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ABSTRACT

Quantifying spatial and temporal heterogeneity in ecosystem processes presents a challenge for conserving ecosystem function across landscapes. In particular, many ecosystems contain small features that play larger roles in ecosystem processes than their size would indicate; thus, they may represent ‘‘hotspots’’ of activity relative to their surroundings. Biogeochemical hotspots are characterized as small features within a landscape that show comparatively high chemical reaction rates. In northeastern forests in North America, vernal pools are abundant, small features that typically fill in spring with snow melt and precipitation and dry by the end of summer. Ephemeral flooding alters soil moisture and the depth of the soil's oxic/anoxic boundary, which may affect biogeochemical processes. We studied the effects of vernal pools on leaf-litter decomposition rates, soil enzyme activity, and denitrification in vernal pools to assess whether they function as biogeochemical hotspots. Our results indicate that seasonal inundation enhanced leaf-litter decomposition, denitrification, and enzyme activity in vernal pools relative to adjacent forest sites. Leaves in seasonally flooded areas decomposed faster than leaves in terra firme forest sites. Flooding also influenced the C, N, and P stoichiometry of decomposing leaf litter and explained the variance in microbial extracellular enzyme activity for phosphatase, β-$_{D}$-glucosidase, and β-*N*-acetylglucosaminidase. Additionally, denitrification rates were enhanced by seasonal flooding across all of the study pools. Collectively, these data suggest that vernal pool ecosystems may function as hotspots of leaf-litter decomposition and denitrification and play a significant role in decomposition and nutrient dynamics relative to their size.

Key words: ephemeral wetland; biogeochemical hotspot; leaf-litter decomposition; denitrification; soil enzymes.

INTRODUCTION

Accounting for heterogeneity in ecosystem processes across landscapes presents a great challenge for ecologists and natural resource managers (Cardinale and others 2002; Lovett and others 2005; Ostojic and others 2013). This is particularly true because the rates of many processes vary in both time and space, producing hotspots (small areas) and hot moments (brief periods) that can be responsible for much of the activity at the landscape scale (McClain and others 2003; Groffman and others 2009). Hotspots and hot moments of ecosystem processes have been acknowledged for decades (McClain and others 2003), and are frequently driven by hydrology. This is especially true at the interface of aquatic and terrestrial environments (McClain and others 2003). For example, carbon (C), nitrogen (N), and phosphorus (P) dynamics, which are linked to organic matter decomposition, are all influenced by the wet–dry periods associated with floodplains and ephemeral freshwater ecosystems (Kelley and Jack 2002; Grimm and others 2003; Baldwin and others 2005; Burt and Pinay 2005; Groffman and others 2009; Harner and others 2009). To promote effective ecosystem-level conservation and maintain ecosystem services, it is imperative to identify hotspots and hot moments of ecosystem processes so that natural variability can be effectively integrated into biogeochemical models and natural resource management activities (Lovett and others 2005; Boyer and others 2006; Groffman and others 2009). However, conservation activities are frequently directed toward maintaining the structure, rather than the function of ecosystems (Palmer and Febria 2012).

The mesofilter approach to conservation (Hunter 2005) is directly relevant to the conservation of hotspots of ecological processes as they are good examples of features with disproportionate importance for maintaining ecosystem integrity at a larger spatial scale. Conservation biologists frequently employ a coarse-filter approach to the conservation of biodiversity and ecosystem processes by protecting a representative array of ecosystems (Hunter 1991); however, this approach may not work to conserve all species. Therefore, fine-filter activities targeting individual species are also employed. Intermediate between coarse and fine-filter approaches is the idea of mesofilter conservation, a strategy designed to conserve ecosystem features that are essential to the success of many species, such as desert springs that provide water for wide ranging animals (Hunter 2005; Crous and others 2013). The cornerstone of mesofilter conservation is the knowledge that ecosystems typically contain features that are essential to the success of many species. Hence, protecting said features could have large, positive effects on entire communities and ecosystems.

Vernal pools are ephemeral wetlands in forested landscapes throughout northeastern North America typically occupying small (<1 ha) depressions (Calhoun and deMaynadier 2008). The primary energy source in undisturbed vernal pools is leaf litter (Earl and Semlitsch 2012, 2013). Previous work has suggested vernal pools may support enhanced biogeochemical cycling and leaf-litter decomposition rates relative to the surrounding forest floor (for example, Palik and others 2006; Brooks 2009; Earl and Semlitsch 2013). Moreover, vernal pools provide essential breeding habitat for a suite of amphibians and macroinvertebrates adapted to development in

temporary waters and thus are a conservation concern (Calhoun and others 2003; Bischof and others 2013). Because of their relatively small size, their known importance to biodiversity conservation, and their potential influence on elemental cycling and decomposition in northeastern forests, vernal pools provide an excellent opportunity to link studies of biogeochemical hotspots and hot moments with a meso-filter framework.

Leaf-litter decomposition and denitrification are two ecosystem processes that are frequently examined using the hotspot/hot moment framework (for example, Fenner and others 2011; Bettez and Groffman 2012). Decomposition of organic matter is a key mediator of energy flow and biogeochemical cycling in both terrestrial and aquatic ecosystems (Petersen and Cummins 1974; Benke and others 1988; Jackson and others 1995). The variability in this process within and among ecosystems (Capps and others 2011), suggests that hotspots and hot moments may be functionally important when considering decomposition at larger spatial scales (Glazebrook and Robertson 1999; Graca and others 2001). Microbes play an important role in decomposition via extracellular enzymes, which acquire the necessary molecular forms of C, N, and P for growth and energy by breaking apart larger organic structures (Chrost 1991). Extracellular enzyme activity (EEA) reflects both a demand for (Sinsabaugh and others 2009; Hill and others 2012) and a response to the available resources (Kirchman and others 2004; Harbott and Grace 2005). Studies examining decomposition across hydrological gradients have produced conflicting results, as decomposition rate is strongly influenced by temperature, moisture, and oxygen

availability (Graca and others 2001; Paul and others 2006; Battle and Golladay 2007; Wieder and others 2009; Yule and Gomez 2009). Sites that experience inundations, such as vernal pools, may exhibit higher rates of decomposition than terrestrial sites (for example, Day 1982; Kelley and Jack 2002); yet, other studies have demonstrated the opposite pattern (Capps and others 2011). These conflicting results can usually be explained either by moisture limitation in terrestrial environments or by hydroperiod and/or sedimentation in aquatic habitats.

Denitrification, or the microbial reduction of N oxides to N gases, is typically controlled by oxygen availability, ambient nitrate concentrations, and carbon composition. It is a difficult process to measure and model because hotspots and hot moments are typically responsible for most denitrification in both terrestrial and aquatic ecosystems (Parkin 1987; Groffman and others 1999). Hotspots and hot moments of denitrification have not been effectively incorporated into N models (Groffman and others 2009); thus, it is difficult to understand the magnitude of the functional role of specific ecological features in N cycling. Together, data from decomposition and denitrification studies suggest features with highly variable hydrologic characteristics, such as ephemeral wetlands, may function as microbially driven biogeochemical hotspots and hot moments in forested ecosystems.

The purpose of this study was to assess whether or not vernal pools function as biogeochemical hotspots within forests in the northeastern United States. Few studies have examined more than one process when considering hotspots and hot moments

in ecosystems, but it is possible that vernal pools may function as hotspots or produce hot moments for multiple ecosystem processes. Thus, in this investigation, we examined how inundation influenced three response variables: leaf-litter decomposition rates, EEA, and denitrification rates across a hydroperiod gradient. We predicted that ecosystem process rates would be greater in vernal pools relative to adjacent uplands, but the timing and duration of flooding within and among pools would influence processes differently. Specifically, we hypothesized sites within pools experiencing intermediate levels of inundation (that is, sites that experienced the greatest numbers of wet–dry intervals) would have greater decomposition rates and sites with the longest hydroperiod would have the greatest denitrification rates (McClain and others 2003; Palik and others 2006).

METHODS

The work was conducted in the University of Maine Demeritt Forest Preserve in Old Town, Maine (44°55′N, 68°41′W; Penobscot County) between March and August of 2013. The forest consisted of mixed deciduous and coniferous stands. Pool volume was roughly estimated by 20 depth measurements and the circumference of the pool and calculating the volume of a cylinder. Leaf-litter decomposition, soil enzyme activity, and denitrification rates were estimated along a flooding gradient in three sites (center, edge, and terra firme) in three vernal pools with variable timing and duration of flooding (Figure 1). Though the edge of the pool varied throughout the study period with the changing water volume, all of the sample sites were held constant throughout the study.

Center sites were located in the deepest part of the pool and remained inundated the longest. Edge sites were inundated at the beginning of the season after spring thaw, but dried down by the beginning of July (Figure 2). Terra firme sites were never inundated throughout the study period. Water samples were filtered through glass fiber filters (Pall A/E), frozen, and subsequently analyzed using standard methods (APHA 1998) for NH_4^+-N, NO_3^--N, and SRP-P on a Lachat Quickchem 8500 at the University of Maine.

Leaf-Litter Decomposition

Leaf-litter decomposition was measured using leaf-litter bags (1 cm mesh Vexar® onion bags) filled with dried, pre-weighed red maple (Acer rubrum) leaves (7 g ± 0.3 g dry mass (DM)). Five replicate bags were deployed on April 28, 2013 for 80 days in a center, edge, and terra firme site (Figure 1B) in each of the three pools and were anchored with tent stakes. At the end of the study, individual litter bags were placed in sealed plastic bags and were transported back to the lab for processing. In the lab, all leaves were gently rinsed to remove detritus and macroinvertebrates and placed in a drying oven at 45°C for 48 h, weighed and ground into a fine powder. Aliquots (rv1 g) of the ground litter were ashed at 550°C for 1 h to estimate ash-free dry mass (AFDM) and additional aliquots (rv1 g) were taken for C, N, and P analysis. Decomposition rates were calculated as the change in AFDM per unit time. Percent C and N were analyzed on a LecoTruMac Series Macro CN-Analyzer and % P was digested in acid and analyzed on an iCap 6000 Series ICP-OES in the Analytical Laboratory and Maine Soil Testing Service at the University of Maine. Change in total C, total N, and total P was estimated

by subtracting the final total C, N, and P (final weight × final % C, N, or P) from the initial total C, N, and P (initial weight × initial % C, N, or P).

Extracellular Enzyme Activity

We measured EEA using a fluorescent microplate approach (Sinsabaugh and Findlay 1995; Hill and others 2006). Briefly, at each site soil cores were collected using a PVC corer on three sample dates in May, June, and July of 2013. Samples were placed into plastic bags, and frozen at −40°C for up to 5 months. Samples were assayed adapting methods from Bell and others (2013) following standard protocols. Briefly, five samples were assayed per microplate (400 µl per well) including reference, sample, substrate, and quench controls. Assayed samples were thawed at room temperature; 1 g of wet sediment was weighed and then homogenized in 40 ml of 50 mM Bis–Tris buffer adjusted to pH 6. A 200 µl aliquot of sample slurry was then added to the appropriate wells for EEA analysis, quench control, and sample control. Plates were then frozen until the day of analysis. On the day of analysis, plates were thawed at room temperature, 50 µl of 100 µM 4-methylumbelliferone was added to reference and quench controls, and 50 µl of 1 mM 4-methylumbelliferyl-linked substrate was added to the substrate control and sample assay wells. The saturating concentration of enzyme substrate was determined by optimizing the sample and 4-methylumbelliferyl-linked substrate concentrations (German and others 2011). Plates were incubated at 25°C and read at regular intervals for (1–8 h). Once the reaction was confirmed to be linear and fluorescence had increased fivefold to tenfold over the

initial value, the pH was raised above 8 by adding 10 µl of 0.5 mM NaOH to each well, and a final reading was made. Separate measurements relating wet weight of sediments to dry weight and EEA were calculated as nmol g DM^{-1} h^{-1}.

We selected α-$_D$-glucosidase (αGLUC) and β-$_D$-glucosidase ($\beta GLUC$)) to measure C-acquiring activity, β-*N*-acetylglucosaminidase (NAG), and leucine aminopeptidase (LAMP) for N-acquiring activity, and phosphatase (PHOS) for P-acquiring activity. Relative differences in absolute enzyme activity may provide evidence of microbially driven biogeochemical hotspots. At the same time, across ecosystems, the log ratio (ln (C):ln (N):ln (P)) of these activities is typically near 1:1:1 (Sinsabaugh and others 2009) and deviations from these ratios may indicate elemental limitation reducing the potential biogeochemical cycling rates of that hot-spot. For example, a ln (C):ln (N) of 1.5 would indicate C limitation relative to N.

Denitrification

Samples for denitrifying enzyme activity (DEA) analysis were collected from three substrate types in each of the three vernal pools to determine variability across a substrate and landscape gradient. Samples were collected on dates in May and July using a PVC corer from terra firme, edge, and center locations with three replications each. Three cores were combined at each sample location to account for local variability within soils. Methods for measurement of DEA were taken from Groffman and others (2009) with slight modifications. Briefly, weighed (10 g) field-moist soil samples were

added to 125-ml mason jars fitted with rubber septa. A chloramphenicol solution (10 ml of 0.01 g l^{-1}) was added to each chamber to cease new enzyme production, and samples were subjected to ambient and plus C (0.04 g C l^{-1}) and N (0.10 g N l^{-1}) treatments, with two replicates each. The DEA was measured under ambient nutrient concentrations to determine rates at the time of sample collection (David and others 2006; Inwood and others 2007), and elevated nutrient concentrations (+CN treatment) to determine potential denitrification rates and any nutrient limitation of C and N (Kaspar 1982; Groffman and others 1999). Chambers were flushed with He, evacuated to 700 mg Hg three times, and brought to atmospheric pressure. To inhibit the conversion of N_2O gas into N_2, 15 ml of acetylene gas was then added (~10% of the headspace). Chambers were placed on a rotary shaker at 120 rpm, and gas samples were taken at 30 and 90 min. Gas samples were analyzed for N_2O on a Shimadzu GC-2014 greenhouse gas analyzer. Standard curves of N_2O in ppmv were made fresh daily and concentrations were converted to ppm using the temperature and atmospheric pressure at the time of sample analysis. DEA was calculated as the rate of accumulation of N_2O in the headspace, standardized by sample dry mass ($\mu g\ N_2O\text{-}N\ g\ AFDM^{-1}\ h^{-1}$).

Statistical Analysis

Leaf-litter decomposition analyses were conducted using two-way ANOVAs (Factors: Pool and Site within Pool). Tukey's post hoc tests were used to identify differences among treatments and contrast tests were run to assess the influence of flooding and

pool identity on decomposition rates (% change in DM and AFDM), leaf-litter nutrient content and stoichiometry (% C, % N, % P, C:N, C:P, N:P, and changes in total C, total N, total P). EEA analysis was conducted using a two-way AN- OVA using P values for pairwise difference and was corrected using Tukey's HSD. For denitrification analysis, pool name, site in pool, nutrient treatment, and all corresponding interaction effects were tested and then removed from the model if they were not significant. Tukey's post hoc tests were used to identify differences among treatments and contrast tests were run to assess the influence of flooding and pool identity on denitrification rates. All statistics were run using JMP© 10 statistical software (SAS Institute 2011).

RESULTS

Physicochemical Properties

The three study pools had variable hydroperiods and ambient soluble N and P concentrations throughout the study period (Figure 2). Pool 1 was completely dried by mid-June and the terra firme, edge, and center sites were dry during the July sampling period. Center sites in pools 2 and 3 remained flooded throughout the study period, but edge sites dried prior to the June (EEA) and July (EEA, leaf-litter decomposition, denitrification) sampling periods. Ambient N and P (NH_4^+-N,NO_3^--N, SRP-P) concentrations increased by at least one order of magnitude in pools 2 and 3, which retained water through the duration of the study (Figure 2).

Leaf-Litter Decomposition

Flooding strongly influenced leaf-litter decomposition (Figure 3). Average decomposition rate was greatest in edge sites (0.038 ± 0.003 g AFDM d^{-1} (mean ± SE)), followed by center sites (0.032 ± 0.001 g AFDM d^{-1} (mean ± SE)), and terra firme sites (0.027 ± 0.004 g AFDM d^{-1} (mean ± SE)). Change in DM and AFDM (%) was significantly greater in edge and center sites when compared to terra firme sites ($P < 0.0001$). There were also significant differences in decomposition among pools; where pool 1 had significantly lower decomposition rates than pools 2 and 3 (DM and AFDM, $P < 0.0001$).

Location within pool and pool identity also influenced % C, N, and P, and the stoichiometry of decomposing leaf litter (Figure 4). Leaf litter % C was greatest in un-flooded sites within pools (terra firme ≥ edge ≥ center, $P = 0.0108$), though it was greater in pools that experienced longer periods of inundation (pool 2 ≥ pool 3 ≥ pool 1; $P = 0.0021$; Figure 4A). Subsequent contrasts also revealed litter in edge and center sites had significantly less % C than terra firme sites ($P = 0.0289$) and litter in pools 2 and 3 had significantly more % C than pool 1 ($P = 0.0015$). Percent N was significantly greater in sites that experienced longer inundation (center > edge > terra firme; $P < 0.0001$) and was greater in pools 2 and 3 than pool 1 ($P < 0.0001$; Figure 4B). Leaf-litter % P exhibited similar patterns (center ≥ edge ≥ terra firme, $P = 0.0026$; pool 2 ≥ pool 3 ≥ pool 1, $P = 0.0556$; Figure 4C) and subsequent contrasts

indicated litter from edge and center sites had significantly more % P than terra firme sites ($P = 0.0018$) and litter from pools 2 and 3 had significantly more P than pool 1 ($P = 0.0341$). The lower N and P content in terra firme sites led to significantly higher C:N (by mass; $P < 0.0001$; Figure 4D) and C:P ($P < 0.0001$; Figure 4E) in litter. There were also significant pool effects on stoichiometry. Litter from pool 1 had greater C:N (by mass; $P < 0.0001$) and C:P ($P = 0.0108$) than litter from pools 2 and 3. However, there were no significant effects of pool ($P = 0.2179$) or site within pool ($P = 0.1126$) on leaf-litter N:P (Figure 4F).

Change in total C, N, and P was also influenced by the degree of flooding (Figure 5), but only total C was significantly influenced by pool identity ($P < 0.0001$; Figure 5A). Loss of total C was greatest in the flooded center and edge sites ($P < 0.0001$). Conversely, total N significantly increased with increasing hydroperiod ($P < 0.0001$; Figure 5B). Total P decreased through time in all sites and among all pools; however, unlike total C and N, the response was greatest in the terra firme sites ($P = 0.0055$).

Extracellular Enzyme Activity

Both spatial and temporal factors explained the variance in EEAs for PHOS ($R^2 = 0.51$, $P < 0.005$), βGLUC ($R^2 = 0.28$, $P < 0.005$), and NAG ($R^2 = 0.30$, $P < 0.005$); other EEAs did not vary significantly among sampling dates or sites. In terms of spatial variability, edge sites that experienced intermittent inundation displayed higher phosphatase activity compared to terra firme ($p_{adj} = 0.01$) and center ($p_{adj} = 0.05$) sites

(Figure 6A). Similarly, NAG and βGLUC activities were highest in edge sites, but were not significantly different from center (p_{adj} = 0.06, 0.09 for NAG and *βGLUC*, respectively) or terra firme sites (p_{adj} = 0.96, 0.26 for NAG and βGLUC, respectively) due to relatively higher standard errors at the edge sites (Figure 6B). In terms of temporal variability, EEA for these three enzymes followed the pattern June $>$ July $>$ May (p_{adj} £0.05) although July activity was significantly greater than May for PHOS. The interaction of habitat and sampling date found that May phosphatase activities were lower than June phosphatase EEA ($p_{adj} < 0.005$) in all sites, but July and June and July and May activities in each site were not significantly different ($p_{adj} > 0.05$).

Denitrification

Denitrification rates were influenced by nutrient treatment ($P < 0.0001$) and site within pool (P = 0.0172; Figure 7A). Ambient treatment had significantly lower denitrification rates than +CN treatments ($P < 0.0001$). Center and edge sites had significantly greater denitrification rates than terra firme sites (P = 0.0049). When analyzed separately from the ambient treatments, the potential denitrification rates (+CN treatment) exhibited the same pattern (center and edge $>$ terra firme; P = 0.0088). However, there were no significant differences in denitrification rates among sites in the ambient treatment (P = 0.1582) and potential denitrification rates in the +CN treatments were not significantly influenced by pool identity (P = 0.6749) or sample date (P = 0.5431; Figure 7).

DISCUSSION

Water is frequently considered a primary driver of biogeochemical processes (McClain and others 2003; Groffman and others 2009). Specifically, sites that experience wet–dry cycles are thought to have great potential to generate biogeochemical hotspots (McClain and others 2003; Seitzinger and others 2006; Mulholland and others 2009), and many investigations have documented the strong, positive influence of seasonal inundation on leaf-litter decomposition (for example, Langhans and others 2006; Battle and Golladay 2007; Langhans and others 2008), EEA (for example, Hill and others 2006; Sinsabaugh and others 2009), and denitrification (for example, Groffman and Hanson 1997; Blackwell and Pilgrim 2011; Bettez and Groffman 2012). Denitrification is the principal process responsible for permanently removing N from terrestrial and aquatic systems (Seitzinger and others 2006; Mulholland and others 2009) and leaf-litter decomposition in the primary source of energy in many ecosystems (Fisher and Likens 1973; Palik and others 2006; Batzer and Palik 2007; Earl and Semlitsch 2013); hence, hotspots of activity of these two processes may influence the structure and function of forests. Our investigation simultaneously considered both decomposition and denitrification, and revealed that vernal pools function as hotspots of both processes in northeastern forests. Leaves and soils exposed to flooding experienced greater decomposition rates and enhanced rates of potential denitrification. Contrary to our hypothesis that biogeochemical processing rates would be greatest in intermittently flooded sites (edge), there were limited differences in leaf-

litter decomposition and denitrification between center and edge sites. As we predicted, pools with longer hydroperiods had faster rates of leaf-litter decomposition. However, there was no difference in potential denitrification rates among pools. Importantly, the patterns in leaf-litter decomposition and potential denitrification, we documented among sites were consistent among pools, suggesting that even brief periods of inundation can stimulate decomposition and denitrification.

Vernal Pools as Hot Spots of Leaf-Litter Decomposition

Flooding enhanced leaf-litter decomposition in our study, but the duration of flooding did not significantly influence decomposition rates among pools (Figure 3). These results are similar to studies documenting enhanced leaf-litter decomposition in permanently flooded areas relative to leaves placed in terrestrial environments (Langhans and others 2006, 2008; Battle and Golladay 2007). In terrestrial sites, leaf-litter decomposition may be limited by moisture (Anderson 1991; Cornejo and others 1994; Cisneros-Dozal and others 2007). Desiccation may have influenced leaf-litter decomposition in our terra firme sites. Additionally, flooded vernal pools are frequently characterized by dense populations of shredding macroinvertebrates (for example, *Diptera, Isopoda, Trichoptera*) and detritivorous tadpoles (Calhoun and de Maynadier 2008). High densities of detritivores in the aquatic relative to the terrestrial habitats may have played an important role in leaf decomposition in this study. Future work should consider the synergistic effects of flooding regimes and detritivores on leaf- litter decomposition across terrestrial and aquatic portions of vernal pools.

Flooding also influenced the nutrient content and stoichiometry of decomposing leaf litter (Figure 4). Typically, leaves that were exposed to longer periods of inundation tended to have greater % N and % P and lower C:N and C:P (Figure 4). Most likely, this pattern was driven by increased colonization of wet leaves by fungal and bacterial communities (Cross and others 2005). Notably, when examined as change in total C, N, and P (Figure 5), it was evident that inundation influenced each element differently. Total change in C was greatest in the center and edge sites, and was negative among all of the sample sites in each of the study pools (Figure 5A). A similar, negative pattern was seen in P; yet, the terra firme sites experienced greater loss relative to the flooded sites (Figure 5C). Conversely, and perhaps most indicative of the potential role of microbial colonization on submerged leaves in influencing leaf stoichiometry, where the increases in total N on all of the leaves exposed to flooding (Figure 5B). These patterns were even evident in the study pool that experienced the shortest hydroperiod, suggesting that even short periods of inundation can also influence leaf-litter chemistry and stoichiometry. Lower C:N, C:P, and N:P indicate leaf-litter of higher food quality (Abenspergtraun 1993; Cross and others 2005); hence, leaves in the aquatic habitats of vernal pools may be of higher quality to both aquatic and terrestrial detritivores than leaves decomposing in terrestrial habitats. Subsequent studies should consider the functional role vernal pools play in transforming the quality and quantity of basal food resources in both aquatic and terrestrial food webs. Moreover, had we measured leaf nutrient content at additional time intervals, we may have documented changes in N and P immobilization rates that were not evident in this study. Future work should

consider examining temporal changes in the immobilization of N and P through time in vernal pools.

Extracellular Enzymes as Indicators of Biogeochemical Hotspots

Soil EEAs were comparable to previously reported values (Hill and others 2006; Sinsabaugh and others 2009) from soil and wetland sediments. The spatial and temporal variability displayed by PHOS, βGluc, and NAG were likely related to seasonal changes in temperature and water level. Their relatively higher activities in the intermittently wetted sites relative to terra firme and permanently inundated sites may have been due to drier conditions in terra firme sites and anoxic conditions in permanently inundated areas inhibiting microbial activity. Temporally, their activities were likely higher in June than May due to soil warming, but decreased again by July as warming soils became desiccated. The high values observed in June, likely represent an optimum balance of temperature and moisture. Alternatively, drying and rewetting periods occurring between June and July may have caused phosphorus desorption from soils (Reddy and others 1999) resulting in a decrease in PHOS EEA. Regardless of the exact mechanism, these results provide further evidence that vernal pools function as both hotspots and hot moments for microbial C, N, and P cycling.

Similar to previous studies (Sinsabaugh and others 2009), C, N, and P-acquiring enzymes increased linearly relative to one another. Our observed slopes for C:N/P (0.86/0.92) were intermediate compared to previously reported lentic sediment

(0.77/0.76) and forest soil (1.09/1.16) slope values (Supplemental Figure 1; Sinsabaugh and others 2009). Interestingly, though the slope of the C:P relationship was consistent with previously observed values (Figure 1B; Sinsabaugh and others 2009), the intercept was much lower. This may have been due to the high aluminum (Al) and iron (Fe) content of Spodosols (Brady and Weil 2007). At low pH (common in Maine Spodosols), inorganic phosphorus readily sorbs and may form insoluble aluminum or iron phosphates (Brady and Weil 2007). This suggests that underlying abiotic processes in the soil chemistry of vernal pools in Maine may naturally inhibit some of their potential for biogeochemical cycling by inhibiting P availability. Future work should consider how P-limitation influences microbial communities responsible for leaf-litter decomposition and other biogeochemical processes in terrestrial and aquatic portions of vernal pools.

Denitrification Hotspots in Forested Landscapes

Denitrification is a process that frequently occurs in relatively small places (hotspots) for brief periods (hot moments) and is notoriously challenging to measure and model (Groffman and others 2006, 2009). For instance, in watersheds total denitrification is typical greater in upland soils; however, on an areal basis, denitrification rates are approximately ten times greater in aquatic environments (Seitzinger and others 2006; Baron and others 2013). Hotspots and hot moments of denitrification are often associated with boundaries between terrestrial and aquatic environments, aerobic/anaerobic boundary in sediments, and areas characterized by decomposing labile organic matter

(Reddy and Patrick 1984; Christensen and Tiedje 1990; Groff- man and others 2009), characteristics of the aquatic environments of vernal pools. In this study, we documented that denitrification in vernal pool soils was nutrient and labile carbon limited (Figure 7A). The potential denitrification rates documented in this study were similar to previously published values in other vernal pools (‘‘woodland pools’’; Groffman and others 1996). Most of our ambient treatments produced N_2O values that were below the level of detection, and there were no significant differences among pools or among sites in pools for ambient samples (Figure 7A). On the contrary, samples amended with C and N suggested a strong effect of flooding on potential denitrification rates, evidenced by greater denitrification rates in center and edge sites from this treatment (Figure 7). Though the pattern was not significant, there was greater potential denitrification in edge sites while they were inundated (May), indicating that denitrification hot moments may occur during flooding (Figure 7C). However, similar to patterns we found in leaf-litter decomposition, soils exposed to any period of inundation had relatively greater potential denitrification rates, and this pattern was evident even after pools had dried for the season. It is important to note, ambient DEA can be underestimated using the acetylene-block method when N is low (Groffman and others 2006), and the potential denitrification measurements (+CN) allowed us to measure the ability of the system to denitrify if conditions were ideal. Although we measured low DEA rates overall, our work suggest vernal pools are capable of high levels of denitrification, when the appropriate conditions are met and flooding increased the potential capacity of the system to denitrify.

Denitrification can be limited by nitrate availability (Groffman and others 2009); thus, increases in ambient nitrate concentrations may enhance denitrification rates. Though it was not a significant relationship, the center sites had greater potential denitrification rates in July than in May (Figure 7), a time which corresponded with the greatest concentrations of ambient nitrate in pools 2 and 3 (Figure 2). The July sampling date also had the greatest concentrations of ammonium, suggesting nitrate increases in the pool may have been due to increased nitrification rates in the aerobic portions of the water column. Greater concentrations of ammonium may have been due to increased temperatures in July that stimulated more N mineralization in soils. Additionally, July also had the greatest organismal density (# macroinvertebrates and tadpoles l^{-1}) in pools (personal observation, K. Capps); hence, increases in ammonium may have been due to waste products excreted by high densities of pool-breeding organisms.

To model the potential landscape-level contribution of vernal pools to denitrification relative to upland forest soils, we coupled our average potential denitrification rates with previously published values of vernal pool size and density in Maine forests (Calhoun and others 2003). We used data from Calhoun and others (2003), as the forests and vernal pools of York County, the focus of their study, were relatively similar to those in Penobscot County and the majority of York County (99%) is forested. The York County study area contained 480 vernal pools (median area of pool: 294 m^2) in mixed forest (white pine [*Pinus strobus*], hemlock [*Tsuga canadensis*], red maple [*Acer rubrum*], and red oak [*Quercus rubura*]; Calhoun and others 2003). Vernal pools made up

approximately 0.39% of the land area (36.4 km^2 total area; 0.14 km^2 vernal pool area). We doubled the published values of total C (Pribyl 2010) in the O horizon of Maine soils (3,800 metric tons of C per km^2; Fernandez 2008) to estimate total AFDM in York County O horizons. We modeled N produced between April and August (153 days) by upland (average DEA in terra firme sites) and vernal pool (average DEA in center and edge sites) soils. By our estimates, upland soils in York County potential denitrification rates would yield approximately 31 metric tons of N, whereas vernal pools would produce approximately 0.31 metric tons of N, or 1% of the N of upland soils. Therefore, during the spring and summer months, vernal pools do seem to be potential hotspots of denitrification relative to upland forests over broader spatial scales. Notably, we assumed that % AFDM and denitrification was uniform through the whole O horizon. However, previous work has demonstrated that in soil cores, most denitrification can occur right at the sediment/leaf-litter boundary (Parkin 1987); thus, our values are most likely over-estimating potential denitrification. More-over, we only modeled potential DEA in spring and summer months. Future studies examining vernal pool denitrification should attempt to measure spatial and temporal changes in denitrification and decomposition for longer periods to see if the initial effects of being flooded are maintained through time.

Challenges for Conserving Ecosystem Function Across Heterogeneous Landscapes

Identifying spatial and temporal heterogeneity in biogeochemical processes is

integral to conserving ecosystem function across landscapes (Cardinale and others 2002; Lovett and others 2005). However, quantifying heterogeneity in ecosystem processes presents a great challenge to ecologists and natural resource managers (Lovett and others 2005). We argue that by employing a mesofilter conservation strategy (Hunter 2005) that focuses on features within ecosystems that have strong potential for generating hotspots and hot moments of biogeochemical processes, natural resource managers could have large, positive effects on the conservation of ecosystem function across sizable landscapes. Moreover, employing a mesofilter approach to conservation may maintain important ecological processes in places that are managed to produce commodities such as livestock forage or fisheries. For example, vernal pools are frequently disturbed as forests are converted into agricultural land or developed landscapes. By creating innovative zoning regulations that avoid disturbing vernal pools, natural resource managers may be able to mitigate degradation of net landscape-level biogeochemical processes. Our data suggest targeted actions designed to conserve vernal pools may play an important role in supporting leaf-litter decom–position and denitrification across forested landscapes. Our work is particularly salient as regulations pertaining to the conservation and the valuation of ecosystem services provided by vernal pools and other types of small ''isolated'' wetlands are frequently debated by policy-makers (Snod-grass and others 2000; Oscarson and Calhoun 2007; Lindquist and others 2013).

Although our recognition of the ecological contribution of small, landscape features are just emerging, examples including coral reefs, prairie potholes, and vernal pools,

illustrate the profound effect they can have on ecological structure and function at broader spatial scales. Integrating the mesofilter approach with the concept of hotspots and hot moments may promote the maintenance of biogeochemical processes across larger areas and maintain functions essential to the structure and stability of targeted terrestrial, freshwater, and marine ecosystems.

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Figure 1. Pool 2 in the University of Maine Dewitt Forest Preserve in Old Town, Maine in 2013 (A). Three sample locations (T terra firme, E edge, and C center) within pool 2 in July 2013 (B).

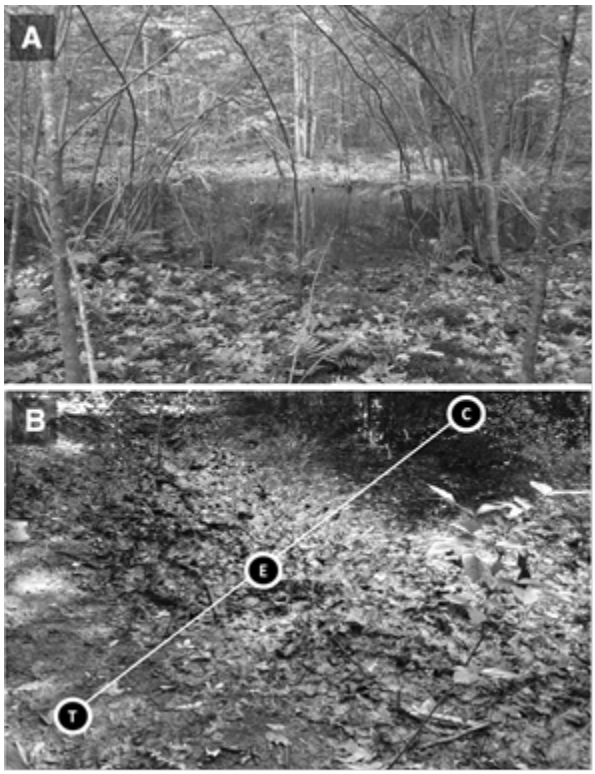


Figure 2. Water column nitrogen (NO^{3-}-N; NH^{4+}- N) and phosphorus (PO_4^{3-}-P) concentrations and pool volume estimate through time in pool 1(A), pool 2 (B), and pool 3 (C). The black arrows in A indicate dates for denitrification and enzyme sampling. The gray arrow in A indicates pool-drying date for pool 1, the time when the edge sites began to dry in pools 2 and 3, and the second sampling date for enzyme sampling.

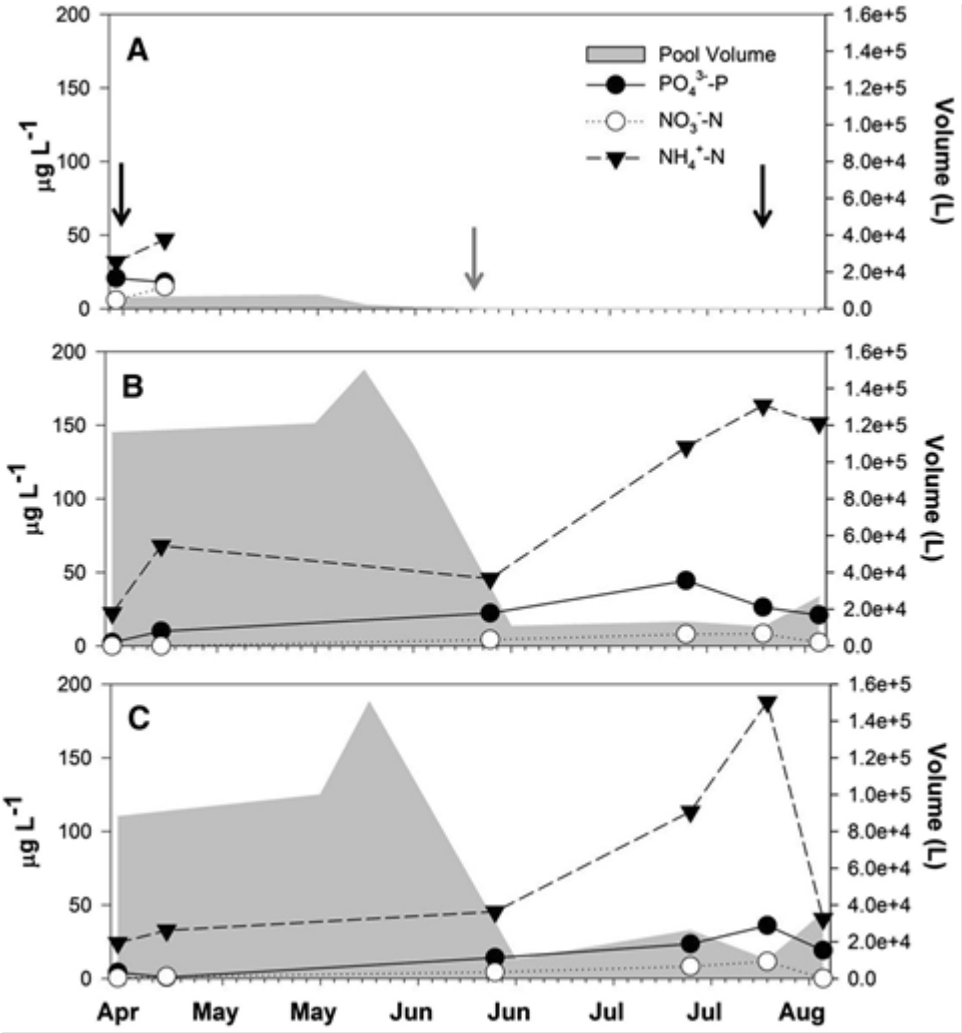


Figure 3. Average leaf-litter decomposition represented by the mass lost (% change of ash-free dry mass (AFDM)) in center, edge, and terra firme locations within each pool. Error bars represent ±1 standard error.

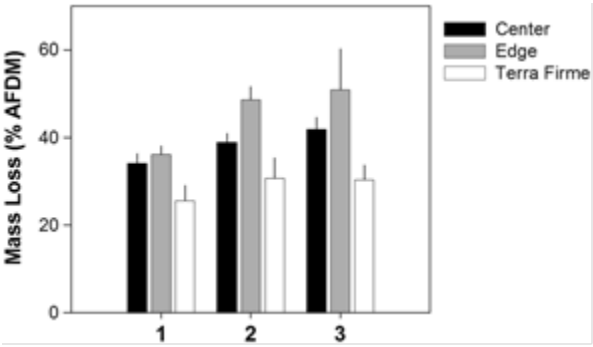
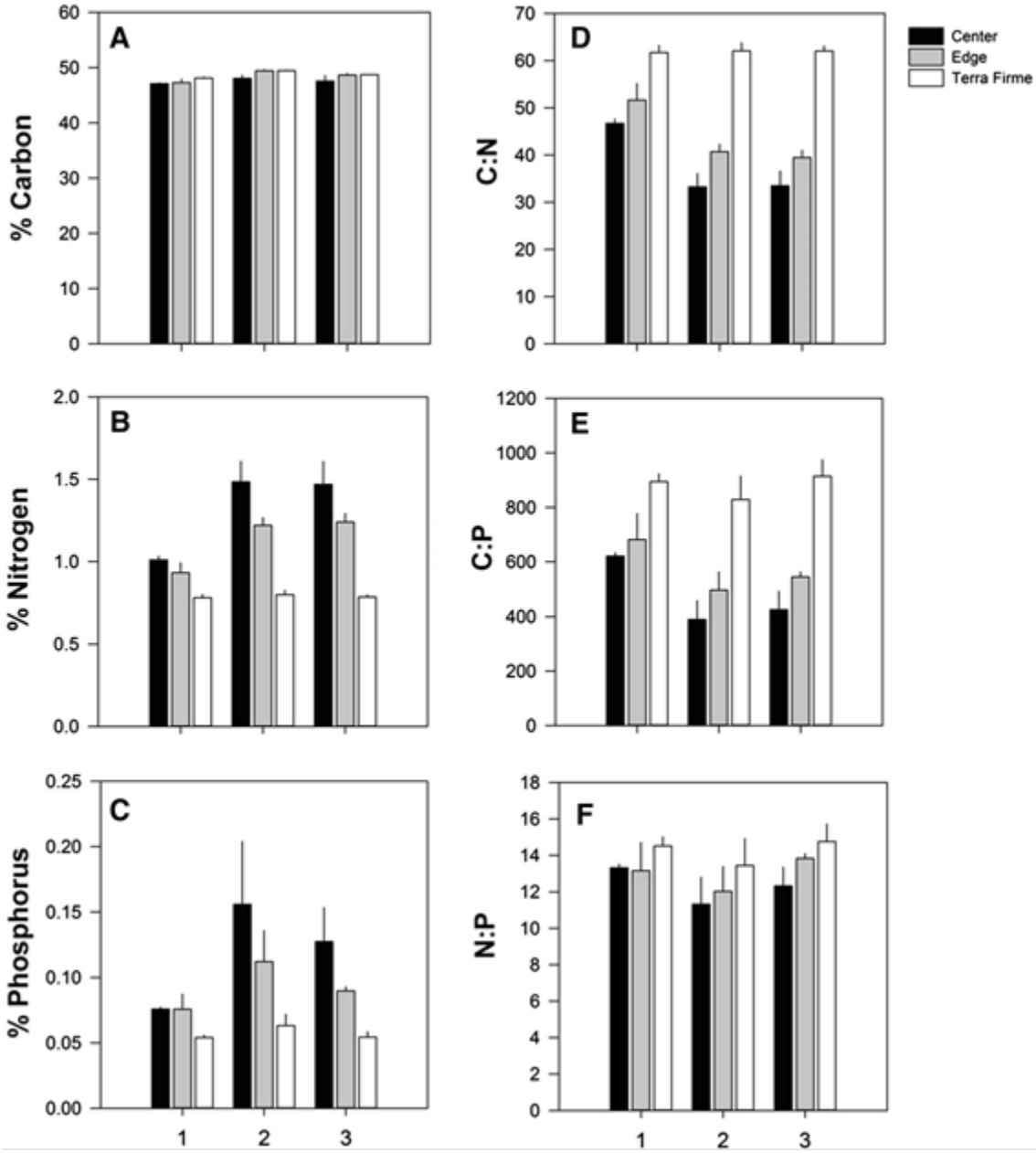
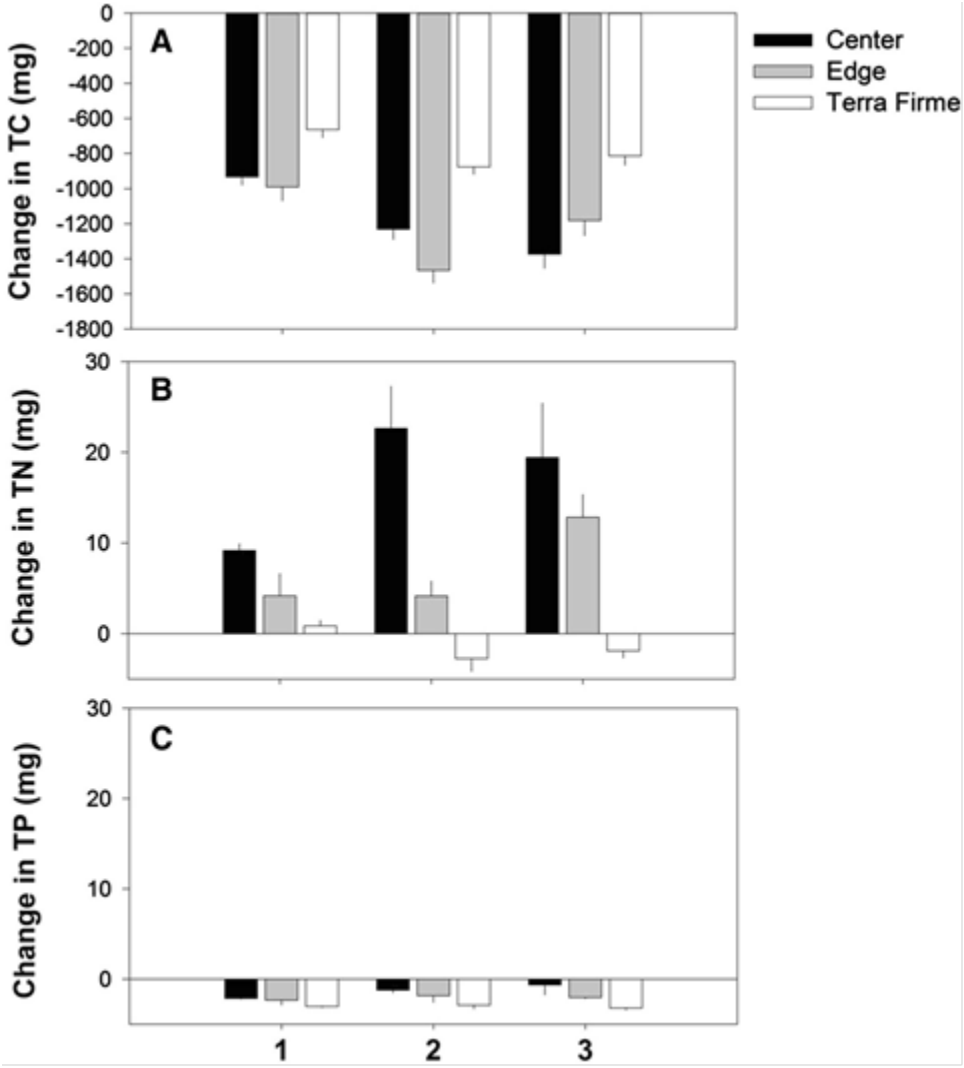


Figure 4. Average final nutrient content (mass%) and stoichiometric ratios of leaf litter collected from center, edge, and terra firme locations within each pool (1–3). Percent carbon (A), nitrogen (B), phosphorus (C), C:N (D), C:P (E), and N:P (F) of decomposed leaf-litter. Error bars represent ±1 standard error.



F igure 5. Average change in total carbon (A), total nitrogen (B), and total phosphorus (C) [final content - initial content] of decomposed leaf-litter in center, edge, and terra firme sites from each of the three study pools (1–3). Error bars represent ±1 standard error.



F igure 6. Average extracellular enzyme activity for per unit ash- free dry mass in center, edge, and terra firme sites (A) and across three sample dates (B) across all three study pools. We sampled α-$_D$-glucosidase (*α*gluc) and β-$_D$-glucosidase (*β*gluc) to measure C-acquiring activity, *β*-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAMP) for N-acquiring activity, and phosphatase (Phos) for P-acquiring activity. Error bars represent ±1 standard error. Note both figures are on log scales.

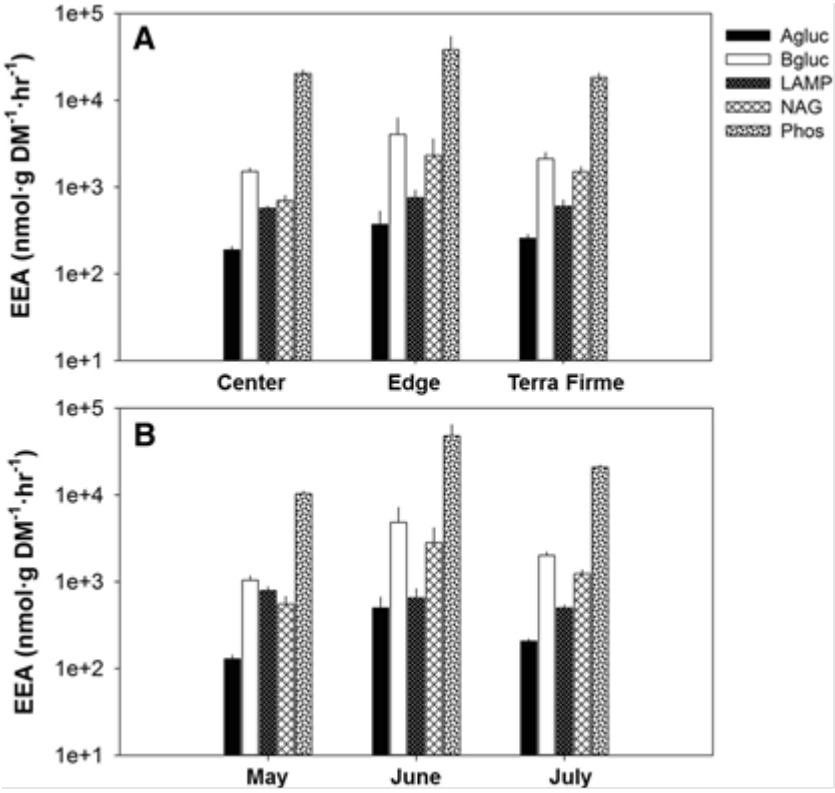


Figure 7. Average denitrification rates in the center (C), edge (E), and terra firme (TF) sites in both ambient and +CN treatments (A), within the C, E, and TF sites in three pools from both sample dates (+CN treatments only; B), and among the three pools during each sample date (+CN treatments only; C). Error bars represent ±1 standard error.