The Effect of Rootstock Selection and Carbon-based Fertility Amendments on Apple Orchard Productivity and Soil Community Ecology

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Abstract

In apple (*Malus ×domestica* Borkh.) orchards, rootstock genotype, and soil fertility management practices impact soil fertility, plant associated soil microbial communities, and orchard productivity. Apple growers select rootstocks to confer beneficial traits, including size control, precocity, and pest and disease resistance. Rootstock genotype may also influence microbial communities, resulting in changes that affect tree health and productivity. Many apple growers apply synthetic nitrogen fertilizers to improve fruit yield and quality. In excess of tree requirements, nitrogen fertilizers may reduce crop yield and quality, as well as contribute to water pollution. The addition of carbon-based amendments, such as yardwaste, chicken litter composts, and biochar, may reduce nitrogen and water loss, while improving soil structure and mineral nutrient availability. Orchard and pot-in-pot experiments were designed to study the following objectives: 1) determine the effects of integrated carbon-based fertilizer amendments on tree growth, productivity, and orchard soil fertility, 2) assess the effects of biochar on tree growth, leaf mineral nutrition, soil physiochemistry, and microbial community structure and activity, and 3) understand how rootstocks and fertilizers alter soil microbial communities. Applications of composts, integrated compost-calcium nitrate fertilizers, and biochar increased soil carbon, organic matter, cation exchange capacity and microbial respiration. In the orchard study, nitrogen fertilizer application did not increase tree growth, fruit quality, or leaf nitrogen concentration. Biochar applied at high rates with nitrogen fertigation increased tree growth and leaf nitrogen concentration similar to nitrogen fertigation. In the pot-in-pot compost study,

chicken litter compost increased tree growth, and integrated compost-calcium nitrate fertilizer applications increased leaf N concentration. Analysis of the microbial community structure of bulk soil samples from the biochar and compost pot-in-pot experiments determined that the community structure was similar for all treatments during the three-year study. Metagenomic sequencing of the rhizosphere bacterial community indicated that compost applications altered community diversity and evenness, and that compost treatments were more similar to each other than to the calcium nitrate treatment. Data from my dissertation research suggests that compost can be used to increase orchard soil fertility, tree growth, and leaf nutrition, and that compost applications increase soil microbial community diversity and activity.

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Abstract

Rootstock genotype and soil fertility management practices in apple (*Malus ×domestica* Borkh.) orchards impact soil health and nutrient status, plant associated soil microbial communities, and tree growth and fruit yield. Growers select specific apple rootstocks for use in their orchards to confer beneficial traits, including size control, earlier fruit production, increased fruit yield, and pest and disease resistance. Many apple growers also apply synthetic nitrogen fertilizers to improve fruit yield and quality. However, in excess of tree requirements, nitrogen fertilizers may reduce crop yield and quality, as well as contribute to environmental pollution. The addition of carbon-based amendments, such as compost and biochar, may reduce nitrogen and water loss, improve soil structure, and make certain mineral nutrients more available to plants and microorganisms. The use of integrated fertilizers, which are carbon-based amendments applied in conjunction with synthetic nitrogen fertilizers, may also enhance edaphic properties while providing plants with adequate nitrogen at times of greater demand. Orchard and pot studies were designed to: 1) determine the effects of carbon-based, and integrated carbon-based fertilizer amendments on tree growth, fruit yield and quality, and orchard soil fertility, 2) determine the effects of rootstocks and fertilizers on soil microbial community composition and activity, and 3) assess the effects of biochar on tree growth, leaf mineral nutrition, soil fertility, and microbial community structure and activity. In this study, applications of composts, integrated fertilizers, and biochar increased soil fertility measures, such as soil

carbon, organic matter, cation exchange capacity and microbial respiration, in fine textured orchard soils. Applying nitrogen fertilizer did not increase tree growth, fruit quality, or leaf nitrogen concentration in the orchard study, however, in the pot study, chicken litter compost increased tree growth, and integrated compost-calcium nitrate fertilizer applications increased leaf N concentration. Biochar applied with synthetic nitrogen fertilizer increased tree growth and leaf nitrogen concentration similar to nitrogen fertilizer alone. Analysis of the microbial community structure of bulk soil samples from the biochar and compost pot experiments determined that the community structure was similar for all treatments during the three-year study. Metagenomic sequencing of the rhizosphere bacterial community indicated that compost applications altered community diversity and evenness, and that compost treatments were more similar to each other than to the synthetic nitrogen treatment. Data from my dissertation research suggests that compost can be used to increase orchard soil fertility, tree growth, and leaf nutrition, and that compost applications increase soil microbial community diversity and activity.

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1.1 Introduction

As Mid-Atlantic apple (*Malus ×domestica* Borkh.) growers increases their plantings of high-density orchards, it is necessary to understand the effects of apple rootstock genotype, soil fertility management practices such as fertilizer applications and soil carbon additions, and their interactions, on soil fertility and nutrient status, plant associated soil microbial community structure and functions, and orchard productivity. The profitability of high-density orchard systems depends upon obtaining high fruit yields as soon as possible after planting. In order to achieve earlier fruiting, trees planted on dwarfing or semi-dwarfing rootstocks must have sufficient growth, which is traditionally achieved through the application of synthetic nitrogen fertilizers. Prior to this dissertation, there were few studies describing the effects of fertigation, composts, and biochar on the growth and productivity, soil fertility, and soil microbial communities in newly planted high-density orchards in the Mid-Atlantic. Additionally, few have studied how synthetic nitrogen (N), compost, and integrated compost-synthetic nitrogen fertilizers, may impact different apple rootstock genotypes.

Currently, apple growers have access to a wide variety of rootstocks and select rootstocks to confer specific beneficial traits, including ease of propagation, mature tree size, precocity, vigor, cold tolerance, pest and disease resistance, yield efficiency, and fruit quality (Fallahi et al., 2002). There is increased interest in planting rootstocks recently released from the joint Cornell-USDA-ARS breeding program because these rootstocks have greater precocity, yield efficiency, and tolerance to cold, fire blight (*Erwinia amylovora*) and replant disease than the more commonly planted Malling and Merton-Malling rootstocks (Russo et al., 2007). Additionally, rootstock below-ground traits, such as anchorage, nutrient and water uptake, and rhizosphere

plant-microbe interactions, vary substantially. Fazio et al. (2013) found that the rootstock 'Geneva 935' from the Cornell-USDA-ARS had greater leaf K and P concentrations than the more frequently planted 'Budagovsky 9', and that these nutrient use efficiency differences are related to rootstock genotype. Geneva rootstocks have microbial communities that are more similar to each other than to other rootstocks with dissimilar genotypes, but it is not known how these communities interact with nutrient sources with different carbon (C):N ratios (Rumberger et al., 2004; St. Laurent et al., 2010). More data is needed to understand the effects of different fertilizer applications, such as synthetic N and C-based amendments, on rootstock mineral nutrition.

Apple trees have low N recovery efficiencies and may recover only 20% of N applied each season (Neilsen and Neilsen, 2002). Applying N in excess of crop demand can increase the vegetative growth and self-shading of the tree which may decrease fruit quality, color, and yield in mature fruit trees, and lead to increased incidence of fire blight. In addition, N applied in the form of nitrate, such as calcium nitrate, is highly soluble and may be leached from the soil following precipitation or irrigation (McKague et al., 2005). Research conducted by Merwin et al. (1996), in a finely textured New York soil, indicates that significant N leaching and runoff can occur in apple orchards. Long term leaching of N and P runoff into the Chesapeake Bay Watershed has resulted in "dead zones" in the Chesapeake Bay. Since the 1980s, the United States Environmental Protection Agency (EPA) has been implementing strategies to decrease pollution in the Chesapeake Bay (EPA, 2010). The Total Maximum Daily Load outlined for the Chesapeake Bay Watershed includes reductions in N and P, as well as sediment loss from agricultural sources, which has a tremendous impact on crop producers that use fertilizers and livestock farmers that produce large quantities of manure. Farmers are now tasked with

developing nutrient management plans to reduce N and P loss. Currently, farmers that produce row crop, such as maize (*Zea mays*) and soy bean (*Glycine max*), and animals are most affected by these policies; however, in the future, tree fruit producers may also be affected. In addition, the cost of synthetic N fertilizers has increased over the past several years due to the rising cost of fossil fuels. Given these challenges, it is important to develop sustainable fertilizer application approaches that enhance soil fertility and fruit tree productivity in Virginia's apple orchards.

Additionally, synthetic and C-based fertilizer applications may alter the soil microbial community. Synthetic N fertilizers have been shown to reduce soil respiration, microbial biomass, and enzyme activity, and increase microbial community diversity (Ramirez et al., 2012; Yeoh et al., 2015; Zhu et al., 2016). Changes in soil physiochemistry, such as pH, and C and N cycling, related to carbon or fertilizer additions have been shown to alter microbial community composition (Grossman et al., 2010; Winston et al. 2014). In apple orchards, compost applications have been associated with increased microbial activity and shifts in rhizosphere microbial community composition; however, these shifts were not related to decreased incidences of apple replant disease (Rumberger et al. 2004; Yao et al., 2006). Additionally, C additions typically increase microbial respiration and biomass; however, these increases are usually lower in more fertile soil with greater OM. Determining the effects of synthetic and compost fertilizers on soil microbial community composition and activity in fertile orchard soils will allow researchers to consider the effect of these additions on soil fertility, and make more informed fertilizer recommendations to apple growers.

Currently, Virginia Cooperative Extension recommends that apple growers apply synthetic N fertilizers, such as calcium nitrate [$Ca(NO_3)_2$], ammonium nitrate (NH_4NO_3), and urea [$CO(NH_2)_2$] (Pfeiffer et al., 2015). There are two widely used approaches that are utilized to

provide trees with N from ground applied synthetic fertilizers, and reduce potential N leaching from synthetic fertilizers: applying smaller amounts of N more frequently and applying N during maximum crop demand. These techniques can be implemented by applying split applications of synthetic N fertilizer during times of high nutrient demand, such as at bloom and mid-season, or through fertigation, a method which dispenses small amounts of solubilized fertilizers through irrigation lines directly to the root zone, and can be scheduled to meet plant N demand. Additionally, other methods, such as the application of compost, which has been shown to increase soil organic matter (OM), cation exchange capacity (CEC), mineral nutrition, plant tissue N, microbial biomass, and microbial activity, has also been reported to reduce N leaching in apple orchards planted in coarse soil and increase fruit yield in peach (Yao et al., 2006; Kramer et al., 2006; Baldi et al., 2010a; Baldi et al., 2010b; Strauss et al., 2014; Rumberger et al., 2014). Sas-Paszt et al. (2014) showed that compost applications in orchards planted in coarse soils did not increase tree growth or apple yield. Applications of integrated fertilizers, such as the application composts in conjunction with synthetic N fertilizers, in a coarse soil reduced N leaching, and increased soil OM, microbial biomass, and microbial enzymatic activity, but did not increase apple tree growth or yield (Kramer et al., 2006). Most fertilizer research in apple orchards has been conducted in arid environments, with coarsely textured, less fertile soil. Results from fertilizer studies conducted in the Shenandoah Valley of Virginia, which has a humid subtropical climate, and finely textured fertile soils, may be highly dissimilar from these studies.

Similarly, there is increasing interest in applying biochar, the solid product of high temperature anaerobic biomass pyrolysis, as an agricultural soil amendment to soil to increase soil fertility, crop productivity, water holding capacity, C sequestration, and reduce N leaching.

Biochar feedstock, maximum heat temperature, pyrolysis heating rate, treatment length at maximum temperature, particle size, and application rate, in addition to initial soil physiochemisty, determines the effects of biochar plant growth and soil quality (Rajkovich et al., 2012; Revell et al., 2012; Sun et al., 2012). Biochar, applied at high application rates, may reduce crop yield due to increase pH and soluble salts (SS), and may have not affect crop yield or biomass when applied to more fertile clayey soils (Revell et al., 2012; Keith et al., 2015). In other studies, conducted in fertile soils, biochar increased plant biomass, mineral nutrition, and cation exchange capacity (Rondon et al., 2007; Chan et al., 2007; Revell et al., 2012; Scharenbroch et al., 2013). In apple orchards planted in coarse soils, applications of 10 and 50 Mg ha^{-1} of biochar did not increase tree yield or leaf mineral nutrition, but did lead to increases in tree growth three years after application (Ventura et al., 2013; Eyles et al., 2015). Currently, most studies focus on using biochar to improve depleted or sandy soils, and there are few studies dedicated to understanding the effects of biochar on perennial crops planted in fertile soils. Thus, it is essential to determine the effects of different biochar types and application rates on apple tree growth and the physiochemistry of fertile, finely textured soils.

In addition, biochar can alter soil microbial community diversity, biomass C and N, and microbial activity, such as respiration and N mineralization, through sorption of compounds that alter microbial activity, or effect plant-microbe interactions through the alteration of pH and mineral nutrient availability or the creation of new microbial habitats (Grossman et al., 2010; Dempster et al., 2012; Spokas et al., 2012; Luo et al., 2013; Masiello et al., 2013; Xu et al., 2016). Changes in microbial community structure following biochar addition have been associated with soil bacterial species known to be plant growth promoters and biocontrol agents, which may lead to an increased tolerance of apple replant disease (Graber et al., 2010; Atucha

and Litus, 2015). However, in studies conducted in more fertile soil, edaphic factors, such as soil texture, C content, and pH, had a greater effect on bacterial community structure then biochar application (Anderson et al., 2011; Quilliam et al., 2012). In some studies, biochar applications increase microbial respiration while in other studies microbial respiration is unaffected by the addition (Grossman et al., 2010; Zavalloni et al., 2011; Ventura et al., 2013). Similarly, biochar applications increased microbial biomass C in some studies, but lead to a reduction of microbial biomass in other studies (Major et al., 2010; Luo et al., 2013; Xu et al., 2016; Steinbeiss et al., 2009; Dempster et al., 2012; Chen et al., 2013; Keith et al., 2015). Biochar applications tend to reduce potentially mineralizable (PMN), and the reduction of PMN is rate dependent; however, reduction is not associated with shifts in microbial populations associated with N cycling (Dempster et al., 2012; Prayogo et al., 2014). Due to the highly variable effects of biochar applications on microbial community structure and function, more research is needed to determine how biochar applications in fertile soil affects microbial community structure and function as related to plant growth and soil fertility.

In order to assess the effects of carbon-based soil amendment, such as compost and biochar, and rootstock genotype on apple tree growth and productivity, and soil fertility with an emphasis on microbial community composition and activity four experiments were devised. The soil amendments evaluated include a commonly used synthetic fertilizer, calcium nitrate [$Ca(NO_3)_2$], locally sourced chicken litter and yardwaste composts, and integrated compost-$Ca(NO_3)_2$ fertilizers, and CQuest biochar. The rootstocks studied were the commonly planted 'Budagovsky 9', 'Malling 9' and 'M.26', and the recently released 'Geneva 41', 'G.214', and 'G.935'.

The objectives of first study, described in Chapter 2, were to evaluate the effects of fertilizers, synthetic N fertilizer, compost and integrated compost-calcium nitrate, on tree growth, fruit yield and quality, soil fertility, and microbial activity in newly planted orchards. I hypothesized that integrated fertilization with applications of composts and calcium nitrate would improve apple tree growth and soil quality after three years, compared to unfertilized trees and compost amended trees because integrated fertilizers provide trees with readily available N when demand is high, and compost provides trees with other essential mineral nutrients not found in synthetic N fertilizers. A field-based study was designed to evaluate the validity of this hypothesis. Fertilizer treatments, a split application of calcium nitrate [$Ca(NO_3)_2$], chicken litter compost, yardwaste compost, a combination of chicken litter compost and $Ca(NO_3)_2$ with equal amounts of N from each fertilizer, a combination of yardwaste compost and $Ca(NO_3)_2$ with equal amounts of N from each fertilizer, and fertigation, were applied at a rate of 67 kg N ha^{-1} to recently planted 'Red Delicious cv Schlect'/'Malling 26' for three years. Tree growth was assessed as trunk cross sectional area (TCSA) at planting, and again in subsequent years. The effects of fertilizer on return bloom was measured as bloom density in 2014 and 2015. Leaf mineral nutrition was measured in 2013, 2014, and 2015. In 2015, fruit were harvested for the first time, and yield efficiency, fruit diameter, weight, firmness, starch, peel color, and soluble solid concentration were assessed. To evaluate soil fertility, soil pH, mineral nutrients, OM, CEC, total C, total N, and C:N ratio were determined. Soil microbial activity was evaluated as microbial respiration, PMN, and microbial biomass C.

The objectives of Chapter 3 were to evaluate the effects of high biochar application rate on apple tree growth and mineral nutrition, soil fertility, and microbial community structure and function. I hypothesized that biochar would increase tree growth and leaf mineral nutrition,

increase soil CEC and total carbon, and stimulate microbial community activity due to its chemical and physical properties. To evaluate the validity of this hypothesis, a pot-in-pot experiment was established. The pot-in-pot system allowed soil physiochemical characteristics to be changed more rapidly by the biochar amendment, prevented migration of the biochar, and allowed trees to be more easily harvested for biomass analysis. CQuest wood-based biochar was applied at 0 Mg biochar ha-1, 50 Mg biochar ha^{-1}, 100 Mg biochar ha^{-1}, 200 Mg biochar ha^{-1} to 'Brookfield Gala'/'M.9'. Trees were fertigated with $Ca(NO_3)_2$ at a rate of 40 kg N ha^{-1}. An un-fertilized container without biochar served as the control. Tree growth was assessed as TCSA at planting, and again in subsequent years, and tree biomass was determined by destructive harvest in 2015. Leaf mineral nutrition was assessed in 2014 and 2015. Soil fertility measurements, including soil pH, mineral nutrients, OM, CEC, total C, total N, and C:N ratio were determined yearly. Microbial respiration, PMN, and microbial biomass C and N assays were used to determine microbial community activity. Additionally, microbial community structure was determined a week after biochar application, and again in Aug. 2013, 2014, and 2015 using terminal-restriction fragment length polymorphisms (T-RFLPs).

In a study similar to that conducted in Chapter 2, in Chapter 4, the effects of synthetic N fertilizer fertigation, compost and integrated compost-calcium nitrate fertilizers applications on commonly used and newly released rootstocks on tree growth and mineral nutrition, soil fertility, and microbial community structure and function were evaluated. Unlike the field study conducted in chapter 2, the study designed in chapter 4 was completed in a pot-in-pot system to alter soil physiochemistry and microbial communities more rapidly than in the field study. I hypothesized that integrated compost-calcium nitrate fertilizer applications will increase tree growth more than the unfertilized control or compost treatments for each rootstock, and compost

applications will increase soil fertility measures, such as OM, CEC, and mineral nutrients, and soil microbial activity, and alter the soil microbial community composition. To determine tree growth, TCSA was measured at planting, and in all subsequent years. In 2015, total tree biomass was determined by destructive harvest. Leaf mineral nutrition was assessed in 2014 and 2015. Soil physiochemical properties including, soil pH, mineral nutrients, OM, CEC, total C, total N, and C:N ratio were measured yearly. Microbial community activity was measured using microbial respiration, PMN, and microbial biomass C and N assays. Microbial community structure was determined using T-RFLPs a week after initial fertilizer application, and in Aug. 2013, 2014, and 2015.

The objective of Chapter 5 was to expand the understanding of the effects of fertilizer and rootstock genotype on rhizosphere microbial communities using metagenomics methods. The T-RFLP method utilized in chapter 4 can indicate shifts in microbial communities in bulk soil due to different treatments, such as different fertilizers and rootstocks; however, this method does not allow bacteria or fungi to be identified, or diversity to be analyzed. I hypothesized both rootstock and fertilizer treatments would cause significant changes in structure and diversity of the rhizosphere microbial community because several previous studies conducted on perennial and annual crops demonstrated that both plant genotype and soil physiochemistry, which can be changed by fertilizer amendments, can change microbial community diversity. The v4 region OF 16 s rRNA gene from soil samples collected from the rhizosphere of M.9 and G.41 trees fertilized with chicken litter compost, yardwaste compost, or calcium nitrate, and the unfertilized control were amplified and sequenced using the Caporaso method and the Illumina MiSeq platform. Data were then analyzed using the QIMME pipeline. Probable family and phyla were assigned to operation taxonomic units (OTUs). Microbial community alpha diversity, the

diversity within the samples, was assessed using the Shannon, Simpson, and Chao1 indices, and beta diversity, the diversity among samples, was measured using Bray-Curtis dissimilarity.

The overall goal of my research was to assess the interactive effects of carbon-based soil amendments, such as composts and biochar, and rootstock genotype on apple tree growth and productivity, and soil fertility. Understanding how soil amendments and fertilizers and rootstock genotypes affect these factors will allow researchers and extension educators to make more informed fertilizer and rootstock recommendations to farmers. These recommendations should take into consideration long-term soil fertility and reducing nutrient loss, as well as tree growth, productivity, and fruit quality.

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2. Composts as Fertilizers for Newly Established Orchards in the Mid-Atlantic Region

2.1 Abstract

In the Mid-Atlantic, synthetic nitrogen (N) fertilizers are applied in high-density apple (*Malus ×domestica* Borkh.) orchards to increase tree vegetative growth and achieve earlier fruiting. However, synthetic N applications may lead to N leaching and ground water pollution, thus it is necessary to explore alternative fertilizers. In apple orchards, compost increases soil mineral nutrition, organic matter (OM), cation exchange capacity (CEC), microbial biomass, and microbial activity. Six fertilizer treatments, a split application of calcium nitrate [$Ca(NO_3)_2$], chicken litter compost, yardwaste compost, a combination of chicken litter compost and $Ca(NO_3)_2$ with equal amounts of N from each fertilizer, a combination of yardwaste compost and $Ca(NO_3)_2$ with equal amounts of N from each fertilizer, and fertigation, were applied to newly planted 'Red Delicious cv Schlect'/'Malling 26' for three years. Nitrogen was applied at 67 kg N ha^{-1}. Non-fertilized trees served as the control. Fertilizer applications did not increase tree growth, leaf N concentration, fruit yield, or quality compared to the control. However, compost applications increased leaf P, K, and B concentrations, and increased soil edaphic properties such as, soil total N, total C, and C:N ,OM, and CEC. Results from this study suggest that applying fertilizers to orchards in a finely textured soil does not increase tree growth or fruit quality within the first three years of orchard establishment. Thus, growers may forgo these applications early in the life of the orchard when trees are not N deficient. Additionally, compost applications increased other leaf nutrients, and increased long-term soil fertility by increasing soil OM and CEC.

2.2 Introduction

The profitability of high-density apple (*Malus ×domestica* Borkh.) orchards depends on obtaining high fruit yields as soon as possible after planting. In order to achieve earlier fruiting, trees must have sufficient vegetative growth. In part, this is achieved through the application of synthetic nitrogen (N) fertilizers. However, there is a lack of research based recommendations for fertilizer use in young apple orchards in the Mid-Atlantic. More research-based information regarding appropriate formulation, timing, and application methods is needed. The effects of N fertilizer timing and concentration on vegetative growth, leaf N concentration, and fruit quality in established high density orchards in arid regions has been well documented (Klein et al., 1989; Neilsen and Neilsen, 2002; Dong et al., 2007; Neilsen et al., 2009). But, there have been few studies describing the effects of carbon-based fertilizers, such as compost, on apple tree growth and productivity in newly planted high density orchards in the Mid-Atlantic.

In the Mid-Atlantic, synthetic N fertilizers, such as calcium nitrate [$Ca(NO_3)_2$], ammonium nitrate (NH_4NO_3), and urea [$CO(NH_2)_2$], are routinely ground or foliarly applied in apple orchards. Ground application of synthetic N fertilizers in orchards has been observed to increase N leaching (Merwin et al., 1996; Dong et al., 2005). This is of growing concern in watershed areas where agricultural N loss has become an environmental problem. For example, the Environmental Protection Agency has enacted Total Maximum Daily Load limits on the amount of N that may enter the Chesapeake Bay Watershed (EPA, 2010).

One potential solution to this problem is fertigation, a method which dispenses solubilized fertilizers through irrigation lines. Fertigation allows growers to apply small amounts of fertilizers directly to the root zone during times of high nutrient demand, and reduces N leaching and runoff. Several studies in arid, apple producing regions with coarse soil, such as

Israel and British Columbia, have demonstrated that fertigation can improve fruit quality and yield, leaf N concentration, and improve tree nitrogen use efficiency (Klein et al., 1989; Dong et al., 2005, Neilsen et al., 2009, Neilsen and Neilsen, 2002). In temperate regions with fertile clay soils, such as the Netherlands, fertigation increased yield, flower bud formation, and shoot growth in established apple orchards (Kipp, 1992). Due to the potential environmental and crop growth benefits of fertigation, more apple producers in the Mid-Atlantic have begun to explore this fertilizer application method.

Additionally, carbon-based amendments, such as mulches, living mulches, woodchips, shredded paper, biosolids, and compost may contribute to more sustainable orchard management in the Mid-Atlantic. Carbon-based amendments have been shown to improve long-term soil, increase soil mineral nutrients, organic matter (OM), soil total carbon (C) and N, microbial activity, tree growth and vigor, and leaf mineral nutrient concentration in orchards established on coarse textured soils in arid environments (Forge et al., 2013; Hoagland et al., 2008; Neilsen et al., 2014). In a study of apple orchards planted on coarsely textured soils, compost and integrated compost and synthetic fertilizer additions increased soil OM, microbial biomass C and N, and increased microbial enzymatic activity (Kramer et al., 2006). However, in another study conducted in a similar soil, wood chip mulch and shredded paper mulch did not improve soil OM, and led to lowered leaf N (Granatstein and Mullinix, 2008). In contrast to that study, applications of bark mulch increased soil OM, and microbial biomass C compared to applications of composted chicken manure in an apple orchard planted in a fine textured silt clay loam soil in New York (Peck et al., 2011). Studies in coarse soils have also demonstrated that carbon-based amendments with high C:N ratios can reduce available plant N, resulting in lower leaf N and P concentrations (Neilsen et al., 2007; Granatstein and Mullinix, 2008; Hoagland et

al., 2008; Sas-Passzt et al., 2014). In newly established orchards planted in sandy loam soil, high C:N ratio amendments, like woodchips, have differing effects on trunk cross sectional area (TCSA). In coarse soil, high C:N amendments did not affect TCSA; however, in another study these amendments increased TCSA (Granatstein and Mullinix, 2008; Hoagland et al., 2008; Sas-Passzt et al., 2014). More data is needed to understand how these amendments affect the fertility of finely textured soils.

Applying compost as a fertility amendment in apple orchards increases soil OM, cation exchange capacity (CEC), soil porosity, aggregate stability, mineral nutrients, microbial biomass, microbial enzymatic activity, and reduce nitrate leaching, and erosion, thus improving soil quality for a variety of soil types under numerous climactic conditions (Yao et al., 2006; Kramer et al., 2006; Baldi et al., 2010; Strauss et al., 2014; Rumberger et al., 2004). Composts made exclusively from plant material, such as yardwastes, have a higher C:N ratio than manure-based composts. Higher C:N ratio compost will initially immobilize N, and then mineralize N slowly, for a longer period of time than manure based composts (Hartz et al., 2000). Manure based composts, such as chicken litter, mineralize N more rapidly and continued to mineralize N throughout the growing season (Hartz et al., 2000). Baldi et al. (2010) found that compost, when tilled into fine textured soil to a depth of 25 cm, in a 7-year-old peach orchard increased soil quality measures, such as OM, mineral nutrition, and microbial biomass C, and fruit yield. In apple orchards, compost applications improved soil edaphic properties, including soil organic matter, microbial biomass C, and microbial respiration, soil mineral nutrition, and leaf mineral nutrients in finely and coarse textured soils (Rumberger et al. 2004; Yao et al., 2006; Forge et al., 2013 Neilsen et al., 2014; Sas-Paszt et al., 2014). However, compost did not increase the TCSA independent of soil type (Rumberger et al. 2004; Yao et al., 2006; Forge et al., 2013 Neilsen et

al., 2014). Applications of compost did not increase the vegetative growth, fruit yield, and quality of apples grown in coarsely textured soils (Forge et al., 2013; Neilsen et al., 2014; Sas-Paszt et al., 2014) These yield and quality results are similar to findings from annual horticultural crops grown with compost as the sole N source, and may be due in part to the low immediate N availability of composts (Stamatiadis et al., 1999; Hernadez et al., 2004).

However, using integrated fertilizer methods, such as the application composts in conjunction with synthetic nitrogen fertilizers, has been shown to increase carbon sequestration, plant nutrient availability, and crop yield and quality (Yu et al., 2012; Stamatiadis et al., 1999; Hernadez et al., 2014). Utilizing an integrated fertilizer approach in apple orchards may provide trees with N when it is most needed, and reduce environmental N loss (Kramer et al., 2006). In a study conducted by Kramer et al. (2006), compost and calcium nitrate [$Ca(NO_3)_2$] increased soil OM, but did not have a significant effect on microbial biomass, potentially mineralizable N, compared to conventional fertilizer application in an established apple orchard planted on coarse, sandy soil in an arid environment.

The objectives of this study were to evaluate the effects of fertilizer application practices on newly planted apple orchard growth and productivity, and how these practices affect orchard soil fertility. I hypothesized that integrated fertilization with applications of composts and calcium nitrate would improve apple tree growth and soil quality after three years, compared to unfertilized trees and compost amended trees.

2.3 Materials and Methods

Three rows of 49 'Red Delicious cv Schlect'/'Malling 26' (M.26) were planted with a tree spacing of 1.5 m x 4.5 m at the Virginia Tech Alson H. Smith, Jr. Agricultural Research and Extension Center in Winchester, VA (39° 06' N, 78° 17' W) in April 2013. The orchard soil was

a Poplimento silt loam, a fine, mixed, subactive, mesic Ultic Hapludalf soil (NRCS, 2001). Treatments were implemented on five-tree sets, each of which was replicated four times in a randomized complete block design. The two end trees in each five-tree set served as buffers and were not used for data collection. All trees were trained as a vertical-axe on a trellis system, and were uniformly treated in regards to pest, disease, and weed control (Pfeiffer et al., 2015). Irrigation was supplied through an in-line drip tube to replace water lost through evapotranspiration. In 2013 and 2014, all flower clusters were removed by hand to prevent fruit set. In 2015, fruitlets were thinned to three per TCSA on each tree to prevent over cropping young trees (Robinson et al., 2013).

Fertilizer treatments were applied to the soil on 13 May 2013, 19 May 2014, and 14 May 2015. All treatments, except the control, were applied at 67 kg of plant available N ha^{-1}. Fertilizer treatments included the following: (1) an unfertilized control (CON), (2) a split application of water soluble calcium nitrate [$Ca(NO_3)_2$] (SYN) (Yara, Oslo, Norway), (3) chicken litter compost (CL) (Valley Pride Compost, Harrisonburg, VA), (4) yardwaste compost (YW) (Loudoun Composting, Chantilly, VA), (5) a combination of chicken litter compost and $Ca(NO_3)_2$ (CL+SYN) with equal amounts of N from each fertilizer, (6) a combination of yardwaste compost and $Ca(NO_3)_2$ (YW+SYN) with equal amounts of N from each fertilizer, and (7) hand fertigation with $Ca(NO_3)_2$ (FGN) for eight weeks beginning in May and ending in July of each year. The first split application of the SYN treatment occurred on 1 June 2013, 28 May 2014, and 28 May 2015, and the second application of SYN occurred on 2 July 2013, 2014, and 2015. Compost was spread in the tree row by hand 83 cm from the trunk, and calcium nitrate was applied around the base of the trees. The amounts of other mineral nutrients and organic matter differed among treatments, and were not controlled for in this experiment. Compost nutrient

analysis was performed by Penn State Agricultural Analytical Services Lab (University Park, PA) prior to compost application each year so that plant available N application rates could be calculated (Table 2.1).

Tree growth was measured by calculating the TCSA at 30 cm above the graft union at the time of planting and in all subsequent years. Flower clusters were counted in 2015 and 2016. Mature fruit were harvested from the three treatment trees on 14 Sept. 2015. Fruit number and fruit weight were recorded, and yield efficiency was calculated. Four fruit were randomly selected from each of the three trees in the middle of the five-tree set. The twelve fruit from each treatment and block were used to determine fruit quality and maturity. Size, weight, and flesh firmness were measured with a fruit texture analyzer (FTA) (GÜSS Manufacturing Ltd., Strand, South Africa) fitted with an 11.1 mm dia tip. Flesh firmness was measured once on each side of the fruit after removing part of the peel. Fruit starch was visually assessed using the Cornell Starch-Iodine Index (Blanpied and Silsby, 1992). Red peel color was visually assessed on 0-100% scale. Fruit internal ethylene was measured in 1 mL of gas drawn from the cavity of the apples using a 7890A gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA). The soluble solid concentration of apple juice was measured using a hand-held digital refractometer (Atago, Tokyo, Japan).

Leaf samples were collected annually on 14 Aug. 2013, 22 Aug. 2014 and 11 Aug 2015 for leaf mineral nutrient analysis. Twenty leaves were removed from the middle of the current year's branch growth on each of the three center treatment trees and combined into a composite sample. Prior to analysis, leaves were oven dried in an oven at 80 °C for 3 d. The concentration of the macronutrients P, K, Ca, and Mg, and the micronutrients Mn, Fe, Cu, B, Al, Zn, and Na were measured using a 730-ES ICP Optical Emission Inductively Coupled Plasma (OES-ICP)

Spectrometer (Agilent Technologies, Santa Clara, CA) using the ICP-dry ash method (Miller, 1998). Leaf N concentration was measured using the combustion analysis method (Horneck and Miller, 1998) on a Vario Max N/C Analyzer (Elementar, Hanau, Germany) at the Penn State Agricultural Analytical Services Laboratory (University Park, PA).

Using a soil auger with a 7-cm diameter, soil samples were collected 30 cm north, south, east, and west from the trunk of the center tree of the five-tree set from the surface to a depth of 15 cm on 8 Sept. 2013, 3 Sept. 2014, and 24 Aug. 2015. Soil was placed in a bag and homogenized by hand. Following collection, soil was sieved using a US number 10 soil sieve (2 mm mesh) and stored at 4°C until use. Prior to analysis, soil samples were air dried and crushed with a hammer mill-type crushing machine (Agvise, Benson, MN). Soil pH, mineral nutrients, and CEC were measured at the Virginia Tech Soil Testing Laboratory (Blacksburg, VA). Soil pH was measured using a pH meter (WP-80D, TPS Pty Ltd., Springwood, Australia) fitted with a combination pH electrode (Orion model 8165BNWP Ross Sure-Flow, ThermoFisher, Waltham, MA). Mehlich 1 solution was used to extract P, K, Ca, Mg, Zn, Mn, Cu, Fe, B, and Al from 4 cm^3 of soil. The mineral nutrient concentration was determined from the extraction by OES-ICP (Acros Spectro, Mahwah, NJ). Cation exchange capacity was estimated by the addition of the non-acid generating cations (Ca, Mg, and K) and Mehlich 1 soil-buffer acidity. Soil OM and soluble salts (SS) were measured at the Virginia Tech Soil Testing Laboratory in 2013 and 2014, and at the Cornell Nutrient Analytical Lab in 2015. An electrical conductivity probe was used to measure SS (3100 Conductivity Instrument, YSI, Yellow Springs, OH). Soil organic matter was measured using the loss on ignition method. Initial soil dry mass was determined by heating soil to 150°C in an electric high temperature, forced air drying oven (Blue M model CW-6680F, New Columbia, PA) for 2 h. This soil was then heated to 360 °C for 2 h, and weighed again to

determine organic matter content. Total C and total N were measured at the Cornell Nutrient Analytical Lab on a CHN Elemental Analyzer-vario EL (Elementar, Hanau, Germany) after soil was ground to a fine powder using a mortar and pestle.

To measure soil respiration, 50 g of soil from each sample was placed in an air-tight jar with a vial containing 20 mL of 0.5 M NaOH solution, to trap the evolved CO_2 gas. The electrical conductivity of the NaOH solution was measured weekly for six weeks using a model 2052 electrical conductivity meter (Amber Science Inc., Eugene, OR). To determine the concentration of evolved CO_2, electrical conductivity of the samples was compared to a blank containing 50 g of autoclaved, dried sand, and a CO_2 saturated standard (Rodella and Saboya, 1999).

Microbial biomass C was measured using the direct chloroform ($CHCl_3$) fumigation extraction method (Fierer and Schimel, 2003). Ten grams of soil from each sample and 40 mL of 0.05 M potassium sulfate (K_2SO_4) were placed in two separate 70 mL glass vials with Teflon-lined lids. Fumigated samples received 0.5 mL of amylated $CHCl_3$. Unfumigated samples did not receive $CHCl_3$, but were otherwise treated the same as fumigated samples. Samples were shaken at 2.5 $r \cdot s^{-1}$on an orbital shaker for 4 h, and allowed to settle for 30 min before being decanted into 50 mL conical tubes. Samples were centrifuged for 10 min at 500 g_n and filtered (FisherBrand G6, Fisher, Waltham, MA). The filtrate was sparged with compressed nitrogen gas for 20 min to remove any remaining $CHCl_3$ from the solution and stored at -20 °C until use. Blank samples with no soil were prepared in the same manner. Prior to analysis, samples were diluted 1:2 (volume/volume) with deionized water. Total carbon was quantified using a Shimadzu (Columbia, MD) carbon analyzer model TOC-VCPH+TNM-1 with an auto-sampler, using high-temperature oxidation catalyzed with platinum-coated alumina beads (temperature 720 °C) in

non-purgeable organic carbon mode (Bird et al., 2003). Non-purgeable organic C was measured using a non-dispersive infrared detector (NDIR). Each sample was run in triplicate. Microbial biomass was calculated by multiplying the difference between the fumigated and unfumigated samples by the k_{ec} value of 0.45 (Joergensen, 1996).

An initial measurement of potentially mineralizable nitrogen (PMN) was made by placing 10 g of soil in a 50 mL conical tube with 40 mL of 2 M KCl. Samples were placed on an orbital shaker for 1 h at 3.3 $r \cdot s^{-1}$, and centrifuged at 500 g_n for 10 min prior to filtration (FisherBrand G6, Fisher). The filtrate was collected in 20 mL scintillation vials. Ten grams of soil were placed in a second 50 mL tube with 10 mL of water (W7-4 Optima Water, Fisher) and incubated for 7 d at 30°C. After 7 d, 30 mL of 2.67 M KCl were added to the samples, which were then shaken on an orbital shaker at 3.3 $r \cdot s^{-1}$ for 1 h. Samples were centrifuged at 500 g_n for 10 min and filtered. The filtrate was collected in 20 mL scintillation vials. All samples were stored at -20 °C until NH_4^+ and NO_3^- concentrations were measured using a Lachat QuickChem 8500 Series 2 Flow Injection Analysis System (Loveland, CO). Ammonia was measured using protocol #12-107-06-2-A, and NO_3^- was measured using protocol #12-107-04-1-B (Lachat Instruments, 2014). Potentially mineralized nitrogen rate was estimated as the difference in the NH_4^+ concentration between the 7-d incubation measurement and initial measurement.

Trunk cross sectional area and fruit quality were analyzed using PROC GLM in SAS 9.4 (SAS Institute Inc., Cary, NC). Significant differences at a $p = 0.05$ level were determined using Tukey's honestly significant differences (HSD) post hoc test. The random effect in both models was Block. Blossom cluster density, yield efficiency, leaf mineral analysis, soil fertility, PMN, and microbial biomass carbon data were analyzed using analysis of variance (ANOVA) and Tukey's HSD post hoc test in JMP Pro 11 (SAS Institute Inc., Cary, NC). Significant differences

were determined at a p = 0.05 level. Slices were used to look at the interaction effects within the Treatment x Year interactions when interactions were significant. The random effect was Block. Soil microbial respiration data were analyzed as a repeated measure using PROC MIXED in SAS 9.4. In this model, Block and Block x Treatment were random effects. Treatment differences were determined at a p = 0.05 level.

Table 2. 1. The C:N ratio, organic matter, total C, organic N, ammonium, nitrate, P, and K content of the chicken litter and yardwaste composts measured in 2013, 2014, and 2015 prior to compost application to ensure plant available N was equivalent among fertilizer treatments.

Compost	C:N	OM (g kg^{-1})	C (g kg^{-1})	Organic N (g kg^{-1})	Ammonium (mg kg^{-1})	Nitrate (mg kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
2013								
Chicken litter	15.8	458	260	16.5	5.0	1,012.62	18.8	7.5
Yardwaste	14.4	538	246	17.1	55.6	21.03	4.60	11.5
2014								
Chicken litter	14.9	475	293	19.6	5.0	501.40	19.0	8.6
Yardwaste	18.8	608	342	18.1	36.8	44.00	4.10	11.2
2015								
Chicken litter	12.9	473	246	19.0	5.0	601.05	16.8	6.5
Yardwaste	18.1	515	291	16.1	48.2	91.52	3.50	7.8

2.4 Results

No differences in TCSA were observed in 2013, 2014, or 2015 (Fig. 2.1). In 2016, trees that received SYN or YW fertilizers had a greater blossom cluster density (blossom clusters $TCSA^{-1}$) than trees that received CL fertilizer (Table 2.2). Blossom cluster density was 47% greater on trees fertilized with SYN, and 54% greater on the trees fertilized with YW compared to trees fertilized with CL. No treatment differences were found for fruit yield efficiency (Table 2.3), and the only fruit quality parameter affected by treatment was red peel color (Table 2.3).

Fruit from trees fertilized with SYN had on average 3% greater red peel color than fruit from FGN trees.

There were no significant differences in leaf N concentration during this study. In 2013, leaf P concentration was greater from trees that received CL+SYN than FGN. In 2015, leaf P concentration was greater from trees that received CL than SYN or FGN. In 2013, YW+SYN increased leaf K concentration compared to CON, SYN, CL+SYN, or FGN. In 2014 and 2015, trees that received CL, YW, or CL+ N had greater leaf B concentrations than those that received CON, SYN, or FGN treatments (Table 2.4).

Within three months after the initial fertilizer application, changes in soil mineral nutrient content were detectable (Table 2.5). Throughout this study, CL increased extractable P, and YW and YW+SYN increased extractable K compared to CON, SYN, and FGN treatments. Soil Zn concentration was increased by applications of CL in 2013 and 2015. Both compost treatments increased soil Ca concentrations in 2015, and soil B concentrations in 2013 and 2015.

In 2013, total C and C:N were higher in soils that received YW than in soils that received CON, SYN, or FGN. There were no statistical differences in total N, total C, or the C:N ratio in 2014. The effects of fertilizer application on soil health factors became apparent in 2015 (Table 2.5). Compost and integrated fertilizer treatments increased total N, total C, and C:N ratios compared to CON, SYN, or FGN treatments. Compost treatments increased CEC 56% compared to CON or SYN fertilized soil, and 67% compared to FGN. Compost increased OM% compared to CON and FGN treatments. Soils amended with CL had 59% more OM than the CON and FERT soils, and YW amended soils had 87% more OM than the CON and FGN soils. Soil amended with YW fertilizer had a higher pH than FGN (Table 2.5).

There were similar trends in soil microbial respiration rate for samples taken in 2013 and 2015 (Fig. 2.2). In both years, the fertilizer treatments had a significant effect on soil microbial respiration, and there were significant Fertilizer x Day interactions. Soil fertilized with YW had greater microbial respiration rates than all other treatments in 2013 ($p = 0.0006$) and 2015 ($p < 0.0001$). Soil microbial respiration was 2.5 times greater in YW fertilized soil than in the CON soils in 2013, and 5 times greater in 2015. Soil microbial respiration was greatest in the YW, CL, and YW+SYN soils (Fig. 2.2). Unlike soil microbial respiration in 2013 and 2015, there were no statistical differences in microbial soil respiration in 2014 (Fig. 2.2); however, microbial respiration was numerically greater in soils fertilized with CL+SYN than the other treatments. Fertilizer application did not affect PMN in 2014 and 2015 (Table 2.6), or microbial biomass carbon in 2015 (Table 2.7). The PMN results for 2014 were similar to soil microbial respiration results that year. Although there were no statistical differences, PMN was numerically greater in soils amended with CL+SYN than the other treatments.

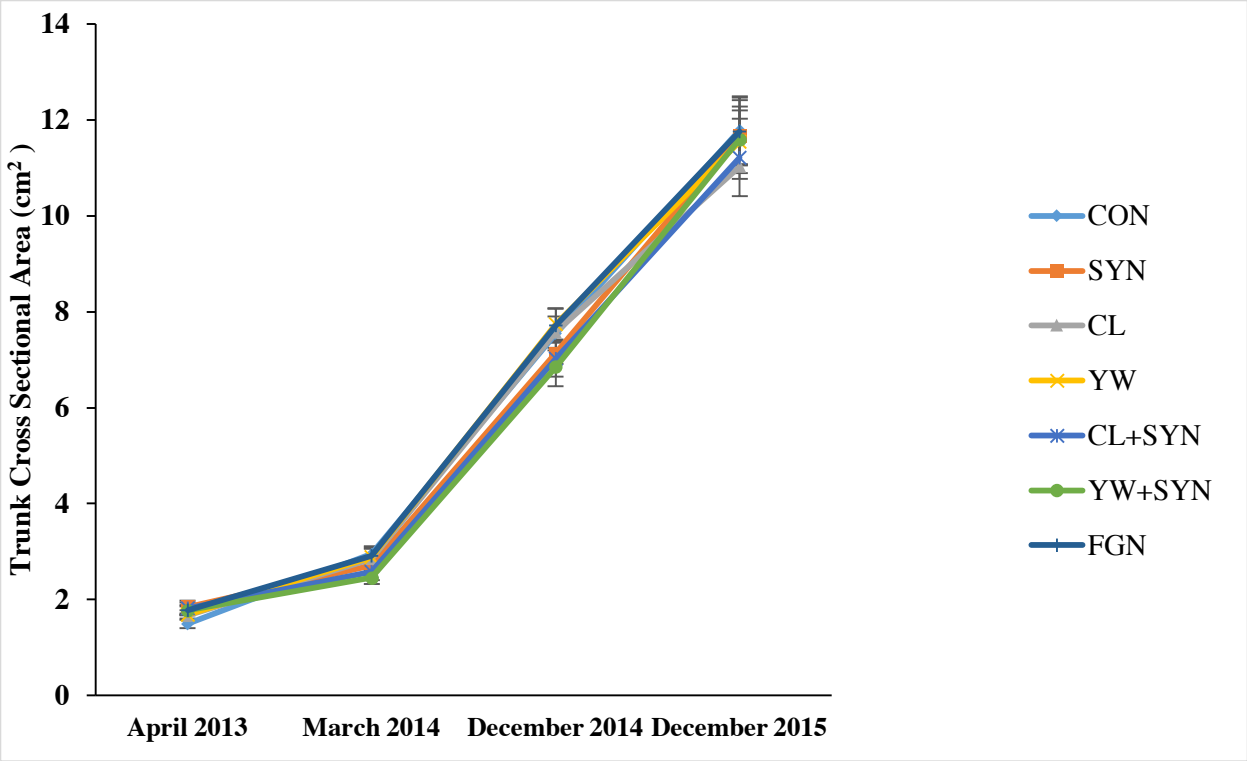


Fig. 2. 1. The effect of fertilizer treatments on tree growth 2013-2015. Tree growth was measured as trunk cross sectional area at planting, and following fertilizer applications in the spring of 2014, the winter of 2014, and the winter of 2015. Each fertilizer treatment was replicated four times on three-tree sets. Data shown represents the mean of 12 trunk cross sectional area measurements per treatment calculated from the trunk caliper diameter. The treatments were: control (CON), split applications of calcium nitrate (SYN), chicken litter compost (CL), yardwaste compost (YW), integrated chicken litter compost and calcium nitrate (CL+SYN), integrated yardwaste compost and calcium nitrate treatment (YW+SYN), and fertigation (FGN).

Table 2. 2. Treatment effects of fertilizers on bloom cluster density in 2015 and 2016. Bloom cluster density differences among fertilizers were determined in 2015 and 2016 in Winchester, VA. Each fertilizer treatment was replicated four times on three-tree sets. Data presented represents the mean number of bloom clusters divided by the trunk cross sectional area during the year the data was collected for the twelve trees in each fertilizer treatment group. The treatments were: control (CON), split applications of calcium nitrate (SYN), chicken litter compost (CL), yardwaste compost (YW), integrated chicken litter compost and calcium nitrate (CL+SYN), integrated yardwaste compost and calcium nitrate treatment (YW+SYN), and fertigation (FGN).

Treatment	**Bloom Cluster Density (Flower Clusters cm^{-2}) (±SEM)[y] 2015**	**Bloom Cluster Density (Flower Clusters cm^{-2}) (±SEM) 2016**
CON	9 (0.89)	11 (0.89) AB[z]
SYN	8 (0.85)	13 (0.75) A
CL	7 (1.16)	8 (0.93) B
YW	6 (0.76)	14 (0.83) A
CL+SYN	8 (1.36)	10 (1.22) AB
YW+SYN	8 (1.13)	11 (0.88) AB
FGN	7 (0.92)	11 (0.76) AB
	$p = 0.6$	$p = 0.005$

[y]SEM is the standard error of the mean
[z]Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.

Table 2. 3. Assessment of the effects of fertilizers on yield efficiency and fruit quality in 2015. Yield efficiency was calculated by weighing the fruit from each tree and dividing the fruit weight by the trunk cross sectional area of the tree. The yield efficiency means shown here were calculated from the twelve experimental trees in each fertilizer treatment. Fruit size, weight, firmness, starch, peel color, soluble solids and ethylene concentration were determined from twelve fruit per experimental unit. Data presented here are the means calculated from 48 apples harvested from each treatment. Fruit were harvested September 14, 2015 in Winchester, VA. Each fertilizer treatment was replicated four times on three tree sets. Four fruit were randomly harvested from each tree in the three-tree set. The treatments were: control (CON), split applications of calcium nitrate (SYN), chicken litter compost (CL), yardwaste compost (YW), integrated chicken litter compost and calcium nitrate (CL+SYN), integrated yardwaste compost and calcium nitrate treatment (YW+SYN), and fertigation (FGN).

Fertilizer	Yield Efficiency (kg cm^{-2})	Fruit Diameter (mm)	Fruit weight (g)	Fruit Firmness (N)	Starch (1-8)	Peel Color (%)	Soluble solid concentration (°Brix)	Ethylene (mg m^{-3})
CON	0.57	85.8	276	68.9	4.3	94 AB[z]	14.1	22.10
SYN	0.51	86.1	287	68.5	4.3	95 A	14.6	26.52
CL	0.38	84.8	269	68.9	4.6	94 AB	13.9	20.23
YW	0.47	86.2	277	68.0	4.5	93 AB	14.3	20.23
CL+SYN	0.49	84.5	262	68.0	4.3	94 AB	13.8	18.44
YW+SYN	0.51	84.1	258	68.0	4.3	93 AB	13.9	23.90
FGN	0.53	83.6	255	69.8	4.3	92 B	13.7	28.74

[z]Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test

Table 2. 4. Leaf nutrient analysis was performed on 60 leaves collected from each experimental unit in August of 2013, 2014, and 2015. Data presented here are the means from four replications of each fertilizer treatment. The treatments were: control (CON), split applications of calcium nitrate (SYN), chicken litter compost (CL), yardwaste compost (YW), integrated chicken litter compost and calcium nitrate (CL+SYN), integrated yardwaste compost and calcium nitrate treatment (YW+SYN), and fertigation (FGN).

Year	Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})
2013	CON	2.7	0.195 AB[z]	1.40 B	1.5	0.33	90	53	3.8	43.3	34.0 B	128	36.5
2013	SYN	2.8	0.200 AB	1.45 B	1.7	0.37	98	51	3.5	45.3	34.6 B	138	20.0
2013	CL	2.8	0.205 AB	1.50 AB	1.5	0.35	94	52	4.0	47.8	35.5 B	137	25.0
2013	YW	2.7	0.193 AB	1.54 AB	1.4	0.30	85	52	4.5	46.3	32.9 B	135	22.8
2013	CL+SYN	2.8	0.210 A	1.45 B	1.7	0.35	95	61	3.8	47.3	41.1 AB	143	20.5
2013	YW+SYN	2.8	0.208 AB	1.59 A	1.7	0.33	106	53	3.5	46.3	35.9 AB	146	19.5
2013	FGN	2.8	0.190 B	1.42 B	1.4	0.35	96	70	3.5	43.6	54.8 A	135	19.5
2014	CON	2.4	0.168	2.19	1.2	0.22	54	52	5.0	42.0 B	30.5	229	14.8
2014	SYN	2.5	0.170	2.16	1.2	0.23	56	50	5.3	41.0 B	26	211	12.8
2014	CL	2.7	0.170	2.30	1.1	0.22	59	52	4.8	47.0 A	27.5	248	14
2014	YW	2.5	0.168	2.41	1.1	0.21	61	47	4.5	47.0 A	23.8	239	12.3
2014	CL+SYN	2.5	0.173	2.25	1.2	0.23	54	50	5.3	46.0 A	25.5	221	12.3
2014	YW+SYN	2.5	0.165	2.32	1.2	0.21	66	47	5.0	44.0 AB	24	249	13.0
2014	FGN	2.5	0.163	2.19	1.3	0.23	63	46	5.0	41.5 B	23.6	218	12.6

Year	Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})
2015	CON	2.4	0.150 ABC	1.45	1.3	0.27	79	41	5.0	36.8 AB	18.8	310	24.5
2015	SYN	2.4	0.143 BC	1.50	1.2	0.26	81	40	5.0	37.8 AB	19.3	285	22.5
2015	CL	2.4	0.155 A	1.49	1.2	0.27	84	40	4.8	38.8 AB	17.5	317	24.8
2015	YW	2.4	0.153 AB	1.57	1.2	0.25	98	43	5.3	40.0 A	22	327	26.0
2015	CL+SYN	2.5	0.148 ABC	1.44	1.2	0.27	88	40	5.3	38.5 AB	17.5	296	24.0
2015	YW+SYN	2.4	0.153 AB	1.59	1.2	0.25	94	40	5.0	39.0 AB	18	318	24.0
2015	FGN	2.4	0.140 C	1.39	1.3	0.27	92	40	5.0	36.0 B	18.8	306	25.0
Treatment		ns	**	***	**	*	*	ns	ns	***	ns	ns	ns
Year		***	***	***	***	***	***	***	***	***	***	***	***
Treatment x Year		ns	ns	ns	ns	ns	ns	*	ns	ns	**	ns	ns
Year effects within Treatment x Year													
2013								**			***		
2014								ns			ns		
2015								ns			ns		
Treatment effects within Treatment x Year													
CON								**			***		
SYN								*			**		
CL								*			***		
YW								ns			**		
CL+SYN								***			***		
YW+SYN								**			***		
FGN								***			***		

[z] Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.

* Significant at $p \leq 0.05$

** Significant at $p \leq 0.01$

*** Significant at $p \leq 0.0001$

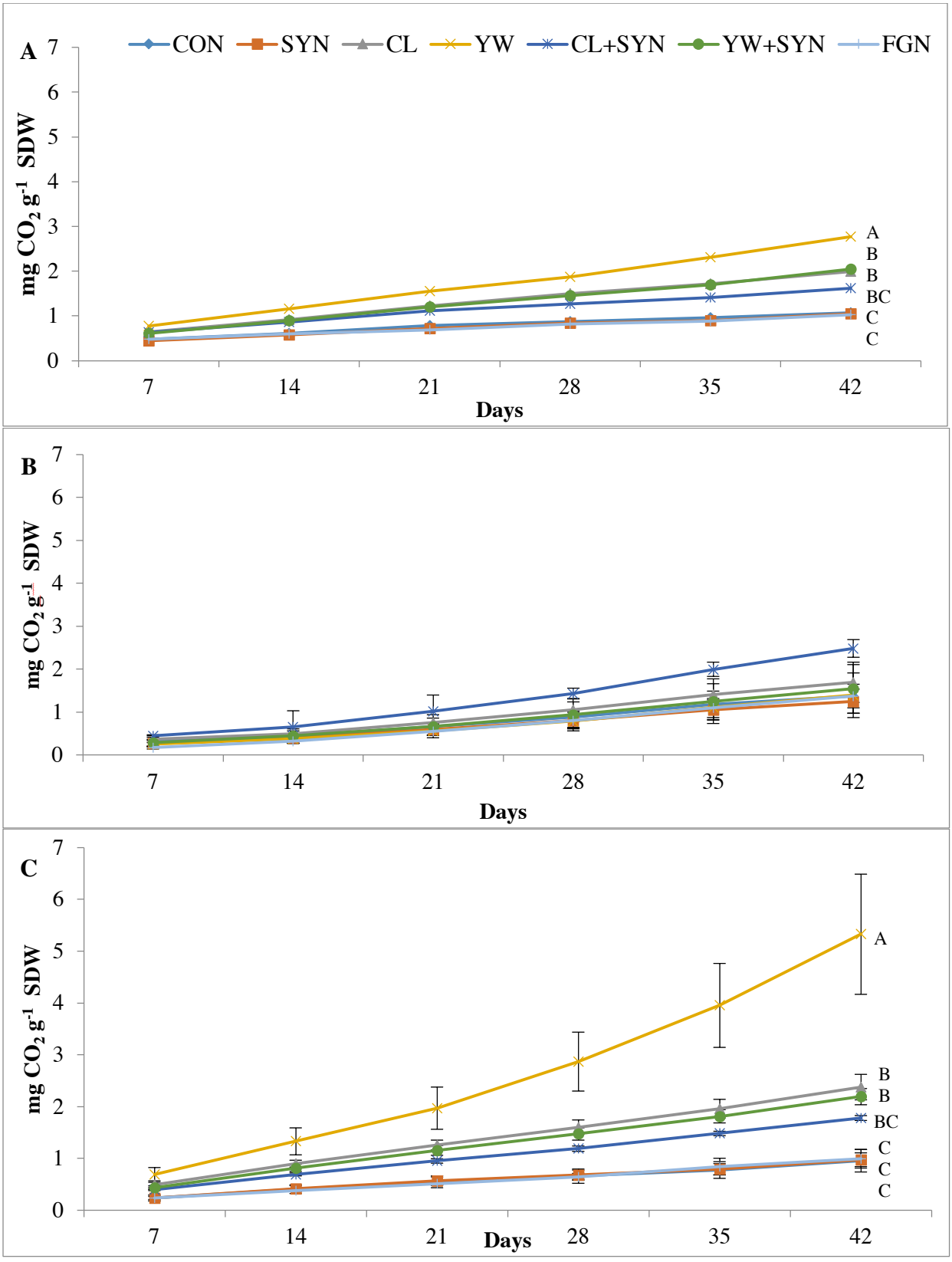
Table 2. 5. Soil mineral nutrients, organic matter, soluble salts, cation exchange capacity, total nitrogen, total carbon and pH measured from soil samples collected to a depth of 15 cm 30 cm from the trunk of the center tree in 2013, 2014, and 2015. Data presented here are the means from four replications of each fertilizer treatment. The treatments were: control (CON), split applications of calcium nitrate (SYN), chicken litter compost (CL), yardwaste compost (YW), integrated chicken litter compost and calcium nitrate (CL+SYN), integrated yardwaste compost and calcium nitrate treatment (YW+SYN), and fertigation (FGN).

Year	Treatment	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	OM[x] (g kg^{-1})	SS[y] (mmos cm^{-1})	CEC[z] (meq $100g^{-1}$)	N (g kg^{-1})	C (g kg^{-1})	C:N	pH
2013	CON	22 B[w]	125 C	115 6	110 C	13 AB	50 AB	0.78	6.05 AB	0.525 BC	29	0.26	7.4	2.6	13.7 B	5.24 BC	6.4
2013	SYN	20 B	90 C	130 7	97 C	10 B	43 AB	0.83	5.05 B	0.550 BC	31	0.48	7.7	2.9	14.3 B	4.91 C	6.5
2013	CL	133 A	144 BC	171 0	161 A	16 A	39 B	0.75	5.15 AB	0.975 A	38	0.27	10.3	2.9	17.8 AB	6.27 ABC	6.7
2013	YW	36 B	241 A	166 5	172 A	12 AB	55 AB	0.53	6.78 A	0.900A	47	0.38	10.4	3.5	28.5 A	7.89 A	6.8
2013	CL+SYN	110 A	144 BC	179 1	152 BC	16 A	54 AB	0.56	4.45 B	0.925 A	43	0.34	10.6	2.9	17.0 AB	5.91 BC	6.7
2013	YW+SYN	33 B	187 AB	165 4	139 ABC	13 AB	58 A	0.45	5.70 AB	0.800 AB	46	0.42	10	3.4	24.1 AB	7.17 AB	6.7
2013	FGN	17 B	113 C	128 8	99 C	11 AB	49 AB	0.75	4.62 B	0.500 C	29	0.58	7.7	2.7	13.8 B	5.07 C	6.3

Year	Treatment	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	OM (g kg^{-1})	SS[y] (mmos cm^{-1})	CEC[z] (meq 100g^{-1})	N (g kg^{1})	C (g kg^{1})	C:N	pH
2014	CON	27 BC	116 BC	1300	114 AB	14	15	1.23 A	5.73	0.570	28	0.20	8.1	1.4	14.6	10.42	6.6
2014	SYN	18 C	77 C	1146	92 AB	9	13	0.95 AB	5.73	0.450	22	0.23	7	1.4	12.2	8.79	6.6
2014	CL	92 A	189 AB	1556	152 AB	15	16	0.80 B	5.55	0.800	29	0.37	9.6	1.7	21.6	13.11	6.8
2014	YW	27 BC	247 A	1490	158 A	11	19	0.70 B	5.95	0.825	39	0.35	9.5	1.9	20.7	11.06	6.8
2014	CL+SYN	69 AB	150 BC	1493	133 AB	13	13	0.72 5 B	4.75	0.775	39	0.35	9	1.6	24.1	13.9	6.9
2014	YW+SYN	25 BC	181 AB	1468	127 AB	11	19	0.68 B	5.45	0.750	35	0.31	8.8	1.7	19.8	11.68	7
2014	FGN	14 C	89 C	1079	88 B	9	11	0.98 AB	5.78	0.425	23	0.40	6.9	0.13	20.7	17.4	6.2
2015	CON	22 C	101 C	1326 BC	111 BC D	14 BC	14 DE	0.95	5.75	0.625 BC	24 C	0.15	8.0 BC	2.5 B	12.6 C	5.08 D	6.9 AB
2015	SYN	18 C	74 D	1383 BC	99 CD	12 BC	15 DE	0.75	5.3	0.600 BC	26 BC	0.44	8.0 BC	2.6 B	14.6 C	5.58 CD	6.7 AB
2015	CL	258 A	217 B	2317 A	253 A	21 A	24 BC	0.55	6.18	1.225 A	44 AB	0.42	14.2 A	3.7 AB	29.9 B	7.95 AB	6.9 AB
2015	YW	50 C	326 A	2267 A	262 A	13 BC	35 A	0.63	7.48	1.325 A	6.1 A	0.36	14.3 A	4.8 A	46.1 A	9.39 A	7.3 A
2015	CL+SYN	146 B	165 BC	2024 AB	179 B	18 AB	19 CD	0.65	5.43	1.075 AB	38 BC	0.37	12.0 AB	3.2 AB	22.1 BC	6.83 BC	7.0 AB
2015	YW+SYN	34 C	196 B	1840 ABC	160 BC	12 BC	26 B	0.43	5.95	0.950 AB	39 BC	0.29	10.9 ABC	3.7 AB	28.4 B	7.74 BC	7.2 AB
2015	FGN	13 C	78 D	1152 C	78 D	11 C	12 E	0.93	4.83	0.500 C	24 C	0.46	7.1 C	2.9 B	12.2 C	4.61 D	6.4 B

	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	OM (g kg^{-1})	SS[y] (mmhos cm^{-1})	CEC[z] (meq $100g^{-1}$)	N (g kg^{-1})	C (g kg^{-1})	C:N	pH
Treatment	***	***	***	***	***	***	***	**	***	***	**	***	***	***	ns	**
Year	***	ns	***	***	**	***	ns	ns	***	**	ns	***	***	*	***	**
Treatment x Year	***	**	ns	**	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns
Year effects within	***	***		***						**				*		
Treatment x Year	***	***		***						**				ns		
2013	***	***		***						***				***		
2014																
2015	ns	ns		ns						ns				ns		
Treatment effects within	ns	ns		ns						ns				ns		
Treatment x Year	***	**		***						**				ns		
CON	ns	**		***						**				***		
SYN	***	ns		ns						ns				ns		
CL	ns	ns		ns						ns				ns		
YW																
CL+SYN																
YW+SYN																
FGN	ns	ns		ns						ns				ns		

[w] Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.
[x] OM is organic matter.
[y] SS is soluble salts
[z] CEC is cation exchange capacity.
ns, nonsignificant, * Significant at $p \leq 0.05$, ** Significant at $p \leq 0.01$, *** Significant at $p \leq 0.0001$


A
CON
SYN
CL
YW
CL+SYN
YW+SYN
FGN
mg CO2 g-1 SDW
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mg CO2 g-1 SDW
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Fig. 2. 2. Fertilizer treatment affected soil microbial respiration in 2013 and 2015. Soil microbial respiration was measured as the evolution of carbon dioxide from soil samples taken a depth of 15 cm 30 cm from the trunk of the center tree in A) 2013, B) 2014, and C) 2015. Soil was incubated for 6 weeks. Data shown here are the means from four replications of each fertilizer treatment. Different letters indicate a means separation at $p \leq 0.05$ level of significance of the least significant differences test. The treatments were: control (CON), split applications of calcium nitrate (SYN), chicken litter compost (CL), yardwaste compost (YW), integrated chicken litter compost and calcium nitrate (CL+SYN), integrated yardwaste compost and calcium nitrate treatment (YW+SYN), and fertigation (FGN).

Table 2. 6. The effect of fertilizer treatment on microbial biomass C in 2015. Microbial biomass carbon was measured from soil samples collected to a depth of 15 cm 30 cm from the trunk of the center 2015. Data presented here are the means from four replications of the control (CON), calcium nitrate split application (SYN), and fertigation (FGN), and three replications of chicken litter compost (CL), yardwaste (YW), chicken litter compost and calcium nitrate (CL+SYN), and yardwaste compost and calcium nitrate (YW+SYN).

Treatment	Microbial biomass μg C g^{-1} SDW[y](±SEM)[z]
CON	30.5 (3.6)
SYN	37.8 (4.9)
CL	43.5 (3.8)
YW	35.2 (4.1)
CL+SYN	39.9 (0.3)
YW+SYN	36.9 (5.2)
FGN	37.6 (11.0)
	p= 0.8

[y]SDW is soil dry weight
[z]SEM is the standard error of the mean

Table 2. 7. The effect of fertilizer treatment on potentially mineralizable N was measured in 2014 and 2015. Potentially mineralizable nitrogen was measured from soil samples collected to a depth of 15 cm 30 cm from the trunk of the center 2014 and 2015. Data presented here are the means from four replications of each fertilizer treatment. The treatments were: control (CON), split applications of calcium nitrate (SYN), chicken litter compost (CL), yardwaste compost (YW), integrated chicken litter compost and calcium nitrate (CL+SYN), integrated yardwaste compost and calcium nitrate treatment (YW+SYN), and fertigation (FGN).

		Potentially Mineralizable Nitrogen	
		***μ*g N g^{-1} SDW[y] (±SEM[z])**	***μ*g N g^{-1} SDW[y] (±SEM[z])**
Year	**Treatment**	**2014**	**2015**
2014	CON	10.3 (0.76)	17 (8.32)
2014	SYN	10.9 (4.49)	19 (8.44)
2014	CL	13.2 (2.09)	30.9 (3.54)
2014	YW	13.7 (1.85)	13.6 (2.20)
2014	CL+SYN	16.3 (1.38)	27.8 (3.41)
2014	YW+SYN	13.5 (1.75)	21.4 (8.06)
2014	FGN	13 (1.49)	25.4 (2.57)
		p = 0.7	p = 0.4
		p = 0.4	
	Treatment	p = 0.2	
	Year	p = 0.0003	
	Treatment x Year	p = 0.5	

[y]SDW is soil dry weight
[z]SEM is the standard error of the mean

2.5 Discussion

During the first three years of apple orchard establishment, none of the fertilizer applications increased tree growth, fruit yield, fruit quality, or leaf N status compared to the unfertilized control (CON). These results are similar to studies in arid environments with coarse soils, where increasing the application rate of fertigated $Ca(NO_3)_2$ in orchards did not increase TCSA, improve fruit quality, yield efficiency or fruit weight (Neilsen et al., 2004a; Neilsen et al., 2009). However, Neilsen et al. (2009) did find increasing N rate increased leaf N concentration compared to lower N rates. In the current study, fertilizer applications did not increase leaf N concentration, but all treatments had adequate leaf N concentration for young bearing trees throughout this study (Stiles and Reid, 1991). This difference may be due to soil type and OM content. Fine textured soils with higher OM content, like those in Virginia, mineralize more N than soils with lower OM content, resulting in increased plant productivity (Bauer and Black, 1994). Trees in this study likely received adequate N nutrition from N mineralized from native soil OM, thus the addition of fertilizer N did not increase leaf N concentration.

Applications of compost did not enhance TCSA, yield, or leaf N concentration in apple orchards planted on coarse or finally textured soil under a variety of climactic conditions (Yao et al., 2006; Kramer et al., 2006; Forge et al., 2013; Sas-Paszt et al., 2014). In contrast, compost applied at a rate of 10 t^{-1} ha^{-1} yr^{-1} increased yield by 29%, and increased microbial biomass carbon compared to a synthetic NPK fertilizer application in a peach orchard planted in a fine textured soil (Baldi et al., 2010). Applying composts, regardless of feed stock, soil type, or crop, enhanced soil edaphic factors, such as OM, microbial activity, and soil mineral nutrition, including increased soil P and K (Kramer et al., 2006; Forge et al., 2013; Baldi et al., 2010). In some instances, increased soil mineral nutrition led to greater leaf mineral nutrient

concentrations. D'Hosa et al. (2014) noted similar plant productivity and soil quality changes following compost application. While compost increased potato yield compared to plots fertilized with conventional fertilizer, the yields of beet, maize, and Brussels sprouts did not differ between the compost fertilized and synthetically fertilized plots. These results are similar to the crop production increases noted by Baldi et al. (2010) for peach, and the unchanged yield observed in apple when compost was applied (Forge et al., 2013). Compost applications also enhanced soil health factors soil organic carbon, total N, and microbial biomass carbon (D'Hosa et al., 2014).

Integrated fertilization methods have been used to increase crop yields while improving soil quality in other horticultural crops. Synthetic N in integrated fertilizer combinations provide plants with readily available N, while the compost amendments slowly mineralize N throughout the growing season. Parsley plants that were fertilized with compost and NH_4NO_3 had 69% more biomass than the control, while plants that received compost alone increased biomass by 18% compared to the control (Mylavarapu and Zinati, 2009). The integrated treatment also increased soil total C and total N as much as compost amendments (Mylavarapu and Zinati, 2009). Similar results have been documented in annual crops, including broccoli and tomatoes (Stamatiadis et al., 1999; Hernadez et al., 2014). In apple orchards, integrated fertilization methods did not improve leaf N concentration (Kramer et al., 2006). The integrated CL+SYN and YW+SYN treatments did not increase tree growth or crop yield, despite providing trees with a burst of N at bloom when N demand is greatest. Apple trees may be less responsive to N applications because they have low N demand compared to other crops, and as a perennial plant, apple trees remobilize N yearly.

Compost and integrated fertilizer applications increased certain soil mineral nutrients, particularly P, K, and B, that are not available from traditional synthetic N fertilizers. Greater leaf P, K, and B concentrations were not associated with increased tree growth, bloom cluster density, fruit yield or quality during this study. Soil Mn and Zn concentration increases associated with compost applications did not increase leaf concentrations of these minerals. Leaf Mn and Zn concentrations were above the adequate level for all treatment. Despite adequate soil Ca and Mg concentrations these minerals were deficient in the leaves throughout all treatments, indicating that compost will not make these nutrients more plant available. Leaf Cu deficiency is most likely due to the high concentration of Zn in the leaves and soil. Thus, foliar applications of Ca, Mg, and Cu would be necessary to prevent nutrient disorders regardless of fertilizer application.

Greater soil P from CL applications were associated with increased leaf P during 2013 and 2015. The lack of increased growth associated with organic P applications from compost is similar to results for ground applications of synthetic P in the form of superphosphate, which increases vegetative growth, but not TCSA (Taylor and Goubran, 1975). Ground applications of P have been shown to increase leaf P concentrations, which has been related to increased fruit set and flower number when leaf P concentration nears 0.3% (Taylor and Goubran, 1975; Neilsen et al., 1990; Neilsen et al., 2008). In addition, trees with higher leaf P concentrations also had higher leaf N concentrations (Neilsen et al., 2008). Although leaf P concentrations were adequate during my study, they remained below 0.25%, even when higher P compost fertilizers like CL were applied for several years. This may explain why higher leaf P concentrations did not correspond to increased yield, bloom cluster density, or leaf N concentrations. Higher leaf P concentrations were not related to improvement in fruit quality such as, size, color, firmness and

soluble solids concentration. These results are consistent with the Neilsen et al. (2008) study, which focused on ground applications of synthetic P fertilizers.

Although fertilization with YW increased soil K throughout the study, leaf K concentration was only greater in 2013. In previous studies, fertilization with K increased shoot growth, but not TCSA in apple orchards (Edgerton, 1948; Neilsen et al., 2004a). Synthetic K fertilizer did not significantly improve apple quality in orchards with adequate K nutrition; however, in K deficient trees or orchard soils fertilization with K increased fruit yield, size, and color (Neilsen and Neilsen, 2011; Neilsen et al., 2004a). Soil and leaf K were adequate for all treatments throughout my study; therefore, increasing available K through applications of YW or YW+SYN did not increase leaf K.

Soil B concentration was greatest in soils fertilized with compost on 2013 and 2015. In 2014 and 2015, boron was higher in the leaves of trees that received compost fertilizers. These results are similar to those of Neilsen et al. (2004b). Ground applied B increase leaf B concentrations (Neilsen et al., 2004b). Adequate B nutrition is essential for flower bud growth and development, and increased pollen germination and pollen tube growth (Stanley and Lichtenberg, 1963). However, applications of composts, containing B, did not increase bloom cluster density or yield. Applying B in conjunction with $Ca(NO_3)_2$ increased plant available soil B, thus increasing leaf B concentration (Wojcik, 2000). However, using integrated fertilizers did not increase soil or leaf B concentrations in this study. Current data suggests ground applied B will not increase fruit set in apple, but may improve fruit quality in B limited soils (Wojcik et al., 1999; Wojcik et al., 2008). Compost applications did not improve fruit quality despite increasing leaf B concentrations. Lack of treatment differences are likely due to adequate soil B prior to fertilizer applications.

Despite significant increases in OM, total C and C:N ratio in 2013 and 2015 when CL and YW amendments were applied, neither amendment increased PMN or microbial biomass carbon. These results were unexpected because soil OM and carbon additions tend to increase microbial biomass carbon and microbial activity (Schnurer et al., 1985; Wardle, 1992). In orchard systems, Peck et al. (2011) demonstrated that additions of organic matter in the form of wood chips increased microbial biomass carbon, but additions of composted chicken litter did not, indicating that the C:N ratio of the organic amendment influences microbial biomass growth. However, soil microbial respiration increased in compost amended soils compared to CON, FERT, and SYN treatments in 2013 and 2015. This indicates that soil microbes utilized the carbon and organic matter in composts to sustain their populations throughout the duration of the experiment, while microbes in treatments lacking added carbon and organic matter were less active as the experiment continued due to decreased availability of a carbon energy source. Soil microbial activity throughout the growing season may mineralize certain mineral nutrients, such as N and P, from organic matter slowly throughout the growing season, leading to increased leaf mineral nutrient content. In 2014, a single CL+SYN soil sample had a higher soil total C and C:N than all other samples collected. This outlier had C:N ratio 66% greater than the average CL+SYN sample taken than year. There were no statistical differences in PMN, microbial respiration, OM, total C, or C:N ratio during this year; however, PMN, microbial respiration, total C, and C:N were numerically greater for the CL+SYN treatment.

In summary, compost applications improved soil edaphic properties, such as mineral nutrition, OM, soil C, C:N, and microbial respiration, compared to the CON and FGN treatments. Compost applications also increased certain leaf nutrients including, P, K, and B.

However, increases in soil and leaf mineral nutrition, following compost application, did not improve TCSA, tree yield efficiency, or fruit quality. In addition, compost applications did not provide trees with adequate Ca, Mg, and Cu, despite containing macro- and micronutrients that are not available from conventional N fertilizers. Foliar applications of Ca, Mg, and Cu would be recommended to commercial apple growers to prevent nutrient disorders independent of N fertilizer source. Thus, this study indicates that applying N fertilizer amendments within the first three years of orchard establishment did not improve tree growth, or fruit quality and yield, and farmers may consider forgoing this additional expense unless they are attempting to improve orchard soil fertility, or provide trees with other nutrients, such as P and K. More research is needed to determine the longer term effects of N fertilization, compost and integrated compost-$Ca(NO_3)_2$ on TCSA, yield, and fruit quality, and soil edaphic properties.

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3. Applying Biochar at High Rates Does Not Increase Apple Tree Growth or Biomass Compared to Calcium Nitrate Fertilizer in a Pot-in-Pot Study

3.1 Abstract

Biochar applications have been shown to increase crop yield, and edaphic properties, such as soil mineral nutrition, water holding capacity, cation exchange capacity (CEC), and organic matter (OM). However, in other studies of the effects of biochar use in established apple (*Malus ×domestica* Borkh.) orchards, biochar applications did not increase tree growth or leaf mineral nutrition. Currently, few studies address the effects of using higher rates of biochar during establishment of a perennial cropping system. In order to further explore the use higher rates of biochar application on newly planted trees, a pot-in-pot study was conducted to determine the effects of biochar application rate on apple tree growth and nutrient status, soil health, and microbial activity. CQuest wood-based biochar was applied at 0 Mg biochar ha^{-}1, 50 Mg biochar ha^{-1}, 100 Mg biochar ha^{-1}, 200 Mg biochar ha^{-1} and fertigated with calcium nitrate [$Ca(NO_3)_2$] at a rate of 40 kg N ha^{-1}. A non-fertilized container without biochar served as the control. Biochar applied with 40 kg N ha^{-1}did not increase tree growth or total biomass compared to fertigation with 40 kg N ha^{-1} alone. Biochar applications increased soil water holding capacity, OM, CEC, total C, C:N, and microbial respiration. However, increases in soil C were not associated with greater microbial biomass or potentially mineralizable N. Bacterial and fungal communities sampled at the same time point had more similar community structures than those that received the same treatment. These results suggest that while high application rates of biochar increased some measures of soil fertility, they did not increase tree growth or biomass.

3.2 Introduction

The use of biochar, the solid product of high temperature anaerobic biomass pyrolysis, as a soil amendment has been shown improve agricultural soil fertility, crop productivity, and C sequestration. Recently, researchers have begun exploring the utility of biochar in apple (*Malus ×domestica* Borkh.) orchards; however, these studies tend to focus on low rates of biochar application in established orchards (Ventura et a., 2013a; Ventura et a., 2013b). Few studies address using higher rates of biochar during orchard establishment (Eyles et al., 2015), and those that do have not addressed the effects of biochar on soil physiochemical properties and microbial community composition and activity.

In depleted soils, biochar application increased soil mineral nutrients, cation exchange capacity (CEC), pH, soil organic matter (OM), total organic carbon (TOC), soil water holding capacity, and plant yield, of agricultural crops such as oats (*Avena sativa* L.) and maize (*Zea mays*), and native grasses, forbs, and legumes, and reduced soil tensile strength (Liang et al., 2006; Major et al., 2010 Schulz and Glaser, 2012; Xiao et al., 2016). Fewer studies have addressed the effects of biochar applications in more fertile soils or in perennial systems, and reports regarding the effects of biochar on plant yield and soil health are conflicting (Spokas et al., 2012). For example, Rajkovich et al. (2012) found that biochar amendments had no effect on maize biomass or soil fertility in an Alfisol soil. Similarly, Tammeorg et al. (2014) observed that biochar applications in a fertile sandy clay loam soil did not increase the biomass of wheat (*Triticum aestivum*), turnip rape (*Brassica rapa*), or faba bean (*Vicia faba*). However, in studies conducted in fertile sandy and silt loam soils, biochar increased the biomass of plants, such as common beans (*Phaseolus vulgaris* L.) and pepper (*Capsicum annum* L.), and increased soil fertility properties, such as mineral nutrition, including soil P, K, and Ca, CEC, TOC, and

reduced soil tensile strength and N leaching (Rondon et al., 2007; Chan et al., 2007; Revell et al., 2012; Scharenbroch et al., 2013; Ventura et al., 2013a). Additionally, biochar applications made at planting increased sugar maple (*Acer saccharum* Marsh.) and honey locust (*Gleditsia triacanthos*) biomass (Scharenbroch et al., 2013). These results are not uniform for all tree species. Responses may also differ between managed and unmanaged ecosystems. In newly planted and established apple orchards, applications of biochar did not increase tree growth, yield, or leaf mineral nutrition, but applying biochar in conjunction with compost increased tree growth significantly (Ventura et al., 2013a; Eyles et al., 2015).

Differences in plant growth and soil quality may also be due to the physiochemical characteristics of the biochar amendments, which depend on feedstock, maximum heating temperature, pyrolysis heating rate, treatment length at maximum temperature and particle size, and soil characteristics, such as OM, CEC, and mineral nutrient content (Rajkovich et al., 2012; Sun et al., 2012). Plant growth differences may also depend on application rate. However, applying some biochars, such as those made from chicken litter feedstock, at high rates increases soil pH and soluble salts, resulting in reduced plant productivity (Rajkovich et al., 2012; Revell et al., 2012). Thus, it is important to know biochar feedstock and production methods prior to agronomic application.

Biochar may be used as a method of C sequestration and reduce soil C emissions due to its recalcitrant nature (Lehmann et al., 2006). Apple orchards already serve as a net C sink, sequestering an average of 14 Mg C ha^{-1} $year^{-1}$ (Wu et al., 2012). Depending upon soil type, biochar may enhance soil C sequestration in orchard systems. Lehmann et al. (2006) notes that applications of biochar can increase soil C sequestration by up to 150% in oxisol soils. Although adding biochar has the potential to increase C sequestration in apple orchards, more research is

needed to understand how biochar applications will impact tree growth and nutrition, soil quality, and soil microbial community structure and activity. Agricultural management techniques, such as the application of synthetic N fertilizers, may increase microbial degradation of biochar, resulting in reduced C sequestration (Schulz and Glaser, 2012). The ability to use biochar to sequester C is also dependent on biochar-C stability, which depends on environmental temperatures, age, feedstock, and pyrolysis method and temperature (Nguyen et al., 2010; Singh et al., 2012). Studies suggest between 0.1 and 27% of biochar C can be mineralized (Nguyen et al., 2010; Singh et al., 2012). Biochar can also have a C priming effect, which is defined as a short-term acceleration in soil organic C mineralization due to the addition of labile C source (Kuzyakov et al., 2000). Luo et al. (2011) observed increased mineralization of native soil OM and labile biochar C following biochar applications. In other studies, biochar applied at a rate of 2% w/w had a negative priming effect, reducing C mineralization (Prayogo et al., 2013; Keith et al., 2015).

Additionally, biochar can alter soil microbial community diversity, microbial biomass C and N, and activity, such as respiration and N mineralization, through sorption of compounds that alter microbial activity, or affect plant-microbe interactions through the alteration of pH and mineral nutrient availability or the creation of new microbial habitats (Grossman et al., 2010; Dempster et al., 2012; Spokas et al., 2012 Luo et al., 2013; Masiello et al., 2013; Xu et al., 2016). These changes may impact plant productivity, health, and nutritional status. Much like the effects of biochar on plant productivity and soil health, the effects of biochar on microbial communities are highly variable, and most likely affected by biochar production methods. Grossman et al. (2010) found that biochar amended soils had significantly different bacterial community structure than unamended adjacent soils, and community structure was similar among the

biochar soils. Shifts in microbial communities following biochar application have also been associated with an increase in soil bacterial species known to be plant growth promoters and biocontrol agents (Graber et al., 2010). These microbial community changes, in addition to chemical sorptive quality of biochar, may reduce incidences and severity of replant disease in newly planted fruit tree orchards. Atucha and Litus (2015) found that applications of biochar increased peach tree trunk growth and root biomass under replant conditions. Increased microbial respiration and enzymatic activity following application has been associated with the labile portion of biochar. Despite this, increased enzymatic activity has not been associated with positive changes to N cycling dynamics, such as increasing soil potentially mineralizable N (PMN), or microbial biomass N (Chan et al., 2006; Ventura et al., 2013a; Ventura et al., 2013b; Anderson et al., 2014).

The study described within this chapter aimed to: 1) assess the effects of high biochar application rates on apple tree growth, biomass, and leaf mineral nutrition, 2) determine the effects of biochar on orchard soil physiochemical properties, and 3) characterize the effects of biochar application on microbial community structure, biomass, and activity. I hypothesized that biochar would increase tree growth and leaf mineral nutrition, increase soil cation exchange capacity and TOC, and stimulate microbial community activity, and change microbial community structure due to its physiochemical properties.

3.3 Materials and Methods

In 2013, a pot-in-pot study was implemented using 'Brookfield Gala'/'Malling 9' trees at the Virginia Tech Alson H. Smith, Jr. Agricultural Research and Extension Center in Winchester, VA (39° 06' N, 78° 17' W). Bench grafted trees were planted in 38 L pots containing native Poplimento silt loam orchard soil, a fine, mixed, subactive, mesic Ultic

Hapludalf (NRCS 2001) mixed with 10% (v/v) Stalite® (Salisbury, NC), a heat expanded slate lightweight aggregate, to improve soil drainage and aeration. A cement mixer was used to mix native orchard soil with Stalite®. Trees received one of five treatments: (1) untreated control (0BC), (2) calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), (3) 50 Mg biochar ha^{-1} (50BC+N), (4) 100 Mg biochar ha^{-1} (100BC+N), and (5) 200 Mg biochar ha^{-1} (200BC+N). The biochar used in this experiment was CQuest fast pyrolysis char produced from wood waste by Dynamotive Energy Systems (Richmond, Canada), a now defunct renewable energy company. Prior to biochar application, mineral nutrient analysis, total C, and total N were conducted at the Cornell Nutrient Analytical Lab (Ithaca, NY) (Table 3.1). A single application of biochar occurred in May 2013. Following application, a hand trowel was used to work soil to a depth of 10 cm. Nitrogen was applied through fertigation with 40 kg N ha^{-1} in the form of $Ca(NO_3)_2$ over 8 weeks beginning in late May and ending in June each year. Trees were irrigated using a micro-spray irrigation system three times a week throughout the spring and summer, hand-weeded when necessary, and uniformly treated for pests and diseases as per regional recommendations (Pfeiffer et al., 2015). Flowers were removed from young trees to prevent fruit set and reduce disease susceptibility. In 2015, gravimetric water content of the pots was determined using the method described by Owen (2007).

Trunk cross sectional area (TCSA) was calculated by measuring the caliper diameter 10 cm above the soil line at planting, and marked with paint in order to measure the tree at the same place in 2014 and 2015. In 2015, trees were destructively harvested to determine biomass. Trees were stripped of leaves in 2015. Leaves were placed in paper bags and oven-dried at 80 °C for 3 d. In 2015, each tree was removed from the pot, and soil was washed from the root system. Trees were cut into five segments: roots, leader, above ground rootstock shank, branches, and below

ground rootstock shank, and oven dried at 80 °C for 5 d until constant mass was achieved. The dry weight of the leaves and segments was recorded. Total tree biomass was determined by summing the dried tree segments for each tree.

Leaf samples for mineral nutrient analysis were collected in Aug. 2014 and 2015. Twenty-five leaves were removed from each tree, placed in paper bags, and oven dried at 80 °C for 3 d. Leaf mineral nutrient analysis was performed at the Penn State Agricultural Analytical Services Laboratory (University Park, PA). Concentrations of P, K, Ca, Mg, Mn, Fe, Cu, B, Al, Zn, and Na were measured using a 730-ES Optical Emission Inductively Coupled Plasma (OES-ICP) Spectrometer (Agilent Technologies, Santa Clara, CA) using the ICP-dry ash method (Miller, 1998). Leaf N concentration was determined using the combustion analysis method (Horneck and Miller, 1998) on a Vario Max N/C Analyzer (Elementar, Hanau, Germany).

Soil samples were collected on 26 June 2013, 24 Sept. 2013, 3 Sept. 2014, and 24 Aug. 2015. Due to the presence of Stalite® in the soil, we were unable to use a traditional soil probe or auger to collect soil samples. Soil was collected 15 cm from the trunk in the north, south, east, and west directions to a depth of 10 cm using a Hori-Hori Japanese soil knife. Soil was placed in a bag and homogenized by hand. A subsample of homogenized soil was stored at -80 °C for microbial community analysis. Soil samples for biological and physiochemical analyses were passed through a US number 10 soil sieve (2 mm mesh) and stored at 4 °C.

Soil pH, mineral nutrients and cation exchange capacity (CEC) were measured at the Virginia Tech Soil Testing Laboratory (Blacksburg, VA). Prior to analysis, samples were air-dried and crushed with a hammer mill-type crushing machine prior to analysis (Agvise, Benson, MN). A pH meter (WP-80D, TPS Pty Ltd., Springwood, Australia) fitted with a combination pH electrode (Orion model 8165BNWP Ross Sure-Flow, ThermoFisher, Waltham, MA) was used to

measure soil pH. The mineral nutrients P, K, Ca, Mg, Zn, Mn, Cu, Fe, B, and Al were extracted from 4 cm^3 of soil with Mehlich 1 solution. Concentrations of the extracted mineral nutrients was determined by OES-ICP (Acros Spectro, Mahwah, NJ). Cation exchange capacity (CEC) was estimated by addition of the non-acid generating cations (Ca, Mg, and K) and Mehlich 1soil-buffer acidity. Soil OM and soluble salts (SS) were measured at the Virginia Tech Soil Testing Laboratory in 2013 and 2014, and at the Cornell Nutrient Analytical Lab in 2015. An electrical conductivity probe (3100 Conductivity Instrument, YSI, Yellow Springs, OH) was used to measure SS. Soil organic matter was measured using the loss on ignition method. Initial soil dry mass was determined by heating soil to 150°C in an electric high-temperature forced air drying oven (Blue M model CW-6680F, New Columbia, PA) for 2 h. This soil was then heated to 360 °C for 2 h, and weighed again to determine organic matter content. Total C and total N were measured at the Cornell Nutrient Analytical Lab on a CHN Elemental Analyzer-vario EL (Elementar) after soil was ground to a fine powder using a mortar and pestle.

For each sample, 50 g of soil was placed in an air-tight jar. A glass vial containing 20 mL of 0.5 sodium hydroxide (NaOH) solution, placed inside the jar, trapped evolved CO_2 gas. The electrical conductivity of the NaOH solution was measured weekly for six weeks using an electrical conductivity meter (Amber Science Inc., Eugene, OR). To determine the concentration of trapped CO_2, electrical conductivity of the samples was compared to a blank containing 50 g of autoclaved, dried sand and a CO_2 saturated standard (Rodella and Saboya, 1999).

In 2015, soil microbial biomass C and N was extracted using the direct chloroform ($CHCl_3$) fumigation extraction method (Fierer and Schimel, 2003). For both fumigated and unfumigated samples, 10 g of soil from each sample and 40 mL of 0.05 M potassium sulfate (K_2SO_4) were placed into 70 mL glass vials with Teflon-lined lids. Fumigated samples were

treated with 0.5 mL of amylated $CHCl_3$. All samples were placed on an orbital shaker for 4 h at 2.5 $r \cdot s^{-1}$. After samples settled for 30 min, they were decanted into 50 mL conical tubes, and centrifuged for 10 min at 500 g_n. Samples were filtered (FisherBrand G6, Fisher, Waltham, MA), and sparged with compressed N_2 gas for 20 min to remove any remaining $CHCl_3$ from the solution, and stored at -20 °C until use. Blank samples with no soil were prepared in the same manner. Samples were diluted 1:2 (volume:volume) with deionized water prior to analysis. Total organic carbon and total dissolved nitrogen were quantified using a carbon analyzer with an auto-sampler (TOC-VCPH+TNM-1, Shimadzu, Columbia, MD) using high-temperature oxidation catalyzed with platinum-coated alumina beads (temperature 720 °C) in non-purgeable organic carbon mode (Bird et al., 2003). Non-purgeable organic C was measured using a non-dispersive infrared detector and chemiluminescence for N. Each sample was run in triplicate. Microbial biomass C was calculated by multiplying the difference between the fumigated and unfumigated samples by the k_{ec} value of 0.45 (Joergensen, 1996). Microbial biomass N was calculated by multiplying the difference between the fumigated and unfumigated samples by the k_{en} value of 0.54 (Brookes et al., 1985).

Potentially mineralizable nitrogen (PMN) was determined by placing 10 g of soil in two separate 50 mL conical tubes. The first tube was used to make an initial measurement of soil N. After soil was placed in the tube, 40 mL of 2 M KCL was added, and the samples were placed on the orbital shaker at 3.3 rs-1 for 1 h. Samples were centrifuged for 10 min at 500 g_n and filtered (FisherBrand G6). Ten grams of soil and 10 mL of water (W7-4 Optima Water, Fisher) were added to the second 50 mL tube. Samples were incubated at 30°C for 7 d. Following incubation, 30 mL of 2.67 M KCl were added to the samples. Samples were then shaken at 3.3 rs-1 for 1 h, centrifuged at 500 g_n for 10 min and filtered. Samples were stored at -20 °C until NH_4^+ and NO_3^-

concentrations were analyzed using a Lachat QuickChem 8500 Series 2 Flow Injection Analysis System (Loveland, CO). The concentration of NH_4^+ was measured using Lachat protocol #12-107-06-2-A, and NO_3^- was measured using Lachat protocol #12-107-04-1-B (Lachat Instruments, 2014). Potentially mineralized nitrogen rate was calculated as the difference in the NH_4^+ concentration between the 7-d incubation measurement and initial measurement.

Changes to bacterial and fungal soil community structure were assessed using the terminal-restriction length polymorphism (T-RFLP) method. A modified protocol was followed to extract total genomic DNA using the MoBio Lab Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA). Approximately 0.25 g of soil were placed in the bead tube provided by MoBio, and heated to 65 °C for 10 min in a water bath. Following this modification, the manufacturer's protocol was followed. The quality and concentration of DNA was determined using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, Massachusetts). DNA was stored at -20°C until duplicate polymerase chain reaction (PCR) amplifications were performed in 50 µL reaction volumes for each sample. Bacterial community DNA was amplified in reactions containing 1 X GoTaq PCR Flexi Buffer (Promega, Madison, WI), 2.5 U GoTaq G2 Flexi DNA Polymerase (Promega), 2 mM $MgCl_2$ solution (Promega), 0.2 mM dNTPs, 0.1 µg μL^{-1} bovine serum albumin (BSA) (New England BioLabs, Ipswich, MA), 0.1 µM fluorescently labeled 27f forward primer (5'-[6FAM] AGA GTT TGA TCC TGG CTC AG-3'), 0.1 µM 1492r reverse primer (5'-GGT TAC CTT GTT ACG ACT T-3'), 40 ng template DNA, and nuclease free water (Qiagen, Hilden, Germany). The reaction conditions for this PCR were a 5 min denaturation step at 95 °C, followed by 27 cycles of 95 °C for 45 s, 56 °C for 45 s, and 72 °C for 1 min, a final extension step of 72 °C for 10 min followed. Fungal community DNA was amplified in reactions containing 1 X GoTaq PCR Flexi Buffer (Promega, Madison, WI), 5 U GoTaq G2 Flexi DNA

Polymerase (Promega), 3 mM $MgCl_2$ solution (Promega), 0.2 mM dNTPs, 0.1 μg μL^{-1} bovine serum albumin (BSA) (New England BioLabs), 0.1 μM fluorescently labeled ITS1f forward primer (5'-[6FAM] CTT GGT CAT TTA GAG GAA GTA A-3'), 0.1 μM ITS4r reverse primer (5'-TCC TCC GCT TAT TGA TAT GC-3'), 40 ng template DNA, and nuclease free water (Qiagen). The reaction conditions for this PCR were a 5 min denaturation step at 94 °C, 30 cycles of 94 °C for 30 s, 51 °C for 45 s, and 72 °C for 45 s, followed by a final extension step of 72 °C for 10 min. Reactions were amplified in a Bio-Rad C1000 Thermal Cycler (Hercules, CA). After amplification, PCR products were visualized on a 1.5% agarose gel in 1 X tris borate EDTA buffer (TBE) stained with GelStar (Lonza, Basel, Switzerland).

Duplicate PCR products were pooled and quantified against a calf thymus DNA standard Curve in an ethidium bromide solution (20 μg in 1 X TBE) using a FOTO/Analyst Investigator gel imaging system (Fotodyne, Hartland, WI) with Quantity One software version 4.4.0 (Bio-Rad, Hercules, CA). Bacterial DNA (600 ng) and fungal DNA (150 ng) were digested in a 50 μL reaction containing 15 U Sau96I (New England BioLabs), 1 X CutSmart Buffer (New England BioLabs), and nuclease free water (Qiagen, Hilden, Germany) at 37 °C for 4 h followed by a restriction enzyme denaturation step at 70 °C for 20 min. Digestion products were visualized on a 2% agarose gel in 1 X TBE stained with GelStar. A purification reaction was performed on digests using Performa DTR plates (EdgeBio, Gaithersburg, MD). Samples were then condensed in a CentriVap Centrifugal Concentrator System (Labconco, Kansas City, MO), and resuspended in 9.5 μL Hi-Di Formamide (Applied Biosystems, Foster City, CA) and 0.5 μL GeneScan 1200 LIZ dye size standard (Applied Biosystems). Fragment size was determined using an Applied Biosystems 3730xl DNA Analyzer at the Cornell University Institute of Biotechnology (Ithaca,

NY). PeakScanner software v1.0 (Applied Biosystems) was used to determine fragment peaks size, height, and area.

Trunk cross sectional area and soil microbial respiration data were analyzed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC). For trunk cross sectional area, the fixed effect was Treatment. For microbial respiration, fixed effects were Treatment and Day. Differences were determined at a $p = 0.05$ level using the least significant differences (LSD). Tree biomass, leaf mineral nutrition, soil fertility, PMN, and microbial biomass C and N data were analyzed using analysis of variance (ANOVA) and Tukey's Honestly Significant Differences (HSD) post hoc test in JMP Pro 11 (SAS Institute Inc., Cary, NC). Significant differences were determined at a $p = 0.05$ level. Slices were used to analyze the interaction effects within the Treatment x Year interactions when interactions were significant.

Terminal-restriction fragment length polymorphisms were analyzed using the T-RFLP Analysis Expedited (T-REX) online software (Culman et al., 2009). This software uses the presence or absences of terminal restriction fragments (T-RFs) of a known base pair length and treatments to construct an additive main effects multiplicative interaction (AMMI) model. The AMMI model, sometimes referred to as a doubly centered principal components analysis (PCA), uses an ANOVA to partition the variation into main effects (T-RFs and treatments) and interactions; PCA is then applied to the interactions to create an interaction principal components analysis. Culman et al. (2008) demonstrated that AMMI is robust analysis for T-RFLP data from a variety of environments.

Table 3. 1. Analysis of biochar pH, organic matter (OM), total N, total C and mineral nutrient content.

	pH	OM (g kg^{-1})	N (g kg^{-1})	C (g kg^{-1})	C:N	B (mg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	P (mg kg^{-1})
CQuest Biochar	6.83	500	3	620	222	3	1,798	1,487	110	81	52

3.4 Results

Soil where the 100BC+N and 200BC+N treatments were applied had greater water holding capacity than the 0BC and 0BC+N treatments (Table 3.2). In September of 2015, trees that received N fertigation had larger TCSAs than 0BC trees. The TCSA of trees that received 200BC+N or 0BC+N were respectively 65% and 45% larger than trees that received the 0BC treatment (Fig. 3.1). Trees that received biochar treatments had greater total tree and leader biomass compared to the 0BC treatment (Table 3.3). The 200BC+N treatment increased tree biomass 47% compared to the control, and increased leader mass by 85%. Leaf biomass was 74% larger from the 200BC+N trees than the 0N trees.

In 2014, there were no differences in leaf N concentration among treatments (Table 3.4). However, 0BC+N and 50BC+N treatment trees had greater leaf N than the 0BC control in 2015. In 2014, trees that received the biochar treatments had greater leaf K concentrations than the 0BC treatment. However, there were no leaf K concentration differences in 2015. In 2014, all biochar treatments increased leaf Ca concentration compared to the 0BC treatment; however, this trend did not continue in 2015. In 2014, trees that received the 200BC+N treatment had increased leaf B concentration by 22% compared to 0BC+N treatments; however, there were no differences in leaf B in 2015.

The effects of biochar treatments on soil mineral content became apparent three months after application (Table 3.5). In 2013, soil B concentration was greater in 200BC+N treated soils than in the 0BC and 0BC+N soils. However, in 2014, soil B was lower in 50BC+N and 100BC+N treated soils than in the control soils. In 2013 and 2014, the 100BC+ N and 200BC+N treatments increased soil K concentration compared to the 0BC and 0BC+N treatments. In 2014 and 2015, biochar treatments affected soil Ca, Mg, and Zn. The 200BC+N treatment increased soil Ca and Mg concentrations compared to 0BC, 0BC+N, and 50BC+N treatments. The 200BC+N treatment decreased soil Zn compared to the control.

Biochar treatments altered soil pH, OM, CEC, total C, and C:N (Table 3.5). Throughout this study, 100BC+ N and 200BC+N treatments reduced soil pH compared to the control. Biochar applications increased OM compared to 0BC and 0BC+N in 2013 and 2014. In 2015, 100BC+ N and 200BC+N increased OM compared to all other treatments. Soil that received the 200BC+N treatment had 173%, 167%, and 137% more OM than the control in 2013, 2014, and 2015 respectively. Organic matter was not statistically compared among years; however, OM decreased from 2013 to 2015 by 68% and 39% in the 200BC+N and 100BC+N treatments, respectively. The 100BC+ N and 200BC+N treatments increased CEC compared to 0BC and 0BC+N throughout the study. The 200BC+N treatment increased CEC by 43%, 42%, and 51% compared to 0BC and 0BC+N in 2013, 2014, and 2015 respectively. Throughout this study, the 100BC+ N and 200BC+N treatments increased total C and, correspondingly, the C:N ratio.

Despite treatment based differences in OM and soil C:N, there were no differences in microbial biomass C among treatments (Table 3.6). However, microbial biomass N was lower in the 0BC and 200BC+N than the 50BC+N treatments. There were differences in PMN in 2014 and 2015. The 100BC+N and 200BC+N biochar applications reduced PMN compared to the

0BC and 0BC+N treatments. The 200BC+N treatment reduced the amount of PMN by 100% in 2014 and 110% in 2015 compared to the 0BC control treatment. The lowest biochar treatment, 50BC+N, also reduced PMN by 57% compared to the 0BC treatment in 2015 (Table 3.7). Biochar treatments had significant effects on soil microbial respiration throughout this study (Fig. 3. 2). In 2013, the cumulative microbial respiration was greater in the 50BC+N, 100BC+ N, and 200BC+N treatments compared to the 0BC and 0BC+N treatment. The 50BC+N, 100BC+ N, and 200BC+N treatments had respiration rates 1.5, 2, and 3.5 times greater than the 0BC and 0BC+N treatments. Microbial respiration results were similar for 2014 and 2015. The 100BC+ N and 200BC+N treatments increased microbial respiration compared to 0BC and 0BC+N; however, the 50BC+N treatment did not have a significant effect on microbial respiration. In both years, the 100BC+ N and 200BC+N treatments had respiration rates 1.7 and 3 times greater than the 0BC treatment.

Amplification and digestion produced 225 unique bacterial T-RFs and 576 unique fungal T-RFs from soil samples collected over three years. The ANOVA produced through AMMI analysis indicates that biochar treatments did not significantly alter soil bacterial or fungal community composition during this study (Table 3.8). However, T-RFs grouped by sampling date for both bacteria and fungi on the IPCA graph (Fig. 3.5-6). Bacterial and fungal T-RFs from soil samples collected June 2013 clustered along the ICPA3 axis, and were separated from communities sampled at the other time points. Bacterial T-RFs from samples collected in August 2013 were less tightly clustered than those from samples collected in August 2014 and August 2015. Fungal T-RFs from samples collected in August 2013 and August 2014 clustered tightly, while samples collected in August 2015 clustered tightly along the negative ends of the ICPA1 and ICPA2 axes. For bacterial samples, ICPA1, ICPA2, and ICPA3 captured 61%, 25%, and 7%

interaction signal variation, respectively. The remaining variation was captured by ICPA4. For fungal samples, IPCA1, ICPA2, ICPA3, and ICPA4 captured 37%, 26%, 20%, and 14% of the interaction signal variation, respectively.

Table 3. 2. The effect of biochar treatment on gravimetric soil water content of the pots was determined in the spring of 2015. Data presented here are the means from four replications of each treatment, untreated control (0BC), calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), 50 Mg biochar ha^{-1} (50BC+N), 100 Mg biochar ha^{-1} (100BC+N), and 200 Mg biochar ha^{-1} (200BC+N).

Treatment	Water (L) (±SEM)[y]
0BC	13.4 (3) C[z]
0BC+N	11.0 (1) C
50BC+N	17.9 (1) BC
100BC+N	23.4 (2) AB
200BC+N	30.5 (2) A
	$p < 0.0001$

[y]SEM is the standard error of the mean

[z]Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.

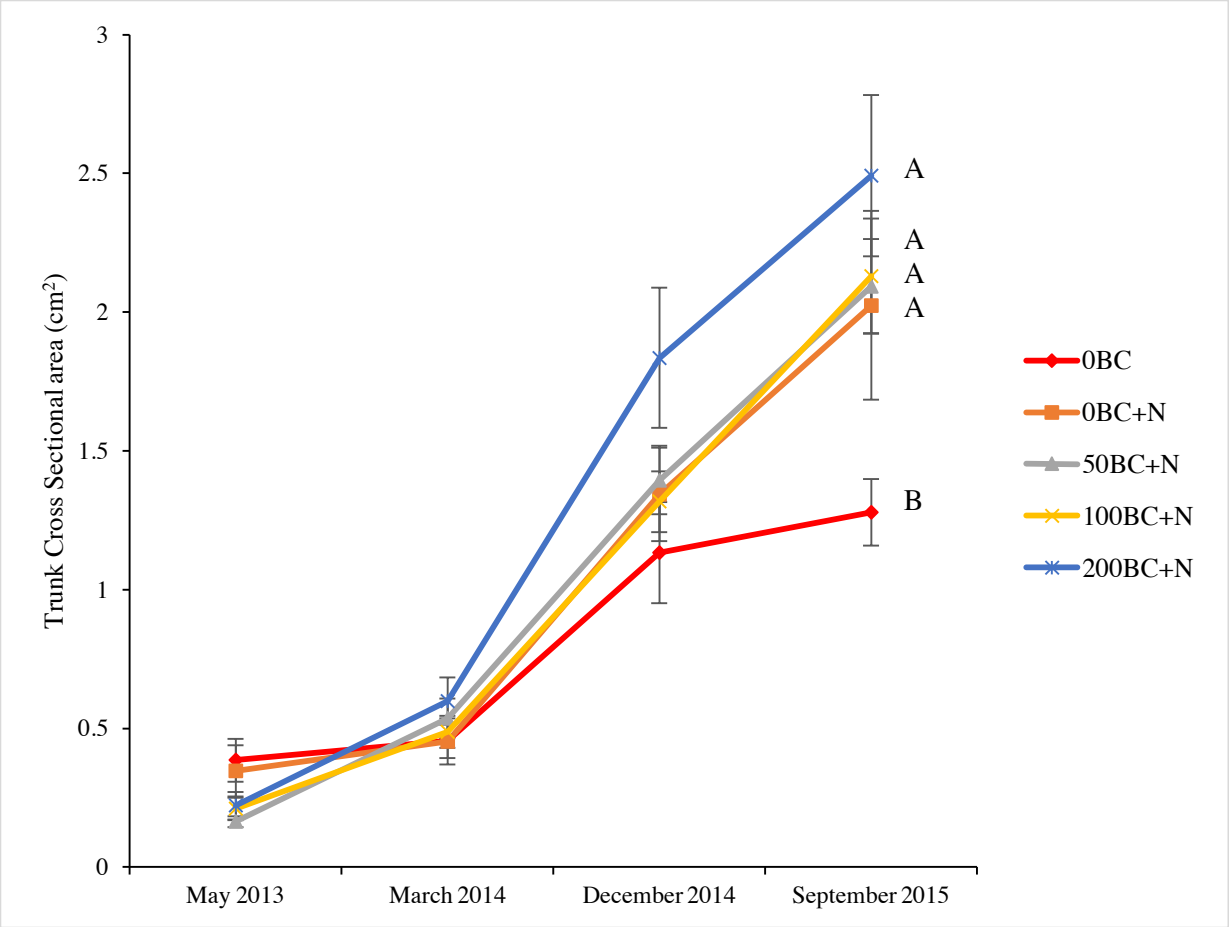

Fig. 3. 1. Tree growth was measured as trunk cross sectional area at planting, and following fertilizer applications in the spring of 2014, the winter of 2014 and the fall of 2015 in Winchester, VA. Data shown represent the mean of 4 trunk cross sectional area measurements per treatment, untreated control (0BC), calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), 50 Mg biochar ha^{-1} (50BC+N), 100 Mg biochar ha^{-1} (100BC+N), and 200 Mg biochar ha^{-1} (200BC+N), calculated from the trunk caliper diameter. Error bars represent the standard error of the mean. Different letters indicate means separation at $p \leq 0.05$ level of significance of the least significant differences test.

Table 3. 3. Tree biomass was determined by destructive harvest in October of 2015. Trees were divided into the following segments: roots, leader, shank, branches, root shank, and leaves. Total mass was determined through summation of the tree segments. Data shown represents the mean dry biomass of 4 replicates per treatment, the untreated control (0BC), calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), 50 Mg biochar ha^{-1} (50BC+N), 100 Mg biochar ha^{-1} (100BC+N), and 200 Mg biochar ha^{-1} (200BC+N).

Treatment	Roots (g) (±SEM)[y]	Leader (g) (±SEM)	Above Ground Rootstock Shank (g) (±SEM)	Branches (g) (±SEM)	Below ground rootstock shank (g) (±SEM)	Leaf (g) (±SEM)	Total mass (g) (±SEM)
0BC	50.9 (9.7)	13.3 (0.6) B[z]	18.1 (1.1)	3.2 (0.7)	21.7 (1.6)	4.6 (0.8) B	111.8 (11.0) B
0BC+N	60.9 (8.4)	27.9 (3.6) A	23.3 (2.1)	3.9 (0.8)	25.2 (4.5)	6.9 (1.1) AB	148.3 (12.0) AB
50BC+N	67.5 (3.5)	31.1 (1.0) A	24.5 (2.1)	4.9 (0.6)	24.6 (1.1)	8.5 (2.1) AB	160.9 (4.9) A
100BC+N	71.9 (6.3)	29.9 (4.4) A	23.9 (2.2)	5.2 (0.3)	20.9 (2.4)	8.2 (1.4) AB	160.1 (2.9) A
200BC+N	79.9 (5.5)	33.1 (1.8) A	23.2 (3.4)	5.8 (1.2)	29.1 (3.9)	10.0 (0.8) A	181.2 (13.2) A
	p = 0.08	p = 0.008	p=0.3	p = 0.2	p = 0.3	p = 0.03	p = 0.002

[y]SEM is the standard error of the mean

[z]Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.

Table 3. 4. Leaf nutrient analysis was performed on 25 leaves collected from each of the four sample tree in August of 2014 and 2015. Data presented here are the means from four replications of each biochar treatment, untreated control (0BC), (2) calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), (3) 50 Mg biochar ha^{-1} (50BC+N), (4) 100 Mg biochar ha^{-1} (100BC+N), and 200 Mg biochar ha^{-1} (200BC+N).

Year	Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})
2014	0BC	1.7	0.158	1.5 B	1.63 A	0.13 B	110	52	4.3	38 AB	44	117	7.8 A
2014	0BC+N	1.9	0.145	2.1 AB	1.15 AB	0.27 A	102	46	5.5	33 B	25	60	5.8 AB
2014	50BC+N	1.9	0.138	2.3 A	1.01 B	0.22 AB	77	48	5.3	35 AB	28	74	5.3 B
2014	100BC+N	19	0.140	2.4 A	0.96 B	0.21 AB	92	52	5.5	40 AB	32	76	5.8 AB
2014	200BC+N	1.9	0.135	2.4 A	1.04 B	0.22 AB	76	49	4.8	41 A	30	96	6.3AB
2015	0BC	1.3 B	0.205	1.5	1.12	0.22	42	50	3.5	35 A	41	76	8.0
2015	0BC+N	1.6 A	0.118	1.4	1.34	0.22	40	50	3.8	26 B	39	62	6.8
2015	50BC+N	1.6 A	0.123	1.7	1.21	0.22	38	50	4.3	30 AB	36	61	6.5
2015	100BC+N	1.5 AB	0.153	1.7	1.03	0.19	42	52	4.5	30 AB	41	54	9.5
2015	200BC+N	1.5 AB	0.135	1.9	1.16	0.22	45	47	3.8	34 AB	37	61	9.8

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})
Treatment	**	ns	**	**	**	ns	ns	ns	**	*	*	ns
Year	***	ns	***	ns	ns	***	ns	**	***	**	**	**
Treatment x Year	ns	ns	ns	*	**	ns	ns	ns	ns	ns	ns	ns
Year effects within Treatment x Year												
2014				**	**							
2015				ns	ns							
Treatment effects within Treatment x Year												
0BC				**	**							
0BC+N				ns	ns							
50BC+N				ns	ns							
100BC+N				ns	ns							
200BC+N				ns	ns							

[x] Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.
* Significant at $p \leq 0.05$
** Significant at $p \leq 0.01$
*** Significant at $p \leq 0.0001$

Table 3. 5. Soil pH, Mehlich 1 extractable mineral nutrients, organic matter, soluble salts, cation exchange capacity, total nitrogen, total carbon, and the carbon to nitrogen ratio were measured from soil samples collected to a depth of 10 cm 15 cm from the trunk in August of 2013, 2014, and 2015. Data presented here are the means from four replications of each biochar treatment, untreated control (0BC), calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), 50 Mg biochar ha^{-1} (50BC+N), 100 Mg biochar ha^{-1} (100BC+N), and 200 Mg biochar ha^{-1} (200BC+N).

Year	Treatment	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	OM (g kg^{-1})	SS (mmhos cm^{-1})	CEC (meq $100g^{-1}$)	N (g kg^{-1})	C (g kg^{-1})	C:N
2013	0BC	7.4 A	4.3	41 C	845	66	8.7	26.1 AB	1.1 AB	5.1 C	0.3 B	19 D	0.13	4.9 C	2.3	22 C	9.1 C
2013	0BC+N	7.2 AB	4.5	45 C	848	68	5.3	25.6 AB	1.4 A	6.1 C	0.3 B	19 D	0.13	4.9 C	2.0	11 C	5.6 C
2013	50BC+N	7.1 AB	4.3	82 C	860	69	4.7	24.4 B	1.1 AB	5.6 C	0.4 AB	46 C	0.11	5.1 BC	1.9	31 C	16 C
2013	100BC+N	6.9 BC	5.3	146 B	960	74	8.1	34.1 AB	0.7 BC	8.4 B	0.4 AB	117 B	0.08	7.0 AB	2.0	80 B	40 B
2013	200BC+N	6.7 C	5.8	232 A	908	74	5.3	39.6 A	0.6 C	11.5 A	0.5 A	260 A	0.08	7.6 A	2.2	140 A	63 A
2014	0BC	7.7 A	4.4	40 D	1103 C	84 C	7.7 A	14.4 D	1.2 A	5.3 B	0.3 A	17 D	0.09	6.3 C	0.9	8 D	9 D
2014	0BC+N	7.7 A	4.5	38 D	1103 C	85 C	6.9 AB	15.9 CD	1.1 AB	5.8 B	0.3 AB	17 D	0.09	6.3 C	0.6	7 D	12 D
2014	50BC+N	7.5 B	4.8	60 C	1315 B	96 B	7.1 AB	23.1 BC	0.7 BC	5.4 B	0.2 B	63 C	0.09	7.5 B	0.8	5 C	65 C
2014	100BC+N	7.3 B	4.5	84 B	1478 AB	100 AB	6.3 AB	26.5 AB	0.5 C	6.3 B	0.2 B	112 B	0.08	8.4 B	0.8	91 B	115 B
2014	200BC+N	7.1 C	5.3	131 A	1666 A	110 A	5.5 B	32.5 A	0.4 C	7.7 A	0.3 AB	186 A	0.09	9.6 A	1.0	163 A	116 A

Year	Treatment	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	OM (g kg^{-1})	SS (mmhos cm^{-1})	CEC (meq 100g^{-1})	N (g kg^{-1})	C (g kg^{-1})	C:N
2015	0BC	7.8 A	3.8	40 C	1121 B	86 B	7.9 A	13.1	0.9 AB	4.7	0.3	24 C	0.10	6.4 B	2.2	8 D	4 D
2015	0BC+N	7.8 A	3.5	40 C	1125 B	83 B	7.7 AB	17.6	1.1 A	5.5	0.3	18 C	0.11	6.4 B	2.1	7 D	3 D
2015	50BC+N	7.7 AB	3.3	48 BC	1239 B	93 B	7.2 AB	14.6	0.8 BC	4.8	0.3	37 C	0.10	7.1 B	2.1	43 C	20 C
2015	100BC+N	7.4 B	3.5	68 AB	1755 A	115 A	8.0 A	17.7	0.6 CD	5	0.3	79 B	0.09	9.9 A	2.3	102 B	46 B
2015	200BC+N	7.1 C	3.0	91 A	1818 A	122 A	6.2 B	15.0	0.4 D	4.8	0.3	128 A	0.10	10.8 A	2.5	186 A	75 A
Treatment		***	ns	**	***	***	ns	***	***	***	ns	***	**	***	**	***	***
Year		***	ns	**	***	***	ns	**	*	***	***	***	**	***	***	**	***
Treatment x Year		ns	ns	**	***	**	ns	*	ns	***	***	***	*	ns	ns	**	***
Year effects within Treatment x Year																	
	2013			**	ns	ns		***		***	***	***	**			***	***
	2014			*	***	***		***		***	*	***	*			***	***
	2015			**	***	***		ns		ns	ns	***	ns			***	***

	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	OM (g kg^{-1})	SS (mmhos cm^{-1})	CEC (meq 100g^{-1})	N (g kg^{-1})	C (g kg^{-1})	C:N
Treatment effects within Treatment x Year																
0BC			ns	***	***		**		ns	ns	ns	*			ns	ns
0BC+N			ns	***	**		*		ns	ns	ns	**			ns	ns
50BC+N			**	***	***		*		ns	***	*	ns			ns	***
100BC+N			***	***	***		**		***	***	***	ns			ns	***
200BC+N			***	***	***		**		***	***	***	ns			***	***

[w] Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.
[x] OM is organic matter.
[y] SS is soluble salts
[z] CEC is cation exchange capacity.
ns, nonsignificant
* Significant at $p \leq 0.05$
** Significant at $p \leq 0.01$
*** Significant at $p \leq 0.0001$

Table 3. 6. Fertilizer treatment effects on microbial biomass C and N in 2015. Microbial biomass carbon and nitrogen were measured from soil samples collected to a depth of 10 cm 15 cm from the trunk of the center in August of 2014 and 2015. Data presented here are the means from four replicates of the 100 Mg biochar ha^{-1} (100BC+N), and 200 Mg biochar ha^{-1} (200BC+N) treatments, three replicates of untreated control (0BC) and 50 Mg biochar ha^{-1} (50BC+N) treatments, and two replicates of the calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N) treatment.

	Microbial Biomass	
Treatment	**μg C g^{-1} SDW[x] (±SEM)[y]**	**μg N g^{-1} SDW (±SEM)**
0BC	1,244 (103)	68 (11) B[z]
0BC+N	1,704 (110)	85 (1) AB
50BC+N	1,634 (138)	109 (10) A
100BC+N	1,673 (120)	84 (66) AB
200BC+N	1,404 (92)	54 (8) B
p value	0.08	0.009

[x]SDW is soil dry weight
[y]SEM is the standard error of the mean
[z] Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.

Table 3. 7. The effects of fertilizers on potentially mineralizable nitrogen was measured from soil samples collected to a depth of 10 cm 15 cm from the trunk of the center in August of 2014 and 2015. Data presented here are the means from four replications of each biochar treatment, untreated control (0BC), calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), 50 Mg biochar ha^{-1} (50BC+N), 100 Mg biochar ha^{-1} (100BC+N), and 200 Mg biochar ha^{-1} (200BC+N).

Year	Treatment	μg N g^{-1} SDW[x]
2014	0BC	197 (47) A[z]
2014	0BC+N	147 (29) AB
2014	50BC+N	89 (6) AB
2014	100BC+N	71 (8) B
2014	200BC+N	65 (6) B
		p = 0.009
2015	0BC	460 (45) A
2015	0BC+N	413 (60) AB
2015	50BC+N	256 (22) BC
2015	100BC+N	171 (30) C
2015	200BC+N	133 (18) C
		p <0.0001
	Treatment	p <0.0001
	Year	p <0.0001
	Treatment x Year	p = 0.01
	Year effects within Treatment x Year	
	2014	*
	2015	***
	Treatment effects within Treatment x Year	
	0BC	***
	0BC+N	***
	50BC+N	**
	100BC+N	*
	200BC+N	ns

[x]SDW is soil dry weight
[y]SEM is the standard error of the mean
[z]Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.

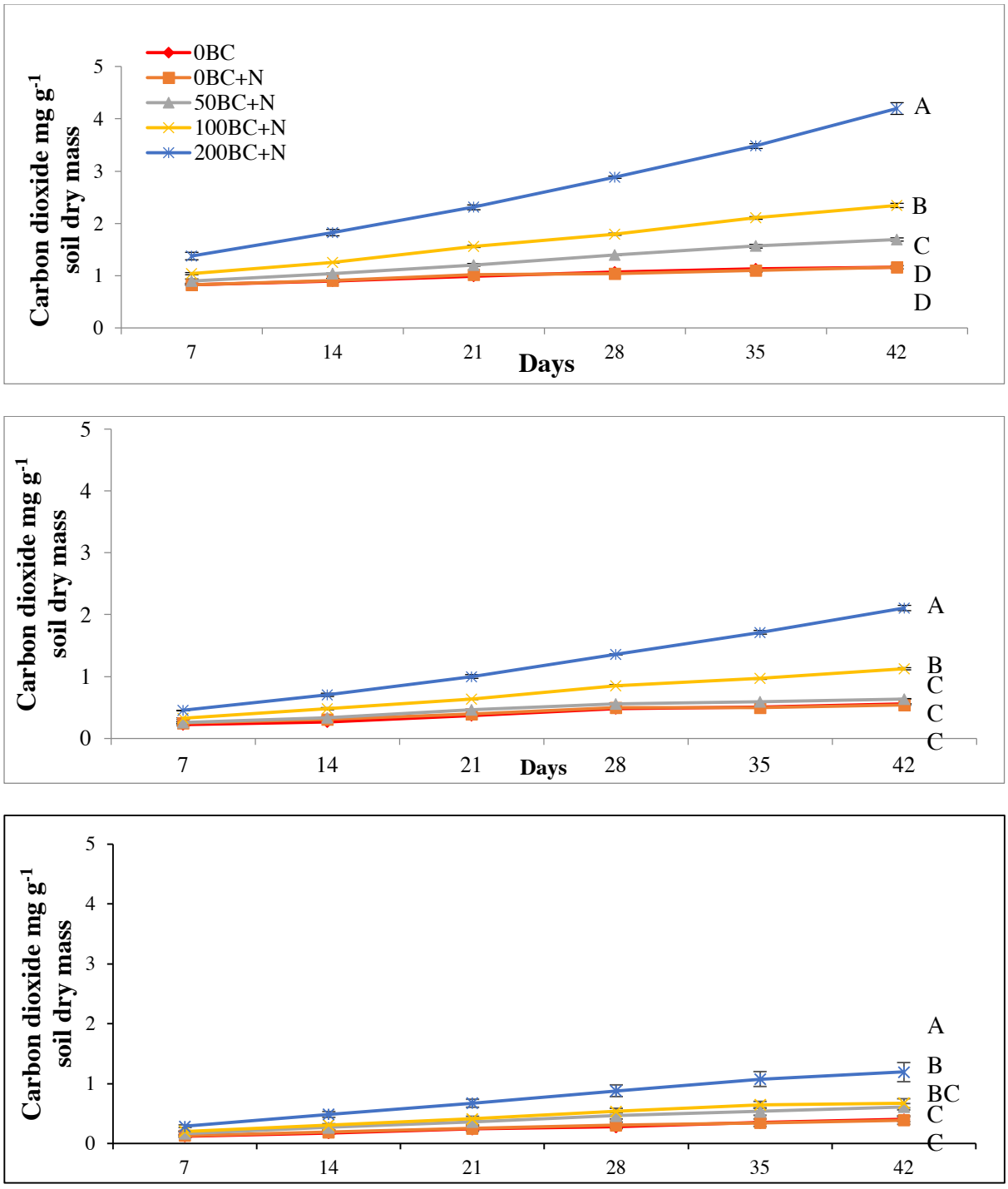

Fig. 3. 2. Soil microbial respiration was measured as the evolution of carbon dioxide from soil samples taken a depth of 10 cm 15 cm from the trunk in Aug 2013 (A), 2014 (B), and 2015 (C), and incubated for 6 weeks. Data shown here are the means from four replications of each treatment, the untreated control (0BC), calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), 50

Mg biochar ha^{-1} (50BC+N), 100 Mg biochar ha^{-1} (100BC+N), and 200 Mg biochar ha^{-1} (200BC+N). Different letters indicate means separation at $p \leq 0.05$ level of significance of the least significant differences test. Error bars represent the standard error of the mean.

Table 3. 8. ANOVA table generated by T-REX analysis of terminal-restriction length polymorphisms (T-RFLPs) produced though amplification of bacterial 16s rRNA and the fungal ITS genes from soil samples taken a depth of 10 cm 15 cm from the trunk. IPCA is the interaction principle components analysis.

Source	Degrees of Freedom	Sums of Squares	Mean Squares
Bacteria			
Total	17,627	2,843	0.16
Treatment	4,519	1,993	0.44
T-RFs	225	817	3.62
Environments	19	88	4.62
T-RFs x Environments	4,275	1,089	0.25
IPCA 1	243	494	2.03
IPCA 2	241	202	0.83
IPCA 3	239	59	0.25
IPCA 4	237	54	0.23
Residual	3,315	281	0.08
Error	13,108	849	0.06
Fungi			
Total	43,274	4,184	0.09
Treatment	11,539	2,450	0.21
T-RFs	576	1,073	1.86
Environments	19	67	3.49
T-RFs x Environments	10,944	1,311	0.12
IPCA 1	594	265	0.45
IPCA 2	592	186	0.31
IPCA 3	590	144	0.24
IPCA 4	588	98	0.17
Residual	8,580	618	0.07
Error	31,735	1,734	0.05

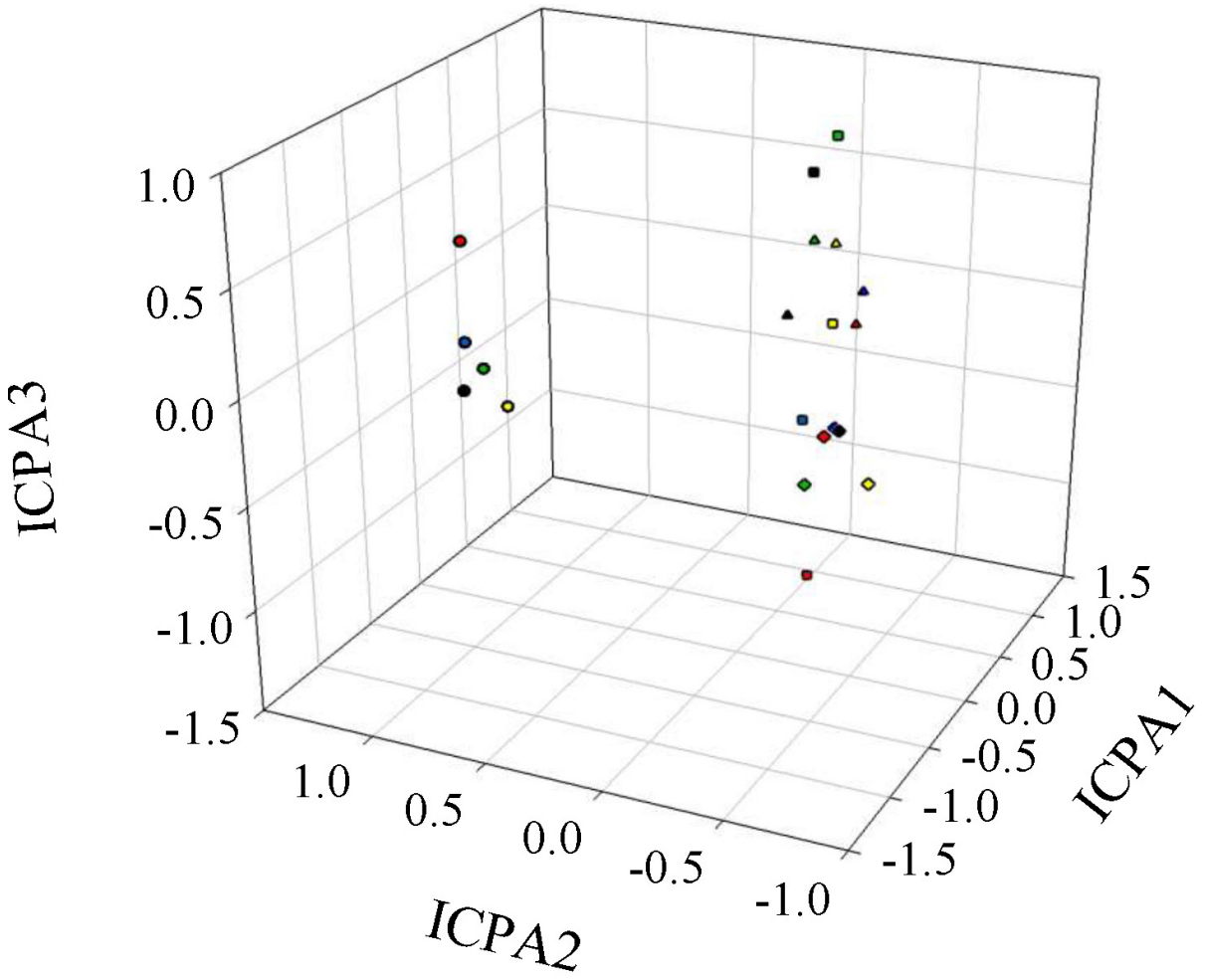

Fig. 3. 3. An interaction principal components analysis plot generated by AMMI analysis of terminal-restriction length polymorphisms (T-RFLPs) produced though amplification of the fungal ITS gene from soil samples taken a depth of 10 cm 15 cm from the trunk. Shapes represent sampling time following initial treatment application: circles = June 2013, squares = August 2013, triangle = August 2014, and diamonds = August 2015. Shape fill indicates treatment: green = the untreated control (0BC), black = calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), yellow = 50 Mg biochar ha^{-1} (50BC+N), blue = 100 Mg biochar ha^{-1} (100BC+N), and red = 200 Mg biochar ha^{-1} (200BC+N). Symbols represent the means of four replicates for each treatment.

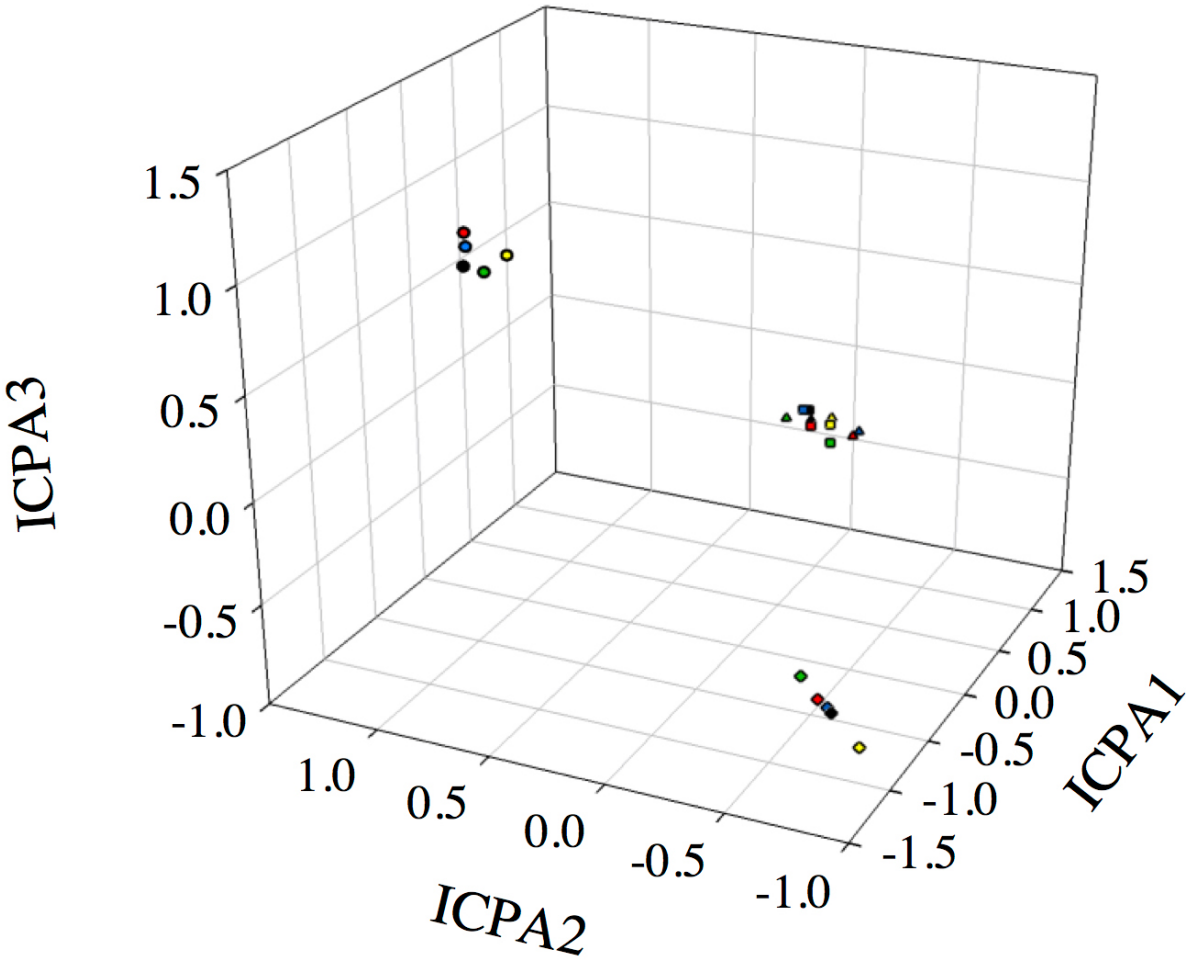

Fig. 3. 4. An interaction principal components analysis plot generated by AMMI analysis of terminal-restriction length polymorphisms (T-RFLPs) produced though amplification of bacterial 16s rRNA gene from soil samples taken a depth of 10 cm 15 cm from the trunk. Shapes represent sampling time following initial treatment application: circles = June 2013, squares = August 2013, triangle = August 2014, and diamonds = August 2015. Shape fill indicates treatment: green = the untreated control (0BC), black = calcium nitrate [$Ca(NO_3)_2$] at 40 kg N ha^{-1} (0BC+N), yellow = 50 Mg biochar ha^{-1} (50BC+N), blue = 100 Mg biochar ha^{-1} (100BC+N), and red = 200 Mg biochar ha^{-1} (200BC+N). Symbols represent the means of four replicates for each treatment.

3.5 Discussion

Biochar applied in conjunction with 40 kg ha^{-1} N increased tree growth and total biomass similarly to yearly applications on N fertigation at a rate of 40 kg ha^{-1} (0BC+N) compared to the unfertilized control (0BC). The 100 Mg ha^{-1} and 200 Mg ha^{-1} biochar application rates were much greater than the agronomic rates, which are usually between 10 and 50 Mg ha^{-1}, used in fruit tree studies (Ventura et al., 2013a; Ventura et al., 2013b; Eyles et al., 2015; Atucha and Litus, 2015). Applying high rates of biochar in annual horticultural crop systems has been shown to increase pH and SS, resulting in decreased plant yield (Revell et al., 2012). In this study, biochar application did not increase soil pH or SS. Despite high biochar application rates, differences in TCSA were not apparent until year three of this study. Eyles et al. (2015) reported similar increases in growth for newly planted apple trees three years after planting when biochar was applied at 47 Mg ha^{-1}. In a pot study using replant soils, biochar improved the TCSA and below ground biomass of newly planted peach trees within the first year (Atucha and Litus, 2015). Similar growth and biomass results were reported for annual crops. Biochar applied at 23 Mg ha^{-1}, slightly less than half the lowest rate used in my study, improved plant biomass by over 1,000% for legumes (Major et al., 2010). However, in more fertile clayey soils, biochar applications had no effects on root or shoot biomass of soybean (Keith et al., 2015).

Trees were N and Mg deficient in 2014 and 2015 (Stiles and Reid, 1991). Initially, the high K content of the biochar amended soil resulted in increased leaf K concentrations; however, this increase was transient. By 2015, there were no differences in leaf K concentration among treatments. Although the biochar increased soil Ca, trees that received biochar had lower leaf Ca content in 2014. In 2015, all trees except the 0BC+N trees were Ca deficient, and there were no significant differences in leaf Ca concentration among biochar treatments. Trees amended with

the 200BC+N treatment had greater leaf B content than the 0BC+N trees in 2014; however, by 2015, trees that received N were B deficient, while the leaf B concentration of 0BC treated trees remained at an adequate concentration. The effects of biochar additions on plant nutrition appears to be highly variable, and most likely dependent on biochar feedstock and pyrolysis method. This is apparent in other studies of orchard crops. In peach, biochar increased leaf P, K, and Mg concentrations, but reduced concentrations of Zn and B, two minerals essential for flower bud growth and development (Atucha and Litus, 2015). Ventura et al. (2013a) also found that biochar application reduced leaf Zn content, but had no effect on other macro- or micronutrients in apple leaves.

Unlike several other studies in which biochar increased soil alkalinity, in my study biochar application decreased soil pH, causing soil to become more acidic than the 0BC and 0BC+N soils. Changes in pH may also affect CEC, which affects retention of K, Ca, and Mg. Due to the nutrient content and CEC of the biochar, soil K, Ca, and Mg concentrations were greater in the biochar treated soils. Soil nutrient increases were not necessarily associated with increased leaf nutrition. Atucha and Litus (2015) observed increased soil Ca, but decreased soil Mg and B. Despite other studies where reduced NO_3^-N leaching was observed, there were no treatment effects on soil total N during this study (Major et al., 2010; Ventura et al., 2013a; Atucha and Litus, 2015; Xu et al., 2016).

Biochar applications increased OM, total C, and C:N. However, Soil OM decreased in the biochar amended soil over the course of three years, but total C did not decrease during my study. Reduction in OM during my study suggests microbes were mineralizing OM due to soil priming effects, which is defined as the accelerated soil organic C mineralization following the addition of labile C source (Kuzyakov et al., 2000). Luo et al. (2011) observed that soil priming

effects resulted in increased mineralization of OM and the labile fraction of biochar. Similarly, Wardle et al. (2008) observed that biochar applications accelerate degradation of OM and simple carbohydrates. The use of N fertilizers in this study may have also increased microbial degradation of OM and labile biochar C (Schulz and Glaser, 2012).

During my study, higher rates of biochar application increased soil microbial respiration, suggesting that microbes in the biochar amended soil had access to more carbon as an energy source than microbes in the 0BC and 0BC+N treated soil. Farrell et al. (2013) found that up to 51% of C from bacteria during phospholipid fatty acids analysis was from the biochar amendment, indicating biochar serves as an important energy source when added to soil. In contrast, Kuzyakov et al. (2014) determined that only 4% of biochar C was transformed to microbial biomass, or respiration after 1.7 years. Major et al. (2010) observed increases in microbial soil respiration in biochar amended soil compared to the control. Similar to my study, the amount of respired C was higher in the first year than in the second year (Major et al., 2010). Major et al. (2010) hypothesized that increased microbial respiration following biochar application was a result of increased microbial biomass, and that only a small proportion of microbial respiration was due to carbon from biochar. Increased soil respiration following biochar applications was not associated with greater microbial biomass in other studies, as well (Zavalloni et al., 2011; Ventura et al., 2013b). However, in other studies, biochar applications do not necessarily lead to increased microbial respiration despite increasing soil total C content (Keith et al., 2015; Liu et al., 2016).

Biochar usually increases microbial biomass C, similar to other C based amendments (Schnurer et al., 1985; Major et al., 2010; Luo et al., 2013; Xu et al., 2016). However, biochar additions may result in little change, or a reduction in microbial biomass C (Steinbeiss et al.,

2009; Dempster et al., 2012; Chen et al., 2013; Keith et al., 2015). This may be due to variation in the labile C portion of biochar, or the sorptive properties of biochar reducing the microbial availability of certain mineral nutrients. Biochar was not associated with increased microbial biomass C in my study, thus increased respiration rates were most likely not related to microbial biomass, and may be attributed to soil priming effects from biochar treatments. Durenkamp et al. (2010) found that using lower concentrations of K_2SO_4 extractant, such as the 0.05 M of K_2SO_4 extractant used in my study, for microbial biomass fumigation assays yielded lower total organic C and N than K_2SO_4 extractant solutions with a greater salt concentration. Thus, using a lower concentration of K_2SO_4 extractant may have led to a significant underestimation of microbial biomass.

Biochar, applied at 100 Mg ha^{-1} and 200 Mg ha^{-1}, significantly reduced PMN during this study. Reduction of PMN following biochar applications has been reported in several other studies. Dempster et al. (2012) observed biochar applications as low as 5 Mg ha^{-1} significantly reduce PMN in a sandy soil. Similarly, Prayogo et al. (2014) reported that greater biochar application rates reduced N mineralization more than lower rates. Biochar and biochar with N fertilizer applications have not been shown to significantly alter the structure or diversity of microbial communities associated with ammonia oxidation (Dempster et al., 2012). Lower PMN indicates net N immobilization by soil microbes. Increase in microbial biomass N only occurred in the 50 Mg ha^{-1} treatment, indicating that microbial immobilization of N may not be responsible for the lower PMN values observed in the 100 Mg ha^{-1} and 200 Mg ha^{-1} biochar treatments. Despite lower PMN in biochar amended soil, there were no observed differences in soil total N among treatments.

Throughout my study, bacterial and fungal communities sampled at the same time point were more similar than communities that received the same treatment. Phospholipid-derived fatty acids, T-RFLP, and automated ribosomal intergenic spacer analysis (ARISA) data revealed similar short-term time-based trends (Anderson et al., 2011; Quilliam et al., 2012; Farrell et al., 2013). These results indicate that environmental factors, such as soil texture, C content, and pH, may have a greater effect on bacterial community structure then biochar application. In other studies, biochar applications had limited effects on soil microbial communities in more fertile soils (Anderson et al., 2011; Quilliam et al., 2012). When bacterial communities were analyzed at a single time point, biochar amended soils were more similar to each other than soils that were not amended with biochar (Grossman et al., 2010; Chen et al., 2013; Xu et al., 2016). Microbial community differences between biochar and unamended soils are most likely due to pH changes (Grossman et al., 2010; Xu et al., 2016). In many studies, soil become more alkaline following biochar applications. However, in my study, soils initially became slightly more acidic, but returned to a similar, neutral pH three years after application. Time-based similarities in microbial community structure may be a result of similar soil pH among biochar amended and unamended soils throughout my study.

In summary, biochar, even when applied at very high agronomic rates, did not improve tree growth or biomass more so than synthetic N fertilizer applications, and the effects of biochar application on leaf mineral nutrition was variable across years. Due to these factors, long-term studies may be necessary to determine the effects of biochar applications on perennial crops. Biochar may have less of an effect on the growth and nutrition of trees planted in the Shenandoah Valley region of Virginia due to the high OM and CEC content and water holding capacity present in the native silty loam soil. These soil characteristics may provide trees with

adequate mineral nutrition and water, thus the effects of biochar on tree growth and mineral nutrition may be negligible. In this study, biochar treatments did not significantly alter the structure of bacterial or fungal communities, or increase microbial biomass or soil PMN. There are several factors that may have affected microbial biomass and PMN. Given the recalcitrant nature of biochar, microbial population number, and in turn biomass, may have decreased as the biochar rate increased. The addition of biochar could have had a priming effect, which lead to increase C and N use by copiotrophic bacteria, causing greater N immobilization. Thus, soils amended with more biochar may have had less N mineralization. Finally, the sorptive properties of biochar may have inhibited full extraction of microbial biomass C and N, and ammonia. Overall, biochar applications may not provide significant plant growth and soil health improvements for orchards in fertile soils, and I would not suggest perennial fruit growers in the Mid-Atlantic apply this amendment until further research regarding best practices for production and application rate have been established.

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4. Compost Applications Increase Young Apple Tree Growth and Soil Fertility in a Pot-in-Pot Study

4.1 Abstract

In apple (*Malus ×domestica* Borkh.) orchards, rootstock genotype and soil fertility management practices may impact soil health and tree nutrient status, plant associated soil microbial communities, and orchard productivity. Rootstocks may influence microbial communities, resulting in changes to microbial community structure and function in ways that affect tree growth and productivity. Many apple growers apply synthetic N fertilizers to improve fruit yield and quality. In excess of tree requirements, nitrogen fertilizers may decrease crop yield and quality, and pollute ground and surface waters. The addition of compost has been shown to reduce N loss and increase soil fertility in orchard systems. In 2013, a pot-in-pot study was implemented to determine the effects of yardwaste and chicken litter composts, fertigation with calcium nitrate [$Ca(NO_3)_2$], and integrated compost and $Ca(NO_3)_2$ fertigation combinations on apple tree growth and nutrient status, soil health, and microbial activity. The scion 'Brookfield Gala' was grafted onto five rootstocks, 'Budagovsky 9', 'Malling 9', 'Geneva 41', 'G. 214', and 'G. 935'. Trees were planted in pots containing native orchard soil. Four tree replicates of each rootstock were treated with 40 kg ha^{-1} N from either chicken litter compost, yard waste compost, $Ca(NO_3)_2$ fertigation, or integrated compost- $Ca(NO_3)_2$ combination fertilizer, which were comprised of 20 kg ha^{-1} from compost and 20 kg ha^{-1} from $Ca(NO_3)_2$. Non-fertilized containers served as a control. After three years, trees fertilized with compost and the integrated compost treatments had larger trunk cross sectional areas than fertigated, or control trees. Tree biomass was greater than the control by 35%, 32%, 27%, and 22% for the chicken litter, yardwaste- $Ca(NO_3)_2$, chicken litter- $Ca(NO_3)_2$, and yardwaste, respectively. In 2015, leaf

N concentration was greatest in 'M.9', 'G.41,' and 'B.9' rootstocks. Trees that received chicken litter, the integrated compost-$Ca(NO_3)_2$, and fertigation had greater leaf N concentrations than the control. Compost and integrated compost-$Ca(NO_3)_2$ treatments increased soil OM and CEC compared to the CON treatments. In 2015, the amount of potentially mineralizable nitrogen and microbial respiration was greater in soils that received compost or integrated compost-$Ca(NO_3)_2$ than in soils that were fertigated or the control. These results indicate that compost and integrated compost-$Ca(NO_3)_2$ fertilizers may be used to increase soil fertility, leaf mineral nutrition, plant growth and microbial activity in newly established orchards.

4.2 Introduction

In apple (*Malus ×domestica* Borkh.) orchards, rootstock genotype and soil fertility management practices potentially impact environmental health, soil health and nutrient status, plant associated soil microbial communities, and orchard productivity. As more rootstock genotypes are released from breeding programs and become commercially available, and growers seek alternative fertilizer and ground cover management methods, understanding how these factors and their interactions affect plant and soil health and productivity is essential for successfully managing newly planted high-density orchards.

Growers select apple rootstock cultivars for use in their orchards to confer beneficial traits, including ease of propagation, mature tree size, precocity, vigor, cold tolerance, and pest and disease resistance. Rootstocks also confer above ground traits, such as fruit yield, weight, and quality (Fallahi et al., 2002). Roots are essential for anchorage, nutrient and water acquisition. Rootstock cultivars can vary considerably for these traits, possibly due to their specific rhizosphere plant-microbe interactions. Some apple and grape (*Vitis vinifera*) rootstocks have improved macro- and micronutrient use efficiency and drought tolerance related to

genotype (Aguirre et al., 2001; Chang et al, 2014; Fazio et al., 2013; Rolli et al., 2015). In the Mid Atlantic, there is increased interest in planting Cornell/Geneva series rootstocks because these rootstocks have greater yield efficiencies and tolerance to fire blight (*Erwinia amylovora*) and replant disease than the more commonly planted Malling and Merton-Malling rootstocks (Russo et al., 2007).

Rootstock cultivars may also confer benefits through interactions with the soil microbiome. Studies of perennial crops, such as apples and grapes, indicate rootstock genotype can alter the soil microbial communities, which in turn affects plant health and productivity (Rumberger et al., 2004; St. Laurent et al., 2010; Rolli et al., 2015; Song et al., 2015). Apple rootstocks from the Cornell/Geneva series enhance disease suppressive soil qualities, leading to reduced incidence of apple replant disease in newly planted trees, and replant disease resistant rootstock varieties had similar microbial communities (Rumberger et al., 2004; St. Laurent et al., 2010). In a study of the grape rootstock rhizosphere, Rolli et al. (2015) observed that bacteria, such as Pseudomonas and Acinetobacter species, in the rhizosphere conferred increased plant growth and drought tolerance during times of water stress. Apple rootstocks also form symbiotic relationships with arbuscular mycorrhizal fungi that result in increased tree mineral nutrition and biomass (Miller et al., 1985; An et al., 1993). Differences in microbial colonization of rootstocks are possibly caused by rootstock genotypes producing distinct exudate, rhizodeposits, and small C molecules (Haichar et al., 2008; Hartmann et al., 2009).

Nitrogen fertilizers may improve fruit yield and quality. However, applying an excessive amount of N fertilizers may reduce crop yield and quality, and may cause environmental problems such as the contamination of ground and surface waters. Currently, ground and foliar applications of synthetic N fertilizers, such as calcium nitrate [$Ca(NO_3)_2$], ammonium nitrate

(NH_4NO_3), and urea [$CO(NH_2)_2$], are recommended N fertilizers for commercial apple growers in Virginia (Pfeiffer et al., 2015). However, apple growers in arid regions commonly use fertigation, a method of mixing soluble N fertilizer with water and applying it to the root zone though a drip irrigation system. Fertigation with N can improve fruit quality and yield, leaf N concentration, and improve tree nitrogen use efficiency while reducing N leaching and runoff (Klein et al., 1989; Neilsen and Neilsen, 2002; Dong et al., 2005, Neilsen et al., 2009). Compost applications have been shown to increase soil organic matter (OM), cation exchange capacity (CEC), mineral nutrition, plant tissue N, root lifespan and length, microbial biomass, and microbial activity, and reduce N leaching in orchard systems (Yao et al., 2006; Kramer et al., 2006; Baldi et al., 2010a; Baldi et al., 2010b; Strauss et al., 2014; Rumberger et al., 2014). In several orchard studies, compost applications improved soil health factors, including soil organic matter and microbial biomass C, soil mineral nutrition, and leaf mineral nutrients, but compost did not improve plant productivity or fruit quality in apple orchards (Yao et al., 2006; Forge et al., 2013; Neilsen et al., 2014; Sas-Paszt et al., 2014). Additionally, integrating C-based amendments with synthetic nitrogen fertilizers has been shown to improve C sequestration and plant nutrient availability while simultaneously increasing crop yield and quality in annual crops, such as broccoli and tomato (Stamatiadis et al., 1999; Yu et al., 2012; Hernadez et al., 2014).

Plant genotype, soil type, and soil pH influence the composition of bacterial and fungal communities in bulk and rhizosphere soil (Marschner et al., 2001; Hartmann et al., 2009). Plants alter rhizosphere microbial communities through the production of C-based exudates and rhizodeposits, and other exudates that alter soil pH in the rhizosphere or antimicrobials (Hartmann et al., 2009). The effect of different apple rootstock cultivars on soil microbial community composition can persist after the tree has been removed (St. Laurent et al., 2010).

Chemical additions to the soil, such as the addition of synthetic or carbon based fertilizers, may also alter the microbial community. The use of synthetic N fertilizers consistently reduces soil respiration, microbial biomass, and enzyme activity, and shift microbial community composition resulting in reduced C sequestration compared to unfertilized soils (Ramirez et al., 2012). Applications of synthetic N and P at high rates may also result in reduced plant-mycorrhizal fungi associations and less diverse microbial communities (Entz et al., 2004; Jumpponen et al., 2005). Fertilizing grassland soil with synthetic N promotes root colonization of arbuscular mycorrhizal fungi (AMF) that are poor mutualists and exploit plants for C (Jumpponen et al., 2005). Van Geel et al. (2015) observed decreased AMF richness and diversity in apple orchards were related to increased plant available P. In other studies, synthetic N and P applications increased the cultured fungal and bacterial population (Styla and Sawicka et al. 2010). In orchards, compost applications increased soil microbial respiration, and altered microbial community composition compared to the unamended control (Rumberger et al. 2004; Yao et al., 2006). However, in several studies, apple rootstock genotypes had a greater effect on microbial community composition than did compost (Rumberger et al. 2004; Yao et al., 2006). Soil microbial community and activity changes related to compost applications do not necessarily increase tree productivity or growth (Rumberger et al. 2004; Yao et al., 2006).

The aims of my study were to: determine how nitrogen fertilizers affect soil fertility, and apple tree growth and nutrient status, and understand how rootstocks and fertilizer amendments affect the soil microbial community. I hypothesized that integrated compost-calcium nitrate fertilizer applications will increase tree growth within each rootstock treatment and compost applications will increase soil fertility measures, such as OM, CEC, and mineral nutrients, increase soil microbial activity and alter the soil microbial community composition.

4.3 Materials and Methods

In 2013, a pot-in-pot experiment was implemented as a completely randomized design at the Virginia Tech Alson H. Smith, Jr. Agricultural Research and Extension Center in Winchester, VA (39° 06' N, 78° 17' W). 'Brookfield Gala' scions were grafted onto five rootstocks, 'Budagovsky 9', 'Malling 9', 'Geneva 41', 'G.214', and 'G.935' and planted in 38 L pots. Pots contained native Poplimento silt loam orchard soil, a fine, mixed, subactive, mesic Ultic Hapludalf (NRCS 2001). The soil in each pot was mixed with 10% (v/v) Stalite® (Salisbury, NC) to improve drainage and aeration. Native orchard soil and Stalite® were mixed using a cement mixer. Four individual tree replicates of each rootstock were treated with 40 kg ha^{-1} of plant available N from locally sourced chicken litter compost (CL) (Valley Pride Compost, Harrisonburg, VA), locally sourced yardwaste compost (YW) (Loudoun Composting, Chantilly, VA), fertigation with calcium nitrate [$Ca(NO_3)_2$] (FERT) (Yara, Oslo, Norway) for 8 weeks, or a combination of 20 kg N ha^{-1} from chicken litter and fertigation with 20 kg N ha^{-1} from $Ca(NO_3)_2$ for 8 weeks (CL+FERT) or 20 kg N ha^{-1} from yardwaste compost and fertigation with 20 kg N ha^{-1} from $Ca(NO_3)_2$ for 8 weeks (YW+FERT). Non-fertilized plants served as a control (CON). Fertigation applications began in late May and ended in July. New compost was acquired each year. Prior to compost application, compost analysis was performed by Penn State Agricultural Analytical Services Laboratory (University Park, PA) to ensure plant available N was applied at an equal rate among fertilizer treatments (Table 4.1). Although other mineral nutrients and organic matter differed among treatments, they were not controlled for in this experiment. Fertilizer were repeated in 2013, 2014, and 2015. Following fertilizer applications each year, a hand trowel was used to work the soil to a depth of 10 cm. Trees were irrigated using a micro-spray irrigation system three times a week throughout the spring and summer,

hand-weeded when necessary, and uniformly treated for pests and diseases (Pfeiffer et al., 2015). Flowers were removed from young trees to prevent fruit set and reduce disease susceptibility. In 2015, gravimetric water content of the pots was determined using the method described by Owen (2007).

The trunk cross sectional area was calculated by measuring trunk caliper diameter 10 cm above the soil line at planting in 2013, and marked with paint to ensure the tree was measured in the same place in 2014 and 2015. In Oct. 2015, trees were destructively harvested to determine dry weight biomass. Leaves were removed from trees, placed in paper bags, and oven dried for 3 d at 80 °C. Trees were removed from the pot and soil was rinsed from the root system. Trees were then cut into five segments: roots, leader, above ground rootstock shank, branches, and below ground rootstock shank. Segments were oven dried at 80 °C for 5 d until constant mass was achieved.

Leaf mineral nutrient analysis was performed at the Penn State Agricultural Analytical Services Laboratory (University Park, PA) in Aug. 2014 and Aug. 2015. Twenty-five leaves were removed from each tree, placed in paper bags, and dried at for 3 d at 80 °C. A 730-ES Optical Emission Inductively Coupled Plasma Spectrometer (Agilent Technologies, Santa Clara, CA) was used to measure concentrations of P, K, Ca, Mg, Mn, Fe, Cu, B, Al, Zn, and Na using the ICP-dry ash method (Miller, 1998). Leaf N concentration was measured using the combustion analysis method (Horneck and Miller, 1998) on a Vario Max N/C Analyzer (Elementar, Hanau, Germany). In 2013, leaves were collected as a composite sample from the four tree treatment set because there were not enough leaves on each tree to obtain a representative sample.

Soil samples were collected in 2013, 2014, and 2015. A Hori-Hori Japanese soil knife was used to collect soil, rather than a traditional soil probe or auger, due to the presence of Stalite. Soil was collected to a depth of 10 cm in the north, south, east, and west directions 15 cm from the tree trunk. Soil was placed in a bag and hand homogenized. Immediately following sampling, a subsample of soil was frozen at -80 °C for microbial community analysis. The remaining soil was sieved through a US number 10 soil sieve (2 mm mesh) and stored at 4 °C until biological and physiochemical analyses were completed.

Soil pH, mineral nutrients, and CEC were measured at the Virginia Tech Soil Testing Laboratory (Blacksburg, VA). Soil samples were air-dried and crushed with a hammer mill-type crushing machine (Agvise, Benson, MN) prior to analysis. Soil pH was measured with a pH meter (WP-80D, TPS Pty Ltd., Springwood, Australia) fitted with a combination pH electrode (Orion model 8165BNWP Ross Sure-Flow, ThermoFisher, Waltham, MA). Mehlich 1 solution was used to extract P, K, Ca, Mg, Zn, Mn, Cu, Fe, B, and Al from 4 cm^3 of soil. Mineral nutrient concentration was determined by OES-ICP (Acros Spectro, Mahwah, NJ). Cation exchange capacity (CEC) was estimated by summation of the non-acid generating cations (Ca, Mg, and K) and Mehlich soil-buffer acidity. In 2013 and 2014, soil organic matter (OM) and soluble salts (SS) were determined at the Virginia Tech Soil Testing Laboratory, and at the Cornell Nutrient Analytical Lab in 2015. Soluble salts were measured with an electrical conductivity probe (3100 Conductivity Instrument, YSI, Yellow Springs, OH). The loss on ignition method was used to measure OM. The initial soil dry mass was determined by heating soil to 150°C in an electric high temperature, forced air drying oven (Blue M model CW-6680F, New Columbia, PA) for 2 h. Soil samples were then heated to 360°C for 2 h, and reweighed to calculate organic matter content. A subsample of soil from samples collected in 2013, 2014, and 2015 was ground to a

fine powder using a mortar and pestle for total C and total N analysis on a CHN Elemental Analyzer-vario EL (Elementar, Hanau, Germany) at the Cornell Nutrient Analytical Lab.

Soil microbial respiration was measured using the conductimetric method (Rodella and Saboya, 1999). For each sample, 50 g of soil and a glass vial containing 20 mL of 0.5 sodium hydroxide (NaOH) were sealed in an air-tight jar. The vial of NaOH was used as a carbon dioxide (CO_2) gas trap inside the jar. A model 2052 electrical conductivity meter (Amber Science Inc., Eugene, OR) was used to measure the electrical conductivity of the NaOH solution weekly for six weeks. The concentration of the evolved CO_2 was measured by comparing the electrical conductivity of the samples to a blank containing 50 g of autoclaved, dried sand, and a CO_2 saturated standard of sodium bicarbonate.

Microbial biomass C and N were extracted from soil samples collected in 2015 using the direct chloroform ($CHCl_3$) fumigation extraction method (Fierer and Schimel, 2003). Ten grams of soil from each sample and 40 mL of 0.05 M potassium sulfate (K_2SO_4) were added to two separate 70 mL glass vials with Teflon-lined lids. Fumigated samples received 0.5 mL of amylated $CHCl_3$. Nonfumigated samples did not receive $CHCl_3$, but were otherwise treated the same as fumigated samples. Samples were agitated for 4 h on an orbital shaker at 2.5 $r \cdot s^{-1}$ and allowed to settle for 30 min. Samples were decanted into 50 mL conical tubes, centrifuged for 10 min at 500 g_n, and filtered (FisherBrand G6, Fisher, Waltham, MA). Remaining $CHCl_3$ was removed from the filtrate via sparging with compressed N_2 gas for 20 min. The solution was stored at -20 °C prior to analysis. Blank samples with no soil were prepared in the same manner. Samples were diluted 1:2 (volume/volume) with deionized water prior to analysis. Total organic C and total dissolved N were quantified using a Shimadzu (Columbia, MD) carbon analyzer model TOC-VCPH+TNM-1 with an auto-sampler, using high-temperature oxidation catalyzed

with platinum-coated alumina beads (temperature 720°C) in non-purgeable organic carbon mode (Bird et al., 2003). Non-purgeable organic C was measured using a non-dispersive infrared detector and chemiluminescence for N. Each sample was run in triplicate. Microbial biomass C was calculated by multiplying the difference between the fumigated and nonfumigated samples by the k_{ec} value of 0.45 (Joergensen, 1996). Microbial biomass N was calculated by multiplying the difference between the fumigated and nonfumigated samples by the k_{en} value of 0.54 (Brookes et al., 1985).

Potentially mineralizable nitrogen (PMN) was estimated as the difference in the NH_4^+ concentration between the 7-day incubation measurement and initial measurement. The initial measurement of PMN was taken by placing 10 g of soil and 40 mL of 2 M KCL to a 50 mL conical tube. Samples were agitated on an orbital shaker for 1 h at 3.3 $r \cdot s^{-1}$, centrifuged for 10 min at 500 g_n, and filtered (FisherBrand G6). Ten grams of soil and 10 mL of water (W7-4 Optima Water, Fisher) in a 50 mL conical tube were incubated for 7 d at 30 °C. After incubation, 30 mL of 2.67 M KCl were added to samples, which were then agitated on an orbital shaker for 1 h at 3.3 $r \cdot s^{-1}$. Samples were centrifuged for 10 min at 500 g_n and filtered (FisherBrand G6). Prior to analysis, the filtrate for the initial and incubated samples were stored at -20 °C. A Lachat QuickChem 8500 Series 2 Flow Injection Analysis System (Loveland, CO) was used to analyze NH_4^+ and NO_3^- concentrations. The concentration of NH_4^+ was measured using #12-107-06-2-A, and NO_3^- was measured using protocol #12-107-04-1-B (Lachat Instruments, 2014).

Changes to bacterial and fungal soil community structure due to treatments were assessed using the terminal-restriction length polymorphism (T-RFLP) method. A modified protocol was followed to extract total genomic DNA using the MoBio Lab Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA). Approximately 0.25 g of soil were placed in the bead tube provided by

MoBio, and heated to 65 °C for 10 min in a water bath. Otherwise, the manufacturer's protocol was followed. DNA quality and concentration was assessed using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, Massachusetts). DNA was stored at -20 °C until duplicate polymerase chain reaction (PCR) amplifications were performed in 50 μL reaction volumes for each sample. Bacterial community DNA was amplified in reactions containing 1 X GoTaq PCR Flexi Buffer (Promega, Madison, WI), 2.5 U GoTaq G2 Flexi DNA Polymerase (Promega), 2 mM $MgCl_2$ solution (Promega), 0.2 mM dNTPs, 0.1 μg μL^{-1} bovine serum albumin (BSA) (New England BioLabs, Ipswich, MA), 0.1 μM fluorescently labeled 27f forward primer (5'-[6FAM] AGA GTT TGA TCC TGG CTC AG-3'), 0.1 μM 1492r reverse primer (5'-GGT TAC CTT GTT ACG ACT T-3'), 40 ng template DNA, and nuclease free water (Qiagen, Hilden, Germany). The reaction conditions for this PCR were a 5 min denaturation step at 95 °C, followed by 27 cycles of 95 °C for 45 s, 56 °C for 45 s, and 72 °C for 1 min, a final extension step of 72°C for 10 min followed. Fungal community DNA was amplified in reactions containing 1 X GoTaq PCR Flexi Buffer (Promega, Madison, WI), 5 U GoTaq G2 Flexi DNA Polymerase (Promega), 3 mM $MgCl_2$ solution (Promega), 0.2 mM dNTPs, 0.1 μg μL^{-1} bovine serum albumin (BSA) (New England BioLabs), 0.1 μM fluorescently labeled ITS1f forward primer (5'-[6FAM] CTT GGT CAT TTA GAG GAA GTA A-3'), 0.1 μM ITS4r reverse primer (5'-TCC TCC GCT TAT TGA TAT GC-3'), 40 ng template DNA, and nuclease free water (Qiagen). The reaction conditions for this PCR were a 5 min denaturation step at 94 °C, 30 cycles of 94 °C for 30 s, 51 °C for 45 s, and 72 °C for 45 s, followed by a final extension step of 72°C for 10 min. Reactions were amplified in a Bio-Rad C1000 Thermal Cycler (Hercules, CA).

PCR products were visualized on a 1.5% agarose gel in 1 X tris borate EDTA buffer (TBE) stained with GelStar (Lonza, Basel, Switzerland).

Polymerase chain reaction products were pooled and quantified against a calf thymus DNA standard Curve in an ethidium bromide solution (20 μg in 1 X TBE) using a FOTO/Analyst Investigator gel imaging system (Fotodyne, Hartland, WI) with Quanity One software version 4.4.0 (Bio-Rad, Hercules, CA). Restriction enzyme digests containing either 600 ng of bacterial DNA or 150 ng fungal DNA, 15 U Sau96I (New England BioLabs), 1 X CutSmart Buffer (New England BioLabs) and nuclease free water (Qiagen, Hilden, Germany) were performed at 37°C for 4 h followed by a restriction enzyme denaturation step at 70°C for 20 min. Digestion products were visualized on a 2% agarose gel in 1 X TBE stained with GelStar. Performa DTR plates (EdgeBio, Gaithersburg, MD) were used to purify the digests. After purification, samples were condensed using a CentriVap Centrifugal Concentrator System (Labconco, Kansas City, MO), and resuspended in 9.5 μL Hi-Di Formamide (Applied Biosystems, Foster City, CA) and 0.5 μL GeneScan 1200 LIZ dye size standard (Applied Biosystems). Fragment size was determined using an Applied Biosystems 3730xl DNA Analyzer (Foster City, CA) at the Cornell University Institute of Biotechnology (Ithaca, NY). PeakScanner software v1.0 (Applied Biosystems) was used to quantify fragment peaks size, height, and area.

Trunk cross sectional area and soil microbial respiration data were analyzed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC). For analysis of trunk cross sectional area, fixed effects were Fertilizer, Rootstock, and Fertilizer x Rootstock. For analysis of microbial respiration, fixed effects were Fertilizer, Rootstock, Day, Fertilizer x Rootstock, Rootstock x Day, Fertilizer x Day, and Fertilizer x Rootstock x Day. Differences were determined at a $p = 0.05$ level using the least significant differences (LSD). Soil gravimetric water content, tree

biomass, leaf mineral nutrition, soil fertility, PMN, and microbial biomass C and N data were analyzed using analysis of variance (ANOVA) and Tukey's Honestly Significant Differences (HSD) post hoc test in JMP Pro 11 (SAS Institute Inc., Cary, NC). Significant differences were determined at a $p = 0.05$ level. Slices were used to analyze the interaction effects within Rootstock x Fertilizer, Year x Fertilizer, Year x Rootstock, and Rootstock x Fertilizer x Year interactions when interactions were significant.

Terminal-restriction length polymorphism data were analyzed using the T-RFLP Analysis Expedited (T-REX) online software (Culman et al., 2009). This software uses the presence or absences of terminal restriction fragments (T-RFs) of a known base pair length and treatments to construct an additive main effects multiplicative interaction (AMMI) model. The AMMI model, sometimes referred to as a doubly centered principal components analysis (PCA), uses an ANOVA to partition the variation into main effects (T-RFs and treatments) and interactions, then a PCA is applied to the interactions to create an interaction principal components analysis. Culman et al. (2008) demonstrated that AMMI is a robust analysis for T-RFLP data from a variety of environments.

Table 4. 1. The C:N ratio, organic matter, total C, organic N, ammonium, nitrate, P, and K content of the chicken litter and yardwaste composts were measured in 2013, 2014, and 2015 prior to compost application to ensure plant available N was equivalent among fertilizer treatments.

Compost	C:N	OM (g kg^{-1})	C (g kg^{-1})	Organic N (g kg^{-1})	Ammonium (mg kg^{-1})	Nitrate (mg kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
2013								
Chicken litter	15.8	458	260	16.5	5.0	1012.62	18.8	7.50
Yardwaste	14.4	538	246	17.1	55.6	21.03	4.60	11.5
2014								
Chicken litter	14.9	475	293	19.6	5.0	501.40	19.0	8.60
Yardwaste	18.8	608	342	18.1	36.8	44.00	4.10	11.2
2015								
Chicken litter	12.9	473	246	19.0	5.0	601.05	16.8	6.50
Yardwaste	18.1	515	291	16.1	48.2	91.52	3.50	7.80

4.4 Results

In 2015, after two seasons of fertilizer treatment applications, there were no differences in soil gravimetric water content among treatments. At planting, the apple rootstock cultivar G.214 had significantly larger TCSA than B.9, G.935, and M.9, and remained so throughout the duration of the study (Fig.1). In December of 2014, trees fertilized with compost and the integrated compost treatments had larger TCSAs than FERT trees or CON trees ($p = 0.0002$) (Fig. 4.2). There were also Fertilizer x Rootstock interactions ($p = 0.007$). In October of 2015, CL trees had larger TCSAs than the than FERT trees or CON trees ($p = 0.002$) (Fig.2). In 2015, there were no Fertilizer x Rootstock interactions.

Trees with the G.214 rootstocks had the largest total biomass and root biomass of all trees (Table 4.3). Total biomass of the G. 214 rootstock trees was 28% greater than the M.9 rootstock trees, which had the lowest total biomass. The trees that received the CL, CL+FERT, YW and

YW+FERT fertilizers had total biomasses respectively 35%, 32%, 27%, and 27% greater than the CON trees, and root biomasses 37%, 35%, 27%, and 26% greater than the CON trees. Trees that received the CL, CL+FERT, YW and YW+FERT fertilizers had 54%, 56%, 51% and 60% greater leaf biomass than the CON trees. There were no Fertilizer x Rootstock interactions for tree segment biomass.

In all three years of this experiment, the compost and integrated compost-calcium nitrate treatments had greater soil P, K, Ca, Mg, Mn, and B compared to FERT and CON treatments (Table 4.4). CL increased soil P more so than any other treatment. Compared to the CON, CL had 152%, 170%, and 191% greater soil P in 2013, 2014, and 2015, respectively. The YW treatment had greater soil K compared to all other treatments, and greater soil K of 78%, 88%, and 104% compared to the control in 2013, 2014, and 2015, respectively. In all three years, compost treatments had greater soil Ca concentration than the other fertilizer treatments. Compared to the control, soil Ca was 43%, 65%, and 78% greater for CL, and 47%, 58%, and 74% greater for YW, in 2013, 2014, and 2015, respectively. In 2013, YW increased soil Mg compared to all other treatments; however, in 2014 and 2015, both compost treatments increased soil Mg compared to all other treatments. The CL treatment increased soil Zn concentration and the YW treatment increased soil Mn concentration throughout this study. The compost and integrated compost fertilizers increased soil B concentration compared to the CON and FERT treatments in 2013, 2014, and 2015. Relative to the control, CL increased soil B by 50%, 84%, and 88%, and the YW treatment increased soil B by 71%, 81%, and 85% in 2013, 2014, and 2015, respectively.

The rootstock cultivar treatments also had an effect on soil mineral nutrition, but these effects were inconsistent during the three years of this study (Table 4.4). In 2013, soil K

concentration was 13% and 15% greater for B.9 and G.214, respectively, compared to M.9, which had the lowest soil K concentration. In 2014, soil where G.41 rootstocks were planted contained 23% more K than soil where G.935, which had the lowest soil K concentration. There were no differences in soil K concentration among the B.9, G.214, and M. 9 rootstock treatments. In 2013, soil Mg concentration was 10% greater where G.214 had been planted than where G.935 or M.9 had been planted. In 2014, soil Mg concentration was greatest where G.41 had been planted. In 2013, soil where B.9 and G.214 rootstocks were planted had 16% more soil B than soil where G.41 or M.9 rootstocks were planted. However, in 2014, soil where B.9 and M.9 rootstocks were planted had greater soil B concentrations than G.935 soils.

Compost and the integrated compost-calcium nitrate treatments increased total soil N and C in 2013 and 2015, but not in 2014 (Table 4.4). In 2013, the compost and integrated compost-calcium nitrate treatments increased total soil N compared to CON. In 2015, the CL, CL+FERT, YW, and YW+FERT treatments increased total soil N% by 66%, 54%, 46%, and 78%, respectively compared to CON. In 2013, the CL, CL+FERT, YW, and YW+FERT treatments increased total soil C by 33%, 14%, 52%, and 89% respectively compared to CON. In 2015, the CL, CL+FERT, YW, and YW+FERT treatments increased total soil C by 62%, 60%, 64%, and 96% respectively compared to CON. In 2013 and 2015, the soil C:N ratio was greatest in the YW and YW+FERT treatments. Compost and integrated compost-calcium nitrate treatments increased soil OM compared to the CON treatments in all years. In 2013, there was 24% and 66% more OM in the CL and YW amended soil than the CON. In 2014, CL and YW increased 71% and 89% respectively compared to the CON, and in 2015, 79% and 102% CL and YW increased 71% and 89% respectively compared to the CON.

Compost and integrated compost treatments had greater soil CEC compared to the FERT and CON treatments in all years. Cation exchange capacity was greatest in soils amended with the compost treatments. In 2013, CEC was 41% and 49% greater in the CL and YW fertilized soil respectively than the CON. In 2014, CEC was 66% and 57% greater in the CL and YW fertilized soil respectively than the CON. In 2015, CEC was 79% an 76% greater in the CL and YW fertilized soil respectively than the CON.

The rootstock cultivar treatments had inconsistent effects on total N and C, C:N ratio, OM, and CEC (Table 4.4). In 2013, soil where G.935 rootstocks were planted had the lowest soil total N, which was 94% less than soil where B.9 rootstocks were planted. In 2014, soil where G.935 rootstocks were planted had 46% less total N than where B.9 rootstocks were planted. In 2013, soil total C was greatest in soil where B.9 rootstocks were planted. In 2014, soil where G.935 rootstocks were planted contained 49% lower total C than soils where B.9 rootstocks, which had the highest soil total C, were planted. However, in 2015, soils where B.9 rootstocks were planted had the lowest soil total C. These soils were 35% lower in total C than soils G.214 rootstock with the greatest soil total C, G.214, were planted. In 2013, soil C:N ratio was 97% greater in the soils where G.214 rootstocks were planted than where G.41 were planted. In 2015, soil C:N ratio was 17% lower in soil where B.9 rootstocks were planted than where G.41 rootstocks were planted. In 2013, soil where B.9 rootstocks were planted had 13% more soil OM than soil where the G.935 rootstocks were planted; however, there were no differences in OM among the rootstocks treatments in 2014 or 2015.

There were no treatment differences in leaf N in 2014; however, in 2015 CL+FERT and YW+FERT increased leaf N concentration compared to all other treatments (Table 4.5). Trees that received CL+FERT and YW+FERT had 27% more leaf N than leaves from CON trees. In

2014 and 2015, compost treatments increased leaf P concentration compared to all other treatments. In 2014, the CL and YW treated trees had 79% and 64% greater leaf P than the control. In 2015, the CL and YW treated trees had 94% and 76% greater leaf P than the control. In 2014, leaves from YW fertilized trees had the greatest K concentration, and increased leaf K concentration 30% compared to the control. In 2015, CL treatment increased leaf K concentration by 36%, and the YW treatment increased leaf K concentration by 35% relative to the CON treatment. In 2014, FERT increased leaf Ca concentration compared to CL+FERT. However, in 2015, FERT and CL increased leaf Ca concentration compared to CON, but there were no significant differences between either treatment and the other compost and integrated compost treatments. The CL and CL+FERT treatments increased leaf Mg concentration in 2014 and 2015. The YW treatment increased leaf B concentration by 17% and 20% in 2014 and 2015, respectively, compared to the control.

Leaf N concentration was not different among the rootstock treatments in 2014 (Table 4.5). However, in 2015, leaves B.9 and M.9 contained 8% more N than leaves from G.214. In 2014, leaves from G.41 and G.214 rootstocks had P concentrations 43% and 33% greater than leaves from the B.9 rootstocks. In 2015, the leaf P concentrations of G.41, G.214, and G.935 were 34%, 22%, and 19%, respectively, greater than leaves from the B.9 rootstock. In 2014 and 2015, the K concentration was greater in leaves from the G.41 rootstock than from the B.9 rootstock by 28% and 22%, respectively. There were no differences in Ca in 2014, but in 2015 leaves from the G.214 rootstocks had the greatest leaf Ca concentration. In 2014, leaves from G.214 rootstocks contained more Mg than all other rootstocks; however, this trend did not occur in 2015. In 2014 and 2015, leaves from G. 41 and G.935 had higher leaf B concentrations than leaves from B.9 or M.9 rootstocks.

In 2015, microbial biomass C and N were affected by fertilizer and rootstock; there were no fertilizer x rootstock interaction effects (Table 4.6). The CL, YW, and YW+FERT treatments had 75%, 81%, and 63% more microbial biomass C than the CON treatment, and 103%, 97%, and 78% more microbial biomass N than the CON treatment. Soil where the B.9 rootstock was planted had 36% more microbial biomass C and 55% more microbial biomass N than soil where the M.9 rootstock was planted.

Potentially mineralizable N was affected by both the rootstock and fertilizer treatments in 2014 and there were no significant Fertilizer x Rootstock interactions (Table 4.7). Soil where the G.214 rootstock was planted had 40% more PMN than soil where G.41 rootstocks were planted. Soils that received the CL and YW treatments had 60% and 52% more PMN than the CON. In 2015, only fertilizer affected PMN. Soil that received the CL, CL+FERT, YW, and YW+FERT had 90%, 74%, 96%, and 75% more PMN than the CON, respectively.

In 2013, YW increased the cumulative microbial respiration compared to all other treatments ($p < 0.0001$), and rootstock increased microbial respiration ($p = 0.03$); however, there were no significant Fertilizer x Rootstock interactions (Fig. 4.3). Results were similar for microbial respiration in 2014 and 2015, the compost and integrated compost fertilizer treatments increased the cumulative microbial respiration compared to FERT and CON treatments ($p < 0.0001$) (Fig. 4.2). There were no rootstock or Fertilizer x Rootstock effects in either year.

Amplification and digestion produced 717 unique bacterial T-RFs and 769 unique fungal T-RFs from soil samples collected over the three years of this experiment. The ANOVA produced through AMMI analysis indicated that fertilizer and rootstock treatments did not significantly alter soil bacterial or fungal community composition during this study (Table 4.8). However, T-RFs associated with fertilizer and rootstock treatments grouped together by

sampling date for both bacteria and fungi on the IPCA graphs (Fig. 4. 4-5). Bacterial and fungal T-RFs from soil samples collected June 2013 clustered towards the positive end of the ICPA 1 axis, and were separated from communities sampled at the other time points. Bacterial T-RFs from samples collected in August 2013 were clustered towards the negative end of the IPCA 1 axis and the positive end of the ICPA 2 axis. Fungal T-RFs from samples collected in August 2014 and August 2015 clustered together along the negative ends of the ICPA1 and ICPA2 axes. For bacterial samples, ICPA1, ICPA2, ICPA3, and ICPA4 captured 33%, 24%, 8%, and 5% interaction signal variation, respectively. For fungal samples, IPCA1, ICPA2, ICPA3, and ICPA4 captured 29%, 14%, 10%, and 7% of the interaction signal variation, respectively.

Table 4. 2. Gravimetric soil water content of the pots was determined in the spring of 2015. Data presented here are the means from four replications of each treatment combination, untreated control (CON), calcium nitrate (FERT) at 40 kg N ha^{-1}, chicken liter compost (CL), chicken litter with calcium nitrate (CL+SYN), yardwaste compost (YW), yardwaste with calcium nitrate (YW+SYN), Budagovsky 9 (B.9), Geneva 41 (G.41), Geneva 214 (G.214), and Malling 9 (M.9).

	Water (L) (±SEM)
Fertilizer	
CON	20.5 (1)
FERT	20.9 (1)
CL	18.9 (1)
CL+FERT	19.1 (1)
YW	20.4 (1)
YW+FERT	22.8 (1)
	p = 0.2
Rootstock	
B.9	18.4 (1)
G.41	22.1 (1)
G.214	20.0 (1)
G.935	21.5 (1)
M.9	19.3 (1)
	p = 0.06
Fertilizer x Rootstock	p = 0.03
Fertilizer	
CON	ns
FERT	*
CL	ns
CL+FERT	ns
YW	ns
YW+FERT	ns
Rootstock	
B.9	ns
G.41	ns
G.214	ns
G.935	ns
M.9	ns

ns, nonsignificant, * Significant at $p \leq 0.05$

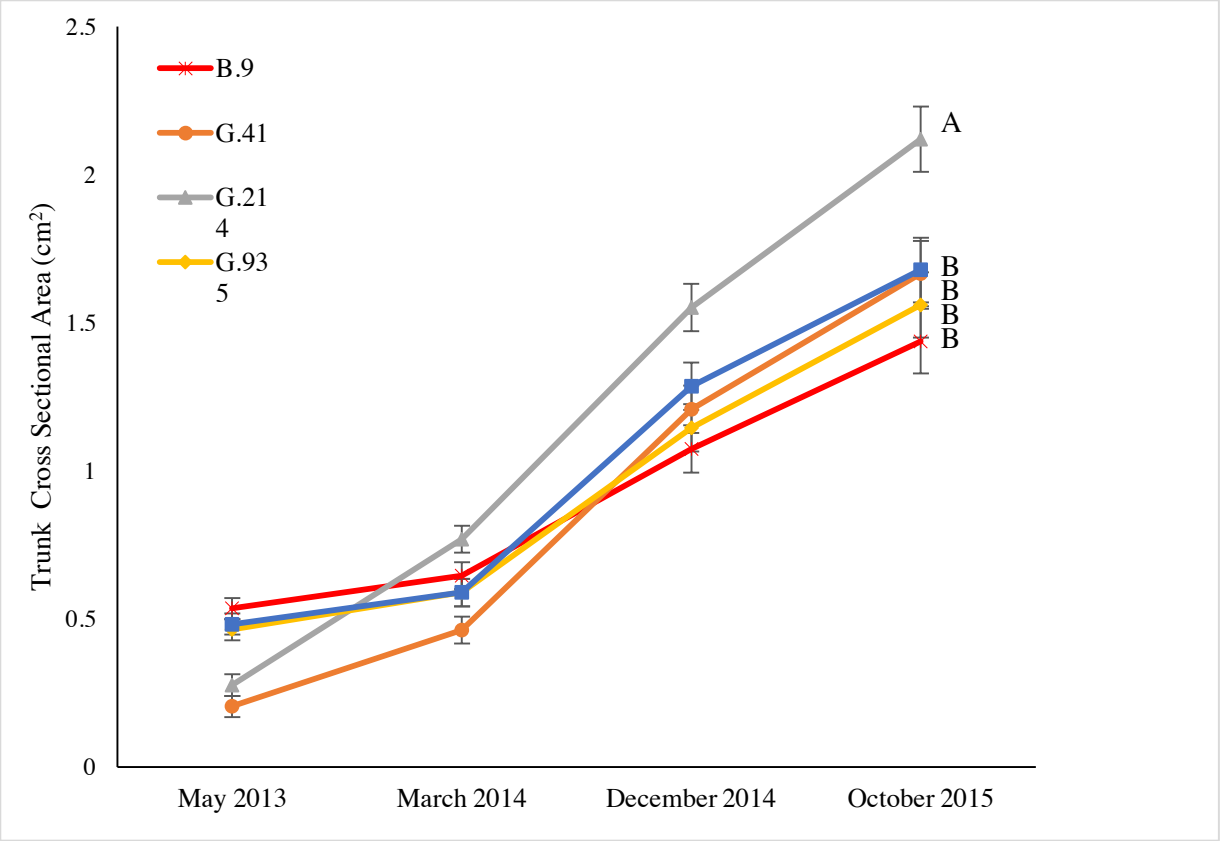

Fig. 4. 1. Tree growth was measured as trunk cross sectional area at planting, and following fertilizer applications in the spring of 2014, the winter of 2014 and the fall of 2015 in Winchester, VA. Data shown represents the mean of 4 trunk cross sectional area measurements per treatment, Budagovsky 9 (B.9), Geneva 41 (G.41), Geneva 214 (G.214), and Malling 9 (M.9). Different letters indicate means separation at $p \leq 0.05$ level of significance using least significant differences test. Error bars represent the standard error of the mean.

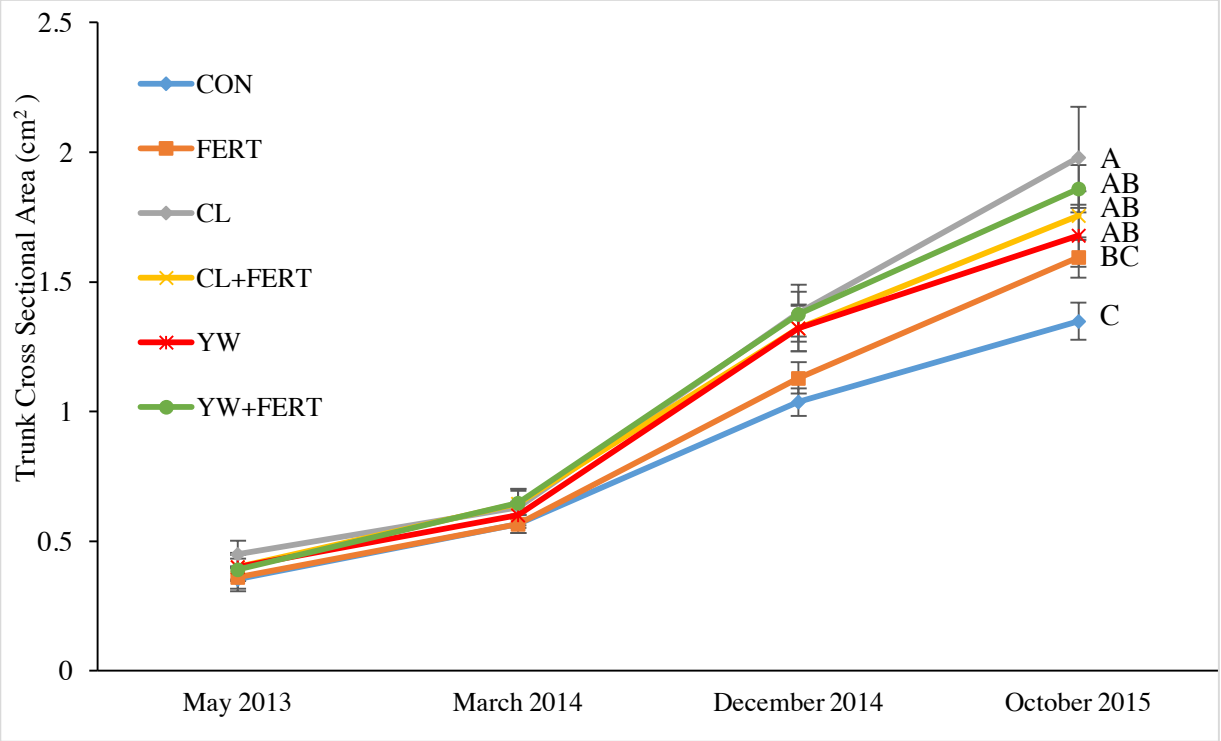

Fig. 4. 2. Tree growth was measured as trunk cross sectional area at planting, and following fertilizer applications in the spring of 2014, the winter of 2014 and the fall of 2015 in Winchester, VA. Data shown represents the mean of 4 trunk cross sectional area measurements per treatment, untreated control (CON), calcium nitrate (FERT) at 40 kg N ha^{-1}, chicken liter compost (CL), chicken litter with calcium nitrate (CL+FERT), yardwaste compost (YW), and yardwaste with calcium nitrate (YW+FERT). Different letters indicate means separation at $p \leq 0.05$ level of significance using least significant differences test. Error bars represent the standard error of the mean.

Table 4. 3. The effects of fertilizer on tree biomass were determined by destructive harvest in October of 2015. Trees were divided into the following segments: roots, leader, shank, branches, root shank, and leaves. Total mass was determined through summation of the segments. Data shown represents the mean dry biomass of 4 replicates per treatment, untreated control (CON), calcium nitrate (FERT) at 40 kg N ha^{-1}, chicken liter compost (CL), chicken litter with calcium nitrate (CL+FERT), yardwaste compost (YW), and yardwaste with calcium nitrate (YW+FERT).

Rootstock	**Roots (g)**	**Leader (g)**	**Above ground shank (g)**	**Branches (g)**	**Below ground shank (g)**	**Leaf (g)**	**Total mass (g)**
B.9	59.3 B	28.0 C	21.0	8.2 A	29.3 A	7.7	153 B
G.41	70.0 B	39.9 B	20.8	5.3 B	15.5 C	9.9	161 B
G.214	89.5 A	48.1 A	24.7	6.8 AB	17.8 BC	8.1	195 A
G.935	63.5 B	37.6 B	20.8	5.1 B	24.6 A	7.9	159 B
M.9	67.1 B	28.0C	25.2	5.6 AB	22.8 AB	7.4	152 B
	p <0.0001	p <0.0001	p = 0.03	p = 0.02	<0.0001	p = 0.09	p <0.0001
Fertilizer							
CON	54.6 B	25.2 C	19.6	5.4	19.8	5.1 B	129 B
FERT	65.6 AB	32.4 BC	21.3	5.5	19.6	7.9 AB	152 AB
CL	79.0 A	42.2 A	23.7	6	23.3	8.9 A	183 A
CL+FERT	77.6 A	40.8 A	22.9	7.2	22.2	9.1 A	179 A
YW	71.7 A	35.8 AB	23.6	6.8	24.0	8.6 A	170 A
YW+FERT	70.8 AB	36.6 AB	23.8	6.3	23.1	9.5 A	170 A
	p = 0.003	p <0.0001	p = 0.2	p = 0.4	p = 0.4	p = 0.003	p <0.0001
Rootstock x Fertilizer	p = 0.7	p = 0.1	p = 0.08	p = 0.2	p = 0.26	p = 0.7	p = 0.3

[x] Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant differences.

Table 4. 4. Soil pH, Mehlich 1 extractable soil mineral nutrients, total nitrogen, total carbon, the carbon to nitrogen ratio, organic matter, soluble salts, cation exchange capacity measured from soil samples collected to a depth of 10 cm 15 cm from the trunk of the center tree in August of 2013, 2014, and 2015. Data presented here are the means from four replications of each treatment combination, untreated control (CON), calcium nitrate (FERT) at 40 kg N ha^{-1}, chicken liter compost (CL), chicken litter with calcium nitrate (CL+SYN), yardwaste compost (YW), yardwaste with calcium nitrate (YW+SYN), Budagovsky 9 (B.9), Geneva 41 (G.41), Geneva 214 (G.214), and Malling 9 (M.9).

	pH	**P (mg kg^{-1})**	**K (mg kg^{-1})**	**Ca (mg kg^{-1})**	**Mg (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Fe (mg kg^{-1})**	**B (mg kg^{-1})**	**Total N (g kg^{-1})**	**Total C (g kg^{-1})**	**C:N**	**OM (g kg^{-1})**	**SS (mmhos cm^{-1})**	**CEC (meq $100g^{-1}$)**
2013																
Fertilizer																
CON	7.43	4.4 D	42.1 D	902 C	72 D	5.58 C	14.1 B	0.99 AB	5.18 B	0.27 D	1.6 B	7.4 D	6.5 CD	17.5 E	0.10 B	5.2 D
FERT	7.38	4.4 D	42.2 D	904 C	69 D	5.58 C	15.6 B	1.02 A	5.31 B	0.26 D	1.6 B	7.5 D	6.9 BC	19.1 DE	0.09 B	5.2D
CL	7.54	32.5 A	51.2 C	1390 A	97 B	7.13 A	16.3 B	0.80 CD	5.10 B	0.44 B	0.22 A	1.03 C	5.6 D	22.3 C	0.12 AB	7.9 B
CL+FERT	7.52	16.5 B	43.8 D	1149 B	83 C	6.49 B	15.7 B	0.83 BC	5.16 B	0.36 C	2.3 A	8.5 CD	5.5 D	20.3 CD	0.10 B	6.5 C
YW	7.56	13.6 B	96.2 A	1452 A	132 A	7.03 AB	29.9 A	0.64 D	7.05 A	0.56 A	1.9 AB	12.6 B	9.3 A	34.8 A	0.13 A	8.6 A
YW+FERT	7.49	8.4 C	71.3 A	1206 B	98 B	6.48 B	25.9 A	0.75 CD	6.66 A	0.42 B	2.3 A	19.2 A	7.9 B	25.8 B	0.12 AB	7.0 C

	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	Total N (g kg^{-1})	Total C (g kg^{-1})	C:N	OM (g kg^{-1})	SS (mmhos cm^{-1})	CEC (meq 100g^{-1})
Rootstock																
B.9	7.42	14.3	60.8 A	1182	93 AB	6.37	18.9	0.84	5.82	0.42 A	2.4 A	12.5 A	4.9 C	25.2 A	0.11	6.8 AB
G.41	7.52	13.1	57.1 AB	1161	92 AB	6.32	21.4	0.89	5.83	0.35 B	1.7 B	10.7 B	9.5 B	28.3 AB	0.11	6.7 AB
G.214	7.49	13.4	61.8 A	1219	98 A	6.31	19.3	0.79	5.89	0.42 A	2.6 A	10.7 B	4.1 C	23.9 AB	0.10	7.0 A
G.935	7.48	13.4	56.1 AB	1135	88 B	6.50	17.9	0.89	5.73	0.37 B	0.9 C	10.4 B	11.8 A	22.1 B	0.10	6.5 B
M.9	7.51	12.2	53.3 B	1139	88 B	6.40	20.4	0.79	5.41	0.35 B	2.3 A	10.2 B	4.3 C	24.6 B	0.10	6.5 B
Fertilizer	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	**	***
Rootstock	ns	ns	**	*	**	ns	ns	ns	ns	***	***	**	***	**	ns	**
Fertilizer x Rootstock	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns
2014																
Fertilizer																
CON	7.73 B	13.0 C	40.8 D	1130 C	84 C	7.62 C	17.3 C	2.25 AB	5.76	0.265 C	0.10	11.9 AB	11.8 AB	17.9 D	0.11 D	6.4 C
FERT	7.71 B	4.5 C	39.5 D	1064 C	80 C	7.69 C	18.3 C	3.04 A	6.00	0.245 C	0.10	10.7 B	10.9 B	17.1 D	0.11 D	6.1 C
CL	7.72 B	162.1 A	74.5 B	2222 A	176 A	13.90 A	25.0 AB	0.67 B	6.25	0.645 A	0.13	15.3 A	11.9 AB	37.4 B	0.20 A	12.7 A
CL+FERT	7.77 AB	69.1 B	52.5 CD	1651 B	120 B	10.43 B	19.5 BC	1.30 AB	6.25	0.435 B	0.11	12.5 AB	11.9 AB	26.4 C	0.14 BC	9.3 B
YW	7.83 A	17.9 C	105.1 A	1983 A	174 A	8.62 BC	30.9 A	0.52 B	5.86	0.627 A	0.13	15.9 A	12.7 A	46.6 A	0.16 B	11.6 A
YW+FERT	7.79 AB	9.5 C	63.0 BC	1558 B	119 B	8.24 C	25.7 AB	1.30 AB	6.22	0.415 B	0.12	15.2 A	12.3 A	30.8 C	0.12 CD	8.9 B

	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	Total N (g kg^{-1})	Total C (g kg^{-1})	C:N	OM (g kg^{-1})	SS (mmhos cm^{-1})	CEC (meq 100g^{-1})
Rootstock																
B.9	7.73	59.0	68.8 AB	1702	135 A	10.04	24.5	1.44	5.93 AB	0.472 A	1.2 A	15.4 A	12.1	31.6	0.15 AB	9.8
G.41	7.81	51.6	70.2 A	1657	133 A	9.62	23.6	1.18	5.77 B	0.450 AB	1.2 A	14.5 A	11.9	30.5	0.13 BC	9.5
G.214	7.74	46.1	59.5 AB	1610	125 AB	9.62	22.7	2.49	6.42 A	0.437 AB	1.3 A	15.0 A	11.4	29.8	0.14 ABC	9.2
G.935	7.79	29.7	55.7 B	1507	115 B	8.75	21.1	1.26	5.77 B	0.379 B	0.8 B	9.3 B	11.8	26.6	0.12 C	8.6
M.9	7.73	43.7	58.5 AB	1531	119 AB	9.03	22.0	1.25	6.40 A	0.454 A	1.1 A	13.8 A	12.3	28.1	0.16 A	8.8
Fertilizer	**	***	***	***	***	***	***	**	ns	**	*	**	**	***	***	***
Rootstock	**	*	**	ns	**	ns	ns	ns	**	***	***	***	ns	ns	***	ns
Fertilizer x Rootstock	ns	***	***	**	***	**	ns	ns	ns	***	***	***	0.01	0.01	***	**
2015																
Fertilizer																
CON	8.05 A	6.4 C	47.4 CD	1073 C	94 C	7.58 DE	13.7 E	1.08 A	5.04 B	0.350 C	0.9 C	10.1 C	10.9 C	19.6 D	0.11 D	6.2 C
FERT	8.09 A	3.5 C	35.6 D	1057 C	82 C	7.09 E	12.1 E	1.04 A	4.75 B	0.290 C	0.81 C	8.4 C	10.4 C	19.0 D	0.10 D	6.0 C
CL	7.92 B	291.6 A	108.1 B	2453 A	234 A	19.68 A	27.6 C	0.51 CD	6.49 A	0.905 A	1.85 AB	19.2 B	10.5 C	41.2 B	0.19 B	14.4 A
CL+FERT	8.04 A	132.9 B	64.7 C	1758 B	154 B	13.59 B	20.8 D	0.68 B	5.28 B	0.575 B	1.62 B	18.8 B	11.3 BC	27.2 C	0.14 C	10.2 B
YW	8.08 A	25.4 C	150.5 A	2335 A	216 A	10.59 C	40.9 A	0.35 D	6.72 A	0.868 A	1.49 B	19.6 B	12.5 AB	54.8 A	0.24 A	13.8 A
YW+FERT	8.07 A	16.2 C	102.3 B	1789 B	154 B	9.26 CD	33.3 B	0.60 BC	7.28 A	0.645 B	2.12 A	28.8 A	13.0 A	39.9 B	0.18 B	10.5 B

	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	Total N (g kg^{-1})	Total C (g kg^{-1})	C:N	OM (g kg^{-1})	SS (mmhos cm^{-1})	CEC (meq 100g^{-1})
Rootstock																
B.9	8.03	81.0	89.7	1838	162	11.3	26.1	0.72	5.86 AB	0.647	1.27	13.0 B	10.2 B	35.2	0.18 A	10.7
G.41	8.07	95.4	79.3	1819	164	11.86	25.2	0.72	6.08 A	0.587	1.71	21.7 A	12.1 A	34.5	0.17 AB	10.6
G.214	8.04	75.6	86.8	1761	159	11.49	25.3	0.68	5.91 AB	0.583	1.52	18.5 A	11.6 A	34.7	0.15 AB	10.3
G.935	7.98	71.8	85.9	1619	148	10.9	24.9	0.74	6.57 A	0.597	1.39	16.6 AB	11.6 A	31.9	0.14 B	9.5
M.9	8.08	72.8	82.0	1685	149	10.93	22.3	0.67	5.19 B	0.612	1.46	17.7 AB	11.7 A	31.7	0.15 AB	9.8
Fertilizer	**	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Rootstock	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	**	**	ns	**	ns
Fertilizer x Rootstock	ns	**	ns	**	*	***	*	ns	ns	ns	***	***	**	ns	*	**
2013-2015																
Fertilizer																
CON	7.73 B	7.9 C	43.4 D	1035 C	88 C	6.9 D	15.0 E	1.42 AB	5.35 C	0.293 C	0.117 C	0.98 D	9.7 B	1.8 D	0.10 C	6.0 C
FERT	7.73 B	4.1 C	39.1 D	1008 C	77 C	6.8 D	15.4 E	1.70 A	5.33 C	0.263 C	0.114 C	0.89 D	9.4 B	1.8 D	0.10 C	5.8 C
CL	7.72 B	162.1 A	77.9 B	2022 A	169 A	13.6 A	22.9 C	0.66 C	5.9 B	0.663 A	0.177 AB	1.49 BC	9.3 B	3.4 B	0.17 A	11.7 A
CL+FERT	7.77 AB	72.8 B	53.7 C	1519 B	119 B	10.1 B	18.7 D	0.93 BC	5.6 BC	0.457 B	0.167 AB	1.33 C	9.6 B	2.5 C	0.13 B	8.7 B
YW	7.82 A	18.9 C	117.3 A	1923 A	174 A	8.7 C	33.9 A	0.50 C	6.72 A	0.683 A	0.157 B	2.10 A	11.5 A	4.5 A	0.18 A	11.3 A
YW+FERT	7.78 AB	11.4 C	78.9 B	1518 B	125 B	7.9 C	28.3 B	0.91 BC	6.54 A	0.491 B	0.187 A	1.60 B	11.1 A	3.2 B	0.14 B	8.8 B

	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	Total N (g kg^{-1})	Total C (g kg^{-1})	C:N	OM (g kg^{-1})	SS (mmhos cm^{-1})	CEC (meq 100g^{-1})
Rootstock																
B.9	7.72	51.5 AB	73.1	1573 A	130 A	9.2	23.2	1.00	5.87 AB	0.512 A	1.65 AB	13.6 AB	9.0 B	31 A	0.15 A	9.1 A
G.41	7.79	53.4 A	68.9	1546 AB	129 A	9.3	23.4	0.93	5.89 AB	0.464 AB	1.53 B	15.6 A	11.1 A	29 AB	0.14 ABC	8.9 AB
G.214	7.75	45.0 AB	69.4	153 ABC	127 AB	9.1	22.4	1.32	6.08 A	0.479 AB	1.80 A	14.7 A	9.0 B	29 AB	0.13 BC	8.8 ABC
G.935	7.75	38.3 B	65.9	1420 C	117 B	8.7	21.3	0.96	6.02 AB	0.447 B	1.02 C	12.1 B	11.7 A	27 B	0.12 C	8.2 C
M.9	7.77	42.9 AB	64.6	1451 BC	118 B	8.8	21.7	0.90	5.67 B	0.474 AB	1.64 AB	13.9 AB	9.4 B	27 B	0.14 AB	8.4 BC
Fertilizer	**	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Rootstock	ns	*	ns	**	**	ns	ns	ns	*	*	***	**	***	**	***	**
Year	***	***	***	***	***	***	***	***	**	***	***	***	***	***	***	***
Fertilizer x Rootstock	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	***	***	***	ns	ns	ns
Fertilizer x Year	**	***	***	***	***	***	***	*	***	***	***	***	***	***	***	***
Rootstock x Year	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	***	***	ns	**	ns
Fertilizer x Rootstock x Year	ns	***	**	***	***	***	ns	ns	ns	***	***	***	***	*	**	***

	pH	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})	Total N (g kg^{-1})	Total C (g kg^{-1})	C:N	OM (g kg^{-1})	SS (mmhos cm^{-1}[1])	CEC (meq 100g^{-1})
Interaction Effects Fertilizer x Rootstock																
CON		ns	ns								***	**	***			
FERT		ns	ns								***	*	***			
CL		***	*								***	***	***			
CL+FERT		*	ns								***	*	***			
YW		ns	***								**	***	***			
YW+FERT		ns	*								**	***	***			
B.9		***	***								***	***	**			
G.41		***	***								***	***	***			
G.214		***	***								*	***	**			
G.935		***	***								***	***	**			
M.9		***	***								***	***	**			

[v]Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.
[w] ppm is parts per million
[x] OM is organic matter.
[y] SS is soluble salts
[z] CEC is cation exchange capacity.
ns, nonsignificant
* Significant at $p \leq 0.05$
** Significant at $p \leq 0.01$
*** Significant at $p \leq 0.0001$

Table 4. 5. Leaf nutrient analysis was performed on 25 leaves collected from each tree in August of 2014 and 2015. Data presented here are the means from four replications of each treatment combination, untreated control (CON), calcium nitrate (FERT) at 40 kg N ha^{-}, chicken liter compost (CL), chicken litter with calcium nitrate (CL+FERT), yardwaste compost (YW), yardwaste with calcium nitrate (YW+FERT), Budagovsky 9 (B.9), Geneva 41 (G.41), Geneva 214 (G.214), and Malling 9 (M.9).

	N (%)	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Mn (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Cu (mg kg^{-1})**	**B (mg kg^{-1})**	**Al (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Na (mg kg^{-1})**
2014 Fertilizer												
CON	1.83	0.18 C	1.76 D	1.29 AB	0.17 C	75.9	52.4	4.6	38.9 BC	35.1 A	66.3	6.0
FERT	1.93	0.16 C	1.83 CD	1.36 A	0.18 BC	77.4	51.2	4.7	35.3 C	30.9 AB	75.6	6.5
CL	1.98	0.41 A	2.29 AB	1.22 AB	0.24 A	73.0	48.0	4.9	42.4 AB	24.9 B	60.5	6.1
CL+FERT	1.97	0.23 B	1.99 BC	1.13 B	0.21 AB	79.2	45.6	4.9	39.0 BC	24.8 B	54.3	5.8
YW	1.95	0.35 A	2.39 A	1.25 AB	0.20 ABC	83.9	51.9	4.7	45.9 A	28.1 AB	67.8	6.5
YW+FERT	1.85	0.20 BC	2.06 BC	1.18 AB	0.20 ABC	86.9	53.4	4.3	38.8 BC	33.5 A	67.2	6.6
Rootstock												
B.9	1.95	0.20 C	1.81 C	1.23	0.18 B	106.2 A	55.0	5.0	36.0 B	34.3 A	66.9 AB	6.9 A
G.41	1.89	0.31 A	2.39 A	1.27	0.24 A	79.4 B	49.1	4.5	43.8 A	27.9 ABC	64.9 AB	5.6 B
G.214	1.85	0.28 A	2.10 B	1.21	0.17 B	66.2 B	48.3	4.2	41.9 A	26.8 BC	61.2 AB	6.8 AB
G.935	2.0	0.26 AB	2.07 B	1.29	0.20 B	65.0 B	50.7	4.8	43.3 A	26.1 C	55.7 B	5.8 AB
M.9	1.88	0.22 BC	1.89 BC	1.19	0.19 B	79.9 B	48.9	4.9	35.1 B	32.6 AB	77.7 A	6.2 AB
Fertilizer	ns	***	***	*	***	ns	ns	ns	***	***	ns	ns
Rootstock	ns	***	***	ns	**	***	ns	ns	***	**	*	**
Fertilizer x Rootstock	ns	*	**	ns	ns	ns	ns	ns	*	**	*	ns

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})
2015												
Fertilizer												
CON	1.21 D	0.17 D	1.39 C	1.21 B	0.2 ABC	29.7	44.9	3.1	33.3 CD	42.7 A	46.8 AB	6.0
FERT	1.44 BC	0.11 D	1.27 C	1.40 A	0.21 AB	31.4	43.0	3.6	28.6 E	36.4 AB	52.6 AB	6.8
CL	1.53 AB	0.47 A	2.01 A	1.43 A	0.22 A	30.6	45.2	6.1	38.1 AB	34.3 B	45.6 AB	5.4
CL+FERT	1.59 A	0.28 C	1.71 B	1.34 AB	0.22 A	29.5	47.5	3.9	32.8 D	34.9 B	36.5 B	5.4
YW	1.34 CD	0.38 B	1.97 AB	1.28 AB	0.19 BC	29.9	42.0	3.4	40.9 A	38.0 AB	55.4 A	7.4
YW+FERT	1.59 A	0.28 C	1.94 AB	1.35 AB	0.18 C	33.1	45.8	3.8	36.3 BC	36.8 AB	51.3 AB	5.7
Rootstock												
B.9	1.49 AB	024 C	1.68 B	1.30 BC	0.20	44.4 A	42.8 B	3.9	32.3 BC	33.8 B	42.8	4.6 B
G.41	1.50 A	0.34 A	2.04 A	1.29 BC	0.21	26.9 B	44.9 B	3.8	38.5 A	36.5 AB	49.7	4.9 B
G.214	1.33 C	0.30 A	1.66 B	1.60 A	0.20	19.7 C	41.7 B	4.8	35.1 B	37.1 AB	51.4	7.0 AB
G.935	1.37 BC	0.29 AB	1.55 B	1.34 B	0.20	23.6 BC	43.7 B	3.3	38.3 A	38.1 AB	50.1	7.8 A
M.9	1.55 A	0.24 BC	1.63 B	1.55 C	0.21	38.8 A	50.7 A	4.1	30.5 C	40.3 A	46.2	6.2 AB
Fertilizer	***	***	***	**	**	ns	ns	ns	***	**	*	ns
Rootstock	***	***	***	***	ns	***	***	ns	***	*	ns	**
Fertilizer x Rootstock	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})
2014 and 2015 Fertilizer												
CON	1.52 C	0.17 D	1.57 C	1.25 B	0.18 C	52.8	48.7	3.9	36.1 C	38.9 A	56.5 AB	5.9
FERT	1.68 AB	0.13 D	1.55 C	1.38 A	0.20 BC	54.4	47.1	4.2	31.9 D	33.7 BC	64.1 A	6.6
CL	1.75 AB	0.44 A	2.15 A	1.32 AB	0.23 A	51.8	46.6	5.5	40.2 B	29.6 C	53.1 AB	5.7
CL+FERT	1.78 A	0.26 C	1.85 B	1.24 B	0.22 AB	54.3	46.6	4.4	35.9 C	29.9 C	45.4 B	5.6
YW	1.64 BC	0.37 B	2.18 A	1.27 AB	0.20 BC	56.9	46.9	4.0	43.3 A	33.0 BC	61.6 A	6.9
YW+FERT	1.71 AB	0.24 C	1.99 AB	1.27 AB	0.19 BC	60.0	49.6	4.0	37.5 BC	35 AB	59.2 AB	6.1
Rootstock												
B.9	1.72 A	0.22 C	1.74 B	1.27 BC	0.19 B	75.3 A	48.9	4.5	34.2 C	34.0 AB	54.8	5.7 AB
G.41	1.70 AB	0.32 A	2.22 A	1.28 BC	0.23 A	53.1 BC	47.0	4.1	41.1 A	32.2 B	57.3	5.2 B
G.214	1.59 B	0.29 AB	1.88 B	1.41 A	0.19 B	43.0 D	45.0	4.5	38.5 B	31.9 B	56.3	6.9 A
G.935	1.69 AB	0.28 B	1.81 B	1.32 AB	0.20 B	44.3 CD	47.2	4.0	40.9 AB	32.1 B	52.9	6.6 A
M.9	1.71 A	0.23 C	1.26 B	1.17 C	0.20 B	59.3 B	49.8	4.5	32.8 C	36.4 A	61.9	5.7 AB

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})
Fertilizer	***	***	***	**	***	ns	ns	ns	***	***	**	ns
Rootstock	**	***	***	***	***	***	ns	ns	***	*	ns	**
Year	***	**	***	**	ns	***	***	ns	***	***	***	ns
Fertilizer x Rootstock	*	**	ns	ns	*	ns	ns	ns	ns	**	***	ns
Fertilizer x Year	**	***	*	**	**	ns	*	ns	ns	ns	ns	ns
Rootstock x Year	**	ns	**	***	***	**	**	ns	ns	**	*	**
Fertilizer x Rootstock x Year	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
Interaction	ns				ns					***	**	
Effects	ns				***					ns	ns	
Fertilizer x	**				ns					ns	**	
Rootstock	ns				*					ns	ns	
CON	**				**					ns	ns	
FERT	ns				ns					*	**	
CL												
CL+FERT	**				ns					**	ns	
YW	ns				ns					ns	**	
YW+FERT	*				*					ns	**	
B.9	**				*					ns	**	
G.41	*				***					*	*	
G.214												
G.935												
M.9												

[x] Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.
ns, nonsignificant
* Significant at $p \leq 0.05$
** Significant at $p \leq 0.01$
*** Significant at $p \leq 0.0001$

Table 4. 6. The effects of fertilizer and rootstock treatments on microbial biomass carbon and nitrogen were measured from soil samples collected to a depth of 10 cm 15 cm from the trunk of the center in Aug. 2015. Data presented here are the means from four replicates of each treatment combination, untreated control (CON), calcium nitrate (FERT) at 40 kg N ha^{-1}, chicken liter compost (CL), chicken litter with calcium nitrate (CL+FERT), yardwaste compost (YW), yardwaste with calcium nitrate (YW+FERT), Budagovsky 9 (B.9), Geneva 41 (G.41), Geneva 214 (G.214), and Malling 9 (M.9).

Microbial Biomass		
Rootstock	***μ*g C g^{-1} SDW[x] (±SEM)[y]**	***μ*g N g^{-1} SDW (±SEM)**
B.9	5912 A (847)	490 A (70)
G.41	4760 AB (531)	371 AB (53)
G.214	5364 AB (442)	408 AB (48)
G.935	5132 AB (469)	391 AB (43)
M.9	4118 B (361)	279 B (41)
	p = 0.04	p = 0.008
Fertilizer		
CON	3051 C (242)	194 C
FERT	3068 C (321)	192 C
CL	6704 A (517)	609 A
CL+FERT	4472 BC (376)	333 BC
YW	7162 A (703)	559 A
YW+FERT	5884 AB (554)	440 AB
	p < 0.0001	p < 0.0001
Rootstock x Fertilizer	p = 0.2	p = 0.5

[x]SDW is soil dry weight

[y]SEM is the standard error of the mean

[z]Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.

Table 4. 7. Potentially mineralizable nitrogen was measured from soil samples collected to a depth of 10 cm 15 cm from the trunk of the center in August of 2014 and 2015. Data presented here are the means from four replications of each treatment combination, untreated control (CON), calcium nitrate (FERT) at 40 kg N ha^{-1} (0BC+N), chicken liter compost (CL), chicken litter with calcium nitrate (CL+FERT), yardwaste compost (YW), yardwaste with calcium nitrate (YW+FERT), Budagovsky 9 (B.9), Geneva 41 (G.41), Geneva 214 (G.214), and Malling 9 (M.9).

Potentially mineralizable nitrogen	**2014**	**2015**	**2014 and 2015**	
	***μ*g N g^{-1} SDW[y]**	***μ*g N g^{-1} SDW**	***μ*g N g^{-1} SDW**	
Rootstock				
B.9	285 AB[z] (25)	499 (32)	383 AB (21)	
G.41	213 B (23)	430 (31)	319 B (20)	
G.214	321 A (24)	532 (31)	427 A (19)	
G.935	245 AB (24)	425 (33)	335 B (21)	
M.9	285 AB (23)	479 (32)	387 AB (20)	
	p = 0.02	p = 0.09		
Fertilizer				
CON	240 BC (26)	237 C (36)	238 C (22)	
FERT	193 C (25)	260 C (35)	226 C (21)	
CL	359 A (26)	624 AB (34)	492 A (21)	
CL+FERT	376 ABC (26)	513 B (33)	394 B (22)	
YW	327 AB (26)	675 A (37)	501 A (22)	
YW+FERT	219 BC (27)	521 B (36)	370 B (22)	
	p < 0.0001	p < 0.0001	Year	p < 0.0001
Rootstock x Fertilizer	p = 0.7	p = 0.5	Year x Rootstock	p = 0.9
			Year x Fertilizer	p < 0.0001

Fertilizer effects within Year x Fertilizer	
CON	ns
FERT	ns
CL	***
CL+FERT	***
YW	***
YW+FERT	***
Rootstock x Fertilizer	p = 0.6
Year x Rootstock x Fertilizer	p = 0.6

[z]Different letters in a column indicate means separation at $p \leq 0.05$ level of significance using Tukey's honest significant difference test.
[y]SDW is soil dry weight
ns, nonsignificant
*** Significant at $p < 0.0001$

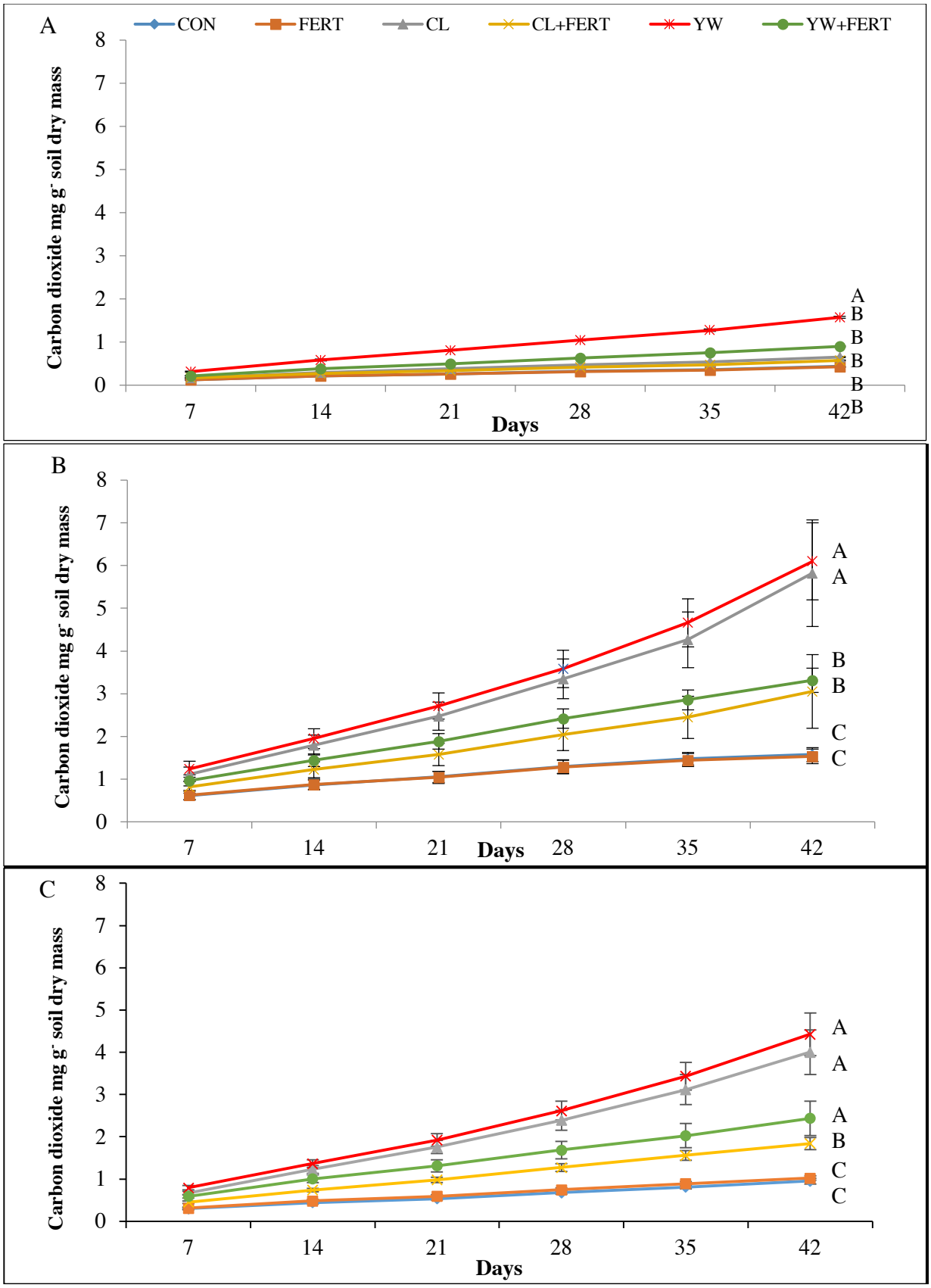
A
CON
FERT
CL
CL+FERT
YW
YW+FERT
Carbon dioxide mg g- soil dry mass
Days
A
B
B
B
B
B
B
Carbon dioxide mg g- soil dry mass
Days
A
A
B
B
C
C
C
Carbon dioxide mg g- soil dry mass
Days
A
A
A
B
C
C

Fig. 4. 3. Soil microbial respiration was measured as the evolution of carbon dioxide from soil samples taken a depth of 10 cm 15 cm from the trunk, and incubated for 6 weeks. Graphs are as follows: A= 2013, B = 2014, C = 2015. Data shown here are the means from four replications of each treatment combination, untreated control (CON), calcium nitrate (FERT) at 40 kg N ha^{-1}, chicken liter compost (CL), chicken litter with calcium nitrate (CL+FERT), yardwaste compost (YW), and yardwaste with calcium nitrate (YW+FERT). Different letters indicate means separation at $p \leq 0.05$ level of significance of the least significant differences test. Error bars represent the standard error of the mean.

Table 4. 8. ANOVA table generated by T-REX analysis of terminal-restriction length polymorphisms (T-RFLPs) produced though amplification of bacterial 16s rRNA gene and the fungal ITS1 gene from soil samples taken a depth of 10 cm 15 cm from the trunk. T-RFs are the terminal-restriction fragments. IPCA is the interaction principal components analysis.

Source	Degrees of Freedom	Sums of Squares	Mean Squares
Bacteria			
Total	302,277	27,709	0.09
Treatment	85,441	20,890	0.24
T-RFs	717	7,701	10.74
Environments	118	906	7.68
T-RFs x Environments	84,606	12,283	0.15
IPCA 1	834	3,193	3.82
IPCA 2	832	2,350	2.82
IPCA 3	830	762	0.92
IPCA 4	828	521	0.63
Residual	81,282	5,458	0.07
Error	216,836	6,819	0.03
Fungi			
Total	333,409	19,070	0.06
Treatment	91,629	12,193	0.13
T-RFs	769	4,061	5.28
Environments	118	231	1.96
T-RFs x Environments	90,742	7,901	0.09
IPCA 1	886	1,508	1.7
IPCA 2	884	792	0.89
IPCA 3	882	547	0.61
IPCA 4	880	393	0.45
Residual	87,210	4,661	0.05
Error	241,780	6,877	0.03

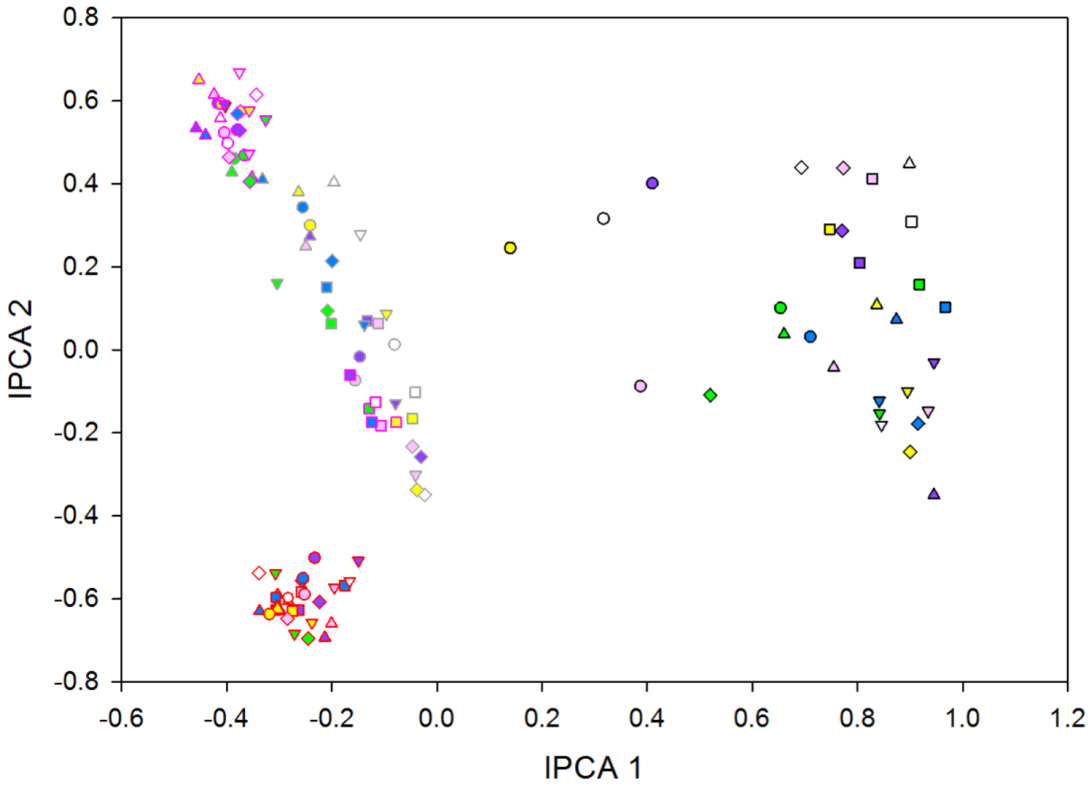

Fig. 4. 4. An interaction principal components analysis (IPCA) plot generated by AMMI analysis of terminal-restriction length polymorphisms (T-RFLPs) produced though amplification of bacterial 16s rRNA gene from soil samples taken a depth of 10 cm 15 cm from the trunk. Outline color indicates sampling date: black = June 2013, gray = August 2013, red = August 2014, and hot pink = August 2015. Shapes represent rootstock treatment: triangle = B.9, square = G.41, diamond = G.214, inverted triangle = G.935, and circle = M.9. Shape fill indicates fertilizer treatment: white = control (CON), yellow = calcium nitrate (FERT), light pink = chicken litter (CL), purple = chicken litter with calcium nitrate (CL+FERT), blue = yardwaste (YW), and green = yardwaste + calcium nitrate (YW+FERT). Each symbol represents the means of the four treatment replicates

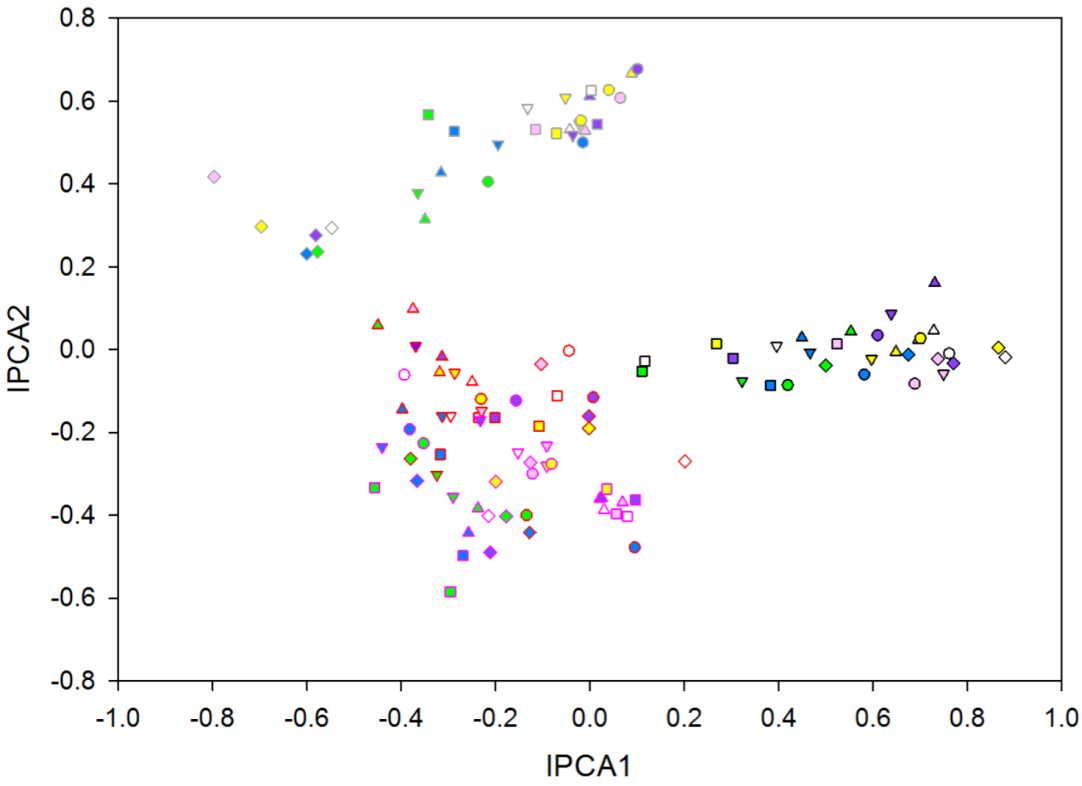

Fig. 4. 5. An interaction principal components analysis (IPCA) plot generated by AMMI analysis of terminal-restriction length polymorphisms (T-RFLPs) produced though amplification of the fungal ITS gene from soil samples taken a depth of 10 cm 15 cm from the trunk. Outline color indicates sampling date: black = June 2013, gray = August 2013, red = August 2014, and hot pink = August 2015. Shapes represent rootstock treatment: triangle = B.9, square = G.41, diamond = G.214, inverted triangle = G.935, and circle = M.9. Shape fill indicates fertilizer treatment: white = control (CON), yellow = calcium nitrate (FERT), light pink = chicken litter (CL), purple = chicken litter with calcium nitrate (CL+FERT), blue = yardwaste (YW), and green = yardwaste + calcium nitrate (YW+FERT). Each symbol represents the means of the four treatment replicates.

4.5 Discussion

Compost and integrated compost-calcium nitrate fertilizer treatments increased TCSA compared to the CON and FERT treatments by the second year of my study. During the third year of my study, there were no significant differences between the CL+FERT, YW, YW+FERT, and FERT treatments. However, the trees fertilized with the CL+FERT, YW, YW+FERT treatments had greater TCSAs than CON trees, and the CL fertilizer continued to increase TCSA compared to CON and FERT treatments. In contrast to similar studies that demonstrated that the use of fertigation with $Ca(NO_3)_2$, compost, or integrated compost fertilizers do not increase apple tree TCSA, my results indicate that composts and integrated compost fertilizers increase TCSA (Neilsen et al., 2004; Kramer et al., 2006; Yao et al., 2006; Neilsen et al., 2009; Baldi et al., 2010a; Forge et al., 2013; Sas-Paszt et al., 2014). The reasons for this difference may be that the pot-in-pot design of this study allowed the soil system to change more rapidly, and that working the compost treatment into the soil to a depth of 10 cm put the compost and integrated compost-calcium nitrate treatment into direct contact with the roots. As others have found, the G.214 rootstock TCSAs were larger than the B.9, G.41, and M.9 (Fazio et al., 2016). The G.935 rootstock was smaller than expected at planting and in the first two years following planting, but was similar in size to the G.214 rootstock by the third year.

The CL, CL+FERT, YW, and YW+FERT treatments increased total tree biomass and leaf biomass, and CL, CL+FERT, and YW increased root biomass compared to the CON treatment. Similarly, nitrogen fertilization has been shown to increase root production in sweet cherry, and compost fertilized peach trees had more cumulative root production and greater root survival than unfertilized trees (Bladi et al., 2010a; Artacho and Bonomelli, 2016). Composts add OM to soil, which often increases soil porosity and water holding capacity and reduces bulk

density, thus resulting in increased root and shoot growth (Passioura, 2002; Mathers et al., 2007). Humic acids extracted from composts have also been shown to increase growth of the wood perennial plant chicory (*Cichorium intybus*) (Valdrighi et al., 1996). The G.214 rootstock had greater root biomass and total tree biomass than the other rootstocks. Atucha et al. (2014) observed that apple replant disease (ARD) tolerant rootstocks from the Geneva series had greater root biomass than more susceptible rootstocks. There were no differences in root biomass among ARD tolerant rootstocks, G.41, G.214, and G.935, and susceptible rootstocks, B.9 and M.9, in this study. However, the rootstock with the greatest total biomass, G.214, also had the greatest root biomass.

Applying composts enhances soil fertility factors such as, plant-available minerals, OM, and CEC in orchards (Kramer et al., 2006; Forge et al., 2013; Baldi et al., 2010; Neilsen et al., 2014). Similarly in my study, compost and integrated compost-calcium nitrate fertilizer treatments increased plant available P, K, Ca, and B mineral nutrition, and the CL, CL+FERT, YW, and YW+FERT treatments also increased soil total N, total C, OM, and CEC.

During 2014 and 2015, trees were N and Mg deficient regardless of fertilizer or rootstock treatments (Stiles and Reid, 1991). In 2015, the CL+FERT and YW+FERT treatments increased leaf N concentration compared to the CON and FERT treatments, indicating that integrated compost-calcium nitrate fertilizer treatments can be used to increase leaf N concentrations. These results are contrary to those reported by Kramer et al. (2006), which demonstrated that integrated compost fertilizer applications did not increase leaf N concentrations. The integrated compost-calcium nitrate fertilizer treatments may have increased leaf N concentration in this study while similar treatments did not improve leaf N in other studies because 1) the pot-in-pot design confined the roots to a smaller area resulting in increased N uptake, and reduced N loss, 2) the

pot-in-pot design changed the soil physiochemistry more quickly than applying compost over a larger area in a field study, and 3) using a hand trowel to work the compost into the soil to a depth of 10 cm put the apple tree roots in direct contact with the compost amendment. The FERT treatment also increased leaf N concertation compared to CON, which did not receive N fertilizer during this study. These results are similar to those reported in other studies (Neilsen et al., 2009). The CL treatment increased leaf N concentration compared to the CON treatment. Sas-Paszt et al. (2014) reported a similar increase in leaf N concentration when compost was applied to a sandy loam soil. In 2014, only trees that received the FERT treatment had adequate leaf Ca concentrations (Stiles and Reid, 1991). However, in 2015, the Ca concentration was adequate in leaves from the FERT, CL, CL+FERT, and YW+FERT treatment. In 2015, leaves from the FERT treatment were P deficient, and leaves from the CON and FERT treatments were B deficient. Compost and integrated compost-calcium nitrate fertilizer applications provided trees with adequate macro- and micro-nutrient nutrition that were not available from synthetic N fertilizers during this study due to the nutrient content of the compost. In this study, compost applications increased soil P, K, B, and Mn, leading to increased leaf P, K, and B. In some studies, the application of compost, especially those with high C:N, lead to a reduction in leaf P, K, B, and Mn, thus determining compost mineral content prior to application is necessary to meet crop nutritional requirements (Yao et al., 2006; Sas-Paszt et al., 2014; Neilsen et al., 2014). In addition, applying compost fertilizers can decrease root suberization, which may lead to increased mineral nutrient uptake (Baldi et al., 2010a).

Differences in microbial community associating with these rootstocks due to root exudates and rhizodeposits may be responsible for differences in microbial biomass C and N. The CL, YW, and YW+FERT treatments increased microbial biomass C and N compared to the

CON and FERT treatments. Microbial biomass C increases are due to increases in available soil C and humic acid compounds from the compost additions (Wardle, 1992; Valdrighi et al., 1996). Compost additions increase microbial biomass C and N in a variety of soil types and cropping systems; however, the greatest increases tend to be observed in less fertile, coarse, C limited conditions, compared to the soil in which this study took place (Borken et al, 2002; Baldi et al., 2010b; Aira et al., 2010; Cytryn et al., 2011; Hernandez et al., 2014). In a sandy loam soil, applications of compost and integrated compost + synthetic N fertilizer increased microbial biomass compared to the unfertilized control (Hernandez et al., 2014). Similarly, low OM soil amended with compost had 3-4 times more microbial biomass (Cytryn et al., 2011). While microbial biomass in soils with greater OM, such as Cambisol soils, were less affected by compost applications (Baldi et al., 2010b). Despite increases in soil OM and total soil C, CL+FERT treatments in my study did not have the same effect as the CL, YW, and YW+FERT fertilizer treatments on microbial biomass because the C:N ratio of the compost treatment alone was already low, and the addition of synthetic N fertilizer further lowered the soil C:N ratio. In 2015, the soil C:N ratio where the CL+FERT treatments was applied was the same as the CON and FERT treatments. Peck et al. (2011) demonstrated the importance of the C:N ratio in increasing microbial biomass C in fertile soils; when additions of organic matter in the form of wood chips increased microbial biomass carbon, but additions of composted chicken litter, which has a lower C:N ratio, did not. Plant genotype can potentially increase microbial biomass. In maize (*Zea mays*), Aria et al. (2010) determined that maximum bacterial biomass in the maize rhizosphere was determined by both plant genotype and fertilizer type. Schweitzer et al. (2008) hypothesized that microbial communities are heritable traits, and plant genotype selects for soil microbial characteristics, including microbial biomass. Different genotypes of the poplar tree

species *Populus angustifolia* had greater microbial biomass N than others perhaps due to fine root production or phytochemical concentrations (Schweitzer et al., 2008). Larger plants were not associated with larger microbial biomass (Aria et al., 2010). Aria et al. (2008) hypothesized that microbial biomass is dependent on plant root exudate quantity and quality. In my study, the B.9 rootstock, the rootstock with the smallest root system, was associated with greater microbial biomass C and N than soil where the M.9 rootstock was planted.

The CL and YW treatments increased PMN compared to the CON treatment in 2014, and in 2015, the compost and integrated compost-calcium nitrate treatments increased PMN compared to CON and FERT treatments. Higher PMN rates indicate net N mineralization by soil microbes, indicating that the compost and integrated compost-calcium nitrate are mineralizing more N from the compost OM additions. This increased N mineralization may have improved leaf N concentration. In 2014, leaves from trees fertilized with the CL+FERT and YW+FERT treatments had the greatest leaf N content. Additionally, the compost and integrated compost fertilizer treatments had higher soil total N concentrations than CON or FERT treatments in 2013 and 2015, which may be related to increase PMN from these treatments. In 2014, PMN was greater where the G.214 rootstock was planted than where the G.41 rootstock was planted. The G.214 rootstock had the greatest overall tree biomass and root biomass; however, in 2014 and 2015 G.214 had the lowest leaf N concentration while G.41 had the greatest leaf N concentration. The G.214 rootstock should have greater leaf N content if microbes associating with rootstock mineralize more N. This discrepancy could be related to a potential dilution effect because of the increased biomass of the G.214 rootstock compared to the other rootstocks analyzed.

The YW treatment consistently increased cumulative microbial respiration compared to the CON, FERT, and CL+FERT treatments, and was related to increased microbial biomass C. Like microbial biomass, microbial respiration is related to additions of soil C and OM (Schnurer et al., 1985; Wardle, 1992). Similar increases in cumulative microbial respiration following compost or C based amendment application in orchard systems have been reported in multiple studies (Yao et al., 2006; St. Laurent et al., 2008; Peck et al., 2011). In my study, the YW treatment had the greatest total soil C and OM concentrations, and the greatest soil C:N ratio in 2013 and 2015. Microbes in the YW treated soil had greater cumulative microbial respiration because C was readily available as an energy source throughout the experiment. Treatments without added OM or C had the lowest cumulative microbial respiration levels because microbes had limited access to a C energy source resulting in less respiration.

The time (year) in which sampling took place, was a better predictor of bacterial and fungal community similarities, than the rootstock or fertilizer treatments. The IPCA for the bacterial and fungal communities explained relatively little of the variation seen in these communities. These results indicate that environmental conditions at sampling time, such as plant age, soil type, soil texture, and pH, may have a greater influence on community structure than the treatments (Marschner, 2001). Results from annual and perennial cropping systems measured at a single time point indicate that plant genotype significantly affects microbial community composition (Rumberger et al., 2004; Yao et al, 2006; Schweitzer et al., 2008; Aira et al., 2010). Rumberger et al. (2004) and Yao et al. (2006) observed that microbial communities of apple rootstocks were dependent upon the rootstock genotype, and that the rhizosphere of rootstocks with similar genotypes, such as replant resistant rootstocks, were colonized by similar microorganisms. Similar results were reported in the rhizosphere of maize and poplar trees

(Schweitzer et al., 2008; Aira et al., 2010). Other studies, conducted on several different soil types, found that ground cover management and fertilizer regime alters microbial community composition (Strauss et al., 2014; Bonilla et al., 2012; Peck et al., 2011; Cytryn et al., 2011). Composts increased the bacterial species diversity compared to other ground cover management methods in avocado orchards, and the bacterial communities associated with different composts, such as vegetable- or manure-based composts, were different from each other, and from the unamended control (Bonilla et al., 2012). These differences are most likely due to the microbial communities associated with the compost amendments. Cytryn et al. (2011) found that soils amended with compost had microbial communities more similar to the compost amendment than the unamended soil.

In summary, the chicken litter compost increased TCSA compared to the fertigation treatment, or the unfertilized control, and the yardwaste compost and integrated compost-calcium nitrate treatments increased TCSA compared to the unfertilized control. Applications of the integrated compost-$Ca(NO_3)_2$ treatments also increased leaf N concentration. These results differ significantly from the conclusions of several other studies. However; I hypothesize that working the compost treatments into the soil put the compost and integrated compost-calcium nitrate treatment into direct contact with the roots, allowing the roots to capture more mineralized nutrients. Trees that received the compost and integrated compost-$Ca(NO_3)_2$ amendments also had greater total tree biomass and leaf biomass compared to the control. It is possible that the trees that received the compost and integrated compost-$Ca(NO_3)_2$, had greater overall biomass and TCSA due to increase photosynthetic capabilities because of their greater leaf biomass. In addition, the compost and integrated compost fertilizer applications provide the added benefited of increased soil fertility measures, such as increased microbial activity and biomass,

OM, CEC, and soil mineral nutrition. Despite increases in microbial biomass and activity, fertilizer applications did not significantly alter microbial community structure of the bulk soil compared to the control. Increases in microbial biomass and PMN may have occurred in this study, but not in the field study, because the pot-in-pot system more rapidly changed the soil physiochemistry. Rootstocks from Cornell/Geneva series had a greater nutrient use efficiency then more widely planted B.9 and M.9 rootstocks; therefore, apple growers in P and K limited soils should consider using these rootstocks. Differences in leaf mineral nutrition among rootstocks does not appear to be related to the slight differences in soil mineral nutrition associated with rootstocks that were observed during this study. The rootstock treatments in this study did not alter microbial community structure, but they did have an effect on microbial biomass and N mineralization. Overall, more research is necessary to determine the effects of tilling or disking compost into the vegetation free strip under apple trees. This can be accomplished prior to planting to reduce the risk of injury to the tree. My data suggests this method may increase tree growth and mineral nutrition compared to ground applied compost.

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5. Compost Applications Increase Bacterial Community Diversity in the Apple Rhizosphere

5.1 Abstract

Apple (*Malus ×domestica* Borkh.) rootstock genotype and soil fertility management practices potentially plant associated soil microbial communities, and orchard productivity. Current data suggests certain apple rootstock cultivars can change microbial community structure, and cultivate microbial communities that make these trees more tolerant of apple replant disease. Additionally, C based amendment, such as compost, can stimulate microbial activity and change microbial community structure. In 2013, a pot-in-pot study was implemented to determine the effect of rootstock genotype and fertilizer formulation on microbial community structure and diversity of the rhizosphere in the apple rootstock rhizosphere. Nitrogen rate was applied at 40 kg ha^{-1} in the form of: chicken litter compost, yardwaste compost, or calcium nitrate. Non-fertilized containers served as a control. The v4 region of the bacterial 16s rRNA extracted from rhizosphere soil was sequences using the Illumina MiSeq platform. Of the 1,320,878 operational taxonomic units (OTUs) sequences, it was possible to identify 612,963 to the family level and 0.06% of these were identifiable at the phylum level. Alpha diversity, as reported by the Shannon diversity index, was significantly different among fertilizer treatments, with the yardwaste treatment having a lower H' value than all other fertilizers. Beta diversity was also significantly different among fertilizer treatments. The results of this study suggest that fertilizer application has a greater effect on microbial community diversity than rootstocks selection. More research regarding how changes in microbial community diversity potentially impact microbial community function are necessary to understand how changes in community diversity may affect plant growth and health.

5.2 Introduction

Apple (*Malus ×domestica* Borkh.) growers select rootstocks to confer specific beneficial traits, including ease of propagation, mature tree size, precocity, vigor, cold tolerance, pest and disease resistance, yield efficiency, and fruit quality (Fallahi et al., 2002). Below-ground traits, such as anchorage, nutrient and water uptake, and rhizosphere plant-microbe interactions vary substantially among rootstock genotypes. There is increased interest in planting rootstocks recently released from the joint Cornell-USDA-ARS breeding program because these rootstocks have greater yield efficiency, as well as tolerance to fire blight (*Erwinia amylovora*) and replant disease than the more commonly planted Malling and Merton-Malling rootstocks (Russo et al., 2007).

Numerous factors, including plant genotype, soil type, and soil pH, influence bacterial rhizosphere colonization (Marschner et al., 2001; Hartmann et al., 2009). According to several studies, soil characteristics, such as pH and texture, are the main determinants of rhizosphere microbial communities (Girvan et al., 2013; Winston et al., 2014; Yeoh et al., 2015; Zarraonaindia et al., 2015). However, apple orchard soil has been shown to be dominated by three phyla, Acidiobacteria, Actinobacteria, and Proteobacteria, regardless of soil type, recent replant status, or rootstock selection (St. Laurent et al., 2008; Zhang et al., 2013; Sun et al., 2014; Franke-Whittle et al., 2015). Zarraonaindia et al. (2015) hypothesized that these phyla, which appear to be ubiquitous in the rhizosphere of woody perennial plants, are attracted to the rhizosphere from neighboring bulk soil by carbon-based exudates.

Plant genotype is also a significant factor affecting the rhizosphere microbial communities through the production of different C-based exudates, rhizodeposits, antimicrobials, and other exudates that alter soil pH in the rhizoplane (Hartmann et al., 2009). Winston et al.

(2014) found that some *Cannabis* strains affected bacterial endophyte diversity, and abundance, including bacteria associated with plant health such as Pseudomonadales, and Rhizobiales. In contrast, the bacterial community composition of sugarcane (*Saccharum officinarum* × *spontaneum* L.) was most significantly affected by soil type and sampling time while sugarcane genotype did not affect rhizosphere community composition (Yeoh et al., 2015). In perennial systems, rootstock genotype can alter the soil microbiome in ways which improve plant health and productivity (Rumberger et al., 2004; St. Laurent et al., 2010; Rolli et al., 2015; Song et al., 2015). For instance, apple and grape (*Vitis vinifera*) rootstocks enhance plant productivity through the cultivation of microbial communities that improve disease resistance and drought tolerance (Rumberger et al., 2004; St. Laurent et al., 2010; Rolli et al., 2015). Apple rootstocks with similar genotypes and parentages have more similar microbial community compositions than less related rootstocks, and rootstock selection has been shown to alter microbial communities in ways that result in greater tolerance to apple replant disease (Rumberger et al., 2004; St. Laurent et al., 2010). Rootstock genotype may also influence the presence of functional genes in the rhizosphere. Zarraonaindia et al. (2015) found several functional genes, including genes responsible for mineral nutrient metabolism and uptake, motility, and chemotaxis, associated with the grape rhizosphere; however, it is important to note that only one grape rootstock genotype (3309) was studied.

The addition of synthetic or carbon-based fertilizers to the soil may also alter the microbial community. In both managed and unmanaged ecosystems, applications of synthetic N fertilizers have been shown to reduce soil respiration, microbial biomass, and enzyme activity, and to shift microbial community composition resulting in reduced C sequestration compared to unfertilized controls (Ramirez et al., 2012). In maize (*Zea mays* L.), Zhu et al. (2016) found that

increasing synthetic N fertilizer application rate increased the overall abundance of soil bacteria, and gene copies associated with N cycling. The N fertilized maize rhizosphere contained more Bacillales, Nitrosomonadles, and Rhodocyclales, and fewer Cloroflexales, Gemmatimonadetes, and Phycisphaerae compared to the unfertilized control. In sugarcane, lower rates of N fertilizer application increased the bacterial community diversity in the maize rhizosphere; however, soil type and when soil was sampled had a larger effect on bacterial community composition (Yeoh et al., 2015). Nitrogen fertilizer rate did not affect the microbial populations responsible for N fixation (Yeoh et al., 2015). In both managed and unmanaged ecosystems, Fierer et al. (2012) observed that N additions increased the number of copiotrophic bacteria, but reduced oligotrophic soil bacteria.

Changes in soil physiochemistry, such as those that occur when compost is applied, are associated with changes in microbial community composition. Winston et al. (2014) observed that soil total nitrogen (N) strongly shifted microbial community associated with *Cannabis*, and Zarraonaindia et al. (2015) found that soil physiochemical factors, such as pH and soil total C and N, and the C:N ratio, altered the microbial community structure associated with grape roots. Despite applications of manure to apple trees planted in a sand culture, the same phyla remained dominant in the soil samples; however, manure application did decrease the soil *Actinobacteria* and *Proteobacteria* content, but lead to an overall increase in bacterial diversity and increased soil *Sinobacteraceae* and *Arthrobacter* content compared to the unfertilized control soil (Zhang et al., 2013). In contrast, Shade et al. (2013) observed that soil mineral nutrient differences in apple orchard soil did not affect bacterial community composition. In apple orchards, the use of compost as a fertilizer increase soil mineral nutrition, organic matter (OM), cation exchange capacity (CEC), microbial biomass, and microbial activity (Kramer et al., 2006; Yao et al., 2006;

Strauss et al., 2014; Rumberger et al., 2004). Compost applications in tomato fields increased the number plant growth promoting bacteria that are antagonistic to plant pathogens, such as *Fusarium*, *Pythium*, *Phytopthora*, and *Rhizoctonia* species (de Brito Alvarez et al., 1995). *Fusarium* has been strongly correlated with reductions in apple tree growth under replant conditions, thus applications of compost may reduce replant disease incidence (Franke-Whittle et al., 2015). In replant apple orchards, compost applications are associated with increased microbial activity and shifts in rhizosphere microbial community composition (Rumberger et al., 2004; Yao et al., 2006). However, these shifts in microbial community composition were not related to decreased incidence of replant disease symptoms.

The objective of this study was to identify rhizosphere bacteria associated with widely used 'Malling 9' rootstock and recently released 'Geneva 41' rootstock, and how these cultivars interact with carbon-based N and synthetic N fertilizers. I hypothesized that both rootstock and fertilizer treatments would cause significant changes in structure and diversity of the rhizosphere bacterial community.

5.3 Materials and Methods

A pot-in-pot experiment was implemented in 2013 at the Virginia Tech Alson H. Smith, Jr. Agricultural Research and Extension Center in Winchester, VA (39° 06' N, 78° 17' W). 'Brookfield Gala' scions were grafted onto two rootstocks, 'Malling 9' and 'Geneva 41'and planted in 38 L pots containing native Poplimento silt loam orchard soil, a fine, mixed, subactive, mesic Ultic Hapludalf (NRCS, 2001). Soil in each pot was mixed using a cement mixer with 10% (v/v) STALITE® lightweight soil aggregate (Salisbury, NC) to improve drainage and aeration. Four individual tree replicates of each rootstock received 40 kg ha^{-1} plant available N from (1) locally sourced chicken litter compost (CL) (Valley Pride Compost,

Harrisonburg, VA), (2) locally sourced yardwaste compost (YW) (Loudoun Composting, Chantilly, VA), (3) fertigation with calcium nitrate [$Ca(NO_3)_2$] (FERT) (Yara, Oslo, Norway) for 8 weeks, or (4) served as an unfertilized control (CON). Fertilizer was applied in May 2013, 2014, and 2015, and new composted material was acquired each year. Compost analysis was performed by Penn State Agricultural Analytical Services Laboratory (University Park, PA) prior to application to ensure plant available N was applied at an equal rate among fertilizer treatments. Application of other mineral nutrients and organic matter differed among treatments, and were not controlled for in this experiment. Trees were irrigated using a micro-spray irrigation system three times a week throughout the spring and summer, hand-weeded when necessary, and uniformly treated for pests and diseases (Pfeiffer et al., 2015).

On 26 May 2015, two weeks after the final compost application, a total of six roots were harvested from each pot using a sterile Hori-Hori Japanese soil knife. Of the six roots sampled, three were removed from the south side of the tree, and three were removed from the north side of the tree. Harvested roots measured 10 cm in length from the root tip, and were cut from each rootstock and treatment combinations using sterile hand pruners. After sampling, excess soil was removed from roots by gentle shaking. Roots were stored at -80 °C prior to DNA extraction.

Soil was removed from three roots using a sterile paintbrush and then homogenized. A modified protocol was followed to extract total genomic DNA using the MoBio Lab Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA). Approximately 0.25 g of soil were placed in the bead tube provided by MoBio, and heated to 65 °C for 10 min in a water bath. Following this modification, the manufacturer's protocol was followed. DNA quality and concentration were assessed using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, Massachusetts). DNA was stored at -20°C until polymerase chain reaction (PCR) amplifications were performed

in triplicate 25 μL reaction volumes for each sample. The bacterial 16s rRNA v4 region was amplified using the method described by Caporaso et al. (2012). Polymerase chain reactions contained 2 μL template DNA, 11.875 μL nuclease free water (MoBio), 1X 5 Prime HotMasterMix (Gaithersburg, MD), 10 μM 515f forward primer, 10 μM 806r reverse primer, and 0.1 $\mu g\ \mu L^{-1}$ bovine serum albumin (BSA). The reverse primer contained a unique Golay barcode sequence (Caporaso et al., 2011). The reaction conditions for this PCR were a 3 min denaturation step at 94 °C, followed by 35 cycles of 94 °C for 45 s, 68 °C for 60 s, and 72 °C for 90 s, and a final extension step of 72 °C for 10 min. Reactions were amplified in a Bio-Rad C1000 Thermal Cycler (Hercules, CA). PCR products were visualized on a 1.5% agarose gel in 1 X tris borate EDTA buffer (TBE) stained with GelStar (Lonza, Basel, Switzerland). The triplicate amplifications were pooled and quantified using a QuBit High Sensitivity DNA quantification system (Invitrogen, Carlsbad, CA). A single, pooled sample was formed by adding 250 ng of DNA from each PCR. The Qiaquick PCR Purification Kit (Quiagen, Valencia, CA) was used to purify the pooled sample. After purification, the quality and concentration of the sample was assessed using a Nanodrop spectrophotometer. Sequencing using 250-bp paired-end reads was completed at the Biocomplexity Institute at Virginia Tech using the Illumina MiSeq platform (San Diego, CA).

Barcodes, adapters, and primers were trimmed from the reads, and the quality was analyzed. A mean quality score of 30 was used for quality assessment. The QIIME pipeline was used to analyze bacterial sequence data. Reads were clustered into operational taxonomic units (OTUs) based on 97% similarity. Taxonomy was assigned to OTUs using GreenGenes. Alpha and beta diversity analyses were completed using the VEGAN package for R.

Table 5. 1.The C:N ration, organic matter, total C, organic N, ammonium, nitrate, P, and K content of the chicken litter and yardwaste composts were measured in 2013, 2014, and 2015 prior to compost application to ensure plant available N was equivalent among fertilizer treatments.

Compost	C:N	OM (g kg^{-1})	C (g kg^{-1})	Organic N (g kg^{-1})	Ammonium (mg kg^{-1})	Nitrate (mg kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
2013								
Chicken litter	15.8	458	260	165	5.0	1,012.62	18.8	7.50
Yardwaste	14.4	538	246	171	55.6	21.03	4.60	11.5
2014								
Chicken litter	14.9	475	293	196	5.0	501.40	19.0	8.60
Yardwaste	18.8	608	342	181	36.8	44.00	4.10	11.2
2015								
Chicken litter	12.9	473	246	190	5.0	601.05	16.8	6.50
Yardwaste	18.1	515	291	161	48.2	91.52	3.50	7.80

5.4 Results

Following normalization, a total of 1,320,878 valid reads were obtained for all Rootstock x Fertilizer combinations and their replicates. The M.9 rootstock treatment had 681,744 valid reads, and the G.41 rootstock treatment had 639,135 valid reads. The CL, YW, FRT, and CON treatments yielded 298,263, 340,872, 340,872, and 340,871 valid reads, respectively. The Shannon, Simpson, and Chao1 diversity indices indicated that there were no significant differences in alpha diversity between the two rootstocks (Table 5.2). There were no significant differences in the Simpson or Chao1 indices among fertilizer treatments, but the Shannon diversity index H value was significantly different among fertilizer treatments. The YW treatment had the lowest H value. The dendrogram of Chao1 distances indicates that the rootstock treatments did not have a significant effect on microbial community, but the CL and YW treatments were clustered separately and were different from the FERT and CON

treatments, which clustered together (Fig. 5.2). Beta diversity, calculated as Bray-Curtis dissimilarity, was not significantly different between rootstock treatments, but was significantly different among fertilizer treatments (Table 5.2). The Bray-Curtis dissimilarity distances used to make the nonmetric multidimensional scaling (NMDS) plot further illustrate beta diversity differences among the communities associated with each treatment (Fig. 5.3). The CL and YW treatments clustered separately along the positive side of the X axis with the CL treatment clustering toward the negative side of the Y axis while the YW treatment clustered along the positive end of the Y axis. The FERT and CON treatments clustered together along the negative end of the X axis. The X axis explains 27.6% of the variation and the Y axis explained 12.8% of the variation.

Operation taxonomic units (OTUs) were identified as representing 41 different phyla, and only 0.06% of the sequences were unidentifiable at the phylum level. Most OTUs belonged to one of 12 phyla, Acidiobacteria, Actinobacteria, Armatimomadetes, Bacteroidetes, Chloroflexi, Cyanobacteria, Euryarchaeota, Gemmatimonadetes, Nitrospirae, Plantomycetes, Proteobacteria, and Verrucomicrobia (Fig. 5.4). Acidiobacteria were the most prevalent phylum identified in the rhizosphere of both rootstock treatments, and made up 17% of the community associated with both rootstocks (Fig. 5.4). Proteobacteria were 8% and 6% of the M.9 and G.41 rhizosphere communities, respectively. Plantomycetes were 6% of community for both rootstocks. Chloroflexi were 5% and 6% of the M.9 and G.41 rhizosphere communities, respectively. Verrucomicrobia were 6% and 5% of the M.9 and G.41 rhizosphere communities, respectively. Acidiobacteria were also the most common phylum identified among all treatments, and were 8% of the rhizosphere bacterial communities associated with the CL, YW, and FERT treatments, and 9% of the community associated with the CON treatment. Similar to the rootstock

treatments, Proteobacteria, Plantomycetes, Chloroflexi, and Verrucomicrobia were common phyla among all fertilizer treatments (Fig. 5. 5). Acidiobacteria, Bacteroidetes, Chloroflexi, Plantomycetes, Proteobacteria, and Verrucomicrobia were the most prevalent phyla associated with all Rootstock x Fertilizer treatment combinations (Fig. 5.6).

I identified 612,963 OTUs, 46% of the valid sequences, to the family level. Most of the identified OTUs belong to one of 25 families (Fig. 5.7) The families Chthoniobacteraceae, Planctomycetaceae, A4b, Chitinophagaceae, and RB40 were the most common families in the rhizosphere of both rootstock treatments. Chthoniobacteraceae were most common family present in the rhizosphere of both rootstock treatments, and were 9% of the community (Fig. 5.7). Similarly, the family Chthoniobacteraceae was the most common family present in the rhizosphere of all fertilizer treatments and the CON treatment (Fig. 5.8). In the CL and YW treatments, the families A4b, Planctomycetaceae, RB40, and Pirellulaceae were the most common. In the FERT treatment, A4b, Chitinophagaceae, Planctomycetaceae, and Sphingomonadaceae were the most common. In the CON treatment, the families Chitinophagaceae, Planctomycetaceae, Sphingomonadaceae, and Pirellulaceae were most common. For the Rootstock x Fertilizer interaction, Chthoniobacteraceae was also the most common bacterial family in the rhizosphere (Fig. 5.9).

Table 5. 2. Comparisons of the normalized operation taxonomic unit (OUT) count alpha and beta diversity and richness indices for the rootstock treatments Geneva 41 (G.41) and Malling 9 (M.9), and the fertilizer treatments chicken litter (CL), control (CON), fertigation (FERT), and yardwaste (YW).

	Shannon	**Simpson**	**Chao1**	**Bray-Curtis**
Rootstock				
G.41	5.758	0.986	1,907	
M.9	5.907	0.991	2,022	
p value	0.1	0.2	0.2	0.1
Fertilizer				
CL	5.927	0.987	1,942	
CON	5.919	0.992	1,984	
FERT	5.944	0.991	1,992	
YW	5.541	0.983	1,939	
p value	0.01	0.2	0.9	0.001

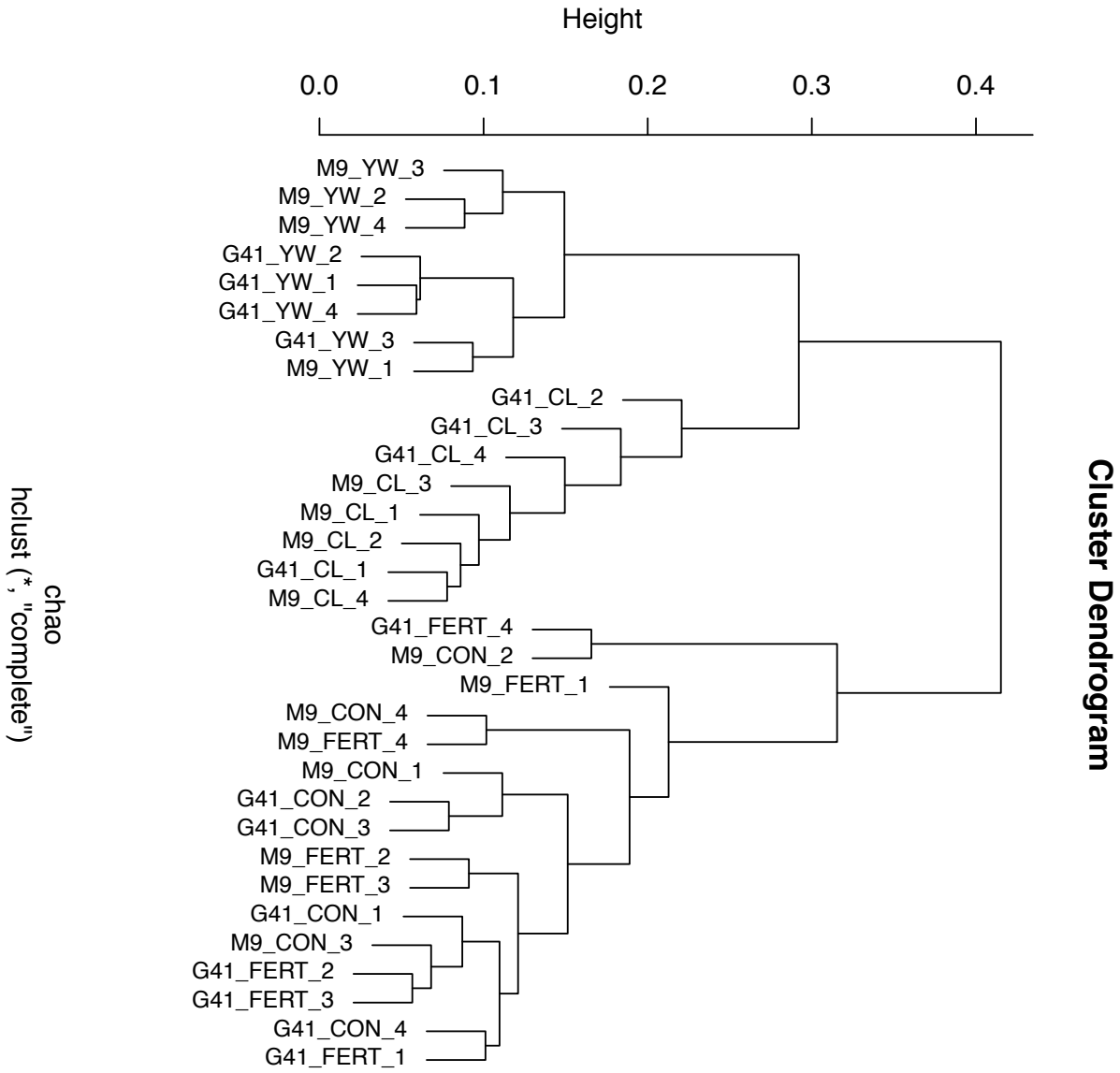

Fig. 5. 1. A cluster dendrogram created using the Chao1 distances to illustrate bacterial community similarity. The abbreviations were as follows for fertilizer treatments: chicken litter compost (CL), yardwaste compost (YW), fertigation (FERT), and control (CON) fertilizer treatments. Root stock treatments abbreviations were: Geneva 41 (G41) and Malling 9 (M9).

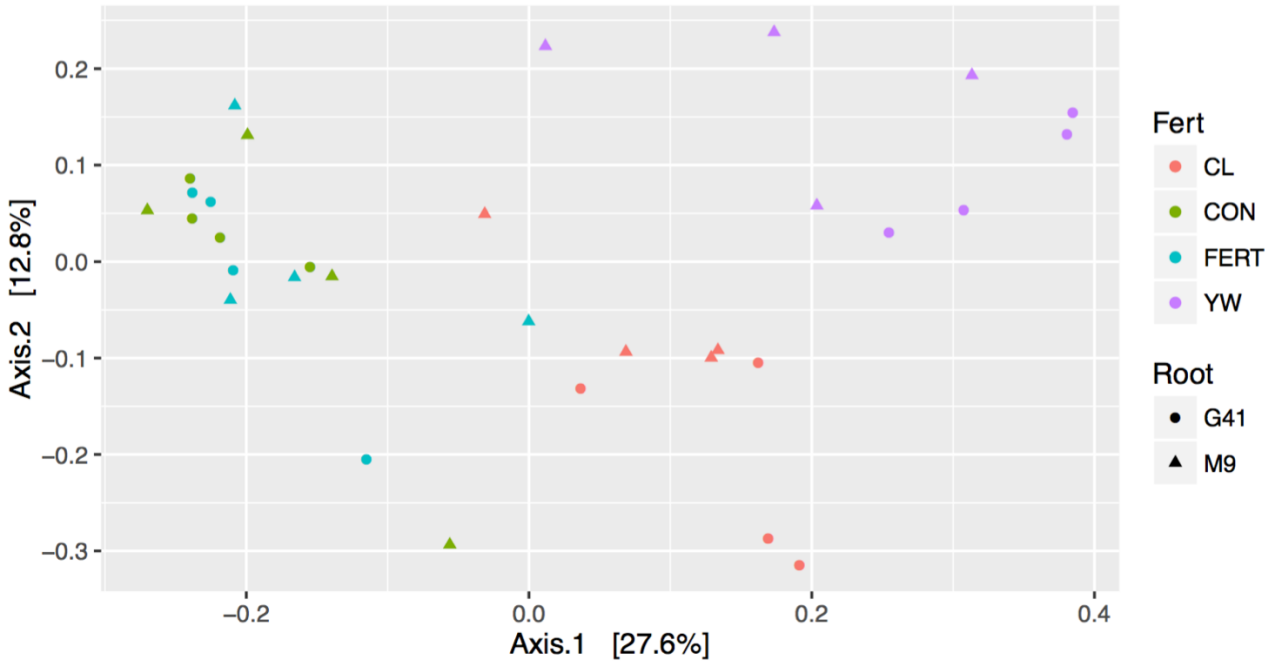

Fig. 5. 2. Nonmetric multidimensional scaling (NMDS) plot of microbial community similarity using log transformed Curtis-Bray dissimilarity. Each symbol represents four replicates of each treatment. Fertilizer treatment abbreviations are as follows, chicken litter compost (CL), CON (control), fertigation (FERT), and yardwaste (YW), Geneva 41 (G41), and Malling 9 (M9).

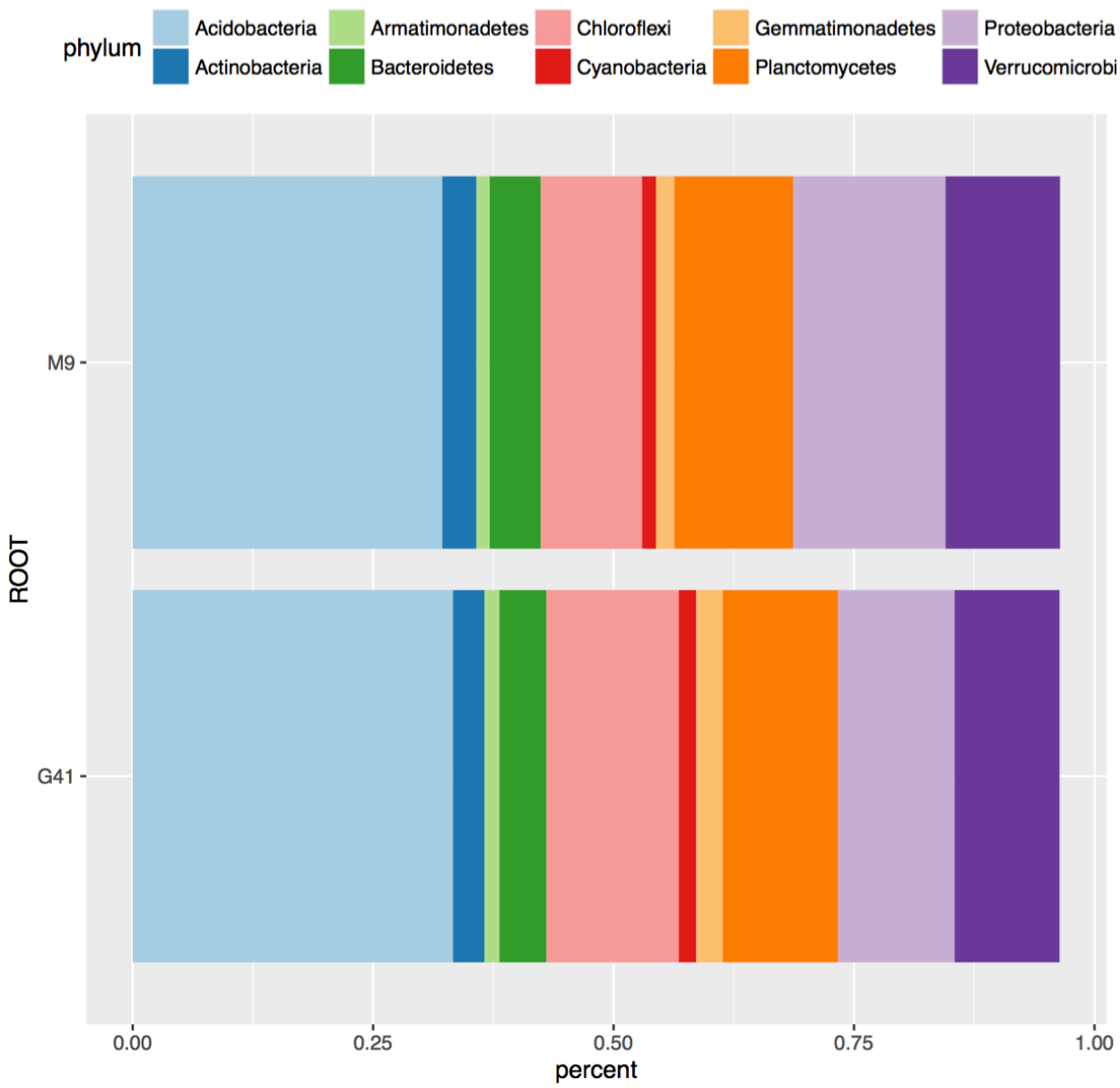

Fig. 5. 3. Comparison of the relative read abundance of bacterial phyla associated with the Geneva 41 (G41) and Malling 9 (M9) rootstocks.

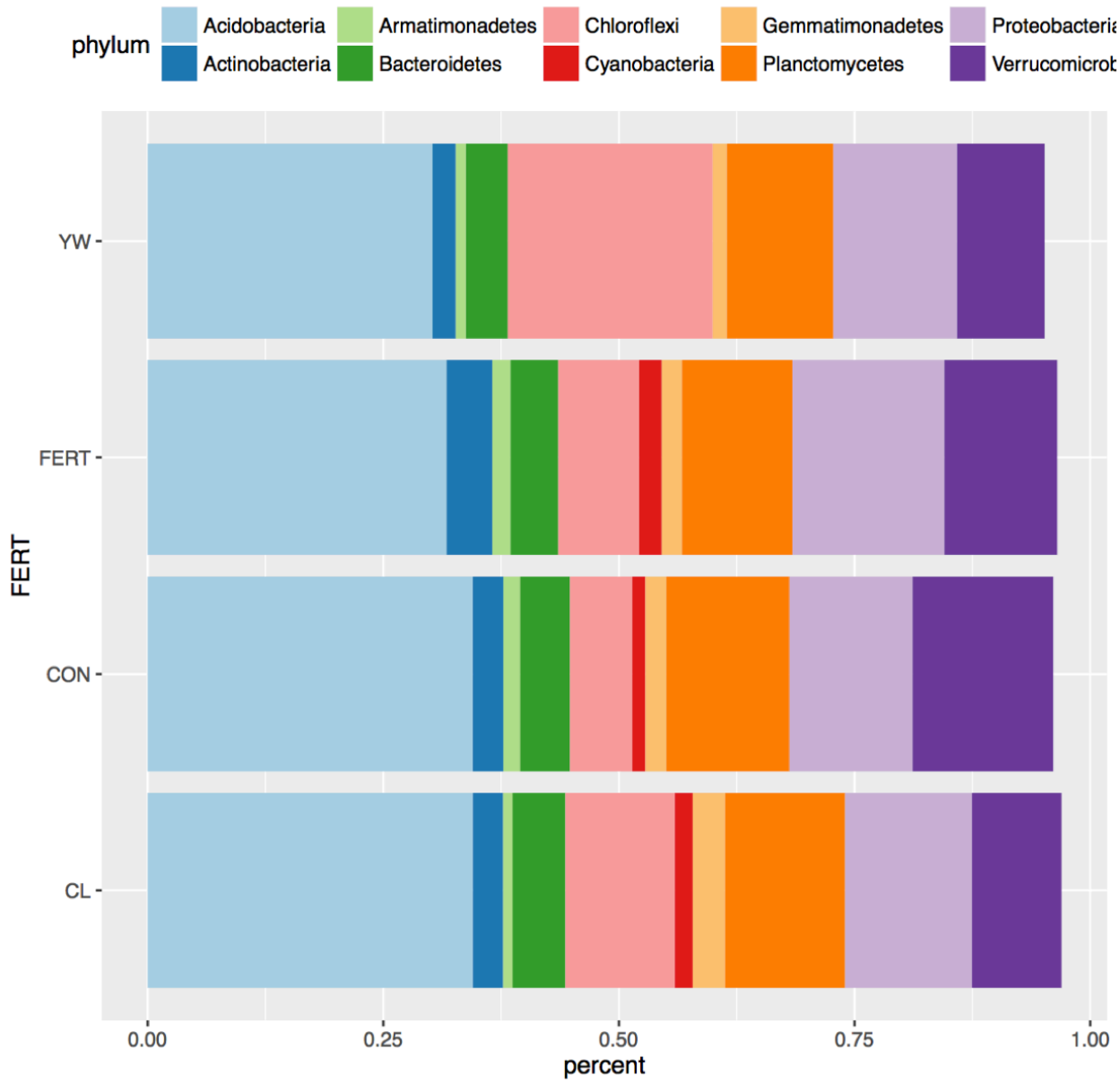

Fig. 5. 4. Comparison of the relative read abundance of bacterial phyla associated with the chicken litter compost (CL), yardwaste compost (YW), fertigation (FERT), and control (CON) fertilizer treatments.

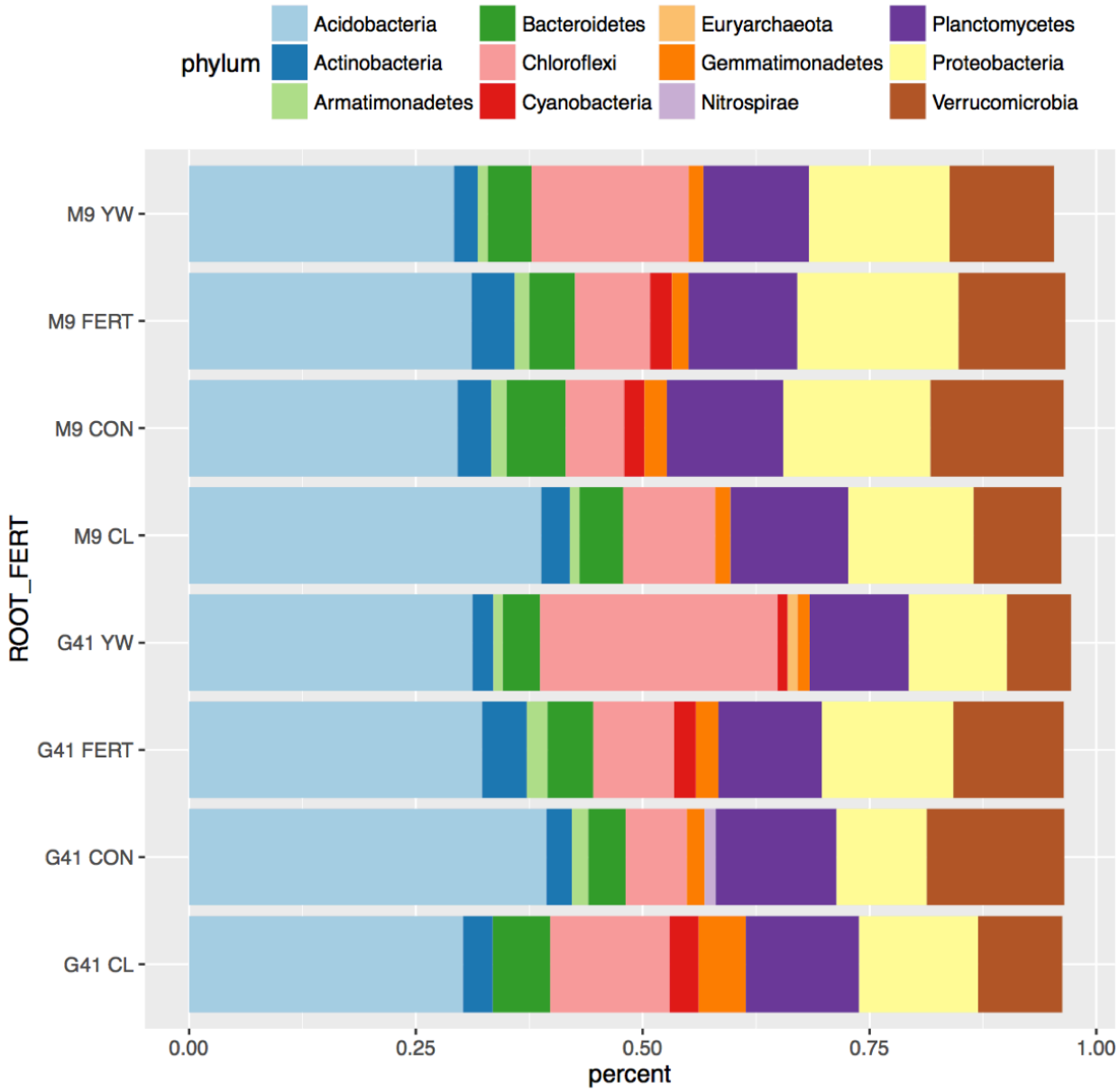

Fig. 5. 5. Comparison of the relative read abundance of bacterial phyla associated with Rootstock x Fertilizer treatments. Treatment abbreviations were: chicken litter compost (CL), yardwaste compost (YW), fertigation (FERT), and control (CON), Geneva 41 (G41) and Malling 9 (M9) rootstocks.

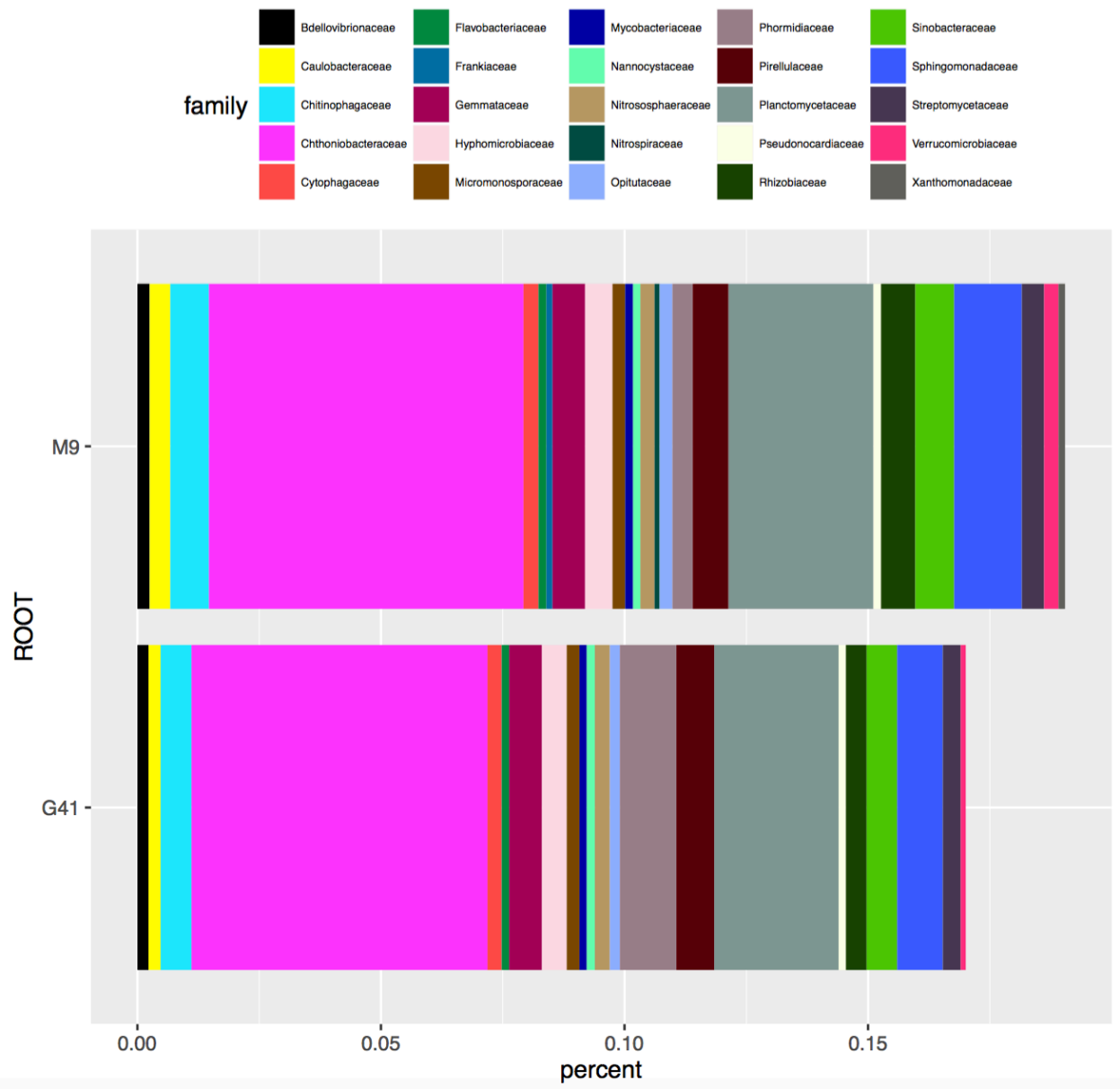

Fig. 5. 6. Comparison of the relative read abundance of bacterial families associated with the Geneva 41 (G41) and Malling 9 (M9) rootstocks.

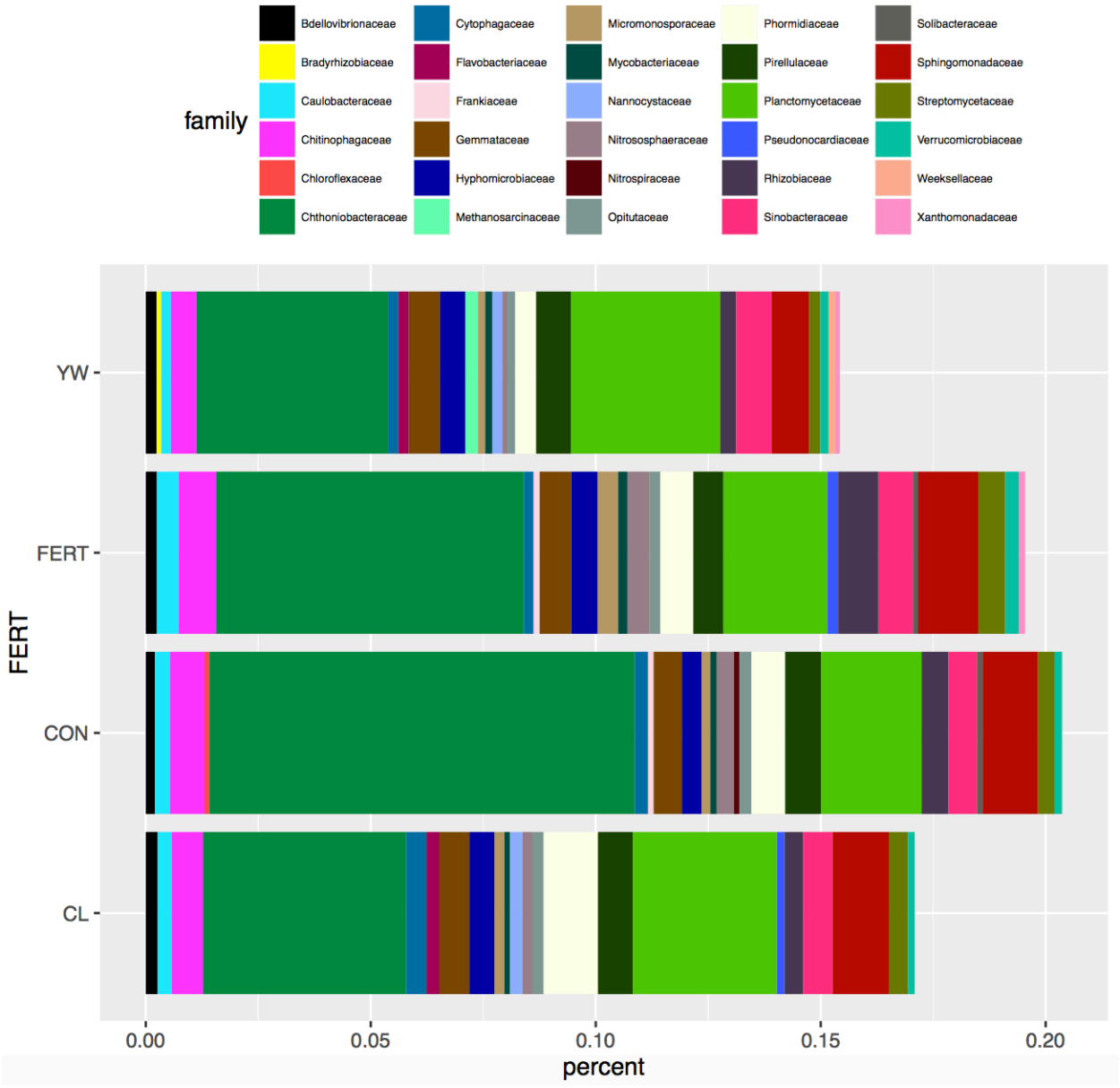

Fig. 5. 7. Comparison of the relative read abundance of bacterial families associated with the chicken litter compost (CL), yardwaste compost (YW), fertigation (FERT), and control (CON) fertilizer treatments.

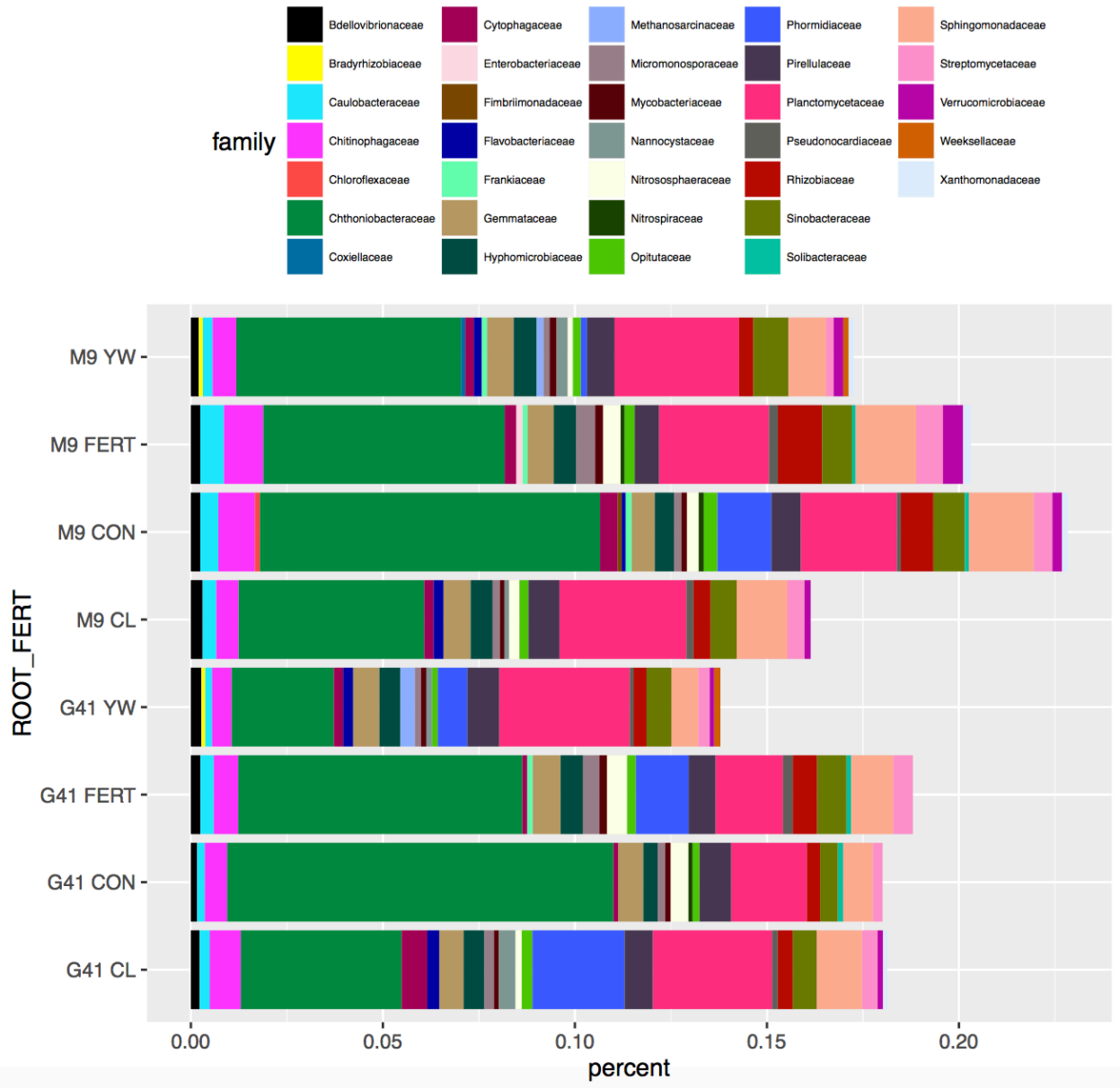

Fig. 5. 8. Comparison of the relative read abundance of bacterial families associated with Rootstock x Fertilizer treatments. Treatment abbreviations were: chicken litter compost (CL), yardwaste compost (YW), fertigation (FERT), and control (CON), Geneva 41 (G41) and Malling 9 (M9) rootstocks.

5.5 Discussion

The alpha diversity indices, Simpson and Chao1 were similar for rootstock and fertilizer treatments; however, the Shannon diversity index was significantly different for the fertilizer treatment. The Simpson diversity index, which considers species dominance, D' values were similar for rootstock and fertilizer treatments. However, the Shannon diversity index H' values, which characterize diversity and species evenness were similar for rootstock treatments, but were significantly different among fertilizer treatment. The Shannon H' values were greater in the CL, CON, and FERT treatments than the YW treatment, indicating increased OTU richness, diversity, and evenness in the CL, CON, and FERT treatments. Despite differences in Shannon values, there were no differences in Chao1 indices among treatments, indicating that the number of rare OTUs were similar regardless of rootstock or fertilizer treatment.

Zhang et al. (2013) found that manure applications did not affect OTU richness and diversity as measured by Chao or Shannon indices for apple trees grown in a sand culture system amended with different amounts of manure. However, the Simpson value of the control treatment, containing no manure, and the highest (25% w/w manure addition) treatment had greater OTU diversity than the other manure treatments (Zhang et al., 2013). Unlike the results of my study, or those presented by Zhang et al., 2013, Fierer et al. (2012) did not observe changes in bacterial community diversity related to synthetic N fertilization rate in either unmanaged or managed grassland ecosystems.

Bacterial species richness and diversity has been correlated with levels of soil organic carbon (SOC) and nutrient availability (Fierer et al., 2012; Sul et al., 2013). Sandy loam agricultural soils containing less SOC have lower bacterial community richness and diversity (Sul et al., 2013). In my study, the treatment with the highest total soil C, the YW treatment, had

lower bacterial community diversity than the FERT or CON fertilizer treatments, which did not add C to the soil like the CL and YW treatments. The silt loam soil analyzed used for my study contained a greater concentration of native SOC and organic matter than the sandy loam studied by Sul et al. (2013) or Zhang et al. (2013), thus increased soil C in this study may not have had the same dramatic effect on bacterial community diversity. Soil nutritional differences, such as those related to compost mineral nutrition, were not related to differences in microbial community diversity or richness (Peiffer et al., 2013; Shade et al., 2013). However, edaphic properties, such as soil total N and C, C:N and pH, can have significant effects on the rhizosphere community diversity and abundance of horticultural crops, such as grape and cannabis (Winston et al., 2014; Zarraonaindia et al., 2015). I hypothesize that greater soil C and C:N in the CL and YW treatments, compared to the CON or FERT treatments, increased microbial community alpha and beta diversity.

In studies of the apple root rhizosphere bacterial communities using terminal restriction length polymorphisms (T-RFLPs) replant disease resistant rootstock varieties had more similar microbial communities than susceptible rootstocks (St. Laurent et al., 2010; Rumberger et al., 2004). The rootstocks used in this study, M.9 and G.41, are not related, and the M.9 rootstock is not replant disease tolerant, while the G.41 rootstock is apple replant disease tolerant. Despite these differing characteristics, the M.9 and G.41 rootstocks had similar alpha and beta diversity in the rhizosphere. Plant genotype affected the alpha diversity in the maize plant rhizosphere (Peiffer et al., 2013). Similarly, studies of annual plants in a variety of soil types, genotype had a slight, but significant effect on beta diversity in the rhizosphere (Peiffer et al., 2013; Yeoh et al., 2015). However, plant genotype did not have a significant effect on bacterial rhizosphere beta diversity of cannabis plants, but plants with different genotypes had different endophytes

(Winston et al, 2014). This further suggests that sampling location is extremely important to consider.

The beta diversity measures, as estimated by Bray-Curtis dissimilarity, were significantly different among the fertilizer treatments, but not between rootstock treatments. Differences in beta diversity among the fertilizer treatments indicates that fertilizers changed the rhizosphere microbial community diversity between communities. Similar differences in community diversity were observed across N fertilization gradients in managed and unmanaged ecosystems (Fierer et al., 2012). Additionally, soil fertility treatments that increase SOC had microbial communities that were more similar to each other than treatments with less SOC (Sul et al., 2013). Compost treatments, which increase total soil C and OM, had communities that were more similar to each other than to the CON and FERT treatments in this study.

Acidiobacteria, Proteobacteria, Plantomycetes, Chloroflexi, and Verrucomicrobia were the most common phyla associated with the apple rhizosphere independent of rootstock or fertilizer treatment in this study. Similarly, Acidiobacteria, Actinobacteria and Proteobacteria dominated both rhizosphere and bulk soil in apple orchards independent of soil type or rootstock choice (St. Laurent et al., 2008; Zhang et al., 2013; Sun et al., 2014; Franke-Whittle et al., 2015). However, in this study, the phylum Actinobacteria, which was one of the dominant phyla in several other studies, was not dominant. Sul et al. (2013) observed that the abundance of Actinobacteria was greatest in soils with low SOC, thus the native C associated with the higher organic matter in the silty loam soil in Virginia may have reduced the number of Actinobacteria. Synthetic N amendments have been shown to increase the number of Proteobacteria and Bacteroidetes, and decrease Acidiobacteria in managed and unmanaged ecosystems (Fierer et al., 2012). The abundance of Bacteroidetes was greater in the FERT and CON treatments than in the

compost treatments. Applications of composts and FERT treatments decreased soil acidiobacteria slightly compared to the CON treatment.

Acidiobacteria, the most dominate phylum in this study, are extremely diverse in their habitat and function, and are frequently referred to as oligotrophs although their ecological function has not been fully determined or understood (Kielak et al., 2016). Although Acidiobacteria abundance has been negatively correlated with SOC and increased soil mineral nutrition, this phylum was abundant in all treatments, regardless of C or soil mineral nutrient content, in my study (Kielak et al., 2016). Proteobacteria were the second most dominate phylum in the apple rhizosphere. Similar to Acidiobacteria, Proteobacteria are extremely diverse in their habitat and function, and include plant growth promoting bacteria, such as Bradyrhizobiales (Spain et al., 2009). The phylum Verrucomicrobia was the fifth most abundant in the rhizosphere. This phylum is considered one of the less abundant phyla in soil compared to Acidiobacteria and Proteobacteria, although it is considered ubiquitous, and it is also considered to be oligotrophic (Bergmann et al., 2011). The family Chthoniobacteraceae, a member of the phylum Verrucomicrobia, was the most abundant family in all treatments. The most abundant families, A4b, Planctomycetaceae, RB40, and Pirellulaceae, in the compost treatments were the same. The families associated with the FERT and CON treatments were different from the compost treatments. In the FERT treatment the families A4b, Chitinophagaceae, Planctomycetaceae, and Sphingomonadaceae were the most abundant, and in the CON treatment Chitinophagaceae, Planctomycetaceae, Sphingomonadaceae, and Pirellulaceae were most abundant. The Chitinophagaceae and Sphingomonadaceae, which were dominant in the FERT and CON treatments were not dominant in the compost treatments.

In summary, apple rootstock did not significantly alter the rhizosphere bacterial community diversity or abundance; however, fertilizer treatment significantly altered both alpha and beta diversity. It is necessary to understand the function of these changing rhizosphere communities so that commercial growers can make more informed management decisions regarding fertilizer use in apple orchards.

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6. Conclusion

As Virginia apple growers increase their plantings of high-density apple orchards, it is necessary to develop research-based fertilizer recommendations for young trees planted on dwarfing and semi-dwarfing rootstocks. The profitability of high-density orchard systems depends upon obtaining high fruit yields as soon as possible after planting. In order to achieve earlier fruiting, trees planted on dwarfing or semi-dwarfing rootstocks must have sufficient growth, which is often achieved through the application of synthetic nitrogen fertilizers. However, in excess of tree requirements, nitrogen fertilizers may reduce crop yield and quality. Excessive nitrogen (N) application and low N recovery by apple trees may also result in N leaching, runoff and volatilization, which can have negative environmental consequences, such as eutrophication and air pollution. Finally, synthetic N sources are often costly for farmers because they require large energy inputs during production. As fossil fuel costs increase, the cost of synthetic N products produced via the Haber-Bosch process have concomitantly become costlier.

Prior to the research described within this dissertation, there were few studies describing the effects of fertigation, composts, and biochar on tree growth, productivity, and leaf mineral nutrition, soil fertility, and soil microbial communities in newly planted high-density orchards in the mid-Atlantic. Few studies focused on understand the effects of various fertilizer types, such as synthetic N, compost, and integrated compost-synthetic N fertilizers, on popular and newly released apple rootstock cultivars. Even fewer studies have addressed the use of high rates of biochar on young, newly planted trees in fertile finely textured soils. The goal of my dissertation research was to determine the effects of carbon (C)-based soil amendment, such as compost and biochar, and rootstock genotype on apple tree growth and productivity, and soil fertility with an

emphasis on microbial community composition and activity. To accomplish this, a series of experiments were designed to evaluate fertilizers, including a commonly used synthetic fertilizer, calcium nitrate, locally sourced chicken litter and yardwaste composts, and integrated compost-calcium nitrate fertilizers, and rootstocks, including the commonly planted 'Budagovsky 9', and 'Malling 9', and the recently released 'Geneva 41', 'G.214', and 'G.935'.

The use of synthetic N fertilizer, compost and integrated compost-calcium nitrate fertilizers to increase the growth, fruit yield and quality, and soil fertility in newly planted orchards was evaluated in Chapter 2. I hypothesized that integrated fertilization with applications of composts and calcium nitrate would improve apple tree growth and soil quality after three years, compared to unfertilized trees and compost amended trees because integrated fertilizers provide trees with readily available N when demand is high, and compost provides trees with other essential mineral nutrients not found in synthetic N fertilizers. To evaluate the effects of these fertilizers on growth, fruit yield and quality, trunk cross sectional area (TCSA), blossom density, yield efficiency, and fruit diameter, weight, firmness, starch, peel color, and soluble solid concentration were analyzed. During this study, none of the fertilizer applications increased tree growth, fruit yield, or fruit quality compared to the unfertilized control in a newly established orchard. The effects of different fertilizers on leaf mineral nutrition was also assessed. Fertilizer applications did not increase leaf N during this study. This is most likely due to a high native soil organic matter content, which caused sufficient N to be mineralized. However, compost applications increased other leaf mineral nutrients, such as P, K and B, that are typically associated with increased tree growth, bloom cluster density, fruit yield or quality. However, in this study, increases in these nutrients were not associated to increased tree growth, bloom cluster density, fruit yield. Lack of treatment differences are likely due to adequate soil P,

K, and B prior to fertilizer application. Although treatments did not affect growth, fruit yield and quality, compost treatments increased soil total C, organic matter (OM) and cation exchange capacity (CEC) following application. Soil C additions lead to increased soil microbial respiration in 2013 and 2015; however, compost applications did not increase microbial biomass carbon or potentially mineralizable nitrogen (PMN). This study indicates that nitrogen fertilizer amendments do not initially improve young tree growth. However, more research is needed to determine the longer term effects of N fertilization on TCSA, yield, and fruit quality.

The effects of high biochar application rate on tree growth and mineral nutrition, soil fertility, and microbial community structure and function were assessed in Chapter 3. As an agricultural amendment, biochar has been shown to increase crop yield and biomass, CEC, soil mineral nutrition, and water holding capacity, and reduce N leaching. Given these characteristics, biochar applications have the potential to reduce fertilizer applications while supporting apple tree growth and productivity. However, the use of biochar has been shown to be highly variable among crop, soil type, and biochar rate, feedstock, and production method. Additionally, few studies have addressed the effects of biochar on perennial crop growth and mineral nutrition. I hypothesized that biochar would increase tree growth and leaf mineral nutrition, increase soil CEC and total C, and stimulate microbial community activity due to its chemical and physical properties. Performing this experiment in the more controlled pot-in-pot system allowed soil to be altered more rapidly and prevented the biochar amendments from migrating away from the trees. The pot-in-pot system also allowed trees to be harvested for the biomass assessment.

The effects of biochar applied in conjunction with synthetic N fertilizer on tree growth, biomass, and leaf mineral nutrition, soil physiochemical properties, and microbial community structure and function were evaluated. Three years after initial application biochar applied with

40 kg ha^{-1} N did not increase apple tree TCSA compared to 40 kg ha^{-1} N; however, the biochar applied with 40 kg ha^{-1} N treatments and 40 kg ha^{-1} N fertigation treatment increased TCSA compared to the unfertilized control. Similarly, biochar applied with 40 kg ha^{-1} N did not increase apple tree total biomass compared to 40 kg ha^{-1} N. Both the 50 Mg biochar ha^{-1} + 40 kg ha^{-1} N and the 40 kg ha^{-1} N treatments increased leaf N content compared to the unfertilized control, indicating that the N retention capacity of biochar did not increase plant available N during this study. In fact, soils amended with biochar did not have greater total N concentrations than the unamended soils. Biochar did not consistently increase leaf K, Ca, or Mg concentrations despite increasing these mineral nutrients in the soil. This may be due to adequate soil K, Ca, and Mg concentrations prior to biochar application. Biochar applications increased soil OM, CEC, total C and C:N. Increased soil C lead to greater microbial respiration; however, these increases were not associated with greater microbial biomass C. Greater biochar application rates were also associated with reduced PMN. Time had a greater effect on bacterial and fungal community structures then biochar application. Additionally, a low level of variation was captured by the interaction principle components analysis (IPCA) indicating environmental factors, such as soil pH, texture, temperature, or wetness, may have a greater effect on microbial community structure than the soil amendments.

The effects of synthetic N fertilizer, compost and integrated compost-calcium nitrate fertilizers applications on commonly used and newly released rootstocks on tree growth and mineral nutrition, soil fertility, and microbial community structure and function were evaluated in Chapter 4. This research is very similar to the research conducted in Chapter 2; however, performing this experiment in a pot-in-pot system allowed the soil system, such as soil physiochemistry and microbial communities, to be changed more rapidly than the field study

described in Chapter 2. Additionally, performing this experiment in the pot-in-pot system allowed whole trees to be harvested for plant biomass analysis, and for more rootstocks to be studied in a smaller space. During this experiment, tree growth, plant biomass, and leaf mineral nutrition, soil physiochemical properties, and microbial community structure and function were evaluated. I hypothesized that integrated compost-calcium nitrate fertilizer applications would increase tree growth more than the unfertilized control or compost treatments, regardless of rootstock, and compost applications will increase soil fertility measures, such as OM, CEC, and mineral nutrients, and soil microbial activity, and alter the soil microbial community composition.

After three years, the chicken litter compost increased TCSA compared to the fertigation treatment, or the unfertilized control, and the yardwaste compost and integrated compost-calcium nitrate treatments increased trunk cross sectional area compared to the unfertilized control. These results differ from those reported in Chapter 2. I hypothesize that working the compost treatments into the soil put the compost and integrated compost-calcium nitrate treatment into direct contact with the roots, allowing the roots to more easily capture more mineralized nutrients. Similarly, the compost and integrated compost-calcium nitrate amendments increased total tree biomass and leaf biomass compared to the control. Trees with greater leaf biomass may have had greater photosynthetic capabilities, thus increasing overall tree growth. Composts and chicken litter compost with calcium nitrate treatments increased root biomass. Nitrogen fertilization and compost application have also been shown to increase root production in other studies. There were no differences in root biomass among ARD tolerant rootstocks, G.41, G.214, and G.935, and susceptible rootstocks, B.9 and M.9, in this study. In 2015, the integrated compost-calcium nitrate treatments increased leaf N compared to calcium nitrate fertigation or

the control. The pot-in-pot study design may have confined the roots to a smaller area resulting in increased N uptake, and reduced N loss, and working the compost into the soil put plant roots in direct contact with the compost amendment at N was mineralized. Additionally, the integrated compost-calcium nitrate fertilizers may have provided trees with readily available N in addition to slowly mineralizing N from the compost. The B.9 and M.9 rootstocks had greater leaf N concentrations than the G.214 rootstock. This is most likely due to rootstock genotype. Compost and integrated compost-calcium nitrate treatments increased other leaf mineral nutrient concentrations, including P, K, Ca, Mg, Mn, and B, compared to the calcium nitrate fertigation treatment and the unfertilized control. Rootstocks from the Geneva series had greater P, K, Ca, Mg, and B concentrations than the B.9 and M.9. The Geneva rootstock series has been bred for increased nutrient uptake.

Compost and integrated compost-calcium nitrate amendments increased soil total N, total C, C:N, OM, and CEC, in addition to mineral nutrients. The compost treatments increased PMN in 2014 and 2015, and the compost and integrated compost-calcium nitrate treatments increased PMN in 2015, compared to the calcium nitrate fertigation treatment and the unfertilized control respectively, indicating that microbes can mineralize more N from the compost OM additions than the native soil alone. The compost and integrated yardwaste-calcium nitrate increased soil microbial biomass C and N. This is most likely due to increased soil C and C:N ratio from these fertilizer treatments. The B.9 rootstock had the greatest microbial biomass C and N, while the M.9 rootstock had the lowest microbial biomass C and N. These rootstocks may produce different root exudates and rhizodeposites, which may be responsible for attracting dissimilar microbial communities, leading to changes in microbial biomass. The compost and integrated compost-calcium nitrate treatments increased microbial respiration compared to the calcium

nitrate fertigation treatment and the unfertilized control, in 2014 and 2015. This is due to increased carbon additions from the compost and integrated compost-calcium nitrate treatments. Soil bacterial and fungal community composition was more affected by the time at which they were sampled than by the fertilizer or rootstock treatments. Additionally, the IPCA captured a very low level of variation for the bacterial and fungal communities in the study, indicating these communities were more effected by other environmental factors, such as soil pH, texture, temperature, or wetness, during this study.

In Chapter 5, the effects of fertilizer and rootstock selection on microbial community diversity was further explored using metagenomics methods. I hypothesized both rootstock and fertilizer treatments would cause significant changes in structure and diversity of the rhizosphere microbial community. A portion of the variable region 16 s rRNA gene from soil samples collected from the rhizosphere of M.9 and G.41 trees fertilized with chicken litter compost, yardwaste compost, or calcium nitrate, and the unfertilized control were amplified and sequenced using the Caporaso method. There were no significant differences in the Simpson and Chao1 alpha diversity indices for rootstock or fertilizer; however, there were significant differences in the Shannon alpha diversity index for fertilizer. The yardwaste compost treatment had a lower Shannon H' value tan the chicken litter compost, calcium nitrate or fertigation treatment, indicating that these treatments had higher OUT richness, diversity, and evenness that the yardwaste compost treatment. However, there were no differences in the Chao1 among fertilizer treatments; therefore, the number rare OTUs were similar among treatments. Using the Bray-Curtis dissimilarity, beta diversity among the fertilizer treatments was determined to be significantly different, indicating that fertilizers altered the rhizosphere microbial community diversity. The compost treatments had more similar communities than the calcium nitrate

fertigation and control treatments in this study based on the Bray-Curtis dissimilarity and Chao1 distances. Orchard soil was dominated by three phyla, Acidiobacteria, Proteobacteria, Plantomycetes. The phylum Actinobacteria, which has been a dominate phylum in several other studies of orchard soil was, not dominate in this study. However, Actinobacteria may be less dominate in soils with high organic matter, such as the silty loam soil at the Alson H. Smith, Jr., AREC in Virginia.

Given our current understanding of carbon-based soil amendments and rootstocks in Virginia I would make the following suggestions to apple growers in this region:

Apple growers should consider integrating compost into their fertility management plans in order to increase soil fertility characteristics, such as OM, CEC, soil mineral nutrition, microbial respiration and biomass, and potentially mineralizable nitrogen. My data suggests that applying composts or integrated compost-calcium nitrate fertilizers can increase P, K, and B in young trees. Although greater leaf P, K, and B were not correlated to increased fruit quality or greater flower abundance in my study, many studies have shown the importance of these nutrients for increasing flower formation and abundance, and fruit quality. Ground applied compost did not increase trunk cross sectional area (TCSA), fruit yield, or quality; however, working compost into the soil, through mechanical methods like tilling or disking, may lead to increased TCSA and leaf N concentration as observed in the pot-in-pot compost study. More research is needed to determine the effects of working compost into the soil under the vegetation free strip in apple orchards. I would also suggest planting the newly released Geneva series rootstocks such as, G.41, which have been selected for their greater nutrient use efficiency.

Currently, I would not suggest applications of biochar to apple growers attempting to improve soil fertility, and orchard growth and productivity. Biochar applied at high rates in

conjunction with 40 kg ha^{-1} N did not increase tree growth, total biomass, or leaf N concentration more than N fertigation at a rate of 40 kg ha^{-1} after three years. Additionally, biochar significantly reduced PMN, indicating that biochar may have negative effects on soil microbial functions, such as N cycling. Given the high cost, variability and difficulty applying this amendment, it is not a suitable amendment for improving edaphic characteristics at this time.

There are several areas of my dissertation research that could benefit from further explorations. Understanding which fertilizer and rootstock combinations increase plant size, as measured by TCSA, could assist researchers in making informed fertilizer recommendations to growers. This can be accomplished by performing a principal components analysis (PCA) using plant size and fertilizer data that is already available. Changes to microbial function in the rhizosphere following fertilizer applications should also be explored. My data demonstrates that fertilizer applications alter microbial community diversity in the rhizosphere; however, what that means for plant health, in terms of organisms such as, plant growth promoting rhizobacteria or pathogens, is currently unknown. There are many ways to determine what the function of microbes in the rhizosphere might be, such as whole genome shotgun sequencing or amplification of certain genes associated with soil biological function. Additionally, the metagenomic 16 srRNA data from this study can be analyzed using the phylogenetic investigation of communities by reconstruction of unobserved states (PiCRUST) software package. The PiCRUST software allows users to predict metagenome function from the 16s rRNA gene. Understanding if and how microbial community function changes following fertilizer application can also allow researchers to more informed fertilizer recommendations that focus on improving plant health. Additionally, exploring methods to ensure biochar applications are not inhibiting chemical methods for determining potentially mineralizable N or microbial

biomass C and N. Other studies suggest using lower concentrations of potassium sulfate as an extractant can skew microbial biomass data due to the sorptive properties of biochar. In this study, biochar may have sorbed the total organic C and total N in the biomass experiment and the ammonia in the potentially mineralizable N experiment, resulting in lower than expected values.