawns are estimated to cover more than 1.9% of the US (Martinez et al., 2014). This coverage will continue to grow as urbanization stimulates the formation of yards and decorative green spaces (Huyler et al., 2014b; Raciti et al., 2008). Moreover, urbanization has significantly increased mosquito habitats associated with sewers and artificial containers such as bottles and tires (Brown et al., 2008; Dowling et al., 2013). Pollutants from both point or non-point sources such as nitrate and phosphate are commonly found in urban streams (Schoonover and Lockaby, 2006; Urban et al., 2006). (Noori et al., 2015) studied the effect of nutrients in water with levels commonly found in urban streams on the survival rate of the *Culex* mosquitoes larvae using leaf litter as a food source. They found that an increase of nitrate and phosphate in the water improved mosquito survival rates.

The objectives of this study were to: 1) determine the survival rate of *Culex quinquefasciatus* in containers using turfgrass (*ryegrass*) and abscised leaves from three common tree species in the southeastern United States including sweetgum (*Liquidambar styraciflua* L.), water oak (*Quercus nigra* L.), and loblolly pine (*Pinus taeda* L.) as carbon sources; and 2) document how the initial nitrate and phosphate concentrations in water affect the survival rate of mosquitoes. These nutrients are commonly associated with urban streams in the southeastern United States.

Material and Method

A laboratory microcosm study consisting of 75, 1.25 liter containers was designed to examine the effects of initial nitrate and phosphate concentrations in the water and vegetation type on the survival rate of *Culex* mosquitoes. Each microcosm contained two grams of air-dried vegetation input as the carbon source and one-liter of water collected from a stream flowing through the undisturbed forested land. The leaves were abscised (consequently, the foliar nutrient concentrations were reduced) but the grass clippings were fresh (green) so that a differential existed between the chemistry of the leaves vs. grass. The reason for the inclusion of abscised leaves and fresh grass was that these materials are likely to accumulate in containers or concave microsites in residential areas and affect the physicochemical habitat of the mosquito larvae. Stream water analysis showed a minimum level of dissolved organic carbon (10.61 ± 0.20 mg/L), dissolved organic nitrogen (0.09 ± 0.01 mg/L) and other nutrients (< 0.06 mg/L).

Vegetation treatments were studied at five nutrient levels: low nitrate (NO_3^- = 3 mg/L); high nitrate (NO_3^-= 12 mg/L); low phosphate (PO_4^{3-}= 1 mg/L); high phosphate (PO_4^{3-}= 6 mg/L); and a control treatment with no nutrient added (Table 1). Initial nitrate and phosphate concentrations were adjusted using sodium nitrate and monopotassium phosphate, respectively.

One hundred *Culex quinquefasciatus* first instar larvae were obtained from a laboratory in Department of Entomology, Auburn University and added to each container. Microcosms were checked twice daily for pupae until day fifty-five at which time there were no more larvae. Following pupation, the pupae were isolated in vials with the same water as that of the initial container. Pupae that developed into adults were sexed and weighed in the laboratory one week after their death. The average temperature in the laboratory was 25.5 ^{o}C (±2.6 ^{o}C). Evaporation was monitored using control containers and was compensated using stream water every week.

Total carbon and nitrogen in abscised leaves and turfgrass were measured using thermal combustion Perkin Elmer 2400 Series II CHNS/O analyzer (Perkin Elmer, Waltham, MA). The water chemistry in each container was tested before addition of larvae and subsequently every two weeks. Water samples were filtered through 0.45 µm filters before analysis. A Shimadzu TOC-V, TNM-1 analyzer (Shimadzu, Norcross, GA.) was used to measure dissolved organic carbon (DOC) and total dissolved nitrogen (TDN). Nitrate, nitrite, ammonium, and phosphate were measured using a Thermo Scientific Dionex ICS-1500 ion chromatography system (Waltham, MA.). Dissolved organic nitrogen (DON) was calculated by subtracting TDN from inorganic nitrogen including nitrate, nitrite, and ammonium (Chow et al., 2012). The pH was monitored by a BASIC pH meter Denver Instrument (Gottingen, Germany). The UV absorbance (UVA) at 254 nm was measured using a Shimadzu UV-1800 at room temperature in a 1 cm quartz cell. UVA at 254 nm was normalized by the DOC concentration to calculate the specific ultraviolet absorbance at 254 nm (SUVA), which is an indicator of aromaticity in DOC. Higher SUVA values (L mg-C^{-1} m^{-1}) indicate a higher aromaticity of the DOC (Majidzadeh et al., 2015; Wang et al., 2015).

Statistical Analysis

A factorial design with three blocks as a random effect was used to study the effects of vegetation type and nutrients on the *Culex* mosquitoes life cycle. All statistical analysis in this study were performed using R software, version 3.1.2. Effects of vegetation type and nutrients on the number of pupae and adults (female and male) were studied using generalized linear mixed models (GLMMs) with a Poisson distribution. The Poisson family calculates relative abundance or the rate ratio using a link function of $e^{(\text{coefficient})}$ (Johnson et al., 2015). The rate ratio was calculated and reported within 95% of the confidence interval (C.I.). GLMMs are considered as one of the best approaches to analyze non-normal data including count data (i.e. number of mosquitoes) with a random effect which is replicated in blocks (Johnson et al., 2015). Ordinary least square regressions are not appropriate for the count data since they can be biased (Coxe et al., 2009). Linear mixed-effects models using the nlme package of R software was used to study the effect of vegetation type and nutrients on water quality over time. Tukey's honestly significant difference (HSD) following an analysis of variance (ANOVA) test was used to differentiate the means.

Results

Chemical characteristics of abscised leaves and turfgrass

The concentration of carbon (C), nitrogen (N), and lignin were determined in three abscised leaf samples from each treatment and fresh turfgrass prior to the experiment (Table 2). The carbon percentage was statistically different among treatments (*p*-value <0.001) and followed the order of 50.58% ± 0.24 (loblolly pine) > 47.07% ± 0.024 (water oak) > 45.69% ± 0.11 (sweetgum) > 42.85% ± 0.12 (turfgrass). Abscised leaves had a higher percentage of carbon than turfgrass. However, nitrogen concentrations were significantly higher in turfgrass in comparison to abscised leaves (*p*-value < 0.001, Table 2). The lignin percentage, and lignin: N ratio which may negatively correlate with decomposition rates (Mafongoya et al., 2000) were significantly higher in abscised leaves in comparison to turfgrass. This lower ratio in the latter was expected since abscised leaves were already dead while the turfgrass was green. The C: N ratio were significantly lower in turf grass in comparison to abscised leaves, confirming the lignin: N ratio results.

Effect of vegetation type and nutrients on water quality

Water samples were collected from all treatments every two weeks for forty days. The sweetgum treatment had the highest DOC (*p*-value < 0.001) followed by turfgrass, water oak, and loblolly pine. Temporally, DOC concentrations within the pine treatment increased 0.12 ± 0.04 mg/L per day (*p*-value = 0.03) (Figure 4.1). This increase may be due to the low solubility of organic carbon and high levels of lignin and lignin: N ratio in pine leaves. The DOC levels of other treatments did not change significantly over time.

Specific UV absorbance at 254 nm (SUVA), an indicator of aromaticity in DOC, was measured for all treatments. The measurement of SUVA was to determine the carbon quality of different vegetation as well as the DOC. The turfgrass and sweetgum treatments had higher aromaticity in comparison to water oak and loblolly pine treatments (*p*-value < 0.001). The aromaticity of treatments did not change over time.

DON concentrations were significantly higher in turfgrass treatments in comparison to abscised leaf treatments (*p*-Value <0.001). Among abscised leaf treatments, sweetgum had higher DON concentrations compared to water oak and loblolly pine. The DOC: DON ratio of the sweetgum treatment was higher than other treatments (167.58 ± 6.41; 95% C.I.; *p*-value <0.001) despite its high levels of DOC and DON. The DOC: DON ratio in other treatments statistically ranked as follows: loblolly pine (99.48 ± 4.35) > water oak (77.75 ± 6.15) > turfgrass (62.19 ± 6.15) (*p*-value < 0.001).

The initial nitrate concentration in the water had no significant effect on DOC leaching from abscised leaves. However, for the turfgrass treatments, a 0.66 ± 0.17 mg/L increase in DOC concentration was observed for each 1 mg/L increase in initial nitrate concentration. This increase may be due to an enhanced decomposition rate following the nitrate addition. Initial phosphate concentration had no effect on the DOC level leaching from abscised leaves and grass.

Effect of vegetation type and water quality on number of pupae

No pupae emerged in control containers, suggesting that water and nutrients were inadequate for pupae development in the absence of a carbon source. Since no pupae developed in the control treatment, this treatment was excluded from the analysis. Turfgrass treatments developed a significantly higher number of pupae with an average of 28.5 ± 5.75 % development at all levels of nutrients and were followed by loblolly pine (3.93 ± 2.29 %), water oak (2.64 ± 1.85 %) and sweetgum (2.25± 1.84 %) (Figure 4.2). The low number of pupae in loblolly pine and water oak was expected due to the lack of food as indicated by the low levels of DOC leaching from these species. However, the low number of pupae in the sweetgum treatments was not expected considering its chemical characteristics. High levels of DOC, DON, and DP in the sweetgum treatments should have created ample food sources for microorganisms. Despite that, most larvae died within the first week of the sweetgum treatment except for a few that developed to the pupae stage.

A generalized linear mixed model was used to study the effect of water quality parameters including DOC, SUVA, DON, and DP on the number of pupae. Since most of the pupae in the sweetgum treatment died within the first week except for a few, this treatment was not included in this analysis. However, the model with sweetgum is presented in the appendix. The number of pupae was positively correlated with DOC concentrations and negatively correlated with DP concentrations (Table 3).

In addition to vegetation inputs, the effect of initial nitrate and phosphate concentrations in the water on the number of pupae was examined. Initial nitrate concentrations were correlated positively with the number of pupae in the grass and sweetgum treatments. A 1 mg/ L increase in nitrate concentrations resulted in 1.04 (1.06-1.01; 95% C.I.; *p*-value = 0.005) and 1.13 (1.22-1.05; 95% C.I.; *p*-value = 0.005) times more pupae per container for turfgrass and sweetgum treatments, respectively. Pupae in water oak and loblolly pine treatments were not affected by nitrate concentrations. Initial phosphate concentrations in water had no significant effect on the number of pupae in any treatment.

Effect of vegetation and water quality on number of adult mosquitoes

The number of adults in the turfgrass treatments was significantly higher than that of the abscised leaf treatments (*p*-value < 0.001, Figure 4.2). In turfgrass treatments 22.54 % (± 4.79; 95% C.I.) of the first instar larvae developed to the adult stage while only 1.17% (± 1.39; 95% C.I.), 1.01% (± 0.96; 95% C.I.), and 0.43% (± 0.79; 95% C.I.) of the first instar larvae developed to the adult stage in the sweetgum, loblolly pine, and water oak treatments, respectively. Although the sweetgum treatments were associated with the low development of pupae, 88% (± 20; 95% C.I.) of developed pupae became adult mosquitos. This dropped to 67% (± 15; 95% C.I.) in turfgrass treatments, 17% (± 1.8; 95% C.I.) in loblolly pine, and 16% (± 2.4; 95% C.I.) in water oak (*p*-value < 0.001).

The same statistical model was also utilized to test the effects of water quality on number of adults. The number of female mosquitoes was positively correlated with DOC and DON concentrations while the number of adult males was positively correlated with DOC concentrations and negatively correlated to DP concentrations (Table 3).

There was a positive interaction between initial nitrate concentration and the number of adult females and males in both the turfgrass and sweetgum treatments. In the grass treatment, there were 1.08 (1.03-1.12; 95% C.I.; *p*-value < 0.001) times more adult females, and 1.05 (1.01-1.08; 95% C.I.; *p*-value = 0.01) times more adult males with a 1 mg/ L increase in nitrate concentration. In the sweetgum treatment, the same increase in nitrate concentration raised the number of adult females and males by 1.12 (1.01-1.24; 95% C.I.; *p*-value = 0.03), and 1.16 (1.02-1.32; 95% C.I.; *p*-value = 0.02) times, respectively. The number of adults in water oak and loblolly pine treatments were not affected by the initial nitrate and phosphate concentrations.

Initial phosphate concentrations were negatively correlated with the number of adult males only in the turfgrass treatment and had no significant effect on other treatments. For each 1 mg/L decrease in phosphate concentration there were 1.08 (1.00-1.18; 95% C.I.; *p*-value = 0.06) times as many adult males. Initial phosphate concentrations had no significant effect on the other treatments.

Weight, time to pupation, and sex correlation

Weights of adult females ranged from 0.59 g to 0.1g with an average of 0.20 ± 0.07 g and were 0.06 ± 0.05 g higher than those of adult males (*p*-value <0.001). Time to pupation varied among different vegetation inputs (Figure 4.3). Turfgrass and sweetgum treatments showed a significantly shorter time to pupation, averaging 16.6 ± 0.3 and 15.3 ± 1.3 days, respectively, in comparison to water oak and loblolly pine treatments where the average time to pupation was 39.4 ± 1.1 and 30.0 ± 0.9 days, respectively. Shorter pupation times for sweetgum and grass could be due to high DOC concentrations, which provide more energy for the larvae while the growth is slower in the other treatments due to lack of food. Moreover, time to pupation varied with the sex of the mosquitoes and number of dead pupae. The longer the time to pupation, the fewer adult males developed ($r^2 = 0.73$) and the percentage of dead pupae increased ($r^2 = 0.86$, Figure 4.4). The percentage of female mosquitoes initially increased over time but declined after day 17 ($r^2 = 0.89$). The longer time to pupation due to lack of food increased the ratio of adult females suggesting adult females may be more tolerant to a shortage of food. For a brief interval, water oak, and loblolly pine treatments with significantly higher times to pupation developed a higher number of adult females than adult males (Figure 4.2).

Discussion

The sweetgum treatment had the highest level of DOC among all treatments, despite the high lignin and lignin: N ratios. It has been shown that sweetgum leaves have more than fifty percent of phenolic compounds (Majidzadeh et al., 2015). Phenolic and humic substances are not a good source of food for microorganisms (Kaufman et al., 2002), suggesting that despite high levels of DOC in the sweetgum treatment, the phenolic portion of it is not usable for microorganisms and, as a result, for *Culex* larvae. However, even at very low levels of DOC in the other treatments, pupae did not die within the first week, and many lived for more than forty days. The death of larvae in the first week in sweetgum treatments suggests the presence of chemicals that prevent the growth of *Culex* larva. Some plant-born molecules have been documented to effectively prevent the growth of *Aedes, Ochlerotatus, Anophele*s and *Culex* larvae (Benelli et al., 2015; Benelli and Mehlhorn, 2016).

Turfgrass may have higher amounts of soluble organic matter in comparison to abscised leaves in both the laboratory and natural conditions. The solubility of organic matter depends on vegetation type, the degree of decomposition, and fluctuation in temperature (Harris and Safford, 1996). Turfgrass lawns in urban areas may be fertilized and irrigated while soil with hardwoods is usually covered with dead, decomposed leaf litter. Moreover, the fluctuation of temperature in the forest floor is less than that of urban grassed areas due to the moderating effect of tree cover (Harris and Safford, 1996, p. 29). As a result of high amounts of soluble organic matter, the fresh turfgrass treatment developed significantly higher numbers of pupae in comparison to the abscised leaf treatments. The number of pupae was positively correlated with DOC concentrations and negatively correlated with DP concentrations. It has been shown that higher phosphate concentrations can increase the time to pupation (Noori et al. 2015), which will result in higher numbers of dead pupae (Figure 4.4).

The number of female and male adults were positively correlated with DOC concentrations as expected, but only the number of adult females was positively correlated with DON concentrations. Since DON increases the algal development in the water (Berman, Tom and Chava, Sara, 1999) it can provide the additional source of food required for females to develop.

Initial nitrate concentrations were positively correlated with the number of pupae, female and male adults only in the turfgrass and sweetgum treatments which did not lack a carbon source. In other words, elevated pollutants such as nitrate and phosphate can increase the number of *Culex* pupae and adults but only in the presence of an adequate source of carbon.

Moreover, in the turfgrass and sweetgum treatment, the time to pupation was significantly shorter probably due to high DOC concentrations. Shorter time to pupation would increase the survival rate of the mosquitoes in natural environments. The longer time to pupation endangers the larvae and pupae through water evaporation, infections, and other aquatics that can use them as a food source (Maleknejad et al., 2014).

These results provide some insights into the *Culex* life cycle in container habitats with different sources of organic matter and nutrients. Urban grass clippings may produce a suitable habitat for urban mosquitoes and translate to a significantly higher survival rate in comparison to forested conditions. This is primarily due to the different chemistries for turfgrass vs. abscised tree leaves. Most of the forested areas in this region typically exhibit forest floors composed of abscised leaves while the turfgrasses in a residential area provide fresh lawnmower clippings most of the year. Water pooling in urban lawns may form due to the microsite variation (i.e. depressions in the urban yard) and provide habitat for mosquitoes. Additionally, grass clippings may be transported by water or the wind into pools, bird baths, and containers thereby providing additional nutrients and a carbon source which could be used by mosquitoes.

In this experiment, the effect of nutrients on the mosquito life cycle was highly correlated with vegetation type. Further studies are required to understand how various species affect the microorganism community in containers. A deeper insight into the effects of vegetation on water quality including phenolic, humic, and fluvic compounds and how they affect mosquito development is of great importance especially in light of the diseases these vectors transmit to humans.

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Tables and graphs

Table 4.1. The experimental design was factorial with five types of vegetation and five levels of nutrients with three replicates.

Vegetation	Nutrient levels				
Input					
no vegetation	No nutrients added	NO_3^- = 3 mg/L	NO_3^- = 12 mg/L	PO_4^{3-} = 1 mg/L	PO_4^{3-} = 6 mg/L
sweetgum	No nutrients added	NO_3^- = 3 mg/L	NO_3^- = 12 mg/L	PO_4^{3-} = 1 mg/L	PO_4^{3-} = 6 mg/L
water oak	No nutrients added	NO_3^- = 3 mg/L	NO_3^- = 12 mg/L	PO_4^{3-} = 1 mg/L	PO_4^{3-} = 6 mg/L
loblolly pine	No nutrients added	NO_3^- = 3 mg/L	NO_3^- = 12 mg/L	PO_4^{3-} = 1 mg/L	PO_4^{3-} = 6 mg/L
turfgrass	No nutrients added	NO_3^- = 3 mg/L	NO_3^- = 12 mg/L	PO_4^{3-} = 1 mg/L	PO_4^{3-} = 6 mg/L

Table 4.2. Carbon (n = 2), nitrogen (n = 2) and lignin (n = 1) concentrations in turfgrass and abscised leaves used in the mosquito microcosm experiment. Column means ± standard are significantly different based on Tukey's honest significant difference (HSD) tests at α = 0.05

Vegetation input	Carbon (g Kg^{-1})	Nitrogen (g Kg^{-1})	C: N	Lignin (g Kg^{-1})
sweetgum	45.69 ± 0.11c	0.59 ± 0.031b	76.55 ± 4.09 c	23.65
water oak	47.07 ± 0.024 b	0.72 ± 0.04 b	65.85 ± 3.71 b	29.79
loblolly pine	50.58 ± 0.24 a	0.34 ± 0.005 c	148.56 ± 1.69 a	31.11
turfgrass	42.85 ± 0.12 d	1.37 ± 0.033 a	31.30 ± 0.84 d	3.73

Table 4.3. Results of a generalized mixed model for parameters related to number of pupae, adult female and male mosquitoes without sweetgum treatments. Estimates (Rate ratios) are calculated using the link function of $e^{(\text{coefficient})}$. Significant parameters depicted in bold (*p*-value < 0.1).

Mosquito stage		Rate ratio	95% Confidence Level		*p*-value
Pupae	(Intercept)	0.40	0.13	1.28	0.120
	DOC	1.06	1.02	1.10	**0.001**
	SUVA	1.01	0.95	1.07	0.790
	DON	0.80	0.23	2.75	0.720
	DP	0.95	0.88	1.02	**0.081**
Female adult	(Intercept)	0.01	0.00	0.10	0.000
	DOC	1.07	1.00	1.15	**0.046**
	SUVA	1.05	0.93	1.18	0.410
	DON	7.42	0.67	81.53	**0.094**
	DP	0.93	0.81	1.06	0.260
Male adult	(Intercept)	0.01	0.00	0.13	0.000
	DOC	1.09	1.03	1.16	**0.003**
	SUVA	1.00	0.90	1.10	0.942
	DON	0.21	0.03	1.64	0.130
	DP	0.88	0.77	1.00	**0.048**

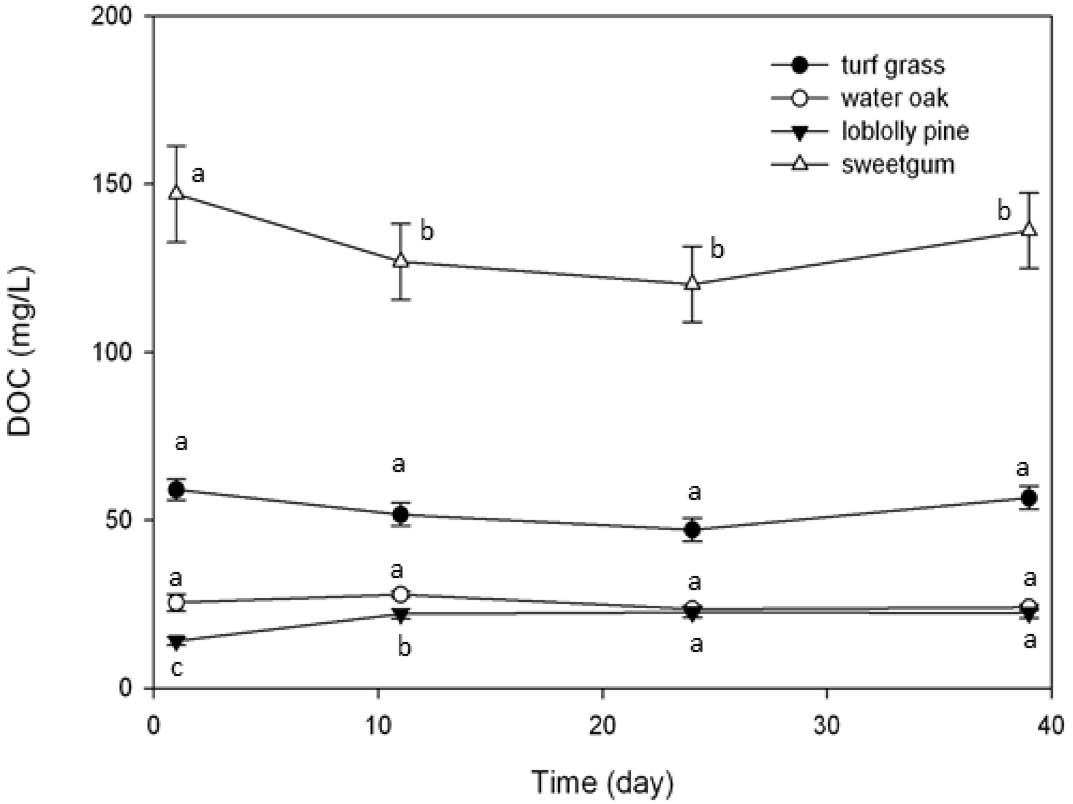

Figure 4.1. Variation in DOC over time (40 days, n = 3 at each measurement). Means ± standard error followed by the same letter are not significantly different based on Tukey’s honest significant difference (HSD) tests at α = 0.05.

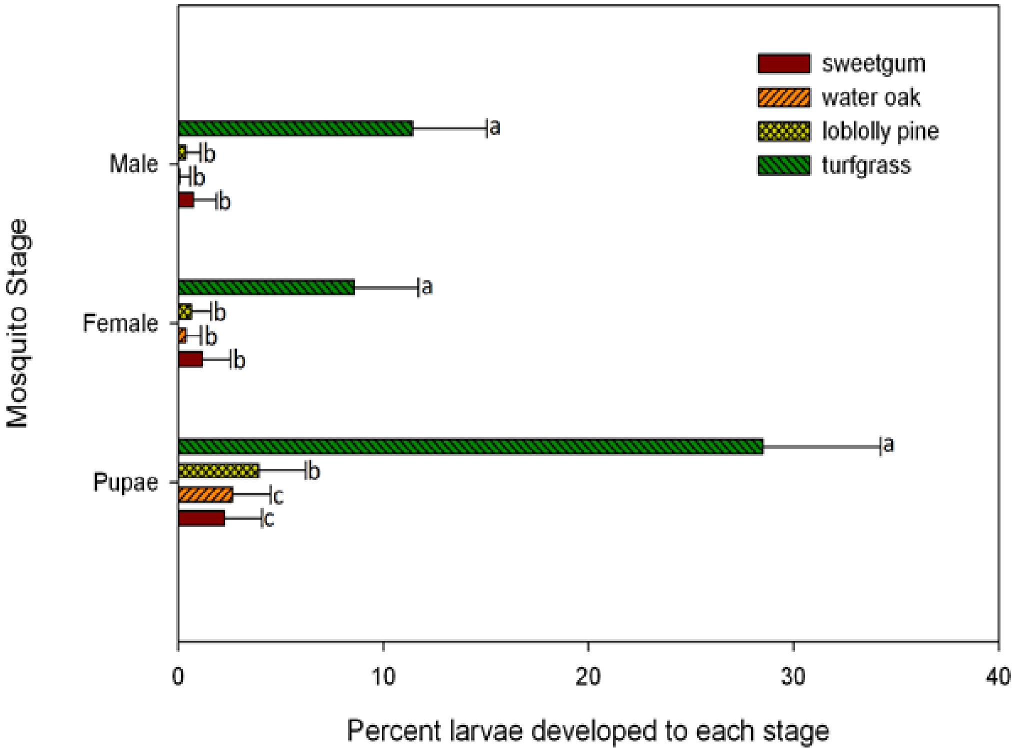

Figure 4.2. Percent larvae developed to pupae, female, and male adults. Means followed by the same letter are not significantly different based on Tukey's honest significant difference (HSD) tests at α = 0.05.

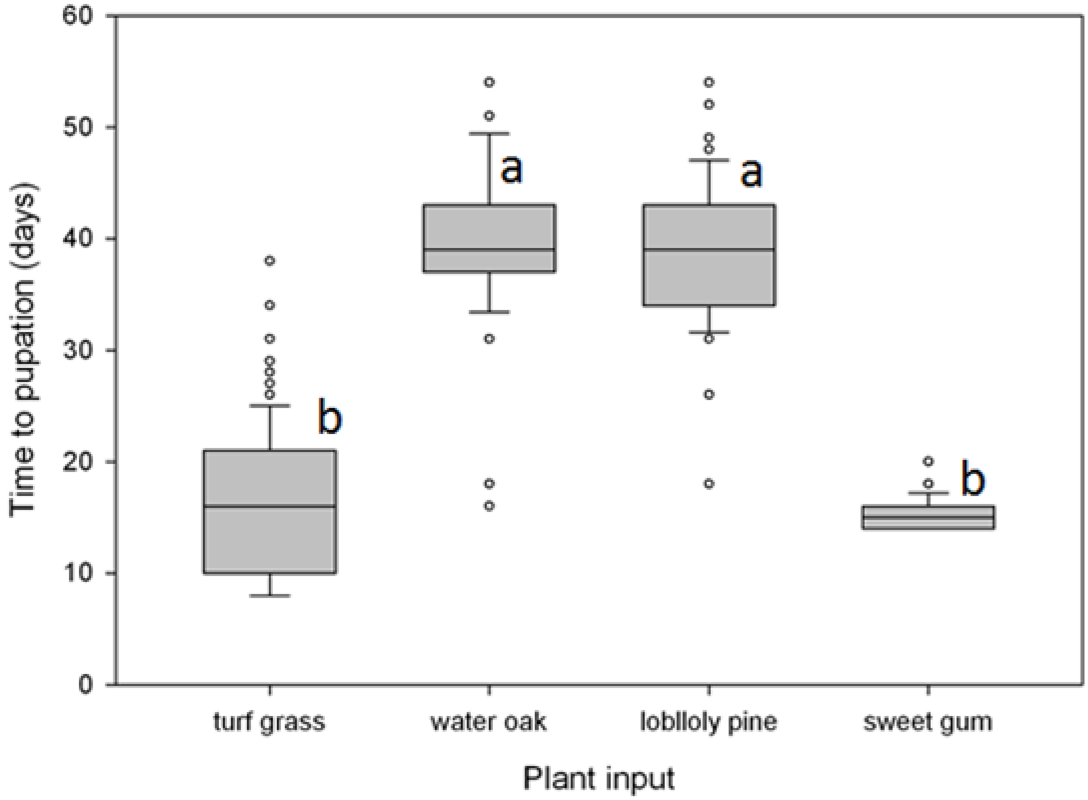

Figure 4.3. Comparison of time to pupation among different vegetation treatments. Means ± standard error followed by the same letter are not significantly different based on Tukey's honest significant difference (HSD) tests at α = 0.05.

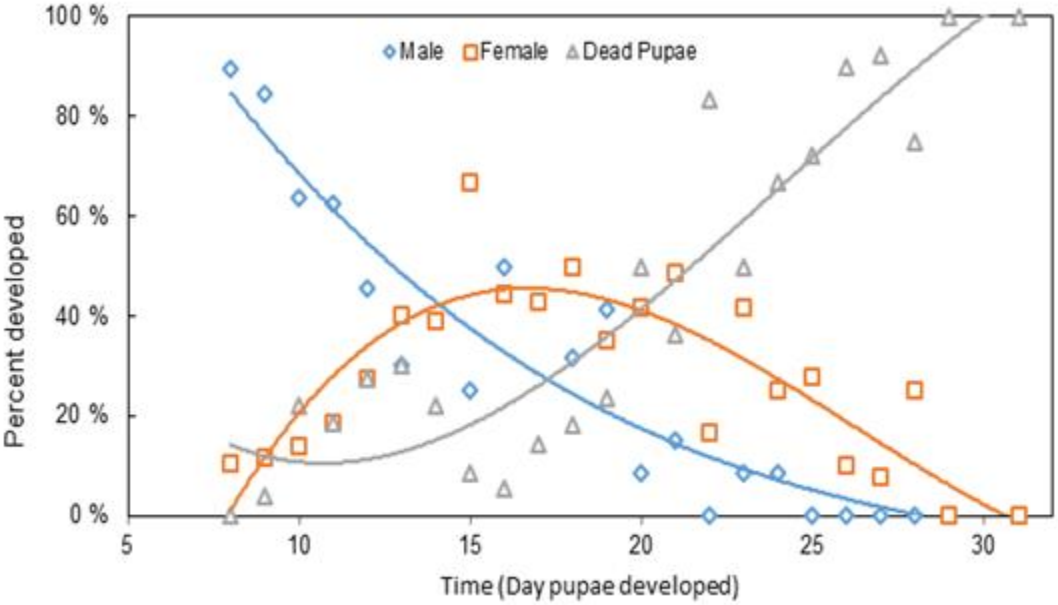

Figure 4.4. The percent of male, female, and dead pupae developed during the first 30 days of experiment averaged across all treatments.

Appendix

Results of a generalized mixed model for parameters related to number of pupae, adult female and male mosquitoes with sweetgum treatments included. Estimates (Rate ratios) are calculated using the link function of $e^{(\text{coefficient})}$. Significant parameters depicted in bold (p-value < 0.1).

Mosquito stage		Rate Ratio	95% Confidence Level		*p*-value
Pupae	(Intercept)	0.71	0.27	1.87	0.48
	DOC	1	0.98	1.03	0.79
	SUVA	1.06	1	1.12	**0.05**
	TDN	2.21	0.72	6.79	0.15
	DP	0.91	0.85	0.97	**0.005**
Female adults	(Intercept)	0.05	0.01	0.39	0.003
	DOC	0.97	0.92	1.02	0.172
	SUVA	1.11	1	1.23	**0.054**
	DON	1.36	1.12	1.66	**0.002**
	DP	1.02	0.91	1.14	0.782
Male adults	(Intercept)	0.03	0	0.4	0.006
	DOC	0.98	0.92	1.03	0.39
	SUVA	1.14	1.03	1.26	**0.01**
	DON	0.29	0.05	1.81	0.18
	DP	0.95	0.84	1.06	0.329

Chapter 5: Conclusions and Summary

This research had two primary objectives: to determine effects of urbanization on A) soil carbon and nitrogen dynamics beneath impervious surfaces, and B) reproduction of urban mosquito populations as a global threat to public health (West Nile virus). In both cases, the land use and land cover change were the drivers of the negative impacts on the ecosystem. A rapid expansion of impervious surfaces and urban lawns affected soil carbon storage and sequestration rates. However, changes in soil carbon and nitrogen dynamics are not the only consequences of urbanization. Urbanization may increase the survival rates of the mosquitoes by creating suitable breeding habitats.

To address the first objective and understand the soil carbon and nitrogen dynamics beneath impervious surfaces a field controlled study, and a field chronosequence study was conducted. Both studies revealed a significant decrease in soil carbon beneath the impervious surfaces. In the controlled study, soil carbon content decreased by 48.39 % (± 16.29 %) and 56.99 % (± 8.59 %) beneath the concrete and homes respectively compared to reference plots in top 10 cm. Temporally, soil carbon content decreased by 0.04 kg m^{-2} and 0.02 kg m^{-2} per month beneath the concrete plots at depth 0-10, and 10-20 cm respectively. Top soil removal was the primary cause of the decrease in soil carbon and nitrogen content beneath impervious surfaces. Moreover, the decrease in carbon sequestration rates or decrease in carbon content over time can increase the difference between open soils and soil beneath impervious surfaces as observed in the field chronosequence study (second study). In the second study, the soil beneath the homes ranging from 11 to 114 years in age was sampled and compared to adjoining urban lawns. The differences in C content beneath the homes and adjoining lawns increased over time and reached its maximum (more than 90 %) at age 53 years. Following that, it decreased between ages 53 and 114 years. The results from both studies are suggesting that soil beneath the homes built on crawl spaces is not as sealed as the soil beneath the slabs. This was further confirmed by higher volumetric water content beneath the homes at depth 10-20 cm compared to concrete plots in the controlled study. Homes had higher carbon content at depth 10-20 cm than that in concrete plots. Similar carbon content beneath the home and concrete plots at top 10 cm is probably due to high carbon loss beneath the homes from oxidation since the top soil is not protected by vegetation or concrete. This pattern was further confirmed by higher C_{mic}: C ratio beneath the homes compared to other treatments at depth 0-10 cm. Higher C_{mic}: C ratio beneath the homes compared to urban lawns also observed in the field study. Both the controlled and filled study revealed that soil carbon dynamics beneath different impervious surfaces (homes built on crawl space vs. concrete in this study) are not the same, and parameters such as the type of structure, topsoil removal depth, age, and climate would affect these dynamics.

A significant decrease in soil total nitrogen was observed in both studies. The soil total nitrogen decreased by 42.43 % (± 16.90 %) and 53 % (± 13.40 %) beneath the concrete and homes at layer one in the controlled study. The decrease in soil total nitrogen in the field study was higher 65.77 % (± 5.65 %) which is probably due to the higher age of those structures and reduced sequestration rates. This was expected because negative, or zero net ammonium production rates were observed beneath impervious surfaces in the controlled study which result in a decrease in soil total nitrogen over time.

The second objective of this research was addressed through a laboratory microcosm study. One means through which urbanization may affect mosquito habitat is through changes in vegetation. Water quality may be affected by the surrounding vegetation which serves as a source of organic matter for mosquito larvae. The effect of vegetation on water quality may be magnified when native forests are replaced with lawns due to urbanization. The forest floor, naturally covered with dead and decomposed leaves, is replaced by highly managed lawns (i.e. fertilized, weed removal, clipped regularly). We found that the number of pupae and adult males was positively correlated with dissolved organic carbon (DOC) and negatively correlated with dissolved phosphate (DP). The number of adult females was similarly affected by DOC and DP while dissolved organic nitrogen (DON) was also a key factor for their development. Abscised leaf treatments had significantly lower survival rates than grass treatments with maximum pupae development of 3.93 ± 2.29 % in the pine treatment and 1.92 ± 1.72 % adult development in the sweetgum treatment. Grass treatments developed 28.50 ± 5.75 % and 22.50 ± 4.79 % pupae and adult mosquitoes respectively. This study highlights the importance of variation in litter quality in the ponded water on survival rates of the *Culex* mosquitoes. Urban grass clippings may produce a suitable habitat for urban mosquitoes and translate to a significantly higher survival rate in comparison to forested conditions. This is primarily due to the different chemistries for turfgrass vs. abscised tree leaves. Most of the forested areas in this region typically exhibit forest floors composed of abscised leaves while the turfgrasses in residential areas provide fresh lawnmower clippings most of the year. Water pooling in urban lawns may form due to the microsite variation (i.e. depressions in the urban yard) and provide habitat for mosquitoes. Additionally, grass clippings may be transported by water or the wind into pools, bird baths, and containers thereby providing additional nutrients and a carbon source which could be used by mosquitoes.