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Stable aggregate dynamics and carbon storage in acidified forest soils: Influence of atmospheric deposition and conifer conversion at the Fernow Experimental Forest

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ABSTRACT

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J. E. Kemner

Understanding anthropogenic changes to forested ecosystems is crucial to appropriately manage forests for overall ecosystem health, soil quality, water quality, and carbon (C) sequestration potential. This research explored the effects of ecosystem change caused by increased atmospheric nitrogen (N) deposition and stand conversion from mixed-species hardwood to monoculture Norway spruce. Research activities for this thesis occurred in three experimental watersheds at the USDA Forest Service Fernow Experimental Forest (FEF), West Virginia, USA. I measured soil aggregation and organic matter (OM) content in a paired watershed study to investigate the influence of forest fertilization in Watershed 3 at FEF, a native hardwood forest that was clear-cut concluding in 1969 and has received 35 kg nitrogen (N) as $(NH_4)_2SO_4$ fertilizer per year since 1989. Results were compared to those from Watershed 7 at FEF, a natural hardwood regrowth following clear cut harvest concluding in 1969, which is considered an appropriate reference for this study. Furthermore, I compared the results from Watershed 7 to nearly adjacent Watershed 6, which was converted to a monoculture of Norway spruce (*Picea abies*) following clearcut harvest, and planting in 1973. Both watersheds were maintained barren via herbicide from 1967 to 1969. In Watersheds 6 and 7, I also quantified select ecosystem pools of C and N, including vegetation biomass, forest floor, and mineral soil to create an ecosystem budget for C and N. Long-term atmospheric deposition and stream export of nitrate (NO_3-N) were also quantified. Soil from both the fertilized watershed and the spruce watershed demonstrated dispersion of macro-aggregates and greater weight in the <53 μm soil aggregate size class – unassociated clay particles or free microaggregates relative to the reference watershed, which was associated with lower soil pH. Soil from the reference watershed has greater aggregate weight in the macro-aggregate size classes (between 250 - 2000 μm), and in the B-horizon, greater organic matter (OM) content in macro-aggregates. Soil from the fertilized watershed exhibits greater intra-aggregate OM beneath arbuscular mycorrhizal-associated (AM) tree species with labile litter, while soil in the reference watershed contains more intra-aggregate OM beneath ectomycorrhizal-associated (ECM) tree species. The spruce watershed conversion has significantly impacted N cycling, as system atmospheric inputs of NO_3-N are equal, yet streamflow exports from Watershed 6 are negligible, and spruce soil inorganic N content is more than 5 times lower relative to that of the reference watershed. In the A-horizon soil in the spruce watershed, microbially-active C is significantly lower, measured as permanganate oxidizable carbon (POXC). As many parts of the world have been exposed to long-term atmospheric deposition of N and sulfur (S)-based compounds and been converted to coniferous planted forests, further study to determine how these systems will respond to our changing global climate is necessary.

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Stable aggregate dynamics and carbon storage in acidified forest soils: Influence of atmospheric deposition and conifer conversion at the Fernow Experimental Forest

Chapter 1. Introduction

Significant areas of forest soils acidify as a result of natural and anthropogenic influences. Natural sources of soil acidification include acidic parent material, abundant rainfall, and vegetation such as conifers. Some of the more profound mechanisms of anthropogenic acidification are via addition of outside material and induced species change. Both of these are notable impacts to the Appalachian forests of the eastern United States, which has experienced extensive atmospheric acidic deposition of nitrogen (N) and sulfur (S) from air pollution, and conversion of native hardwood forests to planted conifer forests. These large-scale landscape influences have various impacts on ecosystem dynamics, including changes to carbon dynamics, nutrient cycling, and physical structure of the underlying soils.

Due to prevailing wind patterns, pollution from more western states such as Ohio, Indiana, and Illinois is carried eastward towards the Appalachian Mountains, where much of it then is captured in rainfall and deposited on the western side of the mountains due to the orographic effect (Smith 1979). Before the Clean Air Act of 1970, and its subsequent revisions in 1977 and 1990, significant amounts of sulfate $(SO_4)^{2-}$ – formed by the oxidation of sulfur dioxide (SO_2) – and nitrate $(NO_3)^-$ – formed by oxidation of nitrogen dioxide (NO_2), which itself is formed by nitric oxide (NO) released by high temperature combustion of fossil fuels (Kinugawa et al. 2011) – fell on the forests of the Appalachian Mountains via dry deposition and precipitation. These depositions not only influence N and S dynamics in the ecosystems they encounter, but the rain carrying them is also reduced in pH, to the point that the most severe acid rain recorded in the United States was in Wheeling, WV with a pH of 1.5 (Chrzan et al. 1989; Tietenberg 1989). Ecosystems have been exposed to both elevated nutrient deposition and reduced pH, resulting in increased growth (Thomas et al. 2010) and soil acidification (Grennfelt and Hultberg 1986); and in some cases, enhanced storage of below-ground C (Frey et al. 2014; Zak et al. 2019).

Another common anthropogenic impact is species conversion, particularly conversion of native hardwood to coniferous stands, which can also alter forest and soil biogeochemistry, including acidification of soil and water draining the system and altered nutrient availability. Planted forests make up 11% (22 million hectares) of total US forest timberland (Stanturf and Zhang 2003) and in the South of the United States, the 14 million hectares of loblolly pine (*Pinus taeda*) makes up ~50% of the world's industrial planted forest. Coniferous trees generally have more recalcitrant litter (e.g. high carbon:nitrogen (C:N) ratio) and more organic acid exudation from roots and associated fungi than deciduous hardwood trees (Reich et al. 2005; Yeung et al. 2019) which can slow nitrification and decomposition rates of the litter layer and underlying humus material. This acidification and altered nutrient cycling influences ecosystem C dynamics, soil organic matter (SOM) turnover, and storage.

Soil plays an important role in global C management, containing approximately 1500 Pg of organic C, twice the amount of C in the atmosphere (Smith 2004), therefore, a more complete understanding of soil processes is paramount to managing our global C budget. As soils continue to be influenced by anthropogenic changes to ecosystems, if we are to effectively predict

ecosystem C dynamics, a more complete mechanistic understanding of C storage and flux is needed.

Soil aggregation is an important indicator of soil health and is integral to physical protection of soil C. Soil aggregates give soils stability, maintain healthy pore volume and size variability – which then maintain air and water exchange, and ensures that the soil is a sufficient substrate for plant root development, water infiltration and storage, and microbial habitat. There are three main processes that form soil aggregates: bacteria and fungi binding soil particles; gelatinous organic materials holding particles together, and clay particle adherence and binding resulting in larger particle entrapment. This clay particle adherence can be caused by the formation of organo-mineral composites or the differing isoelectric points of iron oxides and clay minerals, resulting in positive and negative charges (respectively) and adherence (Martin et al. 1995; Pochet et al. 2007). Within larger soil aggregates, soil C is protected physically from bacterial degradation (Elliott 1986; Plante and McGill 2002; Mikha and Rice 2004). This protected C is especially important for soil C sequestration potential, and the strength of the aggregate against dispersion forces determine a soil's efficacy as a C sink.

Acidification of soil systems may alter aggregation forces in multiple ways, including changes to microbial activity and the amount and chemistry of organic byproducts (Alexander 1980; Power et al. 2000), changes in pH-dependent charges on mineral surfaces, or through dispersion processes associated with particular cations. For example, with accumulation of NH_4^+ and H^+, soil aggregation may be reduced due to dispersion of soil colloids (Haynes and Naidu 1998; Boix-Fayos et al. 2001; Bronick and Lal 2005) due to displacement of Ca^{2+} from clay particles and reduction of microorganism activity. A loss of aggregation capacity is then related to a decline in soil C storage potential as C can no longer be physically protected within large macro-aggregates. Additionally, dominant tree species may further alter forest soil aggregation processes, with a potential influence on aggregation dynamics derived from differences in lability and decomposition of plant litter (Chan et al. 2001), which has been linked to tree mycorrhizal fungal association (arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungi) (Chapman et al. 2006; Cotrufo et al. 2013; Midgley et al. 2015; Craig et al. 2018). ECM-associated species are associated with more recalcitrant leaf litter and slower decomposition rates relative to AM-associated tree species (Chapman et al. 2006; Pritsch and Garbay 2011), resulting in lower production of microbial byproducts for organo-mineral binding processes to occur.

The research activities described in this thesis occurred within three watersheds at the Fernow Experimental Forest (FEF), managed by the USDA Forest Service, in Parsons, WV, USA. Watershed 3 (WS3) is a nearly 50-year-old forest that has received long-term fertilization of 35 kg N ha^{-1} yr^{-1} in the form of $(NH_4)_2SO_4$ as aerial deposition three times a year since 1989 after undergoing clearcut harvests in sections, the last being in 1972. Vegetation in Watershed 6 (WS6) was converted to a monoculture of Norway spruce in 1973 following forest harvest concluded in 1967 and maintained with herbicide treatment in 1977 and 1980. Watershed 7 (WS7) is considered the reference watershed for both studies presented in this thesis, allowed to regrow to a native mixed hardwood stand after being clearcut in sections from 1963-1967 and maintained barren with herbicide from 1967 to 1969. Forest vegetation in WS7 was allowed to naturally regenerate to native hardwood species following forest harvest concluding in 1967 and herbicide treatment concluding in 1969.

In Chapter two of this thesis, I explored how atmospheric deposition of N and S has altered soil aggregate weight distribution, aggregate-associated OM, and microbially-active C in soil from WS3 and WS7 at FEF. Additionally, the influence of four dominant tree species on aggregation dynamics was investigated and compared between watersheds. Soil aggregation was measured from A- and B-horizons in soil across watersheds tree species. Results were then linked to mycorrhizal fungal association, measures of microbially-active C, and soil pH.

In particular, I addressed the following questions:

- How does long-term fertilization with ammonium sulfate affect soil aggregate weight and size class distribution, and the distribution of intra-aggregate OM storage within macro- and micro-aggregates?
- How do tree species and fungal associations influence aggregation capacity and the distribution of intra-aggregate OM?
- How will long-term fertilization with ammonium sulfate alter the aggregation dynamics influenced by differing tree species and fungal associations?

I hypothesized that 1) fertilization negatively affects soil aggregation capacity and a greater proportion of soil C will remain un-associated with mineral soil (POM); 2) AM-associated tree species promote greater aggregation and greater amounts of C in the micro-aggregate size classes than ECM-associated species; and 3) aggregates in soil influenced by AM tree species will be more sensitive to acidification than those influenced by ECM tree species because aggregates are formed by microbial by-products of decomposition.

Results of this study indicated that soil from the fertilized WS3 experienced dispersion of macro-aggregates and dominance in the micro-aggregate size fraction (53-250 μm) and soil particles <53 μm. Additionally, OM content in the macro-aggregate size class was depleted in soil in WS3, especially in sub-surface soil. These effects were related to both lower soil pH and greater fraction of microbially-active C. AM-associated species (specifically black cherry with more easily decomposable litter) produced greater soil aggregation and aggregate-associated OM in WS3, while ECM-associated species produced greater aggregation and aggregate-associated OM in WS7. We hypothesize these soil aggregate dynamics may be due to increased mycorrhizal activity in WS3, due to influence on the plant-fungi relationship induced by the increased N availability.

In chapter three of this thesis, I explored the influence of a nearly 50-year-old stand conversion of WS6 from native Appalachian hardwoods to a monoculture of Norway spruce on select pools of ecosystem C and N. I measured and compared the C and N content in soil, forest floor, and tree biomass; long-term atmospheric deposition and stream NO_3-N export; the distribution of aggregates and aggregate-associated organic matter; and inorganic N and oxidizable C to values from the reference WS7. Values quantified here are compared to values measured twelve years prior and reported in Kelly (2010).

In particular, I addressed the following questions:

- After 50 years of contrasting vegetative influence, how do C and N pools in vegetation biomass, forest floor, and mineral soils compare between WS6 and WS7?
- How is N mineralization in soil related to long-term divergent values of stream NO_3-N export between the two watersheds?
- How do C and N pool sizes compare to previous measurements 12 years ago?

I hypothesized that 1) relative to WS7, WS6 would still exhibit lower amounts of C and N in below-ground biomass and in mineral soil after 50 years of contrasting vegetative influence; and 2) relative to WS7, there would be a lower inorganic N flux in spruce-influenced soils, leading to accumulating C and N pool sizes in the forest floor and lower pool sizes in the mineral soil, ultimately resulting in low NO_3–N export to the stream.

Results of this study indicate that, compared to the previous assessment of C and N dynamics in these two watersheds, WS6 is accruing C at an accelerated rate relative to WS7 and ecosystem C pools are now approximately equal between watersheds. This implies that in the early stages of forest growth, a hardwood forest may store more C than a conifer forest, but as the forests age, the rate of C storage of the conifer increases more rapidly than the hardwood forest. However, ecosystem N is still somewhat lower in WS6. With calculation of the atmospheric inputs and stream export of NO_3-N, we are still unable to account for the large long-term discrepancy of N export from the two watersheds. Spruce vegetation has also altered soil aggregation, where soil in WS6 is dominated by micro-aggregate size classes and intra-aggregate OM is reduced relative to soil from the hardwood watershed, likely due to lower soil pH and the recalcitrant litter derived from spruce.

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Chapter 2. Fertilization and tree species influence on stable aggregate dynamics in forest soil

Abstract

Aggregation and structure play key roles in water-holding capacity and stability of soils and are important for the physical protection and storage of soil carbon (C). Forest soils are an important sink of ecosystem C, though capacity to store C may be disrupted by elevated atmospheric deposition of nitrogen (N) and sulfur (S) compounds by dispersion of soil aggregates via acidification or altered microbial activity. Furthermore, dominant tree species and the lability of litter they produce can influence aggregation processes. We measured water-stable aggregate size distribution and aggregate-associated organic matter (OM) content in soils from two watersheds and beneath four hardwood species at the USDA Forest Service Fernow Experimental Forest in West Virginia, USA where one watershed has received $(NH_4)_2SO_4$ fertilizer since 1989 and one is a reference/control of similar stand age. Bulk soil OM, pH, and permanganate oxidizable carbon (POXC) were also measured. Fertilized soil exhibited decreased macro-aggregate formation and a greater proportion of smaller micro-aggregates or unassociated clay minerals, particularly in mineral soil. This shift in aggregation processes to soil more dominated by the smallest (<53 μm) fraction is associated with both acidification (soil pH) and increased microbially-processed C (POXC) in fertilized soil. Intra-aggregate OM was also depleted in the fertilized soil (52% less OM in 53-2000 μm fraction), most strongly in subsurface B-horizon soil. We also document that tree species can influence soil aggregation, as soil beneath species with more labile litter contained greater OM in the micro-aggregate size class (<250 μm), especially in the fertilized watershed, while species with more recalcitrant litter promoted greater OM in the macro-aggregate size classes (500-2000 μm) in the reference watershed. Long-term fertilization, and likely historic atmospheric deposition, of forest soils has weakened macro-aggregation formation, with implications for soil stability and storage of below-ground C.

Introduction

Soil aggregation is an important indicator of soil health. The process of aggregation results in consolidation of soil particles into peds and increased pore size variability. With a wide variety of pore sizes (micro- and macropores), air and water exchange are more quickly facilitated, and plant roots more easily penetrate through the soil profile. Additionally, pore size variability regulates and restricts soil organism movement, as well as the water needed for biological function (Elliott and Coleman 1988). Martin et al. (1955) describes three aggregate-forming processes: 1) bacteria and fungi binding particles together; 2) gelatinous organic materials hold particles together; 3) clay particles cohere either due to organo-mineral interactions or flocculation around iron-oxides and entrap larger particles. All three of these processes, and their interplay, determine the distribution of macro- vs micro-aggregates in soil. These processes has been well-studied more recently to define macro-aggregates (>250 μm) as being made up of a conglomeration of smaller micro-aggregates (<250 μm), as well as particulate organic matter (OM), fungal hyphae, organic binding agents, and pore space (Tisdall and Oades 1982; Elliott 1986; Jastrow and Miller 1997; Six et al. 2000). The process of aggregation can also result in positive feedback, with mycorrhizal and other fungi able to grow in macroaggregate pore spaces (Miller and Jastrow 2000). In contrast, microaggregates provide habitat space for up to 70% of soil bacterial species (Ranjard et al. 2000; Wilpiszeski et al. 2019). Within aggregates, especially micro-aggregates, soil carbon (C) is also physically protected from bacterial decomposition through strong organo-mineral interactions

with polyvalent cations (Elliott 1986; Plante and McGill 2002; Mihka and Rice 2004), rendering what is referred to as stabilized soil organic C (SOC). This protected C is especially important for C sequestration, as the strength of aggregation and the stability of aggregate-associated SOC determines the efficacy of the soil as a C sink (Lynch and Bragg 1985). Differing factors, including biological influences, soil parent material, degree of weathering and clay minerology, and soil acidity and ionic composition can all affect aggregation of soil particles.

The clay minerology of soil, specifically the proportion of 2:1 clays (such as montmorillonite or vermiculite) relative to proportion of 1:1 clays (kaolinite or serpentine), significantly alters the soil aggregation mechanism and capacity. In systems with limited C inputs, 1:1 clays tend to form more stable macro-aggregates, while in systems with greater C input, 2:1 clays tend to form more stable macro-aggregates (Denef et al. 2002). This is due to the negative charges of organic matter (OM) and clay particles being concurrently bound to charged cations, while 1:1 clays can have both positive and negative charges, and are more readily mineral-bound (Tombácz et al. 2004). 2:1 clays have greater specific surface area and cation exchange capacity (CEC), leading to stronger aggregation forces at mineral surfaces (Hepper et al. 2006), while 1:1 clays have relatively lower CEC values and less aggregation capacity. Finally, 2:1 clays also have a permanent surface charge (usually net negative) that is not influenced by soil pH conditions, while 1:1 clays have a pH-dependent net surface charge (Denef et al. 2002). In acidic soils, soils made up of predominantly 1:1 clays are expected to exhibit less aggregation capacity than would occur in higher pH soil due to changes in surface charge capacity, whereas 2:1 clays likely would not exhibit changes in aggregate formation as a function of acidification (Tombácz et al. 2004).

Tree species influence on aggregation:

Soil aggregation may be altered by biological influences such as soil microbial biomass and activity, as well as characteristics of tree species. Disparities in the mineral association of soil C between arbuscular mycorrhizae (AM-) associated species and ectomycorrhizal mycorrhizae (ECM-) associated species have been demonstrated due to differences in litter composition and lability, as well as differences in the soil microbial community (Chapman et al. 2006; Cotrufo et al. 2013; Midgley et al. 2015; Craig et al. 2018). ECM-associated species tend to have "closed" nutrient cycles, in which leaf litter is made up of relatively recalcitrant and high C:nitrogen (N) ratio materials, resulting in ECM fungi "mining" nutrients (especially N) directly from organic materials via enzyme secretion (Pritsch and Garbay 2011). AM-associated species tend to have "open" nutrient cycles, in which leaf litter is comparatively labile and more available for microbial decomposition, and the AM fungus takes up inorganic N after it has been microbially processed (Chapman et al. 2006).

AM fungi have previously been shown to be positively correlated to soil aggregate development (Rillig et al. 2002; Leifheit et al. 2014) through abundant hyphal biomass and secretion of glomalin-related soil protein (GRSP), though the extent of influence may depend upon both fungal and plant species (Piotrowski et al. 2004). As described by Six et al. (2004), bacterial decomposition products aid mostly in the formation of microaggregates while fungi aid in the formation of macroaggregates due to the "sticky string bag" formed by hyphae (Jastrow and Miller 1997). Following the concept of the Microbial Efficiency Matrix Stabilization (MEMS) framework (Cotrufo et al. 2013), labile plant litter associated with AM tree species promotes more

rapid microbial activity and a greater amount of microbial products, which may create greater organo-mineral interactions and greater aggregate formation (Waksman and Martin 1939; Harris et al. 1966). With both of these concepts, we expect that there is enhanced microbial activity in areas influenced by AM-associated tree species and, as a result, more C protection and storage in the smaller size class of soil aggregates. However, in forested ecosystems that have received decades of elevated atmospheric deposition of nitrogen and sulfur compounds (Zhang et al. 2018), these expected C and aggregate dynamics may be disrupted (Driscoll et al. 2001).

Acidity and ionic composition influence on aggregation:

In ecosystems experiencing elevated N deposition, soil microbial communities and activity are altered (Söderström et al. 1983; Treseder 2008; Carrara et al. 2018; Zak et al. 2019) resulting in decreased enzymatic activity and decomposition rates, and increased soil C (DeForest et al. 2004; Treseder 2008). While more microbially-processed OM can facilitate soil aggregation, acidification processes (e.g. oxidation via nitrification, leaching of base cations such as Ca^{+2}) due to elevated atmospheric deposition of N may result in decreased aggregation (Stătescu and Pavel 2013), either through inhibition of microbial decomposition processes, changes in pH-dependent charges on mineral surfaces, or through dispersion processes associated with particular cations. For example, with accumulation of NH_4^+ and H^+, soil aggregation may be reduced due to dispersion of soil colloids (Haynes and Naidu 1998; Boix-Fayos et al. 2001; Bronick and Lal 2005) as the relative concentrations of these ions increase, whereas presence of abundant Ca^{+2} ions would result in flocculation, or creation of aggregates (Heiland and Sposito 1993; Emerson 1990).

West Virginia and the Central Appalachian region in the eastern US have historically experienced high levels of atmospheric N deposition. The decline in N deposition across the region resulting from policies stemming from the Clean Air Act (Groffman et al. 2018) raises questions relating to ecosystem recovery, particularly the fate of the C that has accumulated in soil as a result of acidification processes (DeForest et al. 2004; Carrara et al. 2018). If the accumulated soil C does not reside in protected aggregates – the result of acidification processes that decrease microbial decomposition – recovery from historic deposition levels may result in rapid decomposition as microbial process rates resume. This poses risks at the ecosystem level (loss of soil C, soil structure, and the resultant trophic effects on the ecosystem as a whole) as well as at the global biome level (C emissions).

Here, we investigate the role of fertilization and tree species (and mycorrhizal fungal associations) in soil aggregation dynamics in two experimental watersheds at the US Forest Service Fernow Experimental Forest (FEF). The main objectives for this study were to compare size class distribution of water-stable soil aggregates and OM distribution throughout aggregate size classes in soils from a watershed receiving long-term elevated deposition of ammonium sulfate $(NH_4)_2SO_4$ and a reference watershed. Specifically, we aimed to 1) determine if experimental fertilization and documented acidification affects soil aggregation capacity and C distribution throughout the aggregate size fractions; 2) determine if select tree species differentially influence soil aggregation capacity and C distribution throughout the aggregate size fractions; and 3) determine if the effect that acidification has on soil aggregation dynamics depends upon the tree species influencing the soil. To address these objectives, we tested the following hypotheses: 1) fertilization negatively affects soil aggregation capacity and a greater proportion of soil C will remain un-associated with

mineral soil (POM); 2) AM-associated tree species promote greater aggregation and greater amounts of C in the micro-aggregate size classes than ECM-associated species; and 3) aggregates in soil influenced by AM tree species will be more sensitive to acidification than those influenced by ECM tree species because aggregates are formed by microbial by-products of decomposition.

Methods

Site description

This study utilized two gaged watersheds within the USDA Forest Service FEF near Parsons, West Virginia, USA. The 1900 ha FEF was established in 1934 within the Monongahela National Forest. Annual precipitation is evenly distributed per annum and averages 145.8 cm (Kochenderfer 2006). Average monthly precipitation peaks in June (144 mm) and typically reaches its lowest value in October (97 mm). Average yearly temperature is 9.2° C, with an average monthly maximum in July (20.6° C) and minimum in January (-18° C) (Kochenderfer 2006).

The hardwood reference watershed (WS7; 24 ha; elevation range 730-860 m) was clearcut logged in sections, beginning in 1964 and continuing to 1967, it was then maintained barren with herbicides until 1969, and since has been allowed to regrow naturally. WS7 has hillslopes with north/south aspects along the stream. Soils in this watershed are dominantly Calvin (Calvin channery silt loam), with small ridgeline areas of Dekalb series (Dekalb channery loam and Dekalb extremely stony loam; Dekalb loamy-skeletal, siliceous, active, mesic typic Dystrudept), derived from acidic sandstone parent material (Soil Survey Staff USDA NRCS web soil survey 2019). Dominant vegetation in WS7 is yellow poplar (*Liriodendron tulipifera*), Northern red oak (*Quercus rubra*), and sugar maple (*Acer saccharum*), with an understory of dogwood (*Cornus florida*), striped maple (*Acer pensylvanicum*), cucumber magnolia (*Magnolia acuminata*), and several species of fern.

The hardwood watershed that receives fertilizer application (WS3; 34.3 ha, elevation range 735-860 m) was clearcut between July 1969 and May 1970, excluding a 2.99 ha buffer strip along the stream channel, where a light selection cut was made. In 1972 the buffer strip was clearcut and all debris in or near the channel was removed. Soils in this watershed are Calvin channery silt loams (loamy-skeletal, mixed, mesic typic Dystrudept, derived from acidic sandstone and Hampshire formation shale parent material (Soil Survey Staff USDA NRCS). The predominant clays of the FEF have been cursorily described as muscovite (2:1) and vermiculite (2:1) (Lusk 1998). The watershed receives 35 kg N ha^{-1} yr^{-1} in the form of $(NH_4)_2SO_4$ as aerial deposition three times a year via helicopter. Fertilizer applications began in 1989, when standing trees were approximately 19 years old. Dominant vegetation in WS3 is black cherry (*Prunus serotina*), red maple (*Acer rubra*), black birch (*Betula lenta*), and American beech (*Fagus grandifolia*) (Adams et al. 2007).

Soil sample collection

To investigate how tree species influences aggregation size distribution and associated OM distribution, four live individuals of each tree species (yellow poplar, Northern red oak, black cherry, and black birch) were selected throughout the watershed, each on a mid-slope section of each watershed, and each having a minimum DBH of 16.25 cm. Using each selected tree as plot-center, we determined surrounding stand basal area by species using a BAF-10 prism. To understand how differing mycorrhizal associations may affect aggregation and carbon storage

processes, two tree species investigated are AM-associated species (black cherry and yellow poplar), and two are ECM-associated species (black birch and Northern red oak).

In May and June 2019, soil samples were collected via auger from below each selected tree. Soil samples were taken at each cardinal direction around each tree within the dripline. A-horizon samples were collected between 0-10 cm depth and B-horizon samples collected between 15-30 cm depth. B-horizon soil is distinct from the A-horizon visually by lighter color, greater silt content content, and subangular blocky structure (NRCS Soil Survey Staff). Samples were bulked at each sampling point to produce one mixed sample per location (n = 2 watersheds; n = 4 species; n = 4 individual trees; n = 2 soil horizons; N = 64 bulk soil samples).

Water-stable aggregate and organic matter analyses

Soil samples were air dried following collection, then sieved to 2 mm. One hundred g of air-dried soil was used for slaking and wet-sieving utilizing an apparatus similar to that by Yoder (1936) and Ekwue et al. (2018) and following the methodology of Mikha and Rice (2004) and Kelly et al. (2014). This procedure utilizes stacked sieves to separate and recover all particle size fractions greater than 53 μm. Sieve mesh sizes were 2000, 1000, 500, 250, and 53 μm. Particles smaller than 53 μm were considered free micro-aggregates or unassociated clay particles. To slake the soils, samples were slowly submerged within the nested sieves into a 2.5 m*M* solution of $CaCl_2$, to prevent aggregate dispersion. Yoder (1936) discusses that pure water exerts dispersion forces on soil colloids, and further discusses Demolon and Henin (1932), who suggest to use a solution of $Ca(NO_3)_2$. Due to the intent to measure nitrogenous compounds, $CaCl_2$ was used instead to the same effect. Soils were soaked for 10 minutes. The machine was then run, agitating soils for 10 minutes at a 4 cm stroke length at 30 rpm. Samples were then carefully washed and transferred from the sieves into drying tins and placed in a drying oven at 50 ºC until fully dried, then weighed. Subsamples of each size class were dried at 105 ºC for 24 hours for correction to dry weight.

To express aggregate size fractions in a sand-free weight basis, subsamples (2-5 g) of each aggregate size class were weighed and 10-25 ml of 5 g L^{-1} of sodium hexametaphosphate ($Na_6P_6O_{18}$) (SHMP) was added as a dispersing agent. Samples were left overnight, shaken for 4 hours on an orbital shaker, then passed through a 53 μm sieve, washed with deionized water, and dried at 105 °C for 24 hours. All aggregate weights are presented as sand-free corrected weights.

To determine soil pH, approximately 5 g of air-dried soil were placed in 10 mL 0.01 *M* $CaCl_2$, shaken, and allowed to settle for 1 hour before reading by a pH electrode (GENERAL digital, Taiwan). To determine OM content, subsamples (0.5 - 10 g) of dried soil were weighed in aluminum tins, placed into a muffle furnace at 550°C for 8 hours. Upon removal, samples were allowed to cool, then weighed to determine the percent loss of mass due to ignition of OM (Bao et al. 2011).

As an estimate of soil microbial activity and the active pool of soil C, we measured permanganate oxidizable carbon (POXC; Culman et al. 2012). Five g of dried soil were mixed with 2 ml of 0.2 *M* $KMnO_4$ solution and 18 ml H_2O, then placed on an orbital shaker for 2 minutes and allowed to settle for 10 minutes, before 0.5 ml of the supernatant was diluted to 50 ml with deionized water. An aliquot (100 μL) was pipetted into a clear 96-well plate, along with a duplicate set of standards

prepared from the $KMnO_4$ stock solution and deionized water control and read at 550 nm using a Synergy HTX plate reader (Biotek, Winooski, VT).

Clay mineralogy within watersheds was examined by X-ray diffraction (XRD) analyses. Soil samples from three locations across three watersheds and horizons were composited such that we analyzed one A-horizon and one B-horizon sample for each watershed (n=6). Composited samples were pretreated with 30 % hydrogen peroxide to remove organic matter, sieved to 250 μm, then air dried. Clay mineralogy was analyzed using a PANalytical X'Pert Pro X-ray Diffractometer (XRD) at the Shared Research Facility at West Virginia University.

Data analysis

Differences in aggregate weight, aggregate OM content, soil pH, and POXC attributed to fertilization or tree species were analyzed using nonparametric tests. When comparing two distinct groups (Watershed comparison), the Wilcoxon two-sample test was applied, using a normal approximation. When greater than two groups were compared (Tree species comparison), a pairwise Wilcoxon two-sample test was applied, using a normal approximation. All data was analyzed using SAS-JMP Statistical Software 14.0 (SAS Analytics, Cary, NC) and a significance level of $\alpha = 0.05$ was applied. Using two watersheds to compare makes this experimental design pseudoreplicated, with an effective sample size of one. We are, however, confident that differences between watersheds are a result of the fertilization treatment, as Adams and Angradi (1996) showed that soil conditions were similar before fertilization treatment began.

Results

Clay mineralogy

XRD analysis from soil samples across three watersheds and two soil horizons exhibit a similar mixed clay mineralogy, with presence of both 1:1 and 2:1 clays (Fig. 1). Results indicate the presence of chlorite (a weathered form of muscovite and vermiculite which are 2:1 clays; 6.2 2-θ), kaolinite (a 1:1 clay; 12.5 2-θ), and illite (a 2:1 clay; 8.9 2-θ).

Bulk soil OM, pH, and POXC by watershed and species

Differences in bulk soil OM, measured as loss on ignition (LOI) were evident between watersheds in the B-horizon, but not in the A-horizon (Table 1). In the B-horizon, WS7 had greater mean bulk OM than WS3 ($p = 0.004$). Within WS3, bulk soil OM also varied by tree species in the B-horizon but not in the A-horizon (Table 1). Bulk soil OM did not vary significantly by species in either the A- or B-horizon in WS7. In WS3, B-horizon, mean OM beneath black cherry was significantly greater than red oak and tulip poplar ($p = 0.030$ for both), while not being significantly different from black birch. In both watersheds and in both horizons, bulk soil OM did not significantly differ among the AM- and ECM-associated species here, though there was a trend that AM-associated species had greater bulk soil OM than ECM-associated species in WS3, B-horizon ($p = 0.066$) (tulip poplar and black cherry are AM-associated species, and black birch and northern red oak are ECM-associated species).

Soil pH varied by watershed in the A-horizon, but not in the B-horizon (Table 1). In WS3 A-horizon, soil pH was 3.57 and 3.97 in WS7 ($p = 0.005$). In WS7 A-horizon, soil pH beneath tulip

poplar was greater than all other species in pairwise comparisons ($p = 0.030$; 0.029; and 0.030, respectively). There were no statistically significant differences in soil pH between species in WS7 B-horizon or in either horizon in WS3. Soil beneath AM-associated species exhibited a significantly lower pH in WS3 relative to WS7, in both the A- and B-horizons, while soil pH beneath ECM-associated species did not vary significantly between watersheds. In the A-horizon, soil beneath AM-associated species had a pH of 3.51 in WS3 and 4.21 in WS7 ($p = 0.003$), and in the B-horizon, a pH of 4.02 in WS3 and 4.3 in WS7 ($p = 0.031$).

Mean POXC values were not statistically different between watersheds in either the A- or B-horizons, ($p = 0.080$ and 0.169, respectively), though there is a trend that POXC is greater in WS3 (mean: 311 g kg^{-1} soil) than in WS7 (mean: 217 g kg^{-1} soil) in the A-horizon (Table 1). No significant differences occurred in POXC were detected as a function of tree species or fungal association type.

Total sand-free aggregate weight

Total aggregate sand-free weight (contained within aggregates between 53-2000 μm) varied between watersheds, and greater total aggregate sand-free weight occurred in WS7 in the B-horizon ($p = 0.005$) relative to WS3, though no differences occurred in total aggregate weight between watersheds in the A-horizon (Fig. 2). When analyzed by size fraction, aggregate sand-free weight varied between watersheds and horizons. Consistently, in both the A- and B-horizons, WS3 soil contained greater aggregate sand-free weight in both the 53-250 and <53 μm size classes relative to WS7 (A-horizon $p = 0.014$ and <0.001, respectively; B-horizon $p = 0.007$ and < 0.001, respectively) (Fig. 2). Comparing among tree species, in both WS3 and WS7, soil beneath differing tree species varied in sand-free weight across aggregate size classes (Fig. 3). In WS7 A-horizon, significant differences between tree species occurred in the 500-1000, 250-500, and 53-250 μm size fractions and in 500-1000 μm fraction in the B-horizon. For example, in the 53-250 μm size fraction in WS7, soil beneath black cherry contained significantly smaller sand-free weight than soil beneath black birch ($p = 0.030$), while no species were significantly different from red oak or tulip poplar. Variation by tree species on aggregate weight in WS3 occurred only in >2000 μm in the A-horizon and 500-1000 in the B-horizon (Fig. 3).

The influence of watershed on sand-free aggregate weight varied by tree species. Soil beneath birch in WS3 A-horizon had greater sand-free weight in the <53 μm size class than birch in WS7 A-horizon ($p = 0.030$). Soil beneath oak in WS3 A-horizon had greater sand-free aggregate weight in the <53 μm size fraction than oak in WS7 A-horizon ($p = 0.030$). Also beneath oak in the B-horizon, a greater sand-free weight was evident in both the 53-250 and <53 μm size classes in WS3 relative to WS7 ($p = 0.030$ for both). Soil beneath poplar in WS3 (both horizons) had greater sand-free aggregate weight in the <53 μm size class ($p = 0.030$) than in WS7.

Aggregate-associated organic matter

Total aggregate OM (contained within aggregates between 53-2000 μm) varied between watersheds only in the B-horizon, where 52% more aggregate-associated OM occurred in soil from WS7 relative to WS3 ($p = 0.001$). However, intra-aggregate OM content was similar in the A-horizon between watersheds. Specific to aggregate size class, aggregate-associated OM varied

between watersheds in the >2000 μm and the 53-250 μm classes in the A-horizon and in the 1000-2000 μm and 500-1000 μm classes in the B-horizon (Fig. 4). For example, in the A-horizon, soil from WS3 had 42% more OM in the 53-250 μm size class ($p = 0.011$) than soil from WS7.

Within aggregate size classes, OM varied by tree species within watersheds, and the influence of species on aggregate-associated OM was not consistent between watersheds (Fig. 4). Most often, significant differences within a watershed between species were detected in the A-horizon between black cherry and the other species – this effect occurred in both watersheds. In WS7 A-horizon, in each of the 500-1000, 250-500, and <53 μm size fractions, soil aggregates beneath black cherry contained significantly less OM than soil beneath black birch and red oak, though was not statistically distinct from tulip poplar. Additionally, in WS3 B-horizon, in the 1000-2000 and 53-250 μm size fractions, soil beneath black cherry contained greater aggregate OM than soil beneath black birch ($p = 0.030$), though neither black birch nor black cherry soil were significantly different from red oak or tulip poplar.

The influence of watershed on aggregate-associated OM varied by tree species. For example, for soil beneath red oak in the A-horizon, WS3 soil contained greater OM in the 53-250 μm size fraction relative to WS7 ($p = 0.030$) and soil beneath black cherry in WS3 A-horizon contained more aggregate OM in the 1000-2000 and 500-1000 μm size class relative to WS7 ($p=0.030$). Aggregate OM was similar between WS3 and WS7 in soil beneath tulip poplar in every size class (Fig. 5).

Mycorrhizal fungal effects on aggregate sand-free weight distribution

Mycorrhizal fungal association significantly influenced aggregate sand-free weight distribution within watersheds, though the influence of fungal association was distinct by watershed (Fig. 6). Most commonly, differences in aggregate weight between fungal association occurred in WS7 A-horizon soil, where ECM soil exhibited significantly greater aggregate sand-free weight in the 53-250, 250-500, and 500-1000 fractions (Fig. 6). Statistical differences between fungal association were not evident in WS7 B-horizon in any size class. This was distinct from soil within WS3, where AM soil exhibited greater aggregate sand-free weight in the 1000-2000 size fraction (A-horizon) and the 500-1000 size fraction (B-horizon). In WS3 B-horizon, soil beneath ECM-associated species consistently have lower aggregate weight in the larger size classes with a shift of aggregate sand-free weight to the <53 μm size class relative to AM soil (Fig. 6).

In the A-horizon, comparing between watersheds, soil beneath AM-associated species from WS3 had larger aggregate sand-free weight in both the 53-250 ($p = 0.018$) and <53 μm ($p = 0.024$) size classes relative to WS7. Soil beneath ECM-associated species had larger aggregate sand-free weight in WS3 in the <53 μm size class relative to WS7 ($p = 0.005$). In the B-horizon, comparing between watersheds, soil beneath AM-associated species had greater aggregate sand-free weight in WS3 than WS7 in the <53 μm size class ($p = 0.003$), though beneath ECM-associated species, WS3 contained greater aggregate sand-free weight than WS7 in the <53 μm size class ($p = 0.002$).

Mycorrhizal fungal effects on aggregate-associated OM

Mycorrhizal fungal association influenced aggregate OM within watersheds though the effect that fungal type had on aggregate OM distribution varied by watershed. In WS3, AM fungi had significantly greater total intra-aggregate OM in both the A- and B-horizon ($p = 0.024$ and 0.014, respectively); in WS7, ECM fungi trended to have greater intra-aggregate OM in the A-horizon ($p= 0.083$), with no significant differences in the B-horizon. By aggregate size class, in WS7 A-horizon, soils beneath ECM-associated species contained more aggregate OM than AM-associated species in the 53-250, 250-500, and 500-1000 μm size classes ($p = 0.018$; Fig. 7). Statistical differences between fungal association were not evident in WS7 B-horizon in any size class. Again, this was distinct from soil in WS3, where AM-associated soil contained greater OM in the 1000-2000 and >2000 size class (A-horizon) and in the 250-500, 500-1000, and 1000-2000 size classes (B-horizon) (Fig. 7). Interestingly, no significant differences occurred in OM in the <53 μm size class between fungal association in either watershed.

Relationship between microaggregate proportion and pH or POXC

Generally, lower soil pH was associated with both greater sand-free aggregate weight and OM content in microaggregate fractions (<53 and 53-250 μm) (Fig. 8). In the A-horizon, in both the <53 and the 53-250 μm size class, higher soil pH is associated with lower sand-free weight ($p = 0.004$, $R^2 = 0.25$ and $p = 0.041$, $R^2 = 0.13$, respectively) and the 53-250 μm size class exhibits lower aggregate-associated OM at higher soil pH ($p = 0.003$, $R^2 = 0.26$). It should be noted that all soils sampled in this study had a pH of 3.5-4.5, classified as extremely acid (Soil Science Division Staff 2017).

Greater POXC values are associated with both greater sand-free aggregate weight and OM content in the <53 μm fraction (Fig. 8). In both the A- and B-horizon across watersheds, within the <53 μm size class greater POXC values are associated with greater OM content ($p = 0.037$, $R^2 = 0.142$ – graph e, and $p = 0.018$, $R^2 = 0.18$ – graph f). Higher POXC is also associated with greater sand-free weight in the <53 size class ($p = 0.037$, $R^2 = 0.14$). Relatedly, in the larger 500-1000 μm size class, higher POXC values are associated with lower sand-free weight ($p = 0.048$, $R^2 = 0.12$).

Discussion

Bulk soil OM, pH and POXC

Our measures of bulk soil OM show significantly greater OM in WS7 in the B-horizon relative to WS3, though a similar, not statistically significant, pattern occurs in the A-horizon between watersheds (Table 1). This result from those reporting an increase in soil C following fertilization (Nave et al. 2009; Janssens et al. 2010; Frey et al. 2014). These studies report that soil C is greater in fertilized soils, due to a decline in microbial extracellular enzymatic activity related to OM degradation and lower overall soil respiration (DeForest et al. 2004; Carrara et al. 2018). However, this response of increased soil C following N fertilization is not universal (Janssens et al. 2010) and other studies reflect our results, as Fowler (2014) reported similar soil C content between fertilized and reference plots at the Long-Term Soil Productivity (LTSP) study at the FEF.

Our measure of bulk soil OM varied by tree species, to the largest extent in WS3 B-horizon, where cherry exhibited the greatest bulk soil OM content, significantly different from soil beneath both oak and poplar, but similar to soil beneath birch (Table 1). It was expected that soil OM would

vary by tree species, as AM-associated species tend to have a more rapid, inorganic nutrient cycle and labile litter (Chapman et al. 2006), which is supported by our measures of soil OM beneath cherry, but not poplar. However, Adams and Angradi (1996) reported tulip poplar litter decomposition rates to be more similar to black birch than black cherry litter, and the average bulk soil OM values measured here for soil beneath black birch are indeed less than from soil beneath black cherry, though these are not statistically distinct.

Bulk soil pH values were significantly higher in WS7 than WS3 in the A-horizon, though B-horizon pH values did not differ between watersheds (Table 1). This is congruent with the findings by Adams et al. (2007) of the fertilization regime resulting in acidification of soil and stream water in WS3. Soil pH also varied among tree species, but only in WS7. Differences in pH due to species has also been reported by Binkley and Giardina (1998). Additionally, Shear and Stewart (1934) reported that soil beneath silver maple (an AM-associated species) was less acidic than soil beneath larch, pine, and oak (ECM-associated species). Soil pH has also been linked to mycorrhizal association, where in Indiana, USA, ECM-dominated plots also have lower soil pH relative to AM-dominated plots (Brzostek et al. 2015) and Yin et al. (2014) reported AM species to have a neutralizing effect on rhizosphere soil, while ECM species had acidifying effects via greater amounts of organic acid exudates for nutrient acquisition. Strickland and Rousk (2010) state that the homogeneity of broadcast fertilizer applications likely negate variations due to fungal association, and in our study, the differences in soil pH as influenced by tree species disappears in WS3 as a function of fertilization.

We measured POXC to gain an estimate of soil microbial activity and the active pool of soil C. Our results suggest little difference between watersheds in this active soil C, with the exception of a trend towards greater POXC in WS3 B-horizon relative to WS7 B-horizon (Table 1). Because POXC reflects a heavily processed, labile fraction of soil C (Culman et al. 2012; Calderon et al. 2014), we hypothesized that POXC would be greater in WS7, as microbial extra-cellular enzyme activity has been shown to be lower in WS3 soil relative to WS7 (Carrara 2018). Our results of greater POXC in WS3 B-horizon may reflect processes linked to increased AM fungal growth resulting from fertilization (Dai et al. 2012), as well as the effect of ECM-associated tree species reducing fungal C allocation under N fertilization regimes (Vallack et al. 2012). Cullings et al. (2008) demonstrate that ECM fungi can utilize their saprotrophic capabilities to secrete OM-degrading enzymes when receiving limited C from host trees, which would result in the greater POXC we report in soil from fertilized WS3.

Aggregate weight

The largest significant differences in total sand free aggregate weight between the watersheds were found in the B-horizon, where WS7 sand-free aggregate weight was greater than that of WS3. The A-horizon values were not significantly different (Fig. 3), which lends support to our hypothesis that fertilization would result in reduced aggregation forces. Aggregation forces in soil are influenced by changes in soil chemistry via pH-dependent change in mineral surface charge or ionic composition, causing dispersal or flocculation of soil particles (Chorom et al. 1994). Another plausible mechanism by which fertilization would reduce aggregate weight in forest soils may be that bacterial enzymatic activity is reduced under N fertilization (shown by Carrara et al. 2018), and that reduced bacterial processes result in reduced soil aggregation (Lynch et al. 1981).

Furthermore, Kallenbach et al. (2016) demonstrated that bacterial processes increase stable SOM and promote aggregation. This contrasts however, with Plaza-Bonilla et al. (2013) who reported no significant differences in aggregate weight following long-term fertilization in agricultural soil.

When separated by size fraction, soil from WS7 has greater aggregate weight in the macro-aggregate size classes relative soil from to WS3, and the 53-250 and <53 μm micro-aggregate size fractions were distinctly greater in soil from WS3 (Fig. 3). It was expected that lower soil pH would act to disperse soil aggregates as a result of altered surface charge and reduced flocculation (Nguetnkam & Dultz 2014). Our results suggest that acidification processes resulted in strong formation of 53-250 μm aggregates and a large proportion of soil particles in the <53 um. In WS7, where soil has a higher soil pH, larger macro-aggregates were dominant.

The influence of tree species on soil aggregate weight is highlighted with black cherry, whereby soil beneath black cherry exhibited the largest change in aggregate weight distribution between watersheds. Perplexingly, soil beneath black cherry consistently had more macro-aggregates than other species in WS3, though consistently fewer macro-aggregates than other species in WS7 (Fig. 3). In general, due to AM-associated species (particularly black cherry) having more labile and easily decomposed litter (Adams and Angradi 1996; Chapman et al. 2006), microbial activity and organic byproducts are greater under AM-associated species, presumably leading to greater aggregation capacity. However, the hypothesis that AM-associated tree species would lead to greater overall aggregation was not supported, as evidenced by the greater aggregation that occurred beneath ECM species in WS7. The related hypothesis that acidification and resultant reduced microbial processes would more significantly reduce stable aggregate formation beneath AM species was also not supported. We suggest that while the effects of mycorrhizal fungi are a significant force on aggregate formation, the mechanisms are still not fully understood.

Aggregate-associated organic matter

Total aggregate-associated OM varied to the greatest extent between watersheds in the B-horizon, where there was greater aggregate-associated OM in soil from WS7, while aggregate-associated OM content was similar between watersheds in the A-horizon. This is as expected, and supported by Carrara et al. (2018), who reported that bacterial enzymatic activities were reduced in WS3 compared to WS7. Furthermore, Rousk et al. (2010) showed bacterial populations decrease under decreased pH, as occurs in soil from WS3. The more distinct differences in aggregate-associated OM that occur in the B-horizon are likely a function of OM interacting with the accumulated clay minerals in the subsurface soil, and is supported by Craig et al. (2018), who reported that AM-associated soil contains greater OM than ECM-associated soil only in the subsoil to 1 m depth, where microbial byproducts from AM litter accumulated on clays in the subsoil. A greater intra-aggregate OM content in the B-horizon is also likely related to the greater sand-free aggregate weight that occurs in WS7 in the B-horizon, due to processes related to pH-dependent dispersion and/or depressed bacterial aggregate-forming activity in the fertilized system.

When comparing the influence of tree species on aggregate OM, the data suggest a similar pattern as sand-free aggregate weight, where soil beneath black cherry contained greater aggregate OM than other species in WS3, and less aggregate OM than other species in WS7 (Fig. 5). This lends support to the concept that litter decomposability plays a large role in accumulation of intra-

aggregate OM, which may be particularly enhanced under N fertilization. In the case of black cherry, AM-fungi experience increased growth under increased N conditions (Dai et al. 2012) and AM-associated species tend to have more easily decomposable litter (Chapman et al. 2006), resulting in greater aggregate OM in fertilized soil. At first, this theory may seem to be contradicted by the similarity of aggregate OM in soil beneath tulip poplar and the ECM-associated species. However, Adams and Angradi (1996) examined the decay rates of litter from multiple tree species in WS3 and WS7 – including black birch, black cherry, and tulip poplar – and found that tulip poplar litter decomposed at a rate more akin to black birch (an ECM-associated species) than to black cherry (AM-associated species) on both a 1- and 2-year timescale. Previously, AM-associated fungi have been shown to positively correlate to development of soil aggregates (Rillig et al. 2002; Leifheit et al. 2014) due to greater hyphae biomass and production of glomalin-related soil protein, however, this effect may vary depending on plant or fungal species (Piotrowski et al. 2004). As litter decomposition affects the amount of OM available to be bound within aggregates, litter decomposition rates would result in tulip poplar influencing the soil differently than black cherry, which has more labile litter resulting in greater aggregate OM.

Relationship between pH, POXC, and micro-aggregate size fraction

Soil pH was inversely related to both microaggregate weight and microaggregate OM content in A-horizon soil across watersheds, where low soil pH resulted in greater micro-aggregate weight and micro-aggregate OM (Fig. 8). While this does not support our initial hypothesis that lowered soil pH would induce dispersion forces and prevent aggregation, a similar effect was documented by Bethlenfalvay et al. (1999), where AM growth was the primary influencer of stable aggregate formation. In that study, water-stable aggregate weight and AM fungal hyphae growth both increased with N addition, as soil pH decreased. This provides further support to the idea that mycorrhizae may play a more fundamental role in aggregate formation than bacterial activity. Bethenfalvay et al. (1999) also found that increased bacterial metabolic activity resulted in a temporary decrease in the amount of water stable aggregates (Bethenfalvay et al. 1999; Jastrow 1996). Rousk et al. (2010) report that bacterial population size positively correlated with soil pH, while fungal abundance was unaffected by pH in a wheat-producing agricultural system in the U.K. Further, Emerson and Dettmann (1960) found that clay-clay attractive forces in predominantly illite soils are stronger in acidic systems due to positively charged clay edges, leading to greater aggregation in acidified systems.

The relationship of aggregate sand-free weight and OM with POXC were contrary to what was expected, and soils with a larger POXC had greater OM content and sand-free aggregate weight in the <53 μm size class. At the outset of this experiment, particles that small were assumed to contain both unassociated clay particles and free microaggregates. Based on the POXC results reported here, we contend that this size class is predominantly made up of free micro-aggregates <53 μm and not unassociated clay particles. Culman (2012) stated that POXC is predominantly related to smaller sized (53-250 μm) particulate organic carbon (POC) and the pool of processed, labile soil C. We are still presented with the question of why there is more OM in this size class in the acidified watershed than in the reference.

Carrara et al. (2018) incubated soil samples from sites receiving long-term N fertilization and also found that with increased N, microbial C use efficiency increased, causing greater microbial

turnover and reduced respiration, resulting in increased stabilized SOM and soil carbon stocks, and our study further supports this conclusion. This contrasts with the conclusions of Frey et al. (2014) and Carrara et al. (2018) who stated that the effect of increased SOC under N addition is due to reduced OM decomposition. Our results of increased POXC correlating to increased OM in the <53 μm size class implies that in the fertilized system, there is increased, not decreased, OM decomposition.

Another relevant factor for POXC was the influence of surrounding tree species' fungal association type. When POXC was examined by %ECM influencing the plots, POXC increased under increasing influence of ECM in WS3, especially in the B-horizon ($p = 0.029$). There were no significant trends between POXC and surrounding %ECM in WS7. This result further supports the conclusions by Vallack et al. (2012) and Cullings et al. (2008) that ECM-associated tree species reduce belowground C allocation when under a fertilization regime, resulting in increased decomposition by ECM fungi.

Conclusions

Our data show that 30 years of forest fertilization with $(NH_4)_2SO_4$ has altered soil aggregation, as fertilized soil exhibits decreased macro-aggregate formation and a greater proportion of smaller micro-aggregates or unassociated clay minerals, particularly in subsurface soil. This shift in aggregation processes to soil more dominated by the smallest <53 μm fraction is associated with both acidification (soil pH) and increased microbially-processed C (oxidizable C) in fertilized soil. Macro-aggregate-associated OM was also significantly reduced in the fertilized soil, most strongly in subsurface soil. We also document that tree species can influence soil aggregation and suggest this is may be related to decomposability of litter material, as tree species influence was further impacted by fertilization. On a larger scale, soil C beneath tree species with more readily decomposable litter – most often AM-associated tree species – increases under increased N conditions, and this has resulted in the soil of the eastern U.S. forests to more strongly function as a C sink. Because of the decreased capacity for macro-aggregate formation induced by fertilization documented here, as the atmospheric deposition of N and S compounds continues to decline following implementation of the Clean Air Act, there is potential for the labile, unprotected C in the smallest <53 μm fraction to be further decomposed as the fungal and bacterial communities shift in response to the changing environment.

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Figures

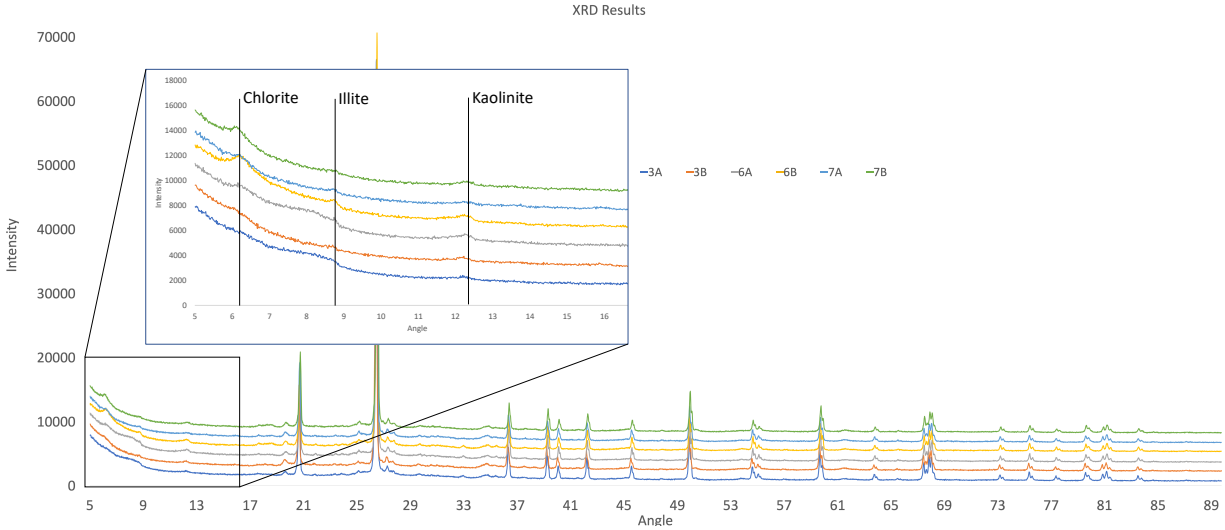

Figure 1. XRD results of testing watershed clay mineralogy. Clays were determined in the 5-15° 2-θ range, which has been expanded and peaks labeled. Sample 3A is unchanged, though each subsequent sample has had an increase of 1500 to aid in ease of viewing. 3A refers to WS3, A-horizon, 3B refers to WS3, B-horizon, 6A refers to WS6, A-horizon, 6B refers to WS6, B-horizon, 7A refers to WS7, A-horizon, and 7B refers WS7, B-horizon.

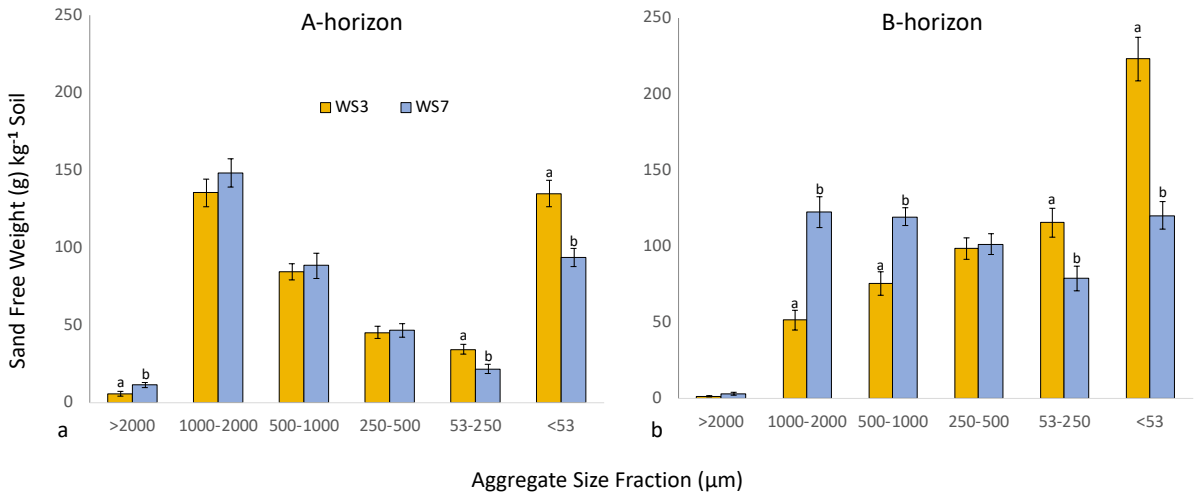

Figure 2. Sand-free weight within each aggregate size class by watershed in A-horizon and B-horizon. For each horizon, bars with different letters within each aggregate size fraction are significantly different according to Kruskal-Wallace means comparison ($p < 0.05$).

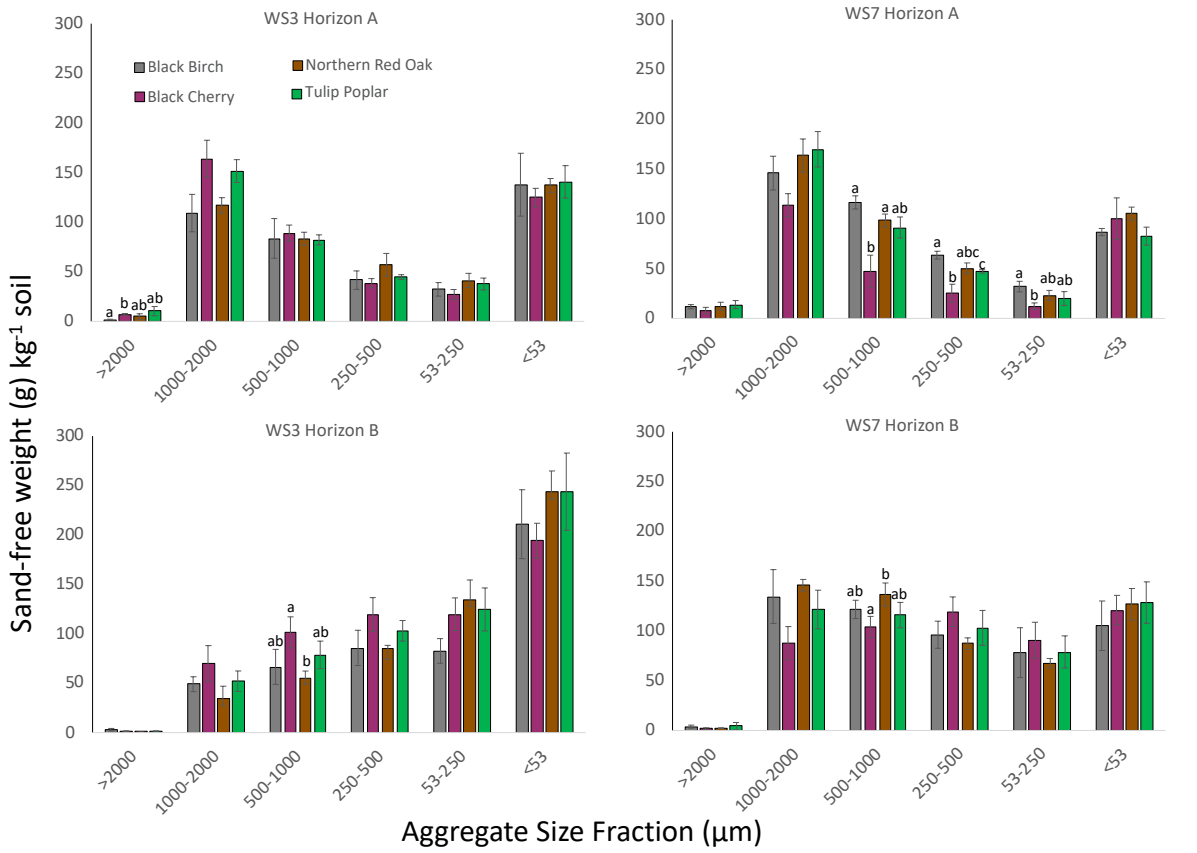

Figure 3. Sand-free weight within each aggregate size class by tree species in A-horizon and B-horizon. For each horizon, within each watershed, bars with different letters within each aggregate size fraction are significantly different according to Kruskal-Wallace pairwise means comparison ($p < 0.05$).

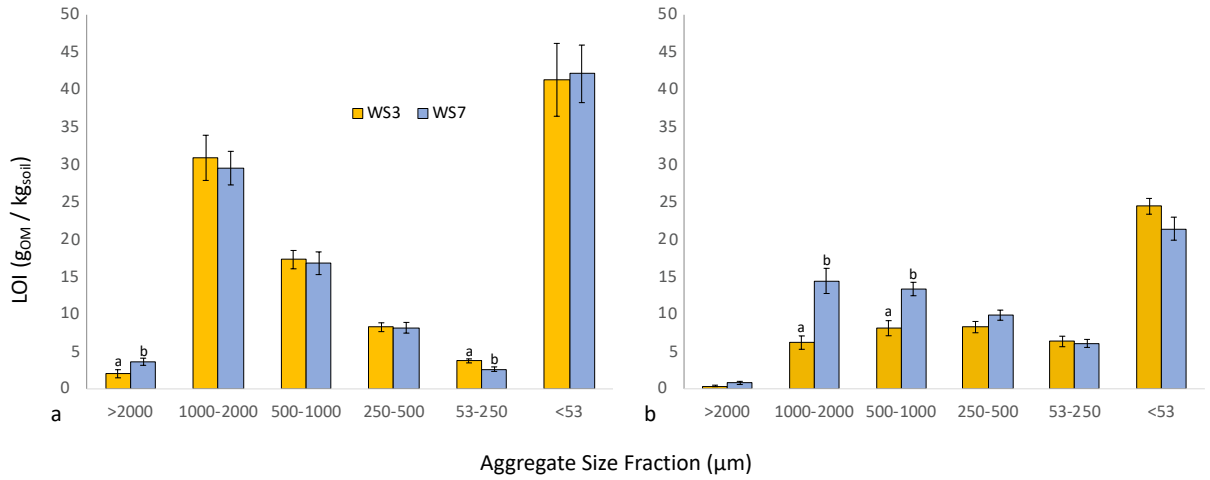

Figure 4. Organic matter within each aggregate size class by watershed in A-horizon and B-horizon. For each horizon, bars with different lowercase letters within each aggregate size fraction are significantly different according to Kruskal-Wallace means comparison ($p < 0.05$).

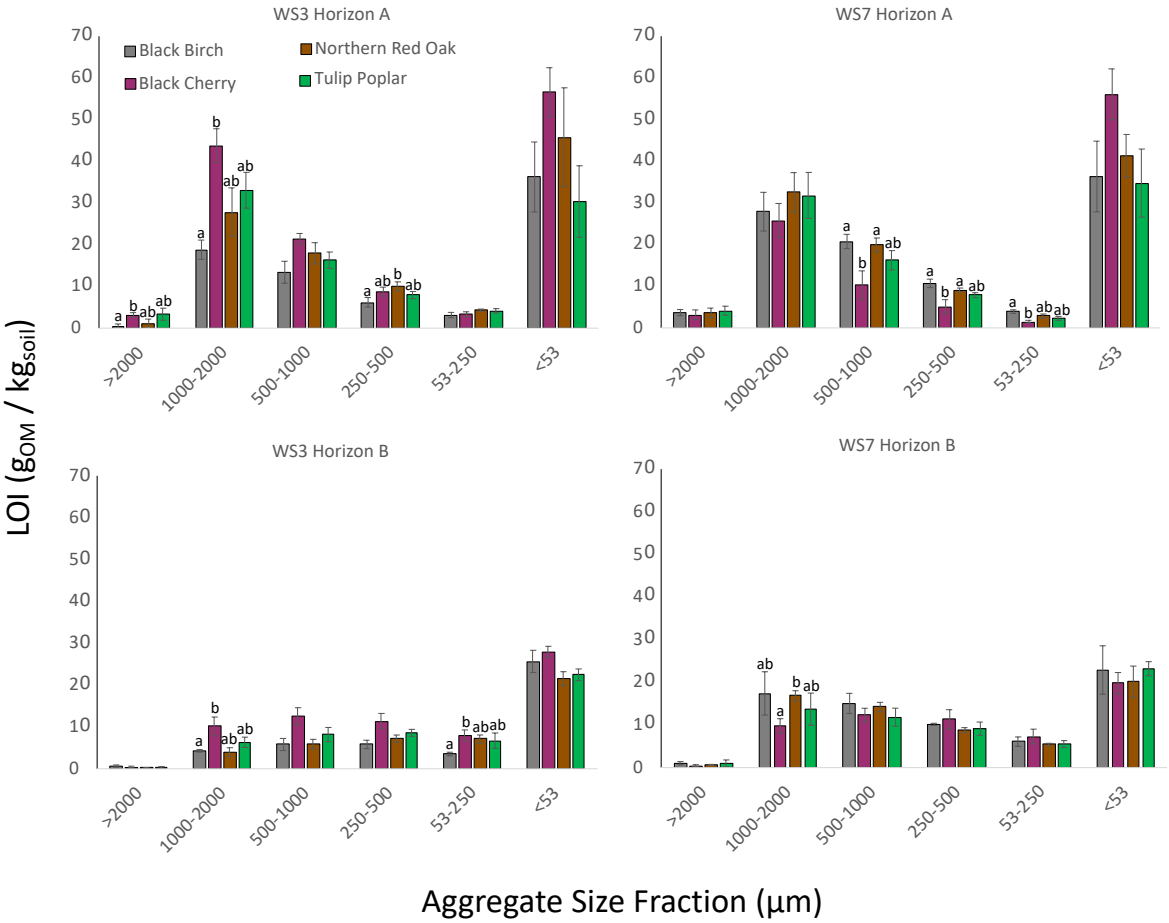

Figure 5. Organic matter within each aggregate size class by tree species in A-horizon and B-horizon. For each horizon, within each watershed, bars with different letters within each aggregate

size fraction are significantly different according to Kruskal-Wallace pairwise means comparison ($p < 0.05$).

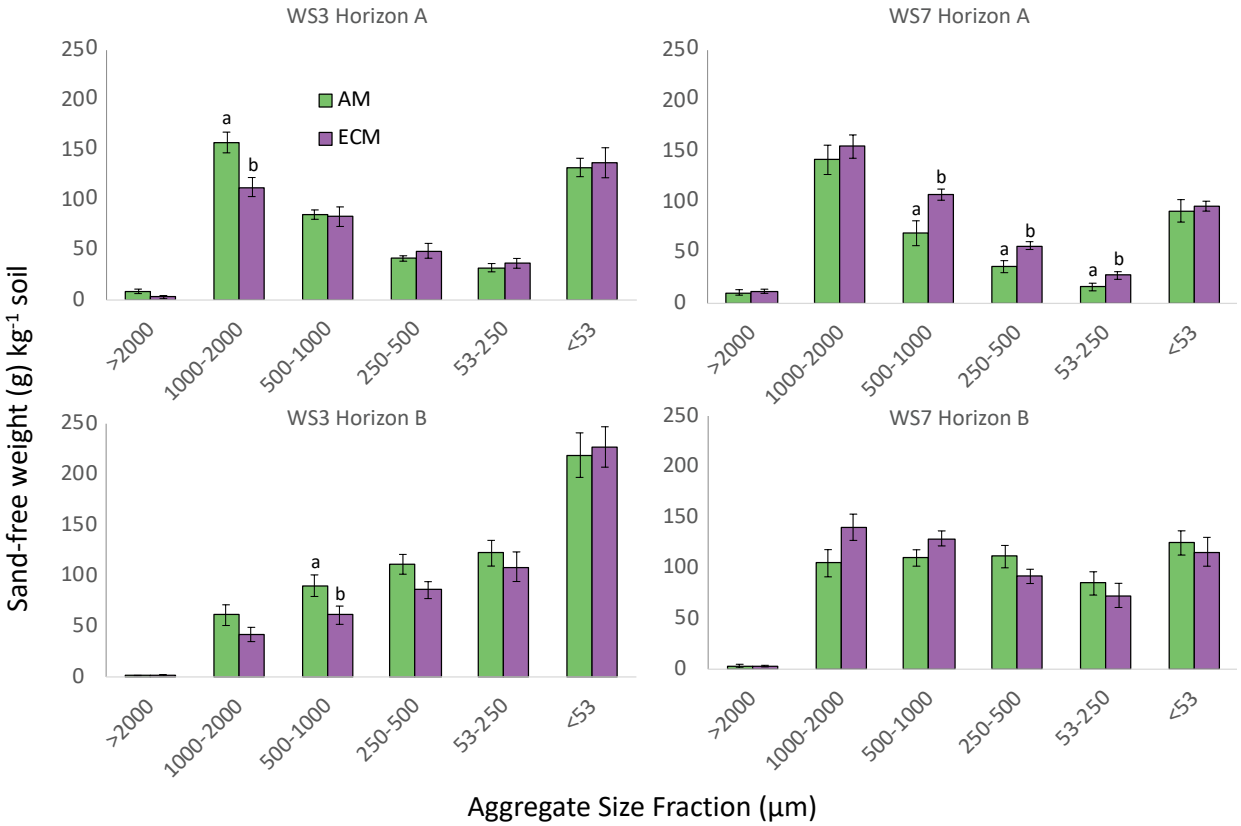

Figure 6. Sand-free weight within each aggregate size class by watershed in A-horizon and B-horizon. For each horizon, bars with different letters within each aggregate size fraction and watershed are significantly different according to Kruskal-Wallace means comparison ($p < 0.05$).

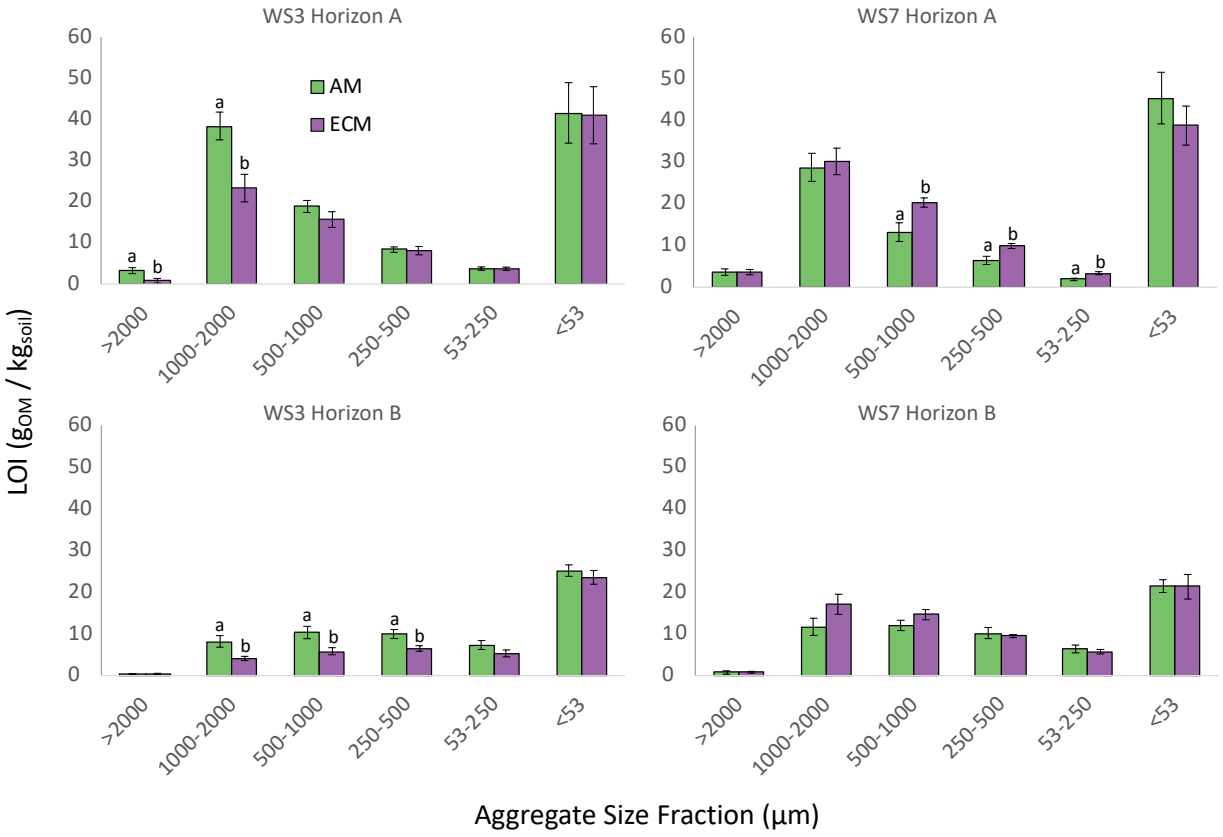

Figure 7. Organic matter within each aggregate size class by watershed in A-horizon and B-horizon. For each horizon, bars with different letters within each aggregate size fraction and watershed are significantly different according to Kruskal-Wallace means comparison ($p < 0.05$).

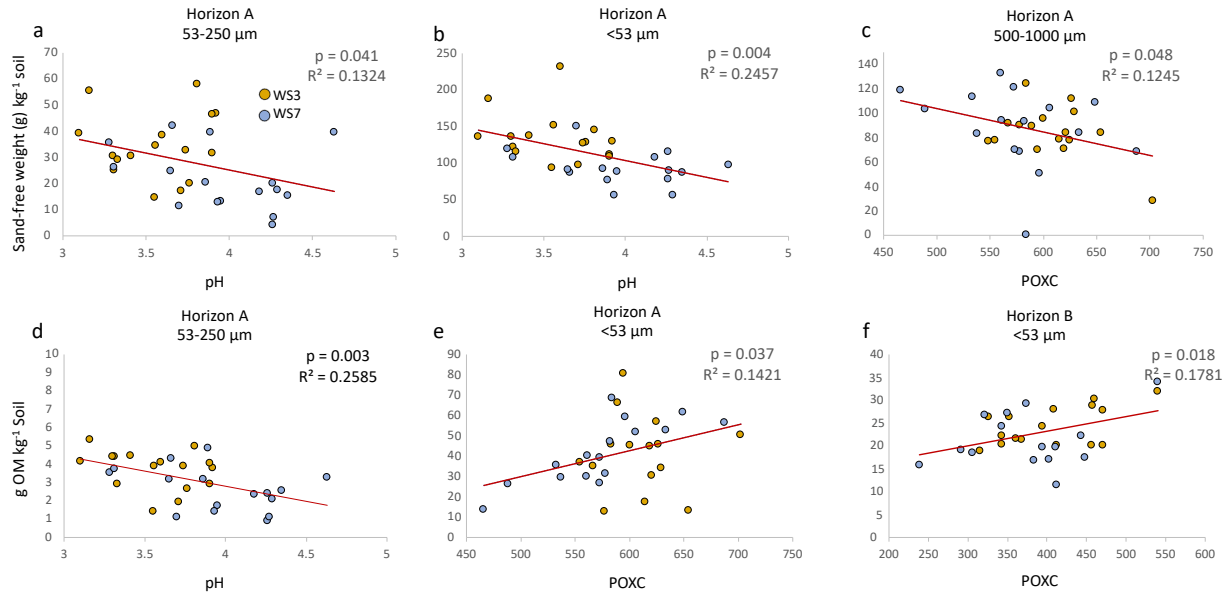

Figure 8. Significant relationships between soil pH_{CaCl2} and microaggregate size fractions weight and OM (a, b, d) and POXC and aggregate size fractions weight and OM (c, e, and f). Colored dots refer to data within a watershed. R^2 and p–values are denoted for each panel for all data across watersheds.

Table 1. Mean bulk soil organic matter (OM), soil pH_{CaCl_2}, and POXC values from each watershed and beneath the four tree species. Values indicate mean (± standard error). Values with different letters indicate statistically significant difference according to Wilcoxon pairwise means separation ($p < 0.05$). Significance is notated between watersheds within the respective horizons and between species within the respective watershed and horizon.

Watershed and Horizon	**Bulk soil OM (g kg^{-1})**	**Soil pH_{CaCl_2}**	**POXC (mg kg^{-1})**	**Tree species**	**Bulk soil OM (g kg^{-1})**	**Soil pH_{CaCl_2}**	**POXC (mg kg^{-1})**
3A	198.65 (*13.29*)	3.57[a] (*0.07*)	606.70 (*9.72*)	Birch	170.14 (*17.62*)	3.60 (*0.12*)	607.58 (*31.80*)
				Cherry	221.08 (*36.18*)	3.50 (*0.14*)	597.22 (*14.98*)
				Oak	212.07 (*32.34*)	3.65 (*0.14*)	599.92 (*16.80*)
				Poplar	191.29 (*18.77*)	3.53 (*0.18*)	622.10 (*16.07*)
7A	209.89 (*9.52*)	3.97[b] (*0.10*)	575.61 (*13.85*)	Birch	195.44 (*18.83*)	3.77 (*0.06*)[b]	567.79 (*32.82*)
				Cherry	240.78 (*21.04*)	4.04 (*0.14*)[b]	610.14 (*26.25*)
				Oak	208.36 (*2.32*)	3.68 (*0.23*)[b]	564.73 (*17.71*)
				Poplar	195.00 (*23.39*)	4.39 (*0.08*)[a]	559.77 (*34.83*)
3B	86.68[a] (*4.12*)	4.09 (*0.06*)	404.70 (*16.27*)	Birch	78.74 (*8.70*)[ab]	4.07 (*0.09*)	424.11 (*49.05*)
				Cherry	107.04 (*3.23*)[a]	3.98 (*0.09*)	429.90 (*24.52*)
				Oak	79.08 (*3.58*)[b]	4.25 (*0.09*)	387.23 (*34.11*)
				Poplar	81.85 (*7.36*)[b]	4.07 (*0.14*)	377.56 (*30.53*)
7B	106.91[b] (*4.44*)	4.16 (*0.06*)	371.23 (*18.74*)	Birch	107.25 (*15.14*)	4.04 (*0.08*)	341.98 (*67.56*)
				Cherry	107.04 (*6.75*)	4.23 (*0.11*)	396.03 (*20.23*)
				Oak	109.31 (*5.28*)	4.01 (*0.15*)	397.67 (*8.90*)
				Poplar	104.04 (*9.32*)	4.38 (*0.08*)	349.23 (*32.92*)

Table 2. Statistical test values for each factor by soil horizon. Values represent z-scores for watershed and fungal association factors and chi-squared values for species factor. P-values are given in parenthesis. Significance is accepted at $p < 0.05$ and significant relationships are bolded.

			Aggregate Size Fraction (µm)					
Parameter	**Soil Horizon**	**Factor**	>2000	1000-2000	500-1000	250-500	53-250	<53
Sand-free weight (g) kg^{-1} soil	A	Watershed	2.34 **(0.019)**	0.89 (0.376)	0.62 (0.534)	0.58 (0.559)	-2/47 **(0.014)**	-3.75 **(<0.001)**
		Species	2.57 (0.462)	3.19 (0.363)	7.89 **(0.048)**	11.26 **(0.010)**	4.76 (0.190)	2.21 (0.530)
		Fungal Association	-0.89 (0.375)	-1.87 (0.235)	2.24 **(0.025)**	2.77 **(0.006)**	1.68 (0.094)	0 (1.000)
	B	Watershed	1.74 (0.082)	4.28 **(<0.001)**	3.49 **(<0.001)**	0.24 (0.807)	-2.69 **(0.007)**	-4.35 **(<0.001)**
		Species	3.08 (0.380)	0.13 (0.988)	0.43 (0.935)	4.77 (0.189)	1.56 (0.669)	1.13 (0.771)
		Fungal Association	0.70 (0.485)	0.32 (0.749)	-0.51 (0.611)	-1.94 (0.0523)	-0.89 (0.376)	0.05 (0.955)
LOI (g_{OM} / kg_{soil})	A	Watershed	2.02 **(0.043)**	-0.17 (0.865)	-0.17 (0.865)	0.09 (0.925)	-2.54 **(0.011)**	-0.18 (0.859)
		Species	2.57 (0.462)	4.91 (0.178)	1.21 (0.751)	5.04 (0.168)	3.41 (0.333)	9.07 **(0.028)**
		Fungal Association	-1.42 (0.157)	-1.79 (0.073)	0.92 (0.356)	1.94 (0.052)	1.49 (0.137)	1.01 (0.314)
	B	Watershed	1.49 (0.136)	3.71 **(<0.001)**	3.26 **(0.001)**	1.87 (0.062)	-0.21 (0.836)	-1.88 (0.060)
		Species	2.73 (0.435)	0.42 (0.936)	1.40 (0.705)	4.53 (0.210)	4.70 (0.195)	1.55 (0.670)
		Fungal Association	0.59 (0.558)	-0.36 (0.720)	-0.58 (0.559)	-1.68 (0.094)	-1.11 (0.266)	-0.53 (0.594)

Chapter 3. Carbon and nitrogen budgets in two Fernow Experimental Forest watersheds: Influence of planted Norway spruce

Abstract

Conversion of native forests to planted coniferous stands is a common anthropogenic landscape alteration. Understanding ecosystem biogeochemistry in these altered systems is necessary for management of ecosystem services and to forecast future ecosystem processes. Alterations to canopy architecture, litter chemistry and mass, and root exudation processes affect the chemical and biological processes in forest soils, which may influence ecosystem carbon (C) and nitrogen (N) storage or loss. We utilized a paired-watershed study at the Fernow Experimental Forest in West Virginia, USA to quantify ecosystem differences between a 50-year old Norway spruce (*Picea abies*) stand conversion and a reference watershed of native vegetation in soil, forest floor, and tree biomass C and N pools; atmospheric deposition and stream NO_3-N export; the distribution of aggregates and aggregate-associated organic matter; and inorganic N and oxidizable C. Values quantified here are compared to those measured twelve years prior. In 2008, total ecosystem C and N content were both more than 30% less in the spruce watershed relative to the hardwood watershed; however, current estimates of ecosystem C and N show that C pools are now similar between watersheds, though total ecosystem N is still 13.4% less in the spruce watershed, driven largely by lower mineral soil N content. Soil from the hardwood watershed exhibits greater macro-aggregate formation and intra-aggregate organic matter (OM), which is likely related to acidification and lower microbial activity in soil from the spruce watershed. Both watersheds show surprising stream N exports, with the spruce watershed having nearly zero export of NO_3-N, and exports from the hardwood watershed exceeding inputs. C and N pools appear to be greater in hardwood systems in the early stages of stand development, but spruce-converted areas are likely to recover to similar C and N storage as stand age approaches 50 years.

Introduction and Background

Utilization of paired-watershed studies allows for examination of the effects of anthropogenic activities on biogeochemical processes, nutrient cycling, and ecosystem storage of carbon (C; Hewlett and Helvey 1970; Swank and Douglass 1975; Likens 1985; Adams et al. 1993). Long-term measures of stream chemistry at gauged watershed outlets integrate ecosystem functions and allow for quantification of net biogeochemical responses of the total watershed (above- and below-ground) to alteration. Nutrient mass-balance studies have been used to study differences in stream chemistry and explore processes influencing storage or export of nutrients and C in ecosystems. For example, in a study in the Hubbard Brook Experimental Forest, New Hampshire, it was estimated that roughly 95% of the 83.5 kg N ha^{-1} yr^{-1} that was added to the inorganic N pool through mineralization was stored in the ecosystem, and only 5% was exported in streamflow (Bormann et al. 1977). Of the N held in long-term storage, they found that roughly 54% was held in living biomass and 46% was held in organic material associated with the forest floor.

A common anthropogenic landscape alteration is vegetation type conversion via establishment of planted forests, with the most common choice for planting being rapidly growing coniferous species (Carnus et al. 2006). Replacing native vegetation with monoculture stands of coniferous species may alter biogeochemical cycling of C and nitrogen (N) (Guo and Gifford 2002), promote soil acidification (Miles 1985), and result in significant losses of C and N from the soil profile

(Guo and Gifford 2002; Zinn et al. 2002; Solomon et al. 2007; Diochon et al. 2009). Tree species can differentially alter soil biogeochemistry through altered inputs of organic matter, via both above- and below-ground litter production and rhizosphere exudate processes (Finzi et al. 2015), which varies by species in quantity and chemical composition (Melillo et al. 1983; Binkley 1995; Lovett et al. 2004). Organic matter comprising this litter is the main driver for microbial and/or sorption processes by which nutrient bioavailability and/or rates of C storage are determined (Bhattarai et al. 2015).

Soil organic carbon (SOC) is one of the building blocks of basic soil health. It provides a substrate for soil biota (Boyle 1989) and promotes soil structure (Cambardella et al. 1996) and nutrient- and water-holding capacity for plants. Further, soils play an important role in global C management, containing approximately 1500 Pg of organic C, twice the amount of C in the atmosphere (Smith 2004). The amount of SOC is naturally a function of climate (temperature, precipitation) and related vegetation type, where cool, moist climates result in greatest accumulation of SOC (Ontl 2012). However, land management practices also influence storage of SOC. As humans alter ecosystems, it is necessary to understand how these changes will influence SOC dynamics and storage to better predict atmospheric C sequestration potential, and resulting site productivity, nutrient cycling, and hydrologic dynamics

The mechanisms by which individual tree species influence C and N dynamics are still being resolved (Hobbie et al. 2006; Mueller et al. 2012; Vesterdal et al. 2013), but may in part be related to mycorrhizal relationships and associated soil microbial activity and nutrient acquisition pathways (Phillips et al. 2013; Averill et al. 2014; Taylor et al. 2016). Many studies have aimed to link vegetation-mediated soil characteristics such as soil pH, soil C:N ratio, lignin:N ratio, and phenol concentrations to C storage and N retention in forest soils (e.g. Melillo et al.1983, Ste-Marie and Paré 1999; Ross et al. 2004). However, these studies have often produced weak correlations and results are highly variable across a scope of ecosystems (Robertson 1982; Ross et al. 2004). This suggests that C storage and N cycling, retention, and export processes may be influenced by specific tree species as well as harvest practices, past land use, age of the stand, and/or disease outbreaks (Lovett et al. 2004), and their interactions.

Tree species can additionally affect C and N dynamics through alteration of the microecosystems beneath them, through variability in canopy cover (Mackay and Band 1997), amount and chemistry of roots and their exudates (Rasse et al. 2005) and above-ground litter, ease of microbial decomposition of plant material (Chapman et al. 2006), root structure and shape (Hishi 2007). Canopy structure and density will alter the hydrologic inputs to a catchment through alterations of the ratio of rainfall:throughfall:stemflow, and relatedly, the amount and composition of atmospheric deposition into a catchment (Mina 1967; Johnson and Lehmann 2006). Root and fungal exudates alter the chemistry of C and other nutrient input to the soil, influencing the bacterial and fungal communities beneath, as well as soil nutrient holding capacity, while root structure influences soil physical characteristics, structure, and water movement. Because leaf litter chemistry varies among tree species, decomposition also varies, resulting in different nutrient inputs to soil (Müller 1889, cited in Gast, 1937; Miles 1985).

Watershed 6 (WS6) and Watershed 7 (WS7) are two adjacent watersheds within the USDA Forest Service Fernow Experimental Forest (FEF) in West Virginia, USA that provide a unique

opportunity to investigate the specific role of tree species in ecosystem C and N cycling and retention. Observations from long-term stream chemistry sampling in these watersheds include divergent NO_3-N export at the stream outlets. WS7, a 50-yr-old hardwood stand, exports approximately 8 kg $ha^{-1}yr^{-1}$ NO_3-N, while NO_3-N exports from WS6, a 48-yr-old Norway spruce stand have been nearly zero for 40 years (mean ≈ 0.6 kg ha^{-1} yr^{-1}). Ecosystem C and N mass-balance budgets were first measured in these watersheds in 2008 (Kelly et al. 2011), indicating that the spruce watershed contained 35% less ecosystem C than the adjacent hardwood watershed at stand age ~40 years. This work reassesses the budgets for select pools of C and N 12 years later to further our understanding of the vegetation influence on ecosystem C and N dynamics and storage. Quantification and comparison of pool size of C and N may help account for nearly 50 years of differences in stream NO_3-N export in these two adjacent, gauged watersheds at the FEF. Utilizing a paired-watershed study, we are able to quantify the effect that this vegetation conversion has had on the C and N biogeochemistry of these forested watersheds. Long-term atmospheric deposition records, along with streamflow and stream chemistry data, further allow for investigation of the inputs and export of nutrients from each watershed over time.

Our specific objectives were to: 1) measure select ecosystem pools of C and N within WS6 and WS7; 2) quantify inputs and exports of ecosystem NO_3-N; and 3) compare pool sizes of C and N and NO_3-N export to the previous measurements to quantify changes over time. It was hypothesized that: 1) relative to WS7, WS6 would still exhibit lower amounts of C and N in below-ground biomass and in mineral soil after 50 years of contrasting vegetative influence; and 2) relative to WS7, there would be a lower inorganic N flux in spruce-influenced soils, leading to accumulating C and N pool sizes in the forest floor and lower pool sizes in the mineral soil, ultimately resulting in low NO_3–N export to the stream.

Methods

Site description

The watersheds investigated in this study are within the FEF, managed by the USDA Forest Service, in Parsons, WV, USA. The 1,900-ha forest was established in 1934 within the Monongahela National Forest. Annual precipitation is evenly distributed per annum and averages 145.8 cm (Kochenderfer 2006). Average monthly precipitation peaks in June (144 mm) and reaches its lowest value in October (97 mm). Average yearly temperature is 9.2° C, with an average monthly maximum in July (20.6° C) and minimum in January (-18° C) (Kochenderfer 2006).

Both WS6, the spruce watershed, and WS7, the hardwood reference watershed, were clearcut logged in sections, beginning in 1964 and concluding in 1967, and maintained barren with herbicides until 1969. Additional aerial herbicide was applied to WS6 in 1977 and again in 1980 to prevent hardwood re-growth (Adams et al. 2020). Initial herbicide applications and harvesting through 1969 were done to evaluate the effects of complete deforestation on water yield, and Norway spruce was planted in 1973 to investigate how conifer conversion of hardwood stands affects water yield (Kochenderfer et al. 1990). After nearly 50 years of growth, WS6 has a closed canopy and dense stand structure (basal area = 46 m^2 ha^{-1}). Annual runoff in WS6 is considerably lower than the adjacent WS7, and lower than pre-treatment (Kochenderfer et al. 1990; Adams, USFS, unpublished data). This has been attributed to greater amounts of interception and transpiration in conifers relative to hardwood species (Kochenderfer et al. 1990).

The spruce watershed (WS6; 22 ha; elevation range 730-830 m) has hillslopes with east/west aspects along the stream. Soils in this watershed are Calvin series (Calvin channery silt loam; Calvin loamy-skeletal, mixed, active, mesic typic Dystrudept) (Soil Survey Staff USDA NRCS web soil survey 2019), derived from sandstone, siltstone, and shale parent material. Due to the influence of Norway spruce, a mor-type litter layer has developed, with a thick horizon of un-decomposed needles (Oi) above further-decomposed spruce organic material (Oe and Oa). There is also evidence of development of a spodic E-horizon in some locations in the watershed. There are few other tree species in the watershed, but individuals of black locust (*Robinia pseudoacacia*), yellow poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), and sourwood (*Oxydendron arboreum*), and patches of greenbrier (Smilax sp.) are present in WS6.

The hardwood reference watershed (WS7; 24 ha; elevation range 730-860 m) has hillslopes with north/south aspects along the stream. Soils in this watershed are dominantly Calvin (Calvin channery silt loam), with small ridgeline areas of Dekalb series (Dekalb channery loam and Dekalb extremely stony loam; Dekalb loamy-skeletal, siliceous, active, mesic typic Dystrudept), derived from acidic sandstone parent material (Soil Survey Staff USDA NRCS web soil survey 2019). This watershed is dominated by yellow poplar, sugar maple (*Acer saccharum*), and Northern red oak (*Quercus rubra*), with an understory of dogwood (*Cornus florida*), striped maple (*Acer pensylvanicum*), cucumber magnolia (*Magnolia acuminata*), and several species of pteridophyte.

In the initial study design established by Kelly (Kelly 2008) transects were established perpendicular from the stream on both sides of the stream. Six transects per watershed were established, three on each side of the main stream draining each watershed to explore potential effects of aspect and spatial variation on soil characteristics. Transects were stratified into zones along the entire length of the stream channel (low, middle, high) in order to compare transects between watersheds and to examine variability along the stream gradient within each watershed. Soil samples were collected initially in July 2008 from five sites (1, 8, 15, 30, and 60 m upslope from the stream) along each transect and from the A- and B-horizons (0 to 10 cm depth and 10 to 45 cm depth, respectively, with a field determination of horizon change) at each of the five sampling sites within a transect (n = 6 transects; n = 2 horizons; n = 5 distances from stream; N = 60 total soil sampling sites per watershed). Generally, bedrock is encountered at approximately 45 cm. All data from the previous sampling will be referred to as "2008".

Soil and forest floor collection

From sampling points (18 per watershed, previous five locations from each transect were reduced to three) previously established in 2008 in WS6 and WS7, soil samples were collected by auger at 0–10 and 10-45 cm depth (A- and B-horizons). Within 24 hours of sampling, subsamples were sieved through 2 mm mesh for determination of inorganic N content and moisture content. Moist samples were stored at 4 °C prior to extraction for N or dried at 105 ºC to determine moisture content via mass loss. Remaining soil was air-dried then sieved to 2 mm to determine total C and N content. Forest floor (organic horizon) samples were collected in October 2019 at each transect location in triplicate using a standardized template of 273.16 cm^2, then composited, for a total area sampled of 0.082 m^2. Forest floor samples were oven-dried at 105 ºC for analysis.

Atmospheric deposition and stream export of NO_3-N

Atmospheric dry and wet NO_3-N deposition data were acquired from the EPA CASTnet PAR107 monitoring site and the National Atmospheric Deposition Program (NADP) monitoring site WV18, respectively. Records are available beginning in 1979 and inputs prior to 1979 are assumed to be equal to the first year of data available in order to calculate a complete annual budget between 1973-2019. Weekly NO_3-N concentration and streamflow data for both watersheds were retrieved from the USDA Forest Service Timber and Watershed Lab, Parsons, WV.

Above-ground and below-ground biomass and C and N pools

Diameter at breast height (DBH) of all trees within 0.004 ha plots in WS6 and 0.04 ha plots in WS7 was measured by the USFS in 2018 in both watersheds. Individual tree measurements were converted to kg dry weight biomass using allometric equations for above-and below-ground biomass following the methods of Kelly 2010. Spruce height was determined from DBH using the equation from Huang et al. (1992), (species group 5; function 13) (Table 1) before applying biomass allometric equations. For above-ground biomass for Norway spruce, equations from Fehrmann and Kleinn (2006) – which use both DBH and tree height – were applied. To estimate biomass below-ground in the spruce watershed, we applied the equation from Drexhage and Gruber (1999). Individual tree biomass was then scaled up to total kg ha^{-1} for both WS6 and WS7.

For WS7, above-ground biomass was determined using the equation from Jenkins et al. (2003). This equation uses species-group-specific variables, and the following was applied: soft maple/birch equation was applied to red maple and black birch (*Betula lenta*); hard maple/oak/hickory/beech equation was applied to sugar maple, Northern red oak, black cherry (*Prunus serotina*), yellow poplar, and American beech (*Fagus grandifolia*); Mixed hardwood equation was applied to all other species. Below-ground biomass was estimated using the equation by Vandeboncouer et al. (2007) for all northern hardwood species at mid-elevation, developed from data from the Hubbard Brook Experimental Forest in New Hampshire, USA.

Using above-and below-ground biomass estimate, tree compartment mass and %N (roots, bole wood, twigs and branches, bole bark, foliage) were estimated using published values from Whittaker et al. (1974) for WS7 and Feng et al. (2008) for WS6. C concentration was assumed to be 50% of biomass within each compartment. C and N content by each tree compartment mass (kg) was summed to estimate total biomass C and N values (Whittaker et al. 1979: Feng et al. 2008).

Soil C and N pools

To determine C and N content, soil samples were ground with a mortar and pestle, while forest floor samples were kiln dried to 105 ºC and passed through a Wiley mill grinder. Ground soil and forest floor samples were weighed on a microgram scale, packed into tin capsules, and total C and N were measured in a Carlo Erba NA 1500 N, C, S elemental analyzer. Coarse fragment and rock content were determined on a subset of sample to determine percentage of fine material. Corrections for bulk density and coarse fragments and rock content were applied to final calculations of C and N to convert concentration to kg ha^{-1}. To determine inorganic N content, fresh soil was extracted using a 2 *M* KCl solution, shaken for one hour, and filtered. Extracts were

analyzed for NO_3-N and NH_4-N concentration via a microplate sulfanilamide colorimetric analysis (DeForest 2011).

As an estimate of the active C pool in mineral soil, we measured permanganate oxidizable carbon (POXC; Culman et al. 2012). Five g of dried soil were mixed with 2 ml of 0.2 *M* $KMnO_4$ solution and 18 ml H_2O, then placed on an orbital shaker for 2 minutes, and allowed to settle for 10 minutes, before 0.5 ml of the supernatant was pipetted and then diluted to 50 ml with deionized water. An aliquot (100 µL) was pipetted into a clear 96-well plate, along with a duplicate set of standards and deionized water control and read at 550 nm using a Synergy HTX plate reader (Biotek, Winooski, VT).

To determine water-stable aggregate distribution, we utilized a wet-sieving method. Air-dried soil samples were sieved to 2 mm, then a 100 g subsample was placed in a device based on those designed by Yoder (1936) and Ekwue et al. (2018), utilizing the methodology of Mikha and Rice (2004) and Kelly et al. (2014). This device uses tiered sieves to stratify and collect all soil size fractions greater than 53 µm. Sieve sizes were 2000, 1000, 500, 250, and 53 µm. Any soil particles not captured by the sieves, and thus smaller than 53 µm were considered free micro-aggregates or unassociated clay particles. To slake subsamples, the nested sieves were slowly submerged into a 2.5 m*M* $CaCl_2$ solution to prevent aggregate dispersion. Yoder (1936) explains that pure water causes dispersion forces on soil particles, citing Demolon and Henin (1932) who suggest a solution of $Ca(NO_3)_2$. Because we intended to later measure for N-based compounds, we used $CaCl_2$ instead for the same effect. After 10 minutes of soaking, the apparatus is run for ten minutes, agistating soils at a 4 cm stroke length at 30 rpm. After agitation, samples are washed and transferred from sieves to drying tins and dried in an oven at 50 ºC until fully dry, then weighed. Subsamples of each stratified size class were dried at 105 ºC to correct to dry weight.

To determine OM content in each aggregate fraction, bulk samples and sieved subsamples (0.5 – 10 g) of dried soil were weighed in aluminum tins, placed into a muffle furnace at 550°C for 8 hours. Upon removal, samples were allowed to cool, then weighed to determine the loss of mass due to ignition of OM (Bao et al. 2011).

Data Analysis

The experimental design of this study is an example of pseudo-replication, with an effective sample size of one (Gilliam, 1994; Hurlbert 1984). Differences in data are assumed to be a result of vegetative effects because 1) the soil descriptions in each watershed are predominantly the same, and similar in clay mineralogy, and 2) previous data of stream conductivity indicate that changes in stream chemistry and soils primarily occurred after the clear-cut harvests in 1967 and the planting of Norway spruce in WS6 (Kelly 2011). To determine differences in C and N pool sizes, inorganic N, POXC, and aggregate weight and organic matter distribution between each watershed, data were analyzed using a nonparametric Wilcoxon two-sample test. Statistical analyses were performed using SAS-JMP version 14.0 (SAS Institute, Cary, NC) at α=0.05 significance level.

Results

Soil and forest floor C and N pools

In 2008, mineral soil from WS7 contained significantly more C and N than soil from WS6 in both A- and B-horizons (Fig. 2). For example, in 2008, A-horizon soil WS7 contained approximately 20% more C (kg ha^{-1}) than spruce soil from WS6. In the 2020 assessment, no significant differences were detected in soil C or N content between watersheds in either soil horizon. In the A-horizon, WS7 previously had greater C content, though current estimates show that WS6 now has similar C content in A-horizon soil (WS6 = 47,053; WS7 = 42,784 kg C ha^{-1}; $p = 0.150$). In the B-horizon, WS7 had significantly more C in 2008, but now, C in the B-horizon is similar between watersheds (WS6 = 68,294; WS7 = 70,156 kg C ha^{-1}; $p = 0.812$). Regarding N, WS7 soil had a larger N pool than WS6 in 2008 in both the A- and B-horizons, but now the differences are no longer significant (A-horizon: WS6 = 2,083; WS7 = 2,868 kg N ha^{-1}; $p = 0.085$; B-horizon: WS6 = 3678; WS7 = 4,594 kg ha^{-1}; $p = 0.200$) (Fig. 3). Between 2008 and 2020, both watersheds experienced increasing mineral soil C and N.

In 2008, forest floor biomass in WS6 was 43% greater than in WS 7 (Fig. 4), resulting in greater C and N content in forest floor material from WS 6. In 2020, forest floor biomass had increased in both watersheds, though to a much greater extent in WS 6 (Fig. 4). WS7 increased by 253%, while WS6 increased by 482% from 2008 to 2020. Forest floor biomass in WS6 is now 206% greater than in WS7. In both the 2008 study and 2020, C and N pools in the forest floor of WS6 ($p = 0.001$ and 0.003, respectively) were significantly greater than in WS7. Both C and N pools increased in both watersheds between studies, though WS6 increased by a greater amount than WS7 (C: WS6 = 24,034; WS7 = 8,322 kg C ha^{-1}; N: WS6 = 842; WS7 = 294 kg N ha^{-1}).

Tree biomass pools

In 2008, total above-ground biomass estimates were approximately 30% less in WS6 (116,800 kg ha^{-1}) relative to WS7 (166,000 kg ha^{-1}). Estimates of below-ground biomass were approximately 45% lower in WS6 (21,000 kg ha^{-1}) relative to WS7 (38,000 kg ha^{-1}) (Fig. 4). Total biomass of both watersheds increased since 2008, though live tree biomass in WS6 increased more (136%) compared to WS7 (49%). Current total biomass values are not significantly different between watersheds ($p = 0.937$).

Aboveground biomass increased in both watersheds between 2008 and 2020, with more above-ground biomass accruing in WS6 (increased by 88%) relative to WS7 (increased by 46%) (Fig. 4). In 2020, above-ground biomass did not significantly differ between the watersheds ($p = 0.130$) (Fig. 4) (WS6 = 220,471; WS7 = 242,660 kg ha^{-1}). Within above-ground biomass, WS7 had a greater pool of N ($p = 0.048$) while no significant difference occurred between C pools (WS6 = 110,235; WS7 = 121,330 kg C ha^{-1}) (WS6 = 19,283; WS7 = 25,918 kg N ha^{-1}). In 2008, vegetation in WS7 contained 30% greater N and C in above-ground biomass.

Below-ground biomass increased in both watersheds over time, where below-ground biomass in WS6 increased by 85% and by 38% in WS7 (Fig. 4). WS7 maintains a significantly greater amount of below-ground biomass than WS6 in 2020 (WS6 = 38,565; WS7 = 51,836 kg ha^{-1}; $p < 0.001$). Between 2008 and 2020, below-ground C and N pools increased in both watersheds. The relative increase was greater in WS7 (38%) than WS6 (27%). WS7 had more C in the below-ground biomass pool than WS6 in 2008 and in 2020. Vegetation in WS6 increased in below-ground biomass N to a greater extent relative to vegetation in WS7 between 2008-2020. In 2008, WS7

had greater below-ground biomass N than WS6; in 2020, WS6 had greater below-ground biomass N. WS6 increased in below-ground biomass N by 85% and WS7 increased by 38%. In the below-ground biomass pool, WS7 had greater amounts of C (WS6 = 19,283; WS7 = 25,918 kg C ha^{-1}), while WS6 had greater N (WS6 = 463; WS7 = 368 kg N ha^{-1}) ($p < 0.001$ and = 0.001, respectively). This contrasts with the 2008 assessment in which vegetation in WS7 contained greater C and N in below-ground biomass.

Atmospheric inputs and stream exports

Wet and dry atmospheric deposition inputs of NO_3-N are assumed to be equal for both watersheds; NO_3-N exports from both watersheds are depicted in Fig. 5. Inputs of NO_3-N totaled 179.8 kg ha^{-1} during 1973-2018. Total NO_3-N stream export from WS6 since 1973 is 47.27 kg N ha^{-1}. This is only 12% of the NO_3-N that was exported from WS7 in the same time (399.06 kg N ha^{-1}). WS6 has continued the pattern of very low stream NO_3-N export.

N mineralization and inorganic N

Previous measures of total net N mineralization annual flux between 2007-2009 in A-horizon soils was approximately three times greater in the WS7 than in WS6. Current inorganic N content as extracted from field-fresh soil are 5.4 times larger in WS7 than WS6 (WS6 = 0.97; WS7 = 5.3 mg inorganic N kg^{-1} soil; $p < 0.001$). When total N is separated into NO_3-N and NH_4-N pools, soil from WS7 has significantly greater NO_3-N (WS6 = 0.25; WS7 = 4.3 mg kg^{-1} soil; $p < 0.001$), though there is no significant difference in NH_4-N between watersheds (Fig. 6).

Aggregate size distribution, OM, and POXC

Total aggregate sand-free weight (53-2000 μm) did not vary between watersheds in either the A- or B-horizon, though soil from WS6 had greater sand-free weight in the micro-aggregate size classes (53-250 and <53 μm) in both the A- and B-horizons ($p = 0.004$ and 0.052, respectively, in the A-horizon and $p = 0.035$ and 0.007 in the B-horizon; Fig. 7). Total aggregate OM did not differ between watersheds in the A-horizon ($p = 0.741$), but in the B-horizon, soil from WS7 contained greater aggregate OM than WS6 (WS6 = 21.7; WS7 = 44.5 g kg^{-1}; $p = 0.006$). While there were no significant differences in aggregate OM content between watersheds in the A-horizon within any aggregate size classes, in the B-horizon, soil from WS7 has significantly greater OM content in the macro-aggregate size fractions (500-100, 100-2000, >2000 μm) and slightly more OM in the smaller aggregate size classes (Fig. 7).

Soil in WS7 had greater POXC values in the A-horizon relative to WS6 (WS6 = 483.8; WS7 = 575.6 mg kg^{-1} soil; $p = 0.029$) and POXC was slightly greater in WS7 in the B-horizon (WS6 = 290.4; WS7 = 371.2 mg kg^{-1} soil; $p = 0.063$) (Fig. 8).

Discussion

Soil and forest floor C and N pools

Biogeochemical differences between hardwood and conifer forests are most often related to litter and forest floor dynamics. Globally, mean annual litterfall mass is greater in temperate hardwood forests (range 5.0 - 6.3 t ha^{-1} yr^{-1}) relative to temperate conifer forests (range 3.0 - 3.7 t ha^{-1} yr^{-1}); Landsberg and Gower 1997; Adams et al. 2019). However, standing forest floor mass is commonly greater in conifer forests relative to hardwood forests (mean 45 and 18 t ha^{-1}, respectively). Conifer forests commonly exhibit a mor-type forest floor (which is present in WS6 at FEF), while a mull-

type forest floor in common in hardwood forests. Mor-type forest floors exhibit an accumulation of intact plant material, a relatively low pH, dominant fungal decomposition, and strong boundaries between the organic and mineral soil horizon. Mull-type forest floor exhibits a gradual mixing of organic material with mineral soil, dominated by bacterial decomposition, and strong granular structure (Fisher and Binkley 2000; Adams et al. 2019). Turnover, or decomposition rate of plant litter in hardwood forests is approximately 4 years, and about 15 years in conifer forests (Waring 2002).

Between the 2008 and 2020 samplings, below-ground pools of C and N increased more in WS6 relative to WS7. S soil profiles influenced by conifer species often contain greater OM relative to soil of hardwood forests (e.g., 943 and 631 g kg^{-1} soil for 0 - 50 cm soil depth, respectively, in Maine, USA; Ohno et al., 2017) and supported by Chiti et al. (2012) who reported that, when comparing coniferous, broadleaf, and evergreen broadleaf systems, coniferous systems had the greatest SOC storage in both the 0-30 and the 30-100 cm soil depths tested. Contrary to our hypothesis that WS6 would still contain less C and N content than WS7, as was the case in 2008, soil C and N pools from WS6 increased at a faster rate than soil from WS7. While WS7 may have previously had a greater amount of C and N in the soil and forest floor pools, WS6 is on a trajectory to overtake it.

Forest harvest may result in loss of soil C, caused by disturbance and changes in soil temperature and moisture and resulting in increased decomposition and erosion, but C stores generally recover to original levels after several decades, especially if the stand regenerates to similar species composition as the pre-existing stand (Harrison et al. 1995, Chen et al. 2000; Kashian et al. 2006). However, species conversion may alter soil C recovery rates following harvest. A chronosequence study of soil C content beneath red spruce (*Picea rubens*) vegetation in northeastern North America (Diochon et al. 2009) showed that soils contain increasingly smaller stocks of C from 1-, 15-, and 45-year-old stands, reaching a minimum of approximately 76 Mg C ha^{-1} soil C after 45 years of regrowth. Soil C stocks then began to increase after 45 years and were considerably higher in 80- and 125-year-old stands. Carbon loss (decrease in C concentration and content) from young stands in the Diochon et al. (2009) study was reported to occur through enhanced mineralization of organic compounds (verified with stable C isotopic analysis), especially in the deeper soil horizons. This pattern of soil C depletion from younger conifer stands, followed by soil C accumulation after 45 years is reflected in WS6 at FEF, as earlier studies demonstrated reduced C pools, and more recent studies show marked increases.

Biomass

Much like the comparison of C and N pools in the forest floor and soil, total biomass of WS6 is increasing at a faster rate than WS7. While total biomass in WS7 was greater in 2008, WS6 now has grown to the point that there is not a significant difference in the above-ground biomass pool. This aligns with Keeton et al. (2011), who reported that when comparing the amount of biomass in medium- to old-growth forests and the trajectory of biomass through time, the percent of the stand that was made up of conifer species was a secondary predictor value. From our studies and those presented by Keeton et al. it appears that as a stand ages, conifers tend to accelerate in biomass accumulation at a greater rate than hardwoods, but that this effect may not significantly affect the stand until it begins to reach maturity. Based on the age of the stand (~50 years) this also agrees with Pearson et al. (1987) who found that in another conifer species - lodgepole pine (*Pinus*

contorta ssp. *latifolia*) – in an even-aged stand, maximum biomass accumulation rates are reached between 40 and 60 years, while in an uneven-aged stand, maximum biomass accumulation rate occurred after 80 years of development, implying that stand development is more influential on biomass accumulation rate than age.

Soil inorganic N

Inorganic N content was more than 5 times greater in WS7 A-horizon soil relative to WS6 (Fig. 6). Greater inorganic N availability is common in hardwood soils relative to conifer soils. Reich et al. (1997) reported that N mineralization was greater in oak hardwood forests when compared to natural conifer forests. Greater inorganic N content in WS7 aligns with our expectation that the conifer system has low rates of nitrification and N mineralization (Kelly et al. 2011), and is likely reflected in the significantly lower POXC values measured in the A-horizon of WS6 (Fig. 8). The lower POXC in the spruce system may be a result of increased C:N ratio of litter or decreased pH (Waring 2002; Francis 1982). Interestingly, while there is a significant difference between watersheds in NO_3-N, NH_4-N content is similar. Francis (1982) stated that nitrification occurs in more neutral to alkaline soils and cites examples of studies in which acidifying conditions reduced nitrate formation; as WS6 has a lower pH (though not statistically significant) compared to WS7, this can potentially have caused reduced nitrification, as demonstrated by Kelly et al. (2011), who attributed the effect to organic substrate suitability. Increased C in the forest floor layer can also suppress nitrification (Van Miegroet et al. 1990), and with the increased C:N ratio of conifer litter, this could also be a cause of the decreased NO_3-N in soil from WS6. Compton and Boone (2000) also reported that NO_3-N accumulation in a conifer system was depressed compared to a hardwood system.

Atmospheric deposition and stream export of NO_3-N

WS6 exports have remained at nearly zero NO_3-N, while exports from WS7 remain relatively high in a pattern related to atmospheric inputs. Currie et al. (1996) found that at the Harvard Experimental Forest in Massachusetts, USA, when comparing a conifer stand (red pine, *Pinus resinosa*) that was ~70 years old to a hardwood stand ~50 years old, the conifer stand had greater inorganic N leaching to the mineral soil from the forest floor. Kelly et al. (2011) previously explained that WS6 has a lowered nitrification rate compared to WS7, potentially due to high soil C:N ratio and the degradability of the OM, and this decreased nitrification can reduce stream NO_3-N export.

Aggregate weight and OM distribution and POXC

Soil aggregate distribution in WS6 exhibited a strong shift to dominance by micro-aggregates (53-250 μm and <53 μm relative to soil from WS7, and this occurred in both A- and B-horizon. This may be a function of macro-aggregate dispersion via acidification of soil (A-horizon soil pH WS6 = 3.85; WS7 = 3.97; B-horizon soil pH WS6 = 4.03; WS7 = 4.16) and lower microbial activity and byproducts (A-horizon POXC WS6 = 483.8; WS7 = 575.6; $p = 0.029$; B-horizon POXC: WS6 = 290.4; WS7 = 371.2 mg kg^{-1}; $p = 0.063$). We anticipated that the more recalcitrant litter and acidic conditions in WS6 would result in decreased bacterial activity, and therefore a greater proportion of OM occurring in larger aggregate size classes or as particulate organic matter unassociated with soil particles, and that the dispersion forces of acidification in WS6 would lead to greater fraction weight in the <53 μm size class. With reduced decomposition, a greater proportion of OM within WS6 resides in the larger macro-aggregates or unassociated <53 μm

fraction. In WS6, within decreasing aggregate size classes, there is reduced OM content, potentially due to reduced decomposition resulting in larger particulates of OM – too large to be incorporated in smaller microaggregates, while the greater amount of OM in the <53 μm size class is likely made up of fungal hyphae and organic exudates. We did not expect that WS6 would have greater OM content in the <53 μm size class, and while it is not significantly different, there is a strong trend ($p = 0.065$).

WS7 has significantly greater OM content in the macro-aggregate size class, and trends towards greater OM content within the smaller aggregate size classes as well. If macro-aggregation is increased in soil with higher pH, this would provide physical protection of OM and greater accumulation of intra-aggregate OM in WS7 due to the more labile litter, and our results support that conclusion. This implies that larger aggregates were dispersed in soil from WS6 and the amount of aggregate-protected OM (Elliott 1986; Plante and McGill 2002; Mihka and Rice 2004) is greater in WS7. Because soil from WS7 has significantly greater POXC values in the A-horizon and slightly greater POXC values in B-horizon soil, as well as 5.4 times greater inorganic N content, we can surmise that hardwood vegetation allows for more rapid microbial activity and organic matter turnover than spruce vegetation, as expected.

Conclusion

Many questions remain concerning the ecosystem changes resulting from conversion of native hardwood forests to conifer monocultures. Our data show that that differences in the rate of C and N accumulation in the spruce watershed are in an accelerating pattern relative to the hardwood system, with the rate of accumulation of C and N increasing over time in the spruce watershed. There likely occurred a significant loss of both C and N immediately after conversion from hardwood to Norway spruce, and that as WS6 ages, this spruce watershed may store more biomass, C, and N relative to the hardwood forest of the same age. We are still unable to account for the large long-term discrepancy of N export from the two watersheds. Spruce vegetation has altered soil aggregation, where spruce soil is significantly dominated by micro-aggregate size classes and intra-aggregate OM is reduced relative to soil from the hardwood watershed, likely due to the recalcitrant litter from spruce and lower microbial activity. This information may help inform management of existing conifer planted forests, as well as for species selection in management efforts for C and N storage or stream N management in the future.

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Figures

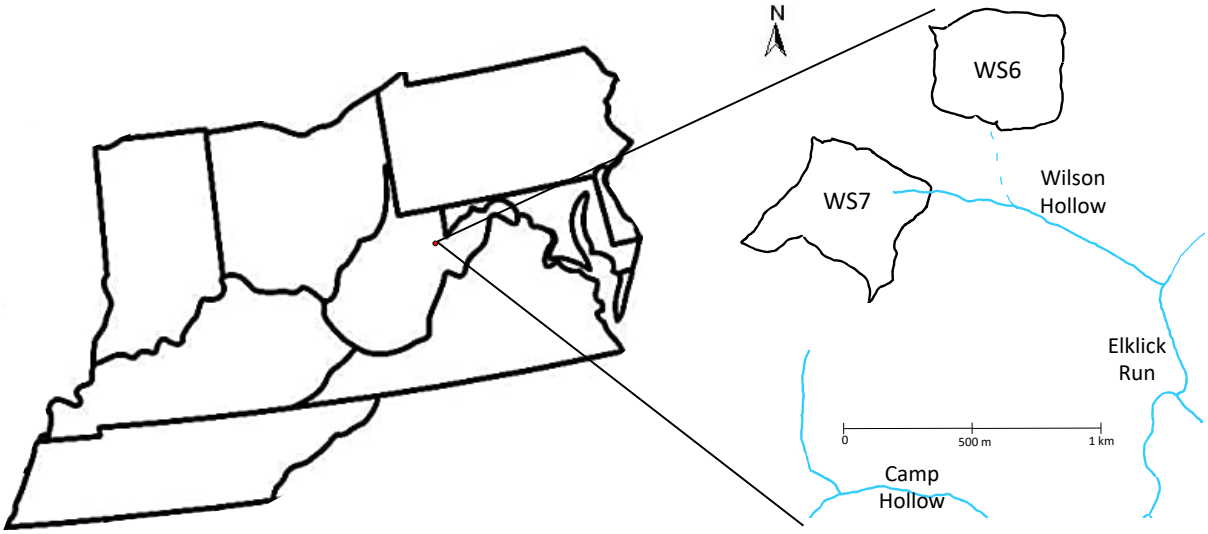

Figure 1. Location of the Fernow Experimental Forest and view of WS6 and WS7, within the Monongahela National Forest in Parsons, WV.

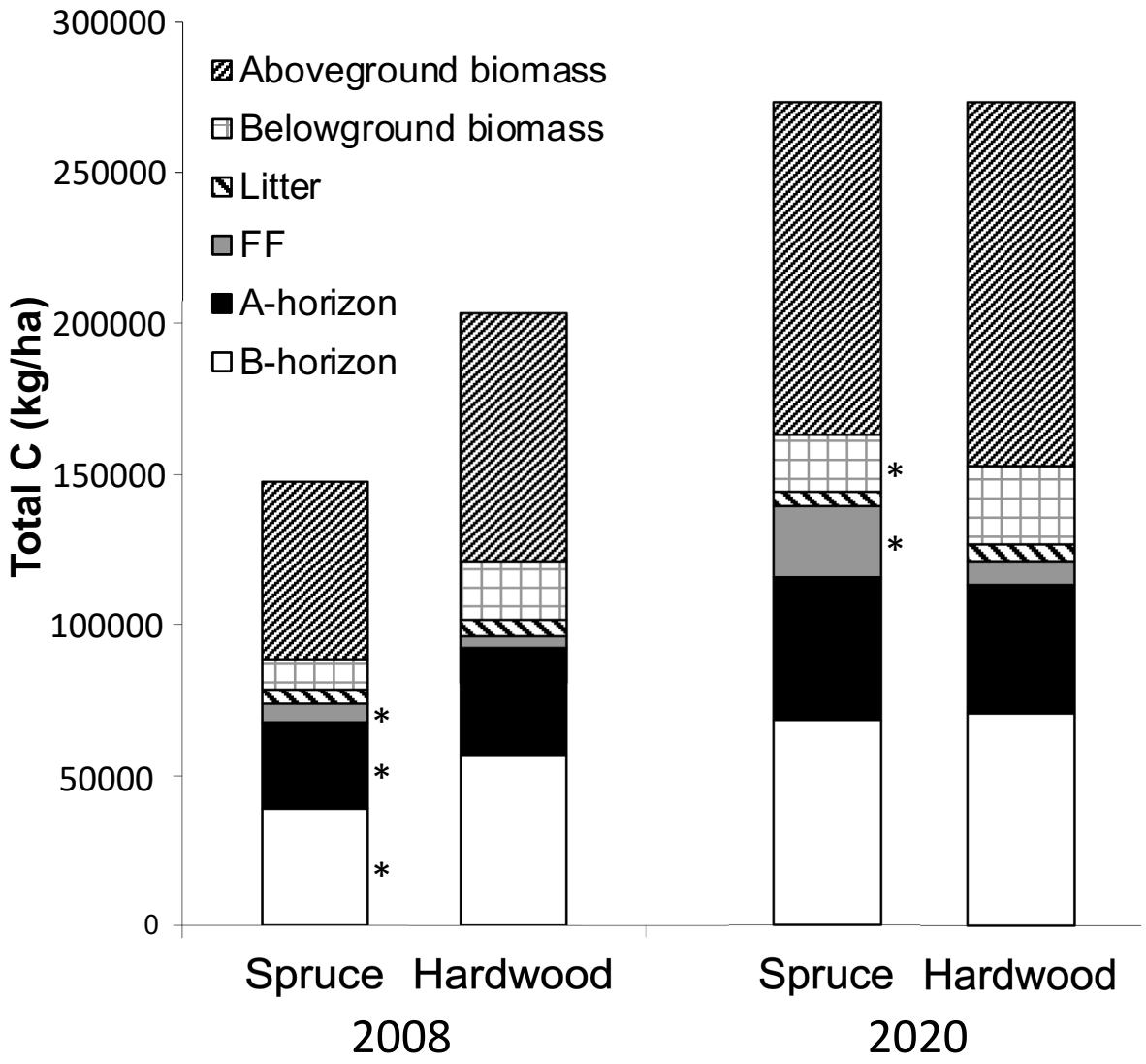

Figure 2. Watershed budget depicting C content contained within each soil and biomass compartment in the spruce (WS6) and hardwood (WS7) watersheds. Asterisks denote significant differences between watersheds in the associated pool in the respective year ($\alpha = 0.05$).

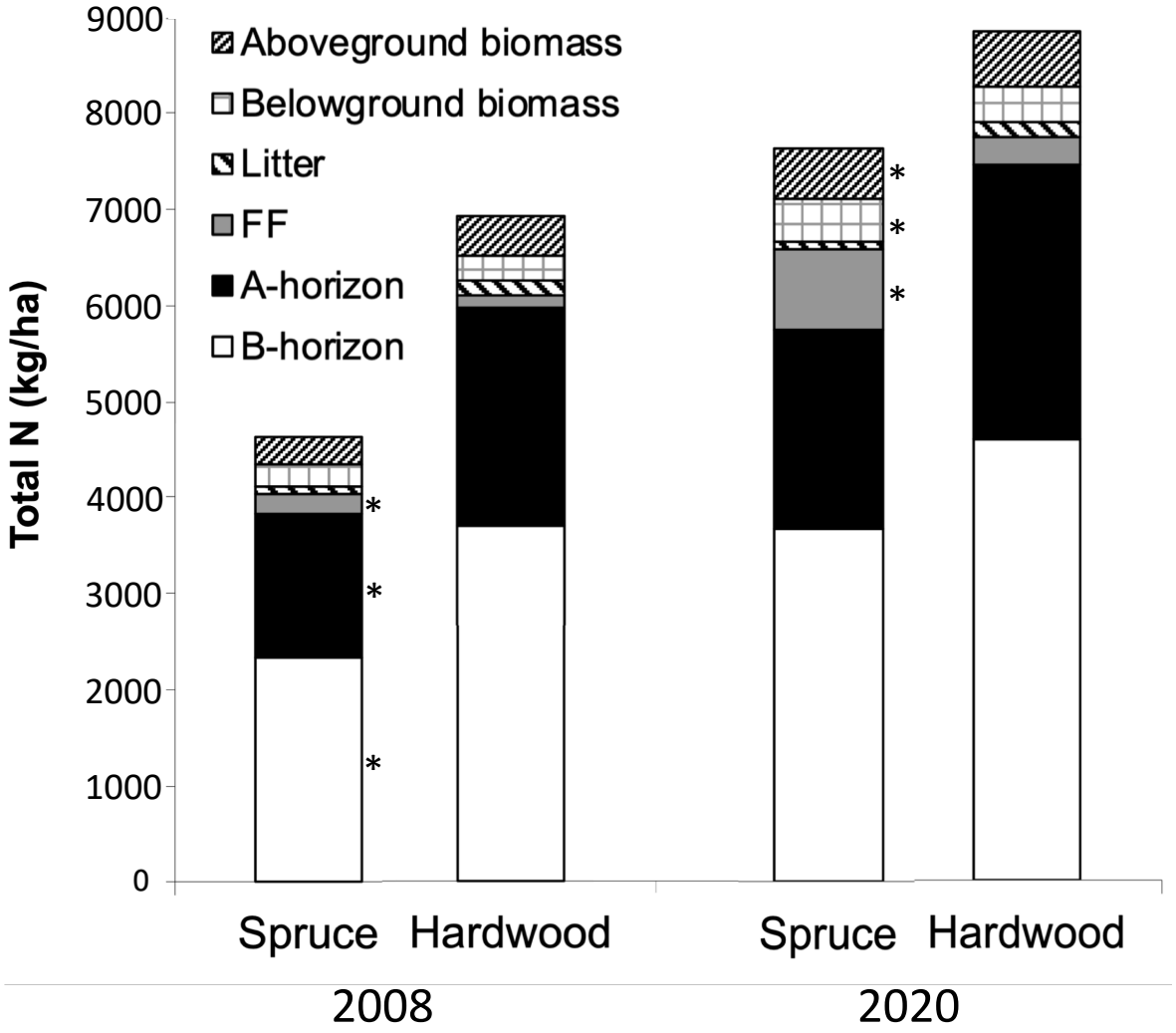

Figure 3. Watershed budget depicting N content contained within each soil and biomass compartment in the spruce (WS6) and hardwood (WS7) watersheds. Asterisks denote significant differences between watersheds in the associated pool in the respective year ($\alpha = 0.05$).

Figure 4. Watershed budget depicting biomass contained in the spruce (WS6) and hardwood (WS7) watersheds. Asterisks denote significant differences between watersheds in the associated pool in the respective year ($\alpha = 0.05$).

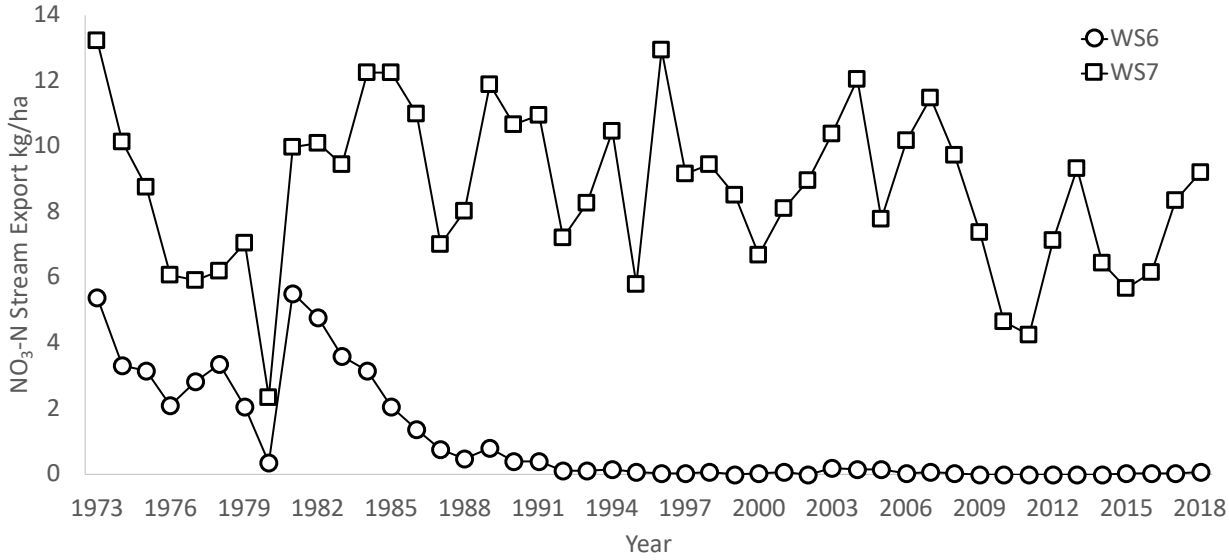

Figure 5. Depiction of yearly stream NO_3-N export of WS6 and WS7. Data courtesy of USFS Timber and Watershed Lab, Parsons, WV.

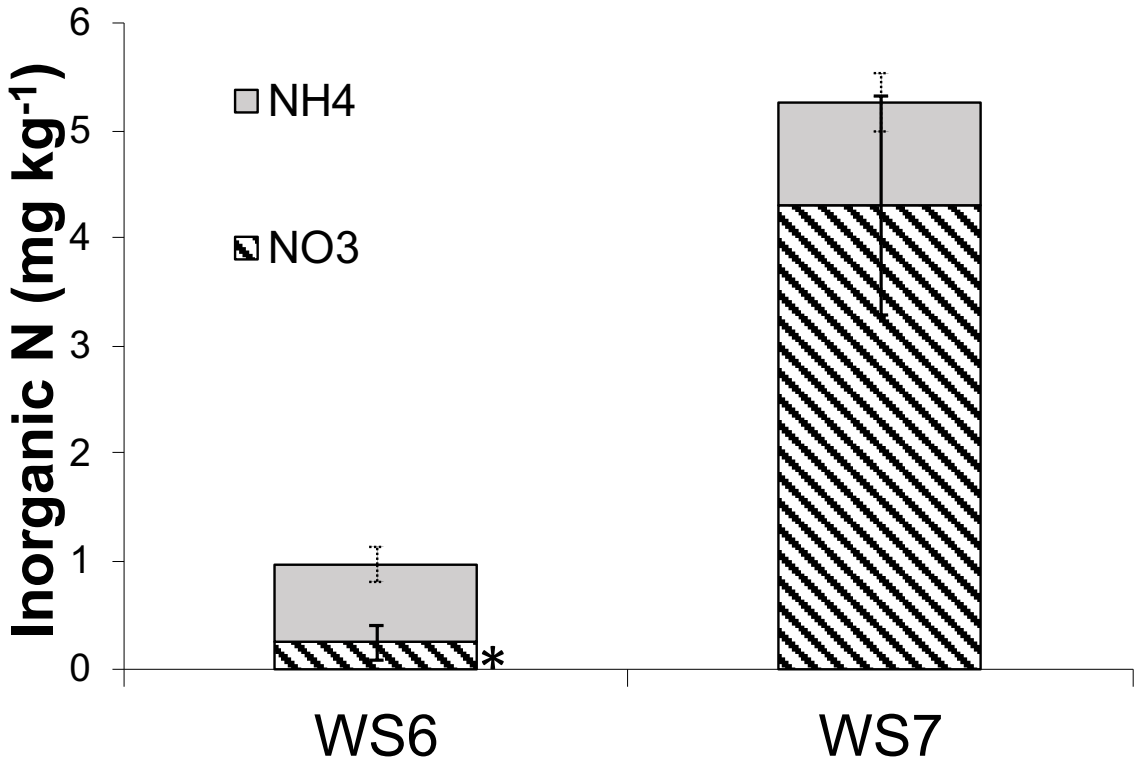

Figure 6. NO_3-N and NH_4-N content from A-horizon soils (n = 18). Asterisks denote significant difference between watersheds as determined by Wilcoxon signed-rank test ($p < 0.05$). Solid error bars denote standard error for NH_4 values, dashed error bars denote standard error for NO_3 values.

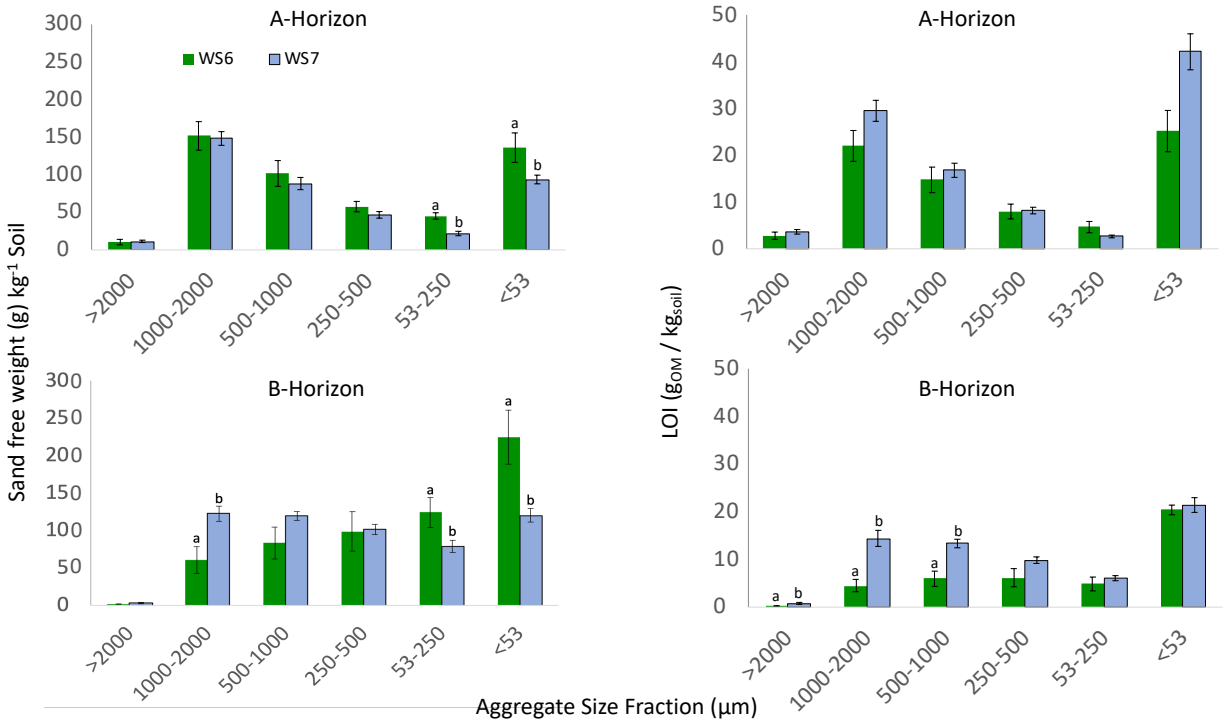

Figure 7. Sand-free and OM weight within each aggregate size class by watershed in A-horizon and B-horizon. For each horizon, bars with different letters within each aggregate size fraction are significantly different according to Kruskal-Wallace means comparison ($p < 0.05$).

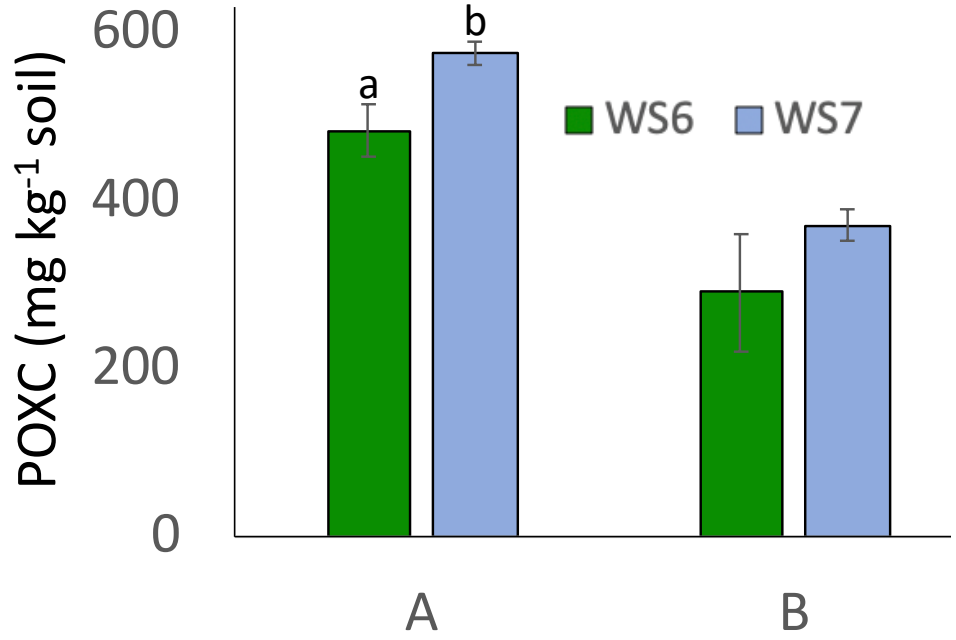

Figure 8. Mean POXC values within each soil horizon by watershed. Within each horizon, bars with different lowercase letters are significantly different according to Wilcoxon signed-rank test ($p < 0.05$).

Table 1. Equations and variables used for estimations of above and below-ground biomass pools (adapted from Kelly (2010)).

Compartment	Equation	Variables	Reference
Hardwood above-ground biomass (kg) (agb)	agb = Exp ($\beta_0 + \beta_1$ ln D)	Tree species: Soft Maple/birch: β_0 = -1.9123; β_1 = 2.3651 Hard maple/oak/hickory/beech: β_0 = -2.0127; β_1 = 2.4342 Mixed hardwood: β_0 = -2.4800; β_1 = 2.4835 D = DBH Exp = exponential function	Jenkins et al. (2003)
Hardwood below-ground Biomass (g) (bgb)	bgb = A + B log (D)	A = 1.5766 B = 2.3407 D = DBH (cm)	Vandeboncouer et al. (2007)
Spruce height H = height (m)	H = 1.3 + a(1-e^{-bDc})	a = 24.5127 b = 0.0308 c = 1.1361 D = DBH (cm) e = exponential function	Huang et al. (2003)
Spruce above-ground biomass agb = above-ground biomass (kg)	ln (agb) = ln a + b (ln D)	Tree height class (m): 4.2-8.0: a = 0.155 b = 2.061 8.0-11.7: a = 0.585 b = 1.643 11.7-15.5 a = 0.194 b = 2.205 19.3-23.0 a = 0.420 b = 1.519 23.0-26.8 a = 1.229 b = 1.711 26.8-30.6 a = 1.146 b = 1.772 D = DBH (cm)	Fehrmann and Kleinn (2006)
Spruce below-ground biomass bgb = below-ground biomas (kg)	bgb = β_0 * (D) $^{\beta 1}$	β_0 = 0.02 β_1 = 2.36 D = DBH (cm)	Drexhage and Gruber (1999)

Table 2. Values for biomass and N concentration by tree compartment for *Acer saccharum* and *Picea abies* used for estimation of above- and below-ground N content (adapted from Kelly (2010)).

	Tree Compartment	**Biomass**	**N Concentration**
		% of total	%
Acer saccharum[1]	Branch and bark wood	31	0.37
	Stem bark	7.5	0.55
	Stem sapwood	59.6	0.098
	Twigs and leaves	1.5	2.19
	Roots	--	0.71
Picea abies[2]	Needles	5.0	1.30
	Twigs and Branches	0.6	0.67
	Bole bark	5.0	0.52
	Bole wood	90.0	0.16
	Roots		1.20

[1]From Whittaker et al. (1974 and 1979)
[2]From Feng et al. (2008)

Chapter 4

Conclusions

Alterations to forested systems carried out in the Fernow Experimental Forest (FEF) have long-lasting and notable impacts. Soil from a hardwood-dominated forest that has experienced long-term fertilizer deposition exhibits dispersion of macro-aggregates and dominance by micro-aggregates and soil particle <53 μm relative to the reference watershed. Additionally, greater soil aggregation and aggregate-associated organic matter (OM) storage under tree species with more labile litter occurred with fertilization, compared to an adjacent natural hardwood watershed in which soils under trees with more recalcitrant litter show greater soil aggregate formation and aggregate-associated OM. Soil beneath a monoculture of Norway spruce (*Picea abies*) established ~50 years ago displayed decreased soil pH, and stream N export than the adjacent reference hardwood watershed.

Results from an analysis of soil aggregates presented in Chapter 2 indicate that fertilizer deposition results in reduced pH, reduced aggregation and aggregate OM storage under tree species with more recalcitrant litter, and reduced total aggregate-associated OM content, when compared to an equal-aged reference watershed. However, soil in the fertilized watershed exhibited greater aggregate OM storage and aggregate weight in the micro-aggregate size class (53-250 μm), especially under tree species with more labile litter. It was hypothesized that in the fertilized watershed, the reduced pH would limit bacterial degradation of litter, resulting in less OM storage and aggregation in the microaggregate size class, however, the effects of fertilization on mycorrhizal fungi may account for that discrepancy. Therefore, as ecosystems respond to decreasing N and sulfur (S) deposition, as a result of the Clean Air Act, there is potential for the labile, unprotected C in the <53 μm size class to be further decomposed due to the recovering bacterial and fungal activity.

In Chapter 3, an investigation of watershed N and C budgets and pool size comparison between equal-aged and neighboring watersheds of monoculture Norway spruce (*Picea abies*) and native mixed hardwood show reduced total C and N pools in the spruce watershed, but that the spruce watershed is accumulating C and N at a faster rate than the hardwood watershed. A significant loss of both C and N likely resulted from initial stand conversion. We are still unable to resolve the case of reduced stream NO_3-N export from the coniferous system. Initial hypotheses were that increased recalcitrance of spruce litter and decreased soil pH would result in decreased decomposition processes, reduced aggregation, limited nitrification, and lowered C and N pool sizes. Our findings show that C and N accumulation rates are in a constant state of flux compared to the hardwood system, as C and N accumulation rates increase over time in the spruce system. Soil from the spruce watershed contained significantly greater weight in micro-aggregate size classes and is reduced in intra-aggregate OM compared to the hardwood system, likely due to the more recalcitrant litter from spruce and lower microbial activity.

Findings shown in this thesis emphasize the impact that above-ground alterations to ecosystems can have on below-ground processes, especially in systems influenced by increased N and S deposition or conversion to coniferous species. Carbon storage potential and nutrient dynamic repercussions should be fully taken into account when making forest management decisions.