Development of Ecosystem Structure and Function on Reforested Surface-Mined Lands

Bethany N. Avera

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Forestry

Brian D. Strahm, chair

W. Lee Daniels

Mark A. Williams

December 9, 2014

Blacksburg, VA

Key words: reforestation, surface coal mining, ecological function, forest ecosystem development, forest soils, carbon and nutrient cycling, soil respiration, microbial biomass

Development of Ecosystem Structure and Function on Reforested Surface-Mined Lands

Bethany N. Avera

ABSTRACT

Surface mining in the central Appalachian coalfield disturbs landscapes. Post-mining reforestation efforts now achieve successful reestablishment and growth; however, it is unclear whether reforestation efforts also restore the native forest ecosystem functions. We quantified rates of return of key ecosystem functions and structural attributes of the post-mining forested ecosystem. A chronosequence of four reforested mine sites and an unmined reference stand were studied in southwestern Virginia. Total soil nitrogen (N) and component (mineral soil, forest floor, root, and aboveground biomass) ecosystem carbon (C) pools were quantified. Throughout the growing season, soil gas fluxes [i.e., carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4)], soil inorganic-N [nitrate (NO_3^-) and ammonium (NH_4^+)], and total and active microbial biomass were measured. Soil organic C (SOC) and total ecosystem C are returning to the mined landscape. Ecosystem C was correlated with N ($r= 0.80$; $p= 0.0003$) and with total and active microbial biomass ($r= 0.92$; $p=<.0001$ and $r= 0.86$; $p=<.0001$). Available soil inorganic-N and CO_2 and N_2O fluxes showed no significant differences among study sites; however, the reforested mine soils showed a diminished capacity for CH_4 uptake. Although some ecosystem components and functions rapidly returned to the mined landscape, others did not. Our results indicate that reforestation on surface mined lands is largely successful at restoring many ecosystem functions, yet certain functions are decoupled from the redeveloping ecosystem structure. Improved understanding of relationships between ecosystem functions and structural measures in this context can aid development of ecosystem restoration science and mine reclamation practice.

Acknowledgements

First and foremost I would like to thank my adviser, Dr. Brian Strahm, for the opportunity to pursue this master's degree; for his guidance, instruction and most of all his patience. I'd like to thank my committee, Dr. W. Lee Daniels and Dr. Mark A. Williams. Dr. Carl Zipper for help in site identification, guidance and accompaniment on the first of several field trips. Patricia Donovan for GIS support and potential site identification.

My project required extensive time in the field and lab. I would like to thank Dave Mitchem and Lara Nichols for their help and training in the lab. I could not have completed all my field sampling without the help of numerous people: Maura Leveroos, Kim Coleman, Amy Werner, Daniel DeBruler, Brian Strahm, Brian Badgley, Shan Sun, Raj Shrestha, Pamela Braff, Brett Rivers, Kristin McElligott, and Carrie Green.

I was supported throughout the process by my family and friends. Thank you for sticking with me, continuing to motivate and encourage me.

Table of Contents

List of Figures

List of Tables

LITERATURE REVIEW

Mining History, Legislation, and Reclamation

Coal mining has been a way of life in the Appalachian region since the late 18th century. As European settlers advanced westward across the state of Virginia, they discovered the three coal-bearing regions of the state. The first to be encountered was the Richmond Coalfield Basin on the Piedmont physiographic province. Roughly 150 square miles in area, this coalfield is situated in the east-central portion of the state, where it is estimated that there were 1 billion tons of Triassic age bituminous coal (Hibbard and Clutter 1990). Early diaries of Colonel William Byrd II, the founder of Richmond, VA, record commercial coal mining in Goochland County of this coalfield region as early as 1709 (Hibbard and Clutter 1990). As settlers continued west, semi-anthracitic coal was discovered in Montgomery and Pulaski Counties of the west central valley region. Here, within approximately 26,000 ha, the region boasts 1 billion tons of Mississippian age coal, accessed primarily through punch-in coal mines (Hibbard and Clutter 1990). Exposed mine shafts and piles of spoil remain today, as evidence of these punch in coal mines. However, the southwestern Virginia coal region is where the modern coal industry in Virginia was truly able to gain its footing.

In 1751, Christopher Gist, a colonial explorer and surveyor, discovered coal in Wise County at Pound Gap (Hibbard and Clutter 1990). Then, over a century later, the Norfolk and Western Railroad company extended the line to southwest Virginia, completing the Clinch River Branch in 1891 from the main line in Bluefield to Norton in Wise County (Hibbard and Clutter 1990; Yarnell 1998). At the same time, Norton was reached by the Louisville and Nashville Railroad from the west (Hibbard and Clutter 1990). Ninety percent of Wise County lies over these coal seams (Hibbard and Clutter 1990). Thus with the expansion of the railroad, the

modern coal industry was ushered into southwest Virginia to take advantage of the greater than 400,000 ha of coal seams underlying the region (Hibbard and Clutter 1990; Ruppert 2001).

With over 600,000 ha of Appalachian forestland drastically disturbed in the pursuit of coal since 1977, the mark of mining has been left on the landscape (Zipper et al. 2011). Of that area, 300,000 ha lie in the Southern Appalachian Forest Region, which includes southwestern Virginia (Acton et al. 2011). Virginia produces 30-40 million tons of coal annually, all from Buchanan, Dickenson, and Wise Counties (U.S. Energy Information Administration 2013). Virginia coal is characterized as having high energy and low-sulfur contents making it ideal for both energy production and fit for metallurgical industrial uses (Ruppert 2001; U.S. Energy Information Administration 2013). Thus, the markets for Virginia coal are steel production companies mostly within the Great Lakes region and electric utilities in the southeastern states (U.S. Energy Information Administration 2013).

In 1980, the Appalachian mines produced 438 of the 825 million tons of coal produced in the U.S. with western mines contributing only 210 million tons. In 2000, the Appalachian region was now producing significantly less at 419 million tons, but production in the west more than doubled in the 20 intervening years resulting in the market share of the Appalachian region being considerably less (U.S. Energy Information Administration 2013). A significant percentage of this trend may be attributed to market costs. The western region can mine a higher proportion using surface mining practices over underground practices than is possible in the Appalachian region, resulting in cheaper operation costs. Thus, mining companies operating in Virginia face additional costs associated with the underground mining process, steep slopes, and the pressure of dwindling state reserves; challenges not typical of mining in the western states.

Official political recognition of the need to reclaim surface mined lands was first made in 1939 when West Virginia passed the first state law regulating surface mining. Additionally during the 1930's, the Illinois Coal Strippers Association, Indiana Coal Producers Association, and the Ohio Reclamation Association all formed in response to the accumulating spoil banks and rapid increase in mining disturbed land (Plass 2000). Other state followed with their own laws or regulations, with Indiana in 1941, Ohio in 1948, and Kentucky in 1954 (Plass 2000). Surface coal mining escalated due to increased demand during World War II, bringing to light several problems especially inherent to the weak regulations. Grassroots movements in opposition to surface coal mining were able to gain footing in the 1960's, culminating in a proposal to outlaw surface mining for coal. With this proposal, national attention was brought to the controversy of surface coal mine regulations.

States quickly tightened up legislation in response, increasing compliance requirements and the penalties for noncompliance (Plass 2000). In the years that followed, more states enacted legislation (Pennsylvania in 1964, Virginia in 1966, Tennessee in 1967, and Alabama in 1969). Yet the public was still not satisfied and the movement headed toward federal legislation. After two Congressional hearings (1967 and 1969), two failed bills (one for a mining ban in 1971 and the other for federal regulations in 1972), and after President Ford vetoed two bills passed by both houses of Congress (in 1974 and 1975), Public Law 95-87 was finally signed into law by President Carter in 1977 (Plass 2000). Public Law 95-87, more commonly called the Surface Mine Control and Reclamation Act (SMCRA), became the first piece of federal legislation to specifically regulate surface coal mine reclamation.

SMCRA focused on three problems-land instability, sedimentation, and surface water contamination due to poor placement of mine spoils on the steep Appalachian terrain (Zipper et

al. 2011). SMCRA is based upon a permit system in which mining companies are required to put up a sum of money called a bond to the U.S. Office of Surface Mining (OSM), the regulatory body that oversees coal surface mining, in order to receive a mining permit. The bond is meant to ensure that the land will be reclaimed following the requirements of the law. Included with the permit are standards for the planning, operational and reclamation steps of the mining process, such that the mine can be closed and the bond money returned. Therefore, in order to have the bond returned, the mining company must return the land to approximate original contour (AOC) and have 70% vegetative cover for 5 continuous years.

To facilitate bond release, mining companies have turned to the practice of establishing a cover of grasses and legumes on all disturbed lands. Areas of exception include those returned to agricultural crops, pasture land or development (Plass 2000). Therefore, while the intent of the law is to minimize land instability and erosion, the law has promoted the establishment of grasslands, not the replacement of native forests (Angel et al. 2009; Burger and Zipper 2011). Landslides were a common occurrence prior to SMCRA due to reclamation methods of the day, therefore SMCRA required improved land stability. An unintended secondary impact of this policy is compaction due to the nature of the reclamation process. Establishment of a dense herbaceous ground species cover has developed as another way to provide land stability as it protects the soil from erosion. Challenges of compaction and dense herbaceous ground cover manifest themselves by inhibiting the colonization of native species and growth of planted trees (Angel et al. 2009; Burger and Zipper 2011). Research over the last few decades has focused on overcoming these obstacles to reestablish forests underneath the framework of SMCRA. Through this research, the Forestry Reclamation Approach (FRA) has been developed, outlining a set of best management practices to reclaim coal-mined land to forest land uses (Angel et al.

2009). While the FRA has been largely successful at resstablishing native tree species on reclaimed coal-mined land, questions remain as to whether the replanted forest is able to provide the same ecosystem services as the native forests they are replacing.

Forest Ecosystem Services and Functions

Appalachian forests are characterized by a myriad of forest functions, such as elemental cycling and retention and the fixation of atmospheric carbon dioxide (CO_2) into organic carbon (C) compounds. Functions provided by forests that benefit humans are more commonly referred to as ecosystem services. Services provided by these Appalachian forests include modulating climate by sequestering and storing C, cycling nutrients, providing habitat for diverse flora and fauna, producing high quality timber, providing aesthetic value, improving soil quality through organic matter inputs, improving water quality and protecting the watershed (Burger and Zipper 2011).

Either by directly releasing greenhouse gases (GHG; e.g., CO_2, CH_4, and N_2O), or disturbing uptake and cycling mechanisms within forest ecosystems, human-induced perturbations and disturbances are changing the composition of the atmosphere at unprecedented rates (Holland et al. 1999). The most well-recognized example is the burning of fossil fuels, but deforestation also negatively impacts these cycles (Vitousek et al. 1997). Quantifying the sources, fate and balance of GHG exchange on the post-mining landscape can serve as a metric for extent of functional replacement of the native mixed hardwood forests present prior to mining.

Forest soils modulate the atmosphere and are significantly the largest known biogenic sink for methane (CH_4). Forest soils are also central to nutrient transformations [e.g., C and

nitrogen (N) mineralization] and translocations responsible for nutrient bioavailability within the forest ecosystem. Soils serve as the medium of plant growth, store and provide nutrients and water to the roots of the growing plants. Soils host thousands of species of microbes. Bacteria and fungi colonize the soil and litter to digest the incumbent nutrient compounds, making them available to plants.

Mine Soil Development and Role of Soils in Ecosystem Restoration

Soil focused research in ecosystem based restoration science is lacking. Callaham et al. (2008) reports that of the studies published in *Restoration Ecology* from 1993 to 2006 (volumes 1-14) fertilization, the most frequent soil-manipulation, occurred only 15 times over the 14 years. Topsoil addition experiments, herbicide and mulch/compost amendments fell shortly behind with 10, 6, and 6 occurrences respectively. Mine soil research has had a very similar focus of topsoil substitutes, organic amendments such as sawdust or biosolids, and herbicide and fertilizer applications. However, the impact of reclamation and reforestation on mine soil functional development, has been largely overlooked. As Callaham et al. (2008) concludes, it is encouraging that soil-ecological related material is beginning to be included in restoration literature. Yet, soil ecological and functional development in mine soil needs to be understood and prioritized such that reclamation and reforestation plans are intentional in restoring soil functionality.

Using soil functions as benchmark for forest soil and ecosystem recovery provides a more inclusive approach to understanding soil development or recovery post-disturbance, as soil physical, chemical, and biological properties must work in tandem to provide each function (Callaham et al. 2008). Pedogenic processes act quickly on parent materials, releasing elements

to the soil solution, accumulating organic matter and biological activity commences in the soil (Sobek et al. 2000).

Parent material, landscape position, climate, biological activity and time are the soil forming factors that develop and differentiate soil properties. New mine soils are classified at "time 0" in the process of pedogenic and ecosystem development (Jenny 1941; Sobek et al. 2000). Time has significantly limited the action of climate and weather, and therefore parent material and final land use design (i.e., placement) are the most influential soil forming factors in young mine soils (Sencindiver and Ammons 2000). Mine soil textures are often coarser than native soils, exacerbated by the high volume of rock fragments (up to 70%). As a result, bridging voids (e.g., large air-filled gaps) are formed in mine soils. The overlapping rocks of bridging voids alter the hydrologic flow through the soil; potentially causing lower water holding capacity, differential settling, or creating space for root growth (Sencindiver and Ammons 2000). Often, mine soils are characterized by a higher bulk density than the native soil, limiting aeration, root penetration and water infiltration into the soils. Morphological differences such as these between native soils and mine soils may have profound implications on the functioning of soils in the post-mining landscape.

Mine soil development as it pertains to forest ecosystem development has been studied in the Appalachian region via the quantification of soil organic C (SOC) and other ecosystem C pools. In 2008 Amichev et al. (2008) concluded that mined soils have a great potential for C sequestration as they are "empty cups" as the reforested plots showed similar productivity to the unmined reference, with less SOC. Acton et al. (2011) showed that SOC stocks are returning but are not at unmined levels. In eastern Kentucky, Littlefield et al. (2013) found that in the first 10 cm of the mineral soil that SOC increased from 0.1% to 0.8% within the 8 years following

reforestation. Sperow (2006) reported mined forest soil C sequestration rates ranging from 0.6-0.8 Mg C ha^{-1} yr^{-1}, slightly slower than the rates of Littlefield et al. (2013). Amichev et al. (2008) also found that low-quality sites actually had the potential to have more tree C per unit area and thus were more productive; thus, leaving more questions unanswered about the controlling factors of forest growth and services on reforested coal mined land.

Soil respiration rates from mine soils are poorly documented. Particularly, the impacts of reforestation or time post-reforestation. In other concepts of human influence or disturbance the impact of soil respiration has been variable among the few sites it has been documented (Raich and Schlesinger 1992). Even in light of variability with other human disturbances, mine land restoration is rather unique as it is primary succession. For example, cultivation usually increases temperature and moisture improving conditions for decomposition and thus decreasing the amount of soil organic matter. In many ways, this could be true for the mined cohorts; however, cultivation also disrupts soil aggregates making more stable adsorbed organic matter susceptible to decomposition and likely this is not occurring in the mined soils (Schlesinger and Andrews 2000).

Reforestation Initiatives

Forests cover approximately 4 billion hectares of land on earth, which is roughly 30% of the earth's surface. Within the Appalachian region, 80% of land cover is forest (Zipper et al. 2011), however, this percentage is decreasing with deforestation for land uses such as mining.

Re-growth of forests on mine soils is defined as primary succession as no previous soil development exists (Jenny 1994; Dobson 1997). Through the process of natural forest succession, the structure and function of the forest can be returned (Burger and Zipper 2011;

Zipper et al. 2011). However, it is estimated on this landscape that it could take several hundred years for mid- to late-successional species to become dominant (Burger and Zipper 2011; Zipper et al. 2011) and more advanced community interactions could take millennia (Dobson 1997).

Questions remain concerning the relationship between the accelerated reestablishment of forest composition and structure through human-mediated reforestation and the associated ecosystem functions of the native forest. Reestablishment of these ecosystem functions should be part of the definition of a successful reclamation outcome. However, research to date has widely failed to address the health and functionality of the mine soils under reforested land (Acton et al. 2011).

Objectives

To investigate the development of forest ecosystem functions, this study focused on addressing the following two objectives primarily within the forest soils: 1) to identify and quantify ecosystem functions occurring on the previously coal-mined and reforested landscape, and 2) to relate the development of ecosystem function to structural attributes of the system.

Literature Cited

Acton P, Fox J, Campbell E, Jones A, Rowe H, Martin D, Bryson S (2011) Role of Soil Health in Maintaining Environmental Sustainability of Surface Coal Mining. Environmental Science and Technology 45:10265-10272. doi: 10.1021/es202764q

Amichev B, Burger J, Rodrigue J (2008) Carbon sequestration by forests and soils on mine land in the Midwestern and Appalachian coalfield of the U.S. Forest Ecology and Management 256:1949-1959. doi: 10.1016/j.foreco.2008.07.020

Angel P, Burger J, Davis V, Barton C, Bower M, Eggerud S, Rothman P (2009) The Forestry Reclamation Approach and the Measure of its Success in Appalachia. *Revitalizing the Environment: Proven Solutions and Innovative Approaches*, ASMR, Billings, MT

Burger JA and Zipper CE (2011) How to Restore Forests on Surface-Mined Land. Virginia Cooperative Extension. Blacksburg, VA. Virginia Polytechnic and State University. https://pubs.ext.vt.edu/460/460-123/460-123_pdf.pdf. Accessed 25 Oct 2012

Callaham M, Rhoades C, Heneghan L (2008) A Striking Profile: Soil Ecological Knowledge in Restoration Management and Science. Restoration Ecology 16(4):604-607. doi: 10.1111/j.1526-100X.2008.00490.x

Dobson, Andy P., Bradshaw, A.D. and A.J.M. Baker (1997) Hopes for the Future: Restoration Ecology and Conservation Biology. Science 277:515-522.

Hibbard W Jr, Clutter T (1990) Virginia coal: an abridged history and complete data manual of Virginia coal production/consumption from 1748 to 1988. Blacksburg, VA. Virginia Center for Coal & Energy Research, Virginia Polytechnic Institute and State University.

Jenny H (1941) Factors of Soil Formation A System of Quantitative Pedology. McGraw-Hill Book Company, Inc., New York

Littlefield T, Barton C, Arthur M, Coyne M (2013) Factors controlling carbon distribution on reforested minelands and regenerating clearcuts in Appalachia, USA. Science of the Total Environment 465:240-247.

Plass WT (2000) History of Surface Mining Reclamation and Associated Legislation. In: Barnhisel RI, Darmody RG, Daniels WL (ed) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Madison, WI, pp 1-20.

Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44B:81-99.

Ruppert LF (2001) Chapter A—Executive Summary—Coal Resource Assessment of Selected Coal Beds and Zones in the Northern and Central Appalachian Basin Coal Regions. In: Northern and Central Appalachian Basin Coal Regions Assessment Team, 2000 resource assessment of selected coal beds and zones in the northern and central Appalachian Basin coal regions: U.S. Geological Survey Professional Paper 1625-C, CD-ROM, version 1.0.

Sencindiver JC, Ammons JT (2000) Minesoil Genesis and Classification. In: Barnhisel RI, Darmody RG, Daniels WL (ed) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Madison, WI, pp 595-613.

Schlesinger WH, Andrews JA (2000) Soil respiration and the global carbon cycle. Biogeochemistry 48:7-20.

Sobek AA, Skousen JG, Fisher SE Jr (2000) Chemical and Physical Properties of Overburdens and Minesoils. In: Barnhisel RI, Darmody RG, Daniels WL (ed) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Madison, WI, pp 77-104.

Sperow M (2006) Carbon Sequestration Potential in Reclaimed Mine Sites in Seven East-Central States. Journal of Environmental Quality 35(4):1428-1438.

Torbert JL, Burger JA (2000) Forest Land Reclamation. In: Barnhisel RI, Darmody RG, Daniels WL (ed) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, WI, pp 371-398..

U.S. Energy Information Administration (2013) Annual Coal Report 2012. U.S. Department of Energy. http://www.eia.gov/coal/annual/pdf/acr.pdf. Accessed 9 July 2014.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human Domination of Earth's Ecosystems. Science 277:494-499.

Yarnell SL (1998) The Southern Appalachians: A History of the Landscape. US Department of Agriculture Forest Service. Southern Research Station General Technical Report SRS-18. Asheville, NC

Zipper CE, Burger JA, Skousen JG, Angel PN, Barton CD, Davis V, Franklin JA (2011) Restoring Forests and Associated Ecosystem Services on Appalachian Coal Surface Mines. Environmental Management 47:751-765.

Development of Ecosystem Structure and Function on Reforested Surface-Mined Lands

Abstract

Surface mining in the central Appalachian Basin coal region drastically disturbs the landscape. Post-mining reforestation efforts have reached reliable tree survivability and growth; however, it is unclear whether these reforestation efforts also restore the ecological functions associated with the native forest ecosystem. The objectives of this study were to quantify the rate at which key ecosystem functions return to the landscape, and to relate the development of those functions to structural attributes of the ecosystem. A chronosequence of four reclaimed and reforested stands (ages 5, 11, 21 and 30 years) and an unmined reference stand representing pre-mining conditions, were identified on the Appalachian Plateau in southwestern Virginia. Total soil nitrogen (N) and component (mineral soil, forest floor, root, and aboveground biomass) ecosystem carbon (C) pools were quantified. Throughout the growing season, monthly samples for soil gas fluxes [i.e., carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4)], available inorganic-N [nitrate (NO_3^-) and ammonium (NH_4^+)], and total and active microbial biomass were measured. As expected, soil organic C (SOC) and total ecosystem C returned to the mined landscape, although at levels still less than half of the unmined reference after 30 years. Ecosystem C accumulation was significantly correlated with N ($r=0.80$; $p=0.0003$) as well as total and active microbial biomass ($r=0.92$; $p=<.0001$ and $r=0.86$; $p=<.0001$, respectively). Surprisingly, available inorganic-N and gas fluxes of CO_2 and N_2O showed no significant differences among any of the mined and unmined stands; however, the reforested mined soils showed a significantly diminished capacity for CH_4 uptake, where upland soils typically contribute to the largest global biogenic sink of atmospheric CH_4. Thus, although many ecosystem components (e.g., forest and microbial biomass) and functions (e.g., N cycling), rapidly returned to the reclaimed landscape, some critical ecosystem functions (e.g., methanotrophy) exhibited a fundamentally different rate of return, if present at all. Our results indicate that reforestation of native hardwoods on reclaimed surface mined lands is largely successful at restoring many ecosystem functions, but that certain functional attributes are decoupled from the observed redevelopment of ecosystem structure. Thus, reforestation and forest ecosystem restoration are not necessarily the same thing, and a better understanding of

potential disconnects between the two concepts can be critical in guiding both the science and the practice into the future.

Introduction

Forests play a vital role on the landscape. They sequester and store C, cycle nutrients, provide habitat for diverse flora and fauna, produce high quality timber, provide aesthetic value, improve soil quality through organic matter inputs, and modulate water quality (Burger and Zipper 2011; Zipper et al. 2011). Within the temperate deciduous forest biome of the eastern US, the Appalachian mixed mesophytic forest ecoregion reaches from northwest Alabama to southwestern Pennsylvania, on the western side of the Appalachian Mountains (Bailey 1983; Omernik 1987). The mixed mesophytic forest is one of the most biologically diverse temperature forest ecosystems in the world with up to 30 distinct canopy tree species and a rich understory composition of plant, animal and microbial communities (Braun 1950, McEwan et al. 2005).

Geologically, the Appalachian mixed mesophytic forest region is underlain by the Appalachian Coal Basin, the predominant coal resource in the eastern US (Ruppert 2001). Resultant surface coal mining is deforesting and drastically disturbing the landscape within the central Appalachian region. Bernhardt and Palmer (2011) report that over 1.1 million ha of forested land has been converted to surface mines in the Appalachian region. The nature of the mining process involves using ammonium nitrate and iron oxide to remove the surface layers, called overburden, sitting above the coal seams. Once the overburden is removed, the coal is extracted. This iterative process of removing overburden and the coal continues until all the economically feasible seams have been mined. The overburden is then used as part of the reclamation process to recreate the approximate original contour of the local landscape (Zipper et al. 1989). Because local soils are so thin, SMCRA allows for the use of topsoil substitute rather than saving topsoil. The blasted overburden is used as topsoil substitute and as a result the

system to restore/reforest is in many ways the old system inverted. As a result mine soils are characteristically very distinct from undisturbed soils.

Mine soils are very rocky, with coarse fragments frequently exceeding 50% (Torbert and Burger 2000). As a result, bridging voids can be formed in mine soils. The overlapping rocks of bridging voids alter the hydrologic flow through the soil; potentially causing lower water holding capacity, differential settling, or creating space for root growth (Sencindiver and Ammons 2000). Compaction during the reclamation process generally causes mine soils to have higher bulk density than the native soil, limiting aeration, root penetration and water infiltration into the soils. These challenges of mine soils, together with the desire of mining companies to quickly establish 70% vegetative cover as required by the Surface Mine Control and Reclamation Act (SMCRA) encourages mining companies to use a mixture of grass and legumes (Torbert and Burger 2000). In the face of these challenges, researchers have been working since the early 1980's to develop methods that allow for reliable survivability and growth of mixed hardwood tree species.

Native hardwood reforestation efforts have largely focused on seedling establishment and growth, including many species trials to determine the most successful species on the post-mining landscape (Torbert et al. 1985; Burger et al. 2005; Burger et al. 2008; Burger and Fannon 2009). Through the Appalachian Regional Reforestation Initiative (ARRI), a partnership between university scientists, the US Office of Surface Mining, citizen groups, and the coal mining industry, researchers have put together a set of best management practices called the Forestry Reclamation Approach (FRA) that is a set of best management practices for the reforestation of native hardwoods.

Secondary to establishing native tree species, an underlying assumption of reforestation on surface mined land in the Central Appalachian Coal Basin is that return of trees (e.g., species density) will necessarily lead to the development of a forest ecosystem that mimics the functionality of the local unmined forest ecosystem. Research to date has largely failed to address this question of reconciling reforestation with forest ecosystem restoration. This study sought to investigate the relationships between reforestation with native hardwoods and the associated functions of forest ecosystems by comparing predominant ecosystem functions (e.g., C sequestration, nutrient cycling, biosphere-atmosphere regulation) occurring on both the previously coal-mined and reforested landscape, and relating those measured ecosystem functions with more commonly observed metrics of ecosystem structure (e.g., species composition, density, productivity).

Materials and Methods

Study location and sites

This study was conducted on the Appalachian Plateau physiographic province in Wise County in southwestern Virginia on a chronosequence of four reclaimed and reforested stands (ages 5, 11, 21, and 30 years) and an unmined reference stand. Local vegetation is primarily of the Appalachian mixed mesophytic forest ecotype prior to mining. Local climate is characterized by a mean annual precipitation averaging 123 cm with the lowest mean annual temperatures occurring in January averaging 5.4°C and the warmest temperatures occurring in July and August averaging 27°C.

All mined stands in the chronosequence were surface-mined and reclaimed by Red River Coal following SMCRA requirements. This included recontouring slopes using a mix of

weathered and unweathered sandstone and siltstone overburden (with some shale and coal fragments), as a topsoil substitute. In selecting chronosequence sites, other relevant site characteristics (e.g., slope, initial revegetation seed mix, grading/compaction), were constrained to the best of our ability whenever possible (Table 1). Each of the reclaimed stands was intentionally planted with native hardwoods following reclamation (Table 2). Detailed land use history was not available for the unmined reference stand; although given patterns in land use history regionally and current stand conditions, it is likely that the unmined sits had been harvested for timber more than 50 years ago.

Site and stand characterization

Three replicate sampling plots (5 m radius) were established in each age cohort for site and stand characterization. In the spring of 2013, three quantitative soil pits were excavated 5 m from each plot center (Vadeboncoeur et al. 2012), at 0°, 120° and 240° relative to an azimuth of 0°. Within a 25 cm x 25 cm sampling frame the O horizon was clipped and removed. Mineral soils were excavated by depth increment (0-5, 5-10, and 10-25 cm). A flat edged tool was used to scrape the sides and bottom of the pits to the precise dimensions. Soil was brushed off of large rocks excavated from the pit and the rocks were left in the field. Likewise, roots that were too thick to be cut with a small folding saw were brushed off and left in situ. Collected material was oven-dried at 60°C. Dried mineral soils were gently broken up manually and dry sieved through a 2-mm sieve to remove rocks, roots, and other debris. Of the >2 mm fraction, roots and coal were separated for further analysis. Volumetric density (VD) of the fine, soil fraction (<2 mm) was calculated for each depth increment by dividing the dry mass of the fine fraction by the total volume of the excavated depth increment.

Mineral soil organic C (SOC) and mineral soil N concentration were quantified at each depth of the quantitative pits using an Elementar vario Micro Cube elemental analyzer connected to an Elementar IsoPrime100 isotope ratio mass spectrophotometer (IRMS) (Elementar, Hanau, Germany) using a two end-member mixing model based on the different $\delta^{13}C$ isotopic compositions of geogenic (i.e., coal) and pedogenic (i.e., plant) C sources. Geogenic OC (GOC) in these soil and overburden materials are derived from coal and are not considered to be a portion of the cycling SOC pool. Therefore to dry combustion techniques to quantify the available SOC pool, must account for GOC. Following Acton et al. (2011), O horizon and coal were used as end-members in the analysis. O horizon samples were pre-ground using a Wiley Mill (Thomas Scientific Model 4 Miley Mill, Swedesboro, NJ) and coal samples were ground using a mortar and pestle. A subsample of each of the mineral soil, forest floor, and coal samples were individually ground to a fine powder using a Retsch MM200 ball mill (Retsch, Haan, Germany). Following the method of Harris et al. (2001), finely ground soil samples were weighed into silver (Ag) capsules and were fumigated with 100 mL of 12 M hydrochloric acid (HCl) in a vacuum desiccator for 24 h to remove inorganic-C (i.e. carbonates) that would otherwise provide another $\delta^{13}C$ end-member. These soil samples were then dried at 60°C for 48 h to remove any residual chloride (Harris et al. 2001). Silver capsules were closed and packaged within tin (Sn) capsules to preserve sample integrity. O horizon and coal samples were weighed directly into Sn capsules. The isotope mass balance determination of pedogenic organic C (POC) concentration was calculated using the following equations:

$$(1)\delta^{13}C_{TOC} = P_{POC}\delta^{13}C_{POC} + P_{GOC}\delta^{13}C_{GOC}$$

$$(2)\ 1 = P_{POC} + P_{GOC}$$

(3) $C_{corrected} = P_{POC} \times C_{uncorrected}$

Where $\delta^{13}C_{TOC}$ is the isotopic value of the total organic carbon (TOC) in each sample (i.e., air-dried, sieved, untreated) and $\delta^{13}C_{POC}$ and $\delta^{13}C_{GOC}$ are the delta values (‰) of the pedogenic organic carbon and geogenic coal organic carbon stable isotope end-member, respectively. P_{POC} and P_{GOC} represent the proportional amounts of TOC accounted for by the pedogenic and geogenic sources of organic carbon, respectively. Soil C and N content (Mg ha^{-1}) was then calculated from concentration (%) using measured VD for each depth increment.

All trees falling within the 5 m plot radius and having a diameter greater than 2.54 cm at breast height (1.4 m) were measured for diameter at breast height (DBH). For woody plants smaller than these criteria, ground line diameter (GLD) was recorded. Aboveground woody biomass (Mg oven-dry weight ha^{-1}) for each plot was calculated using region- and species-specific DBH and GLD based allometric equations (Bickelhaupt et al. 1973; Day and Monk 1974; MacLean and Wein 1976; Brenneman et al. 1978; Ker 1984; Williams and McClenahen 1984; Clark et al. 1986; Clark et al. 1990; Elliot and Clinton 1993; Ter-Mikaelian and Korzukhin 1997; Jenkins et al. 2004). Aboveground woody biomass was then converted to aboveground biomass C (tree C) content by multiplying biomass by a factor of 0.5.

Soil dynamics

Measurements of total and active microbial biomass, inorganic N availability, and gas fluxes of [carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O)] were taken monthly from spring (April/May) to fall (October/November) 2013. All monthly samples were duplicated within each plot and co-located with gas flux measurement locations. Mineral soil samples (0-10 cm) were collected for total and active microbial biomass C and each sample was a composite of

three locations adjacent to the corresponding gas flux measurement location. Samples were transported on ice and stored at 4°C prior to analysis. Each sample was homogenized and the rocks and roots were manually removed and discarded. Microbial biomass assays were completed within one week using the soils at field moist capacity.

Chloroform fumigation extraction was used to quantify the total microbial biomass C (i.e., chloroform-sensitive fraction; Wardle and Ghani 1995), following the method of Beck et al. (1997). Two 25 g replicates of field moist soil were weighed out from each 10 cm mineral soil sample. One replicate of each soil sample was fumigated with chloroform ($CHCl_3$) in a vacuum desiccator for 24 hours. The second replicate was not fumigated. Post fumigation, both replicates were shaken on the reciprocal shaker with 100 mL 0.5 M K_2SO_4 for 1 hour at 200 rev m^{-1}. After settling, each K_2SO_4-extract was then filtered through Whatman #2 filter paper and the filtrate was collected in scintillation vials. Filtrates were sent to the NC State Environmental and Agricultural Testing Service for TOC analysis on a Shimadzu TOC/TON Analyzer (Shimadzu Scientific Instruments, Inc., Columbia, Maryland). To calculate total microbial biomass C the following equation was used (Parkinson and Paul 1982; Beck et al. 1997):

$$Microbial\ C\ (mg\ C \cdot g^{-1} dry\ soil) = \frac{C_{fum} - C_{non-fum}}{0.45}$$

Substrate-induced respiration (SIR) was used to quantify the metabolically active (i.e., glucose-responsive) fraction of the microbial biomass C (Wardle and Ghani 1995). Ten g of field moist soil were placed into glass vials. D-Glucose (Dextrose anhydrous, F.W. 180.16) solution of 1 g glucose g^{-1} soil was dissolved in deionized water (DI H_2O) and 20 mL glucose solution was added to each sample, vials were then sealed with rubber septa (West and Sparling 1986; Fierer et al. 2003). Samples were incubated at room temperature (~20-25°C) while mixed on a

reciprocal shaker table on low. Headspace CO_2 concentrations were determined immediately after all vials were capped and in 1 h intervals for the duration of the 4 h incubation using a Li-Cor LI-6250 CO_2 Analyzer (LI-COR Biosciences Inc., Lincoln, Nebraska). The CO_2-C flux rate was then calculated using the slope of CO_2-C concentrations evolved over the 4 h incubation period. To convert to final units of μg CO_2-C g^{-1} soil × h^{-1} soil, field moist mass was converted to dry mass equivalent using measured gravimetric water content (Parkinson and Paul 1982; Bailey et al. 2002).

Two ion exchange membranes (IEMs) (GE Osmonics, Inc., Trevose, PA), both an anion and cation, were used *in situ* to quantify nitrate (NO_3^-) and ammonium (NH_4^+) (Subler et al. 1995; Bowatte et al. 2008; Duran et al. 2013). IEMs were cut to a size of 50 cm^2 (5 × 10 cm) and a 7 mm diameter hole was punched at the top. IEMs were submerged in a 1 M solution of sodium chloride (NaCl) to fill all exchange sites with readily exchangeable sodium (Na^+) or chloride (Cl^-) ions. Anion and cation membranes were stored in separate containers, each in 1 M NaCl solution at 4°C prior to field deployment. Immediately prior to field deployment, IEMs were thoroughly rinsed with DI H_2O. The first sets of IEMs were installed in April and IEMs were replaced approximately every four weeks, with the exact duration of field deployment recorded. To install in the field a narrow slit was cut into the soil at a 45° angle using a soil knife. Each IEM pair (anion and cation) were gently placed in the slit with no wrinkles or overlaps between them. Nylon string was tied to the hole punched in each IEM and to a pin flag to identify the location of the membranes. Upon removal from the field each IEM pair was stored in its own small plastic bag and transported on ice back to Virginia Tech. IEMs were stored at 4°C for less than 7 days until inorganic-N was extracted. To extract inorganic-N, IEMs were gently rinsed with DI H_2O to remove soil particles from the surface. Each IEM was individually

submerged in 50 mL of 1 M potassium chloride (KCl) and placed on a reciprocal shaker for 1 hour at 200 rev m^{-1} (Subler et al. 1995; Hangs et al. 2003). Extracts were analyzed for NO_3^--N and NH_4^+-N concentration on a TrAAcs 2000 Analytical Console (Bran+Luebbe, Analyser Division, Norderstedt, Germany) connected to an XY2 Auto sampler (SEAL Analytical, Mequan, Wisconsin).

Gas fluxes were measured using vented, non-steady state static chambers (Holland et al. 1999) installed in two random locations within each plot. The collars were installed at least one month prior to sampling in order to diminish impacts of soil disturbance associated with installation on the measured gas fluxes. Polyvinyl chloride (PVC) collars with a 23.5 cm diameter were situated approximately 5 cm into the ground, with ~12 cm of height aboveground forming the chamber. Volume calculations were made based on chamber-specific measurements where aboveground chamber height was measured at 4 points on the inside of each collar. At the time of sampling, the collars were capped to allow gas accumulation (Holland et al. 1999). Four 7 mL samples were taken from each chamber. Each sample was taken using a 30 mL plastic syringe fitted with a stopcock and 21 gauge needle, then ejected into a glass vial that had been sealed with a rubber septa, purged with dinitrogen gas and evacuated. The first sample was taken immediately after capping to establish the gas concentration prior to accumulation. Subsequent samples were taken at approximately 20, 40 and 60 min, with exact times recorded.

Concentrations of CO_2, CH_4 and N_2O in each of the soil gas flux samples were analyzed simultaneously using a GC-2010 Gas Chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, Maryland) with an AOC-5000plus Autosampler (Shimadzu Scientific Instruments, Inc., Columbia, Maryland). Following the method of Holland et al. (1999), gas concentrations were converted to mass and corrected for field conditions. A flux rate of each individual gas was

calculated based upon the change in concentration over time, relative to the chamber volumes and surface area of the collar.

Statistical Analyses

For all statistical analysis, data was first averaged at the plot level (n=3) for all age cohorts. Differences in volumetric density, C and N concentrations and pool sizes, and C sequestration rates between the age cohorts were detected by using a one-way analysis of variances (ANOVAs), using the statistical computing software R (R Team 2013). For the parameters found to have significant differences ($p<0.1$), multiple comparisons were made using Tukey's HSD post-hoc test ($\alpha=0.1$) to identify which age cohorts were significantly different from each other, calculated with Agricolae R package (R Team 2013; de Mendiburu 2014). Non-normally distributed data was transformed when appropriate prior to statistical analysis. Differences between the age cohorts of the monthly measurements were analyzed through ANOVA with repeated measures, paired with Tukey's HSD post-hoc analysis sliced by time to detect differences between the age cohorts within each month using SAS 9.3 software (SAS Institute 2011). For repeated measures ANOVA, all non-normal monthly data was transformed using logarithmic transformations. Correlation coefficients for all parameters using Spearman's nonparametric rank correlation method with untransformed data were calculated in SAS 9.2 software (SAS Institute 2009).

Results

Ecosystem C and total soil N (TN) are accumulating on the mined reforested landscape. Overall, C accumulation showed a rapid increase early in stand development that began tapering off after the 11-year-old cohort. This trend was very noticeable in tree C which increased 0.3 ±

0.1 Mg C ha^{-1} at the 5-year-old stand to 53.8 ± 8.3 Mg C ha^{-1} at the 11-year-old. Coincident with these observations, the highest C sequestration rate was measured in the 11-year-old cohort (Table 3). Total ecosystem C increased from 9.5 ± 1.7 Mg C ha^{-1} in the 5-year-old cohort to 83.5 ± 16.3 Mg C ha^{-1} in the 30 year cohort; after 30 years 47% of the 178.9 Mg C ha^{-1} of the unmined ecosystem C had returned (Table 3). For all stands other than the 5-year-old cohort, aboveground tree C was the greatest proportion of ecosystem C, accounting for an average of 73% of the total ecosystem C. Mineral soil was the second largest pool for these age cohorts at 12-16% of the total ecosystem C for all mined cohorts and 20% for the unmined. In the 5-year-old cohort, the O horizon accounted for 48% of the total ecosystem C, mineral soil organic C (SOC) 26% and tree C just 3%.

Following trends in aboveground tree biomass, SOC and mineral soil N concentrations also increased with stand age. Perhaps not surprisingly, the unmined cohort had the highest concentration of both SOC and N at all three depths (Fig. 1). Mineral soil N and TN increased in a more gradual stepwise manner from one age cohort to the next than the ecosystem C pools, which had a marked increase from 5- to 11-years and then few significant differences (Table 3). In general, mineral soil N did correlate strongly with ecosystem C (Fig. 2). The strongest correlations were between mineral soil N and SOC ($r=0.93$; $p<0.0001$) and mineral soil N and tree C ($r=0.66$; $p=0.007$).

Ecosystem C pools also correlated strongly with both total and active microbial biomass (Fig. 3). The unmined cohort had significantly more total microbial biomass C than all of the mined cohorts from April-September, with the exception of May when the 21-year-old cohort was not different from unmined (Fig. 4). Similarly, active microbial biomass C was significantly higher in the unmined than the mined cohorts from April-August (Fig. 4). The strongest

individual correlations for both total and active microbial biomass were tree C (r=0.89; p=<.0001 and r=0.80; p=0.0003, respectively) and SOC (r=0.81; p=0.0002 for both). Overall correlations between total ecosystem C and total and active microbial biomass C were r=0.92 (p=<.0001) and r=0.86 (p=<.0001), respectively.

Interestingly, the significant differences in microbial biomass were not observed in many of the measured ecosystem functions that are, at least in part, microbially mediated. There were no significant differences among any of the age cohorts for available soil NO_3^-, available soil NH_4^+, or total inorganic-N (available NO_3^- + NH_4^+; Fig. 5). Likewise, neither the soil CO_2 flux nor N_2O flux varied significantly among cohorts (Fig. 6). As anticipated, CO_2 flux peaked during mid-growing season (June-August), corresponding to the peak in soil temperatures (July-September; Fig. 6 & 7); however, no clear temporal trend in N_2O fluxes were observed. In almost all cases from June-October, the unmined cohorts consumed significantly more atmospheric CH_4 than the mined stands (Fig. 6). During that period, CH_4 consumption in the unmined cohort averaged 70.9 ±8.1 μg CH_4-C m^{-2} h^{-1}, whereas CH_4 consumption in the mined plots averaged 93% less, at only 5.2 ± 1.2 μg CH_4-C m^{-2} h^{-1} (Fig. 6).

Soil temperature and moisture both generally followed a seasonal pattern. Soil temperature showed a clear seasonal pattern, ranging from 10.1-26.8°C (Fig. 7). Notably, the 5-year-old cohort was significantly warmest in all months, similar only to the 11-year-old cohort in April to May, which was the next warmest cohort. The remaining stands, anecdotally with a more closed canopy, had fewer significant differences in soil temperature. Volumetric soil moisture ranged from 8-43% across the age cohorts during the sampling months (Fig. 7). Corresponding with warmer temperatures, the 5-year-old cohort was generally the driest site while the 30-year-old cohort was most moist in all months.

Discussion

C and N accrual patterns

The establishment and growth of trees on the post-mining landscape has significant implications for ecosystem C, as affirmed by the strong correlation between aboveground tree C and total ecosystem C ($r=0.907$; $p<0.0001$). Within the 11-, 21-, and 30-year-old mined cohorts, as well as the unmined forest, aboveground tree C accounted for approximately 70-73% of the total ecosystem C, demonstrating the importance of tree establishment and productivity in ecosystem C accrual (Table 3). Amichev et al. (2008) reported similar results of at least 75% of total ecosystem C contributed by aboveground tree C on reforested mined land. In this regard, the reclaimed mined C pools scales with the unmined forest. Mineral SOC pools also help drive the overall trend in ecosystem C. SOC content tripled from the 5-year-old to the 11-year-old cohort, with the most rapid SOC sequestration rate of 0.7 ± 0.1 Mg SOC ha^{-1} yr^{-1} measured in the 11-year-old stand, the only cohort in our chronosequence with SOC sequestration rates as rapid as those reported on other reclaimed and reforestation mine sites (Sperow 2006).

Ecosystem C accrual was tightly correlated with mineral soil N ($r=0.93$, $p<0.0001$ for mineral SOC; $r=0.66$, $p=0.007$ for tree C) (Fig. 2), demonstrating that productivity on reforested mined sites is driven by N, as is often expressed in most other temperate deciduous forest ecosystems. Thus, the return of soil N to the reclaimed mined landscape is critical for meeting reforestation goals in terms of productivity, and in turn, driving other more stable ecosystem C pools.

Patterns in microbial biomass and microbially mediated processes

As most soil microorganisms are heterotrophic, it was unsurprising that our observations showed strong positive increase in microbial biomass with increases in ecosystem C pools (Fig.

3). What was surprising, however, was there was there were no notable differences in plant available inorganic-N (Fig. 5) despite the differences in both total and active microbial biomass (Fig. 4). Although it is worth noting that the IEMs provide a cumulative net measure of available NH_4^+ and NO_3^-. As such, they are not purely indicative of microbially mediated process (e.g., mineralization and nitrification), but most also be considered in the context of plant uptake which can be assumed to increase proportionally with plant biomass. The same caveat cannot be applied to soil-atmosphere fluxes of N_2O, however. N_2O is a byproduct of nitrification and denitrification, and N_2O fluxes suggested that such microbially mediated N cycling rates are occurring similarly across all of the age cohorts (Fig. 6) despite differences in total N capital (Table 3).

Soil-atmosphere CO_2 efflux is also jointly controlled by microbial (heterotrophic respiration) and plant (autotrophic root respiration) processes. Given increases in plant biomass and soil C, it was expected that CO_2 efflux scale with age. Surprisingly, our observations show little differences for this combined heterotrophic and autotrophic flux, despite statistically significant differences in microbial biomass and ecosystem C pools. Although respiration generally correlates with plant productivity, it is also driven by changes in soil temperature and moisture (Raich and Schlesinger 1992; Holland et al. 1999: Schlesinger and Andrews 2000). Of these three, our soil CO_2 flux measurements correlated most strongly with temperature $r=0.588$ ($p<.0001$), suggesting an indirect biotic control on this important ecosystem-level C flux.

Of the three greenhouse gas fluxes characterized in this study, patterns in CH_4 fluxes, especially soil uptake, showed the largest decoupling between forest ecosystem structure (e.g., microbial and plant biomass) and function. Upland soils are the only known global biogenic sink of atmospheric CH_4, of which forest soils are often demonstrated to be the most efficient (Keller

et al. 1983; Crill 1991; Keller and Reiners 1994; Castro et al. 1995; Le Mer and Roger 2001). In this study, the reforested mined sites are almost completely lacking this methanotrophic function. Even after 30 years of development, the highest measured CH_4 uptake rate was 13.649 μg CH_4-C m^{-2} h^{-1}, contrasted with an uptake rate in excess of seven times higher in the unmined reference (-98.037 μg CH_4-C m^{-2} h^{-1}) during the same month (September). Seasonality is known to highly mediate CH_4 soil-atmospheric fluxes (Crill 1991), yet no clear seasonal patterns explained the lack of methanotropy in the mined plots, where there were no statistical differences in soil temperature and moisture (Figure 8). Nitrification has also been cited as a mechanism for suppressing CH_4 uptake as nitrifying microorganisms compete with methane-mono-oxygenase activity, the enzyme that oxidizes C-H bonds (Le Mer and Roger 2001). Nitrifying activity does not seem like a plausible explanation here, due to the observed lack of trends in available NH_4^+ and NO_3^-. Research to-date investigating CH_4 fluxes under disturbance scenarios has focused on secondary successional environments (e.g., conversion of agricultural pastures or grasslands to forest), not on scenarios of primary succession, such as this study and other surface mining disturbances. Thus, these observations are extremely novel and are an important point for future study regarding the relationships between ecosystem structural and functional development on the more than 1 million ha of surface mined land in the Appalachian Coal Basin as well as disturbances of similar magnitude.

Implications

Reforestation in the post-surface mining context has focused on reestablishing forest structure and productivity. Understanding the effectiveness of these reforestation measures at returning a forest ecosystem to the post-mining landscape, however, also requires an evaluation of forest ecosystem functions. This research shows that the reforestation of native hardwoods in

the Central Appalachian Coal Basin is largely effective in restoring the ecosystem C and N pools, as well as ecosystem functions of C sequestration and N cycling. In a large part it looks like these reforestation efforts are also largely successful at restoring a functioning microbial community, however, the critical microbially mediate function of CH_4 uptake by upland soils is essentially absent from the mined soils. Thus our results affirm that ecosystem structural metrics are not a direct corollary for forest or microbial function in all cases, and specific restoration objectives need to be explicitly considered and monitored.

Acknowledgements

We thank the Powell River Project and the Department of Forest Resources and Environmental Conservation at Virginia Tech for funding. Additionally, funding for this work was provided in part by Virginia Agricultural Experiment Station and the McIntire-Stennis Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

Literature Cited

Acton P, Fox J, Campbell E, Jones A, Rowe H, Martin D, Bryson S (2011) Role of Soil Health in Maintaining Environmental Sustainability of Surface Coal Mining. Environmental Science and Technology 45:10265-10272. doi: 10.1021/es202764q

Amichev B, Burger J, Rodrigue J (2008) Carbon sequestration by forests and soils on mine land in the Midwestern and Appalachian coalfield of the U.S. Forest Ecology and Management 256:1949-1959. doi: 10.1016/j.foreco.2008.07.020

Bailey RG (1983) Delineation of Ecosystem Regions. Environmental Management 7(4):365-373.

Bailey V, Peacock A, Smith J, Bolton H (2002) Relationships between soil microbial biomass determined by chloroform fumigation extraction, substrate-induced respiration and phospholipid fatty acid analysis. Soil Biol. Biochem. 34(9):1385-1389.

Beck T, Joergensen G, Kandeler E, Makeschin F, Nuss E, Oberholzer H, Scheu S (1997) An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol. Biochem. 29(7):1023-2032.

Bernhardt ES, Palmer MA (2011) The environmental costs of mountaintop mining valley fill operations for aquatic ecosystems of the Central Appalachians. Annals of the New York Academy of Scienceis 1223:39-57. doi: 10.1111/j.1749-6632.2011.05986.x

Bickelhaupt DH, Leaf AL, Richards NA (1973) Effect of branching habit on aboveground dry weight estimates of *Acer saccharum* stands. In: Young HE (ed) IUFRO Biomass Studies, College of Life Sciences and Agriculture, University of Maine, Orono, Maine, pp 219-230.

Bowatte S, Tillman R, Carran A, Gillingham A, Scotter D (2008) In situ ion exchange resin membrane (IEM) technique to measure soil mineral nitrogen dynamics in grazed pastures. Biol. Fertil. Soils. 44:805-813. doi: 10.1007/s00374-007-0260-4

Braun EL (1950) Deciduous forests of eastern North America. Blakiston, Philadelphia

Brenneman BB, Frederick DJ, Gardner WE, Schoenhofen LH, Marsh PL (1978) Biomass of species and stands of West Virginia hardwoods. In: Pope PE (ed) Proceedings of Central Hardwood Forest Conference II, West LaFayette, Purdue University pp 159-178

Burger JA, Fannon AG (2009) Capability of Reclaimed Mined Land for Supporting Reforestation with Seven Appalachian Hardwood Species. Barnhisel RI (ed) Revitalizing the Environment: Proven Solutions and Innovative Approaches, ASMR, Billings, MT, 30 May – 5 June 2009

Burger JA, Mitchem DO, Zipper CE, Williams R (2005) Herbaceous Ground Cover Effects on Native Hardwoods Planted on Mined Land, ASMR, Lexington, KY, 19-23 June 2005

Burger JA, Mitchem DO, Zipper CE, Williams R (2008) Hardwood Reforestation for Phase III Bond Release: Need for Reduced Ground Cover. In: Barnhisel RI (ed) *New Opportunities to Apply our Science*, ASMR, Lexington, KY 14-19 June 2008

Burger JA and Zipper CE (2011) How to Restore Forests on Surface-Mined Land. V.C. Extension. Blacksburg, VA. Virginia Polytechnic and State University. https://pubs.ext.vt.edu/460/460-123/460-123_pdf.pdf. Accessed 25 Oct 2012

Castro M, Steudler P, Melillo J, Aber J, Bowden R (1995) Factors controlling atmospheric methane consumption by temperate forest soils. Global Biogeochemical Cycles 9(1):1-10.

Clark A III and Schroeder J (1986) Weight, Volume, and Physical Properties of Major Hardwood Species in the Southern Appalachian Mountains. Southeastern Forest Experiment Station, USDA Forest Service. Asheville, NC.

Crill P (1991) Seasonal patterns of methane uptake and carbon dioxide release by a temperature woodland soil. Global Biogeochemical Cycles 5(4):319-324.

Day F and Monk C (1974) Vegetation Patterns on a Southern Appalachian Watershed. Ecology 55(5):1064-1074.

de Mendiburu F (2014) agricolae: Statistical Procedures for Agricultural Research. R package version 1.1-8. http://CRAN.R-project.org/package=agricolae

Duran J, Delgado-Baquerizo M, Rodriguez A, Covelo F, Gallardo A (2013) Ionic exchange membranes (IEMs): A good indicator of soil inorganic N production. Soil Bio. Biochem. 57:964-968.

Elliot K and Clinton B (1993) Equations for Estimating Biomass of Herbaceous and Woody Vegetation in Early-Successional Southern Appalachian Pine-Hardwood Forests. Southeastern Forest Experiment Station, USDA Forest Service. Asheville, NC.

Fierer N, Schimel J, Holden P (2003) Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35:167-176.

Hangs R, Greer K, Sulewski C (2003) The effect of interspecific competition on conifer seedling growth and nitrogen availability measured using ion-exchange membranes. Can. J. For. Res. 34:754-761. doi: 10.1139/X03-229

Harris D, Horwath W, van Kessel C (2001) Acid Fumigation of Soils to Remove Carbonates Prior to Total Organic Carbon or Carbon-13 Isotopic Analysis. Soil Science Society of America Journal 65:1853-1856.

Holland E, Robertson GP, Greenberg J, Groffman P, Boone R, Gosz J (1999) Soil CO_2, N_2O, and CH_4 Exchange. In: Robertson GP, Coleman D, Bledsoe C, Sollins P (ed) Standard Soil Methods for Long-term Ecological Research, New York Oxford University Press, New York, pp 185-201

Jenkins JC, Chojnacky DC, Heath LS, Birdsey RA (2004) Comprehensive Database of Diameter-based Biomass Regressions for North American Tree Species. Northeastern Research Station, USDA Forest Service. Burlington, VT.

Keller M, Goreau T, Wofsky S, Kaplan W, McElroy M (1983) Production of nitrous oxide and consumption of methane by forest soils. Geophysical Research Letters 10(12):1156-1159.

Keller M, Reiners WA (1994) Soil-atmosphere exchange of nitrous oxide, nitric oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. Global Biogeochemical Cycles 8)4):399-409.

Ker MF (1984) Biomass equations for seven major maritimes tree species. Canadian Forestry Service, Maritime Forest Research Center Inf. Rep. M-X-148.

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: A review. Eur. J. Soil Biol. 37:25-50.

MacLean DA, Wein RW (1976) Biomass of jack pine and mixed hardwood stands in northeastern New Brunswick. Can. J. For. Res. 6:441-447.

McEwan RW, Muller RN, McCarthy BC (2005) Vegetation-Environment Relationships among Woody Species in Four Canopy-Layers in an Old-Growth Mixed Mesophytic Forest. Castanea 70(1):32-46.

Muller RN (1982) Vegetation Patterns in the Mixed Mesophytic Forest of Eastern Kentucky. Ecology 63(6):1901-1917.

Omernik JM (1987) Ecoregions of the Conterminous United States. Annals of the Association of American Geographers 77(1):118-125.

Parkinson D, Paul EA (1982) Microbial biomass. In: Page AL, Miller RH, Keeney DR (ed) Methods of Soil Analysis Part 2. Chemical and Microbiological Properties, American Society of Agronomy, Inc., Madison, WI

R Core Team (2013) R: A Language and Environment for Statistical Computing version 3.0.2 edn. R Foundation for Statistical Computing, Vienna

Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44B:81-99.

Ruppert LF (2001) Chapter A—Executive Summary—Coal Resource Assessment of Selected Coal Beds and Zones in the Northern and Central Appalachian Basin Coal Regions. In: Northern and Central Appalachian Basin Coal Regions Assessment Team, 2000 resource assessment of selected coal beds and zones in the northern and central Appalachian Basin coal regions: U.S. Geological Survey Professional Paper 1625-C, CD-ROM, version 1.0.

SAS Institute, Inc. (2009) edn. 9.2. Cary, NC

SAS Institute, Inc. (2011) edn. 9.3. Cary, NC

Sencindiver JC, Ammons JT (2000) Minesoil Genesis and Classification. In: Barnhisel RI, Darmody RG, Daniels WL (ed) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Madison, WI, pp 595-613.

Schlesinger WH, Andrews JA (2000) Soil respiration and the global carbon cycle. Biogeochemistry 48:7-20.

Sperow M (2006) Carbon Sequestration Potential in Reclaimed Mine Sites in Seven East-Central States. Journal of Environmental Quality 35(4):1428-1438.

Subler S, Blair JM, Edwards CA (1995) Using Anion-Exchange Membranes to Measure Soil Nitrate Availability and Net Nitrification. Soil Biol. Biochem. 27(7):911-917.

Ter-Mikaelian MT and Korzukhin MD (1997). Biomass equations for sixty-five North American tree species. Forest Ecology and Management 97:1-24

Torbert JL Jr, Burger JA, Lien JN, Schoenholtz SH (1985) Results of a Tree Species Trial on a Recontoured Surface Mine in Southwestern Virginia, Southern Journal of Applied Forestry 150-153.

Torbert JL, Burger JA (2000) Forest Land Reclamation. In: Barnhisel RI, Darmody RG, Daniels WL (ed) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, WI, pp 371-398.

Vadeboncoeur MA, Hamburg SP, Blum JD, Pennino MJ, Yanai RD, Johnson CE (2012) The Quantitative Soil Pit Method for Measuring Belowground Carbon and Nitrogen Stocks, Soil Science Society of America Journal 76:2241-2255. doi:10.2136/ssaj2012.0111

Wardle DA, Ghani A (1995) Why is the Strength of Relationships between Pairs of Method for Estimating Soil Microbial Biomass Often so Variable?. Soil Biol. Biochem 27(6):821-828. doi: 0038-0717/95

West AW, Sparling GP (1986) Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. Journal of Microbiological Methods 5:177-189.

Williams RA, McClenahen JR (1984) Biomass Prediction Equations for Seedlings, Sprouts, and Saplings of Ten Central Hardwood Species. Forest Science 30(2):523-527.

Zipper CE, Daniels WL, Bell, JC (1989) Approximate original contour reclamation: An alternative in steep slope terrains. Journal of Soil and Water Conservation 44(4):279-283.

Zipper CE, Burger JA, Skousen JG, Angel PN, Barton CD, Davis V, Franklin JA (2011) Restoring Forests and Associated Ecosystem Services on Appalachian Coal Surface Mines. Environmental Management 47:751-765.

Table 1 Site variables of each stand at time of sampling

Stand Age	Aspect	Slope (%)	Basal Area (m^2 ha^{-1})
5	S	63	0.51 ± 0.1
11	SW, SE & NE	32	19.7 ± 2.6
21	S & W	28	20.0 ± 1.5
30	NW	33	23.2 ± 3.8
Unmined	W & SE	24	36.8 ± 7.9

Table 2 Reclamation treatments and planted species of mined and reforested stands

Stand Age	Reclamation Treatment	Hydroseed Cover Crop Mix	Planted Tree Species
5	Loose-graded Hydroseeded Fertilized with 22 kg ha^{-1} N, 68 kg ha^{-1} phosphorous and 18 kg ha^{-1} potassium 1680 kg ha^{-1} wood cellulose fiber	Rye grain *(Secale cereal)* Orchard grass (*Dactylis glomerata*) Perennial ryegrass (*Lolium perenne*) Korean lespedeza (*Lespedeza cuneata*) Birdsfoot trefoil (*Lotus corniculatus*) White (Ladino) clover (*Trifolium repens*) Redtop (*Agrostis gigantea*) Weeping lovegrass (*Eragrostis curvula*)	*Crop trees*: white ash (*Fraxinus americana*), white oak (*Quercus alba*), sugar maple (*Acer saccharum*), black cherry (*Prunus serotina*), red oak (*Quercus rubra*), chestnut oak (*Quercus prinus*), black oak (*Quercus velutina*), yellow poplar (*Liriodendron tulipifera*), and white pine (*Pinus strobus*) *Wildlife/Nurse trees*: Gray dogwood (*Cornus racemosa*) , red mulberry (*Morus rubra*) Redbud (*Cercis canadensis*) , and shagbark hickory (*Carya ovata*)
11	Lightly graded and left uncompacted Hydroseeded	Orchard grass Birdsfoot trefoil Timothy grass (*Phleum pratense*) Red clover (*Trifolium pratense* L.)	*Crop trees*: white ash, white oak, sugar maple, red oak, chestnut oak, yellow poplar, and white pine *Wildlife/Nurse trees*: Silky dogwood (*Cornus amomum*), crab apple (*Malus spp.*), and bristly locust (*Robinia hispida*)
21	Smooth-graded and tracked Hydroseeded	Kentucky-31 tall fescue (*Festuca arundinacea* Schreb.) Orchard grass Redtop Perennial ryegrass Red clover *Serecia lespedeza*	*Crop trees*: red oak, white oak, white ash), black walnut (*Juglans nigra*), eastern cottonwood (*Populus deltoides* Bartram ex Marsh), American sycamore (*Platanus occidentalis*), and yellow poplar
30	Smooth-graded and tracked Hydroseeded Fertilized with 560 kg ha^{-1} of 10-20-20 1681 kg ha^{-1} wood fiber mulch	Kentucky-31 tall fescue Birdsfoot trefoil Redtop White (Ladino) clover Annual rye (*Lolium multiflorum)*	*Crop trees:* black locust (*Robinia psuedoaccacia*), European black alder (*Alnus glutinosa*), black walnut, chestnut oak, Chinese chestnut (*Castanea mollissima*), yellow poplar, American sycamore, eastern cottonwood, and red oak

Table 3 Nitrogen and carbon pool accrual values

Age	5		11		21		30		Unmined	
Component	Soil N Pools (Mg ha^{-1})									
O Horizon	0.18 ± 0.02	(ab)	0.18 ± 0.02	(a)	0.34 ± 0.04	(b)	0.21 ± 0.04	(ab)	0.21 ± 0.05	(ab)
Mineral Soil	0.72 ± 0.05	(a)	1.09 ± 0.10	(ab)	1.37 ± 0.07	(bc)	1.73 ± 0.16	(bc)	2.54 ± 0.19	(c)
Total Soil	0.9 ± 0.05	(a)	1.26 ± 0.11	(ab)	1.72 ± 0.09	(bc)	1.94 ± 0.20	(c)	2.75 ± 0.23	(d)
	Ecosystem C Pools (Mg ha^{-1})									
Tree	0.27 ± 0.06	(a)	53.80 ± 8.28	(bc)	48.86 ± 7.20	(b)	64.27 ± 13.40	(bc)	125.71 ± 37.93	(c)
O Horizon	4.57 ± 0.48	(a)	5.85 ± 0.41	(ab)	9.11 ± 1.05	(b)	6.86 ± 1.40	(ab)	6.53 ± 1.39	(ab)
Mineral Soil	2.45 ± 1.16	(a)	7.67 ± 0.72	(b)	12.24 ± 1.38	(b)	9.76 ± 1.75	(b)	36.32 ± 0.88	(c)
Root	2.22 ± 0.19	(a)	1.63 ± 0.24	(a)	5.24 ± 0.49	(b)	2.64 ± 0.51	(a)	10.38 ± 2.92	(b)
Total Ecosystem	9.51 ± 1.74	(a)	68.95 ± 8.15	(b)	75.46 ± 5.07	(b)	83.53 ± 16.33	(b)	178.89 ± 39.71	(c)
	C Sequestration Rate (Mg ha^{-1} yr^{-1})									
Tree	0.05 ± 0.01	(a)	4.89 ± 0.75	(b)	2.33 ± 0.35	(c)	2.14 ± 0.45	(c)	-	
O Horizon	0.91 ± 0.10	(a)	0.53 ± 0.04	(b)	0.43 ± 0.05	(bc)	0.23 ± 0.05	(c)	-	
Mineral Soil	0.49 ± 0.23	(a)	0.70 ± 0.07	(a)	0.58 ± 0.07	(a)	0.33 ± 0.06	(a)	-	
Roots	0.44 ± 0.04	(a)	0.15 ± 0.02	(bc)	0.25 ± 0.02	(b)	0.09 ± 0.02	(c)	-	
Total Ecosystem	1.90 ± 0.35	(a)	6.27 ± 0.74	(b)	3.59 ± 0.24	(a)	2.78 ± 0.54	(a)	-	

Mean and standard error (n=3) of soil N pools (Mg ha^{-1}), ecosystem C pools (Mg ha^{-1}) and C sequestration rates for each pool (Mg ha^{-1} yr^{-1}) within each age cohort. Letter groups indicated significant differences between age cohorts within each depth ($p<0.1$).

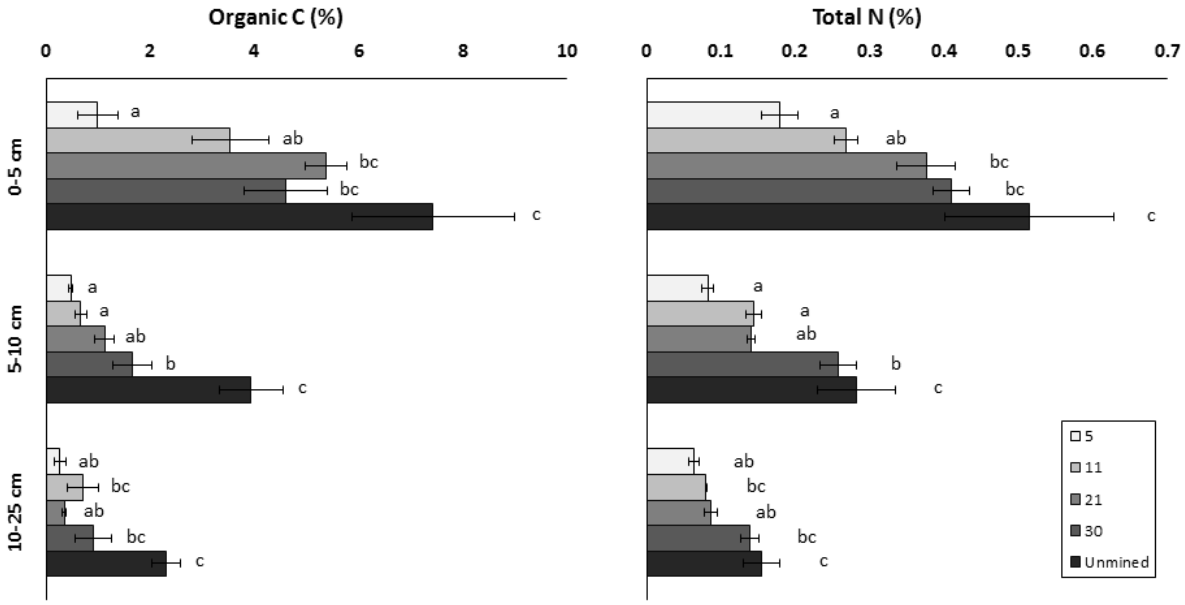


Figure 1

Mean and standard error (n=3) of organic C and mineral soil total N concentration (%) within each age cohort by depth, 0-5, 5-10 and 10-25 cm. Letter groups indicated significant differences between age cohorts within each depth ($p<0.1$).

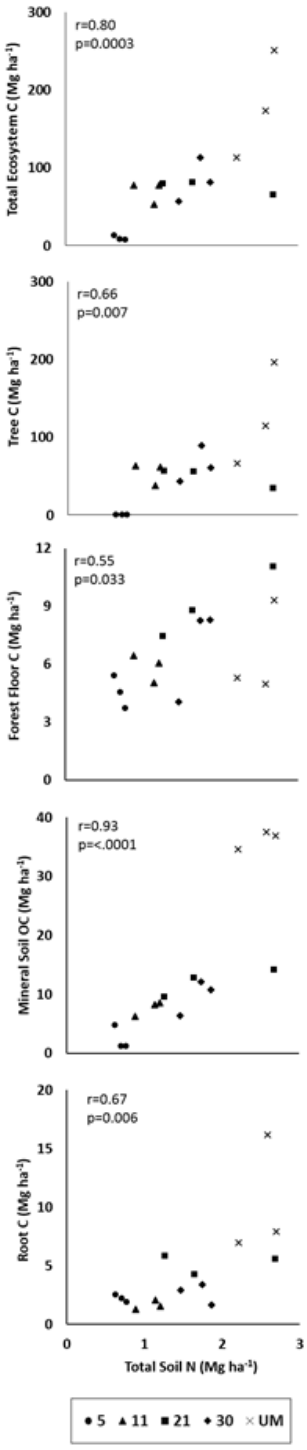


Figure 2

Mean (n=3) total soil N (Mg ha-1) correlated with ecosystem C components of total ecosystem C, tree C, forest floor C, mineral soil C, and root C. Displayed on each graph are the Pearson's Correlation Coefficient (r) and significance value (p) for the relationship.

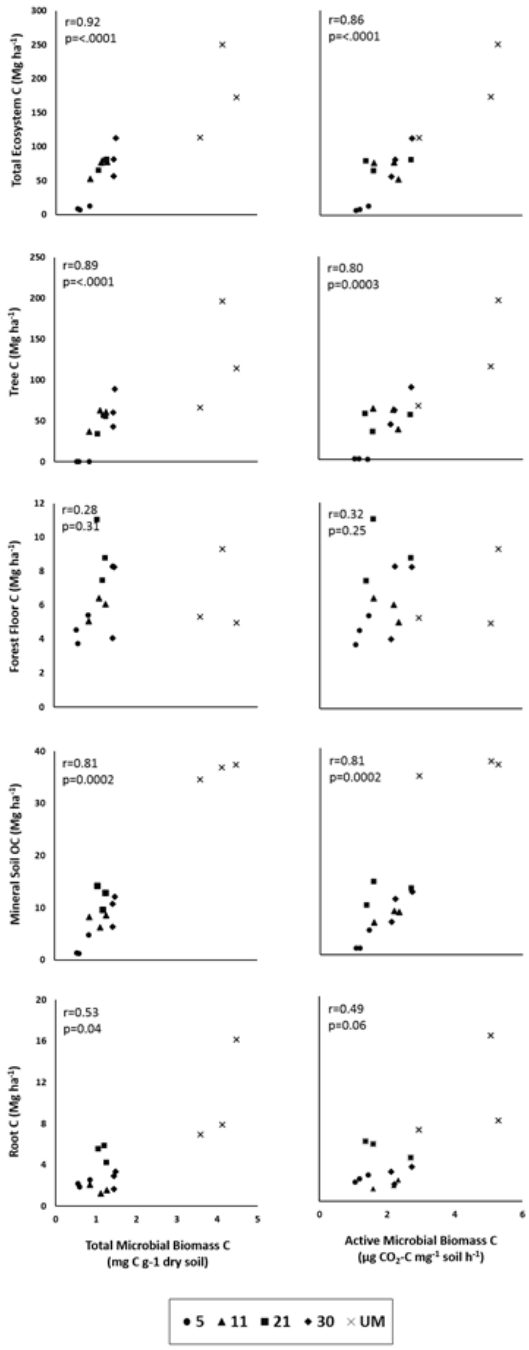
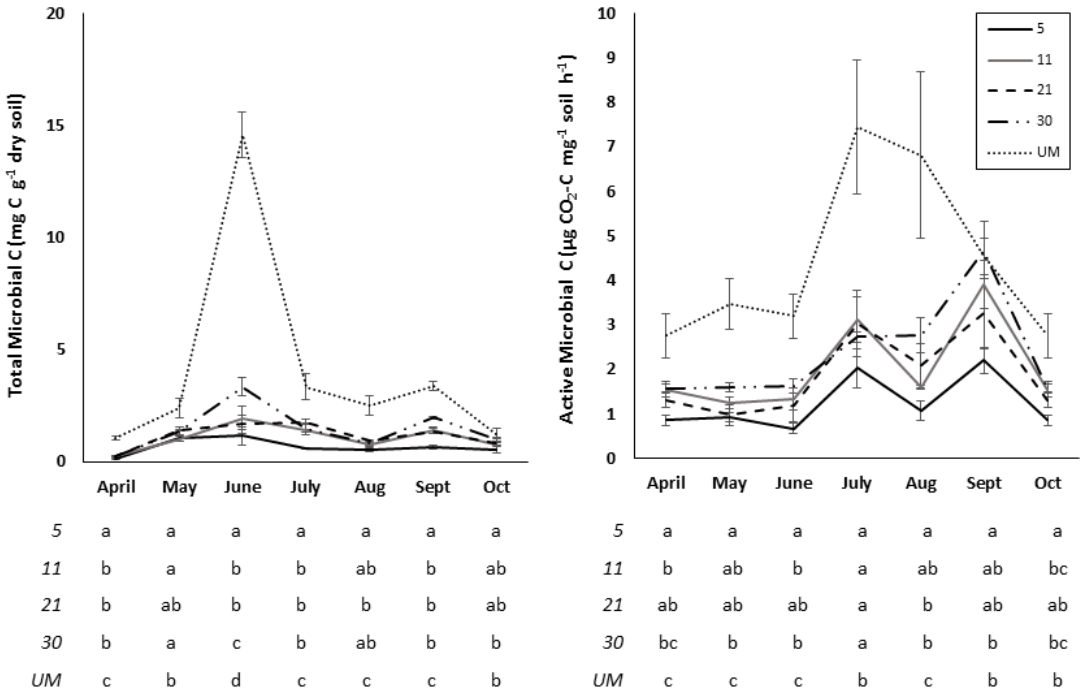


Figure 3

Mean (n=3) total microbial biomass C (left) and active microbial biomass C (right) correlated with ecosystem C components of total ecosystem C, tree C, forest floor C, mineral soil C, and root C. Displayed on each graph are the Pearson's Correlation Coefficient (r) and significance value (p) for that relationship.

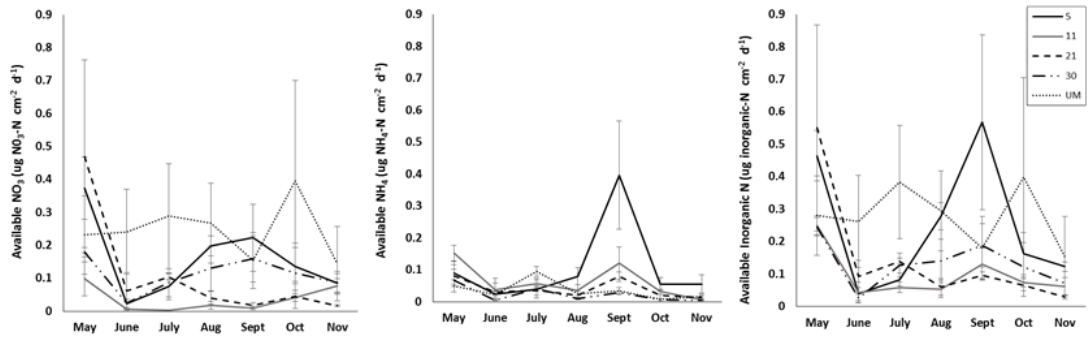


	April	May	June	July	Aug	Sept	Oct
5	a	a	a	a	a	a	a
11	b	a	b	b	ab	b	ab
21	b	ab	b	b	b	b	ab
30	b	a	c	b	ab	b	b
UM	c	b	d	c	c	c	b

	April	May	June	July	Aug	Sept	Oct
5	a	a	a	a	a	a	a
11	b	ab	b	a	ab	ab	bc
21	ab	ab	ab	a	b	ab	ab
30	bc	b	b	a	b	b	bc
UM	c	c	c	b	c	b	b

Figure 4

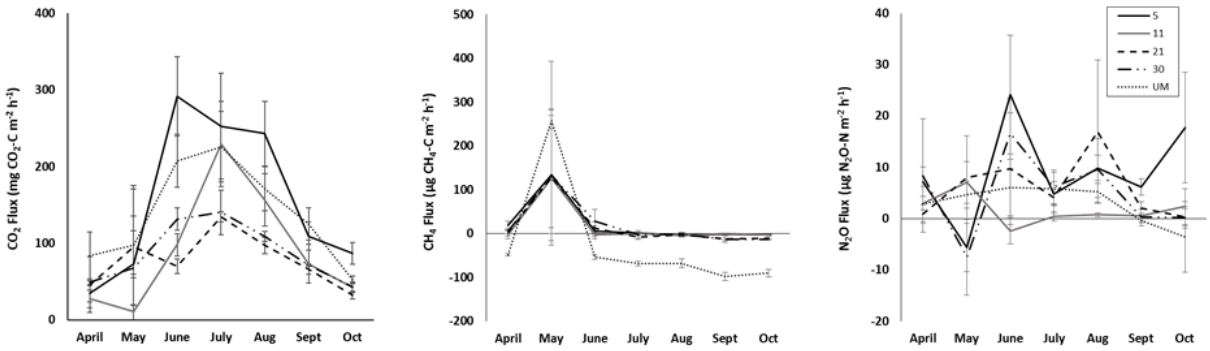
Mean and standard error (n=3) of total microbial biomass C (mg C g-1 dry soil) and active microbial biomass C (µg CO2-C mg-1 soil h-1) within each age cohort measured across the seven sampling months. Letter groups below the graph indicate significant differences between age cohorts within each month ($p<0.1$).



5	ab	ab	a	*5*		a	*5*	a	a
11	b	b	b	*11*		ab	*11*	a	b
21	ab	a	ab	*21*		ab	*21*	ab	b
30	ab	a	ab	*30*		ab	*30*	a	ab
UM	a	a	ab	*UM*		ab	*UM*	b	ab

Figure 5

Mean and standard error (n=3) of nitrate (NO_3^-), ammonium (NH_4^+) and inorganic N (NO_3^- and NH_4^+) availability (mg N cm^{-2} d^{-1}) within each age cohort measured across the seven sampling months. Letter groups below the graph indicate significant differences between age cohorts within each month ($p<0.1$).



	April	May	June	July	Aug	Sept	Oct
5	ab	a	a				
11	a	b	ab				
21	ab	ab	b				
30	ab	ab	ab				
UM	b	ab	ab				

	April	May	June	July	Aug	Sept	Oct
5	ab			a	a	a	a
11	ab			a	a	a	a
21	ab			a	a	a	a
30	a			a	a	ab	ab
UM	b			b	b	b	b

	April	May	June	July	Aug	Sept	Oct
5					ab		
11					b		
21					ab		
30					b		
UM					ab		

Figure 6

Mean and standard error (n=3) of CO_2 flux (mg CO_2-C m^{-2} h-1), CH_4 flux (µg CH_4-C m^{-2} h^{-1}) and N_2O flux (µg N_2O-N m^{-2} h^{-1}) within each age cohort measured across the seven sampling months. Letter groups below the graph indicate significant differences between age cohorts within each month ($p<0.1$).

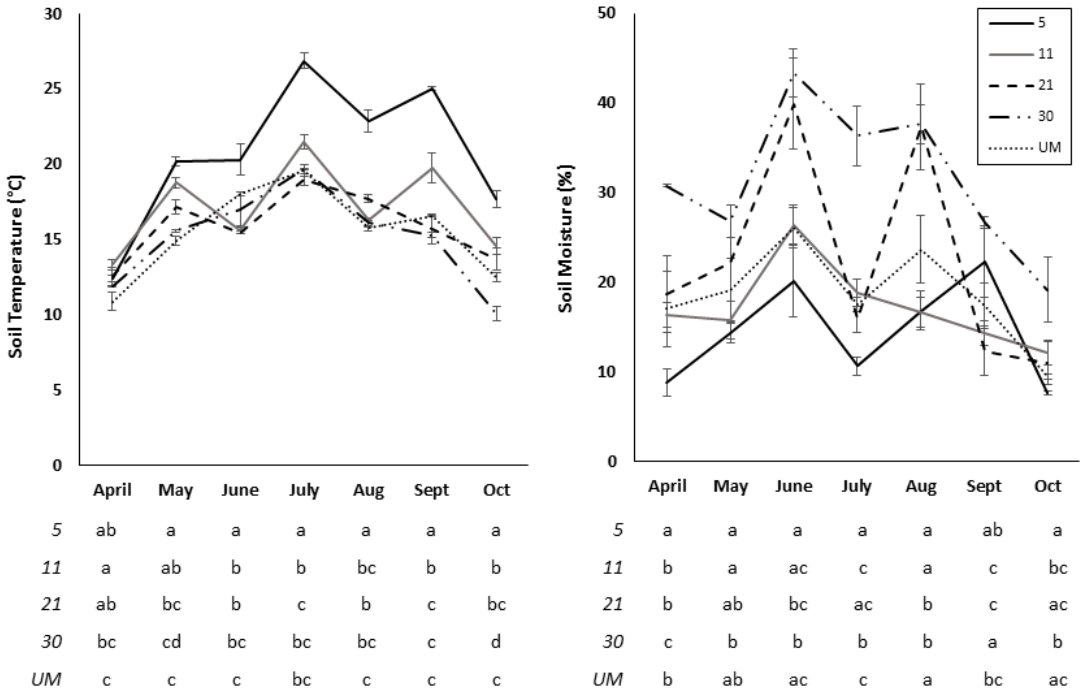


Figure 7

Mean and standard error (n=3) of soil temperature (°C) and moisture (%) within each age cohort measured across the seven sampling months. Letter groups below the graph indicate significant differences between age cohorts within each month ($p<0.1$).

Conclusions

Reforestation in the post-surface mining context has focused on reestablishing forest structure and productivity. Understanding the effectiveness of these reforestation measures at returning a forest ecosystem to the post-mining landscape, however, also requires an evaluation of forest ecosystem functions. The reforested landscape has aesthetic and cultural values as it is reminiscent, at least visually, of the pre-mining forest and helps to hide the scar of the decimated mountain. However, the purpose of this research was to investigate whether or not the functions, and thus the forest ecosystem services, of the pre-mined forest are returning. Traditional mine land reclamation has been heavily studied, including ecosystem processes and function, however the development of ecosystem function post-reforestation is still largely unknown.

In the native forest ecosystem, many of these services originate within the forest soils. Therefore this study focused primarily on the forest soils. A broad yet fairly thorough suite of metrics was taken including ecosystem C pool content, total N concentration and content, pH, volumetric density, C:N, total and active microbial biomass, plant-available inorganic soil N, available soil gas fluxes of CO_2, CH_4 and N_2O, and soil temperature and moisture. By selecting sites that together formed a chronosequence, it was possible to compare metrics across time, in addition to the unmined reference stand. This research shows that the reforestation of native hardwoods in the Central Appalachian Coal Basin is largely effective in restoring the ecosystem C and N pools, as well as ecosystem functions of C sequestration and N cycling. In a large part it looks like these reforestation efforts are also largely successful at restoring a functioning microbial community, however, the critical microbially mediated function of CH_4 uptake by upland soils is essentially absent from the mined soils. Thus, our results affirm that ecosystem structural metrics are not a direct corollary for forest or microbial function.

We recommend that in pursuit of forest ecosystem restoration, that future research focuses on bringing more clarity to the decoupling of structure and function found within our data. Surprisingly, we found no significant differences in plant-available inorganic-N among any of the cohorts, despite significant differences that were present within the mineral soil N capital and microbial biomass. There has been a heavy focus in the literature on establishing grasses and legumes to achieve high vegetation coverage to protect the soil resource from erosion, as well as a focus on simultaneously reducing the competition of the ground cover species with native hardwoods. However, more research to identify the sources and fate of the plant-available inorganic-N early in stand development will help to bring clarity to the early steps of reclamation and reforestation. Most novel, this decoupling was also found between the microbial community and the microbially mediated process of atmospheric CH_4 uptake by upland forest soils. Upland forest soils are the most significant global biogenic CH_4 sink, and even after 30 years, the reforested mined soils show almost no atmospheric CH_4 uptake. Understanding the microbial community dynamics occurring in the mined soils to cause the observed lack of methanotrophy will be essential to moving forward with forest ecosystem restoration. This research provides early support for reforestation post-mining to return forest functioning; however, specific restoration objectives need to be explicitly considered and monitored, to lead to a reforestation method that results in total ecosystem restoration.

APPENDICES

A. Site descriptions and thorough sampling descriptions and methods

Materials and Methods

Study Location and Sites

This study was conducted on the Appalachian Plateau physiographic province in Wise County in southwestern Virginia on a chronosequence of four reclaimed and reforested stands (ages 5, 11, 21, and 30 years) and an unmined reference stand. The 5, 11 and 21-year-old sites, and unmined reference were located on the Powell River Project and the 30-year-old site is along the Roaring Fork River at Kent Junction. Local vegetation is primarily of the Appalachian mixed mesophytic forest ecotype prior to mining. Local climate is characterized by a mean annual precipitation averaging 123 cm with the lowest mean annual temperatures occurring in January averaging 5.4°C and the warmest temperatures occurring in July and August averaging 27°C.

All mined stands were surface-mined and reclaimed by Red River Coal following SMCRA requirements. This included recontouring slopes using a mix of weathered and unweathered sandstone and siltsone overburden (with shale and coal fragments), as a topsoil substitute. In selecting chronosequence sites, other relevant site characterestics (e.g., slope, initial revegetation seed mix, grading/compaction), were constrained to the best of our ability whenever possible (Table A.1). Each of the reclaimed stands was intentionally planted with native hardwoods following reclamation (Table A.2). Detailed land use history is not available for the unmined reference stand; although given patterns in land use history regionally and current stand conditions, it is likely that the unmined sites had been harvested for timber more than 50 years ago.

Table A.1 Site variables at each stand at the time of sampling

Stand Age	Aspect	Slope (%)	Basal Area (m^2 ha^{-1})
5	S	63	0.51 ± 0.1
11	SW, SE & NE	32	19.7 ± 2.6
21	S & W	28	20.0 ± 1.5
30	NW	33	23.2 ± 3.8
Unmined	W & SE	24	36.8 ± 7.9

Table A. 2 Reclamation and reforestation treatments

Stand Age	Reclamation Treatment	Hydroseed Cover Crop Mix	Planted Tree Species
5	Loose-graded Hydroseeded Fertilized with 22 kg ha^{-1} N, 68 kg ha^{-1} phosphorous and 18 kg ha^{-1} potassium 1680 kg ha^{-1} wood cellulose fiber	Rye grain *(Secale cereal)* Orchard grass (*Dactylis glomerata*) Perennial ryegrass (*Lolium perenne*) Korean lespedeza (*Lespedeza cuneata*) Birdsfoot trefoil (*Lotus corniculatus*) White (Ladino) clover (*Trifolium repens*) Redtop (*Agrostis gigantea*) Weeping lovegrass (*Eragrostis curvula*)	*Crop trees*: white ash (*Fraxinus americana*), white oak (*Quercus alba*), sugar maple (*Acer saccharum*), black cherry (*Prunus serotina*), red oak (*Quercus rubra*), chestnut oak (*Quercus prinus*), black oak (*Quercus velutina*), yellow poplar (*Liriodendron tulipifera*), and white pine (*Pinus strobus*) *Wildlife/Nurse trees*: Gray dogwood (*Cornus racemosa*) , red mulberry (*Morus rubra*) Redbud (*Cercis canadensis*) , and shagbark hickory (*Carya ovata*)
11	Lightly graded and left uncompacted Hydroseeded	Orchard grass Birdsfoot trefoil Timothy grass (*Phleum pratense*) Red clover (*Trifolium pratense* L.)	*Crop trees*: white ash, white oak, sugar maple, red oak, chestnut oak, yellow poplar, and white pine *Wildlife/Nurse trees*: Silky dogwood (*Cornus amomum*), crab apple (*Malus spp.*), and bristly locust (*Robinia hispida*)
21	Smooth-graded and tracked Hydroseeded	Kentucky-31 tall fescue (*Festuca arundinacea* Schreb.) Orchard grass Redtop Perennial ryegrass Red clover *Serecia lespedeza*	*Crop trees*: red oak, white oak, white ash), black walnut (*Juglans nigra*), eastern cottonwood (*Populus deltoides* Bartram ex Marsh), American sycamore (*Platanus occidentalis*), and yellow poplar

30	Smooth-graded and tracked Hydroseeded Fertilized with 560 kg ha^{-1} of 10-20-20 1681 kg ha^{-1} wood fiber mulch	Kentucky-31 tall fescue Birdsfoot trefoil Redtop White (Ladino) clover Annual rye (*Lolium multiflorum)*	*Crop trees:* black locust (*Robinia psuedoaccacia*), European black alder (*Alnus glutinosa*), black walnut, chestnut oak, Chinese chestnut (*Castanea mollissima*), yellow poplar, American sycamore, eastern cottonwood, and red oak

The tree species on the 5 and 11-year-old sites were planted the season following initial reclamation and hydroseeding. The tree species on the 21-year-old site were planted one year after reclamation and planting with erosion control grasses and the 30-year-old was planted within two years of initially being recontoured. It is important to note that all four sites were sown with a conventional seed mix. The intention of a conventional seed mix is to establish a quick and hardy cover. However the specific species that comprised this seed mix for each of the age cohorts varied (Table A.2). There are no species that are constant through all four age classes, however there are several species that are common between at least two age classes. Amongst these species, tall fescue and certain clovers (*Trifolium* sp.) are recognized as tree-competitive species. While redtop, timothy, birdsfoot trefoil and white clover are all among the species listed that are recognized as more tree-compatible and utilized by the Forestry Reclamation Approach (FRA) (Zipper et al. 2011).

Figure A.1

Research sites included in this study. From left to right 5, 11, 21, and 30 years sites of the chronosequence and unmined reference.

Site and stand characterization

Three replicate sampling plots (5 m radius) were established in each age cohort for site and stand characterization. A systematic approach was used to choose plot locations, as space was limited in three of the four mined cohorts. Exclusion criteria were used to avoid areas that were not representative of the site as a whole. Areas excluded included those that had atypical canopy cover, noticeably different slope, and uncharacteristic understory vegetation within the site. Cohorts were distinguished by differing levels of canopy cover (or lack thereof), slopes, and by differing understory vegetation compositions, thus exclusion criteria were unique to each site.

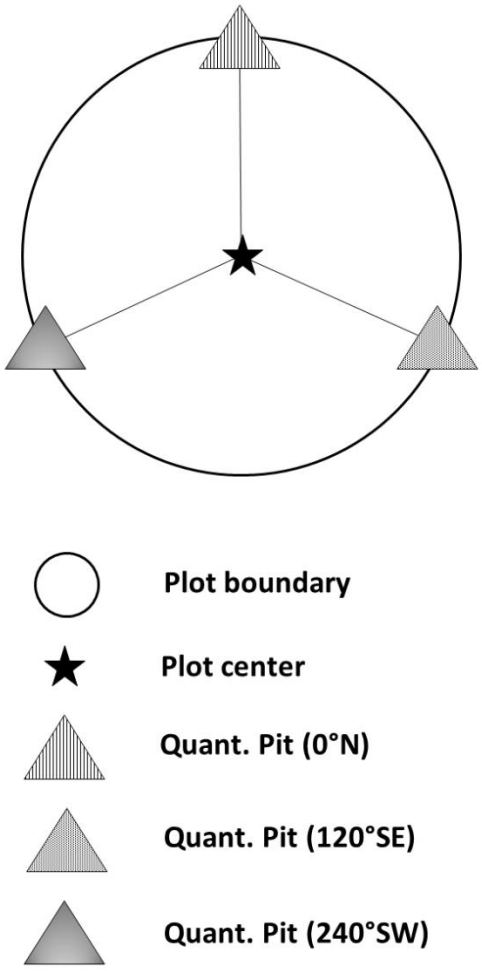


Figure A.2

Sampling schematic of the location of the three quantitative soil pits collected in each plot.

In the spring of 2013, three quantitative soil pits were excavated 5 m from each plot center (Vadeboncoeur et al. 2012), at 0°, 120° and 240° relative to an azimuth of 0° (Figure A.2). Within a 25 cm x 25 cm sampling frame the O horizon was clipped and removed. Mineral soils were excavated by depth increment (0-5, 5-10, and 10-25 cm). A flat edged tool was used to scrape the sides and bottom of the pits to the precise dimensions. Soil was brushed off of large

rocks excavated from the pit and the rocks were left in the field. Likewise, roots that were too thick to be cut with a small folding saw were brushed off and left in situ. Collected material was oven-dried at 60°C. Dried mineral soils were gently broken up manually and then dry sieved through a 2-mm sieve to remove rocks, roots, and other debris. Of the >2 mm fraction, roots and coal were separated for further analysis. Volumetric density (VD) of the fine, soil fraction (<2 mm) was calculated for each depth increment by dividing the mass of the fine fraction by the total volume of the excavated depth increment: $VD\ (g\ cm^{-3}) = soil\ mass\ (g) \div quadrat\ volume\ (cm^{-3})$.

Mineral soil organic C (SOC) and mineral soil N concentration were quantified at each depth of the quantitative pits using an Elementar vario Micro Cube elemental analyzer connected to an Elementar IsoPrime100 isotope ratio mass spectrophotometer (IRMS) (Elementar, Hanau, Germany) using a two end-member mixing model based on the different $\delta^{13}C$ isotopic compositions of geogenic (i.e., coal) and pedogenic (i.e., plant) C sources. Geogenic OC (GOC) in these soil and overburden materials are derived from coal and are not considered to be a portion of the cycling SOC pool. Therefore to dry combustion techniques to quantify the available SOC pool, must account for GOC. Following Acton et al. (2011), O horizon and coal were used as end-members in the analysis. O horizon samples were pre-ground using a Wiley Mill (Thomas Scientific Model 4 Miley Mill, Swedesboro, NJ) and coal samples were ground using a mortar and pestle. A subsample of each of the mineral soil, forest floor, and coal samples were individually ground to a fine powder using a Retsch MM200 ball mill (Retsch, Haan, Germany). Following the method of Harris et al. (2001), finely ground soil samples were weighed into silver (Ag) capsules and were fumigated with 100 mL of 12 M hydrochloric acid (HCl) in a vacuum desiccator for 24 h to remove inorganic-C (i.e. carbonates) that would

otherwise provide another δ^{13}C end-member. These soil samples were then dried at 60°C for 48 h to remove any residual chloride (Harris et al. 2001). Silver capsules were closed and packaged within tin (Sn) capsules to preserve sample integrity. O horizon and coal samples were weighed directly into Sn capsules. The isotope mass balance determination of pedogenic organic C (POC) concentration was calculated using the following equations:

$$(1)\delta^{13}C_{TOC} = P_{POC}\delta^{13}C_{POC} + P_{GOC}\delta^{13}C_{GOC}$$

$$(2)\ 1 = P_{POC} + P_{GOC}$$

$$(3)\ \%C_{corrected} = P_{POC} \times \%C_{uncorrected}$$

Where $\delta^{13}C_{TOC}$ is the isotopic value of the total organic carbon (TOC) in each sample (i.e., air-dried, sieved, untreated) and $\delta^{13}C_{POC}$ and $\delta^{13}C_{GOC}$ are the delta values (‰) of the pedogenic organic carbon and geogenic coal organic carbon stable isotope end-member, respectively. P_{POC} and P_{GOC} represent the proportional amounts of TOC accounted for by the pedogenic and geogenic sources of organic carbon, respectively. Soil C and N content (Mg ha^{-1}) was then calculated from concentration (%) using measured VD for each depth increment using the following equation:

$$(4)\ Mg\ ha^{-1} = \%\times\frac{soil\ mass\ (g)}{625\ cm^2 quadrat}\times\frac{Mg}{10^6\ g}\times\frac{10^6\ cm^2}{ha}$$

Simplified, $Mg\ ha^{-1} = \% \times Density\ (g\ cm^{-3}) \times Depth(cm)$

All trees falling within the 5 m plot radius and having a diameter greater than 2.54 cm at breast height (1.4 m) were measured for diameter at breast height (DBH). For woody plants smaller than these criteria, ground line diameter (GLD) was recorded. Aboveground woody biomass (Mg oven-dry weight ha^{-1}) for each plot was calculated using region- and species-

specific DBH and GLD based allometric equations (Bickelhaupt et al. 1973; Day and Monk 1974; MacLean and Wein 1976; Brenneman et al. 1978; Ker 1984; Williams and McClenahen 1984; Clark et al. 1986; Clark et al. 1990; Elliot and Clinton 1993; Ter-Mikaelian and Korzukhin 1997; Jenkins et al. 2004). Aboveground woody biomass was then converted to aboveground biomass C (tree C) content by multiplying biomass by a factor of 0.5.

Soil dynamics

Measurements of total and active microbial biomass, inorganic N availability, and gas fluxes of [carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O)] were taken monthly from spring (April/May) to fall (October/November) 2013. All monthly samples were duplicated within each plot and co-located with gas flux measurement locations. Mineral soil samples (0-10 cm) were collected for total and active microbial biomass C and each sample was a composite of three locations adjacent to the corresponding gas flux measurement location. Samples were transported on ice and stored at 4°C prior to analysis. Each sample was homogenized and the rocks and roots were manually removed and discarded. Microbial biomass assays were completed within one week using the soils at field moist capacity.

Chloroform fumigation extraction was used to quantify the total microbial biomass C (i.e., chloroform-sensitive fraction; Wardle and Ghani 1995), following the method of Beck et al. (1997). Two 25 g replicates of field moist soil were weighed out from each 10 cm mineral soil sample. One replicate of each soil sample was fumigated with chloroform ($CHCl_3$) in a vacuum desiccator for 24 hours. The second replicate was not fumigated. Post fumigation, both replicates were shaken on the reciprocal shaker with 100 mL 0.5 M K_2SO_4 for 1 hour at 200 rev m^{-1}. After settling, each K_2SO_4-extract was then filtered through Whatman #2 filter paper and the filtrate was collected in scintillation vials. Filtrates were sent to the NC State Environmental and

Agricultural Testing Service and total organic C (TOC) was measured using a Shimadzu TOC/TON Analyzer (Shimadzu Scientific Instruments, Inc., Columbia, Maryland). To calculate total microbial biomass C the following equation was used (Parkinson and Paul 1982; Beck et al. 1997):

$$Microbial\ C\ (mg\ C \cdot g^{-1} dry\ soil) = \frac{C_{fum} - C_{non-fum}}{0.45}$$

Substrate-induced respiration (SIR) was used to quantify the metabolically active (i.e., glucose-responsive) microbial biomass C in each plot (Wardle and Ghani 1995). Ten g of field moist soil was measured into glass vials. D-Glucose (Dextrose anhydrous, F.W. 180.16) solution of concentration 1 g glucose g^{-1} soil was dissolved in deionized water (DI H_2O) and 20 mL glucose solution was added to each sample, vials were then sealed with rubber septa (West and Sparling 1986; Fierer et al. 2003). Samples were incubated at room temperature (~20-25°C) on the reciprocal shaker table on low. Headspace CO_2 concentrations were determined immediately after all vials were capped and in 1 h intervals for the duration of the 4 h incubation using a Li-Cor LI-6250 CO_2 Analyzer (LI-COR Biosciences Inc., Lincoln, Nebraska). The CO_2-C flux rate was then calculated using the slope of CO_2-C concentrations evolved over the 4 h incubation period. To convert to final units of μg CO_2-C g^{-1} soil × h^{-1} soil, field moist mass was converted to dry mass equivalent using measured gravimetric water content (Parkinson and Paul 1982; Bailey et al. 2002).

Two ion exchange membranes (IEMs) (GE Osmonics, Inc., Trevose, PA), an anion and cation, were used *in situ* to quantify nitrate (NO_3^-) and ammonium (NH_4^+) (Subler et al. 1995; Bowatte et al. 2008; Duran et al. 2013). IEMs were cut to a size of 50 cm^2 (5 × 10 cm) and a 7 mm diameter hole was punched at the top. IEMs were submerged in a 1 M solution of sodium

chloride (NaCl) to fill all exchange sites with readily exchangeable sodium (Na^+) or Cl^- ions. Anion and cation membranes were stored in separate containers, each in 1 M NaCl solution at 4°C prior to field deployment. Immediately prior to field deployment, IEMs were thoroughly rinsed with DI H_2O. The first sets of IEMs were installed in April and IEMs were replaced approximately every four weeks, with the exact duration of field deployment recorded. To install in the field a narrow slit was cut into the soil at a 45° angle using a soil knife. Each IEM pair (anion and cation) were gently placed in the slit with no wrinkles or overlaps between them. Nylon string was tied to the hole punched in each IEM and to a pin flag to identify the location of the membranes. Upon removal from the field each IEM pair was stored in its own small plastic bag and transported on ice back to Virginia Tech. IEMs were stored at 4°C for less than 7 days until inorganic-N was extracted. To extract inorganic-N, IEMs were gently rinsed with DI H_2O to remove soil particles from the surface. Each IEM was individually submerged in 50 mL of 1 M potassium chloride (KCl) and placed on a reciprocal shaker table for 1 hour 200 rev m^{-1} (Subler et al. 1995; Hangs et al. 2003). Extracts were analyzed for NO_3^- and NH_4^+ concentration on a TrAAcs 2000 Analytical Console (Bran+Luebbe, Analyser Division, Norderstedt, Germany) connected to an XY2 Auto sampler (SEAL Analytical, Mequan, Wisconsin). Post-extraction, IEMs were placed on low on the reciprocal shaker for 10 minutes with 5% HCl (vol/vol) then rinsed thoroughly twice with DI H_2O and then stored in 1 M NaCl at 4°C.

Gas fluxes were measured using vented, non-steady state static chambers (Holland et al. 1999) installed in two random locations within each plot. The collars were installed at least one month prior to sampling in order to diminish impacts of soil disturbance associated with installation on the measured gas fluxes. Polyvinyl chloride (PVC) collars with a 23.5 cm diameter were situated approximately 5 cm into the ground, with ~12 cm of height aboveground

forming the chamber. Volume calculations were made very specific to each chamber and exact height was measured at 4 points on the inside of each collar in the field. At the time of sampling, the collars were capped to allow gas accumulation (Holland et al. 1999). Four 7 mL samples were taken from each chamber. Each sample was taken using a 30 mL plastic syringe fitted with a stopcock and 21 gauge needle, then ejected into a glass vial that had been sealed with a rubber septa, purged with dinitrogen gas and evacuated. The first sample was taken immediately after capping to establish the gas concentration prior to accumulation. Subsequent samples were taken approximately 20, 40 and 60 min, with exact times were recorded.

Concentrations of CO_2, CH_4 and N_2O in each of the soil gas flux samples were analyzed simultaneously using a GC-2010 Gas Chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, Maryland) with an AOC-5000plus Autosampler (Shimadzu Scientific Instruments, Inc., Columbia, Maryland). Following the method of Holland et al. (1999), gas concentrations were converted to mass and corrected for field conditions, using equation 5:

(5) $C_m = (C_v \times M \times P) \div (R \times T)$;

C_m = the mass to volume concentration
C_v = the volume to volume concentration, e.g. ppm_v CO_2
M= the molecular weight of the trace species, e.g., 12 g CO_2-C per mol of CO_2
P = barometric pressure expressed as atmospheres, made the assumption P=1 atm
T = air temperature within the enclosure at the time of sampling, expressed as °K, we made the assumption 293.15°K
R = the universal gas constant, i.e., 0.0820575 L atm • °K • mole

A flux rate of each individual gas was calculated based upon the change in mass over time, relative to the chamber volumes and surface area of the collar. Sample times for each of the four measurements per collar and chamber volumes were used to fit a curve to the data to determine the flux rate of each individual gas at each cohort (equation 6):

(6) $f = \frac{V \times C_{rate}}{A}$

f = gas flux as mass m^{-2} h^{-1}
V = the internal volume of the enclosure (chamber with lid)
A = the area of soil the enclosure covers
C_{rate} = change in concentration of gas (C_m) over the enclosure period

Statistical Analyses

For all statistical analysis, data was first averaged at the plot level (n=3) at all age cohorts. Differences in volumetric density, C and N pool sizes, and C sequestration rates between the age cohorts of the quantitative soil pits were detected by using a one-way analysis of variances (ANOVAs) at each depth, using the statistical computing software R (R Team 2013). For the parameters found to have significant differences ($p<0.1$), multiple comparisons were made using Tukey's HSD post-hoc test ($\alpha=0.1$) to identify which age cohorts were significantly different from each other, calculated with Agricolae R package (R Team 2013; de Mendiburu 2014). Non-normally distributed data was transformed when appropriate prior to statistical analysis. Differences between the age cohorts of the monthly measurements were analyzed through ANOVA with repeated measures, paired with Tukey's HSD post-hoc analysis sliced by time to detect differences between the age cohorts within each month using SAS 9.3 software (SAS Institute 2011). For repeated measures ANOVA, all non-normal monthly data was transformed using log base 10 transformations. Correlation coefficients for all parameters using Spearman's nonparametric rank correlation method with untransformed data were calculated in SAS 9.2 software (SAS Institute 2009).

B. Quantitative soil pit characteristics

Table B.1

Mean and standard error (n=3) of soil pH, volumetric density (g cm-3), and C:N ratio, Ca (ppm), K (ppm), P (ppm), and cation exchange capacity (CEC) (meq 100 g^{-1} soil) by age and depth.

Age	5	11	21	30	Unmined
Depth			pH		
0-5 cm	5.78 ± 0.199	6.08 ± 0.198	6.12 ± 0.413	5.44 ± 0.226	5.26 ± 0.500
5-10 cm	5.68 ± 0.280	5.92 ± 0.287	5.71 ± 0.548	5.21 ± 0.285	4.85 ± 0.323
10-25 cm	5.84 ± 0.245	6.36 ± 0.339	5.73 ± 0.332	4.89 ± 0.152	4.60 ± 0.062
			Volumetric Density (g cm^{-3})		
0-5 cm	0.317 ± 0.025	0.267 ± 0.010	0.289 ± 0.022	0.206 ± 0.007	0.346 ± 0.066
5-10 cm	0.369 ± 0.050	0.400 ± 0.039	0.526 ± 0.0002	0.381 ± 0.077	0.467 ± 0.057
10-25 cm	0.292 ± 0.029	0.414 ± 0.025	0.364 ± 0.056	0.421 ± 0.021	0.478 ± 0.067
			C:N		
0-5 cm	6.05 ± 2.78	13.97 ± 1.83	14.62 ± 1.03	10.26 ± 1.69	14.47 ± 0.51
5-10 cm	5.46 ± 1.25	4.62 ± 0.65	7.81 ± 1.07	5.80 ± 0.67	14.16 ± 0.67
10-25 cm	4.54 ± 1.28	5.65 ± 2.80	4.72 ± 1.00	4.90 ± 2.16	15.02 ± 0.63
			Ca (ppm)		
0-5 cm	842.89 ± 63.95	1216.22 ± 86.05	1805.22 ± 283.49	1514 ± 138.73	842.89 ± 63.95
5-10 cm	670.33 ± 46.15	879.33 ± 61.78	882.78 ± 127.37	1031.78 ± 96.82	670.33 ± 46.15
10-25 cm	676 ± 31.04	771.94 ± 60.71	632.56 ± 108.05	611.89 ± 35.65	676 ± 31.04
			K (ppm)		
0-5 cm	101.56 ± 8.03	126.78 ± 18.77	165.44 ± 24.16	119.56 ± 4.33	102.33 ± 16.44
5-10 cm	70.78 ± 3.72	89.67 ± 8.39	123.78 ± 13.77	109.11 ± 4.89	59.89 ± 7.84
10-25 cm	55.33 ± 2.87	63.56 ± 7.53	109.56 ± 5.02	103.22 ± 6.81	39.22 ± 5.47
			P (ppm)		
0-5 cm	40.44 ± 1.25	29.89 ± 5.30	21.1 ± 0.62	26.44 ± 2.22	11.44 ± 1.79
5-10 cm	30.89 ± 1.18	33.44 ± 5.50	20.89 ± 6.04	20.44 ± 1.42	9.89 ± 0.99
10-25 cm	26.22 ± 1.24	33.39 ± 7.46	17.44 ± 04.61	14.56 ± 1.44	6.89 ± 0.59
			CEC (meq 100 g^{-1} soil)		
0-5 cm	7.77 ± 0.33	10.01 ± 0.54	13.68 ± 1.16	15.01 ± 0.61	14.47 ± 0.51
5-10 cm	6.28 ± 0.04	7.7 ± 0.09	8.63 ± 0.13	11.81 ± 0.52	14.16 ± 0.67
10-25 cm	6.24 ± 0.23	6.41 ± 0.13	7 ± 0.38	9.3 ± 0.21	6.38 ± 0.48

C. Allometric equations used for each tree species

Table C.1 Diameter-at-breast-height (DBH) based allometric equations and reference source used to calculate aboveground woody biomass

Species1	Equation	Source
	DBH >2.54 cm	
Acer rubrum (red maple)	$M=aD^b$; a=0.1970, b=2.1933	(Ter-Mikaelian and Korzukhin 1997; Ker 1984)
Acer saccharum (sugar maple)	$M=aD^b$; a=0.2064, b=2.3300	(Ter-Mikaelian and Korzukhin 1997; Bickelhaupt et al. 1973)
Alnus glutinosa (European alder)	$M=a(D^2)^b$; a=1.56282, b=1.28807	(Clark et al. 1986)
Fraxinus americana (white ash)	$M=a(D^2)^b$; a=2.71163, b=1.2299	(Clark et al. 1986)
Liriodendron tulipifera (yellow poplar)	$M=a(D^2)^b$; a=0.87948, b=1.40401	(Clark et al. 1986)
Pinus strobus (white pine)	$M=aD^b$; a=0.6298, b=1.3475	(Ter-Mikaelian and Korzukhin 1997; MacLean and Wein 1976)
Platanus occidentalis (American sycamore)	$M=a(D^2)^b$; a=1.99067, b=1.30291	(Clark et al. 1986)
Populus deltoides (eastern cottonwood)	$M=a(D^2)^b$; a=1.56282, b=1.28807	(Clark et al. 1986)
Prunus serotina (black cherry)	$y=a(D^2)^b$; DBH <11 in a=1.51263, b=1.32307; >11 in a=2.3475, b=1.23142	(Clark et al. 1986)
Quercus alba (white oak)	$M=aD^b$; a=1.73738, b=1.33404	(Clark et al. 1986)
Quercus prinus (chestnut oak)	$M=aD^b$; a=0.0554, b=2.7276	(Ter-Mikaelian and Korzukhin 1997; Brenneman et al. 1978)
Quercus rubra (northern red oak)	$M=aD^b$; a=0.1130, b=2.4572	(Ter-Mikaelian and Korzukhin 1997; Brenneman et al. 1978)
Rhododendron maximum	$y=a(D^2)^b$; DBH <11 in a=1.51263 and b=1.32307; >11 in a=2.3475 and b=1.23142	(Clark et al. 1986)
Robinia pseudoacacia (black locust)	$M=a(D^2)^b$; a=1.04649, b=1.37539	(Clark et al. 1986)
General Pine/Softwood	$bm=EXP(\beta_0-\beta_1 \ln D)$; β_0=-2.5356, β_1=2.4349	(Jenkins et al. 2004)

Table C.2 Ground-line diameter (GLD) based equations used with trees of DBH <2.54 cm to calculate aboveground woody biomass

DBH <2.54 cm		
Acer rubrum (red maple)	$Log_{10}Y=1.1891+1.4190(Log_{10}D^2)$	(Williams and McClenahen 1984)
Acer saccharum (sugar maple)	$Log_{10}Y=1.2315+1.6376(Log_{10}D^2)$	(Williams and McClenahen 1984)
Aesculus hippocastanum (horse chestnut)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Alnus glutinosa (European alder)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Amelanchier arborea (downy serviceberry)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Cercis canadensis (eastern redbud)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Cornus florida (flowering dogwood)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Elaeagnus angustifolia (Russian olive)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Fraxinus americana (white ash)	$Log_{10}Y=1.2784+1.4248(Log_{10}D^2)$	(Williams and McClenahen 1984)
Juglans nigra (black walnut)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Liriodendron tulipifera (yellow poplar)	$Log_{10}Y=0.8306+1.5270(Log_{10}D^2)$	(Williams and McClenahen 1984)
Malus coronaria (crabapple)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Populus deltoides (eastern cottonwood)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Prunus serotina (black cherry)	$Log_{10}Y=1.981+1.5876(Log_{10}D^2)$	(Williams and McClenahen 1984)
Quercus alba (white oak)	$Log_{10}Y=1.2840+1.3262(Log_{10}D^2)$	(Williams and McClenahen 1984)
Quercus prinus (chestunut oak)	$\ln(M)=3.92693+2.97911\ln D$	(Elliot and Clinton 1993)
Quercus rubra (northern red oak)	$Log_{10}Y=(-1.8789+2.1716Log_{10}D)+(-1.3620+2.7172Log_{10}D)$	(Day and Monk 1974)
Robinia pseudoacacia (black locust)	$\ln(M)=3.50859+2.81508\ln D$	(Elliot and Clinton 1993)

D. Pearson's correlation coefficient table for all metrics

Table D.1 Pearson's correlation coefficients of all measured variables for all age cohorts

	Mineral SOC	Soil N	Litter C	Litter N	Mineral + Litter SOC	Mineral + Litter N	Root C	Tree C
Mineral SOC		0.932 p<.0001	0.554 p=0.0323	0.461 p=0.0839	0.993 p<.0001	0.929 p<.0001	0.750 p=0.0013	0.718 p=0.0026
Soil N			0.550 p=0.0337	0.464 p=0.0813	0.925 p<.0001	0.993 p<.0001	0.671 p=0.0061	0.661 p=0.0073
Litter C				0.850 p<.0001	0.614 p=0.0148	0.561 p=0.0297	0.218 p=0.4354	0.336 p=0.2212
Litter N					0.511 p=0.0517	0.493 p=0.0620	0.321 p=0.2427	0.214 p=0.4431
Mineral and Litter SOC						0.918 p<.0001	0.721 p=0.0024	0.740 p=0.0016
Mineral and Litter N							0.668 p=0.0065	0.625 p=0.0127
Root C								0.382 p=0.1598

	Total Below-ground C	Total Plant C	Total Eco-system C	Basal Area	Stem Density	Species Richness	pH	Total Microbial Biomass C
Mineral SOC	0.993 p<0.0001	0.746 p=0.0014	0.896 p<.0001	0.804 p=0.0003	-0.312 p=0.2583	-0.809 p=0.0003	-0.461 p=0.0839	0.811 p=0.0002
Soil N	0.904 p<.0001	0.668 p=0.0065	0.804 p=0.0003	0.743 p=0.0015	-0.274 p=0.3231	-0.878 p<.0001	-0.500 p=0.0577	0.793 p=0.0004
Litter C	0.571 p=0.0261	0.400 p0.1396	0.514 p=0.0498	0.493 p=0.0620	-0.129 p=0.6470	-0.533 p=0.0407	-0.004 p=0.9899	0.282 p=0.3083
Litter N	0.489 p=0.0642	0.264 p=0.3412	0.411 p=0.1283	0.411 p=0.1283	0.063 p=0.8244	-0.341 p=0.2137	0.004 p=0.9899	0.139 p=0.6205
Mineral and Litter SOC	0.993 p<.0001	0.768 p=0.0008	0.907 p<.0001	0.814 p=0.0002	-0.367 p=0.1784	-0.832 p=0.0001	-0.443 p=0.0983	0.793 p=0.0004
Mineral and Litter SOC	0.900 p<.0001	0.636 p=0.0109	0.775 p=0.0007	0.718 p=0.0026	-0.224 p=0.4226	-0.872 p<.0001	-0.446 p=0.0953	0.757 p=0.0011
Root C	0.757 p=0.0011	0.450 p=0.0924	0.611 p=0.0156	0.432 p=0.1077	0.016 p=0.9545	-0.439 p=0.1017	-0.532 p=0.0412	0.532 p=0.0412

	Total Below-ground C	Total Plant C	Total Ecosystem C	Basal Area	Stem Density	Species Richness	pH	Total Microbial Biomass C
Tree C	0.732 p=0.0019	0.982 p<.0001	0.907 p<.0001	0.932 p<.0001	-0.467 p=0.0790	-0.651 p=0.0086	-0.539 p=0.0380	0.889 p<.0001
Total Below-ground C		0.768 p=0.0008	0.900 p<.0001	0.804 p=0.0003	-0.329 p=0.2305	-0.780 p=0.0003	-0.418 p=0.1212	0.782 p=0.0006
Total Plant C			0.921 p<.0001	0.932 p<.0001	-0.440 p=0.1003	-0.644 p=0.0096	-0.532 p=0.0412	0.889 p<.0001
Total Eco-system C				0.932 p<.0001	-0.376 p=0.1672	-0.684 p=0.0049	-0.625 p=0.0127	0.918 p<.0001
Basal Area					-0.355 p=0.1948	-0.637 p=0.0107	-0.486 p=0.0664	0.896 p<.0001
Stem Density						0.546 p=0.0351	0.090 p=0.751	-0.340 p=0.2147
Species Richness							0.281 p=0.3102	-0.655 p=0.0081
pH								-0.725 p=0.0022
Total Microbial Biomass C								

	Active Microbial Biomass C	CO2	CH4	N2O	NH4	NO3	Total Inorganic N
Mineral SOC	0.811 p=0.0002	-0.143 p=0.6115	-0.268 p=0.3344	-0.236 p=0.3977	-0.657 p=0.0078	-0.143 p=0.6115	-0.286 p=0.3019
Soil N	0.718 p=0.0026	-0.186 p=0.5075	-0.214 p=0.4431	-0.182 p=0.5159	-0.768 p=0.0008	-0.032 p=0.9095	-0.250 p=0.3688
Litter C	0.318 p=0.2483	-0.743 p=0.0015	-0.261 p=0.3480	0.075 p=0.7905	-0.554 p=0.0323	-0.157 p=0.5760	-0.257 p=0.355
Litter N	0.064 p=0.8199	-0.586 p=0.0218	-0.314 p=0.2539	0.439 p=0.1014	-0.375 p=0.1684	0.146 p=0.6025	0.054 p=0.8496
Mineral and Litter SOC	0.811 p=0.0002	-0.207 p=0.4588	-0.332 p=0.2265	-0.221 p=0.4277	-0.646 p=0.0092	-0.129 p=0.6479	-0.264 p=0.3412
Mineral and Litter SOC	0.686 p=0.0048	-0.182 p=0.5159	-0.161 p=0.5672	-0.148 p=0.6115	-0.782 p=0.0006	-0.057 p=0.8397	-0.289 p=0.2957
Root C	0.489 p=0.0642	0.161 p=0.5672	-0.171 p=0.5413	0.036 p=0.8994	-0.389 p=0.1515	0.139 p=0.6205	-0.057 p=0.8397
Tree C	0.804 p=0.0003	0.289 p=0.2957	-0.432 p=0.1077	-0.268 p=0.3344	-0.521 p=0.0462	-0.043 p=0.895	-0.129 p=0.6479
Total Below-ground C	0.786 p=0.0005	-0.175 p=0.5327	-0.300 p=0.2773	-0.211 p=0.4510	-0.629 p=0.0121	-0.143 p=0.6115	-0.286 p=0.3019
Total Plant C	0.782 p=0.0006	-0.321 p=0.2427	-0.375 p=0.1684	-0.218 p=0.4354	-0.557 p=0.0310	-0.043 p=0.8795	-0.157 p=0.5760
Total Eco-system C	0.857 p<.0001	-0.300 p=0.2773	-0.471 p=0.0761	-0.239 p=0.3904	-0.596 p=0.0189	-0.018 p=0.9496	-0.104 p=0.7134
Basal Area	0.782 p=0.0006	-0.318 p=0.2483	-0.339 p=0.2160	-0.168 p=0.5499	-0.586 p=0.0218	-0.082 p=0.7710	-0.150 p=0.5936
Stem Density	-0.410 p=0.1290	0.057 p=0.8393	0.487 p=0.0656	0.406 p=0.1327	0.138 p=0.6241	-0.057 p=0.8393	-0.106 p=0.7079
Species Richness	-0.678 p=0.0054	0.238 p=0.3939	0.285 p=0.3037	0.243 p=0.3828	0.734 p=0.0018	0.073 p=0.7973	0.317 p=0.2491
pH	-0.571 p=0.0261	-0.089 p=0.7517	0.346 p=0.2059	0.039 p=0.8894	0.357 p=0.1913	-0.443 p=0.0983	-0.318 p=0.2483
Total Microbial Biomass C	0.857 p<.0001	-0.146 p=0.6025	-0.282 p=0.3038	-0.325 p=0.2372	-0.639 p=0.0103	0.004 p=0.9899	-0.11071 p=0.6945

	Active Microbial Biomass C	CO2	CH4	N2O	NH4	NO3	Total Inorganic N
Active Microbial Biomass C		-0.064 p=0.8199	-0.329 p=0.2318	-0.539 p=0.0380	-0.464 p=0.0813	-0.289 p=0.2957	-0.296 p=0.2834
CO2			0.382 p=0.1598	0.029 p=0.9195	0.404 p=0.1358	0.054 p=0.8496	0.096 p=0.7325
CH4				0.093 p=0.7420	-0.004 p=0.9899	-0.429 p=0.1110	-0.529 p=0.0428
N2O					0.068 p=0.8101	0.614 p=0.0148	0.421 p=0.1177
NH4						0.029 p=0.9195	0.421 p=0.1177
NO3							0.857 p<.0001
Total Inorganic N							

E. Measured tree species at each stand

Table E.1

Total list of tree species measured within each stand.

Stand Age	Measured Tree Species
5	Yellow poplar (*Liriodendron tulipifera*), northern red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), chestnut oak (*Quercus prinus*), white pine (*Pinus strobus*), black cherry (*Prunus serotina*), dogwood (*Cornus florida*), white ash (*Fraxiunus americana*), white oak (Quercus alba), European alder (*Alnus glutinosa*), walnut (*Juglans nigra*), Russian olive (*Elaeagnus angustifolia*)
11	Chestnut oak, northern red oak, white ash, sugar maple, white oak, black locust (*Robinia pseudoacacia*), white pine, yellow poplar
21	Northern red oak, black cherry, black locust, red maple (*Acer rubrum*), eastern cottonwood (*Populus deltoides*), sugar maple, American sycamore (*Platanus occidentalis*), crabapple (*Malus coronaria*), downy serviceberry (*Amelanchier arborea*)
30	Red maple, American sycamore, redbud (*Cercis canadensis*), European alder, chestnut oak, yellow poplar, sugar maple, chestnut oak, black locust
Unmined	Yellow poplar, sugar maple, rhododendron (*Rhododendron maximum*), chestnut oak, horse-chestnut (*Aesculus hippocastanum*)