

Article

Fertilization and Tree Species Influence on Stable Aggregates in Forest Soil

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Abstract: *Background and objectives:* aggregation and structure play key roles in the 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 the capacity to store C may be disrupted by the 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. *Materials and methods:* 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 $(\text{NH}_4)_2\text{SO}_4$ 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. *Research highlights:* fertilized soil exhibited decreased macro-aggregate formation and a greater proportion of smaller micro-aggregates or unassociated clay minerals, particularly in the B-horizon. This shift in aggregation to soil more dominated by the smallest ($<53 \mu\text{m}$) 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 the $53\text{--}2000 \mu\text{m}$ fractions), 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 more OM in the micro-aggregate size class ($<250 \mu\text{m}$), especially in the fertilized watershed, while species with more recalcitrant litter promoted more OM in the macro-aggregate size classes ($500\text{--}2000 \mu\text{m}$) in the reference watershed. *Conclusions:* long-term fertilization, and likely historic atmospheric deposition, of forest soils has weakened macro-aggregation formation, with implications for soil stability, hydrology, and storage of belowground C.

Keywords: Fernow Experimental Forest; acidic deposition; organic matter; mycorrhizal fungi; soil aggregation; forest soils



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1. Introduction

Changes in soil aggregation over time may be 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 [1]. Martin et al. [2] described three aggregate-forming processes: (1) bacteria and fungi binding particles together; (2) gelatinous organic materials holding particles together; (3) clay particles cohering either

due to organo-mineral interactions or flocculation around iron and aluminum oxides and entrapping larger particles. All three of these processes and their interplay determine the distribution of macro- vs. micro-aggregates in soil. These processes have been well-studied more recently to define macro-aggregates ($>250\ \mu\text{m}$) as being made up of a conglomeration of smaller micro-aggregates ($<250\ \mu\text{m}$) as well as particulate organic matter (OM), fungal hyphae, organic binding agents, and pore space [3–6]. The process of aggregation can also result in positive feedback, with mycorrhizal and other fungi able to grow in macro-aggregate pore spaces [7]. In contrast, micro-aggregates provide habitat space for up to 70% of soil bacterial species [8,9]. Within aggregates, especially micro-aggregates, soil carbon (C) is also physically protected from bacterial decomposition through strong organo-mineral interactions with polyvalent cations [4,10,11], 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 [12]. Differing factors, including biological influences, soil parent material, degree of weathering and clay mineralogy, and soil acidity and ionic composition can all affect aggregation of soil particles.

The clay mineralogy of soil, specifically the proportion of 2:1 clays (such as montmorillonite or vermiculite) relative to the proportion of 1:1 clays (kaolinite or serpentine), controls aggregation. In systems with limited C inputs, 1:1 clays tend to form more stable macro-aggregates, while in systems with more C input, 2:1 clays tend to form more stable macro-aggregates [13]. 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 [14]. 2:1 clays have greater specific surface area and cation exchange capacity (CEC), leading to stronger aggregation forces at mineral surfaces [15], 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 [13]. 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 [14].

1.1. 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 [16–19]. 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 [20]. 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 [16].

AM fungi have previously been shown to be positively correlated to soil aggregate development [21,22] 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 [23]. As described by Six et al. [6], bacterial decomposition products aid mostly in the formation of micro-aggregates while fungi aid in the formation of macro-aggregates due to the “sticky string bag” formed by hyphae [24]. Following the concept of the Microbial Efficiency Matrix Stabilization (MEMS) framework [17], labile plant litter associated with AM tree species promotes more rapid microbial activity and a greater amount of microbial products, which may create more organo-mineral interactions and greater aggregate formation [25,26]. 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 [27], these expected C and aggregate dynamics may be disrupted [28].

1.2. Acidity and Ionic Composition Influence on Aggregation

In ecosystems experiencing elevated N deposition, soil microbial communities and activity are altered [29–32], resulting in decreased enzymatic activity and decomposition rates and increased soil C [30,33]. While more microbially processed OM can facilitate soil aggregation, acidification processes (e.g., oxidation via nitrification and leaching of base cations such as Ca^{2+}) due to elevated atmospheric deposition of N may result in decreased aggregation [34], either through inhibition of microbial decomposition processes, changes in pH-dependent charges on mineral surfaces, or dispersion processes associated with particular cations. For example, with accumulation of NH_4^+ and H^+ and increases in electrical conductivity (EC) with deposition, soil aggregation may be reduced due to dispersion of soil colloids [35–37] as the relative concentrations of these ions increase, whereas the presence of abundant Ca^{2+} ions would result in flocculation or creation of aggregates [38,39].

West Virginia and the Central Appalachian region in eastern US have historically experienced high levels of atmospheric N deposition in the country. The decline in N deposition across the region resulting from policies stemming from the Clean Air Act [40] raises questions relating to ecosystem recovery, particularly the fate of C that has accumulated in soil as a result of acidification processes [31,33]. 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 long-term fertilization and tree species in soil aggregation 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)₂SO₄ and a reference watershed. Specifically, we aimed (1) to determine if experimental fertilization and documented acidification affects soil aggregation and OM distribution throughout the aggregate size fractions; (2) to determine if selected tree species differentially influence soil aggregation capacity and OM distribution throughout the aggregate size fractions; and (3) to 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 aggregate weight and intra-aggregate OM content; (2) AM-associated tree species, or those with relatively easily degradable litter, promote greater aggregate weight and intra-aggregate OM content, particularly 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.

2. Materials and Methods

2.1. 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 relatively evenly distributed per annum and averages 1458 mm [41]. 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 (−2.8 °C) [41].

The hardwood reference watershed (WS7-REF; 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-REF 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 [42]. Dominant vegetation in WS7-REF is tulip poplar (*Liriodendron tulipifera* Linnaeus), Northern red oak (*Quercus rubra* Linnaeus), and sugar maple (*Acer saccharum* Marshall), with an understory of dogwood (*Cornus florida* Linnaeus), striped maple (*Acer pensylvanicum* Linnaeus), cucumber magnolia (*Magnolia acuminata* Linnaeus), and several species of fern. From the most recent 2018 survey, basal area in this watershed was approximately 13.5 m² ha^{−1}.

The hardwood watershed that receives fertilizer application (WS3 -FERT; 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 [43]). The predominant clays of the FEF have been cursorily described as muscovite (2:1) and vermiculite (2:1) [44]. The watershed receives 35 kg N ha^{−1} yr^{−1} in the form of (NH₄)₂SO₄ 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-FERT is black cherry (*Prunus serotina* Ehrhart), red maple (*Acer rubrum* Linnaeus), black birch (*Betula lenta* Linnaeus), and American beech (*Fagus grandifolia* Ehrhart) [45], with an understory of striped maple, red maple, and increasing abundance of *Rubus* species. Basal area in this watershed is approximately 17.5 m² ha^{−1}.

2.2. Soil Sample Collection

To investigate how tree species influences aggregation size distribution and associated OM distribution, four live individuals of each tree species (tulip 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 a plot-center, we determined the surrounding stand basal area by species using a BAF-10 prism. To further understand how differing mycorrhizal associations may affect aggregation and carbon storage processes, we selected two tree species for investigation as AM-associated species (black cherry and tulip poplar) and two as 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 of the four cardinal directions around each tree within the dripline. A-horizon samples were collected between 0–10 cm depth, and B-horizon samples were collected between 10–45 cm depth. B-horizon soil is distinct from A-horizon soil visually by a lighter color, a greater silt content, and a subangular blocky structure [42]. The 45-cm depth sampling generally represents the extent of the B-horizon in these watersheds and has been described as a BW2 horizon [46]. 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; and $N = 64$ bulk soil samples).

2.3. Water-Stable Aggregate and Organic Matter Analyses

Soil samples were air dried following collection and then sieved to 2 mm. Approximately 100 g of air-dried soil was used for slaking and wet-sieving utilizing an apparatus

similar to that described by Yoder [47] and Ekwue et al. [48] and following the methodology of Mikha and Rice [11] and Kelly et al. [49]. 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-mM solution of CaCl_2 , to prevent aggregate dispersion. Yoder (1936) discussed that pure water exerts dispersion forces on soil colloids, and Demolon and Hénin [50] further suggested using a solution of $\text{Ca}(\text{NO}_3)_2$. Due to the intent to measure nitrogenous compounds, CaCl_2 was used instead to the same effect. Soils were soaked for 10 min. The machine agitated soils for 10 min at a 4-cm stroke length at 30 rpm. Samples were quantitatively transferred from the sieves into drying tins and placed in a drying oven at 50 $^\circ\text{C}$ until fully dried and then were weighed. Subsamples of each size class were dried at 105 $^\circ\text{C}$ for 24 h for correction to dry weight.

To express aggregate size fractions on 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 ($\text{Na}_6\text{P}_6\text{O}_{18}$) (SHMP) was added as a dispersing agent. Samples were left overnight, shaken for 4 h on an orbital shaker, then passed through a 53- μm sieve, washed with deionized water, and dried at 105 $^\circ\text{C}$ for 24 h. All aggregate weights are presented as sand-free corrected weights.

To determine soil pH, 5.0 \pm 0.10 g of air-dried soil was placed in 10 mL 0.01 M CaCl_2 , shaken, and allowed to settle for 1 h 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 and placed into a muffle furnace at 550 $^\circ\text{C}$ for 8 h. Upon removal, samples were allowed to cool and then weighed to determine the percent loss of mass due to ignition of OM [51].

As an estimate of soil microbial activity and the active pool of soil C, we measured permanganate oxidizable carbon (POXC) [52]; 5.0 \pm 0.10 g of dried soil was mixed with 2 mL of 0.2 M KMnO_4 solution and 18 mL H_2O , then placed on an orbital shaker for 2 min, and allowed to settle for 10 min 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 KMnO_4 stock solution and deionized water control and read at 550 nm using a Synergy HTX plate reader (Biotek, Winooski, VT, USA).

Clay mineralogy within watersheds was examined by X-ray diffraction (XRD) analyses. Soil samples from two locations across two 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 , and 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.

2.4. Data Analysis

Differences in aggregate weight, aggregate OM content, soil pH, and POXC attributed to fertilization or tree species were analyzed using nonparametric tests due to a lack of normality of all variables. When comparing two distinct groups (watershed comparison), the Wilcoxon two-sample test was applied using a normal approximation. When more than two groups were compared (tree species comparison), a pairwise Wilcoxon two-sample test was applied using a normal approximation followed by Kruskal–Wallis test to compare the effect of tree species within a watershed. For simplicity of interpretation, the data presented are calculated means and standard errors. All data were analyzed using SAS-JMP Statistical Software (Version 14.0, Cary, NC, USA) and a significance level of $\alpha = 0.05$. The statistical design of this study is an example of simple pseudo-replication, a common characteristic of ecosystem studies at the watershed scale [53]; thus, our data should be interpreted with that in mind and may not be applicable to other locations. It is a reasonable contention, however, that the effects reported here are treatment effects rather

than preexisting differences among watersheds as Adams and Angradi [54] showed that soil conditions were similar before fertilization treatment began.

3. Results

3.1. Clay Mineralogy

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

3.2. Bulk Soil OM, pH, and POXC by Watershed and Species

Differences in bulk soil OM were evident between watersheds in the B-horizon but not in the A-horizon (Table 1). In the B-horizon, WS7-REF had greater mean bulk OM than WS3-FERT ($p = 0.004$). Within WS3-FERT, 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-horizons in WS7-REF. In WS3-FERT, B-horizon, the mean OM beneath black cherry was significantly greater than that beneath red oak and tulip poplar ($p = 0.030$ for both) but was not significantly different from black birch. In both watersheds and in both horizons, bulk soil OM did not significantly differ between the AM- and ECM-associated species here, though there was a trend that AM-associated species had more bulk soil OM than ECM-associated species in WS3-FERT, 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).

Table 1. Mean bulk soil organic matter (OM), soil pH_{CaCl₂}, permanganate oxidizable carbon (POXC), and total aggregate weight and OM from each watershed and beneath four tree species: values indicate mean (\pm standard error). Values with different letters indicate statistically significant differences according to Wilcoxon pairwise means separation ($p < 0.05$). Significance is denoted between watersheds within the respective horizons and among species within the respective watershed and horizon. * Values represent sum of aggregate weight and OM in size classes between 53 and 2000 μ m, respectively.

Watershed and Horizon	Bulk Soil OM (g kg ⁻¹) n = 16	Soil pH _{CaCl₂} n = 16	POXC (mg kg ⁻¹) n = 16	Total Aggregate Sand-Free Weight (g kg ⁻¹) * n = 16	Total Aggregate OM (g kg ⁻¹) * n = 16	Tree Species	Bulk Soil OM (g kg ⁻¹) n = 4	Soil pH _{CaCl₂} n = 4	POXC (mg kg ⁻¹) n = 4
3A-FERT	198.65 (13.29)	3.57 ^a (0.07)	606.70 (9.72)	305.57 (61.52)	62.15 (19.02)	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-REF	209.89 (9.52)	3.97 ^b (0.10)	575.61 (13.85)	316.48 (80.28)	60.73 (16.09)	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-FERT	86.68 ^a (4.12)	4.09 (0.06)	404.70 (16.27)	342.30 ^a (75.54)	29.23 ^a (11.18)	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-REF	106.91 ^b (4.44)	4.16 (0.06)	371.23 (18.74)	425.18 ^b (53.51)	44.47 ^b (10.48)	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)

Soil pH differed by watershed in the A-horizon but not in the B-horizon (Table 1). In WS3-FERT, A-horizon, soil pH was 3.57 and 3.97 in WS7-REF ($p = 0.005$). In WS7-REF, 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 among species in WS7-REF, B-horizon, or in either horizon in WS3-FERT. Soil beneath AM-associated species exhibited a significantly lower pH in WS3-FERT relative to WS7-REF in both the A- and B-horizons, while soil pH beneath ECM-associated species did not differ significantly between watersheds. In the A-horizon, soil beneath AM-associated species had pH of 3.51 in WS3-FERT and of 4.21 in WS7-REF ($p = 0.003$), and in the B-horizon, pH of 4.02 in WS3-FERT and of 4.3 in WS7-REF ($p = 0.031$).

Mean POXC values did not differ statistically 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-FERT (mean: 311 g kg^{-1} soil) than in WS7-REF (mean: 217 g kg^{-1} soil) in the A-horizon (Table 1). No significant differences occurred in POXC as a function of tree species or fungal association type.

3.3. Total Sand-Free Aggregate Weight

Total aggregate sand-free weight (contained within aggregates between 53 and $2000 \mu\text{m}$) differed between watersheds, and greater total aggregate sand-free weight occurred in WS7-REF in the B-horizon ($p = 0.005$) relative to WS3-FERT, though no differences occurred in total aggregate weight between watersheds in the A-horizon (Figure 1). When analyzed by size fraction, aggregate sand-free weight differed between watersheds and horizons. Consistently, in both the A- and B-horizons, WS3-FERT soil contained greater aggregate sand-free weight in both the 53 – 250 and $<53 \mu\text{m}$ size classes relative to WS7-REF (A-horizon $p = 0.014$ and <0.001 , respectively; B-horizon $p = 0.007$ and <0.001 , respectively) (Figure 1). Comparing among tree species, in both WS3-FERT and WS7-REF, soil beneath differing tree species varied in sand-free weight across aggregate size classes (Figure 2). In WS7-REF, A-horizon, significant differences among tree species occurred in the 500 – 1000 , 250 – 500 , and 53 – $250 \mu\text{m}$ size fractions and in the 500 – $1000 \mu\text{m}$ fraction in the B-horizon. Variation by tree species on aggregate weight in WS3-FERT occurred only in $>2000 \mu\text{m}$ in the A-horizon and in 500 – 1000 in the B-horizon (Figure 2).

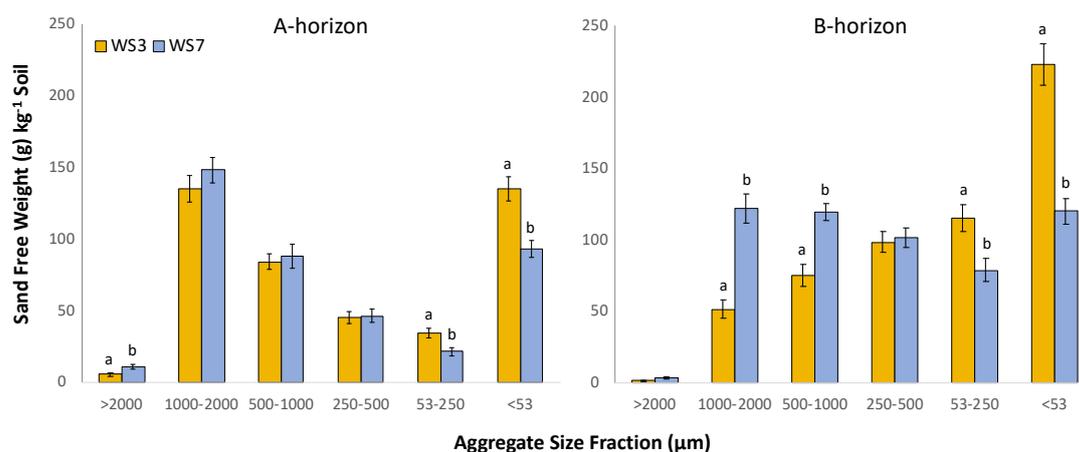


Figure 1. Sand-free weight within each aggregate size class by watershed (WS3-FERT and WS7-REF) in A-horizon and B-horizon: for each horizon, bars with different letters within each aggregate size fraction are significantly different according to Kruskal–Wallis means comparison ($p < 0.05$).

3.4. Aggregate-Associated Organic Matter

Total aggregate OM (contained within aggregates between 53 and $2000 \mu\text{m}$) differed between watersheds only in the B-horizon, where 52% more aggregate-associated OM occurred in soil from WS7-REF relative to WS3-FERT ($p = 0.001$). However, total intra-aggregate OM content was similar in the A-horizon between watersheds. Specific to aggregate size class, aggregate-associated OM differed between watersheds in the $>2000 \mu\text{m}$ and the 53 – $250 \mu\text{m}$ classes in the A-horizon and in the 1000 – $2000 \mu\text{m}$ and 500 – $1000 \mu\text{m}$ classes in the B-horizon (Figure 3). In the A-horizon, soil from WS3-FERT had 42% more OM in the 53 – $250 \mu\text{m}$ size class ($p = 0.011$) than soil from WS7-REF.

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 (Figure 4). Most often, significant differences within a watershed among species were detected in the A-horizon between black cherry and the other species—this difference of black cherry relative to other species occurred in both watersheds, though the effect was reversed

between the two watersheds. In WS7-REF 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 aggregates beneath black birch and red oak. However, in WS3-FERT B-horizon, in the 1000–2000 and 53–250 μm size fractions, the soil beneath black cherry contained more aggregate OM than soil beneath black birch ($p = 0.030$), though other comparisons were not significantly different.

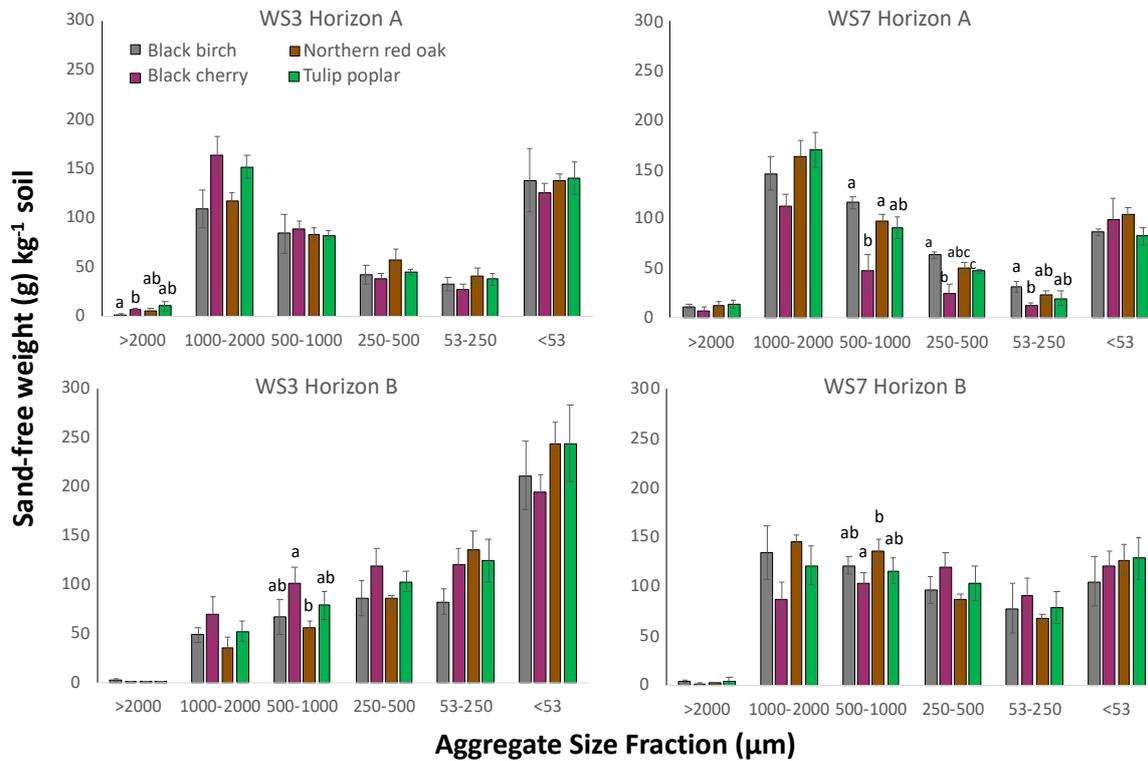


Figure 2. Sand-free weight within each aggregate size class by tree species in A-horizon and B-horizon by watershed (WS3-FERT and WS7-REF): for each horizon within each watershed, bars with different letters within each aggregate size fraction are significantly different according to Kruskal–Wallis pairwise means comparison ($p < 0.05$).

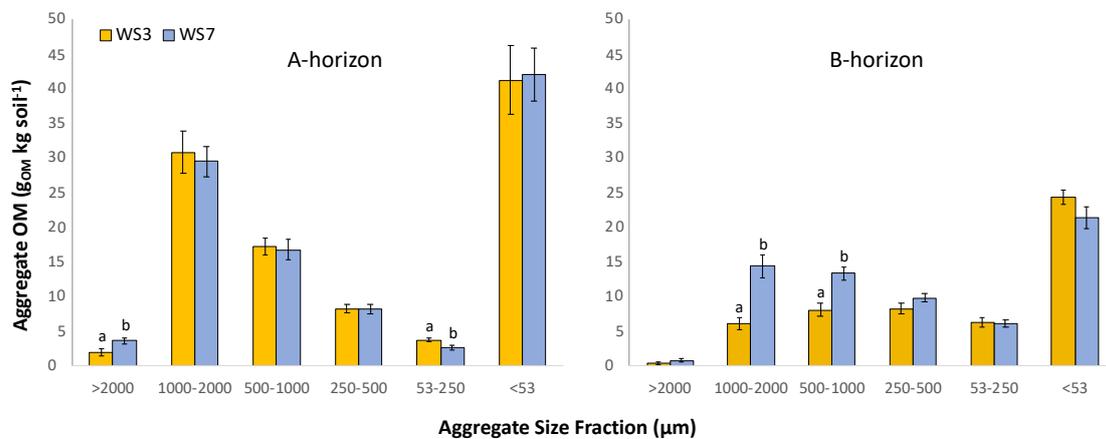


Figure 3. Organic matter within each aggregate size class by watershed (WS3-FERT and WS7-REF) in A-horizon and B-horizon: for each horizon, bars with different lowercase letters within each aggregate size fraction are significantly different according to Kruskal–Wallis means comparison ($p < 0.05$).

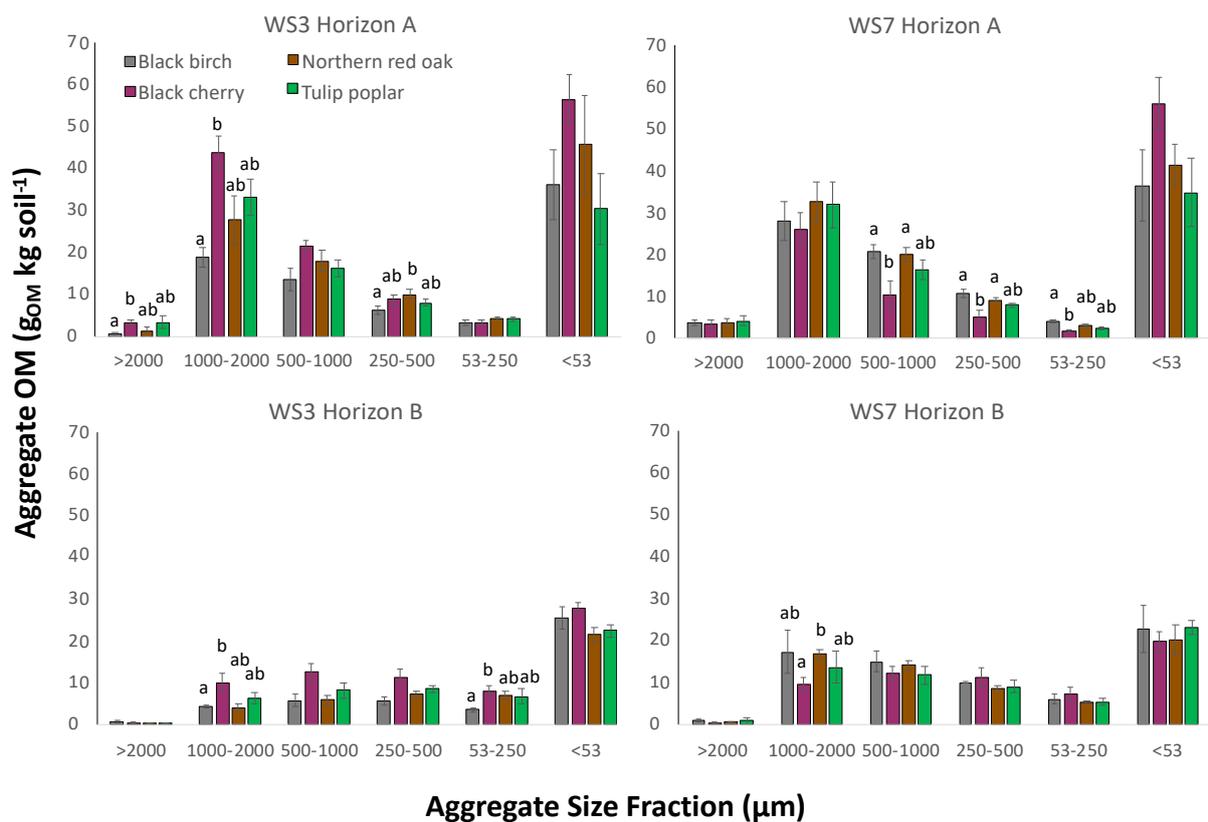


Figure 4. Organic matter within each aggregate size class by tree species in A-horizon and B-horizon (WS3-FERT and WS7-REF); for each horizon within each watershed, bars with different letters within each aggregate size fraction are significantly different according to Kruskal–Wallis pairwise means comparison ($p < 0.05$).

The influence of watershed on aggregate-associated OM varied by tree species (Table A1). For example, in soil beneath red oak in the A-horizon, WS3-FERT soil contained more OM in the 53–250 µm size fraction relative to WS7-REF ($p = 0.030$) and soil beneath black cherry in WS3-FERT contained more aggregate OM in the 1000–2000 and 500–1000 µm size class relative to WS7-REF ($p = 0.030$). In contrast, soil beneath tulip poplar exhibited similar aggregate OM content between WS3-FERT and WS7-REF in every size class (Figure 4).

3.5. Relationship between Micro-Aggregate Proportion and pH or POXC

Generally, lower soil pH was associated with both greater sand-free aggregate weight and OM content in micro-aggregate fractions (<53 and 53–250 µm) (Figure 5). In the A-horizon, in both the <53 and the 53–250 µm size classes, 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 (Figure 5). In both the A- and B-horizons across watersheds, within the <53 µm size class, greater POXC values are associated with more OM content ($p = 0.037$, $R^2 = 0.142$ —panel e, and $p = 0.018$, $R^2 = 0.18$ —panel f). Higher POXC is also associated with greater sand-free weight in the <53 µm 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 ($p = 0.048$, $R^2 = 0.12$).

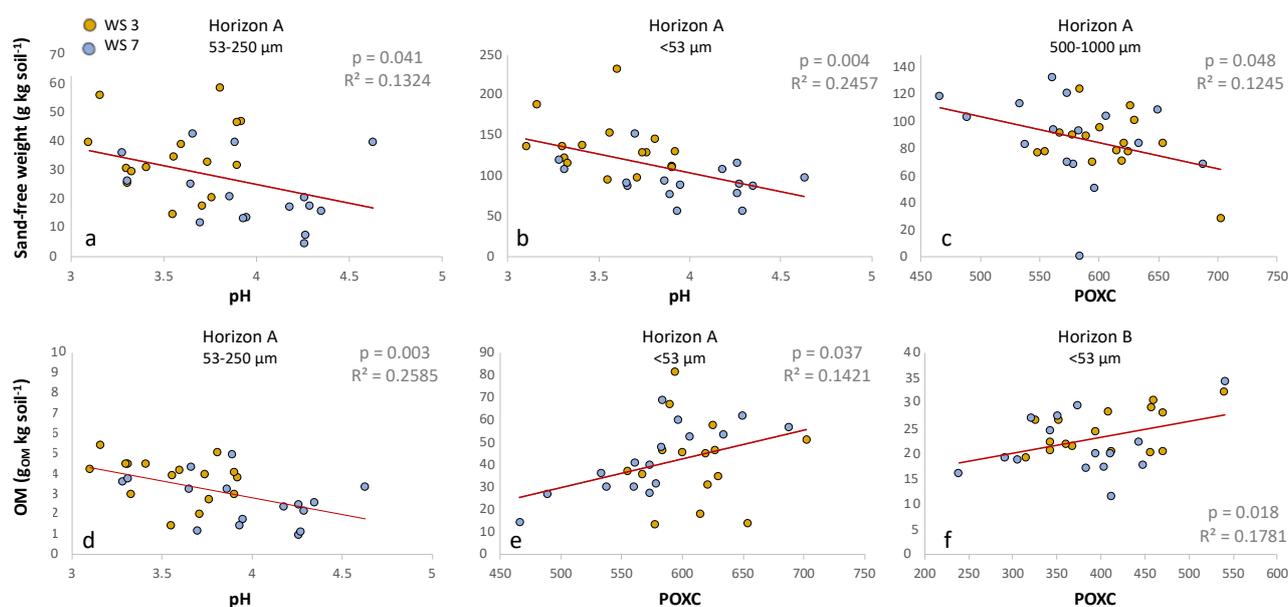


Figure 5. Significant relationships between (a,b,d) soil pH_{CaCl2} with micro-aggregate size fractions weight and OM, and (c,e,f) POXC (g_{POXC} kg soil⁻¹) with aggregate size fractions weight and OM: colored dots refer to data within a watershed. R² and *p*-values are denoted for each panel for all data across watersheds.

4. Discussion

This study builds on previous research at the FEF from this whole-watershed fertilization effort that has documented that the fertilized watershed exhibits altered N cycling [55–58]; slower litter decomposition rates [54]; and altered soil nutrient-acquiring enzyme activity, bacterial community composition, and below-ground allocation of C by vegetation [31] relative to reference watersheds. Here, we further document that fertilization of this forested watershed resulted in altered soil aggregation in soil beneath the four tree species selected here. Soil in the fertilized watershed exhibits less macro-aggregate formation and a greater proportion of smaller micro-aggregates or unassociated clay minerals, particularly in B-horizon soil. This shift in aggregation size distribution 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 reduced in the fertilized soil, most strongly in the B-horizon soil, suggesting less physical protection of C in forest soil receiving elevated deposition of N and S compounds. We contend that soil aggregation and aggregate-associated OM are important ecosystem parameters to monitor in assessment of forest soil quality because they are sensitive to fertilization via atmospheric deposition and are influenced by tree species. Both atmospheric deposition and dominant tree species may be altered as a function of human emissions and a changing climate.

4.1. Bulk Soil OM, pH, and POXC

Bulk soil OM was significantly greater in WS7-REF in the B-horizon relative to the fertilized WS3-FERT, and a similar, not statistically significant pattern occurred in the A-horizon between watersheds (Table 1). The greater OM in WS7-REF soil documented here differs from other studies reporting an increase in soil C following fertilization [59–61], which has been attributed to a decline in microbial extracellular enzymatic activity related to OM degradation and lower overall soil respiration [31,33]. Indeed, rates of soil respiration were approximately 13% lower in WS3-FERT relative to WS7-REF, as measured from June 2016 to May 2018 (Eastman and Peterjohn, unpublished data). However, the response of increased bulk soil C following N fertilization is not universal [60] and other studies reflect our results, as Fowler et al. [62] reported similar soil C content between fertilized

and reference plots at the Long-Term Soil Productivity (LTSP) study at the FEF. Soil OM values may be highly spatially variable, which also may help account for the differences in results between studies of soil OM values following fertilization.

Bulk soil OM also varied beneath different tree species, to the largest extent in WS3-FERT 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 [16], which is supported by our measures of soil OM beneath cherry but not poplar. However, Adams and Angradi [54] reported tulip poplar litter decomposition rates to be more similar to black birch than black cherry litter, and the average bulk soil OM measured here for soil beneath black birch are less than from soil beneath black cherry, though these are not statistically distinct.

Bulk soil pH values were significantly higher in WS7-REF than WS3-FERT 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. [45] of the fertilization regime resulting in acidification of soil and stream water in WS3-FERT. Soil pH also varied among tree species, but only in WS7-REF. Differences in pH due to species has also been reported by Binkley and Giardina [63] and by Shear and Stewart [64], who 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 had lower soil pH relative to AM-dominated plots [65], and Yin et al. [66] reported that AM species have a neutralizing effect on rhizosphere soil while ECM species had acidifying effects via greater amounts of organic acid exudates for nutrient acquisition. In WS7-REF, we measured a similar effect where the ECM species (red oak and black birch) had significantly lower pH in both the A- and B-horizons compared to the AM species. Strickland and Rousk [67] stated that the homogeneity of broadcast fertilizer applications likely negates differences due to fungal association, and in our study, the differences in soil pH as influenced by tree species disappears in WS3-FERT, perhaps related to the soil homogenization effect of fertilization that was documented in measures of soil N processes by Gilliam et al. [68] in WS3-FERT at FEF.

We measured POXC to estimate 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 toward more POXC in the WS3-FERT B-horizon relative to the WS7-REF B-horizon (Table 1), though POXC was significantly correlated to the <53 μm size class in both soil horizons. Due to the high variability of POXC values within a watershed measured here, our sample size may not be sufficient to statistically capture true differences in this parameter. Because POXC reflects a heavily processed, labile fraction of soil C [52], we hypothesized that POXC would be greater in WS7-REF, as microbial extra-cellular enzyme activity has been shown to be lower in WS3-FERT soil relative to WS7-REF [31]. The slightly greater amount of POXC in the WS3-FERT B-horizon may reflect processes linked to the altered bacterial community resulting from fertilization [31], increased AM fungal growth resulting from fertilization [69], and/or the effect of ECM-associated tree species reducing fungal C allocation under N fertilization regimes [70]. Cullings et al. [71] demonstrated that ECM fungi can utilize their saprotrophic capabilities to secrete OM-degrading enzymes when receiving limited C from host trees, which could result in the greater POXC values observed in soil from fertilized WS3-FERT. Overall, POXC may influence aggregate measures, though more studies with larger sample sizes are needed to confirm this.

4.2. Aggregate Weight

Total sand-free aggregate weight in the B-horizon of WS7-REF was consistently greater than that of WS3-FERT, but there were not significant differences in total aggregate weight between the watersheds for the A-horizon (Table 1). The greater aggregate weight docu-

mented in soil from WS7-REF provides support for the hypothesis that fertilization would result in reduced aggregation in fertilized soil. Aggregation in soil may be influenced by changes in soil chemistry via pH-dependent change in mineral surface charge or ionic composition, causing dispersal or flocculation of soil particles [72]. Other studies have documented changes in soil chemical parameters following fertilization in WS3-FERT. Among those parameters, decreases in extractable soil Ca^{2+} and increases in electrical conductivity (EC) of soil solution in lysimeters have been reported. Both a decrease in Ca^{2+} and an increase in EC will promote dispersion of soil aggregates, as noted here in soil from WS3-FERT. 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 [73]. Furthermore, Kallenbach et al. [74] demonstrated that bacterial processes increase stable SOM and promote aggregation. This contrasts however, with Plaza-Bonilla et al. [75], who reported no significant differences in aggregate weight following long-term fertilization in agricultural soil.

It was expected that lower soil pH in the fertilized soil would act to disperse soil aggregates as a result of altered surface charge and reduced flocculation [76]. Our results suggest that acidification processes resulted in a greater abundance of 53–250 μm aggregates and soil particles in the $<53 \mu\text{m}$ (Figure 1). In WS7-REF, where soil pH is higher, larger macro-aggregates were most abundant. The alteration of aggregate size distribution to a greater abundance of the smallest fractions in fertilized forest soil may have important implications for water storage, plant-available water, hydraulic conductivity, and generation of runoff [77]. The size distribution of aggregates influences the size and continuity of pore space, whereby water is held or moves through preferential flow. In WS3-FERT, where soil has a greater abundance of aggregates $< 250 \mu\text{m}$ and particles $< 53 \mu\text{m}$, hydraulic conductivity and infiltration of water may be lower and may potentially result in more runoff generation. Further study on the hydrologic implications of the altered aggregate size distribution in these watersheds is warranted.

The influence of tree species on soil aggregate weight is highlighted with black cherry, whereby soil beneath black cherry exhibited the largest difference in aggregate weight distribution between watersheds. Perplexingly, soil beneath black cherry consistently had more macro-aggregates than other species in WS3-FERT and consistently fewer macro-aggregates than other species in WS7-REF. In general, because AM-associated species (particularly black cherry) have more labile and easily decomposed litter [16,54], microbial activity and organic byproducts are greater under AM-associated species, presumably leading to greater aggregation capacity. Upon further analysis of the role of fungal association on aggregate weight distribution using the limited number of representatives here in this study, we see that the mycorrhizal fungal association significantly influenced aggregate sand-free weight distribution within each watershed, though the influence of fungal association was distinct by watershed (Figure 6). Most commonly, differences in aggregate weight between fungal association occurred in WS7-REF A-horizon soil, where ECM soil exhibited significantly greater aggregate sand-free weight in the 53–250, 250–500, and 500–1000 fractions. Statistical differences between fungal association were not evident in the WS7-REF B-horizon in any size class. This was distinct from soil within WS3-FERT, 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-FERT B-horizon, soil beneath ECM-associated species consistently had lower aggregate weight in the larger size classes, with a shift of aggregate sand-free weight to the $<53 \mu\text{m}$ size class relative to AM soil.

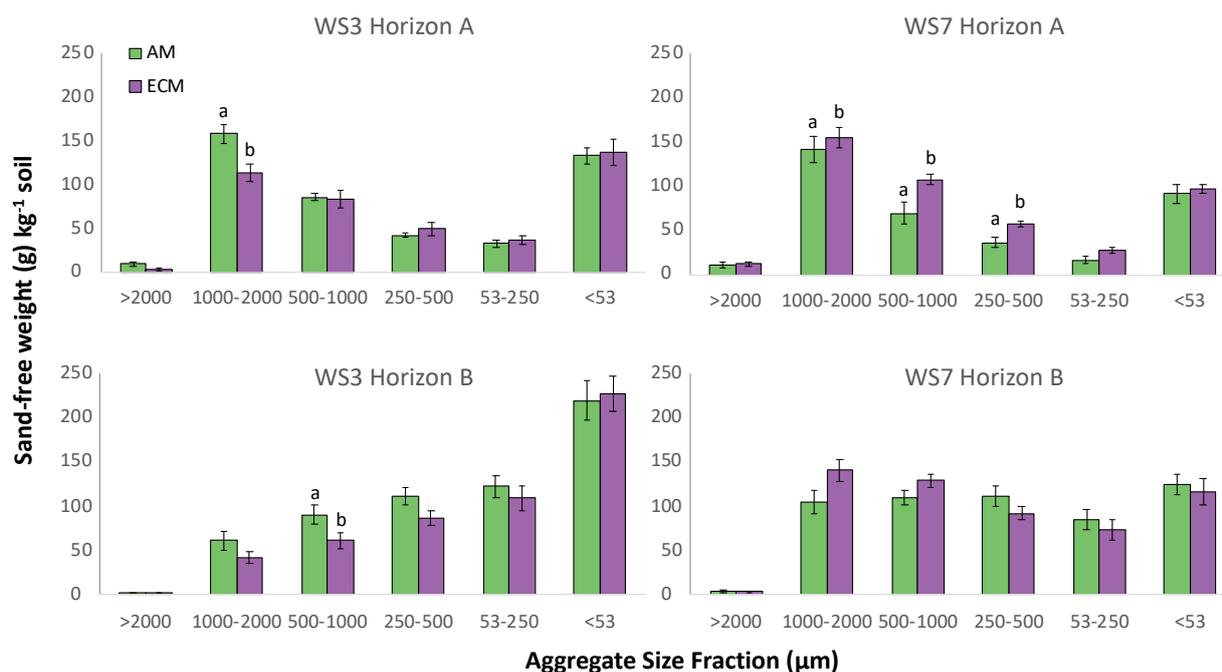


Figure 6. Sand-free weight within each aggregate size class by watershed (WS3-FERT and WS7-REF) in A-horizon and B-horizon: for each horizon, bars with different letters within each aggregate size fraction and watershed are significantly different according to Kruskal–Wallis means comparison ($p < 0.05$).

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-REF (Figure 6). The related hypothesis that acidification and the 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. Additionally, because we only included two of each AM and ECM species in our analysis here, we encourage further study on a larger set of AM and ECM species to provide further insight into the role of fungal association on aggregate measures.

4.3. Aggregate-Associated Organic Matter

Total aggregate-associated OM also differed to the greatest extent between watersheds in the B-horizon, where there was more total aggregate-associated OM in soil from WS7-REF, while aggregate-associated OM content was similar between watersheds in the A-horizon. This is as expected and supported by Carrara et al. [31], who reported that bacterial enzymatic activities were reduced in WS3-FERT compared to WS7-REF. Furthermore, Rousk et al. [78] showed that bacterial populations decrease at lower pH, as in soil from WS3-FERT. The more distinct differences in aggregate-associated OM that occur in the B-horizon are likely a function of the decreased decomposition of litter and soil above, leading to reductions of bulk soil and aggregate-associated OM in the B-horizon of WS3-FERT. In addition, OM interactions with the accumulated clay minerals in the subsurface soil may amplify the differences in aggregate-associated OM, which is supported by Craig et al. [19], who reported that AM-associated soil contains more 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 in WS7-REF 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 to sand-free aggregate weight, where soil beneath black cherry contained

more aggregate OM than other species in WS3-FERT and less aggregate OM than other species in WS7-REF (Figure 4). This lends support to the concept that litter decomposability plays a large role in the 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 elevated N availability [69] and AM-associated species tend to have more easily decomposable litter [16], resulting in more 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 [54] examined the decay rates of litter from multiple tree species in WS3-FERT and WS7-REF—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 1- and 2-year timescales. Previously, AM-associated fungi have been shown to positively correlate to development of soil aggregates [21,22] due to more hyphae biomass and production of glomalin-related soil protein; however, this effect may vary depending on plant or fungal species [23]. Because the relative rate of litter decomposition affects the amount of microbially derived OM available to be bound within aggregates, the different litter decomposition rates of tree species between watersheds likely influenced the differential response of aggregate OM beneath black cherry and the other tree species.

4.4. Relationship between pH, POXC, and Micro-Aggregate Size Fraction

Soil pH was inversely related to both micro-aggregate weight and micro-aggregate OM content in A-horizon soil across watersheds, where low soil pH resulted in greater micro-aggregate weight and micro-aggregate OM (Figure 5). 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 Bethlenfalvai et al. [79], 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 for the idea that mycorrhizae may play a more fundamental role in aggregate formation than bacterial activity. Bethlenfalvai et al. [79] also found that increased bacterial metabolic activity resulted in a temporary decrease in the amount of water stable aggregates [5,79]. Rousk et al. [78] reported 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 [80] found that clay–clay attractive forces in predominantly illite soils are stronger in acidic systems due to positively charged clay edges, leading to more 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 more OM content and sand-free aggregate weight in the <53 μm size class. At the outset of this experiment, small particles were assumed to contain both unassociated clay particles and free micro-aggregates. 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 [52] 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 the <53 μm size class in the acidified watershed than in the reference.

Carrara et al. [31] analyzed soil from the watershed receiving long-term N fertilization and found that, with increased N, belowground plant C allocation decreases, resulting in a cascading effect of decreased extracellular bacterial enzyme activity and arbuscular mycorrhizal colonization. The decrease in bacterial enzyme activity noted by [31] also supports the conclusions of Frey et al. [61], who reported that the increased SOC measured following N addition is due to reduced OM decomposition. Our finding that increased POXC is correlated to increased OM in the <53 μm size class indicates that, in the fertilized

watershed, there may be increased, not decreased, microbial decomposition in contrast to findings by Carrara et al. [31].

Finally, it is also important to consider the influence of surrounding tree species' fungal association on soil microbial activity. POXC increased under increasing influence of surrounding basal area ECM in WS3-FERT, especially in the B-horizon ($p = 0.029$). There were no significant trends between POXC and surrounding %ECM basal area in WS7-REF. This result further supports the conclusions by Vallack et al. [70] and Cullings et al. [71] that ECM-associated tree species may reduce belowground C allocation when under a fertilization regime, resulting in increased decomposition by ECM fungi.

5. Conclusions

Our data show that 30 years of forest fertilization with $(\text{NH}_4)_2\text{SO}_4$ 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 B-horizon soil. This shift in aggregation processes to soil more dominated by the smallest $<53 \mu\text{m}$ fraction is associated with parameters measured in this study of both acidification (soil pH) and increased microbially processed C (oxidizable C) in fertilized soil, though the potential role of added N, S, and greater EC should also be considered. 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 that this may be related to the 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 eastern U.S. forests to more strongly function as C sinks. 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 of 1990 and amendments, there is potential for labile, unprotected C in the smallest $<53 \mu\text{m}$ fractions to be further decomposed in the fungal and bacterial communities shifting in response to the changing environment. This has significant implications for our understanding of C cycling in the landscape as well as concerns relative to hydrologic alteration and nutrient movement in forested ecosystems.

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Appendix A

Table A1. 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 effects are bolded.

Parameter	Soil Horizon	Factor	Aggregate Size Fraction					
			>2000	1000–2000	500–1000	250–500	53–250	>53
Aggregate 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)
Aggregate OM (gOM kg ⁻¹ 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)

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