THE FATE OF

PARTICULATE NITROGEN IN FLUVIAL SYSTEMS

by

Chelsea S. Krieg

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Approved: _____
Shreeram P. Inamdar, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Erik H. Ervin, Ph.D.
Chair of the Department of Plant and Soil Sciences

Approved: _____
Mark W. Rieger, Ph.D.
Dean of the College of Agriculture & Natural Resources

Approved: _____
Ann L. Ardis, Ph.D.
Senior Vice Provost for Graduate and Professional Education

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ABSTRACT

Large storms can erode, transport, and deposit large amounts of allochthonous particulate organic matter (POM) and particulate nitrogen (PN) to the fluvial network. The role of storm-driven POM in the processing of fluvial C and N and its impact on water quality is still poorly understood. This study investigates the fate of C and N from storm-driven POM deposition using a 56-day incubation experiment of five known POM sources from a 79-ha forested watershed. Incubation columns were treated with one of two moisture treatments; Moisture Regime 1: sediment subjected to frequent rewetting treatments, Moisture Regime 2: sediments subjected to dry-wet cycles. Sediment and porewater samples were collected throughout the incubation and analyzed to characterize and compare the solid and solution pool chemistries and the abundances of nitrifying and denitrifying microbial populations. Key findings from this study are: (1) C and N rich sources experienced decomposition, mineralization, and nitrification and released large amounts of dissolved N, but the amount of N released varied by POM source and moisture regime. Drying and rewetting stimulated nitrification and suppressed denitrification in most POM sources. (2) Fluvial Storm Deposits released large amounts of porewater N regardless of the moisture conditions, indicating that they can readily act as N sources under a variety of conditions. (3) Forest Floor Humus was the only POM source to exhibit mineralization and denitrification when frequently rewetted. Under these conditions, this POM source acted as the strongest source of dissolved organic nitrogen. Under the drying and rewetting moisture conditions, Forest Floor Humus became a N source, specifically exhibiting intense decomposition, mineralization, and nitrification, resulting the release of large amounts porewater

nitrate. The inputs and processing of large storm-driven PN inputs becomes increasingly more important as the frequency and intensity of these large storms are predicted to increase due to global climate change. Gaining a better understanding of the fate of the N derived from particulate materials is critical to the development and implementation of effective management practices for controlling water pollution and maintaining healthy waterways.

Chapter 1

INTRODUCTION

Prior to the industrial revolution, the nitrogen (N) cycle was controlled by atmospheric reactions, slow geological processes, and restrictive biological cycles (Canfield et al. 2010; Thamdrup 2012). These robust feedback mechanisms were undermined by the transformation and release of excess N from the combustion of fossil fuels, enhanced biological nitrogen fixation (BNF), and the use of synthetic fertilizer (Galloway et al. 2004; Follett 2008; Canfield et al. 2010). In a study comparing the global N dynamics of the 1990's to that of the 1860's, Galloway et al. (2004) found that the reactive N generated from anthrogenic activities accounted for the production of ~156 Tg N yr^{-1} in the 1990's, which was ~10 times greater than that of the 1860's. In total, it was estimated that out of the ~268 Tg N yr^{-1} released in the 1990's, roughly ~59 Tg N yr^{-1} was delivered to inland and coastal systems via deposition in rivers and fluvial networks (Galloway et al. 2004). The way in which terrestrial and aquatic systems respond to such large N inputs has been of great interest to the scientific and environmental communities and has very practical management and remediation applications, however it can be very difficult to characterize due to its complex and dynamic nature (Howarth et al. 2006; Galloway et al. 2004).

It is estimated that 30 - 70% of N entering the fluvial network in the watersheds of the eastern USA can be removed via denitrification (Seitzinger et al. 2002), especially in the riparian zone of forested ecosystems where organic matter (OM) and

nitrate (NO_3) are plentiful (Steinhardt et al. 2000). It is clear that these fluvial N processing pathways play a key role in regulating excess inputs of N, however these pathways can be disrupted resulting in the release of N into downstream waters (Mulholland & Webster 2010; Aber et al. 1998), which can severly impair water quality (Driscoll et al. 2001; Driscoll et al. 2003; Anderson et al. 2002). Increases in N availability in aquatic ecosystems relax primary production limitations which can lead to increased biotic production, habitat change, harmfula algal blooms (HABs), fishkills, and dangerous toxins release (Driscoll et al. 2001; Michalak et al. 2013). Treatment of nutrient polluted waters is an intensive and expensive process, which can yield poor results, and potentially lead to more problems like the production of carcinogenic disinfectant byproducts (DBPs) (Kraus et al. 2008). Understanding the fate of N in the fluvial network, and the N processing mechanisms driving it, are critical to the development and implementation of effective best management practices.

Historically, research has been focused on the mobilization and availability of dissolved N species, however recent research has begun to identify and argue the importance of particulate N (PN). Recent studies have found that large storms have the erosive power necessary to transport significant PN loads and deposit them in the fluvial network. These storms events, which have primarily been recorded in the tropics and temperate forests, have been shown to transport a substantial portion of the annual N flux in the span of a few hours (Inamdar et al. 2015; Taylor et al. 2015; Lloret et al. 2013). Dhillon & Inamdar (2013) reported as much as 56% of the annual POC and ~33% of annual N flux were transported during the Tropical Storm Irene. Inamdar et al. (2015) found that PN composed 39- 87% of the storm event N export with storms constituting 65% of the 2011 total PN export (Inamdar et al. 2015). Though these

transport events comprise a significant portion of the annual N flux, the fate of the transported PN and its effect on water quality are still poorly understood.

PN that enters the fluvial network is subject to instream processing that can result in the leaching, transformation, and sequestration of N inputs. Low order streams are sites of dynamic instream processing and powerful N removal (Peterson et al. 2001; Seitzinger et al. 2006; Bernhardt et al. 2003), but these processes can be highly variable, and sensitive to environmental conditions that control residence time, temperature, oxygen supply, microbial community structure and function, and moisture conditions (Bernhardt et al. 2003; Mulholland 2004; Seitzinger et al. 2006; Triska et al. 1993; Storey et al. 1999). Instream N processing is particularly sensitive to moisture content; drying and rewetting cycles have been shown to spur intense decomposition, mineralization and release of C and N, and the emission of carbon dioxide (Birch 1958; Cui & Caldwell 2017; Jarvis et al. 2007). This response is largely credited to physical disruption of macroaggregates resulting in the exposure of previously unavailable POM (Utomo & Dexter 1982; Denef, Six, Paustian, et al. 2001), the decomposition of microorganisms that die due to the moisture stress (Bottner 1985), and the increase of productivity and shifts in population dynamics of surviving microorganisms that can facilitate the assimilation, nitrification, or denitrification of N (Fierer & Schimel 2002; Bottner 1985; Fierer et al. 2003; Stark & Firestone 1995). Rates of mineralization and nitrification subject to drying and rewetting have been shown to outperform that of soils which experienced more consistent moisture conditions, though this response decreases with successive drying and rewetting cycles (Borken & Matzner 2008; Fierer & Schimel 2002). Denitrification, which favors low oxygen conditions, is performed at great rates in the hyporheic zone, woody debris dams, and wetlands associated with low

order streams (Peterson et al. 2001; Seitzinger et al. 2006; Triska et al. 1993; Bilby 1981), but it can be limited by the aeration generated by low moisture conditions (Thamdrup 2012; Trimmer et al. 2012; Triska et al. 1993). Though it is clear that drying and rewetting cycles strongly influence physical, biological, and chemical processes of the fluvial network, the impact on specific N processing mechanisms and the resulting N species can be highly variable and rely heavily on POM composition, soil moisture stress history, and transport conditions (Franzluebbers et al. 1994; Triska et al. 1993; Seitzinger et al. 2006). The ability of these instream mechanisms to accommodate and regulate the PN loads associated with large storms may be weakened by reoccurring droughts and high intensity storms, both of which are predicted to increase in severity and frequency due to global climate change (Karl et al. 2009; Melillo 2014). To fully understand the environmental impact of storm driven PN on aquatic ecosystems, it is imperative that we characterize the effect of drying and rewetting on the fate of PN and the processing mechanisms controlling that fate.

To better understand the fate of PN and the implications this may have on downstream water quality and aquatic ecosystems, this study utilized a 56-day incubation experiment of five POM sources from a 79-ha forested watershed. Porewater and sediment samples were collected throughout the incubation and analyzed to characterize and compare the leached and soil pool chemistries and the abundances of nitrifying and denitrifying microbial populations. This data was used to address the questions posed by this study, which are as follows:

***Question 1*:**

(*A*) *How do watershed sources of PN affect N leaching or consumption in fluvial systems? Which PN sources act as sources or sinks of N?*

(B) *How do moisture regimes affect the release or consumption of N?*

***Question 2*:**

What are the key processes (mineralization, immobilization, nitrification or denitrification) affecting PN in stream sediments and what roles do microbes play in these transformations?

Hypotheses associated with these questions are:

(1A) PN sources with elevated N content will more readily leach N into the fluvial network and act as N sources.

(1B) The drying and rewetting moisture regime (as opposed to the frequently rewetted moisture regime) will result in more N release and leaching from watershed PN sources.

(2) Mineralization followed by nitrification will be the primary N processing mechanisms stimulated by the drying and rewetting cycles, resulting in the leaching of N.

Chapter 2

LITERATURE REVIEW

This literature review addresses the environmental significance of POM in the fate of the N transported by large storms in forested headwater catchments. Previous studies suggest that the fate of PN deposited in the fluvial network is driven by (1) watershed hydrology and precipitation patterns, (2) N removal mechanisms in the fluvial network, and (3) moisture stress of deposited POM and PN.

2.1 Particulate Nitrogen Transport due to Large Storm Events

Large storms can erode, transport, and deposit significant amounts of allochthonous DOM and POM within the fluvial network. Dhillon & Inamdar (2013) reported that in a mid-Atlantic forested watershed, Hurricane Irene (August 21, 2100) accounted for 56% of the annual POC, and 19% of the annual DOC exports. POM transport during large storms is equally significant in other climates and land- use regions. Jeong et al. (2012) found that 84% of the annual POC export was mobilized by large storm events, with one event in particular accounting for 62% of that export in a mountaineous headwater catchment in South Korea. Though there have been extensive studies on transport of POM from terrestrial sources to fluvial networks (Jeong et al. 2012; Pawson et al. 2012; Goñi et al. 2013), few studies have investigated the effect of large storms on PN export and how the fate of this N may influence aquatic ecosystems. Past PN studies, which have primarily focused on tropical and coastal regions (Taylor et al. 2015; Garzon-Garcia et al. 2015; Wiegner et al. 2009), with a handful of studies focusing on temperate forested watersheds (Vanni et al. 2017; Akamatsu et al. 2010;

Inamdar et al. 2015), found that large storms also play a significant role in PN transport and annual N flux. Inamdar et al (2015) found that PN comprised 39- 87% of the N export with storms constituting 65% of the 2011 PN export in a forested Piedmont catchment. In tropical regions, PN export is highly variable and could range from <1 to 50 kg ha^{-1} yr^{-1} (Alongi et al. 2013; Taylor et al. 2015). In addition, PN often exceeds the dissolved N yields during these events. In Costa Rica, PN yields were 10-54 times greater than the corresponding nitrate (NO_3) and dissolved organic nitrogen (DON) exported during large storms (Taylor et al. 2015) and similar results were found in study watersheds in Hawaii and the Carribbean (Wiegner et al. 2009; Lloret et al. 2013).

POM composition directly effects the processing of C and N in aquatic environments and influences the impact of the large storm PN contributions to the fluvial network. The composition of storm-delivered allochthonous OM is a function of the factors driving POM transport including, watershed morphology and hydrology (Atkinson et al. 2009; Akamatsu et al. 2010; Inamdar et al. 2015), seasonal hydrological regime (Atkinson et al. 2009; Akamatsu et al. 2011), storm event characteristics (Kaushal et al. 2014; Akamatsu et al. 2010; Rowland et al. 2017), and antecedent moisture conditions (Akamatsu et al. 2010; Bass et al. 2014). Watershed geomorphology and hydrology directly control the quality and amount of the POM that is transported to the fluvial channel by influencing the flowpaths taken during and after precipitation events. Atkinson et al (2009) found that low flow conditions during the summer season reduce POM quality by reducing connectivity between high quality POM sources and the fluvial network in coastal plain watersheds of southwest Georgia. Similarly, POC exports were sourced from less microbially processed surficial material during high intensity storms due to steep slopes found in the tropical study watershed

(Bass et al. 2012). In comparison, Jung et al. (2012) found that the POC mobilized by large storm events in the mountaineous mixed-land use study catchment were sourced from the stream bank and channel resulting in poorer quality POM transport. Even though POM quality may be adversely affected in regions of greater slope steepness, PN has also been found to increase in response to increased storm intensity and magnitude if the region is characterized by greater slope steepness (Hoover & MacKenzie 2009). Though hydrologic connectivity acts as a strong regulating force in POM transport, these controls can be altered by seasonality, storm event charactersitics, and antecedent characterisitics.

In the mid-Atlantic Piedmont region, summer storms are characterized by convective, high-intensity, short-lived storm events, while spring and fall storms are typically characterized as low-intensity, long-duration storms associated with frontal systems (Inamdar et al. 2013). Warm and, potentially dry, antecedent conditions coupled with high-intensity precipitation events give summer storms the erosive forces to significantly disturb forest floor and activate the upland POM sources (Dhillon & Inamdar 2013; Dhillon & Inamdar 2014). During the spring and fall, snow and leaf-fall act as physical barriers between precipitation and upland POM sources, reducing the erosive impact of the seasonal large storms (Dhillon & Inamdar 2013; Inamdar et al. 2015). Additionally, freeze-thaw stresses have the potential to destabilize exposed near stream POM sources in the winter and early spring, resulting in enhanced transport of these POM sources during high flow events and seasonal storms (Gellis & Noe 2013).

Frequent storms or large storms in rapid succession may repeatedly activate POM transport, resulting in pulses and eventual decline of the quality and amount of POM transport (Akamatsu et al. 2010). Similarly, PN export has not been found to be

supply limited, but there is evidence that the source can be exhausted after several storms (Taylor et al. 2015; Alongi et al. 2013; Inamdar et al. 2015). Comparatively, infrequent large storms have been shown to produce a variety of impacts. Inamdar et al (2015) found that Tropical Storm Nicole (09/30/2010) had the same amount of precipitation as some of the highest PN yielding storms of 2010-2011, but produced less than half of the streamflow PN yield due to a month-long drought prior to the disturbance event. To the contrary, Bass et al. (2014) found that infrequent storms coupled with low moisture conditions primed POM sources for large erosive episodes, resulting in larger concentrations of high quality storm POC transport.

Current research clearly demonstrates how extreme and variable storm-driven PN yield can be. Watershed hydrology and hydrologic connectivity (Atkinson et al. 2009; Akamatsu et al. 2010; Inamdar et al. 2015), seasonality (Atkinson et al. 2009; Akamatsu et al. 2011), storm event characteristics (Kaushal et al. 2014; Akamatsu et al. 2010; Rowland et al. 2017), and antecedent moisture conditions (Akamatsu et al. 2010; Bass et al. 2014) all play important roles in determining the quality of POM and amount of PN transported during these storm events. Though there is an understanding to what controls this PN transport, more research is needed to better understand how it impacts downstream waters.

2.2 Nitrogen Cycling Mechanisms and In-stream Processing

Early studies of watershed biogeochemistry utilized the ‘watershed ecosystem concept’, which considered both stream channel and watershed draining into it as one functional unit (Bormann & Likens 1967). Subsequent research has demonstrated that

in-stream processing can act separately to ameliorate or exacerbate nutrient export from the surrounding drainage area by altering timing, magnitude, and the form of nutrient that is transported through the fluvial network (Meyer et al. 1988). In-stream processing plays a key role in N export and N fate by providing both temporary and permanent mechanisms of NO_3^- removal (Wollheim et al. 2001; Bernhardt et al. 2003; Bernhardt et al. 2005; Seitzinger et al. 2006).

During transit, temporary uptake and transformation of N inputs spur nutrient spiraling that drive waterhsed N export (Peterson et al. 2001; Bernhardt et al. 2003). Inorganic N (NO_3^- and NH_4^+) can be temporarily removed from the water column through (1) immobilization from the decomposition of OM, (2) assimilation by plants, microbes, and fungi, and (3) sorption of NH_4^+ to fine clay particles (Seybold et al. 2005; Triska et al. 1993; Peterson et al. 2001). In headwater streams, transient N uptake and removal processes are primarily achieved by sediment and biofilms present in submerged portions of the stream channel (Peterson et al. 2001). NH_4^+ is preferentially removed from solution and stored at these sites through assimilation and sorption (Thamdrup 2012). Ammonium transport is strongly correlated with stream discharge, and the most rapid rates of NH_4^+ uptake and transformation are found in small headwater streams characterized by shallow depths with high surface-to-volume ratios (Peterson et al. 2001). NH_4^+ not retained through the transient mechanisms can be aerobically oxidized to NO_3^- through the process of nitrification (Thamdrup 2012). NO_3^-, which does not readily undergo sorption, is more mobile than NH_4^+ and less sensitive to transient N uptake mechanisms (Peterson et al. 2001), however it can be removed permanently from the system via denitrification (Seitzinger et al. 2006; Thamdrup 2012). Denitrification is a facultative anaerobic respiratory pathway employed by

heterotrophic micro-organisms that convert NO_3^- to N_2O and N_2 gas (Trimmer et al. 2012; Thamdrup 2012). This process is favored in low oxygen soils, is limited by the availability of labile C (Thamdrup 2012; Storey et al. 1999; Triska et al. 1993), and has the potential to mitigate downstream effects of massive N inputs (Seitzinger et al. 2006).

Other N processes, including anammox and dissimulatory nitrate reduction to ammonium (DNRA), have been more recently investigated and serve as pathways for N transformation and removal. Anammox, which is the anearobic oxidation of NH_4^+ in the presence of nitrite (NO_2^-) and NO_3^-, has been found to occur in anoxic ocean waters, and is unlikely in forested freshwater catchments (Thamdrup 2012; Trimmer et al. 2012; Canfield et al. 2010). Conversely, DNRA, which is a fermentive or respiratory process that transforms NO_3^- to NH_4^+, was originally believed to occur primarily in strongly reducing sediments (Thamdrup 2012; Trimmer et al. 2012), but can be found in saturated soils that undergo drying and rewetting cycles (Arce et al. 2015). Recent studies have shown that DNRA can occur in terrestrial and aquatic sediments as well (Dong et al. 2011; Rütting et al. 2011), and may potentially effect fluvial sediments that undergo intense drought stress (Arce et al. 2015). DNRA may be present in forested low-order catchment systems, but annamox is unlikely.

Temporary and permanent N uptake mechanisms are driven by upland N inputs, stability of stream ecosystems, and geomorphology and hydrology of stream channels. Immobilization and assimilation in low order streams are controlled by short-term fluctuations of N input, OM quality, and biotic demand, resulting in significant in-stream cycling between N storage and N regeneration (Bernhardt et al. 2005). Similarly, adsorption is influenced by microbial community structure, which enhances N retention

by increasing sediment stability through biostabalization of fluvial sediments (Vignaga et al. 2013) or diminish N storage by actively mining immobilized or sorbed NH_4^+ from clays, iron oxides, etc. The rate of N uptake is ultimately a function of N demand and N input, but the composition of transitory N load and N export is driven by the morphology and hydrology of the stream channel which apply selective pressures that directly shape uptake mechanism prevalence (Seitzinger et al. 2006). Several studies suggest that low-order streams serve as sites of maximum N retention and removal due to shallow water depths, low average velocities, and high surface-to-volume ratios that result in long residence times (Alexander et al. 2000; Peterson et al. 2001; Wollheim et al. 2001; Seitzinger et al. 2006). Channel morphology can also serve to mitigate the impact of periodic ecosystem turnover and large stochastic disturbances which weaken the long-term efficacy of both temporary and permanent inorganic N retention and removal mechanisms (Bilby 1981; Fisher 1983). Stream channel features, including fluvial channel margin depositis, the hyporheic zone, and the areas surrouding debris dams, are less vulnerable to disturbances that reduce sediment and ecosystem stability, and may serve as sites of more robust N uptake, storage, and removal, especially in low-order headwater reaches (Trimmer et al. 2012; Bilby 1981; Bernhardt et al. 2005; Seitzinger et al. 2006).

Climate, forest age (Bernhardt et al. 2005), and historical and contemporary land uses (Hoover & MacKenzie 2009) can influence the efficacy of watershed N storage and the impact of in-stream processing on N export load. Even if a watershed has proven to be an effective site for N storage, disturbances large enough to disrupt ecological, morphological, and hydrological stream processes directly impact N in-stream processing and watershed N export. As highlighted earlier, Inamdar et al. (2015)

reported that tropical storm Irene produced 42% and 27% of the annual PN and total N exports for 2011 in 59 hours, but the implications of this surge of material is yet to be fully understood. Bernhardt et al (2003) examined the effect of in-stream processing on the N released into the fluvial network after a large ice storm spurred isolated deforestation, and the transport of woody debris, sediment, and OM from terrestrial sources into the fluvial network. The authors found that the disturbance produced a negative feedback mechanism; the storm introduced a large NO_3^- load to the stream and spurred in-stream processing, resulting in muted NO_3^- export in downstream waters. The increased NO_3^- load and in-stream processing associated with this event were highly localized and declined sharply with distance downstream. The ameliorating effect of in-stream processing prevented this large N load from being transported downstream, but different in-stream conditions following the transport of storm material could produce far-reaching results. Future large storm events are predicted to become more frequent and more intense as a result of climate change (Karl et al. 2009; Melillo 2014). Increased periods of drought and intensity of re-wetting events will prime watershed sediment for even larger mass transports and N loads, and may significantly affect the impact of in-stream processing on N watershed export. To better understand the current fate and future fate of N transported by large storms, the role of drying and rewetting must be explored.

2.3 Impact of Drying and Rewetting Events on PN

The drying and rewetting of soils impact soil structure, aeration (Brady & Weil, 2008), water holding capacity (Adu & Oades 1978), microbial activity (Fierer &

Schimel 2002), and nutrient processing (Birch 1958; Bottner 1985). The physical, biological, and chemical changes driven by drying and rewetting spur C and N processing beyond that found in soils without moisture stress. This elevated C and N response, known as the Birch Effect, is rapid, ephemeral, and diminishes with successive moisture stress cycles. Soils that have been impacted by drying exhibit an increase in C and N mineralization rates in minutes to days following rewetting (Birch 1958; Franzluebbers et al. 2000; Borken et al. 2003; Pulleman & Tietema 1999), specifically including pulses of inorganic N (NO_3^- and NH_4^+) in porewater (Birch 1958; Cui & Caldwell 2017) and short-term emission of N gases (Jarvis et al. 2007). The severity of physical, biological, and chemical responses to drying and rewetting are influenced by soil moisture, soil aggregate stability, the presence of resistant and resilient microbial biomass, the soil moisture stress history of the soil source, and the characteristics of the drying and rewetting events (Denef, Six, Bossuyt, et al. 2001; Haynes & Swift 1990; Fierer & Schimel 2002; Denef, Six, Paustian, et al. 2001).

Though stress associated with soil shrinking has been shown to reinforce the stability of microaggregates in clay soils (Kemper & Rosenau 1988), soil shrinking more commonly acts to disrupt macroaggregates (Utomo & Dexter 1982; Denef, Six, Paustian, et al. 2001) and expose previously unavailable OM (Utomo & Dexter 1982). Denef et al (2001) demonstrated that the influence of drying and rewetting on aggregate disruption is restricted to only a few drying and rewetting cycles, and that after several cycles, macroaggregate turnover became similar to that of soils that remained wet and did not experience the moisture fluctuations. Similarly, the intensity of C and N mineralization following rewetting have been found to decrease with successive drying and rewetting pulses (Birch 1958; Fierer & Schimel 2002). Though there are conflicting

characterizations of N release spurred by drying and rewetting cycles, several studies demonstrate that the intensity and duration of the drying and rewetting cycles directly influence subsequent N processing and N loss from stressed soils. A review by Borken & Matzner (2008) found that cumulative rate of C and N mineralization decline as duration and intensity of drying conditions increase. Comparatively, rewetting rainfall duration, not intensity, significantly influences cumulative mineralization rates. Regardless of drying and rewetting cycle characteristics, each N pulse response decreases with successive moisture change cycles, potentially due to a decrease in labile OM availability through consumption and soil aggregate turnover (Denef, Six, Paustian, et al. 2001; Birch 1958), a shift in microbial community structure (Fierer et al. 2003), or a combination of both.

In addition to altering soil structure and OM availabilty, drying and rewetting cycles of various intensities and duration, directly influence soil microbial biomass and community structure (Fierer & Schimel 2002; Bottner 1985; Lundquist et al. 1999), thus effecting N processing potentials (Stark & Firestone 1995; Fierer et al. 2003; Franzluebbers 1999). The reduction of soil moisture lowers soil water potential and can impede the availability of organic and inorganic soluable substrates and the mobility of extracellular enzymes (Stark & Firestone 1995), which diminishes the activity of microbial communities (Van Gestel et al. 1993). Microorganisms utilize osmostic regulation to compensate for the stresses experienced during drying, but once the specific drought response threshold is exceeded, micro-organisms either survive through the formation of dormant endospores and cystes (Chen & Alexander 1973) or perish (Sparling & Ross 1988; Van Gestel et al. 1993). Rewetting is also hazardous for microorganisms who cannot withstand the water potential shock associated with

rehydration (Van Gestel et al. 1993). Febria et al. (2015) characterized two biotic response mechanisms that influence stressed microbial population characteristics; (1) resistance, and (2) resilience. Resistant communities exhibit minimal change when exposed to drying and rewetting cycles, while resilient communities are sensitive to drying, but recover to pre-dry conditions upon rewetting (Febria et al. 2015). Microbial populations may be characterized by one or both response types, and may change under the selective pressure of applied moisture conditions. Chen & Alexander (1973) estalished that microbial populations exposed to greater drought-like conditions during initial development were more drought-tolerant and resistant to moisture stress, indicating that moisture stress history has a direct impact on future microbial response and N yield of soil and POM sources. This is supported by Fierer & Schimel (2002) that found that oak soils, which were less frequently exposed to moisture stress compared to other studied soil types, exhibited greater microbial community shift, long-term net accumulation of biomass, and increased nitrification potential than other studied soil types. Soil moisture stress history directly impacts the physical soil storage of nutrients and OM, N processing potential of soil microbial populations (Lundquist et al. 1999; Fierer et al. 2003; Febria et al. 2015; Bottner 1985), and is strongly linked to soil source type (Fierer & Schimel 2002; Fierer et al. 2003; Lundquist et al. 1999), suggesting that the environmental characteristics typically associated with soil source type may be a strong indicator of soil response to drying and rewetting events.

Though the mechanisms underpinning the physical and biological response to drying and rewetting cycles are well studied, the specific N processing mechanisms driving N yield response are still poorly understood (Borken & Matzner 2008; Xiang et al. 2008). Gomez et al (2012) investigated the underlying N processes and their

regulating mechanisms in Mediterranean intermittant streams which are typically subject to severe drought and flooding conditions. The authors found that stream sediments became NO_3^- enriched due to increases in N mineralization and nitrification coupled with diminished denitrification in response to the applied moisture stress cycle. Additionally, the authors demonstrated that soil moisture (defined as percent water filled pore space) positively correlated with denitrification rates and negatively correlated with sediment extractable NO_3^- concentrations, indicating that the N processing of soils exposed to these conditions were primarily moisture limited. In the following years, Arce et al (2014) conducted a study in the same watershed to determine if reach-scale hydrologic drying constrained the response of N processing for this region. The authors found that the soil in this region produced similar results at the reach-scale, including significant sediment NO_3^- enrichment during drought. These studies reaffirm the constraining affect of soil moisture on N yield and N response mechanisms, specifically emphasizing an enhancement of nitrification and suppression of denitrification during drying, and the enhancement of denitrification upon rewetting, but this is not always the case. Other studies have shown that both nitrification and denitrification can be either enhanced and inhibited during each phase of the drying and rewetting cycle. Franzluebbers (1994) investigated the effect of drying and rewetting in N processing of plant OM-rich soil and found that the significant decline N mineralization due to repeated moisture stress events, was a function of plant tissue N becoming more resistant to decomposition, not an indication of microbial decline. In contrast, Fierer & Schimel (2002) found that nitrification potential increased in response to repeated moisture stresses because the microbial populations were capable of surviving through the drying stress, and were then primed to utilize previously

unavailable N upon rewetting. These results reflect the themes discussed earlier in this literature review and reaffirm that drying and rewetting stress can stimulate various N processing mechanisms and produce significant and variable N yields.

Though these N processing mechanisms have yet to be teased apart to explain the full range of N responses to drying and rewetting cycles, it is apparent that N yield is heavily influenced by; 1) OM, C, and N content of the soil undergoing drying and rewetting (Fierer & Schimel 2002; Lund & Goksøyr 1980; Yoshimura et al. 2010), 2) disruption of soil aggregates and the exposure of previously unavailable OM through soil shrinkage (Denef, Six, Paustian, et al. 2001; Denef, Six, Bossuyt, et al. 2001), 3) the intensity and duration of drying and rewetting events, 4) inhibition and stimulation of microbial functions from fluctuations of soil water potential (Stark & Firestone 1995; Chen & Alexander 1973; Bottner 1985; Van Gestel et al. 1993), and 5) soil moisture stress history which is intimately tied to soil type (Febria et al. 2015; Lundquist et al. 1999; Bottner 1985; Arce et al. 2015).

Chapter 3

SITE DESCRIPTION AND METHODS

3.1 Site Description

This study was conducted within the 79-ha watershed (Figure 3.1) at the Fair Hill Nature Resources Management Area, Cecil County, MD. This Piedmont watershed is predominately forested with mean stand age of 60 years, bordered by managed pasture. The deciduous canopy is principally comprised of *Fagus grandifolia* (American beech), *Liriodendron tulipifera* (yellow poplar), and *Acer rubrum* (red maple). Soils are deep, well-drained, coarse loamy, mixed, mesic Lithic Inceptisols that overlay the Mount Cuba Wissahickon Formation, which is primarily comprised of pelitic gneiss and schist with portions of pegmatite and amphibolite (Blackmer, 2005). This watershed is drained by two first order streams that join to flow into the Big Elk Creek and subsequently drain into the Chesapeake Bay. This region receives a mean annual precipitation of 1205 mm and annual snowfall of 447 mm of snowfall (*Maryland State Climatologist Office Data Page*, http://metosrv2.umd.edu/~climate/weather/marylandnormals.htm, accessed May 9, 2016). Annual mean temperature is 13 ℃, with the highest-mean air temperature registering at 25.7 ℃ (July) and lowest -0.1 ℃ (January) (Maryland State Climatologist Data page, 2016).

Streams are driven by groundwater at baseflow conditions, primarily exhibiting the same chemical signature found at groundwater seeps that occur at the base of hillslopes, but this changes during large storm events (Inamdar et al., 2013). Storm-

event runoff is associated with surficial sources and overland flow. This shift in stream water source, leads to significant changes in water, sediment, and POM export chemistry.

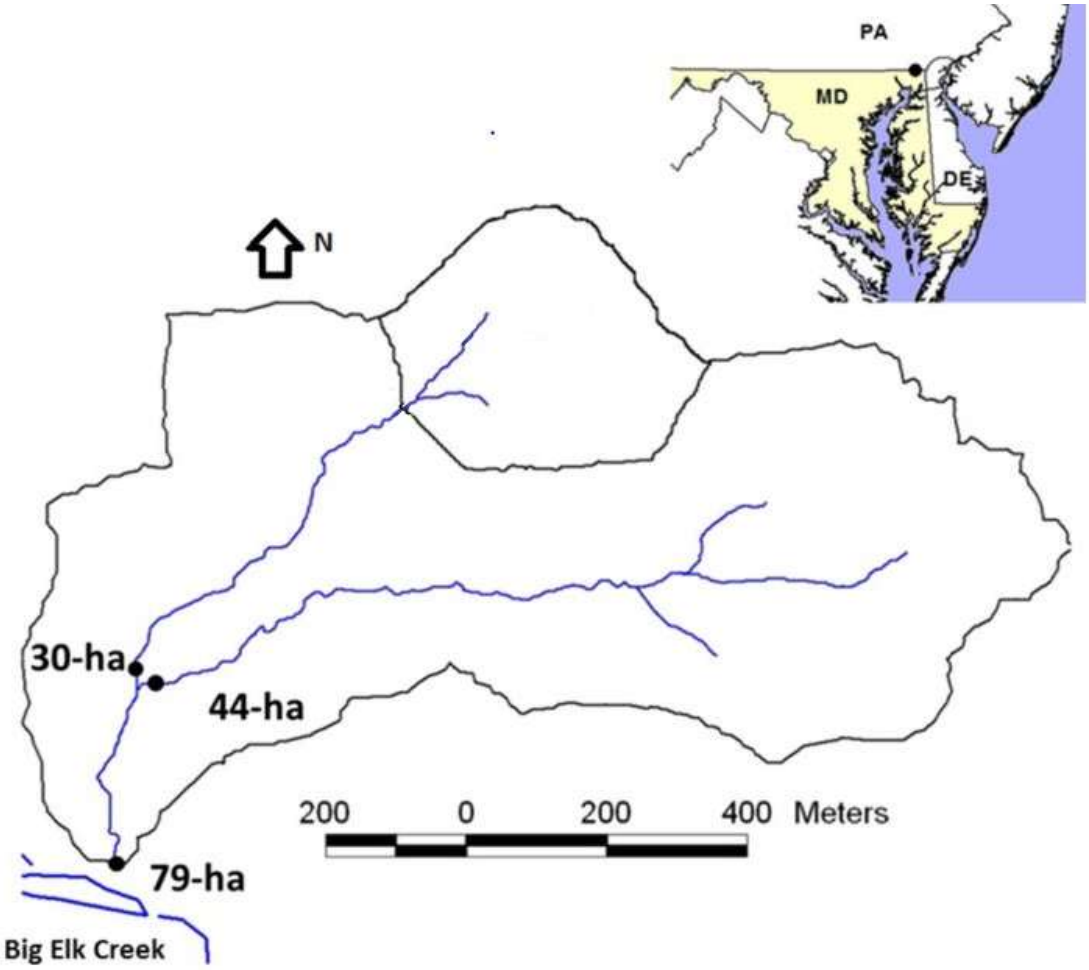

Figure 3.1: Fair Hill NRMA, Cecil County, MD. 79 ha forested Piedmont watershed with two 1st-order streams (in the 30 and 44 ha watersheds) converge at a 2nd-order stream draining to Big Elk Creek.

3.2 PN Incubation Study

An incubation experiment was performed to determine how particulate and leachate pools of various watershed PN sources compared to each other and how they changed when exposed to different moisture treatments. C and N rich and poor POM sources (Forest Floor Humus, Upland Mineral A Horizon, and Stream Bank) and "mixed" POM sources (Stream Bed, and fluvial channel margin deposits, hereafter referred to as -Storm Deposit) were incubated to establish how C and N pools evolve and change for a variety of POM sources and in a mixture. These PN sources were treated with one of two moisture regimes; (1) Regime 1 consisted of frequent rewetting to represent continuously moist in-stream conditions, and (2) Regime 2 consisted of drying and rewetting to represent variable floodplain conditions. This investigation provides insight into how PN source and moisture conditions effect the processing of C and N, which allows for a thorough understanding of how these factors may affect downstream waters.

PN Sources

A 56-day incubation experiment was used to assess the fate of C and N across POM sources in response to different moisture conditions. Specific POM sources, including Forest Floor Humus, Upland Mineral A Horizon, and Stream Bank (combination of exposed A and B horizons), and "mixed" sources, including Storm Floodplain Deposits and Stream Bed were selected for this incubation experiment (Figure 3.2). These POM sources have been previously defined in Dhillon and Inamdar (2014) and Rowland et al. (2017), except for the Storm Deposits, which are fluvial channel margin deposits that are comprised of sediment and OM that underwent

entrainment, transport, and deposition associated with the flows of the large storm events. These POM sources were selected to investigate how particulate and leachate chemistry change and evolve in a mixture vs. an original source.

Storm Deposit and Stream Bed POM sources are presumed to have already undergone the processing associated with the storm event flow-path and represent a mixture of other watershed sources. The remaining three POM sources, Forest Floor Humus, Upland Mineral A Horizon, and Stream Bank, were collected from undisturbed source sites and mixed with stream water during an additional treatment step to simulate storm processing before the generation of the incubation columns. This is described in greater detail below.

Figure 3.2: POM source sites and corresponding incubation columns. The POM sources types are as follows: A) Forest Floor Humus; B) Upland Mineral A Horizon; C) Stream Bank (composite of exposed A and B horizons); D) Storm Deposit; and E) Stream Bed.

Field collection and incubation column design

To account for POM source heterogeneity across the 79-ha watershed, three to six sample sites were selected for each of the five POM sources (Figure 3.3, Figure 3.4). At each site, a composite sample of POM was collected, prepared, and then divided into two incubation columns that would be treated with one of two moisture treatments during the incubation experiment (Figure 3.3). This resulted in the sampling of 20 field sites (note that the same sites were used for Forest Floor Humus and Upland Mineral A), and the production of 40 incubation columns (Figure 3.4, Figure 3.3). Half of the incubation columns were frequently rewetted to represent in-stream conditions and preserve the initial moisture conditions throughout the incubation experiment; this treatment was called Regime 1. Regime 2 incubation sediments were only re-wetted after Day 25 to represent the drying and rewetting episodes characteristic of stream bank conditions. Each set of incubation columns associated with a collection site acted as an experimental replicate for that POM source.

Incubation columns were leached high density polyethylene (HDPE) 2-gallon buckets (9¼" tall, 9¼" in diameter). Preparation of the sediment for the incubation columns occurred in the field at the time of sediment collection (Figure 3.5) and the installation of the sampling apparatuses (e.g., micro-rhizon samplers) occurred shortly thereafter at the site of the incubation experiment. To account for heterogeneity at each sample site, three POM source subsamples were randomly collected using cleaned trowels and placed into an ethanol-wiped mixing tub. Upland POM sources (Forest Floor Humus, Upland Mineral A Horizon, and Stream Bank) underwent an additional homogenization step that the fluvial POM sources (Storm Deposit and Stream Bed) did not, to simulate the storm transport the explicit POM sources had not yet experienced. Once the subsamples of Upland POM source material were combined in the clean

mixing tubs, four gallons of fresh baseflow stream water was added to the mixture. The contents of the mixing tub were gently stirred in a “back and forth” pattern with clean trowel for 5 min, and then allowed to rest for 15 min. At the end of 20 min, the incubation sediment material had settled and floating debris was poured off the top with a portion of the excess water to simulate the entrainment, transport, and deposition associated with storm processing. Care was taken to avoid pouring off visible fine-grained material (Figure 3.6). After excess water was removed, the resulting homogenized POM source was divided between the “Regime 1” and “Regime 2” incubation columns for that site. Once the incubation columns were filled with POM material they were lidded and transported to the incubation experiment site. A similar procedure was used in the homogenization of the fluvial POM material, but no water was added to the composite mixture.

All incubation columns were fitted with Soilmoisture Equipment Corp Micro-Rhizon sampler (1908D series), and one column for each POM source-regime combination (e.g., FFH R1, FFH R2, SBED R1, SBED R2, etc.) were fitted with Decagon 5TM dual moisture and temperature probes. The micro-rhizon samplers were installed approximately 2” below the surface of the incubation sediment at a 45° angle. For select representative replicates, Decagon 5TM dual moisture and temperature probes were installed next to the micro-rhizon samplers, at similar depths and orientations (Figure 3.7). This design facilitated optimal pore water collection and avoided potential moisture pooling, thus providing representative moisture and temperature measurements. Throughout the duration of the incubation, the micro-rhizon samplers and soil moisture probes remained undisturbed.

For the duration of the 56-day incubation, the sediment columns were housed at the Greenhouse at the Stroud Water Research Center, Avondale, PA. They were grouped by sediment type and were arranged so that they alternated between Regime 1 and Regime 2 replicates, to avoid potential bias from varying conditions of the room (e.g., sunlight exposure and temperature) (Figure 3.8). Ambient temperature and humidity were measured and recorded on sample collection days and incubation moisture content and temperature were continuously monitored at a 15-min interval by the Decagon 5TM dual moisture and temperature probes and EnviroDIY Mayfly Data Loggers. Direct sunlight was dampened by the greenhouse screens and there was no automated airflow active in the greenhouse for the duration of the incubation experiment.

Incubation buckets, micro-rhizon samplers, and moisture probes were washed and leached in deionized water to remove readily leachable C in preparation for the incubation. Leachate samples collected from the buckets were tested for C content, and equipment was re-leached until leachate contained negligible amounts of C, thus assuring subsequent incubation measurements would not be influenced by C in this equipment. All equipment used for field collection, and micro-rhizon samplers and moisture probe installation was cleaned using a 70% ethanol solution and gloves were used always to prevent contamination of the incubation columns.

5 POM Sources	Sites (Replicates)	Moisture regimes
Stream Bed (SBed) Stream Bank (SBank) Forest Floor Humus (FF) Upland Min Soil A horizon (UM) Storm Deposit (SD)	3-6 samples of each sediment type per regime (Denoted with A, B, C, D, E, F)	Regime 1 Regime 2 (Denoted with 1 or 2)

Incubation Column Strategy								
POM Source	Regime 1				Regime 2			
Stream Bank	SBed1A	SBed1B	SBed1C	SBed1D	SBed2A	SBed2B	SBed2C	SBed2D
Stream Bed	SBank1A	SBank1B	SBank1C	SBank1D	SBank2A	SBank2B	SBank2C	SBank2D
Forest Floor Humus	FF1A	FF1B		FF1C	FF2A	FF2B		FF2C
Upland Mineral A Horizon	UM1A	UM1B		UM1C	UM2A	UM2B		UM2C
Storm Deposit	SD1A	SD1B		SD1C	SD2A	SD2B		SD2C
	SD1D	SD1E		SD1F	SD2E	SD2E		SD2F

Figure 3.3: Incubation Experiment Strategy included generating incubation columns with three-six replicates from five POM sources for two moisture treatment regimes, and resulted in 40 incubation columns. Each incubation column name (e.g., SBed1A) featured with the *Incubation Column Strategy* table represents one experimental incubation column. Four sites were sampled for Stream Bank and Stream Bed POM sources, three sites for Forest Floor Humus and Upland Mineral A Horizon sources, and six sites for the Storm Deposit POM source.

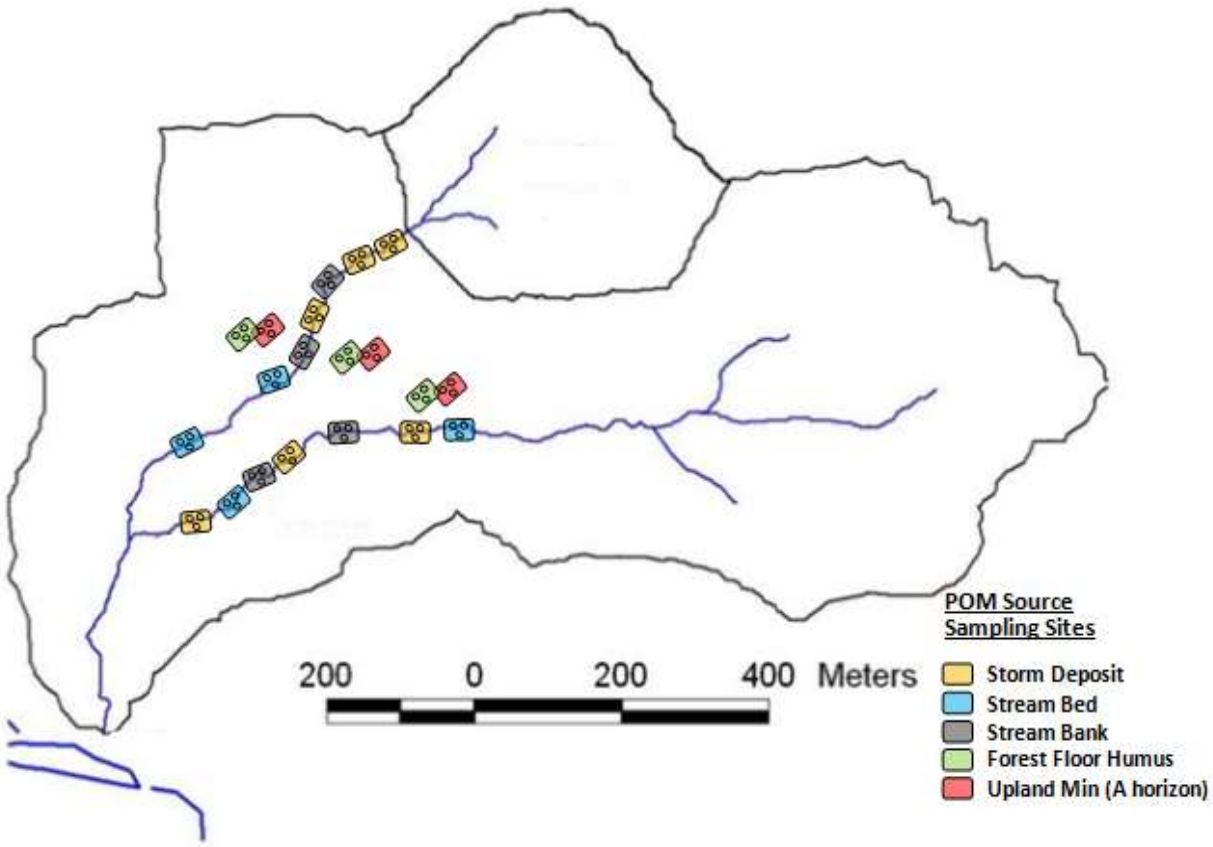

Figure 3.4: Incubation POM source collection map provides the location of all POM source sampling sites. Each box is colored to denote POM source and contains three circles, which represent the subsamples collected at that site. At each site, the subsamples that were collected were homogenized and then split between two incubation columns. Four sites were sampled for Stream Bank and Stream Bed POM sources; two sites on each 1st-order stream in the 79-ha watershed. Similarly, six sites for the Storm Deposit POM source; three sites on each stream branch. Lastly, three sites were sampled for the Forest Floor Humus and Upland Mineral A Horizon sources to account for the slopes contributing to the stream channel of this watershed.

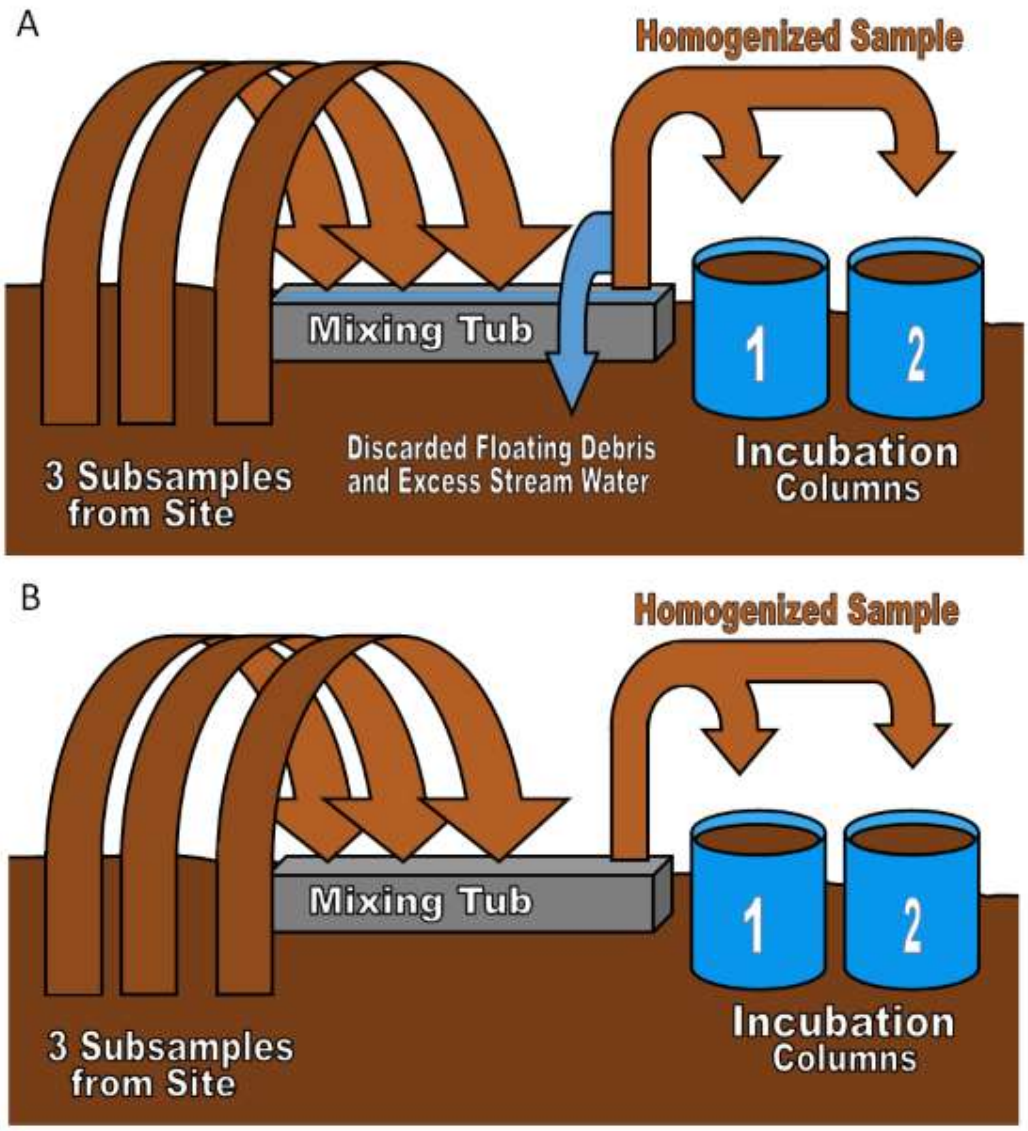

Figure 3.5: Incubation column preparation processes included a homogenization step that differed by POM source. A) Preparation process for Upland POM sources (Forest Floor Humus, Upland Mineral A Horizon, and Stream Bank) includes a water addition of fresh stream baseflow water to the homogenization step. B) Preparation process for Fluvial POM sources (Stream Bed, and Storm Deposit) does not include a water addition. The incubation column preparation process includes, the collection and homogenization of subsamples (brown arrows furthest to the left) in a clean mixing tub with (or without) fresh stream water, followed by the removal of excess water, and placement of composite sample into two clean 2-gallon HDPE buckets.

Figure 3.6: Incubation preparation of Upland POM source material. A-E depict the preparation process represented in Figure 3.4 (A) for an Upland Mineral A horizon incubation columns. A) Three subsamples have been collected and combined in the clean mixing tub. B) Approximately four gallons of fresh baseflow stream water has been added to the mixing tub and gentle mixing was completed. C) Excess water and large floating debris was poured off 20 min after homogenization. Care was given to avoid loss of sediment during this step. D) An example of the floating debris that was removed from this system in step C. E) Final Upland Mineral A Horizon incubation columns.

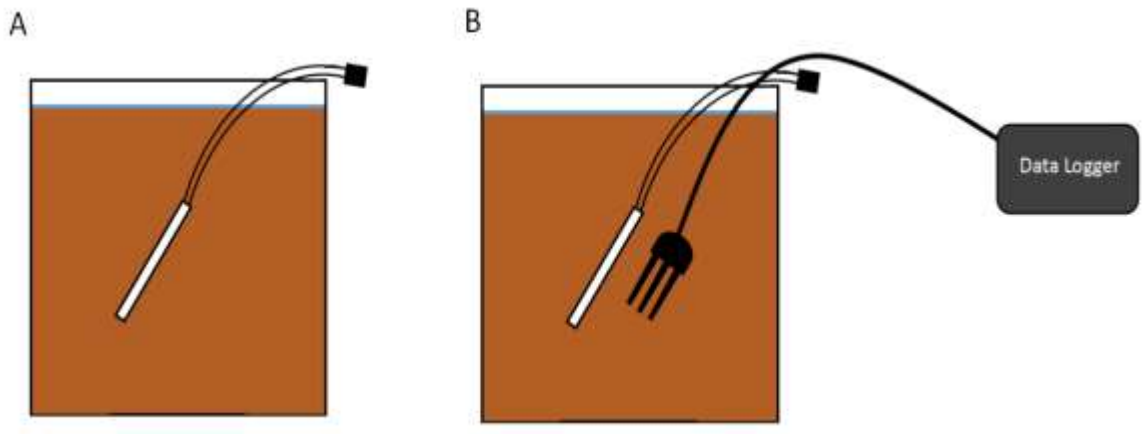

Figure 3.7: Incubation column cross section schematic shows the location and orientation of installed monitoring and sampling equipment. A) All incubation columns were fitted with micro-rhizon samplers (white rectangle). These samplers were installed at a 45° angle approximately 2" below the sediment surface at the beginning of the incubation and remained in place throughout the duration of the experiment. B) Select incubation columns were fitted with micro-rhizon samplers (white rectangle) and soil moisture probes (black 3-pronged probe). The soil moisture probes were also installed at 45° angle approximately 1" below the sediment surface. Moisture probes and the micro-rhizon samplers were installed next to each other, positioned at the same depth with the same orientation.

Figure 3.8: Incubation columns set up at the greenhouse of the Stroud Water Research Center, Avondale, PA. Incubation columns were grouped by sediment type (indicated by top layer of colored tape on each bucket), but Regime treatment columns were in an alternating order (e.g., Column order of Regime 1 (no bottom tape label), Regime 2 (orange tape label), Regime 1, Regime 2, etc.).

Experimental design

Incubation moisture and temperature conditions were recorded at 15 min intervals throughout the experiment by EnviroDIY Mayfly Data Loggers. Mean temperatures ranged between 20- 22°C. Two contrasting moisture treatments were applied to the incubation column replicates: Regime 1 (R1) and Regime 2 (R2). Regime 1 incubation sediments were frequently re-wetted with "small" rewetting events to represent in-stream conditions and preserve the initial moisture conditions (measured at

Day 0 for each POM source) throughout the incubation experiment. Regime 2 incubation sediments were re-wetted at Day 25 with a "large" rewetting event, and then rewetted with the same "small" rewetting events of Regime 1 until Day 56 (Figure 3.9) to represent stream bank conditions. The water volumes associated with "small" rewetting varied due to differences in POM source material characteristics and initial moisture contents. Typically, these watering events ranged between 300 – 500 mL applied to the columns over a period of 30 min. The "large" rewetting event applied on Day 25 represented a substantial precipitation event that would lead to uniform inundation across all sediment types and was applied to both Regime 1 and Regime 2 sediments. To achieve this, all columns were watered until inundated with ½ in standing water for a period of 30 min. Treatment water was added to columns in volume increments of 300 mL at a time. This "large" rewetting event used 600 - 1500 mL per incubation column and took place over a 3-hour period.

Treatment water used for the "small" and "large" rewetting events was filtered (Sterlitech 0.22 µm) stream water collected during baseflow conditions downstream of the confluence of 30 and 44 ha watershed streams, that was then stored at 4 °C walk-in-refrigerator in leached 5-gallon carboys. This filtration and storage approach decreased potential microbial activity and maintained the integrity of the original stream water chemistry. Due to space limitations and the volume of water required, baseflow stream water was collected and filtered 3 times throughout the incubation for treatment water. Subsamples of treatment water for each water addition were collected and tested with the other water samples to take note of any introduced chemical variances.

Two types of samples were collected from the incubation samples throughout the experiment: porewater and sediment cores. Incubation pore water was extracted

with the micro-rhizon samplers that were installed at the beginning of the incubation experiment. Extraction syringes were connected to the micro-rhizon samplers during pore water extraction samplings using a Luer-lock system and clear PVC tubing (Figure 3.10). For pore water collection, a vacuum was applied to the micro-rhizon samplers by locking the extraction syringes in an open position. Once vacuum was lost or the syringe was filled, the sample was decanted to a clean 250 mL HDPE bottle and the vacuum was reapplied until enough sample was collected. Due to incubation sediment texture and moisture, water extraction required 3 - 5 days. Extracted water samples were kept on ice to prevent sample degradation during this multiple day process. Filtration before refrigeration storage was not needed due to the 0.15 µm porosity of the micro-rhizon samplers, which effectively removed microbes from the sample during collection. Porewater collection occurred 4 times (Day 0, Day 26, Day 33, and Day 50) throughout the incubation.

Sediment samples were collected 6 times (Days 0, 4, 25, 26, 45, and 56) throughout this experiment. Sediment cores were extracted using a ¼" inner diameter aluminum pipe. After sample collection, the core hole was filled with heat-treated bentonite to prevent oxygenation without altering the sediment chemistry of the column (Figure 3.11). The sample core was separated into three sections; "Top", "Bottom", and "Middle". The "Top" and "Bottom" samples included the top and bottom 1" of the sediment core and were collected separately for microbial analysis. The "Middle" was collected for sediment chemistry analysis.

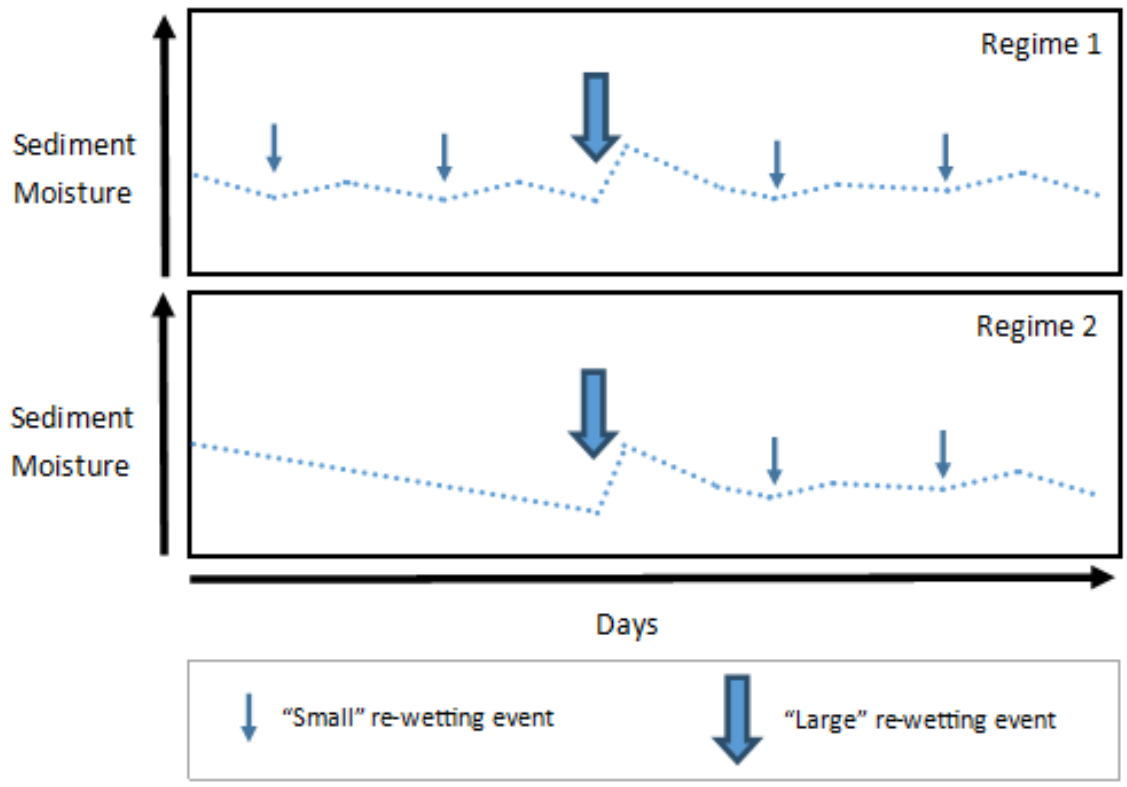

Figure 3.9: Moisture conditions of the incubation columns were manipulated through the volume and timing of water additions. "Small re-wetting events" were characterized by low volume water additions (small arrows), while "Large re-wetting events" were characterized by high volume water additions (large arrows). Regime 1 and Regime 2 only differed in the water additions prior to the "large" re-wetting event on Day 25. Prior to Day 25, Regime 1 received several "small" re-wetting events, while Regime 2 received no water additions prior to the Day 25 "large" re-wetting event. After Day 25, both regimes were treated with the same series of "small" re-wetting events.

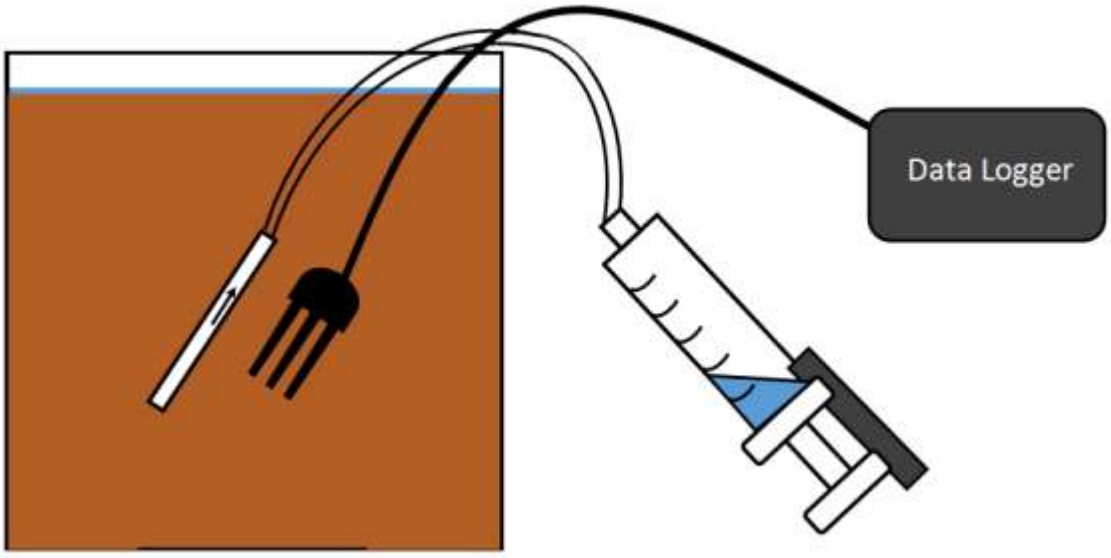

Figure 3.10: Schematic of the incubation column pore water extraction in cross section. Once a vacuum was applied to micro-rhizon sampler (white rectangle) by the extending and locking the extraction syringe, porewater flowed through the sampler and was collected in the syringe. After sufficient water volume was collected, the sample was decanted to storage containers. The moisture probe (black three-pronged probe) remained intact throughout collection process.

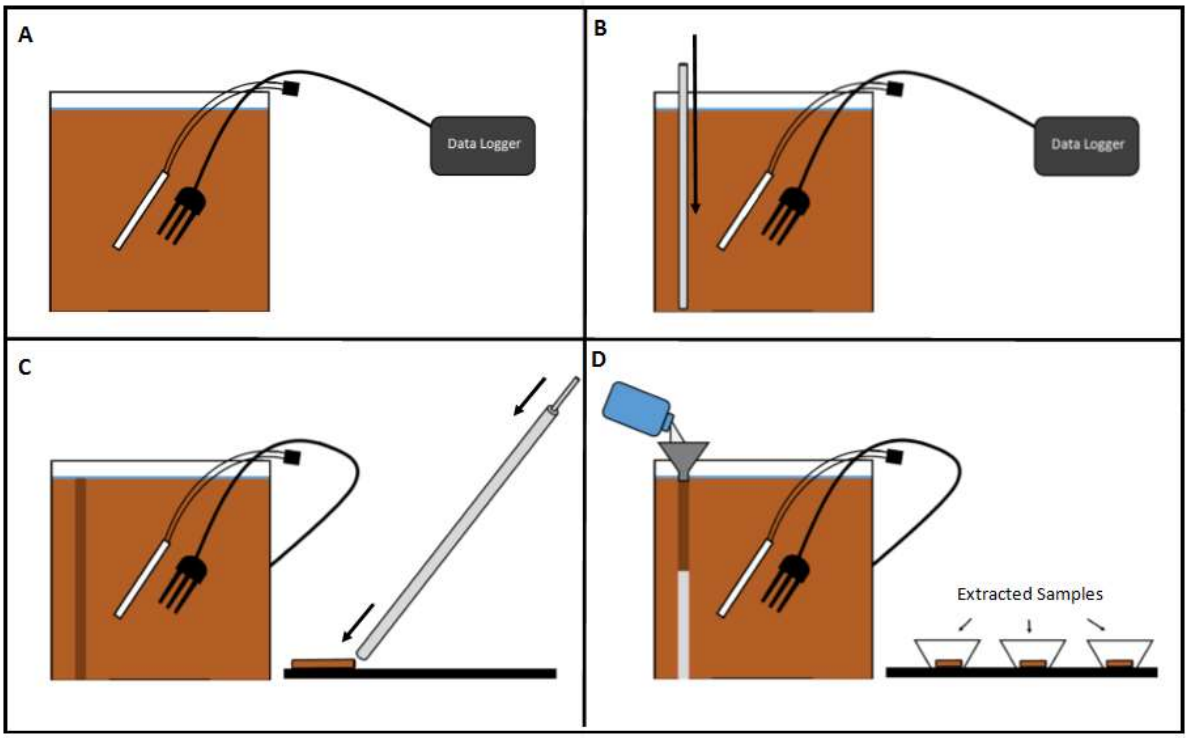

Figure 3.11: Schematic of the incubation column sediment extraction in cross section using an aluminum corer. A) Pre-collection incubation column cross section. B) Sediment extraction corer was used to core out a ¼" core for the entire column height. C) Sample core was deposited and divided into "Top", "Bottom", and "Middle" sections. D) Heat-treated bentonite was used to fill the core hole and subsamples were processed.

3.3 Analyses

Fluorescence spectroscopy, discrete colorimetric analysis, and catalytic thermal decomposition were used to determine porewater C and N quality, species, and concentration. Sediment chemistry analyses were used to determine sediment C and N content. Quantitative PCR was used to determine the abundances of N processing genes present in the microbial populations found in the particulate material. All particulate C and N contents and dissolved C and N concentrations were converted from concentrations to masses using percent volumetric water content, bulk density, and the dimensions of the incubation sediment. The mass calculations are as follows:

$$\mathrm{TC\ (g)} = \%\mathrm{TC} * \frac{1}{100} * \left(\frac{\prod * D^2}{4} * h\right) * BD$$

Figure 3.12: Equation used to convert particulate C and N percentages to masses. Percent TC was determined via chemical analysis, and diameter (*D*), height (*h*), and bulk density (*BD*) were measured for the column during the incubation.

$$\mathrm{TC\ (mg)} = \mathrm{TC}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) * \frac{\left(\frac{\prod * D^2}{4} * h\right)}{1000} * \%VWC$$

Figure 3.13: Equation used to convert dissolved C and N concentrations to masses. Dissolved C or N (denoted as TC(mg/L) in the equation) were determined via chemical analysis, diameter (*D*) and height (*h*) were measured, and percent volumetric water content (%VWC) was recorded by the moisture probes during the incubation.

In addition to calculating C and N masses, the "net change" was determined for both particulate and dissolved C and N. To determine the net change values, the end value was subtracted (i.e., particulate masses and gene abundances collected at Day 56, dissolved masses collected at Day 50) from initial value (masses and abundances collected at Day 0). Positive net change values were interpreted as net increase of masses/abundances throughout the incubation, while negative net change values were interpreted as net decreases. The resulting differences were also examined using ANOVAs and Tukey HSD.

As a quality control measure for the water chemistry analyses, an Incubation Control Standard (ICS) was made by filtering and freezing one uniform water sample set (stream baseflow). An ICS sample was thawed and included in every water chemistry analysis processed for this incubation experiment. The ICS was used to ensure multiple analyses run on the same instrument were truly comparable (e.g., ICS values from multiple runs needed to be within 10% of each other).

Fluorescence spectroscopy

Fluorescent dissolved organic matter (FDOM) of the incubation pore water samples were analyzed using fluorescence spectroscopy on a Horiba Aqualog® fluorometer. This analysis produced a fluorescence Excitation Emission Matrix (EEMs) by using an excitation wavelength range of 700 - 240 nm at 4 nm increments and measuring emission wavelengths of 700 - 240 nm (estimated interval of 4.66 nm). Routine daily checks, including the manufacturer's excitation check, emission check, cuvette check, and Raman water scan, were performed to ensure accurate baseline and high quality data. After the fluorescence measurements were completed, EEMs were

generated through the performance of post-processing procedures in Matlab (Version R2015b V 8.6.0). These post-processing steps corrected for inner filter effects and applied 1st and 2nd order Rayleigh Masking (sum of slit width set to 10). Lastly, the EEMs were run through a PARAFAC model (Singh et al., 2013) to generate quality metrics used in this analysis, including % protein-like, % humic-like, and % fulvic-like fluorescence.

TOC/TN analysis

A Shimadzu TOC-L was used to determine pore water concentrations of Total Carbon (TC), Inorganic Carbon (IC), Dissolved Organic Carbon (DOC) and Total Nitrogen (TN) using a combination of catalytic oxidation, and thermal decomposition methods. Proper cleaning methods and checks were performed prior to each run to ensure appropriate baseline measurements were being recorded. Sample checks, standard checks, replicate samples, reagent blanks, and ICS were analyzed every 15 experimental samples to ensure accurate and precise data within an analysis run. Standard curves were generated for each test (TC, IC, and TN) and measured standard curve values were compared to expected values to ensure precise and accurate measurements.

Nitrate-N and ammonium-N

An AQ2 Discrete Analyzer was used to determine the nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) concentrations of incubation porewater samples using a colorimetric method. AQ2 method EPA-129-A Rev. 8 was used for the nitrate-N

analyses, and AQ2 method EPA-148-A Rev. 2 was used for the ammonia-N analyses. Continuing calibration blanks (CCB), continuing calibration verifications (CCV), standard checks, sample replicates, and incubation control standards (ICS) were analyzed every 15 incubation samples to ensure accurate and precise data. Proper instrument cleaning and quality control checks were performed for every sample run.

Percent carbon, nitrogen, and Melich-3 analyses

The "Middle" sediment core section was sent to the UD Soil Testing Laboratory for sediment characterization. Day 0 and Day 56 "Middle" sediment samples were submitted for the *TC/TN by combustion* analysis and *Research M3-Routine Analysis*, which measured % TC, % TN, and base cations (Ca, Fe, Al, K, P, Mg, Mn, Zn, Cu, B, S). The sediment samples collected on Day 4, Day 25, Day 26, and Day 45 were only submitted for *TC/TN by combustion* analysis (% TC, % TN) since the study focus was primarily on TC and TN.

qPCR analyses

Forest Floor Humus and Storm Deposit were selected for microbial analysis because they had distinctly different particulate and leachate chemistry and they allowed for the comparison of a C and N rich PN source and mixed source. Quantitative PCR (qPCR) analysis for nitrification and denitrification was performed on Forest Floor Humus and Storm Deposit incubation sediments ("Top" and "Bottom" core sections) using QuantStudio^{TM} 6- Flex Real-Time PCR System with SYBR-Green I fluorescent dye. Primers were selected to measure the abundance of genes associated with

nitrification and denitrification, specifically Arch-*amo*Af and Arch-*amo*AR (Francis et al., 2005) for ammonia oxidizing archea (AOA), *amo*A-1F and *amo*A-2R (Rotthauwe et al., 1997) for ammonia oxidizing bacteria (AOB), *nir*S1R and *nir*S6R (Braker et al., 1998) for denitrifying archea (nirS), and *nir*K583f and *nir*K5R (Mosier and Francis, 2010) for denitrifying bacteria (nirK). Gene abundances for nitrification and denitrification were compared across the two POM sources, moisture regimes, and day to address Question 2.

3.4 Statistical Analyses

ANOVAs and post hoc Tukey HSD

Data was analyzed using a series of Repeated Measurement One-way ANOVAs and post hoc Tukey honest significant differences (HSD), which are robust under unequal variances. P values < 0.05 were considered significant. To address Question 1A, data for the full incubation period was pooled for each POM source and compared across the other POM sources for each regime.

Chapter 4

RESULTS

4.1 POM Moisture Patterns for Regime 1 and Regime 2

Small rewetting treatments were administered to only Regime 1 incubation columns at Day 2, 9, 18, and 19, and both Regime 1 and Regime 2 columns at Day 26, 30, 42, 40, 48, 52, 55, and 56. The large rewetting treatment was administered to all incubation columns at Day 25. Moisture profiles for Regime 1 were characterized by small spikes in percent volumetric water content (%VWC), while moisture profiles for Regime 2 exhibited a steady decline from Day 0 to Day 25, increase at Day 25, followed by decline and minor spike for the rest of the incubation (Figure 4.1). Storm Deposit and Stream Bed have similar moisture profiles across both regimes and Regime 2 %VWC appear to be lower than the other POM sources. Forest Floor Humus Regime 1 had greater %VWC values and more readily retained its %VWC than the other POM sources in either Regime 1 or Regime 2 (Figure 4.1). The moisture treatments resulted in significantly different moisture content profiles (e.g., Regime 1 and Regime 2) for Forest Floor Humus ($p = <0.0001$), Storm Deposit ($p = <0.0001$), Stream Bank ($p = <0.0001$), and Upland Mineral A Horizon ($p = 0.0051$, Table 4.1).

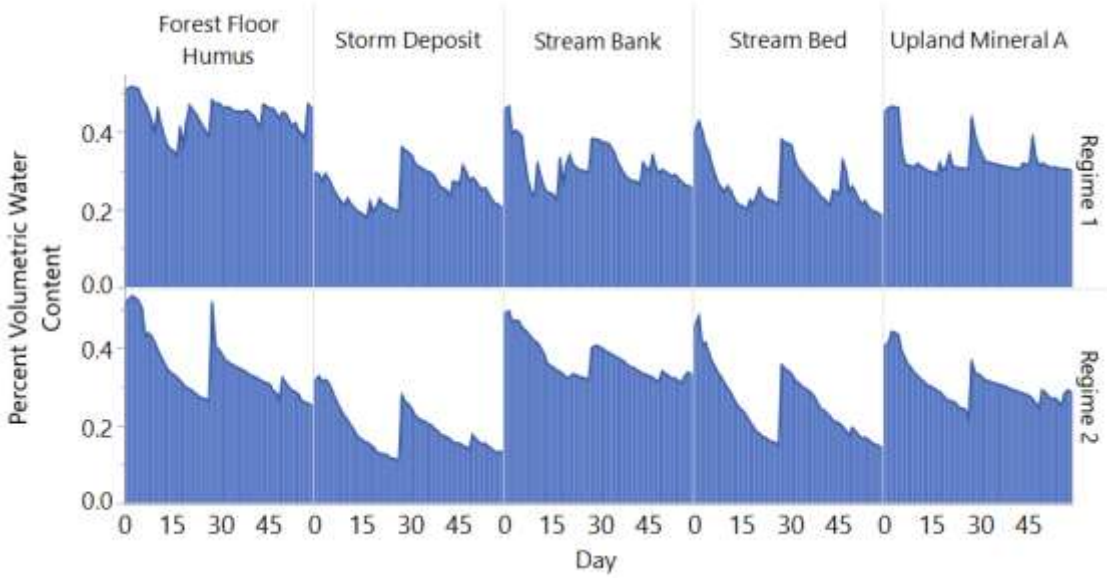

Figure 4.1: Comparison of moisture profiles (%VWC) over time of both Moisture Regimes for all POM sources.

Table 4.1: ANOVA comparison of percent volumetric water content for POM sources between Regime 1 and Regime 2. P values are reported and significant differences are defined as $p < 0.05$.

P-values for Comparison of Percent Volumetric Water Content for Regime 1 and Regime 2	
Forest Floor Humus	<0.0001
Storm Deposit	<0.0001
Stream Bank	<0.0001
Stream Bed	0.3773
Upland Mineral A Horizon	0.0051

4.2 Changes in Particulate TC and TN Masses for Regime 1 and Regime 2

At the beginning of the incubation, Forest Floor Humus had the highest TC and TN, followed by the Upland Mineral A Horizon, Stream Bank, and Storm Deposit and Stream Bed (Figure 4.2, Figure 4.3). In addition, the Forest Floor Humus and Upland Mineral A Horizon had the greatest C:N ratio, followed by Stream Bed and Stream Bank, and then Storm Deposit. All molar C:N ratios fell within the range of 14.25$\pm$ 3.05 to 19.25$\pm$ 0.64 (Table 4.2). At the end of the incubation (Day 56), all POM sources had lower TC and TN than initial conditions in both Regime 1 and Regime 2. The initial ranking of these parameters was maintained; TC and TN were the greatest for Forest Floor Humus, followed by Upland Mineral A Horizon, Stream Bank, and then Storm Deposit and Stream Bed. Additionally, the final C:N ratios of the of both Regime 1 and Regime 2 were slightly lower than the initial ratios.

Table 4.2: Initial and final molar and mass C:N ratios for all POM sources. Mean C:N ratios are in bold, standard deviation is indicated in the , parentheses, and letters denote significant difference.

Moisture Regime	POM Source	Day 0		Day 56	
		Molar C:N ratio	Mass C:N ratio	Molar C:N ratio	Mass C:N ratio
Regime 1	Forest Floor Humus	**19.25** (3.05, A)	**16.50** (2.62, A)	1**8.01** (2.51, A)	**15.43** (2.16, A)
	Storm Deposit	**14.80** (0.97, B)	**12.68** (0.84, B)	**15.67** (1.27, B)	**13.43** (1.09, B)
	Stream Bank	**17.53** (6.79, B)	**15.03** (5.82, B)	**15.37** (3.15, B)	**13.18** (2.70, B)
	Stream Bed	**17.88** (4.08, B)	**15.33** (3.50, B)	**14.58** (1.24, B)	**12.50** (1.06, B)
	Upland Mineral A Horizon	**19.99** (3.78, A)	**17.13** (3.24, A)	**18.24** (3.87, A)	**15.63** (3.32, A)
Regime 2	Forest Floor Humus	**19.25** (3.05, A)	**16.51** (2.65, A)	**18.55** (2.67, A)	**15.9** (2.29, A)
	Storm Deposit	**14.80** (0.97, B)	**12.68** (0.84, B)	**14.25** (0.64, B)	**12.22** (0.55, B)
	Stream Bank	**17.53** (6.79, B)	**15.03** (5.82, B)	**14.73** (2.86, B)	**12.63** (2.45, B)
	Stream Bed	**17.88** (4.08, B)	**15.33** (3.50, B)	**14.76** (0.90, B)	**12.**65 (0.77, B)
	Upland Mineral A Horizon	**19.99** (3.78, A)	**17.13** (3.24, A)	**19.02** (4.14, A)	1**6.3** (3.55, A)

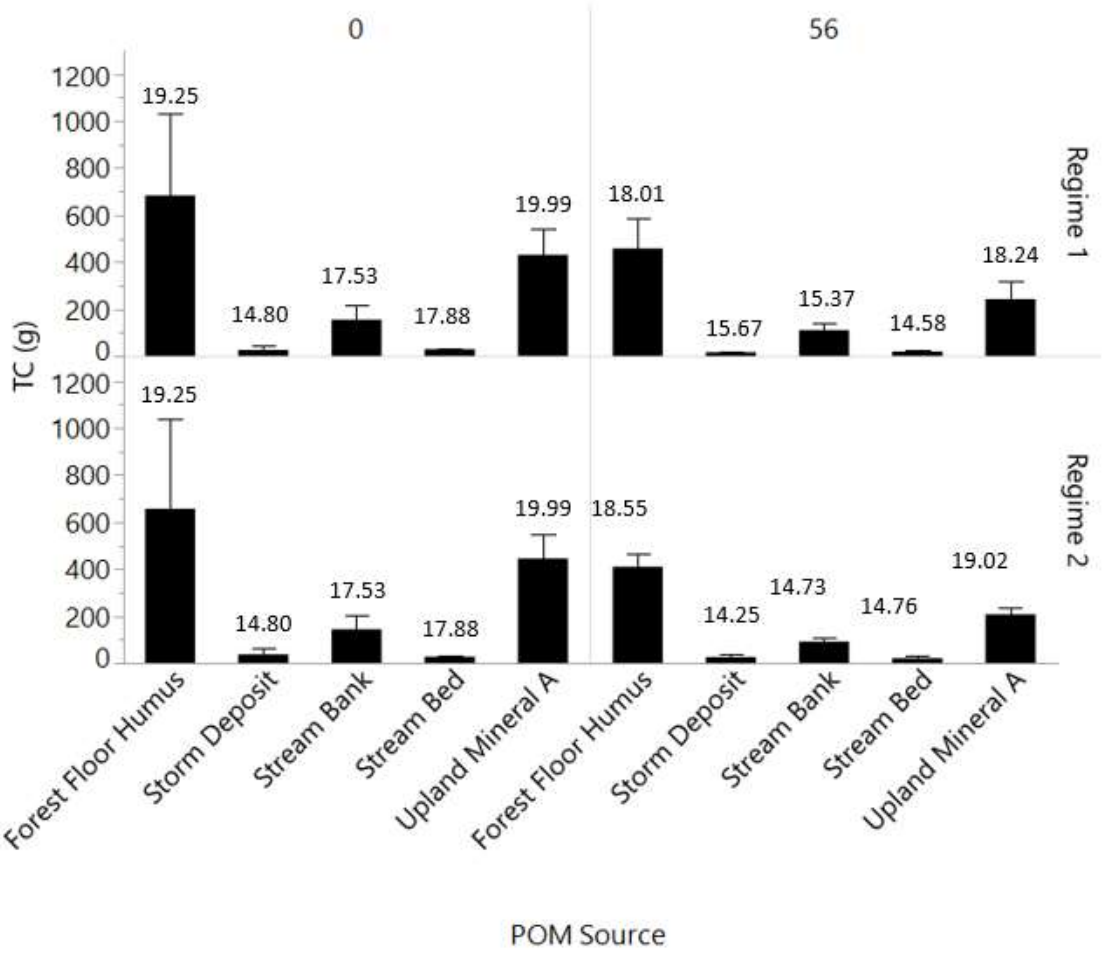

Figure 4.2: Comparison of initial and final TC (g) across POM sources. Initial and final molar C:N ratio for each POM source is listed above the corresponding TC bar. Final TC and C:N ratio of both regimes are lower than initial TC and C:N ratios. Regime 2 TC and C:N ratio are lower than that of Regime 1.

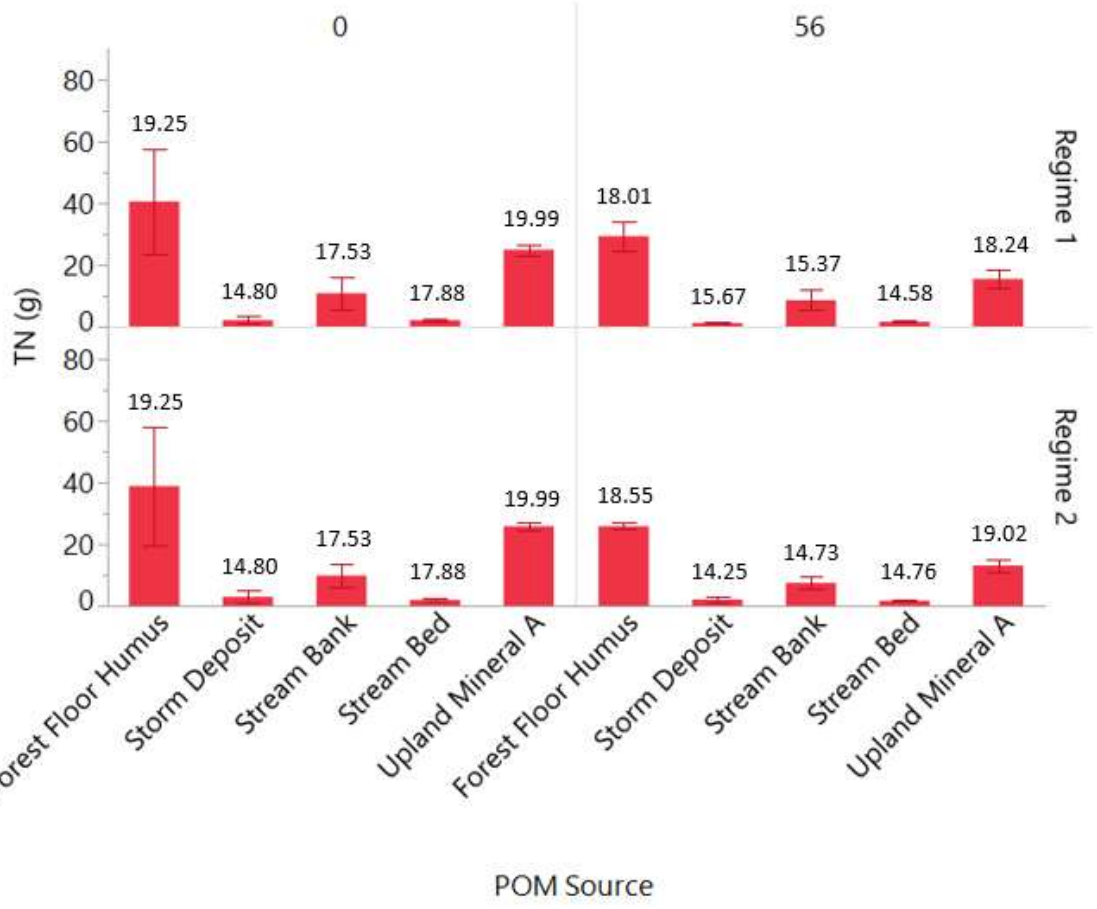

Figure 4.3: Comparison of initial and final TN (g) across POM sources. Initial and final molar C:N ratio for each POM source is listed above the corresponding TN bar. Final TN and C:N ratio of both regimes are lower than initial TN and C:N ratios, and Regime 2 TN and C:N ratio are lower than that of Regime 1.

In Regime 1, Forest Floor Humus and Upland Mineral A Horizon exhibited a decline in particulate TC from Day 0 to Day 4 (Figure 4.4). Forest Floor Humus appears to increase from Day 25 to Day 26, whereas this is not seen in the other POM sources for Regime 1. In Regime 2, Forest Floor Humus exhibits a similar pattern, but instead of

increasing after Day 25, it may have experienced a decline in particulate TC. The temporal changes exhibited by TC are mirrored in particulate TN for all POM sources (Figure 4.5).

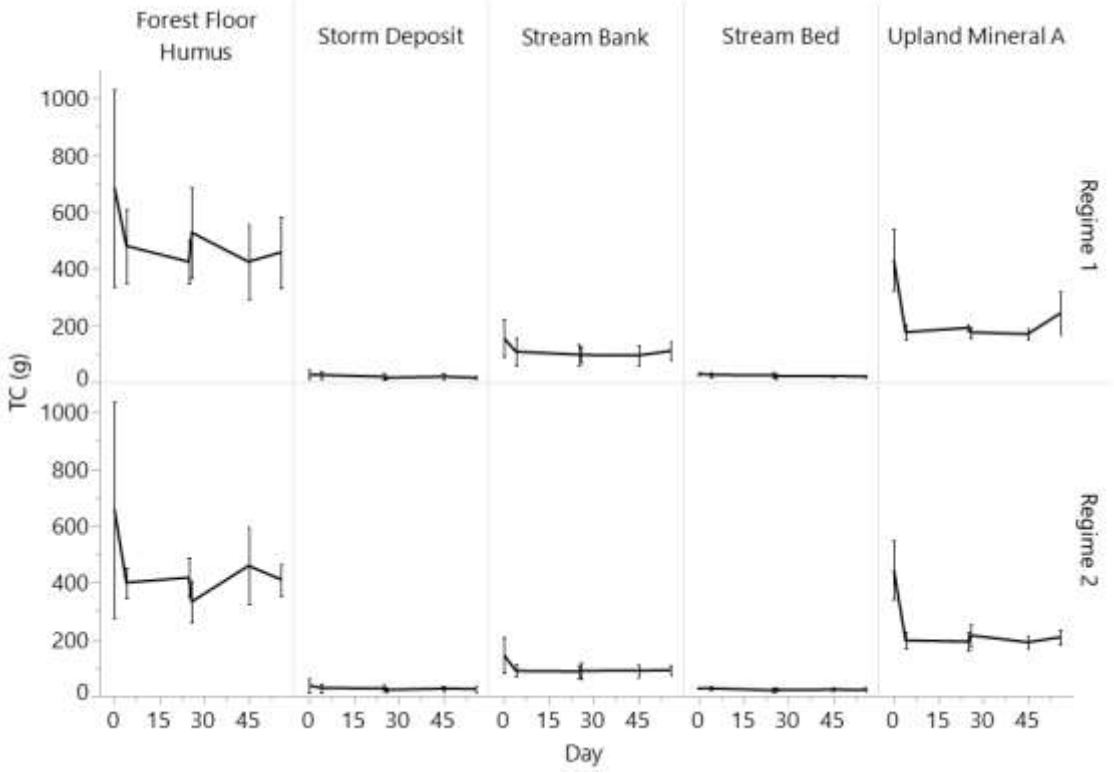

Figure 4.4: Comparison of particulate TC (g) over time for each POM source across Regime. TC measurements were taken on Day 0, 4, 25, 26, 45, and 56 and their means are reported as points on the graph. These data points are connected by a line to denote temporal change and the error bars associated with each mean depict standard deviation.

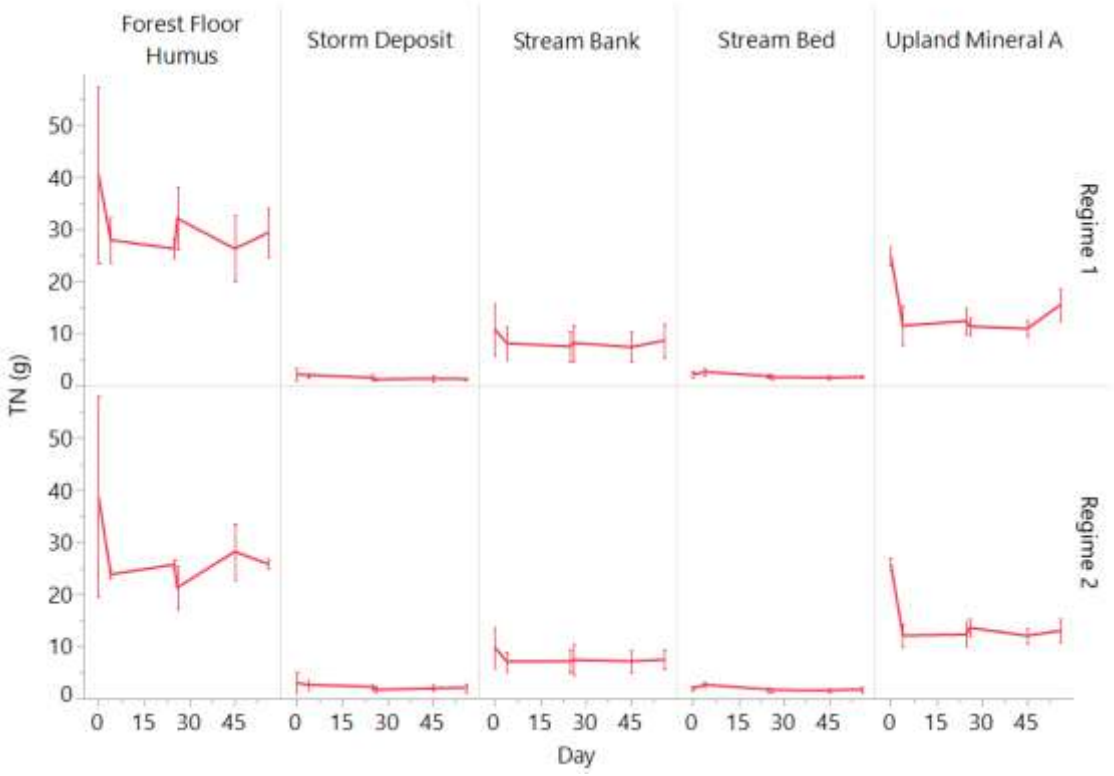

Figure 4.5: Comparison of particulate TN (g) over time for each POM source across Regime. TN measurements were taken on Day 0, 4, 25, 26, 45, and 56 and their means are reported as points on the graph. These data points are connected by a line to denote temporal change and the error bars associated with each mean depict standard deviation.

Total Nitrogen for Forest Floor Humus (R1; 30.42± 8.58g, R2; 27.30± 9.07g) was significantly greater than that for Upland Mineral A Horizon (R1; 14.42± 5.50g, p = <.0001, R2; 14.81± 5.36g, p = <.0001, Table A7), and Upland Mineral A Horizon was significantly greater than that for Stream Bank (R1; 8.42± 3.34g, p = <.0001, R2; 7.67± 2.51g, p = <.0001, Table 4.3, Table A7). Total Nitrogen for Stream Bank was significantly greater than that for Stream Bed (R1; 1.85± 0.57g, p = <.0001, R2; 1.86± 0.87, p = <.0001) and Storm Deposit (R1; 1.54±0.70, p = <.0001, R2 2.24± 1.06g, p =

<.0001, Table A7). Particulate TC exhibited a similar pattern across POM sources (Figure 4.2). Particulate TC for Forest Floor Humus (R1; 499.72± 179.80g, R2; 445.88± 178.79g) was significantly greater than Upland Mineral A Horizon (R1; 14.42± 5.50g, R2;14.81± 5.36g, p = <.0001); Upland Mineral A Horizon was significantly greater than Stream Bank (R1; 8.42± 3.34g, R2;7.67± 2.51g, p = <.0001); Stream Bank was significantly greater than Storm Deposit (R1; 1.54± 0.72g, R2; 2.24± 1.06g, p = 0.0116); and Stream Bed (R1; 1.85± 0.57g, R2; 1.86± 0.55g, p = 0.0161) was not significantly different from both Stream Bank and Storm Deposit in particulate TC.

Table 4.3: Mean masses and standard deviations of TC and TN for watershed sources within each moisture regime. Mean particulate masses are in bold, standard deviation is indicated in the parentheses, and letters denote significant difference.

Moisture Regime	POM Source	TC (g)	TN (g)
Regime 1	Forest Floor Humus	**499.72** (179.80, A)	**30.42** (8.58, A)
	Storm Deposit	**20.95** (10.81, D)	**1.54** (0.72, D)
	Stream Bank	**110.40** (42.52, C)	**8.42** (3.34, C)
	Stream Bed	**24.51** (7.07, D)	**1.85** (0.57, D)
	Upland Mineral A Horizon	**231.53** (106.43, B)	**14.42** (5.50, B)
Regime 2	Forest Floor Humus	**445.88** (178.79, A)	**27.30** (9.07, A)
	Storm Deposit	**28.02** (13.72, D)	**2.24** (1.06, D)
	Stream Bank	**98.55** (35.85, C)	**7.67** (2.51, C)
	Stream Bed	**23.83** (6.54, D)	**1.86** (0.55, D)
	Upland Mineral A Horizon	**240.86** (103.67, B)	**14.81** (5.36, B)

There were significant differences across POM sources for the net change of particulate TC and TN from Day 0 to Day 50 in Regime 1. Net increase of TC for Stream Bed Regime 1 (297.51± 226.46g) was significantly greater than that for Forest Floor Humus (-227.26± 349.76, p = 0.0068) and Upland Mineral A Horizon (-187.80± 75.11, p = 0.0124, Figure 4.6, Table 4.4, Table A8), which exhibited net decreases of TC. The net increase seen for Stream Bed in Regime 1 was not seen in Regime 2. A similar set of patterns were seen in particulate TN; Stream Bed Regime 2 (-16.98± 11.95g) was significantly greater than Storm Deposit (-0.89± 1.01, p = 0.0495), Upland Mineral A Horizon (-9.43± 3.50, p = 0.0113), and Forest Floor Humus (-11.15± 19.12, p = 0.0070, Figure 4.7, Table 4.3, Table A8).

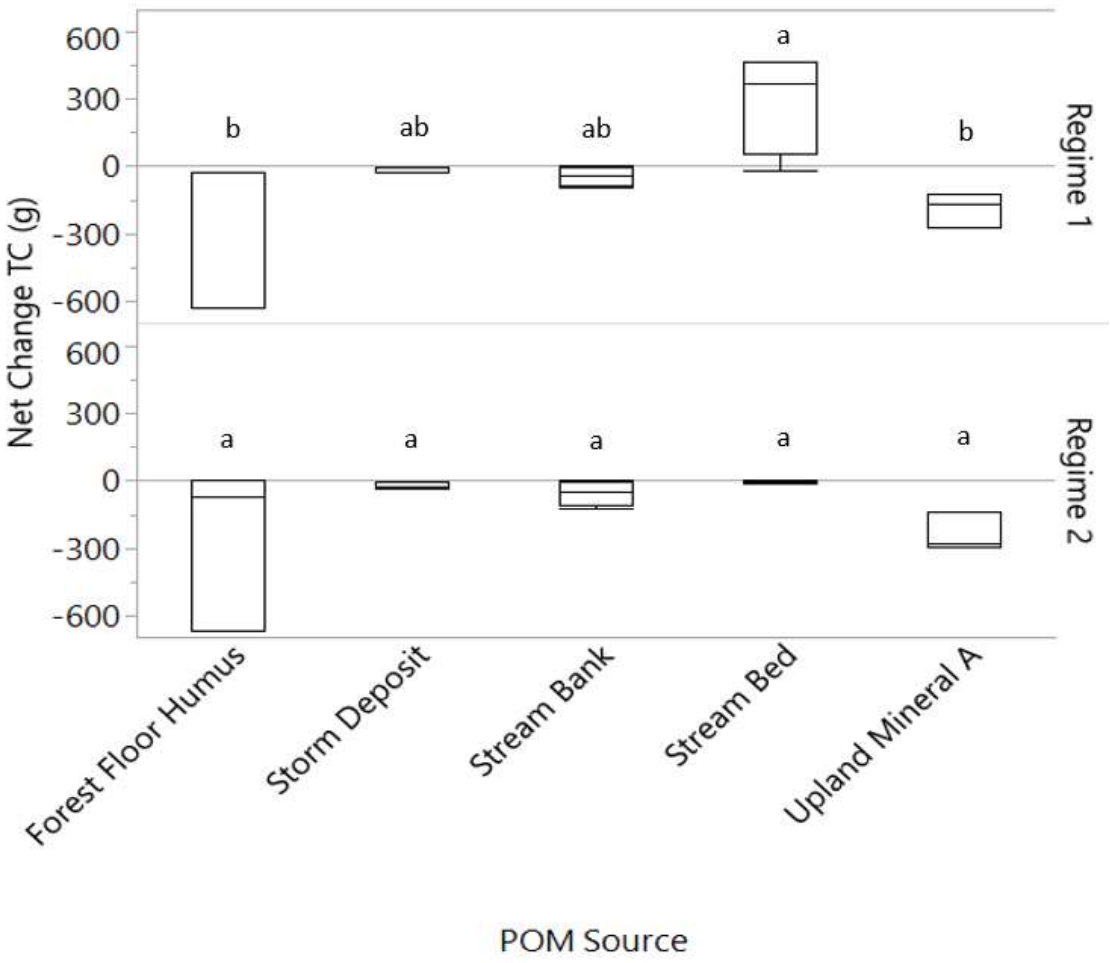

Figure 4.6: Comparison of particulate TC (g) net change from Day 0 to Day 56 for each POM source by regime. Net change means that are greater than zero indicate a net increase in TC over time. Letters denote significant difference.

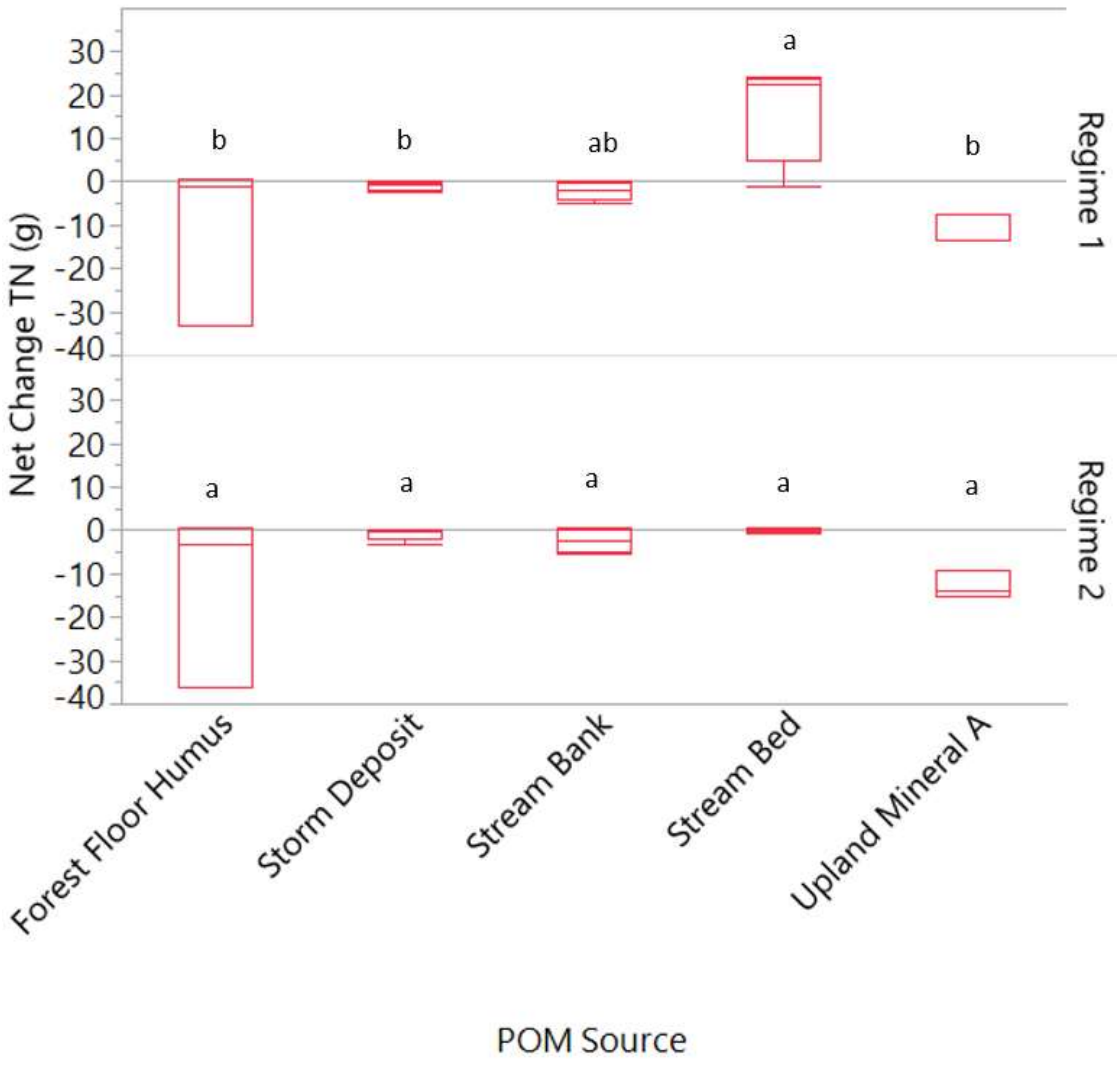

Figure 4.7: Comparison of particulate TN (g) net change from Day 0 to Day 56 for each POM source by regime. Net change means that are greater than zero indicate a net increase in TN over time. Letters denote significant difference.

Table 4.4: Mean net change of masses TC and TN for watershed sources within each moisture regime. Mean net change masses are in bold, standard deviation is indicated in the parentheses, and letters denote significant difference.

Moisture Regime	POM Source	Net Change TC (g)	Net Change TN (g)
Regime 1	Forest Floor Humus	**-227.26** (349.76, B)	**-11.15** (19.12, B)
	Storm Deposit	**-10.10** (11.77, AB)	**-0.89** (1.01, B)
	Stream Bank	**-44.47** (41.19, AB)	**-2.18** (2.03, AB)
	Stream Bed	**297.51** (226.46, A)	**16.98** (11.95, A)
	Upland Mineral A Horizon	**-187.80** (75.11, B)	**-9.43** (3.50, B)
Regime 2	Forest Floor Humus	**-246.25** (366.27, A)	**-12.96** (20.03, A)
	Storm Deposit	**-11.79** (13.55, A)	**-0.911** (1.20, A)
	Stream Bank	**-52.97** (54.43, A)	**-2.41** (2.62, A)
	Stream Bed	**-5.95** (5.71, A)	**-0.20** (0.62, A)
	Upland Mineral A Horizon	**-238.17** (85.65, A)	**-12.84** (3.31, A)

In summary, Regime 1 and Regime 2 are potentially distinguished by the changes of TC and TN for Forest Floor Humus after the large rewetting event between Day 25 and Day 26 (R1; increases in TC and TN, R2; decrease in TC and TN), however these differences are difficult to determine due to wide standard deviation values (Figure 4.4, Figure 4.5). POM sources are clearly distinguished by their C:N ratios, particulate TC and TN content and net change means; the upland sources, Forest Floor Humus and Upland Mineral A Horizon are C and N rich, while the fluvial and mixed sources, Stream Bank, Stream Bed, and Storm Deposit, are C and N poor.

4.3 Changes in Porewater Mass

At the beginning of the incubation, porewater TN for Forest Floor Humus, Stream Bank, Stream Bed, and Upland Mineral A Horizon were primarily comprised of DON and NH_4^+-N; DON and NH_4^+-N accounted for 65-67% and 34-32% of the TN respectively for the POM sources (Figure 4.8). In contrast, NO_3^--N accounted for 75-98% of the TN at Day 0 for Storm Deposit, with DON and NH_4^+-N only contributing 2-22% to the TN. Forest Floor Humus had the greatest TN, DON, and NH_4^+-N of all POM sources. Similarly, Forest Floor Humus initially had the highest DOC (267.97-412.38 $\pm$ 74.45), followed by Upland Mineral A Horizon (59.52- 98.36 $\pm$ 20.00), Stream Bank (19.00- 161.94 $\pm$ 65.91), Storm Deposit (9.11- 23.14 $\pm$ 5.29), and Stream Bed (13.56- 54.23 $\pm$ 19.90, Figure 4.9).

The comparison of % Humic-like fluorescence across POM source followed a pattern similar to that of the particulate TC and TN; greatest % Humic-like fluorescence was found in Forest Floor Humus and Upland Mineral A Horizon, followed by Storm Deposit and Stream Bed, and then Stream Bank (Figure 4.10). Percent Protein-like fluorescence loosely followed the inverse of this pattern; greatest % Protein-like fluorescence was found in Stream Bank, followed by Storm Deposit and Stream Bed, and then Forest Floor Humus and Upland Mineral A Horizon (Figure 4.10). Initially, SUVA was the lowest for Stream Bank, followed by Forest Floor Humus and Upland Mineral A Horizon, and the greatest in Storm Deposit and Stream Bed (Figure 4.11).

By the end of the incubation (Day 50), porewater TN masses increased and became more NO_3^--N rich across all POM sources, except for Regime 1 Forest Floor Humus, which maintained initial TN conditions (Figure 4.8). In contrast, final porewater DOC masses were lower than the initial masses across all POM sources and both regimes (Figure 4.9). At the end of the incubation, there was almost no DOC found in Storm Deposit, Stream Bank, Stream Bed, and Upland Mineral A Horizon porewater. Forest Floor Humus had final DOC masses lower than the initial masses, but still had more than the other POM sources. At the end of the incubation (Day 50), % Protein-like fluorescence in Regime 1 was lower than initial conditions across all POM sources and this decline did not occur in Regime 2 (Figure 4.10). SUVA changed from initial conditions, resulting in Forest Floor Humus and Upland Mineral A Horizon having the greatest SUVA, followed by Storm Deposit and Stream Bed, and then Stream Bank (Figure 4.11), indicating the C and N rich sources ended up being more aromatic than the mixed and C and N poor POM sources.

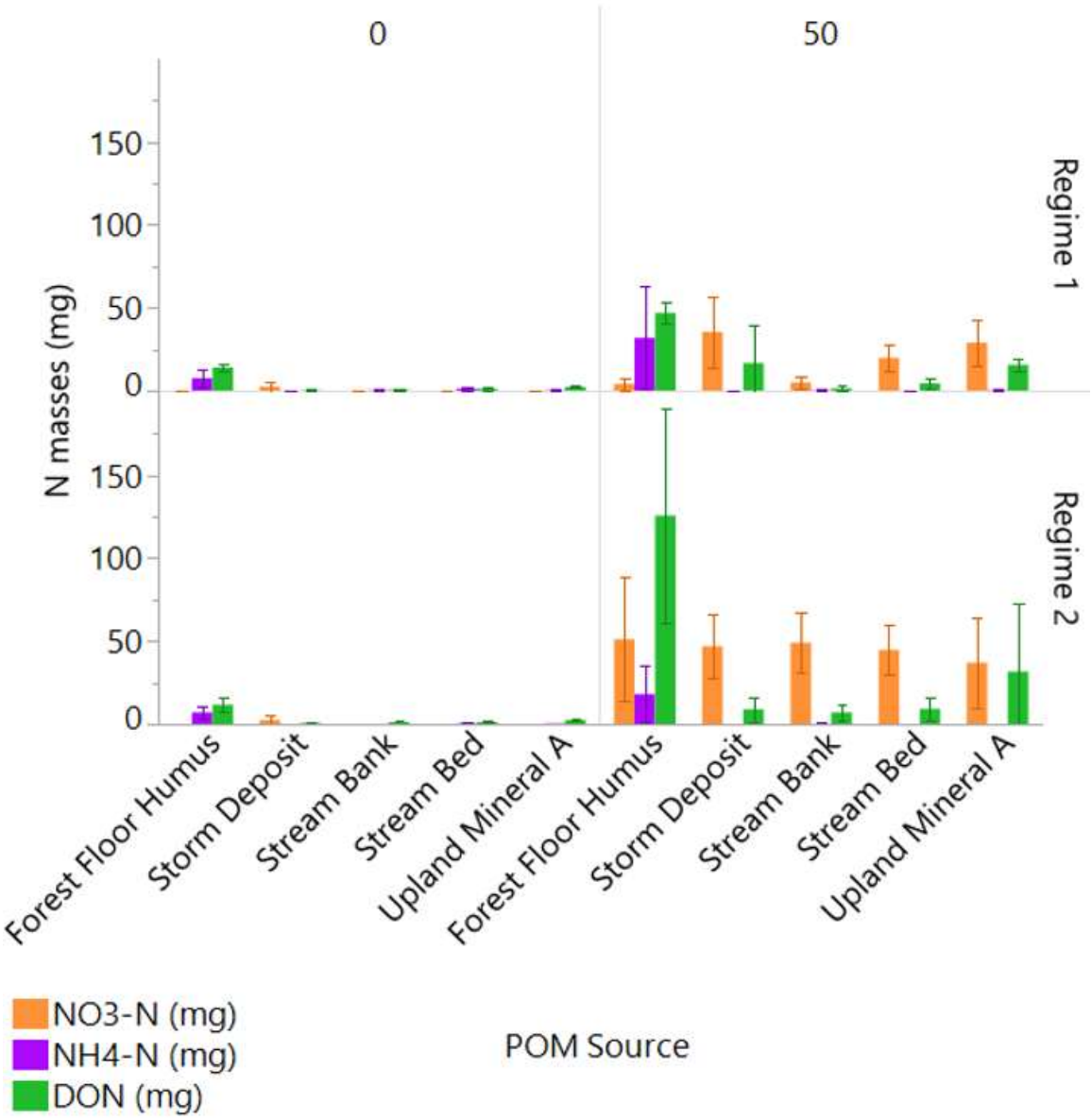

Figure 4.8: Comparison of initial (Day 0) and final (Day 50) N species across all POM sources. TN is comprised of nitrate, measured as NO_3^--N (orange), ammonium, measured as NH_4^+-N (purple), and dissolved organic nitrogen, measured as DON (green). Porewater masses shift and become more NO_3^--N-rich by the end of the incubation.

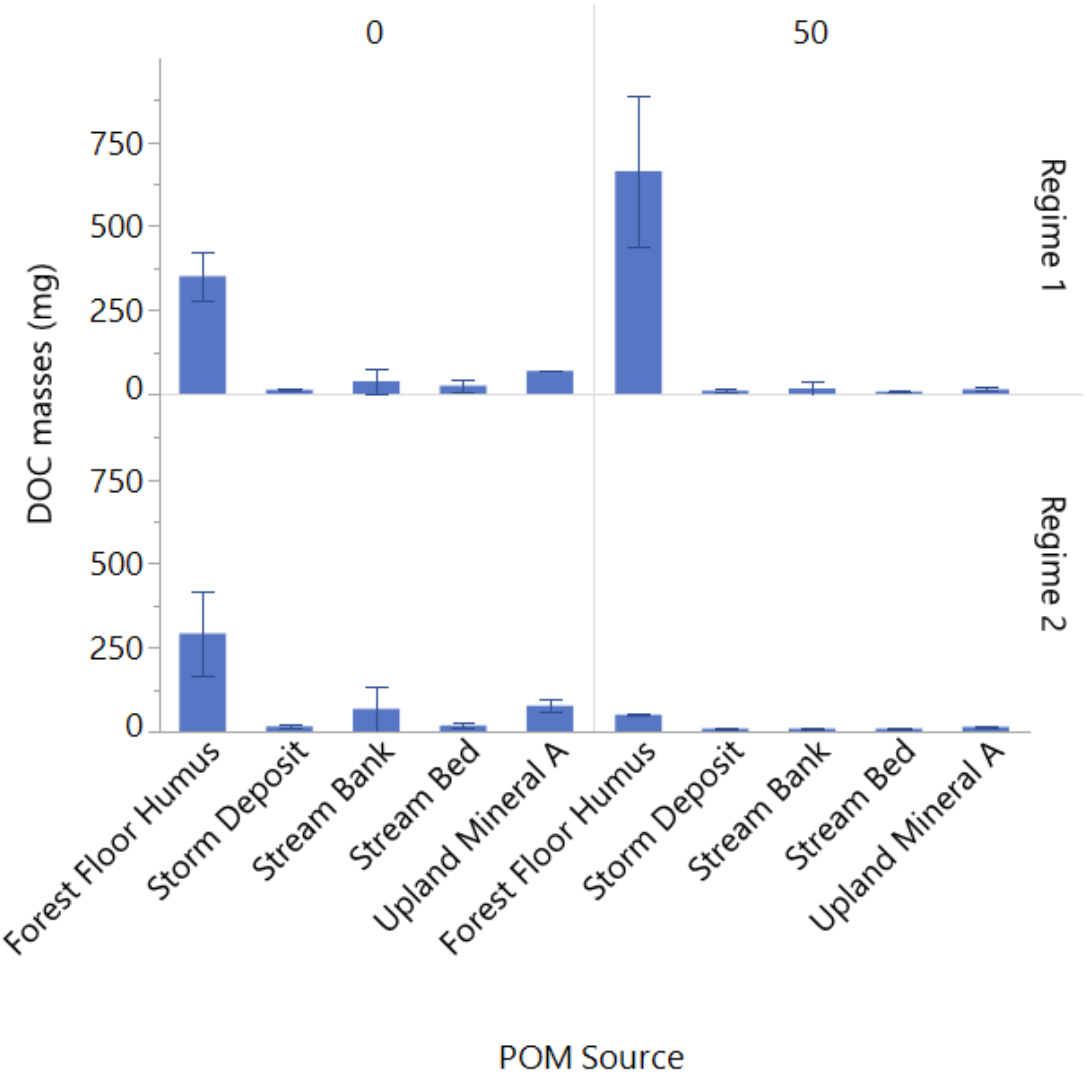

Figure 4.9: Comparison of initial (Day 0) and final (Day 50) DOC across all POM sources. All POM source types had greater porewater DOC at Day 0 than Day 50.

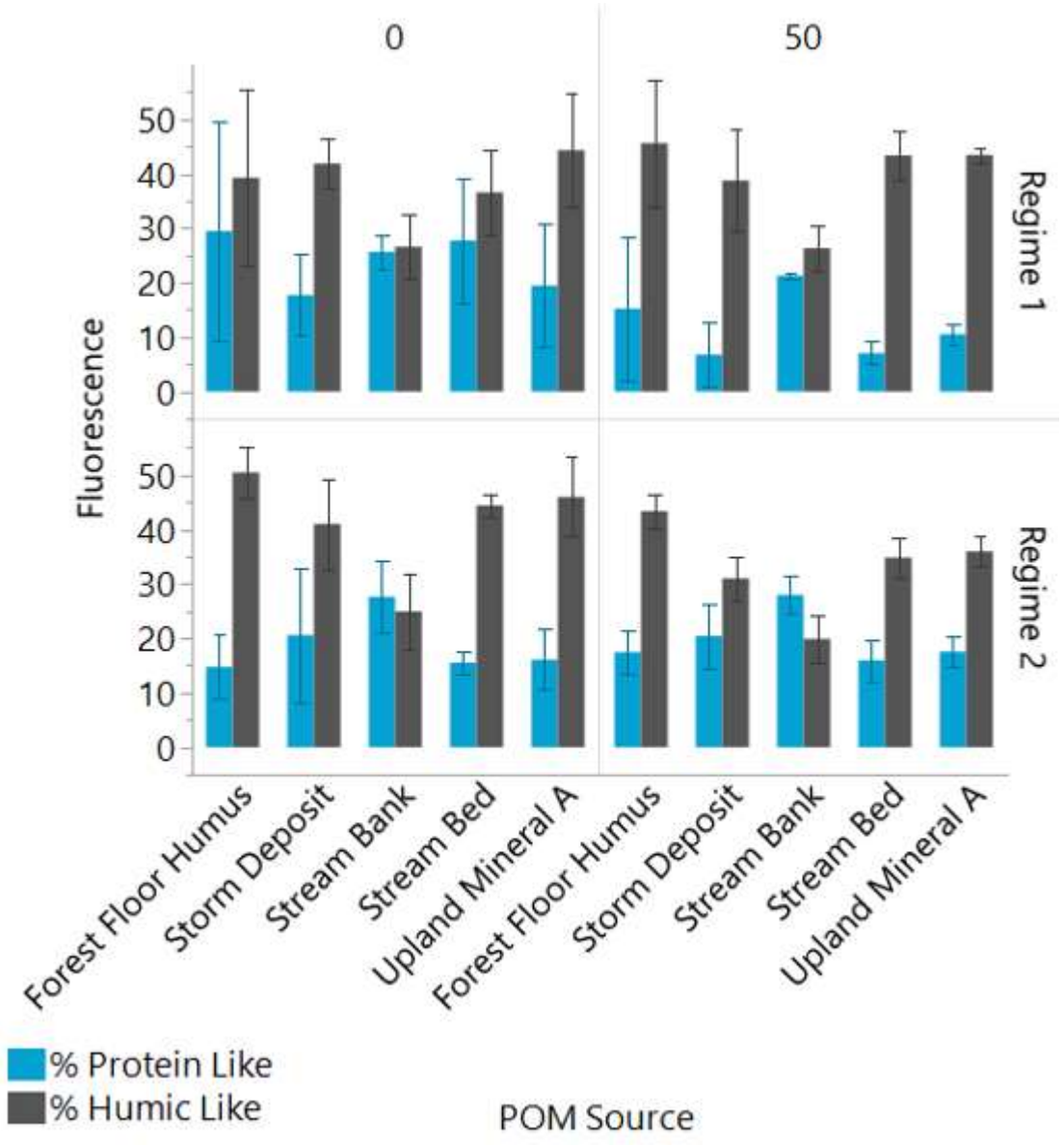

Figure 4.10: Comparison of initial (Day 0) and final (Day 50) of % Protein-like and % Humic-like fluorescence across all POM sources in Regime 1 and Regime 2.

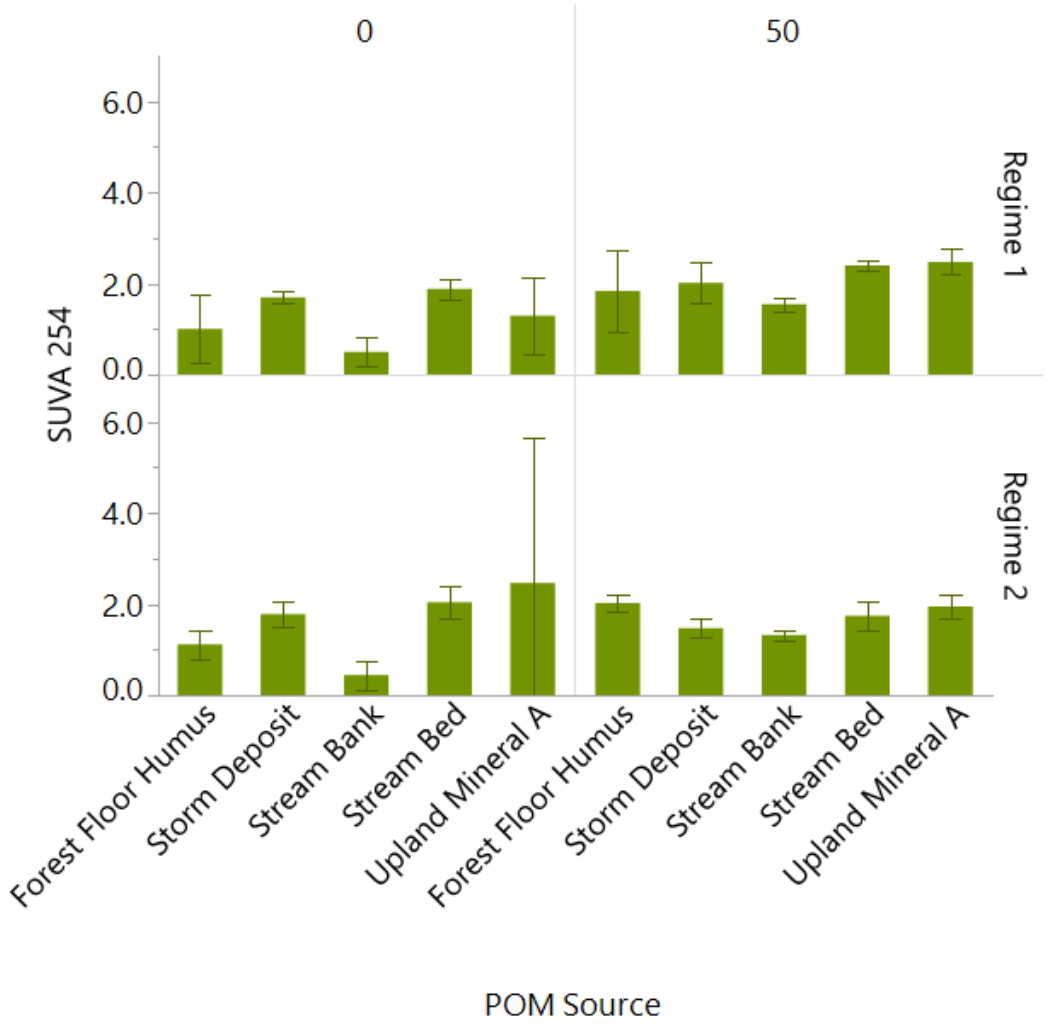

Figure 4.11: Comparison of initial (Day 0) and final (Day 50) of SUVA across all POM source in Regime 1 and Regime 2.

Porewater C and N masses changed greatly throughout the incubation and varied across POM source and moisture Regime. In general, porewater TN increased throughout the incubation across all POM sources, but Forest Floor Humus was distinct in the magnitude of the porewater TN increase. Forest Floor Humus porewater TN for Regime 1 gradually increased between Day 0 and Day 25, while TN for Regime 2

potentially experienced a sharp increase after Day 25 which continued to the end of the incubation (Figure 4.12).

The temporal changes of porewater TN were largely driven by changes in porewater NO_3^--N, so the temporal patterns observed in TN are strong reflections of the temporal changes of NO_3^--N across all POM sources, except for Forest Floor Humus Regime 1. Throughout the incubation substantial changes in porewater NO_3^--N for Forest Floor Humus Regime 2 appear to occur, however large variance makes it difficult to determine the significance and magnitude of these changes (Figure 4.13). In contrast, it is clear that porewater NO_3^--N for Forest Floor Humus Regime 1 remained at low masses throughout the incubation and constituted a small portion of the TN for that POM source (Figure 4.12, Figure 4.13).

Ammonium for Forest Floor Humus Regime 1 increased sharply between Day 0 and Day 25, potentially declined after the large rewetting event at Day 25, and then recovered to pre-rewetting event conditions by the end of the incubation (Figure 4.14). In comparison, Forest Floor Humus Regime 2 exhibited a prominent increase NH_4^+-N in the period between Day 26 and Day 50. Similarly, DON for Forest Floor Humus Regime 2 remained relatively unchanged until Day 26 at which point it dramatically increased by Day 50, from 11.6 $\pm$ 1.05mg to 125.77 $\pm$ 65.04mg, respectively (Figure 4.15). Greater means and wider variances of DON for Stream Bed and Upland Mineral A Horizon, indicate that for Stream Bed Regime 1 there were some incubation columns that experienced increases in DON directly after the large rewetting event at Day 25, while Upland Mineral A Horizon Regime 2 experienced a decrease and then recovery after this event (Figure 4.15).

Porewater DOC for Storm Deposit, Stream Bank, Stream Bed, Upland Mineral A Horizon, and Forest Floor Humus Regime 2 decreased throughout the incubation (Figure 4.16). Though Forest Floor Humus Regime 2 exhibited the same temporal pattern as the other POM sources, the magnitude of the porewater DOC mass for Forest Floor Humus dwarfs that of the other POM sources. Porewater DOC for Forest Floor Regime 1 increases throughout the incubation, increasing from 350.65± 74.45mg at Day 0 to 663.25± 224.37mg at Day 50. For Forest Floor Regime 1, porewater DOC and NH_4^+-N have very similar patterns, while TN ultimately resembles that of NO_3^- -N.

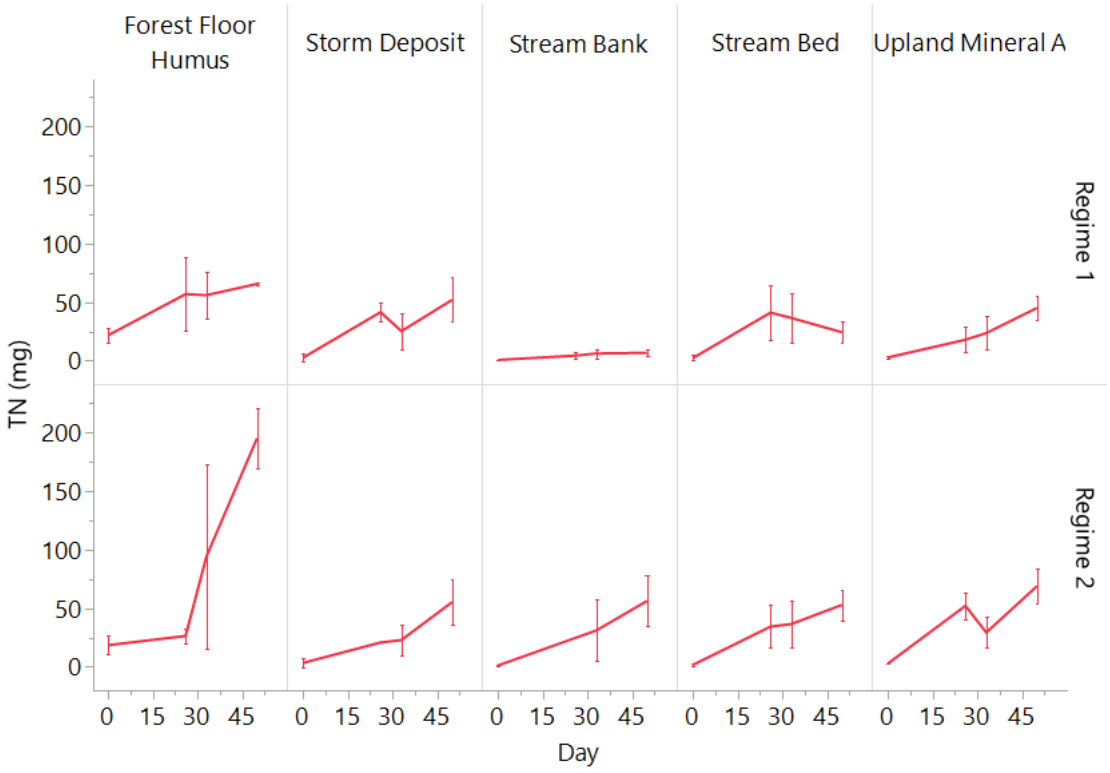

Figure 4.12: Comparison of the porewater TN (mg) means at Day 0, 26, 33, and 50 for each POM source across regime. Reported means are connected by a line to denote change over time. Error bars denote standard deviation of each mean.

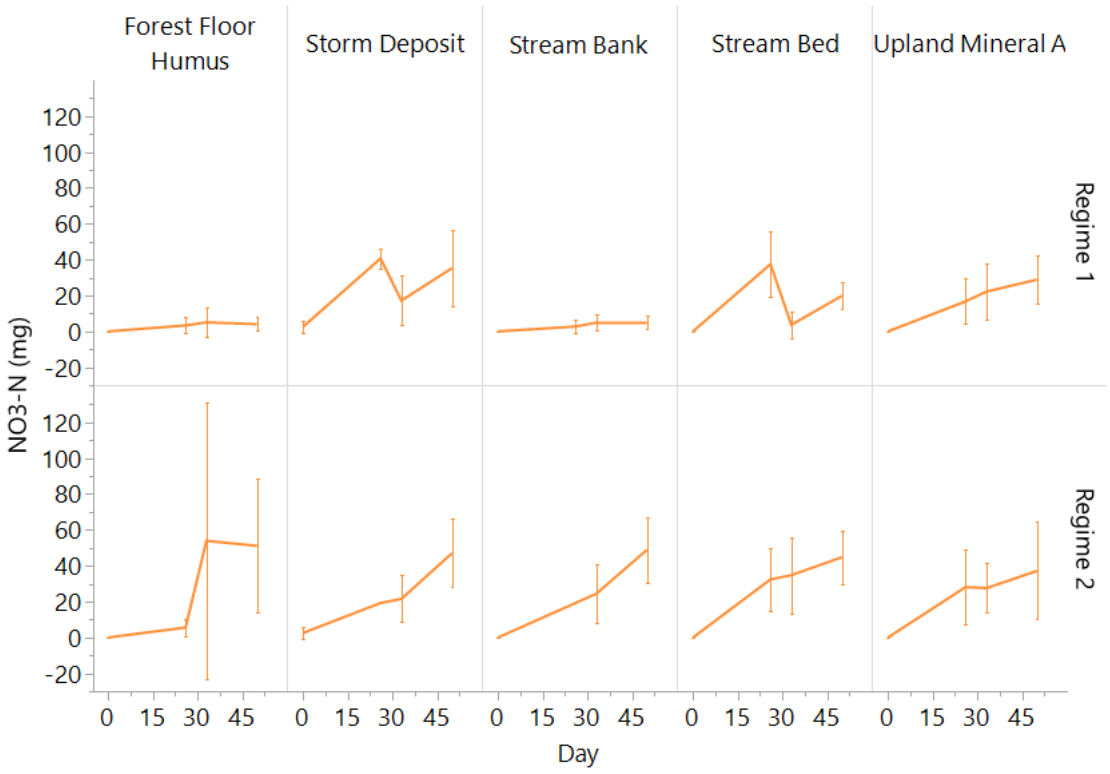

Figure 4.13: Comparison of the porewater TN (mg) means at Day 0, 26, 33, and 50 for each POM source across regime. Reported means are connected by a line to denote change over time. Error bars denote standard deviation of each mean.

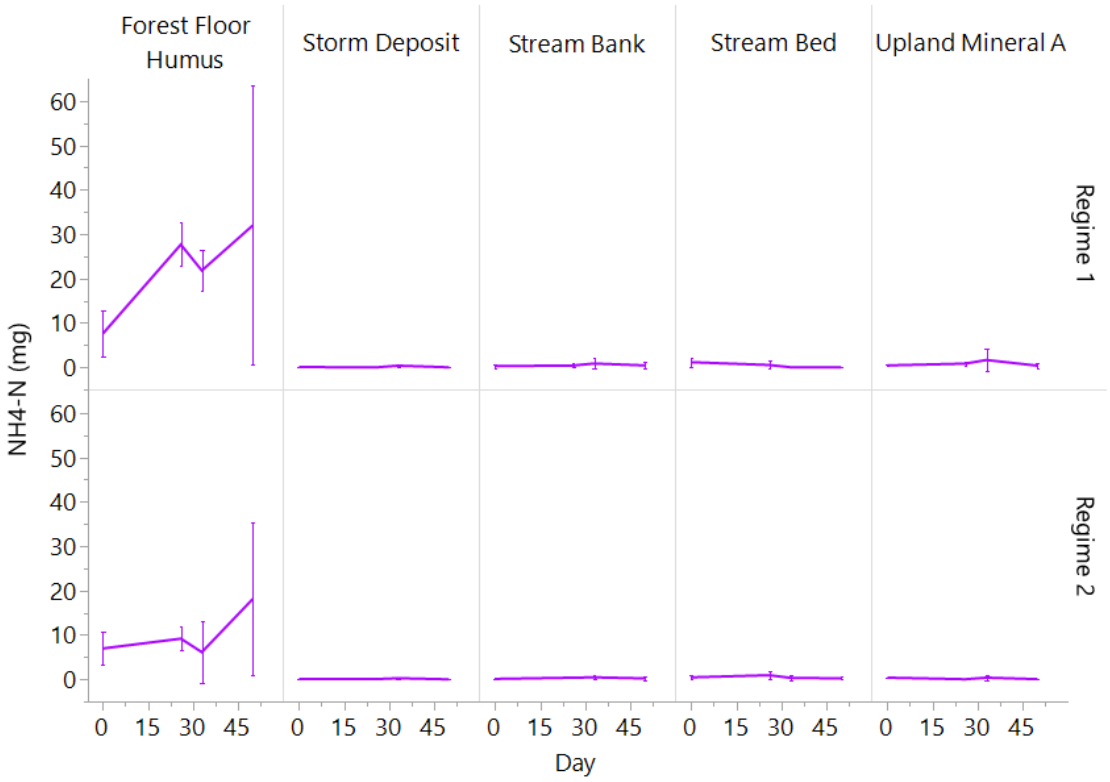

Figure 4.14: Comparison of the porewater TN (mg) means at Day 0, 26, 33, and 50 for each POM source across regime. Reported means are connected by a line to denote change over time. Error bars denote standard deviation of each mean.

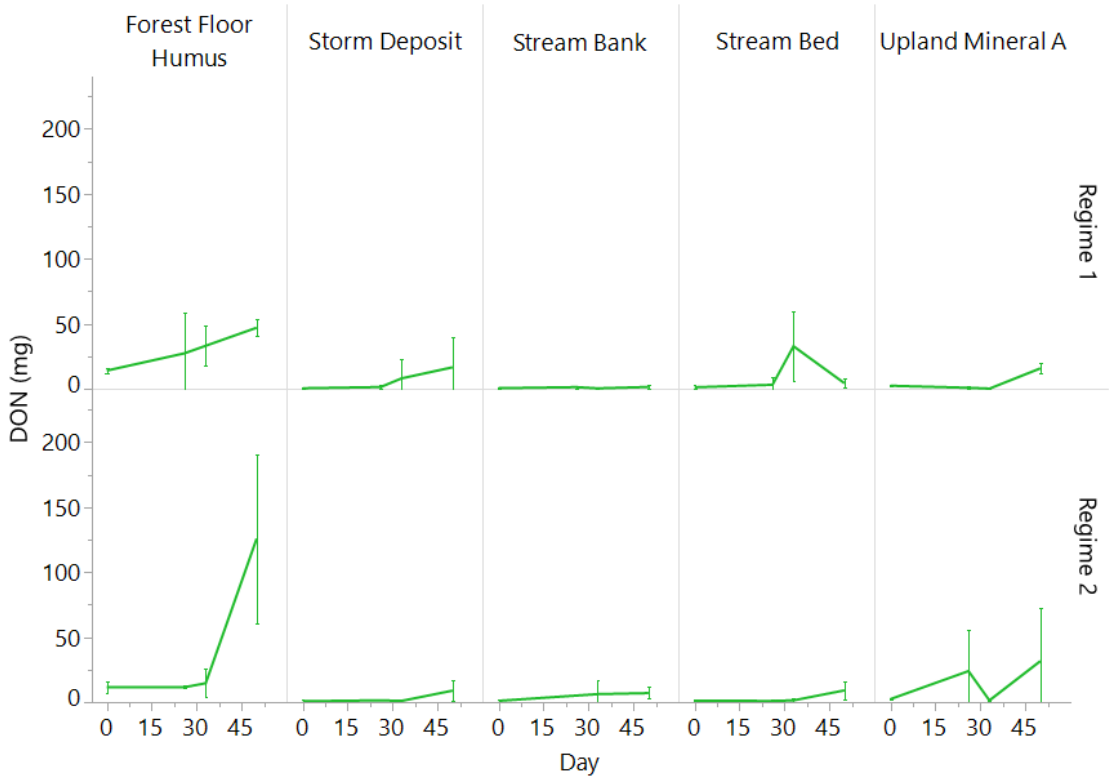

Figure 4.15: Comparison of the porewater TN (mg) means at Day 0, 26, 33, and 50 for each POM source across regime. Reported means are connected by a line to denote change over time. Error bars denote standard deviation of each mean.

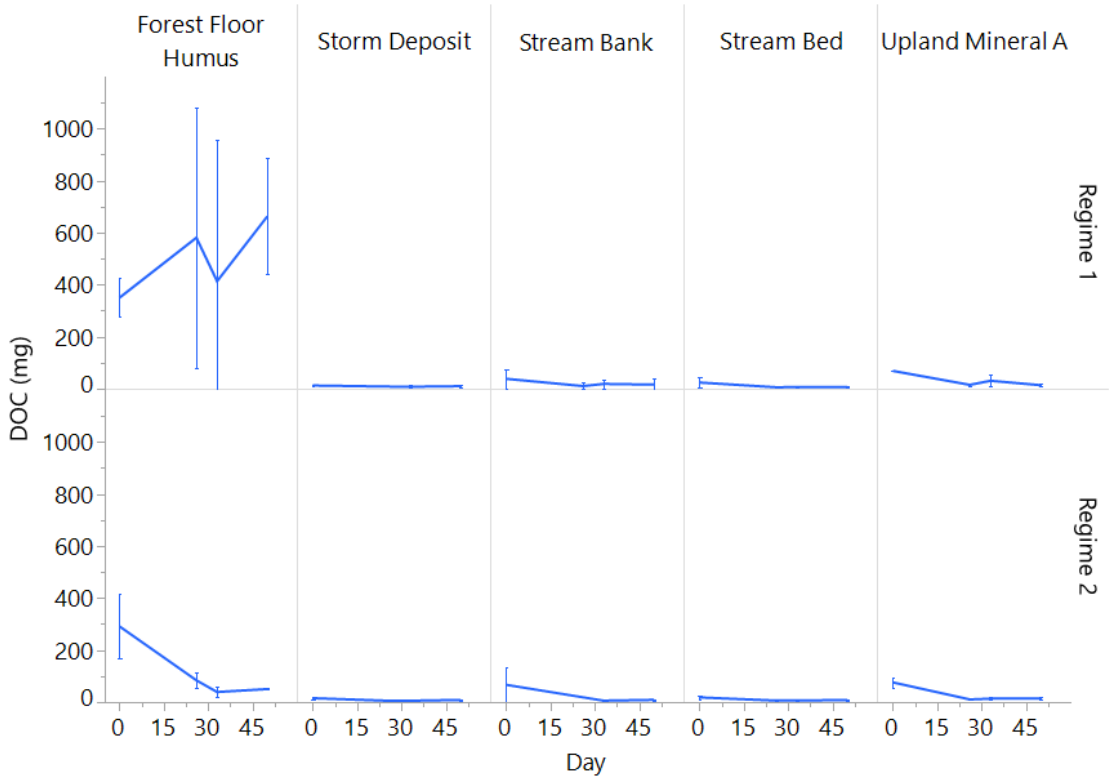

Figure 4.16: Comparison of the porewater TN (mg) means at Day 0, 26, 33, and 50 for each POM source across regime. Reported means are connected by a line to denote change over time. Error bars denote standard deviation of each mean.

In Regime 1, TN for Stream Bed (26.07± 21.38mg) and Forest Floor Humus (48.61± 24.43mg) were significantly greater than TN for Stream Bank (4.52± 3.58mg, Stream Bed- Stream Bank p = <0.0001, Forest Floor Humus- Stream Bank p = 0.0219, Table 4.5). In Regime 2, TN for Forest Floor Humus (88.12± 84.46mg) was significantly greater than TN for Storm Deposit (26.82± 24.70mg, p = 0.0018), Stream Bank (29.51± 29.44mg, p = 0.0087), Stream Bed (31.36± 23.47mg, p = 0.0065), and Upland Mineral A Horizon (38.27± 27.70mg, p = 0.0367, Table 4.5, Table A9). In Regime 1, NO_3^--N for Storm Deposit (23.23± 19.77mg) was significantly greater than

NO_3^--N for Forest Floor Humus (3.11± 4.69mg) and Stream Bank (3.12± 3.65mg). Also in Regime 1, DON for Forest Floor Humus (28.89± 19.60mg) was significantly greater than of Storm Deposit (6.86± 14.64mg, p = 0.0004), Stream Bank (0.93± 0.90mg, p = <.0001 for all comparisons), and Upland Mineral A Horizon (4.79± 6.89mg, 0.0008). Regime 2 reflected this pattern, except DON for Forest Floor Humus (46.43± 8.33mg) was also significantly greater than that of Stream Bed (10.37± 3.47mg).

DOC for Storm Deposit (10.14± 4.03mg), Stream Bank (21.17± 23.94mg), Stream Bed (11.06± 11.97mg), and Upland Mineral A Horizon (32.38± 25.10mg) were all not significantly different from each other, and significantly lower than that of Forest Floor Humus (502.23± 355.66mg, p = <0.0001 for all comparisons, Table 4.5, Table A9). The large difference between DOC for Forest Floor Humus and all other POM sources is reflected in the patterns seen in the POM source comparisons of Regime 2 DOC and both regimes of NH_4^+-N.

Table 4.5: Mean porewater masses for all POM sources by moisture regime. Mean dissolved masses are in bold, standard deviation is indicated in the parentheses, and letters denote significant differences.

Moisture Regime	POM Source	DOC (mg)	TN (mg)	NH_4^+-N (mg)	NO_3^-N (mg)	DON (mg)
Regime 1	Forest Floor Humus	**502.23** (355.66, A)	**48.61** (24.43, A)	**22.27** (16.88, A)	**3.**11 (4.69, B)	2**8.89** (19.60, A)
	Storm Deposit	**10.14** (4.03, B)	**29.82** (22.86, AB)	**0.13** (0.21, B)	**23.22** (19.77, A)	**6.86** (14.64, B)
	Stream Bank	**21.17** (23.94, B)	**4.52** (3.58, C)	**0.50** (0.71, B)	**3.12** (3.65, B)	**0.93** (0.90, B)
	Stream Bed	**11.06** (11.97, B)	**26.07** (21.38, B)	**0.45** (0.79, B)	**15.30** (18.03, AB)	**10.38** (18.13, AB)
	Upland Mineral A Horizon	**32.38** (25.10, B)	**22.37** (18.03, BC)	**0.83** (1.25, B)	**17.00** (15.21, AB)	**4.79** (6.89, B)
Regime 2	Forest Floor Humus	**119.33** (125.30, A)	**88.18** (84.46, A)	**10.18** (9.96, A)	**29.68** (46.56, A)	**46.43** (62.90, A)
	Storm Deposit	**9.70** (5.19, B)	**26.82** (24.70, B)	**0.11** (0.16, B)	**23.49** (22.03,	**3.45** (5.53, B)
	Stream Bank	**27.32** (45.20, B)	**29.51** (29.44, B)	**0.26** (0.33, B)	**24.47** (24.46, A)	**4.78** (6.45, B)
	Stream Bed	**9.99** (6.12, B)	**31.36** (23.47, AB)	**0.49** (0.59, B)	**28.01** (22.31, A)	**3.19** (4.69, B)
	Upland Mineral A Horizon	**28.70** (30.10, B)	**38.2**7 (27.70, B)	**0.22** (0.32, B)	**23.21** (21.40, A)	**14.85** (25.96, AB)

In Regime 1, %Protein-like fluorescence for Stream Bank (20.79± 2.23%) was significantly greater than that of Storm Deposit (10.21± 11.86%, p = 0.0044, Table 4.6). In Regime 2, %Protein-like fluorescence for Stream Bank (25.12± 2.09%, p = 0.0003-0.0212) was significantly greater than that of all other POM sources.

In Regime 1, % Humic-like fluorescence was not significantly different across all POM sources, but this was not the case for Regime2. In Regime 2, % Humic-like fluorescence for Forest Floor Humus (47.44± 2.03%) was significantly greater than that of Storm Deposit (38.47± 1.62%, p = <0.0001), and % Humic-like fluorescence for Storm Deposit was significantly greater than that for Stream Bank (23.76± 2.03%, p = <0.0001).

In Regime 1, % Fulvic-like fluorescence for Stream Bank was (51.73± 2.03%) was significantly greater than Forest Floor Humus (38.23± 2.44%, p = 0.0006). In Regime 2, % Humic-like fluorescence for Stream Bank (51.11± 1.53%) was significantly greater than that for Storm Deposit (45.58± 1.22%, p = 0.0482), and %Humic-like fluorescence for Storm Deposit was significantly greater than that for Forest Floor Humus (37.16± 1.53%, p = <0.0001).

Table 4.6: SUVA and % humic-, fulvic-, and protein-like metrics for all POM sources by moisture regime. Means are in bold, standard deviation is indicated in the parentheses, and letters denote significant difference

Moisture Regime	POM Source	%Protein Like Fluorescence	%Humic Like Fluorescence	% Fulvic Like Fluorescence	SUVA
Regime 1	Forest Floor Humus	**16.4** (14.34, AB)	**44.78** (10.1, A)	**38.82** (13.22, C)	**1.33** (0.84, A)
	Storm Deposit	**10.21** (6.84, B)	**41.19** (6.42, A)	**48.60** (8.07, AB)	**1.99** (0.78, A)
	Stream Bank	**20.79** (4.05, A)	**27.48** (5.18, A)	**51.73** (5.13, A)	**1.00** (0.48, A)
	Stream Bed	**13.77** (4.05, AB)	**39.70** (7.51, A)	**46.54** (7.44, ABC)	**2.12** (0.29, A)
	Upland Mineral A Horizon	**12.25** (6.85, AB)	**45.02** (4.82, A)	**42.73** (4.82, BC)	**1.81** (0.89, A)
Regime 2	Forest Floor Humus	**15.40** (5.65, B)	**47.44** (6.21, A)	**37.16** (4.13, C)	**3.06** (4.23, A)
	Storm Deposit	**15.95** (10.32, B)	**38.47** (8.72, B)	**45.58** (6.24, B)	**1.72** (0.33, A)
	Stream Bank	**25.12** (5.73, A)	**23.76** (5.97, C)	**51.11** (5.44, A)	**1.09** (0.52, A)
	Stream Bed	**12.27** (5.81, B)	**41.46** (6.35, AB)	**46.29** (4.89, AB)	**1.97** (0.36, A)
	Upland Mineral A Horizon	**15.08** (4.24, B)	**42.48** (6.27, AB)	**42.44** (4.96, BC)	**2.14** (1.99, A)

The net change of porewater TN, NO_3^--N, NH_4^+-N, DON, and DOC from Day 0 to Day 50 varied among POM source and regime. Net change of porewater C and N for Stream Bank, Stream Bed, and Upland Mineral A Horizon were positive across both regimes, but Regime 1 resulted in smaller net increases than Regime 2 (Figure 4.17, Figure 4.18, Table 4.7). Porewater NH_4^+-N net change was very small for the majority of POM sources, but there were large net increases in NH_4^+-N for Forest Floor Humus in both moisture regimes (R1; 24.37$\pm$ 32.08mg, R2; 11.15$\pm$ 14.01mg). The net increase in TN for Storm Deposit, Stream Bank, and Stream Bed were driven by NO_3^--N, except for Upland Mineral A Horizon Regime 2 which was also largely influenced by DON (Figure 4.17). The net increase in DON observed for Upland Mineral A Horizon Regime 2 (29.20$\pm$ 41.29mg) is only rivaled by that of Forest Floor Humus across both regimes (R1; 17.26$\pm$ 29.34mg, R2; 114.23$\pm$ 68.60mg), and the greatest DON net increase is for Forest Floor Humus Regime 2. Net increase in DOC was only found for Forest Floor Humus Regime 1 (312.61$\pm$ 234.37mg). All other net change of DOC were net decreases, the greatest of which was for Forest Floor Humus Regime 2 (-240.72$\pm$ 121.72mg, Figure 4.18).

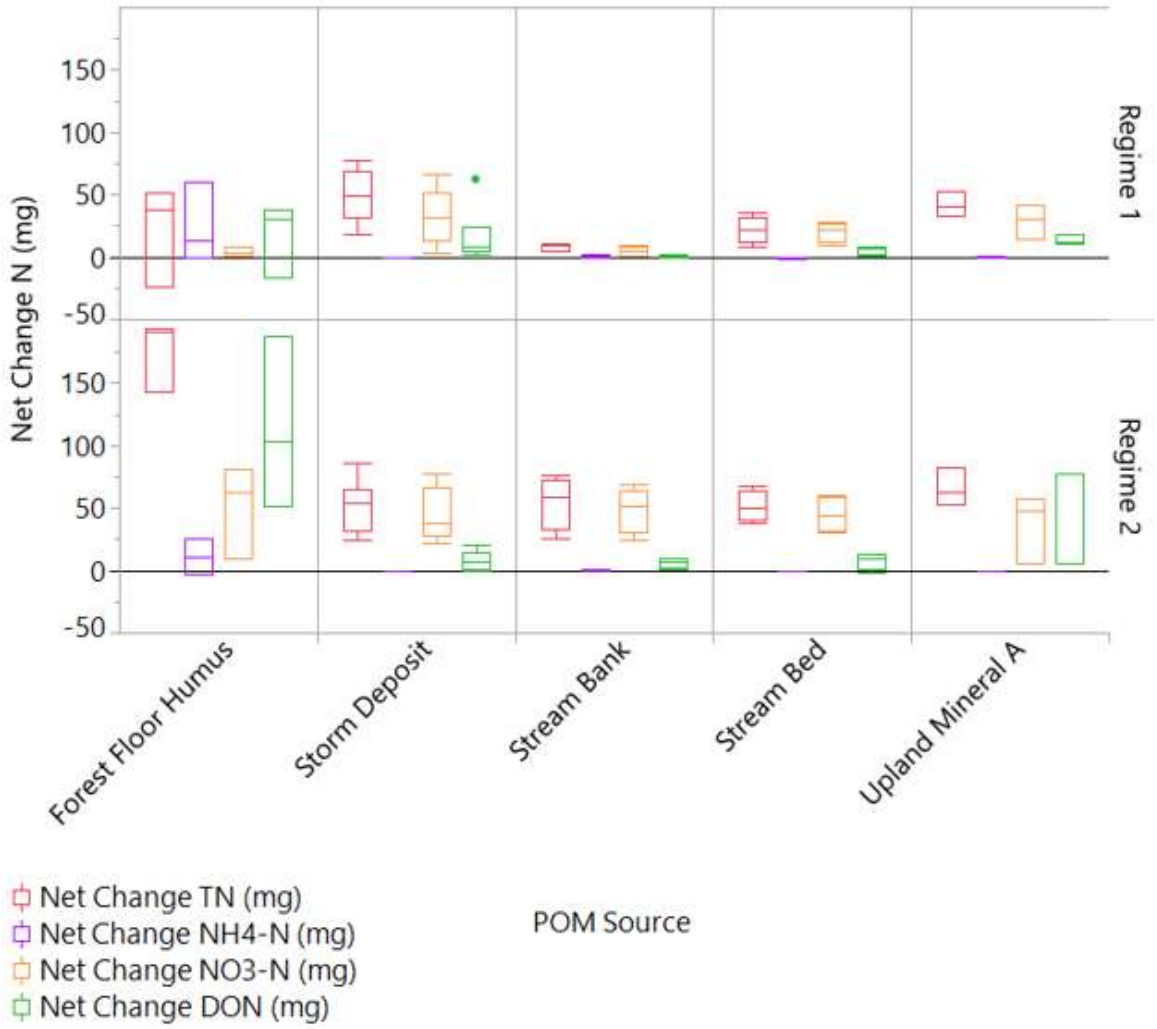

Figure 4.17: Comparison of porewater TN (g), NH_4^+-N (g), NO_3-N (g), and DON (g) net change from Day 0 to Day 56 for each POM source by regime. Net change means that are greater than zero indicate a net increase in TN over time.

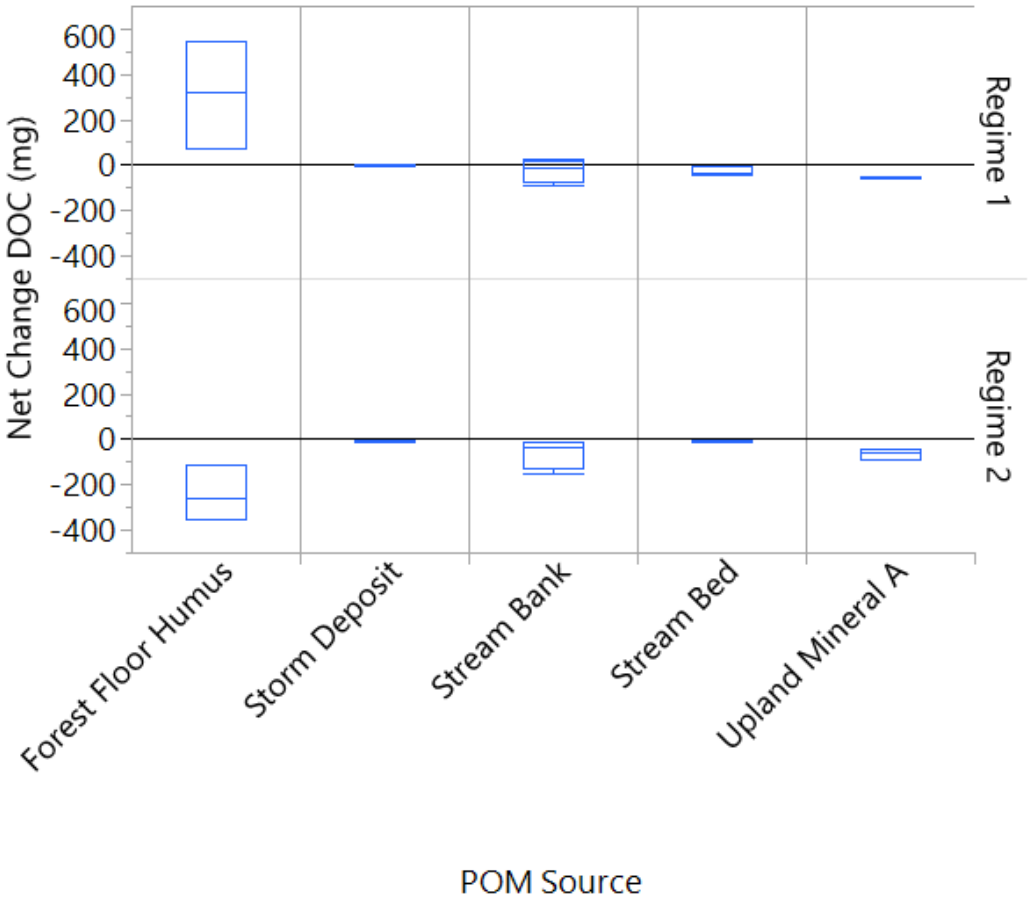

Figure 4.18: Comparison of porewater TC (g) net change from Day 0 to Day 56 for each POM source by regime. Net change means that are greater than zero indicate a net increase in TN over time.

Table 4.7: Net change porewater mass means for POM sources by moisture regime. Mean net change dissolved masses are in bold, standard deviation is indicated in the parentheses, and letters denote significant difference.

Moisture Regime	POM Source	Net Change DOC (mg)	Net Change TN (mg)	Net Change NH_4^+-N (mg)	Net Change NO_3^--N (mg)	Net Change DON (mg)
Regime 1	Forest Floor Humus	**312.61** (234.4, A)	**22.02** (40.49, AB)	**24.37** (32.08, A)	**4.00** (3.64, A)	**17.26** (29.34, A)
	Storm Deposit	**-2.23** (4.61, B)	**49.30** (21.45, A)	**-0.04** (0.07, A)	**32.72** (21.91, A)	**16.10** (22.88, A)
	Stream Bank	**-22.13** (49.12, B)	**6.03** (2.86, B)	**0.17** (1.01, A)	**4.83** (3.86, A)	**0.93** (1.10, A)
	Stream Bed	**-17.37** (18.92, B)	**22.20** (11.04, AB)	**-1.11** (1.00, A)	**19.88** (7.75, A)	**3.18** (3.34, A)
	Upland Mineral A Horizon	**54.91** (5.14, B)	**42.19** (9.99, AB)	**-0.06** (0.51, A)	**28.87** (13.54, A)	**13.38** (4.13, A)
Regime 2	Forest Floor Humus	**-240.72** (121.72, B)	**176.51** (28.65, A)	**11.15** (14.01, A)	**51.13** (37.26, A)	**114.23** (68.60, A)
	Storm Deposit	**-6.80** (3.92, A)	**51.81** (21.63, B)	**-0.08** (0.10, B)	**44.49** (21.42, A)	**8.16** (7.81, B)
	Stream Bank	**-57.75** (67.29, A)	**55.01** (21.28, B)	**0.12** (0.45, AB)	**48.93** (18.26, A)	**5.97** (4.03, B)
	Stream Bed	**-9.93** (4.33, A)	**51.13** (12.44, AB)	**-0.25** (0.27, AB)	**44.79** (14.88, AB)	**787** (6.81, B)
	Upland Mineral A Horizon	**-62.17** (23.54, A)	**65.99** (15.30, B)	**-0.34** (0.03, AB)	**37.13** (27.21, A)	**29.20** (41.29, B)

In summary, Regime 1 and Regime 2 differed substantially in the initial and final masses, temporal patterns, and net changes of porewater C and N. This was particularly evident in Forest Floor Humus. Forest Floor Humus Regime 1 exhibited substantial increases in NH_4^+-N and DOC, whereas Forest Floor Humus Regime 2 exhibited large increases in DON and decreases in DOC (Figure 4.14, Figure 4.15, Figure 4.16). By the end of the incubation, Forest Floor Humus Regime 1 was unique in that it did not become NO_3^--N rich when all other POM source-regime combinations did (Figure 4.8, Figure 4.12, Figure 4.13). Forest Floor Humus Regime 2 was similarly unique in that the net increase of porewater TN was primarily driven by porewater DON increases (Figure 4.12, Figure 4.15), while the other POM sources experienced net TN increases that were due to NO_3^--N enrichment regardless of regime (Figure 4.12, Figure 4.13).

4.4 Nitrification and Denitrification Gene Abundance for Particulate Sources

In the beginning of the incubation (Day 0), both nitrification and denitrification gene abundances were greater for Storm Deposit than Forest Floor Humus across both regimes and this remained true at the end of the incubation (Day 56, Figure 4.19, Figure 4.20). Both nitrification and denitrification gene abundances for Forest Floor Humus exhibited minimal change. In contrast, nitrification gene abundances increased and denitrification gene abundances decreased for Storm Deposit from Day 0 to Day 56 (Figure 4.19, Figure 4.20). It is worth noting that Day 56 nitrification gene abundances for Storm Deposit Regime 2 had greater variance than that of Regime 2 (Figure 4.19).

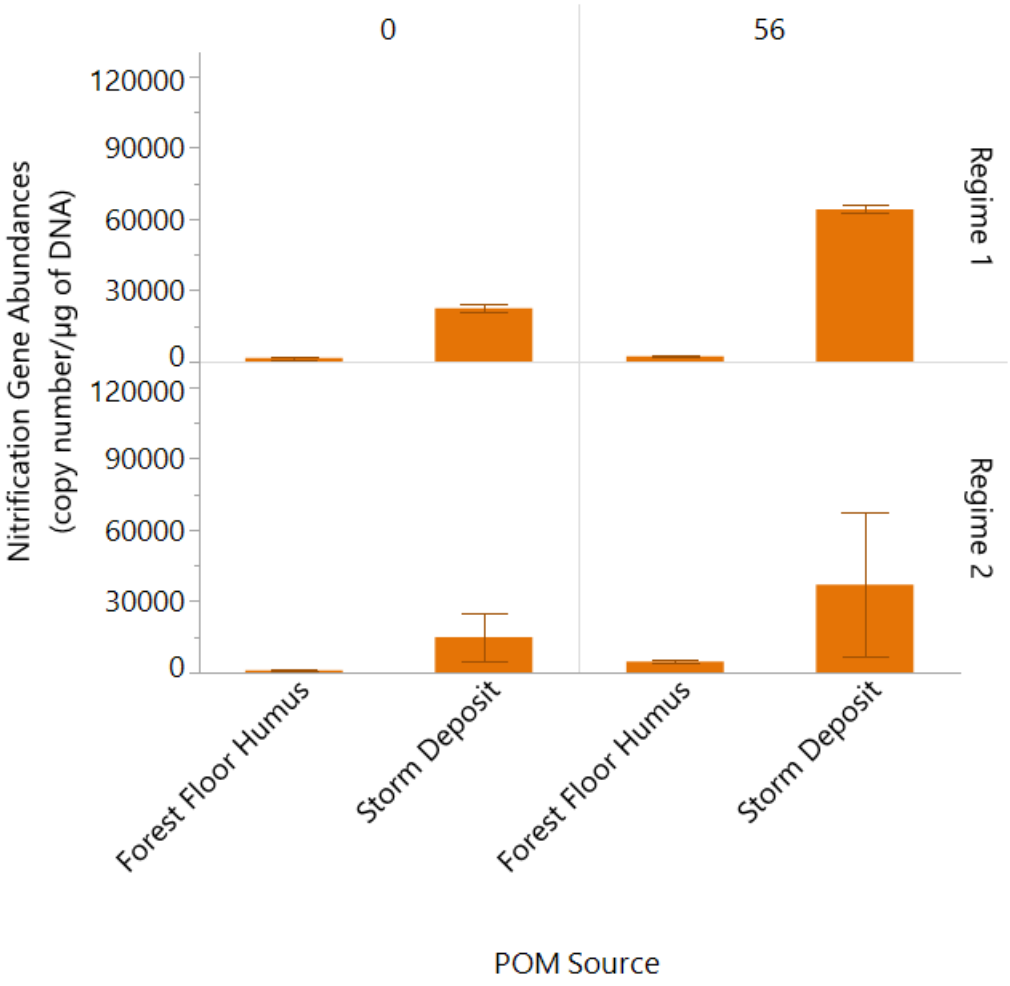

Figure 4.19: Comparison of initial (Day 0) and final (Day 50) nitrification gene abundances for Forest Floor Humus and Storm Deposit by moisture regime. Bars and error bars denote gene abundance means and standard deviation.

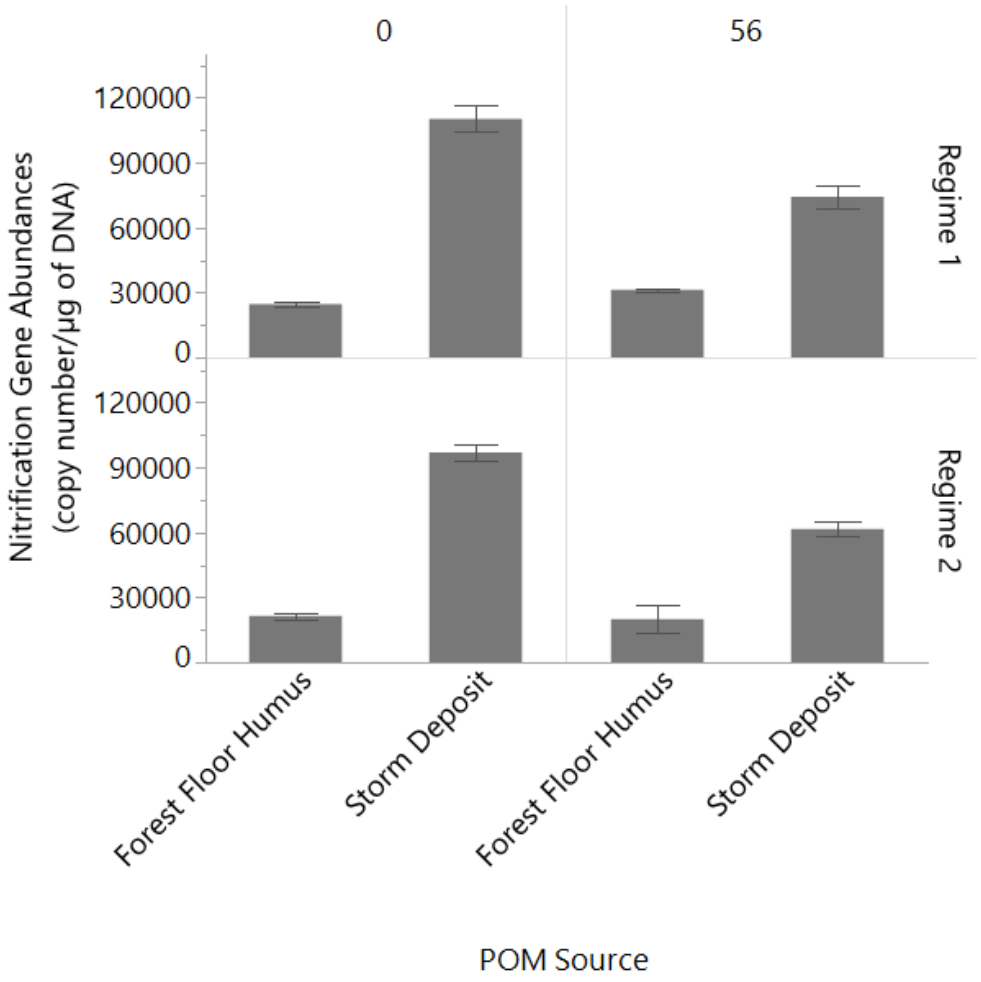

Figure 4.20: Comparison of initial (Day 0) and final (Day 50) denitrification gene abundances for Forest Floor Humus and Storm Deposit by moisture regime. Bars and error bars denote gene abundance means and standard deviation.

The changes of nitrogen processing gene abundances throughout the incubation clearly differed by POM source and Regime. As indicated by the initial and final comparison above (Figure 4.19), Forest Floor Humus exhibited minimal change in nitrification gene abundances (Figure 4.21). Nitrification gene abundances for Storm

Deposit were consistent across Regime until Day 25, which was the day of the 'large rewetting treatment'. From Day 25 to Day 26, nitrification gene abundances increased in Regime 1 and decreased in Regime 2. The opposite occurred between Day 26 and Day 45, where nitrification gene abundances decreased in Regime 1 (thus returning to Day 25 levels) and increased in Regime 2. From Day 45 to Day 56, both regimes increase in a similar fashion (Figure 4.21). It is important to note that the nitrification gene abundances for Storm Deposit Regime 2 have substantial variance. It is possible that the nitrification gene abundances for Storm Deposit Regime 2 may resemble a temporal pattern similar to that of Storm Deposit Regime 1 or even a pattern more in line with that of Forest Floor Humus.

The temporal changes of denitrification gene abundances differed subtly across regime for Forest Floor Humus; Regime 2 exhibited minimal change throughout the incubation, while Regime 1 experienced an incremental increase from Day 25 to Day 26 (Figure 4.22). The difference in temporal denitrification gene abundances change was much stronger for Storm Deposit. Denitrification gene abundances for Storm Deposit increased in Regime 1 and decreased in Regime 2 from Day 25 to Day 26. From Day 26 to Day 45, it is clear that the denitrification gene abundances for Storm Deposit decreased in Regime 1, but in Regime 2 substantial variances associated with the abundances at Day 45 imply that it could have increased, decreased, or stayed the same (Figure 4.22).

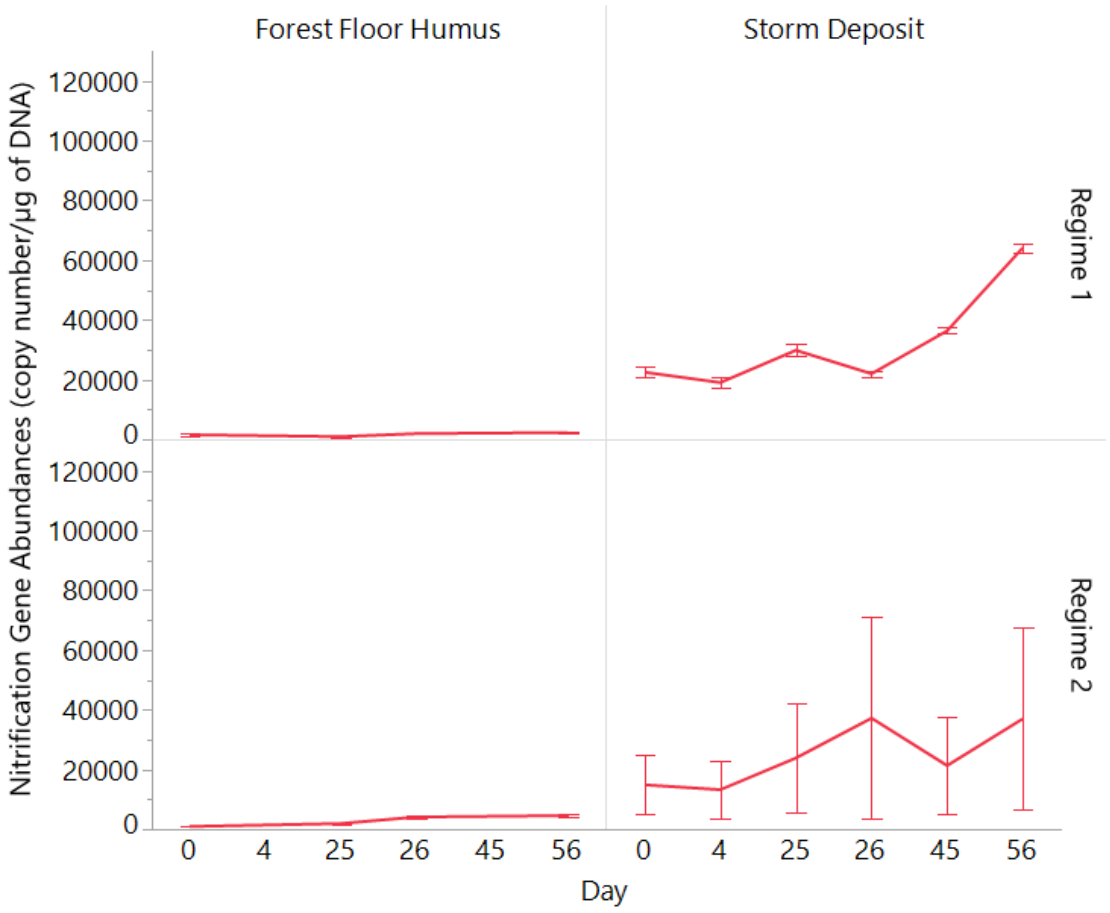

Figure 4.21: Comparison of nitrification gene abundances (copy number/µg of DNA) over time for each POM source across Regime. Nitrification gene abundance measurements were taken on Day 0, 4, 25, 26, 45, and 56 and their means are reported as points on the graph. These data points are connected by a line to denote change over time and standard deviation is denoted with error bars.

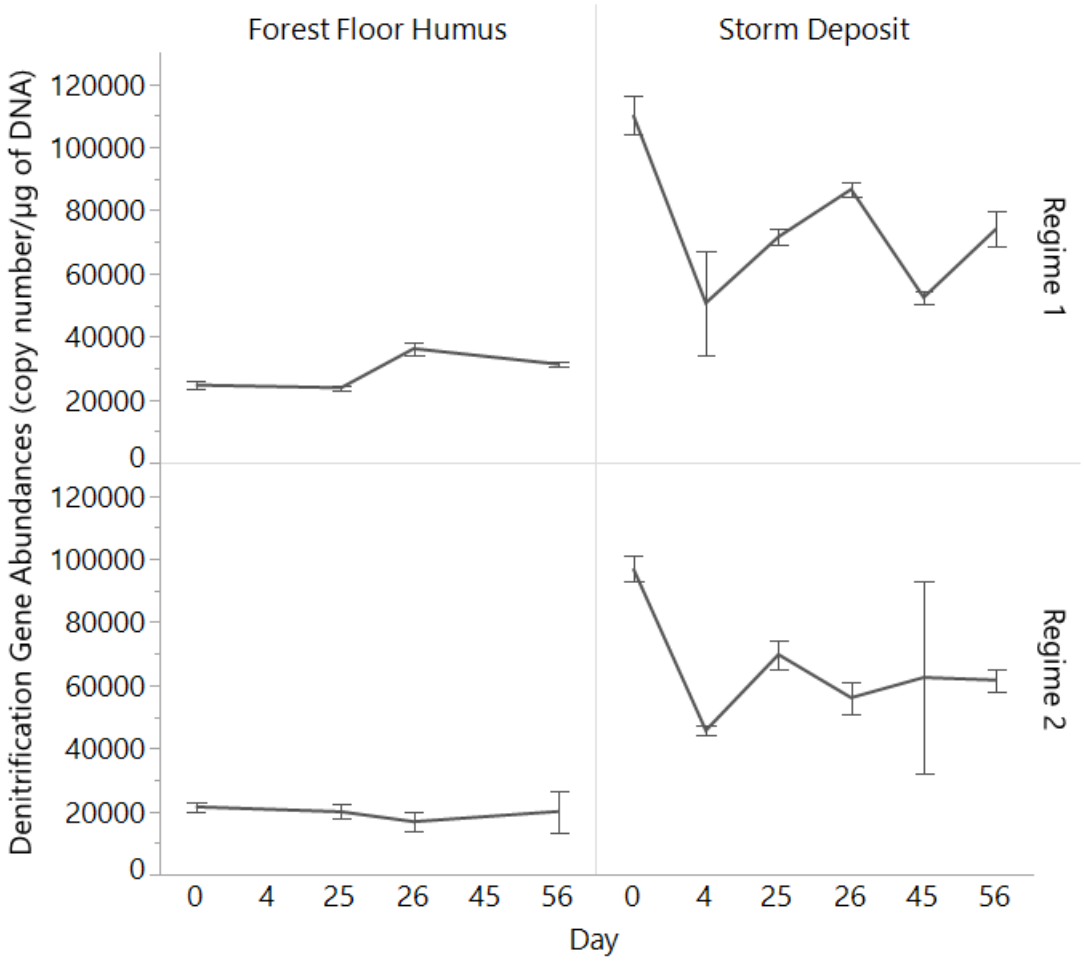

Figure 4.22: Comparison of denitrification gene abundances (copy number/µg of DNA) over time for each POM source across Regime. Denitrification gene abundance measurements were taken on Day 0, 4, 25, 26, 45, and 56 and their means are reported as points on the graph. These data points are connected by a line to denote change over time and standard deviation is denoted with error bars.

Nitrification gene abundances for Storm Deposit (R1; 32310.8± 15854.34 copy number/ µg of DNA, p = <0.0001, R2; 24574.6± 20816.29 copy number/ µg of DNA, p = 0.0013) were significantly greater than that of Forest Floor Humus (R1; 1690.7±

612.08 copy number/ µg of DNA, p = <0.0001, R2; 2885.8± 1595.18 copy number/ µg of DNA, p = <0.0001) in both regimes (Table 4.8, Table A11). Similarly, denitrification potential (R1; 70898.71± 22829.30 copy number/ µg of DNA, R2; 65408.23± 19673.42 copy number/ µg of DNA) for Storm Deposit was significantly greater than that of Forest Floor Humus (R1; 28937.42± 5417.61copy number/ µg of DNA, p = <0.0001, R2; 19512.55± 3761.69copy number/ µg of DNA, p = <0.0001) in both regimes (Table 4.8, Table A11).

Table 4.8: Nitrogen processing gene abundances for POM source by moisture regime. Mean C:N ratios are in bold, standard deviation is indicated in the parentheses, and letters denote significant difference.

Moisture Regime	POM Source	Nitrification Gene Abundances (copy number/µg of DNA)	Denitrification Gene Abundances (copy number/µg of DNA)
Regime 1	Forest Floor Humus	**1690.72** (612.08, B)	**28937.42** (5417.61, B)
	Storm Deposit	**32310.77** (15854.34, A)	**70898.71** (22829.30, A)
Regime 2	Forest Floor Humus	**2885.81** (1595.18, B)	**19512.55** (3761.69, B)
	Storm Deposit	**24574.61** (20816.29, A)	**65408.23** (19673.42, A)

Net change of nitrification for Forest Floor Humus is very close to zero, indicating that it ended the incubation with abundances very similar to the initial conditions of the incubation (Figure 4.23). Net increases of nitrification for Storm Deposit (R1; 41648.17$\pm$ 246.25 copy number/µg of DNA) were significantly greater

than that of Forest Floor Humus for Regime 1 (R1; 743.54± 460.72 copy number/μg of DNA, p = <0.0001, Figure 4.23, Table 4.8, Table A12).

Net change of denitrification gene abundances differed by Regime for Forest Floor Humus, but not Storm Deposit. A net increase in denitrification gene abundances was found in Regime 1 and a net decrease was found in Regime 2 for Forest Floor Humus, while a net decrease was found for Storm Deposit regardless of regime (Figure 4.24, Table 4.9). The net decrease for Storm Deposit (R1;-36043.76± 2954.99 copy number/μg of DNA, R2;-35385.22± 927.30 copy number/μg of DNA, Table 4.9) were significantly greater than the net changes for Forest Floor Humus (R1; 6601.46± 1671.35 copy number/μg of DNA, p = <0.0001, R2; -1438.17± 5232.22 copy number/μg of DNA, p = 0.0004, Figure 4.24, Table 4.8, Table A12).

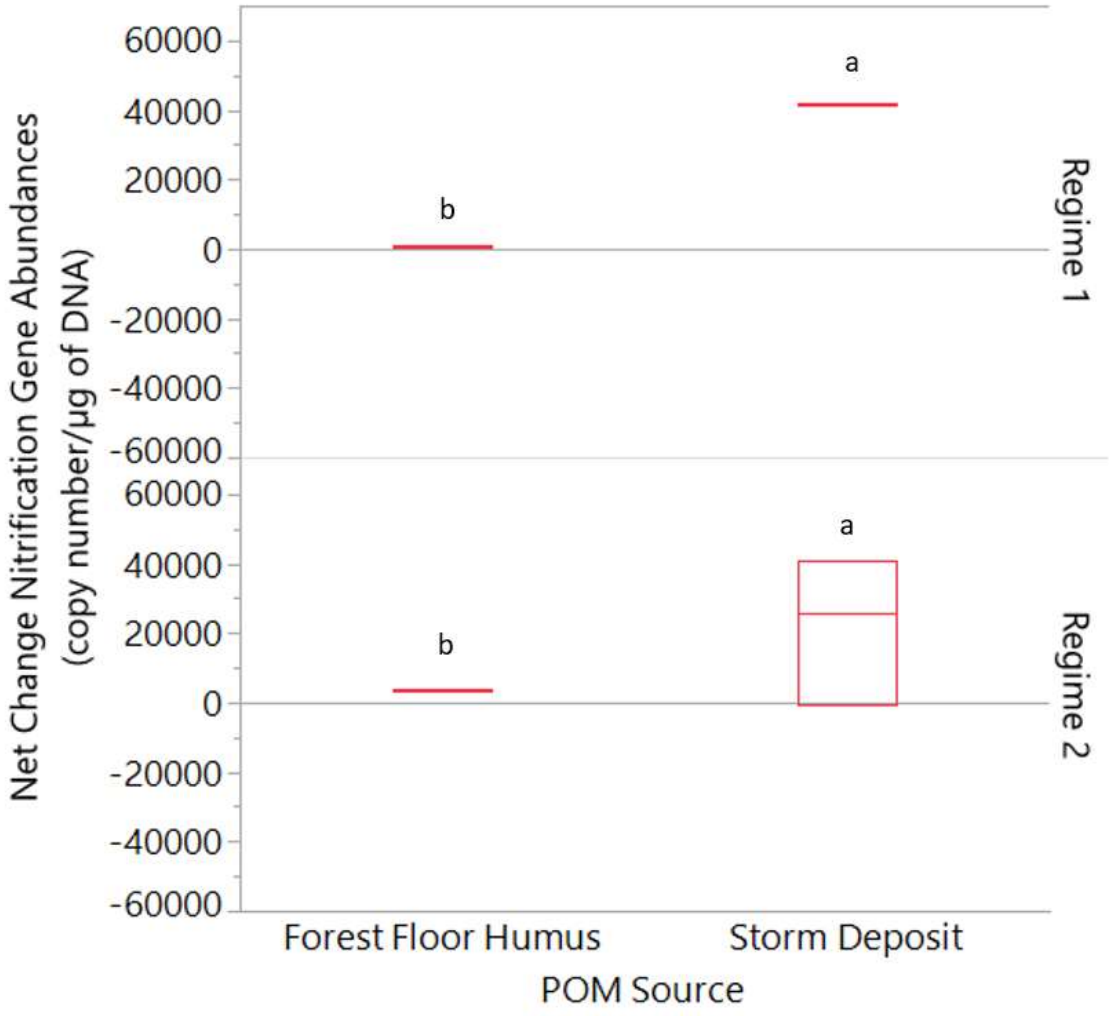

Figure 4.23: Comparison of nitrification gene abundance (copy number/ µg of DNA) net change from Day 0 to Day 56 for each POM source by regime. Net change means that are greater than zero indicate a net increase in TN over time. Letters denote significant difference.

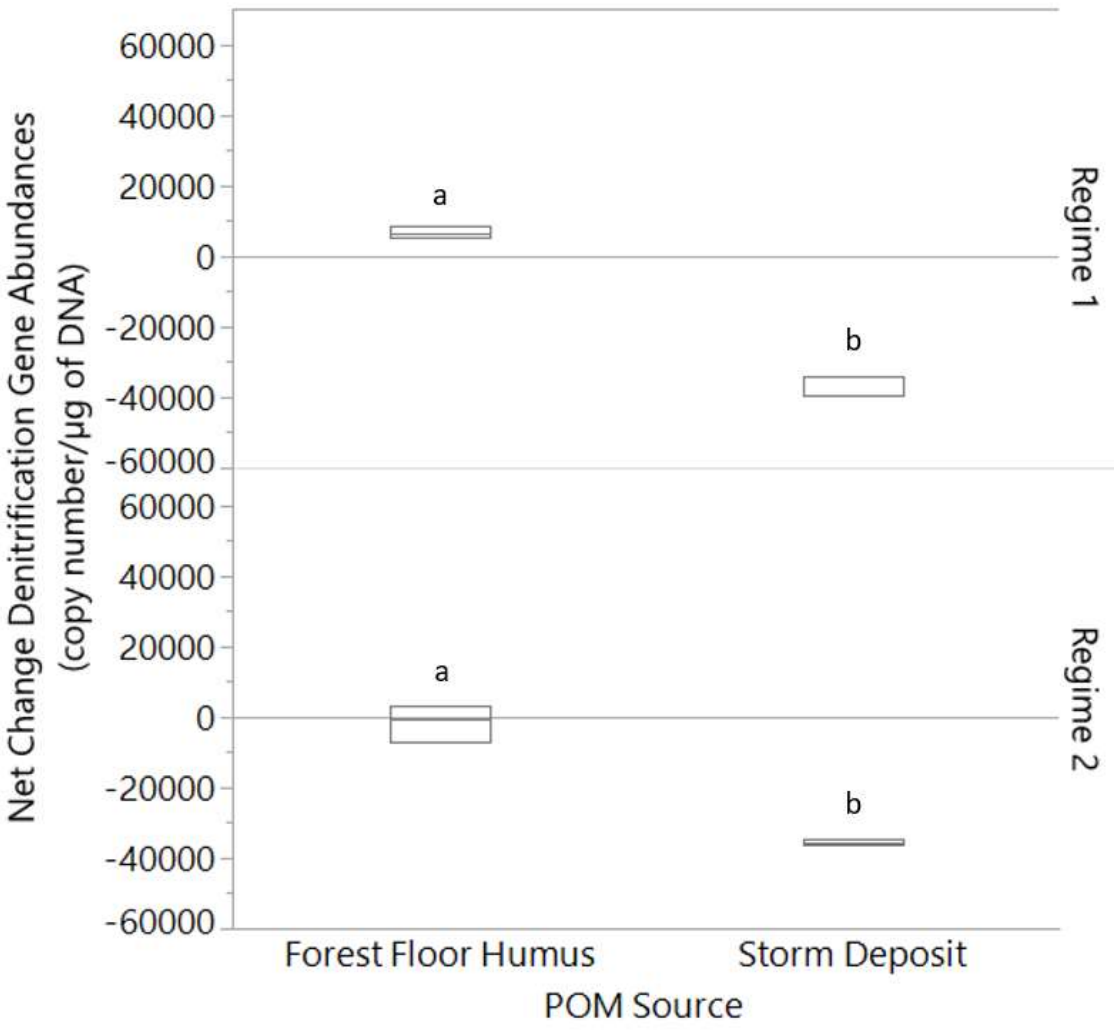

Figure 4.24: Comparison of denitrification gene abundance (copy number/ µg of DNA) net change from Day 0 to Day 56 for each POM source by regime. Net change means that are greater than zero indicate a net increase in TN over time. Letters denote significant difference.

Table 4.9: Net change of nitrogen processing gene abundances for POM source by moisture regime. Mean C:N ratios are in bold, standard deviation is indicated in the parentheses, and letters denote significant difference.

Moisture Regime	POM Source	Net Change Nitrification Gene Abundances (copy number/µg of DNA)	Net Change Denitrification Gene Abundances (copy number/µg of DNA)
Regime 1	Forest Floor Humus	**743.54** (460.72, B)	**6601.46** (1671.35, B)
	Storm Deposit	**41648.17** (246.25, A)	**-36043.76** (2954.99, A)
Regime 2	Forest Floor Humus	**3639.61** (347.93, B)	**-1438.17** (5232.22, B)
	Storm Deposit	**22118.65** (20929.45, A)	**-35385.22** (927.30, A)

Chapter 5

DISCUSSION

5.1 Role of POM Sources for N Fate and Processing in Streams

Results from the incubation have shown there are important differences between POM sources. Though Forest Floor Humus and Upland Mineral A Horizon had significantly greater masses of particulate TC and TN than the other POM sources (Figure 4.2, Figure 4.3, Table 4.3), Forest Floor Humus and the mixed sources (Storm Deposit, and Stream Bed) had comparable amounts of porewater N, specifically porewater NO_3^- in Regime 2 and porewater DON in Regime 1 (Table 4.5). Forest Floor Humus and the mixed sources all had large amounts of porewater TN, NO_3^--N, NH_4^+-N, and DON while Upland Mineral A Horizon and Stream Bank contained moderate to minimal amounts of N (Figure 4.12, Figure 4.13, Figure 4.14, Figure 4.15, Table 4.5). It was unexpected that Forest Floor Humus and the mixed sources would produce similar porewater N masses, since Forest Floor Humus was considerably more particulate C and N rich than the mixed sources (Figure 4.2, Figure 4.3, Table 4.2). Similarly, Forest Floor Humus and Upland Mineral A Horizon were expected to yield similar porewater N because of their similarly high particulate C and N masses, but Upland Mineral A Horizon produced results similar to that of Stream Bank, the poorest C and N source (Table 4.5).

It is hypothesized that the release of large amounts of porewater N for Forest Floor Humus, Storm Deposit, and Stream Bed are due to significant levels of C and N mineralization, enhanced nitrification, and low denitrification. The presence of porewater NH_4^+-N coupled with the net decrease of particulate TN for Forest Floor

Humus indicates that organic N was readily mineralized throughout the incubation in both moisture regimes (Table 4.5, Table 4.6). The lack of ammonium porewater masses for Storm Deposit and Stream Bed (Figure 4.14, Table 4.5) may indicate that it was nitrified or that volatilization actively removed unused NH_4^+-N from solution (Thamdrup 2012; Follett 2008). In an experiment similar to the one employed in this study, drying and rewetting has been found to spur the loss of NH_4^+-N via DNRA, but in that case DNRA occurred only for a short time after rewetting and was accompanied by enhanced denitrification and the reduction of NO_3^- (Arce et al. 2015), which was not seen in this incubation.

Both Forest Floor Humus and Storm Deposit experienced a net increase in nitrification potential throughout the incubation (Figure 4.23, Table 4.9). In contrast, there was a decrease in denitrifying genes for both POM sources, except in Regime 1 for Forest Floor Humus (Figure 4.24, Table 4.9). This combination of gene abundance increase coincided with the net increase of porewater NO_3^--N for Storm Deposit and Regime 2 of Forest Floor Humus (Table 4.79), making a strong argument that elevated porewater NO_3^--N was due to enhanced nitrification and low denitrification. The enhancement of nitrification and reduction of denitrification in C and N rich soils is reflected in other studies. For instance, Fierer & Schimel (2002) showed that oak soils, which are similar in description to the Forest Floor Humus soils defined in this study, had a history of limited moisture stress and exhibited microbial community change, increased short-term respiration rates, and nitrification potential. The authors credit this chemical and biological response to a resistant portion of nitrifying microorganisms that survived the stresses of drying, and then thrived upon rewetting, which appears to be what happened for Forest Floor Humus in Regime 2 and Storm Deposit in both regimes.

As mentioned earlier, it was unexpected that the C and N rich source, Forest Floor Humus, and C and N poor mixed sources, Storm Deposit and Stream Bed, would leach comparable amounts of N. High quality POC, comprised of leaf litter, has been shown to lead to greater rates of nitrate and total dissolved nitrogen retention than lower quality POC inputs due to enhanced denitrification (Stelzer et al. 2014). This strongly supports the findings for the Forest Floor Humus that were frequently rewetted in Regime 1, specifically the removal of NO_3 in concurrence with the enhancement of denitrifying microbial populations and the diminishment of nitrifying microbial populations (Figure 4.23, Figure 4.24, Table 4.9), however this improvement of denitrification and the lack of porewater NO_3 increase was not found for the Forest Floor Humus that underwent drying and rewetting in Regime 2. It appears the lack of N removal for Forest Floor Humus Regime 2 is due to the decline of denitrification potential (Figure 4.24, Table 4.9) coupled with improved nitrification potential (Figure 4.23, Table 4.9). This resulted in Forest Floor Humus Regime 2 acting as a N source similar to the mixed sources, Storm Deposit and Stream Bed.

Similarly, it was unexpected that Upland Mineral A Horizon, was a C and N rich source, did not contain as much porewater N as the other C and N rich source, Forest Floor Humus in either regime (Table 4.3, Table 4.5). Upland Mineral A Horizon and Forest Floor Humus had comparable C:N ratios and particulate TN (Table 4.2, Table 4.3), but Forest Floor Humus released significantly greater amounts of porewater TC and DOC than Upland Mineral A Horizon (Table 4.3, Table 4.4). Though the quality of the leached C did not significantly differ between Forest Floor Humus and Upland Mineral A Horizon (Figure 4.10, Figure 4.11, Table 4.6), the greater concentration of leached C in Forest Floor Humus porewater may have contributed to greater amounts N

processing via enhanced assimilation, and denitrification. Though POM source C and N characteristics are important, it is apparent they are not the only factors controlling N processing and that microbial community characteristics and moisture regime can directly influence the fate of N in these POM sources.

Forest Floor Humus is also distinguished from other POM sources by the amount of leached DON found for both regimes. Denitrification potential and decrease in denitrification gene abundances for Forest Floor Humus were significantly lower in Regime 2 than Regime 1 (Table 4.9). If this decline in gene abundances is assumed to represent the loss of the denitrifying portion of the population, the DON released into solution may be comprised of intracellular organic solutes and enzymes released via lysing (Sparling & Ross 1988; Van Gestel et al. 1993). In contrast, Regime 1 had a significantly smaller decline of denitrification gene abundances, but still leached amounts of DON similar to that of Regime 2, indicating leached DON is likely comprised of a combination of intracellular and extracellular enzymes that have been lost via lysing and osmotic regulation (Chen & Alexander 1973; Van Gestel et al. 1993; Fierer & Schimel 2002).

Forest Floor Humus, which is speculated to mostly contain clay, was finer in texture than most of the other POM sources. Clay, especially in the presence C, can serve as an important site of N retention via the direct sorption of NH_4^+-N (Seybold et al. 2005; Follett 2008), and it can exhibit higher N recovery rates than coarse grained POM (Barrett & Burke 2002). This abiotic N pathway was likely available to the N in Forest Floor Humus and Upland Mineral A Horizon, but not in the coarse, more mineral-rich Storm Deposit, and Stream Bed material. NH_4^+-N not bound by sorption and likely in the presence of oxygen due to the porosity of coarse-grained material,

would be more vulnerable to nitrification than the sorbed NH_4^+-N of Forest Floor Humus.

The results clearly show that POM sources share several N processing mechanisms and are influenced by similar controlling factors, but there are two things that really set Forest Floor Humus apart from the other POM sources; (1) porewater NH_4^+-N and DON, and (2) sensitivity to moisture regime. Forest Floor Humus has demonstrated that it can facilitate significant N storage through C and N mineralization and denitrification under conditions resembling that of submerged stream channel (Regime 1), however it can also faciliate substantial N release through improved nitrification and severely diminished denitrification under conditions resembling that of stream bank drought (Regime 2). In contrast, the remaining POM sources primarily acted as N sources and released a range of porewater N (4.52$\pm$ 3.58mg – 38.27$\pm$ 27.70mg) regardless of moisture conditions. These results clearly show that POM source plays an important role in the type of N processes that occur, but that the fate of N is intricately influenced by both POM source and additional factors, including drying and rewetting conditions and microbial resistance or resilience.

5.2 Influence of Moisture Regime for N Processing

Drying and wetting cycles have been shown to release N (Birch 1958; Franzluebbers et al. 2000; Borken et al. 2003; Pulleman & Tietema 1999), and this appears to be the case for Forest Floor Humus. Forest Floor Humus began the incubation with small amounts of porewater and large amounts of porewater NH_4^+-N

and DON and, unlike the other POM sources, Forest Floor Humus Regime 1 ended the incubation with minimal porewater NO_3^--N and even greater amounts porewater NH_4^+-N and DON (Figure 4.8, Figure 4.15, Figure 4.17). For Forest Floor Humus, Regime 1 had lower nitrification gene abundances and greater denitrification gene abundances than that of Regime 2, indicating that denitrification was being employed more effectively in this source under Regime 1 conditions, resulting in thorough removal of leached NO_3^--N (Figure 4.19, Figure 4.20, Figure 4.13, Figure 4.17). The lack of leached NO_3^--N and net decrease of porewater DOC, and particulate TC and TN (Table 4.7, Table 4.4) further supports the occurrence of denitrification, which is a C limited facultative anaerobic respiratory process (Thamdrup 2012; Trimmer et al. 2012). Denitrification preferentially occurs under low oxygen conditions, but it can occur in anoxic micro-sites in otherwise well oxygenated soils (Storey et al. 1999; Triska et al. 1993), which indicates it should have been able to readily occur in the fine-grained material of Regime 1 for Forest Floor Humus. Additionally, high quality POC inputs can lead to increased biotic consumption resulting in redox conditions favorable for denitrification (Stelzer et al. 2014).

Forest Floor Humus had substantially greater amounts of porewater NO_3^--N and nitrification gene abundances in Regime 2 than in Regime 1 (Table 4.5, Table 4.8), and similar net increases in porewater NH_4^+-N and net decreases in particulate TN masses across regime (Table 4.7, Table 4.4). Assuming the amount of porewater NO_3^--N released during the incubation was ultimately NH_4^+-N limited, then the significantly greater amount of porewater NO_3^--N for Forest Floor Humus Regime 2 would be a function of elevated release of porewater NH_4^+-N via N mineralization (Follett 2008). Additionally, Regime 2 experienced greater net decrease and contained significantly

lower amounts of leached DOC than Regime 1 for Forest Floor Humus (Table 4.5, Table 4.7). Since net decrease in particulate TC did not significantly differ across regimes, and nitrification is an autotrophic oxidation process that is independent of C, it is hypothesized that this net decline of porewater DOC was due to CO_2 release via C mineralization spurred by the drying and rewetting conditions of Regime 2 (Franzluebbers et al. 2000; Jarvis et al. 2007; Kim et al. 2012). This increase in leached inorganic N and decrease in leached C exemplifies the C and N mineralization response expected for the Birch Effect (Birch 1958).

The effect of drying and rewetting on the fate of N is highly dependent on the duration, intensity, and frequency of the applied drying and rewetting cycles. C and N mineralization and denitrification have been shown to decrease when drought intensity or duration increase (Borken & Matzner 2008; Gómez et al. 2012; Seitzinger et al. 2006; Sharma et al. 2006; Schimel et al. 2007), and increase when rewetting duration increase (Borken & Matzner 2008), however the intensity of mineralization decreases over successive drying and rewetting cycles (Birch 1958; Fierer & Schimel 2002). Nitrification was originally believed to have a low tolerance to moisture stress (Stark & Firestone 1995), but has been found to persist during drying and rewetting cycles and prosper under rewetting conditions (Gómez et al. 2012; Fierer et al. 2003; Fierer & Schimel 2002). These findings imply that when exposed to long drought and short rewetting cycles, Forest Floor Humus, would likely experience strong nitrification, weak denitirifcation, and intense osmotic shock microbial death, resulting in large amounts of leached NO_3 and DON. Conversely, Forest Floor Humus exposed to droughts and rewetting events of long duration would likely experience intense nitrification tempered by growing denitrification, resulting in muted NO_3 and DON

release. In comparison, short-lived drought followed by short rewetting events would likely produce the greatest rates of C and N mineralization, though the mineralization would weaken over successive cycles. Lastly, short drought paired with long rewettting conditions would yield the most effective N removal via spurred C and N mineralization and improved denitrification in Forest Floor Humus. Though these scenarios also apply to the other POM sources, Forest Floor Humus are particularly vulnerable to drying and rewetting conditions and have the potential to act as both strong N sources and N sinks.

5.3 Broader Implications for Aquatic Ecosystems with Climate Variability

Low-order streams serve as sites of significant N retention (Alexander et al. 2000; Peterson et al. 2001; Wollheim et al. 2001; Seitzinger et al. 2006), and in a survey of North America streams have been estimated to be responsible for gross removal rates of NO_3^- ranging from 0% to 15% per meter on an annual basis (Peterson et al. 2001). However, N storage via microbial biomass, vegetation, and soils have a limited capacity and these pathways can become N saturated, breakdown over time, or experience stochastic disturbances (Bernhardt et al. 2005; Howarth 2008; Bilby 1981), resulting in the loss of large amounts of N. The controlling mechanisms and ramifications of N pollution in downstream waters are intense, complex, and still poorly understood. It is estimated that approximately 59 Tg N $year^{-1}$ are transported to the world's oceans via rivers annually (Boyer et al. 2006), but there is significant uncertainty in estimating the

deposition of N to coastal systems because terrestrial N retention is highly variable (Howarth et al. 2006; Howarth 2008; Aber et al. 2003). Howarth et al (2006) conducted a study that calculated the predicted net anthropogenic nitrogen input (NANI) under different watershed conditions, and found that NANI increased significantly in regions of cooler temperatures when average precipitation and discharge increased. The authors attributed this enhanced N export to the loss of N storage in low-order streams due to significantly reduced residence times. They predicted that N flux down the Susquehanna River Basin will increase to 17% by 2030 and 65% by 2095, if current landuse and NANI persist and predicted increases in precipitation occur as a result of climate change (Howarth et al. 2006). These results paint a stark picture of future N budgets for the waterbodies of the Northeastern and mid-Atlantic regions of the United States, but they only take anthropogenic N into consideration. Recent studies have begun to investigate and argue the importance of intense episodic PN flux transported during large storms (Inamdar et al. 2015; Taylor et al. 2015; Alongi et al. 2013), which are also expected to increase with climate change and have not historically been included in budget estimates like that of Howarth et al. 2006.

Seasonality plays an important role in shaping large storm PN export, the loss of dissolved N via leaching, and the processing of N in receiving waterbodies. In the mid-Atlantic region, elevated C and N rich POM transported by convective, high-intensity, short-lived summer storms (Inamdar et al. 2013; Rowland et al. 2017) coincide with the annual peak of in-stream nitrification and net NO_3^- release typical of low order streams during the summer season (Starry et al. 2005; Mulholland 2004). A quantification of the seasonal N dynamics of the Chesapeake Bay found that influx of allochthonous N that supports spring aquatic primary production continues to sustain the high N demands of

phytoplankton in the summer via DON and DIN regeneration and recycling (Baird et al. 1995; Bronk et al. 1998). According to the applied Finn Cycling Index (FCI), approximately 70% of the total system activity in the summer is due to the rate of nitrogen recycling in downstream aquatic ecosystems, indicating that N leached from PN derived from large summer storm transport could substantially disturb the seasonal N dynamics in coastal waters by providing fresh allochthonous N inputs that are typically not available (Baird et al. 1995).

In addition to influencing in-stream processing and biotic nutrient utilization, season also shapes the precipitation and moisture conditions of headwater catchments, which directly influence the fate of watershed N. The relative inputs of POM sources have been shown to vary with storms and their seasonal occurrence (Rowland et al. 2017; Johnson et al. 2018), specifically intense summer events typically deliver more C and N rich upland, forest floor material, while winter storms typically erode stream banks and stream bed material. In addition to driving the transport of more C and N rich POM (Rowland et al. 2017; Johnson et al. 2018), high intensity summer storms are often punctuated with periods of substantial drought (Inamdar et al. 2015). As expected, drying and rewetting cycles can substantially alter the fate of PN transported by large storms in the Fair Hill NRMA, Cecil County, MD. The results from this study have demonstrated that drying and rewetting can spur nitrification and diminish denitrification, resulting in the release of leached NO_3^-, NH_4^+, and DON. Specifically, summer drying and rewetting cycles can stimulate an otherwise impressive N sink (i.e., Forest Floor Humus) to act as a substantial N source, during a time at which in-stream nitrification and net NO_3^- release in low-order streams is at its annual peak (Starry et al. 2005; Mulholland 2004), and the demand for N by phytoplankton, bacteria, and algae in

high-order rivers and coastal waterbodies is at its greatest (418 mg N m^{-2} day^{-1}) (Bronk et al. 1998; Baird et al. 1995). This suggests that the drying and rewetting conditions applied to small, headwater catchments exert substantial control over downstream water quality by regulating both the type and magnitude of N lost from storm driven PN deposition in the fluvial network.

The impact of drying and rewetting cycles on fluvial N dynamics is of growing concern as the intensity and frequency of both drought and large storms are predicted to increase with global climate change (Karl et al. 2009; Melillo 2014). For several decades, watersheds draining to the Chesapeake Bay have experienced N fluxes that are on average 10 times higher than the background fluxes (Howarth et al. 1996), and have endured 6-8 fold increases of N inputs due to anthropogenic sources (Boynton et al. 1995). As anthropogenic N inputs in aquatic systems increase, the release of N from small headwater catchments, augmented by drying and rewetting cycles driven by climate change, will continue to magnify an already difficult environmental problem.

Chapter 6

CONCLUSION

This study provided novel insight into the fate of particulate N in stream ecosystems by investigating the role watershed POM sources and moisture regimes. Characterization of particulate and porewater N masses coupled with N functional gene measurements provided information about the how N species and transformations differed across POM sources and moisture regimes.

Key findings from this research are as follows:

(1) POM source comparison: Forest Floor Humus and the mixed sources, Storm Deposit and Stream Bed, yielded comparable amounts of porewater N. Enhanced C and N mineralization, nitrification, and low denitrification spurred these POM sources to release large amounts of porewater NO_3. In comparison, Upland Mineral A Horizon and Stream Bank released minimal amounts of porewater N. Forest Floor Humus was the only POM source to release only porewater NH_4^+-N and DON under moist conditions likely due to C and N mineralization and suppressed nitrification.

(2) Moisture regime: Forest Floor Humus was the only POM source to not act as a N source under the moist conditions. In contrast, Forest Floor Humus that underwent drying and rewetting acted as N source and yielded significantly greater amounts of porewater NO_3 than frequently rewetted Forest Floor Humus. In comparison, the other POM sources acted as N sources regardless of applied moisture conditions.

This study showed that POM can be an important source of porewater N and that the amount of N leached can vary with POM sources. Climate projections indicate that the largest storms will increase in intensity and frequency, which could result in greater amounts of POM, from variable sources, being delivered to the fluvial network. The N released from such sources could be highly variable, as demonstrated by this work. Climate variability will also likely enhance the drying and rewetting cycle, which as shown by this work, may lead to more mineralization and nitrification of POM, resulting in the release of dissolved N. Taken together, these observations suggest there will be important challenges for vulnerable aquatic ecosystems such as the Chesapeake and Delaware Bays, which are already suffering from excess pollution and eutrophication.

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Appendix A

A POM SOURCE INCUBATION MOISTURE PROFILES

At Day 4, 11, 18, and 20, small rewetting additions were applied to Regime 1, but not Regime 2. At Day 25, a large rewetting addition was added to both Regime 1 and Regime 2, followed by small rewetting additions at Day 44 and 49. Though the small water additions added to Regime 1 prior to Day 25 did not maintain initial moisture conditions for all POM sources, they created significantly distinct moisture profiles for all POM sources except Stream Bed (Figure A1; Figure A2; Figure A3; Figure A4; Figure A5).

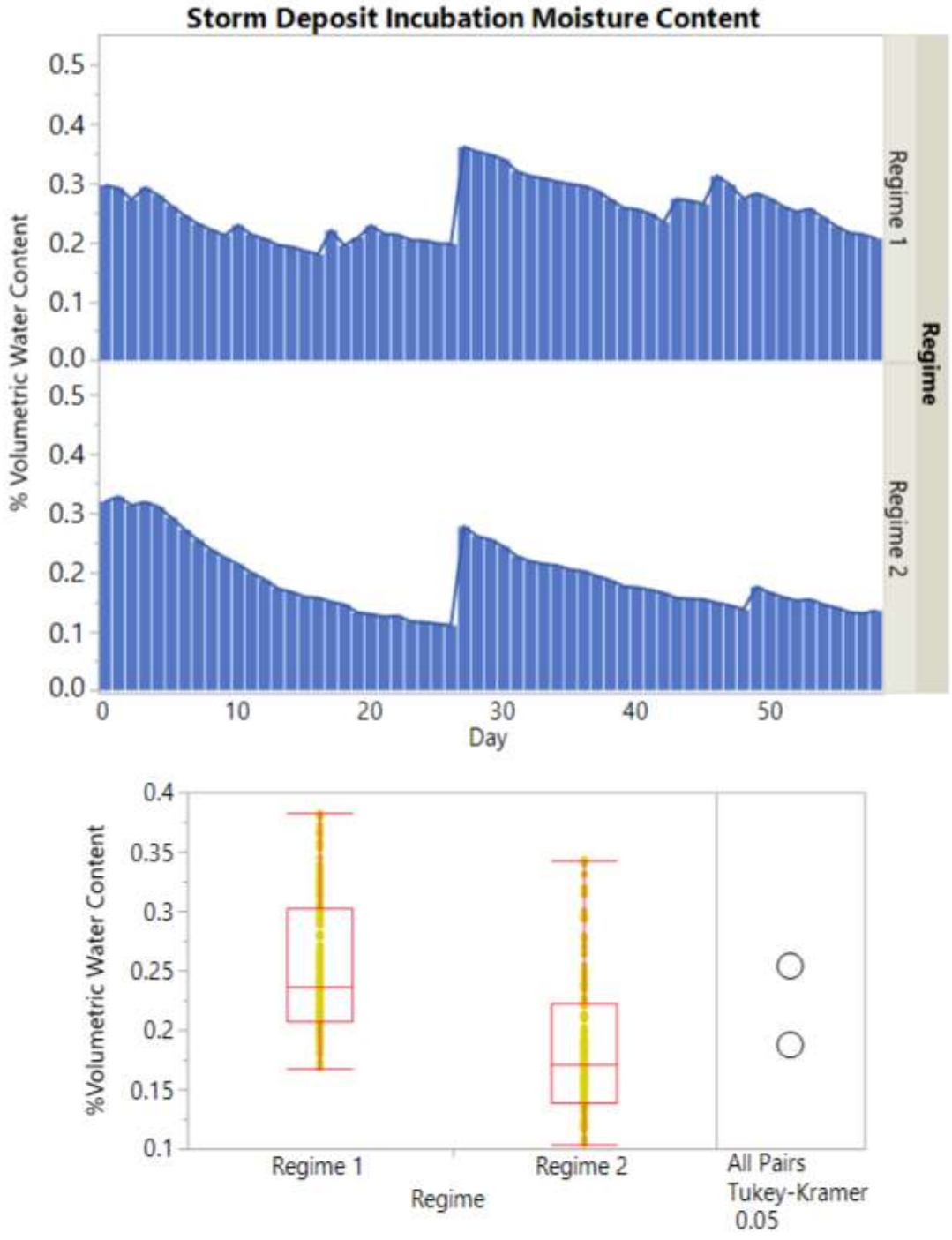

Figure A1: Moisture content over time for Storm Deposit across Regime 1 and Regime 2. Storm Deposit experimental incubation moisture profiles, Regime 1 and Regime 2, were significantly different from each other.

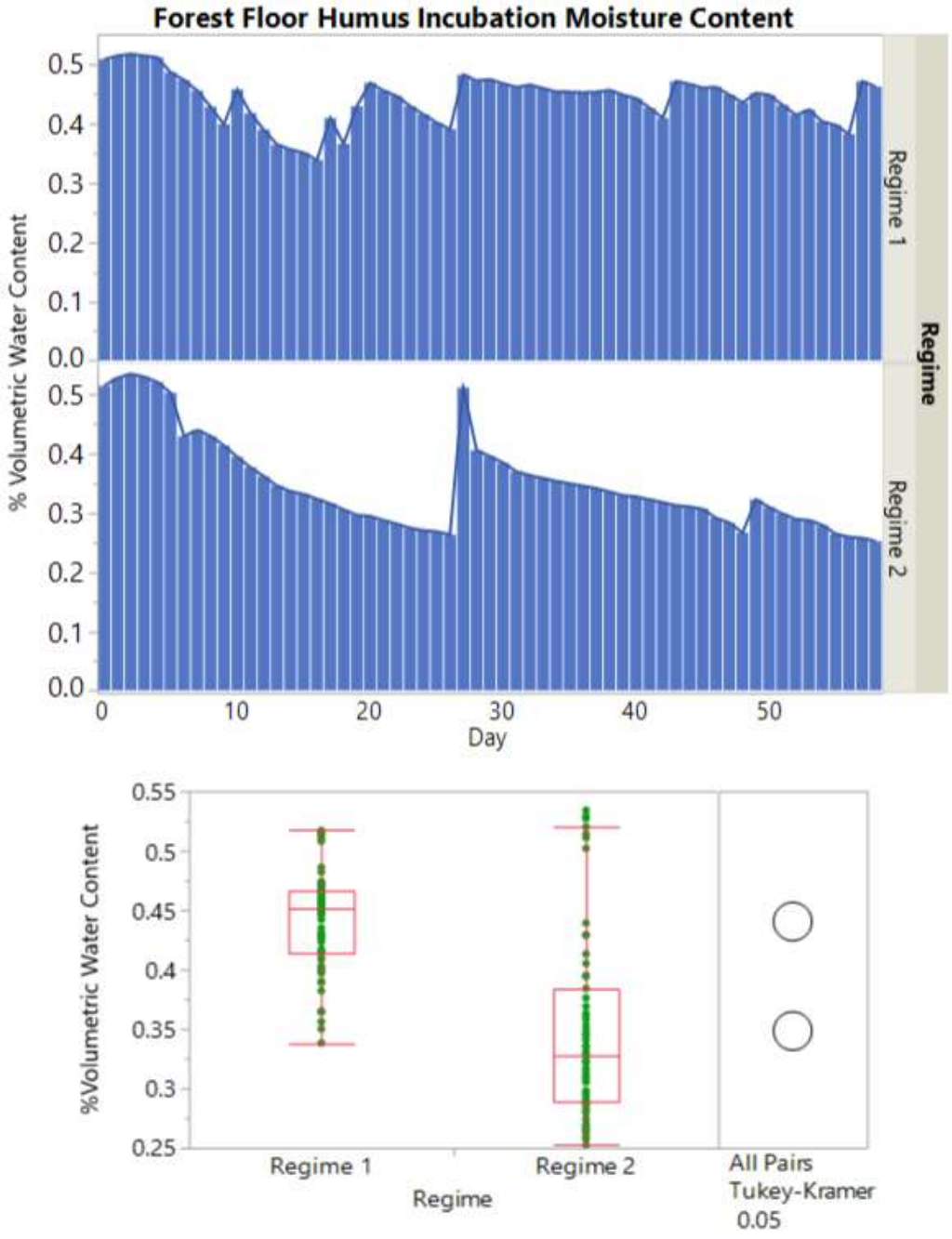

Figure A2: Moisture content over time for Forest Floor Humus across Regime 1 and Regime 2. Forest Floor Humus experimental incubation moisture profiles, Regime 1 and Regime 2, were significantly different from each other. Forest Floor Humus was sensitive to the small rewetting events applied prior to Day 25 in Regime 1.

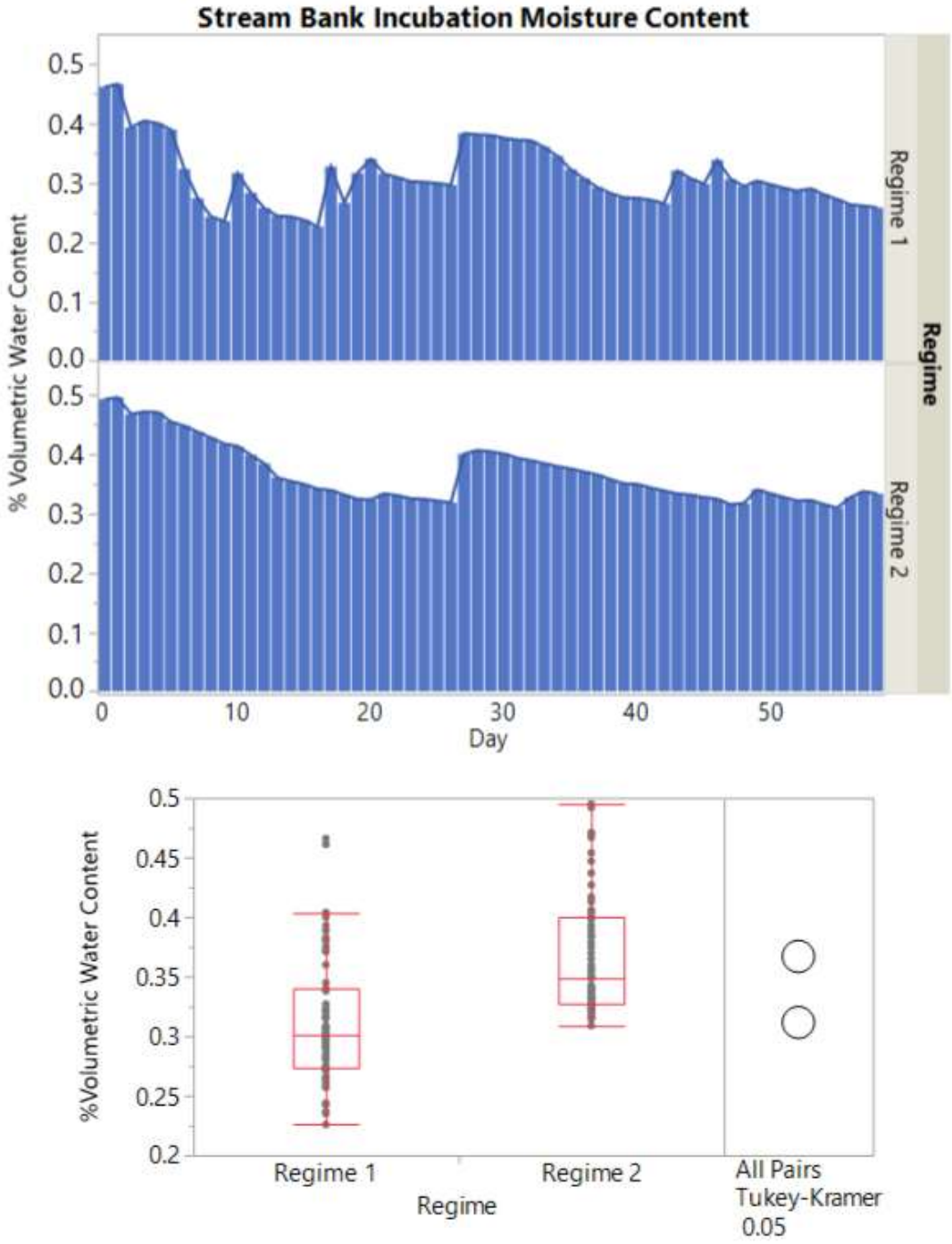

Figure A3: Moisture content over time for Stream Bank for Regime 1 and Regime 2. Stream Bank experimental incubation moisture profiles, Regime 1 and Regime 2, were significantly different from each other. Stream Bank experienced

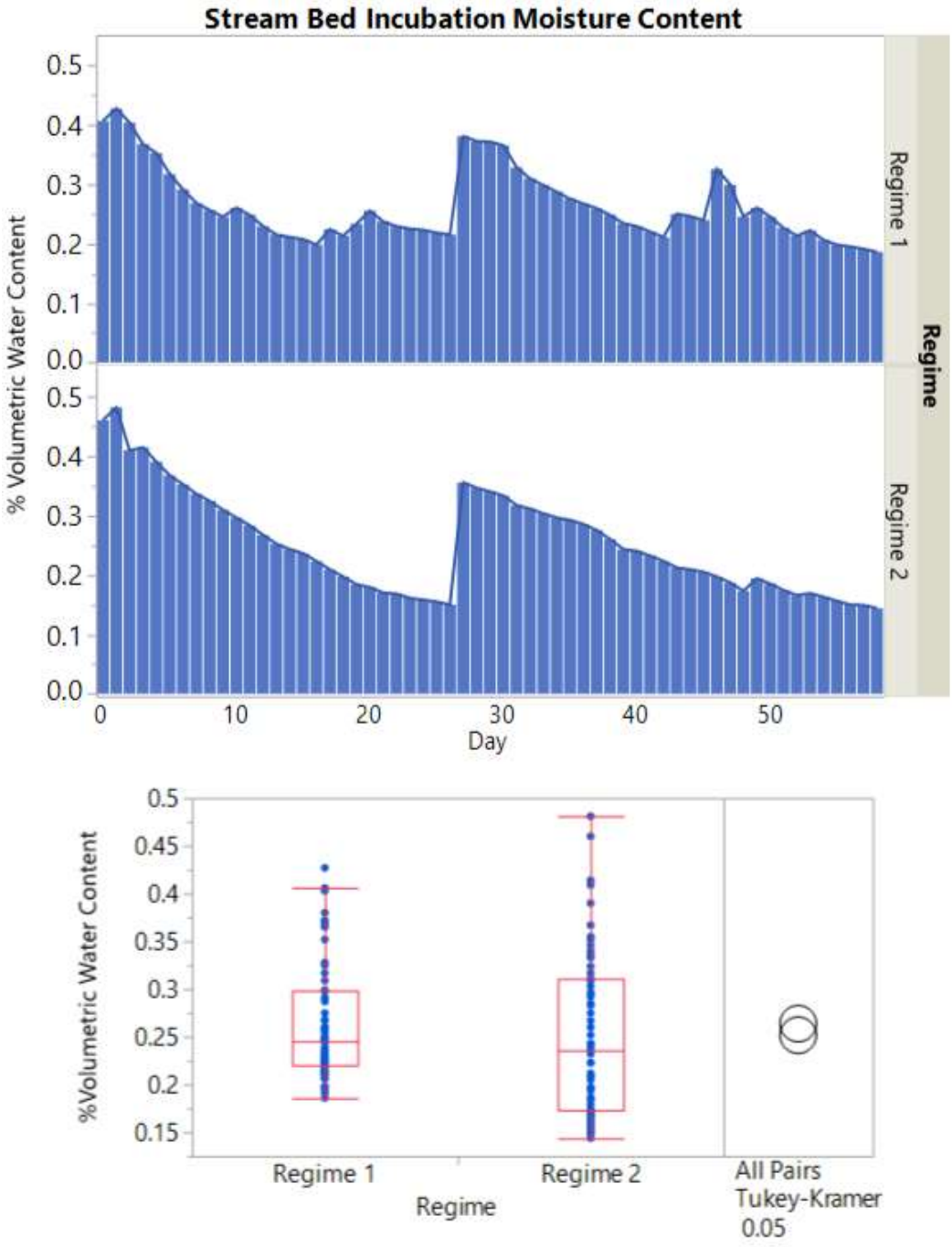

Figure A4: Moisture content over time for Stream Bed across Regime 1 and Regime 2. Stream Bed experimental incubation moisture profiles, Regime 1 and Regime 2, were significantly different from each other. Stream Bed was very insensitive to the small rewetting events applied prior to Day 25 in Regime 1 and experienced drying like that of Regime 2.

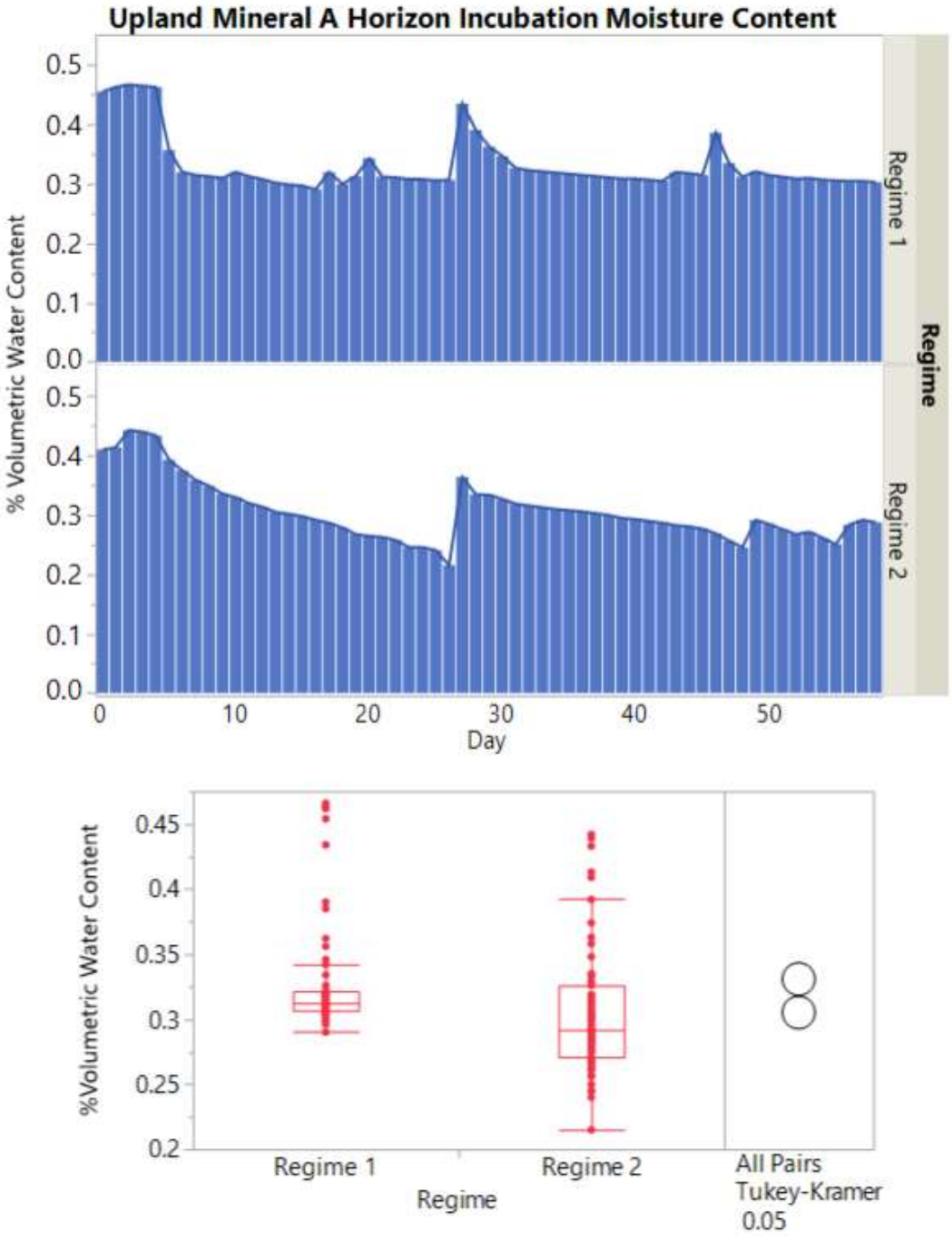

Figure A5: Moisture content over time for Upland Mineral A Horizon across Regime1 and Regime 2. Upland Mineral A Horizon experimental incubation moisture profiles, Regime 1 and Regime 2, were significantly different from each other.

Table A1: ANOVA p-values for POM source comparison by regime for molar and mass C:N ratios. P <0.05 are considered significant.

		Regime 1					Regime 2				
C:N Ratio	**POM Source**	**Forest Floor Humus**	**Storm Deposit**	**Stream Bank**	**Stream Bed**	**Upland Mineral A Horizon**	**Forest Floor Humus**	**Storm Deposit**	**Stream Bank**	**Stream Bed**	**Upland Mineral A Horizon**
Mass C:N Ratio	Forest Floor Humus	1.0000	0.0052	0.0050	0.0094	0.9996	1.0000	<0.0001	0.0004	0.0006	0.9999
	Storm Deposit	0.0052	1.0000	0.9975	1.0000	0.0094	<0.0001	1.0000	0.9245	0.8899	<0.0001
	Stream Bank	0.0050	0.9975	1.0000	0.9995	0.0050	0.0004	0.9245	1.0000	1.0000	0.0002
	Stream Bed	0.0094	1.0000	0.9995	1.0000	0.0184	0.0006	0.8899	1.0000	1.0000	0.0003
	Upland Mineral A Horizon	0.9996	0.0112	0.0102	0.0184	1.0000	0.9999	<0.0001	0.0002	0.0003	1.0000
Molar C:N Ratio	Forest Floor Humus	1.0000	0.0052	0.0050	0.0094	0.9996	1.0000	<0.0001	0.0004	0.0006	0.9999
	Storm Deposit	0.0052	1.0000	0.9975	1.0000	0.0094	<0.0001	1.0000	0.9245	0.8899	<0.0001
	Stream Bank	0.0050	0.9975	1.0000	0.9995	0.0050	0.0004	0.9245	1.0000	1.0000	0.0002
	Stream Bed	0.0094	1.0000	0.9995	1.0000	0.0184	0.0006	0.8899	1.0000	1.0000	0.0003
	Upland Mineral A Horizon	0.9996	0.0112	0.0102	0.0184	1.0000	0.9999	<0.0001	0.0002	0.0003	1.0000

Table A2: ANOVA p-values for POM source comparison by regime for particulate C and N masses. P <0.05 are considered significant.

		Regime 1					Regime 2				
	POM Source	**Forest Floor Humus**	**Storm Deposit**	**Stream Bank**	**Stream Bed**	**Upland Mineral A Horizon**	**Forest Floor Humus**	**Storm Deposit**	**Stream Bank**	**Stream Bed**	**Upland Mineral A Horizon**
TC (g)	Forest Floor Humus	1.0000	<0.0001	<0.0001	<0.0001	<0.0001	1.0000	<0.0001	<0.0001	<0.0001	<0.0001
	Storm Deposit	<0.0001	1.0000	0.0007	0.9998	<0.0001	<0.0001	1.0000	0.0116	0.9997	<0.0001
	Stream Bank	<0.0001	0.0007	1.0000	0.0043	<0.0001	<0.0001	0.0116	1.0000	0.0161	<0.0001
	Stream Bed	<0.0001	0.9998	0.0043	1.0000	<0.0001	<0.0001	0.9997	0.0161	1.0000	<0.0001
	Upland Mineral A Horizon	<0.0001	<0.0001	<0.0001	<0.0001	1.0000	<0.0001	<0.0001	<0.0001	<0.0001	1.0000
TN (g)	Forest Floor Humus	1.0000	<0.0001	<0.0001	<0.0001	<0.0001	1.0000	<0.0001	<0.0001	<0.0001	<0.0001
	Storm Deposit	<0.0001	1.0000	<0.0001	0.9987	<0.0001	<0.0001	1.0000	<0.0001	0.9969	<0.0001
	Stream Bank	<0.0001	<0.0001	1.0000	<0.0001	0.0001	<0.0001	<0.0001	1.0000	<0.0001	<0.0001
	Stream Bed	<0.0001	0.9987	<0.0001	1.0000	<0.0001	<0.0001	0.9969	<0.0001	1.0000	<0.0001
	Upland Mineral A Horizon	<0.0001	<0.0001	0.0001	<0.0001	1.0000	<0.0001	<0.0001	<0.0001	<0.0001	1.0000

Table A3: ANOVA p-values for POM source comparison by regime for net change particulate C and N masses. P <0.05 are considered significant.

		Regime 1					Regime 2				
	POM Source	**Forest Floor Humus**	**Storm Deposit**	**Stream Bank**	**Stream Bed**	**Upland Mineral A Horizon**	**Forest Floor Humus**	**Storm Deposit**	**Stream Bank**	**Stream Bed**	**Upland Mineral A Horizon**
Net Change TC (g)	Forest Floor Humus	1.0000	0.3857	0.6145	0.0068	0.9983	1.0000	0.1764	0.4031	0.2141	1.0000
	Storm Deposit	0.3857	1.0000	0.9975	0.0756	0.5720	0.1764	1.0000	0.9901	1.0000	0.2012
	Stream Bank	0.6145	0.9975	1.0000	0.0700	0.7900	0.4031	0.9901	1.0000	0.9885	0.4433
	Stream Bed	0.0068	0.0756	0.0700	1.0000	0.0124	0.2141	1.0000	0.9885	1.0000	0.2408
	Upland Mineral A Horizon	0.9983	0.5720	0.7900	0.0124	1.0000	1.0000	0.2012	0.4433	0.2408	1.0000
Net Change TN (g)	Forest Floor Humus	1.0000	0.5073	0.6876	0.0070	0.9992	1.0000	0.2187	0.4010	0.2343	1.0000
	Storm Deposit	0.5073	1.0000	0.9994	0.0495	0.6669	0.2187	1.0000	0.9979	0.9999	0.2264
	Stream Bank	0.6876	0.9994	1.0000	0.0560	0.8229	0.4010	0.9979	1.0000	0.9935	0.4118
	Stream Bed	0.0070	0.0495	0.0560	1.0000	0.0113	0.2343	0.9999	0.9935	1.0000	0.2418
	Upland Mineral A Horizon	0.9992	0.6669	0.8229	0.0113	1.0000	1.0000	0.2264	0.4118	0.2418	1.0000

Table A4: ANOVA p-values for POM source comparison by regime for dissolved C and N masses. P <0.05 are considered significant.

		Regime 1					Regime 2				
		Forest Floor Humus	Storm Deposit	Stream Bank	Stream Bed	Upland Mineral A Horizon	Forest Floor Humus	Storm Deposit	Stream Bank	Stream Bed	Upland Mineral A Horizon
TN (mg)	Forest Floor Humus	1.0000	0.0785	<0.0001	0.0166	0.0166	1.0000	0.0018	0.0087	0.0065	0.0367
	Storm Deposit	0.0785	1.0000	0.0016	0.9766	0.8228	0.0018	1.0000	0.9997	0.9972	0.9340
	Stream Bank	<0.0001	0.0016	1.0000	0.0216	0.1316	0.0087	0.9997	1.0000	0.9999	0.9828
	Stream Bed	0.0351	0.9766	0.0216	1.0000	0.9877	0.0065	0.9972	0.9999	1.0000	0.9910
	Upland Mineral A Horizon	0.0166	0.8228	0.1316	0.9877	1.0000	0.0367	0.9340	0.9828	0.9910	1.0000
NO3-N (mg)	Forest Floor Humus	1.0000	0.0028	1.0000	0.2152	0.1651	1.0000	0.9759	0.9912	0.9999	0.9801
	Storm Deposit	0.0028	1.0000	0.0008	0.4801	0.7621	0.9759	1.0000	1.0000	0.9887	1.0000
	Stream Bank	1.0000	0.0008	1.0000	0.1538	0.1188	0.9912	1.0000	1.0000	0.9972	1.0000
	Stream Bed	0.2152	0.4801	0.1538	1.0000	0.9984	0.9999	0.9887	0.9972	1.0000	0.9909
	Upland Mineral A Horizon	0.1651	0.7621	0.1188	0.9984	1.0000	0.9801	1.0000	1.0000	0.9909	1.0000
NH4+-N (mg)	Forest Floor Humus	1.0000	<0.0001	<0.0001	<0.0001	<0.0001	1.0000	<0.0001	<0.0001	<0.0001	<0.0001
	Storm Deposit	<0.0001	1.0000	0.9998	0.9999	0.9981	<0.0001	1.0000	1.0000	0.9986	1.0000
	Stream Bank	<0.0001	0.9998	1.0000	1.0000	0.9999	<0.0001	1.0000	1.0000	0.9999	1.0000
	Stream Bed	<0.0001	0.9999	1.0000	1.0000	0.9981	<0.0001	0.9986	0.9999	1.0000	0.9997
	Upland Mineral A Horizon	<0.0001	0.9981	0.9999	0.9999	1.0000	<0.0001	1.0000	1.0000	0.9997	1.0000
DON (mg)	Forest Floor Humus	1.0000	0.0004	<0.0001	0.0091	0.0008	1.0000	0.0008	0.0040	0.0012	0.0507
	Storm Deposit	0.0004	1.0000	0.6836	0.9357	0.9934	0.0008	1.0000	0.9999	1.0000	0.7658
	Stream Bank	<0.0001	0.6836	1.0000	0.3122	0.9490	0.0040	0.9999	1.0000	0.9999	0.8812
	Stream Bed	0.0091	0.9357	0.3122	1.0000	0.8284	0.0012	1.0000	0.9999	1.0000	0.7736
	Upland Mineral A Horizon	0.0008	0.9934	0.949	0.8284	1.0000	0.0507	0.7658	0.8812	0.7736	1.0000
DOC (mg)	Forest Floor Humus	1.0000	<0.0001	<0.0001	<0.0001	<0.0001	1.0000	<0.0001	0.0012	<0.0001	0.0015
	Storm Deposit	<0.0001	1.0000	0.9992	1.0000	0.9912	<0.0001	1.0000	0.9023	1.0000	0.8753
	Stream Bank	<0.0001	0.9992	1.0000	0.9996	0.9995	0.0012	0.9023	1.0000	0.9177	1.0000
	Stream Bed	<0.0001	1.0000	0.9996	1.0000	0.9942	<0.0001	1.0000	0.9177	1.0000	0.8940
	Upland Mineral A Horizon	<0.0001	0.9912	0.9995	0.9942	1.0000	0.0015	0.8753	1.0000	0.8940	1.0000

Table A5: ANOVA p-values for POM source comparison by regime for net change dissolved C and N masses. P <0.05 are considered significant.

		Regime 1					Regime 2				
		Forest Floor Humus	Storm Deposit	Stream Bank	Stream Bed	Upland Mineral A Horizon	Forest Floor Humus	Storm Deposit	Stream Bank	Stream Bed	Upland Mineral A Horizon
Net change TN (mg)	Forest Floor Humus	1.0000	0.3573	0.8368	1.0000	0.7414	1.0000	<0.0001	<0.0001	<0.0001	<0.0001
	Storm Deposit	0.3573	1.0000	0.0332	0.2765	0.9865	<0.0001	1.0000	0.9991	1.0000	0.8596
	Stream Bank	0.8368	0.0332	1.0000	0.7963	0.1877	<0.0001	0.9991	1.0000	0.9987	0.9527
	Stream Bed	1.0000	0.2765	0.7963	1.0000	0.6940	<0.0001	1.0000	0.9987	1.0000	0.8720
	Upland Mineral A Horizon	0.7414	0.9865	0.1877	0.6940	1.0000	<0.0001	0.8596	0.9527	0.8720	1.0000
Net change NO_3-N (mg)	Forest Floor Humus	1.0000	0.0754	0.5572	0.5979	0.2517	1.0000	0.9939	0.9999	0.9962	0.9454
	Storm Deposit	0.0754	1.0000	0.0539	0.6351	0.9948	0.9939	1.0000	0.9982	1.0000	0.9911
	Stream Bank	0.5572	0.0539	1.0000	0.5772	0.2254	0.9999	0.9982	1.0000	0.9990	0.9620
	Stream Bed	0.5979	0.6351	0.5772	1.0000	0.9714	0.9962	1.0000	0.9990	1.0000	0.9922
	Upland Mineral A Horizon	0.2517	0.9948	0.2254	0.9174	1.0000	0.9454	0.9911	0.9620	0.9922	1.0000
Net change NH_4^+-N (mg)	Forest Floor Humus	1.0000	0.0653	0.1010	0.0782	0.1311	1.0000	0.0491	0.0082	0.0069	0.0093
	Storm Deposit	0.0653	1.0000	1.0000	0.9999	1.0000	0.0491	1.0000	1.0000	1.0000	1.0000
	Stream Bank	0.1010	1.0000	1.0000	0.9999	1.0000	0.0082	1.0000	1.0000	1.0000	1.0000
	Stream Bed	0.0782	0.9999	0.9999	1.0000	1.0000	0.0069	1.0000	1.0000	1.0000	1.0000
	Upland Mineral A Horizon	0.1311	1.0000	1.0000	1.0000	1.0000	0.0093	1.0000	1.0000	1.0000	1.0000
Net change DON (mg)	Forest Floor Humus	1.0000	1.0000	0.7255	0.8163	0.9985	1.0000	0.0012	0.0020	0.0024	0.0231
	Storm Deposit	1.0000	1.0000	0.6540	0.7689	0.9994	0.0012	1.0000	1.0000	1.0000	0.8518
	Stream Bank	0.7255	0.6540	1.0000	0.9997	0.8726	0.0020	1.0000	1.0000	1.0000	0.8419
	Stream Bed	0.8163	0.7689	0.9997	1.0000	0.9330	0.0024	1.0000	1.0000	1.0000	0.8779
	Upland Mineral A Horizon	0.9985	0.9994	0.8726	0.9330	1.0000	0.0231	0.8518	0.8419	0.8779	1.0000
Net change DOC (mg)	Forest Floor Humus	1.0000	0.0012	0.0014	0.0016	0.0011	1.0000	0.0002	0.0040	0.0005	0.0084
	Storm Deposit	0.0012	1.0000	0.9965	0.9988	0.9142	0.0002	1.0000	0.6067	1.0000	0.6135
	Stream Bank	0.0014	0.9965	1.0000	1.0000	0.9878	0.0040	0.6067	1.0000	0.7276	1.0000
	Stream Bed	0.0016	0.9988	1.0000	1.0000	0.9798	0.0005	1.0000	0.7276	1.0000	0.7196
	Upland Mineral A Horizon	0.0011	0.9142	0.9878	0.9798	1.0000	0.0084	0.6135	1.0000	0.7196	1.0000

		Regime 1				
	POM Source	**Forest Floor Humus**	**Storm Deposit**	**Stream Bank**	**Stream Bed**	**Upland Mineral A Horizon**
TC (g)	Forest Floor Humus	1.0000	<0.0001	<0.0001	<0.0001	<0.0001
	Storm Deposit	<0.0001	1.0000	0.0007	0.9998	<0.0001
	Stream Bank	<0.0001	0.0007	1.0000	0.0043	<0.0001
	Stream Bed	<0.0001	0.9998	0.0043	1.0000	<0.0001
	Upland Mineral A Horizon	<0.0001	<0.0001	<0.0001	<0.0001	1.0000
TN (g)	Forest Floor Humus	1.0000	<0.0001	<0.0001	<0.0001	<0.0001
	Storm Deposit	<0.0001	1.0000	<0.0001	0.9987	<0.0001
	Stream Bank	<0.0001	<0.0001	1.0000	<0.0001	0.0001
	Stream Bed	<0.0001	0.9987	<0.0001	1.0000	<0.0001
	Upland Mineral A Horizon	<0.0001	<0.0001	0.0001	<0.0001	1.0000

Comparison of nitrogen processing gene abundances of Forest Floor Humus and Storm Deposit

Regime 1	Nitrification Gene Abundance	<0.0001
	Denitrification Gene Abundance	<0.0001
Regime 2	Nitrification Gene Abundance	0.0013
	Denitrification Gene Abundance	<0.0001

Table A6: ANOVA p-values for POM source comparison by regime for N processing gene abundances. P <0.05 are considered significant.

Comparison of net change of nitrogen processing gene abundances of Forest Floor Humus and Storm Deposit		
Regime 1	Net change nitrification Gene Abundance	<0.0001
	Net change denitrification Gene Abundance	<0.0001
Regime 2	Net change nitrification Gene Abundance	0.2010
	Net change denitrification Gene Abundance	0.0004

Table A7: ANOVA p-values for POM source comparison by regime for net change of N processing gene abundances. P <0.05 are considered significant.