BIOGEOCHEMICAL HOTSPOTS IN FLUVIAL SYSTEMS: WOODY DEBRIS AND BEAVER PONDS

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BIOGEOCHEMICAL HOTSPOTS IN FLUVIAL SYSTEMS: WOODY DEBRIS AND BEAVER PONDS

BY

JULIA GRACE LAZAR

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL SCIENCE

UNIVERSITY OF RHODE ISLAND 2013
DOCTOR OF PHILOSOPHY

OF

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DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND
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ABSTRACT

Through a combination of in-stream incubations, incubation of soil cores, and mesocosm experiments, this dissertation examines denitrification of woody debris in stream settings, and denitrification, soil N transformations and GHG generation of beaver pond sediments.

In the first chapter we examined the effect of instream large wood on denitrification capacity in two contrasting, lower order streams – one that drains an agricultural watershed with no riparian forest and minimal stores of instream large wood and another that drains a forested watershed with an extensive riparian forest and abundant instream large wood. We incubated two types of wood substrates (fresh wood blocks and extant streambed wood) and an artificial stone substrate for nine weeks in each stream. After in situ incubation, we collected the substrates and their attached biofilms and established lab-based mesocosm assays with stream water amended with $^{15}$N-labeled nitrate-N. Wood substrates at the forested site had significantly higher denitrification than wood substrates from the agricultural site and artificial stone substrates from either site. Nitrate-N removal rates were markedly higher on woody substrates compared to artificial stones at both sites. We found nitrate-N removal rates were significantly correlated to biofilm biomass and denitrification capacity accounted for only a portion of nitrate-N removal observed within the mesocosms in both the wood controls and instream substrates. $N_2$ accounted for 99.7% of total denitrification. In terms of management, restoration practices that generate large wood in streams should be encouraged for N removal and do not appear to generate high risks of instream $N_2O$ generation.
In the second chapter we used $^{15}$N tracer additions in soil core mesocosm incubations with a mass-balance approach to address the fate of nitrate in beaver ponds and understand the capacity of beaver ponds to serve as long-term watershed N sinks. We evaluated and quantified different nitrate transformation pathways, including: denitrification, assimilation into soil microbial biomass and organic N, and net generation of ammonium N. Denitrification constituted between 52 and 86 percent of total N transformations under enriched levels of nitrate; approximately 3 to 5 fold higher than the rates ascribed to nitrate assimilation in soil organic N, which constituted the next highest mechanism of nitrate transformation. On average, 0.2% of denitrification is being released as N$_2$O under low nitrate-N concentrations in the three beaver ponds, while under N-enriched conditions, the average was 7%. Our data suggest that under enriched conditions beaver ponds have greater N$_2$O production than streams, but are similar to wetland soils. We estimate that beaver pond denitrification can remove approximately 50 to 450 kg nitrate-N km$^{-2}$ of catchment area, assuming 0.7 beaver ponds per km$^2$ of catchment area. Based on the beaver pond/watershed area ratios, and inter-pond variability in denitrification we estimate that beaver ponds in southern New England can remove 5-45% of watershed nitrate loading from rural watersheds with high N loading (i.e., 1000 kg km$^{-2}$). Thus, beaver ponds represent a proportionally significant sink for watershed N if current beaver populations persist.

In the third chapter we determined the diffusive flux of greenhouse gases (GHGs) — methane (CH$_4$), carbon dioxide (CO$_2$), and nitrous oxide (N$_2$O) — from the air-water interface of three beaver ponds in Rhode Island, USA. We launched five floating static gas chambers on each beaver pond during spring, summer, and fall
seasons, and sampled at 15-minute intervals over one hour. Emission rates were derived for each gas from the linear regression of the change in concentration of the gas over time. Fall had significantly higher CO$_2$ emission than other seasons, mean 9.298 g CO$_2$ m$^{-2}$ day$^{-1}$ versus 3.305g CO$_2$ m$^{-2}$ day$^{-1}$ in spring and 3.188g CO$_2$ m$^{-2}$ day$^{-1}$ in summer. CH$_4$ and N$_2$O emissions did not show seasonal differences: annual means were 174 mg CH$_4$m$^{-2}$ day$^{-1}$ and 1 mg N$_2$Om$^{-2}$ day$^{-1}$, respectively. When flux was expressed in CO$_2$ global warming equivalents, CH$_4$ emissions comprised the majority of the GHG emissions, at 67.5% across all sites and seasons. Significant correlation was found between CO$_2$ emission rates and pond water DOC, while CH$_4$ emissions were significantly correlated to air or water temperature. Our results show that beaver ponds generate high fluxes of CH$_4$ and CO$_2$ emissions per surface area of the pond. However, the relatively small areal footprint of beaver ponds at the watershed scale greatly diminishes their net effect. Thus, at a catchment scale we estimate that the global warming potential of the GHG emissions from the beaver ponds expressed as CO$_2$ equivalents range from 3-26 Mg km$^{-2}$ yr$^{-1}$. Assessment of the net effect of beaver ponds on the greenhouse gas budget of the Northeast U.S. must consider more than the GHG emissions from the ponded areas of the beaver ponds. Studies are warranted on the extent of changes in water tables, and associated changes in GHG emissions, in the lands surrounding the ponds and the fate of the organic soils in abandoned beaver ponds.
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I would like to thank Arthur Gold for being an outstanding advisor. His ability to quickly solve problems, stay enthusiastic, and continually make time for me is remarkable. Art has also created a web of supportive, intelligent, and hardworking people, without whom I would not have had such a positive graduate school experience. Within the Watershed Hydrology Lab, Kelly Addy has provided an immense amount of support, helping with: developing methods, analyzing data, and always being friendly and inviting when answering my questions. I appreciated the chance to engage with many remarkable undergraduate students, forcing me to be better in communicating details of my research and goals, and impressing me with their dedication and intelligence. My committee members, Peter Groffman, Candace Oviatt, Mark Stolt, and Cathy Wigand: for all of their time and energy spent teaching me, reading my drafts, offering suggestions, and improving the research and the final output of these papers. Coastal Institute IGERT Project was a major factor in my decision to attend URI, and made my first two years filled with field trips and the chance to interact more closely with phenomenal faculty members and students, making this campus feel like a close-knit community. My much-loved husband, Louis, whose willingness to always lend an ear, and learn to intricate details of my research over dinner, helped to keep me grounded and to stay positive. My wonderful immediate and extended family, friends, lab mates, and anyone that has showed an interest in this work—thank you for your support and care.
PREFACE

This dissertation is written in manuscript format with three chapters corresponding to the format of the journal articles.

Listed below are the questions (Q) this study will address and specific hypotheses (H).

**Q1:** What is the effect of fresh inputs of woody debris on denitrification in forested and deforested stream channels?

**H1:** Fresh wood will be a source of labile carbon, which promotes elevated rates of denitrification in a range of stream settings.

**Q2:** What is the fate of nitrate in beaver ponds and what is their capacity to serve as long-term watershed N sinks?

**H2:** Seasonality will play a role in how NO$_3^-$ is being transformed. An increase in labile C in the fall, may lead to increased rates of denitrification and uptake of N by microbial biomass. As long as an individual beaver pond remains intact, I predict it will serve as a watershed N sink due to sedimentation and denitrification. Larger ponds, which can trap more sediment, will have higher rates of N retention and transformation.

**Q3:** Are the range and magnitude of beaver pond emission of GHGs, such as N$_2$O, CO$_2$, and CH$_4$ affected by seasonal conditions and/or pond attributes?

**H3:** The magnitude of emissions from different GHGs will display different patterns of seasonal variability. N$_2$O rates will be highest when denitrification rates are highest, which I hypothesize to be in the fall, in response to fresh inputs of labile C
from within-pond vegetation senescing as well as upstream leaf fall. CO$_2$ and CH$_4$ emission rates will be highest in summer when temperatures are most elevated and when the rate of CH$_4$ transport from plant roots is most pronounced. Increased retention time and depth of organic matter within pond will lead to increases in GHG fluxes.

The first manuscript addresses hypothesis 1, the second manuscript addresses hypothesis 2, and the third manuscript addresses hypothesis 3.

The first manuscript has been accepted for publication by the *Journal of American Water Resources* (JAWRA), featured collection on riparian ecosystems.

The second manuscript will be submitted to the *Journal of Environmental Quality* (JEQ) after presenting the research at the Soil Science Society of America's annual meeting from November 3-6, 2013 in Tampa, Florida.

The third manuscript will be submitted to the *Journal of Global Biogeochemical Cycles*.
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CHAPTER 1

Instream Large Wood: Denitrification Hotspots with Low N\textsubscript{2}O Production

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*This has been accepted by the Journal of American Water Resources
We examined the effect of instream large wood on denitrification capacity in two contrasting, lower order streams – one that drains an agricultural watershed with no riparian forest and minimal stores of instream large wood and another that drains a forested watershed with an extensive riparian forest and abundant instream large wood. We incubated two types of wood substrates (fresh wood blocks and extant streambed wood) and an artificial stone substrate for nine weeks in each stream. After in situ incubation, we collected the substrates and their attached biofilms and established lab-based mesocosm assays with stream water amended with $^{15}$N-labeled nitrate-N. Wood substrates at the forested site had significantly higher denitrification than wood substrates from the agricultural site and artificial stone substrates from either site. Nitrate-N removal rates were markedly higher on woody substrates compared to artificial stones at both sites. Nitrate-N removal rates were significantly correlated to biofilm biomass and denitrification capacity accounted for only a portion of nitrate-N removal observed within the mesocosms in both the wood controls and instream substrates. $N_2$ accounted for 99.7% of total denitrification. Restoration practices that generate large wood in streams should be encouraged for N removal and do not appear to generate high risks of instream $N_2O$ generation.

**Key Terms:** riparian ecology, aquatic ecology, biogeochemistry, rivers/streams, nutrients, nitrous oxide, non-point source pollution, algae, biofilm
INTRODUCTION

Restoring riparian forests to reduce waterborne nitrogen (N) pollution has been an objective of many watershed management efforts (Schultz et al., 2004; Hassett et al., 2005; Mitsch et al., 2007). Riparian forests can reduce groundwater N loading to streams through denitrification, plant uptake, and microbial immobilization (Gold et al., 2001; Mayer et al., 2007; Vidon et al., 2010). Riparian forests also support functions that enhance the ecosystems of lower order streams by modulating stream temperature through shading, increasing stream width, and habitat complexity through geomorphic effects on stream banks (Sweeney et al., 2004), increasing species richness, and affecting biogeochemical functions through additions of large wood and organic carbon (C) (Welsh, 1991; Naiman and Decamps, 1997; Bilby, 2003).

Increases in anthropogenic N inputs have led to increased N in riverine systems (Howarth et al., 1996; Galloway et al., 2004), accelerating rates of eutrophication in coastal areas (Turner and Rabalais, 1994). Much effort has been made to understand and manage N loads within aquatic systems in order to improve water quality and other ecosystem services (Galloway et al., 2003). Evidence has pointed to relationships between riparian forests and increased soil denitrification, an anaerobic microbial process that permanently removes nitrate from fluvial systems by returning N to the atmosphere (Alexander et al., 2000; Gold et al., 2001; Mulholland et al., 2008).

Higher fluvial denitrification rates have been found to be associated with increases in the organic content of benthic sediments, respiration rates, and opportunities for contact with the stream bed (e.g., shallow, wide streams, and streams
with extensive hyporheic flow) (Hinkle et al., 2001; Mulholland et al., 2008; Hall et al., 2009; Mulholland et al., 2009). These conditions can be fostered by large wood (e.g., sticks, branches or tree trunks) and debris dams in streams that are derived from a riparian forest. Large wood in streams from a riparian forest can be a direct source of labile C to streams to fuel microbial processes. Large wood also adds structure to the stream channel and creates obstacles that slow the flow of water and extend the residence time of surface water in the stream and facilitate accumulation of finer organic sediments that support biofilms. Instream large wood has the potential to function as microsites or "hotspots" of elevated biogeochemical cycling including denitrification (McClain et al., 2003; Groffman et al., 2005; Groffman et al., 2009). Downstream declines in nutrient concentrations have also been attributed to biofilms (Sabater et al., 1991; Ryhiner et al., 1994 and Mulholland et al., 1995). Biofilm structure, composition, and capacity for biogeochemical cycling is influenced by substrate composition, light penetration, nutrient concentration, flow rates, seasonality, sediment composition, and the community of grazers in the vicinity (Sabater et al., 1988; Rott et al., 1998; Sabater et al., 2002). Biofilms formed on wood substrates have been found to have higher respiration rates and greater N demand than biofilms developed on rock substrates (Sabater et al., 1998).

Here we examine the effect of instream large wood on denitrification capacity in two contrasting, lower order streams – one that drains an agricultural watershed with no riparian forest and minimal stores of instream large wood and another that drains a forested watershed with an extensive riparian forest and abundant instream large wood. The agricultural stream was scheduled for extensive riparian restoration
that is expected to increase the extent of large wood in the channel. We incubated two types of wood substrates (fresh wood blocks and extant streambed wood) and an artificial stone substrate for nine weeks in each stream: After in situ incubation, we collected the substrates and their attached biofilms and established lab-based mesocosm assays with stream water amended with $^{15}$N-labeled nitrate-N. We hypothesized that mesocosms containing wood substrates would have higher denitrification capacity rates than other mesocosms as we expected the labile C to promote conditions that would enhance denitrification. While the agricultural stream had less instream large wood, we hypothesized that the lack of shade and elevated nutrients associated with the agricultural stream would yield higher rates of nitrate removal on substrates in response to increased autotrophic communities that can form under those conditions. This research aims to further our understanding of the effects of riparian forests on fluvial denitrification.

**METHODS**

We used a mesocosm approach to examine denitrification and nitrate-N removal rates of substrates and associated biofilm that were placed within a specific reach of each stream (i.e., the study sites) for nine weeks as well as from bare substrates without biofilm development which were never subjected to field conditions as controls.

**Study Sites**

During the summer of 2009, we used two streams that differed markedly in nitrate concentration, watershed land use and riparian cover: Big Spring Run, located in Lancaster County, Pennsylvania, and Mawney Brook, located in Kent County, Rhode Island (Table 1). The Big Spring Run and Mawney Brook watersheds that drain to the study sites are 4.3 and 4.8 km$^2$, respectively. Based on NLCD geospatial data
(National Land Cover Dataset, 2006, accessed November 12, 2011; http://streamstats09.cr.usgs.gov/), land use in the Big Spring Run watershed is 41% agricultural, 4% forest, and 55% developed (Table 1). Agricultural land cover borders the Big Spring Run riparian zone adjacent to the study reach. Land use in the Mawney Brook watershed is 62% forested, 11% wetlands and 27% developed (Table 1). A mature riparian forest borders the entire length of Mawney Brook along the study reach and upstream from the study site. Hereafter, Big Spring Run is referred to as the “agricultural” site and Mawney Brook is referred to as the “forested” site. USGS gage stations provided all flow data: gage 015765195 and 01116905 for the agricultural and forested sites, respectively.

We computed sinuosity of the 300 m reach upstream of the study sites from 1:24,000 USGS topography maps (Cushing and Allan, 2001; U.S. Geological Survey, Streamstats, accessed November 12, 2011, http://streamstats09.cr.usgs.gov/) as 1.39 and 1.27 for the agricultural and forested sites, respectively (Table 1). Acidic stratified drift deposits and limestone bedrock dominated soils at the forested site and the agricultural site, respectively; these surficial geology differences are reflected in the hardness levels of the two streams (Table 1). Ambient nitrate concentrations in the agricultural stream were more than tenfold higher than in the forested stream (Table 1).

In situ incubation and harvesting of substrates

Treatment substrates of similar size and shape consisted of (1) wood blocks (26 cm x 4.5 cm x 2.2 cm) made from red maple (Acer rubrum); (2) artificial stone made from unglazed clay-fired blocks (25 cm x 5 cm x 1.25 cm); and (3) bundles of sticks (~5 cm
x 25 cm bundle composed of ~1.5 cm diameter sticks) collected within 25 to 75 m of each stream site. Since extant wood at the forested site was widely available, we bundled sticks from within 25 m of the site whereas at the agricultural site, we compiled sticks from approximately 75 m from the site due to less wood being available. In both streams, we placed substrates within a 25 m reach of the stream in early summer. We monitored nitrate concentrations for a year at the forested stream and used flow and nitrate data collected by the EPA for the agricultural stream. At each site, we anchored 16 wood blocks, 10 artificial stone substrates, and 10 extant wood bundles to individual bricks via plastic zip ties. The anchors also kept the substrates submerged in water. After nine weeks, we collected the substrates, their associated biofilms and bricks in 35 cm x 25 cm x 12.8 cm clear plastic bins underwater to minimize exposure to air, taking care to avoid disturbance of biofilms associated with the substrate structure. Nevertheless, slight turbidity in both the stream reach and mesocosm bin was inevitable. These plastic bins containing the extracted substrates are hereafter referred to as “mesocosms.” We added ambient stream water to the mesocosms until they were full and sealed them with dark lids to limit photosynthesis and to minimize exposure of the blocks to air during the 30 minute transportation to the lab. We sampled the ambient stream water, transported samples on ice and stored samples at 4° C until analysis.

Upon arrival at the lab, we removed lids, and sampled a 9 cm² area (< 3.0% of the total surface area of the artificial substrates) of one corner of each wood block and artificial stone. We placed the harvested biofilm into 450 ml of deionized water and stored it at 4° C for further analyses. We did not collect biofilm from the extant wood
bundles due to difficulties in quickly establishing a firm estimate of substrate area before beginning the sealed mesocosm experiment.

Mesocosm experiments

Each mesocosm contained one substrate, attached biofilm, brick, and lid fitted with a #37 Suba-Seal™ rubber septa placed in a drilled sampling port in the center of the lid. We used a $^{15}$N tracer technique to estimate denitrification (Nishio et al., 1983; Jenkins and Kemp, 1984). We amended the forested mesocosms with KNO$_3^-$ and $^{15}$N-KNO$_3^-$ to 20 atom% for a final concentration of 8 mg N l$^{-1}$ of NO$_3^-$.

Average summer nitrate concentrations at the agricultural site (Table 1) were much higher, 9.69 mg l$^{-1}$, and therefore, only 99% $^{15}$N-KNO$_3^-$ was added, for a final concentration of 11 mg N l$^{-1}$ of NO$_3^-$ at 20 atom%. These concentrations ensured NO$_3^-$ was available in excess throughout the incubation. The agricultural site had higher final N concentration than the forested site due to elevated background concentrations in ambient stream water. The computed rates represent denitrification capacity where nitrate is abundant and other factors, such as electron donors or redox conditions control the observed rates (Addy et al., 2005).

Prior to sealing, we collected well-mixed water samples for analysis of initial conditions and stored them at 4° C until analysis. A headspace of 1.5 cm remained between the top of the water and the lid (total headspace volume per mesocosm ~1800 ml) to facilitate gas sampling. We steadily bubbled helium (He) gas into half of the wood block mesocosms with sparge stones until dissolved oxygen (DO) concentrations were below 2 mg l$^{-1}$ to optimize conditions for denitrification.

Dissolved oxygen levels in mesocosms without He added averaged 8.5 mg/l. We
recorded dissolved oxygen and temperature (C°) before securing the dark mesocosm lids. We secured lids onto the bins and sealed with silicone to prevent air from leaving or entering the mesocosms. In mesocosms where He was bubbled into the water before sealing, we added additional He via needle through the sampling port for 5 minutes to replace headspace gases with He. During this addition, a second needle placed in sampling port vented excess gas.

After 1 min of shaking to equilibrate headspace, we extracted 20 ml of initial headspace samples via syringe and placed these samples into 12 ml pre-evacuated Exetainter™ vials (Labco 839W). We repeated 20 ml headspace samples at 1.5, 3, and 18 hrs. In order to prevent negative pressure, we added 20 ml of He back into each mesocosm via the septa after samples were taken. After the last gas sample was taken, we removed lids, measured DO, and collected a final water sample which we stored at 4° C until analysis.

“Blank” mesocosms consisted of ambient stream water from each site, with 20 atom% $^{15}$NO$_3^-$ as KNO$_3^-$ added to reach a desired concentration of 8 mg and 11 mg N/l of NO$_3^-$, for the forested site and the agricultural site, respectively. Control mesocosms consisted of wood blocks or artificial stone substrates, attached to individual bricks, which were kept dry in the lab while the other blocks were submerged in the stream. Biofilms did not develop on these controls. Table 2 provides definitions of each of the mesocosm incubation types for easy reference. On the day when the mesocosms were established, we placed control blocks and attached bricks into in mesocosms with fresh stream water. We treated and sampled blanks and controls as described above for the instream substrates.
Analyses

We filtered the biofilm samples removed from each wood and artificial stone block onto Whatman 42 ashless 90 mm pre-weighed filters, dried the filters and then re-weighed the filters to quantify biomass. The University of California Davis Stable Isotope Facility analyzed these filters for natural abundance of δ\(^{13}\)C and δ\(^{15}\)N isotopes and bulk C and N composition using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

The University of California Davis Stable Isotope Facility analyzed the mesocosm headspace samples for concentrations and isotope ratios of N\(_2\) and N\(_2\)O using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany).

We analyzed water samples from the beginning and end of mesocosm incubations using the open tubular cadmium reduction method (APHA et al., 1995) on an Astoria Pacific Model 303A Segmented Continuous Flow Autoanalyzer (Astoria-Pacific Inc., Clackamas, OR). Samples from Mawney Brook were analyzed for alkalinity using a Hanna Instruments 902 Color Automatic Potentiometric Titrator (Woonsocket, RI). Samples from Big Spring Run were analyzed for alkalinity by manual titration (APHA et al., 1999). A LaMotte Total Calcium & Magnesium Hardness test kit (Code 4824 DR-LT) was used to determine hardness at Mawney Brook, while hardness samples from Big Spring Run were analyzed using the hardness by calculation method after mineral analysis was performed (APHA et al., 1999).
Dissolved oxygen and temperature were measured using a YSI DO-temperature meter, model 55 (YSI, Yellow Springs, Ohio). We measured the length and width of extant large wood after the mesocosm experiment was completed in order to calculate surface area.

**Data Analyses**

We used a $^{15}$N tracer technique to estimate denitrification capacity (Nishio et al., 1983; Jenkins and Kemp, 1984). Denitrification masses of N$_2$O and N$_2$ gases (µmol) in headspace samples were extrapolated to the whole mesocosm scale using Bunsen coefficients from Tiedje (1982) and equation constants from Mosier and Klemedtsson (1994) following the formulas used in Kellogg et al. (2005). The total masses of N$_2$O-N and N$_2$ generated during the incubation period were calculated by dividing the masses of $^{15}$N$_2$O-N and $^{15}$N$_2$ by the dosed NO$_3$$^-$$$-N atom%. The mass of $^{15}$N$_2$O-N and $^{15}$N$_2$ generated was divided by the number of hours that have passed since the last sample time. Samples were taken at time 0, 1.5, 3, and 18. The average denitrification rate from those three time periods is recorded. Gas production rates (N$_2$O-N and N$_2$) were expressed as µg N m$^{-2}$ of substrate hr$^{-1}$.

We use the term nitrate-N removal to reflect reduction in total nitrate per unit time within each mesocosm, calculated by subtracting the post-incubation nitrate concentration from the pre-incubation nitrate concentration. Because the mesocosms were sealed throughout the incubation period, we did not obtain estimates of uptake kinetics. The data from “blank” mesocosms estimated denitrification and nitrate-N removal in the stream water itself. We subtracted the rates of the blanks in all substrate
rates given in the results to highlight the rates associated with the addition of substrates.

Statistical Analyses

Unless otherwise noted, instream mesocosm results are based on the following n values for the forested site: 13 wood blocks, 8 extant wood bundles, and 5 artificial stone blocks. For the agricultural site there were 9 wood blocks, 4 extant wood bundles, and 5 artificial stone blocks. The numbers differ by site because some substrates were lost, presumably during high flows. We also employed the same mesocosm setup to evaluate denitrification capacity and nitrate-N removal on 5 forested control wood blocks, 4 agricultural control wood blocks, 4 control artificial stone blocks, and 5 blanks (mesocosms without substrates).

We tested for differences in biofilm dry mass, denitrification and nitrate-N removal rates, and biomass N and C between substrates and sites. Aside from biomass C, data were normally distributed. We pooled data within a site if they were not significantly different. For biofilm dry mass, denitrification rates, and nitrate-N removal rates from the agricultural site, we used Student’s t-tests to test for differences between substrates within each site. Nitrate-N removal rates from the two woody substrates at the forested site could not be pooled and analysis of variance (ANOVA) was used to test for differences between the three substrates. For pairwise comparisons of denitrification and nitrate-N removal rates between substrates and across sites we used ANOVA with a Tukey’s post hoc test. Biomass C data for the forested site and all biomass δ^{13}C and δ^{15}N data were not normal so we used the Kruskal –Wallis test to determine significant differences between sites and substrates.
To test for differences between substrates and sites of the percent of nitrate-N removal that can be ascribed to denitrification we used ANOVA with a Tukey’s post-hoc test. We evaluated correlation between denitrification rates, nitrate-N removal rates, and biofilm mass using Pearson product-moment correlation coefficients, after log transforming the data. Stream sites were treated separately and only pooled for correlation statistics. Statistical significance was set at $\alpha < 0.05$ for all analyses. All statistical analyses were performed with Analyse-it version 2.26.

**RESULTS**

*Ambient Water Quality*

The agricultural site had an average summer nitrate concentration of 9.69 mg l$^{-1}$, several orders of magnitude greater than concentrations in the forested site, with mean summer nitrate of 0.05 mg l$^{-1}$ (Table 1). Average stream DO concentration during that same time period was 9.0 and 8.0 mg l$^{-1}$ for agricultural and forested sites, respectively. Median flow from June-Oct was 0.013 (n=4) and 0.015 (n=131) m$^3$ sec$^{-1}$ km$^{-2}$ for the agricultural and the forested sites respectively. The agricultural site had an average summer temperature of 16.7°C. Stream temperature data were not obtained at the forested site, but summer stream temperatures at the gaging station at the Beaver River, a neighboring (within 10 km) forested watershed with similar physiography had an average summer (June-August) temperature of 17.5°C.

*Quality and Quantity of Biofilm*

Artificial stone substrates at the forested site had significantly less ($p \leq 0.05$) biofilm mass than wood blocks (Table 3a), while the biofilm masses were not significantly different between the artificial stone and wood block substrates at the agricultural site (Table 3b). Biomass was not measured on extant wood at either site.
Within each site, the appearance of the biofilms was similar across the artificial stone and wood block substrates; however, between sites, the biofilms were visibly different. Green biofilms developed on substrates at the agricultural site, which was without shade, while the substrates at the forested site developed darker biofilms (Table 3). Biofilm C and N did not differ between wood and artificial stone substrates within each site, so the data were pooled for statistical comparison. The mean C:N ratio of the biofilms at the forested site was 16.2 (SD: 5.9) and significantly greater (p ≤ 0.05) than at the agricultural site (mean:8.0; SD: 1.6). The mean biomass N cm\(^{-2}\) was not significantly different between the two sites, but the mean biomass C cm\(^{-2}\) at the forested site was significantly higher (p ≤0.05) than the agricultural site, 531 µg C cm\(^{-2}\) (SD: 410) and 213 µg C cm\(^{-2}\) (SD: 84), respectively.

Biofilms at the agricultural site had significantly more enriched δ\(^{13}\)C values (p ≤0.05) than at the forested site. There were no significant differences in biofilm δ\(^{15}\)N between sites or substrates.

*Mesocosm Denitrification Capacity*

Control wood block substrates generated significantly (p ≤0.05) higher denitrification rates than the control artificial stone substrates (Figure 1). No significant differences in denitrification rates were found between sites for each type of control and blanks, and results were pooled for statistical tests.

Instream artificial stones at the agricultural site had significantly higher denitrification rates than the control artificial stones that were not incubated in the stream. In contrast, at the forested site, the denitrification capacity rates of instream
artificial stones were much lower and did not significantly differ from the control artificial stones.

Within each site, denitrification rates from instream extant wood bundles were not significantly different from instream wood block substrates. The denitrification rates from both wood sources were therefore combined within each site for further statistical comparisons; hereafter referred to as “instream wood substrates.” The instream wood substrates at the forested site had significantly higher denitrification rates (p ≤0.05) than those at the agricultural site (Figure 2). At the forested site, mesocosms of instream wood substrates had significantly higher denitrification rates than instream artificial stones (Figure 2). However, at the agricultural site, denitrification rates from instream wood and artificial stones were not significantly different (p > 0.05), Figure 2).

Wood blocks subject to hypoxic and oxic mesocosms were not significantly different. Hypoxic mesocosms generally remained below 2.2 mg l\(^{-1}\) of DO for the mesocosm assays. Oxic mesocosms which started with DO over 7.0 mg l\(^{-1}\), ended below 3 mg l\(^{-1}\). Although it is expected that the oxygen levels decreased overtime, the \(\text{N}_2\) and \(\text{N}_2\text{O}\) production rates did not significantly differ between sampling times.

Low levels of nitrous oxide were generated through denitrification. Rates of \(\text{N}_2\text{O}-\text{N}\) were consistently <0.02 ug N m\(^{-2}\) hr\(^{-1}\). \(\text{N}_2:\text{N}_2\text{O}\) ratios were >99.7 in all measurements.

**Nitrate-N Removal**

Blanks displayed no evidence of nitrate-N removal (limit of detection on instrument is 0.02 mg N l\(^{-1}\)). Nitrate-N removal trends at the agricultural site followed
the denitrification results; extant wood and wood blocks had similar rates and were significantly higher (p ≤0.05) in nitrate-N removal than instream artificial stones (Figure 3). Ending nitrate-N concentration for the wood blocks and extant wood at the agricultural site was 10.80 mg l⁻¹, a reduction of 1.40 mg l⁻¹. At the forested site, wood blocks had significantly higher (p ≤0.05) nitrate-N removal than instream extant wood. The wood blocks at the forested site had an average ending nitrate concentration of 8.28 mg l⁻¹, a reduction of 1.75 mg l⁻¹. Combined extant wood and wood blocks at the agricultural site had significantly (p ≤0.05) higher nitrate-N removal rates than extant wood from the forested site. Wood blocks from the forested site had significantly (p ≤0.05) higher nitrate-N removal than instream artificial stones (p ≤0.05), which had no nitrate-N removal. Denitrification rates of wood blocks with biofilm were significantly correlated to nitrate-N removal rates (r = 0.57, p≤0.01). Nitrate-N removal rates were also significantly correlated to biofilm mass (r=0.69, p≤0.01); however, no significant correlation was found between biofilm mass and denitrification rates.

**DISCUSSION**

Wood substrates were found to promote denitrification and nitrate removal in starkly contrasting sites with different levels of riparian forest cover, ambient nutrient enrichment, alkalinity and hardness (Figure 2). This evidence follows other studies, which have shown that organic substrates such as riparian forests, organic debris dams, and carbon bioreactors can be hotspots of denitrification (Reisinger et al., 2013; Schipper et al., 2010; Hall et al., 2009; Groffman et al., 2005). Most of the mesocosms with woody substrates displayed high N transformation rates. This study supports the
importance of instream large wood for promoting conditions that stimulate N cycling within streams.

The significantly higher denitrification generated by the control wood block mesocosms (not subjected to instream incubation) compared to the artificial stone substrate controls was expected as wood substrates have been found to generate labile C, promote denitrification, and are used in denitrifying carbon bioreactors – where a carbon substrate is added to the flow path of nitrate enriched water to stimulate denitrification in groundwater and agricultural runoff (Bernhardt and Likens, 2002; Robertson, 2010; Schipper et al., 2010).

Instream wood blocks and extant large wood substrates generated comparable denitrification at both sites implying that the wood blocks created for this mesocosm experiment are comparable to the wood that is already found at these two stream sites. The significantly higher denitrification rates of the instream wood substrates than the wood block controls, suggest the importance of biofilm development for instream cycling of N. Although no significant correlation was found between biofilm mass and denitrification rates of instream substrates, there was a significant correlation between biomass and nitrate-N removal capacity. The lowest biomass was found on the forest artificial stone, which corresponded with lower nitrate-N removal rates. The forest wood blocks had significantly higher biofilm mass than the forest artificial stones, corresponding with the highest nitrate-N removal rates. In contrast, the biofilm masses at the agricultural site were quite similar between wood and stones, potentially obscuring substrate differences in denitrification rates. The biofilm at the agricultural site without a riparian forest received more sunlight and may thus have been more
productive and had higher N turnover rates due to intense sunlight and nutrient availability.

Although we did not identify the type and extent of algal vs. bacterial biomass, we note that the appearance and color of the biofilms contrasted sharply between sites and that the composition of biofilms has been found to alter N cycling (Romani & Sabater, 2000). Measures of $\delta^{13}$C and C:N ratios also show that the biofilm compositions differed by site. Published C:N ratios for epilithon match the C:N ratios found at the forested site and published ratios for filamentous green algae coincide with biofilm results at the agricultural site (Kemp and Dodds, 2002).

The high denitrification capacity of the forested wood blocks compared to the agricultural wood blocks is noteworthy given the high nitrate concentrations in the agricultural stream. Peterson et al. (2011) compared biofilm growth in two streams that differed in nitrate concentrations by an order of magnitude and suggest that in un-enriched nitrate conditions algae influence the denitrifying community due to their dependence on dissolved organics, while in enriched conditions this relationship is disconnected. A clear separation between the two biofilm communities was noted, and the low nitrate stream had increased species diversity, which they suggest leads to increased denitrification rates (Peterson et al., 2011). Similar to our study, Peterson et al., 2011 found no difference in biofilm mass between the enriched and un-enriched biofilm communities. The biofilm at the forested site may have had a more robust denitrifying community leading to higher denitrification rates. Another possibility is that oxygen generated by photosynthesizing algae at the agricultural site could create conditions that limited the extent of denitrifiers in the biofilm.
The high nitrate levels in the agricultural stream are reflected in higher biofilm N content compared to the forested biofilm. The low levels of biomass carbon in the agricultural biofilm may be due to macroinvertebrate grazing (Hillebrand and Kahlert, 2001) or to enhanced rates of microbial degradation. In contrast to our results, Romani et al. (2004) found that C:N molar ratios of biofilms at enriched and non-enriched stream sites were not different. Biofilm δ^{13}C values seen at both study sites fall in the normal range for C3 plants, which ranges from -32 to -22 (Rounick and Winterbourn, 1986). In sites with greater periphyton productivity and less canopy cover δ^{13}C tends to be enriched relative to those with more canopy (Ishikawa et al. 2012). Our agricultural site is exposed to more sunlight and likely supports greater algal standing stock than the shaded forest site and the enriched biofilm δ^{13}C observed in our study. This agrees with a phenomenon observed in Canada where periphyton grown in high light conditions had more enriched δ^{13}C values than in low light (MacLeod and Barton 1998) and in New Zealand where algae in unshaded pasture streams (especially filamentous green algae), were more enriched than algae (diatoms) in shaded forest streams (Hicks 1997).

Although significantly correlated, denitrification capacity accounted for only a portion of nitrate-N removal observed within the mesocosms in both the wood controls and instream substrates. Assimilation, both autotrophic and heterotrophic, generally accounts for a higher proportion of N removal than denitrification (Peterson et al., 2001; Mulholland et al., 2008).

The oxic and hypoxic mesocosms did not have significantly different denitrification rates. However, both oxic and hypoxic mesocosms were hypoxic at the
end of the 18 hr incubation. Mesocosms were covered with dark lids in an effort to limit photosynthesis, thereby decreasing oxygen production. One drawback of this mesocosm technique is that by creating a dark environment we may have increased net respiration rates, decreasing oxygen, and increasing denitrification rates. Microbial respiration was likely responsible for decreasing O$_2$ concentrations.

N$_2$ gas accounted for 99.7% of the total denitrification indicating complete denitrification. Therefore, wood in these two stream ecosystems are not a substantial source for N$_2$O generation. Similarly, in a large study comparing denitrification rates of 49 streams across varying landscapes median N$_2$ production rate was 99.4% of the sum of N$_2$ and N$_2$O (Mulholland et al., 2009), and Mosier et al. (1998) suggested comparable results.

Controlling nitrogen loads from watersheds is a huge problem that will likely require multiple activities, including management of both sources and sinks (Kellogg et al., 2010; Groffman et al., 2011). Planting woody species in riparian buffers next to agricultural lands can be an important component of nitrogen management. Riparian forests have been shown to increase hydrological connectivity, increasing denitrification in groundwater before it enters the stream (Gold et al., 2001). This study further emphasizes the value of restoring mature riparian forests for N management since wood substrates, regardless of the extent of biofilm development, tend to generate higher denitrification than stone substrates.

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TABLE 1. Land use, sinuosity, soil parent material, and ambient stream characteristics for the study sites.

| Stream Name     | Agricultural      | Forested         |
|-----------------|-------------------|------------------|
| Big Spring Run  | 41°59'35.75"N, 76°15'41.73"W | 41°38'37.93"N, 71°31'16.73"W |
| Location        | Lancaster, PA     | East Greenwich, RI |
| % Wetland       | 0                 | 10.6             |
| % Agriculture   | 41                | 0.2              |
| % Forest        | 4.1               | 62.0             |
| % Developed     | 54.7              | 27.1             |
| Sinuosity       | 1.39              | 1.27             |
| Dominant Soil Parent Material | carbonate limestone | acidic stratified drift |
| Average Summer $\text{NO}_3$-N (mg l$^{-1}$) | 9.69 | 0.05 |
| Channel Depth (m) | 1.7 | 0.9 |
| pH              | 7.56              | 6.23             |
| Hardness*       | 343               | 13               |
| Average Alkalinity (ppm) | 223.84 | 6.89 |
| Median Flow June-Oct ($\text{m}^3 \text{sec}^{-1} \text{km}^{-2}$) | 0.013 | 0.015 |

*single data point

Flow rates of Mawney Brook were estimated from the USGS gage (01116905) located at Fry Brook that was down gradient of the study site. Flow rates were adjusted based on the ratio of the watershed area of the study reach to the watershed area of USGS gage.

TABLE 2. Mesocosm terminology defined.

| Mesocosm Terminology | Definition |
|----------------------|------------|
| Blank                | streamwater only, no substrates |
| Control Wood         | streamwater + wood block that has not been incubated in stream |
| Control Stone        | streamwater + artificial stone that has not been incubated in stream |
| Extant Wood          | streamwater + bundle of sticks found in the stream site attached to bricks and incubated in stream |
| Wood Blocks          | streamwater + fresh Red Maple wood blocks attached to bricks and incubated in stream |
| Artificial Stones    | streamwater + clay-fired blocks attached to bricks and incubated in stream |
TABLE 3. Biofilm biomass and characteristics on substrates at the forested (a) and agricultural (b) study reaches. At the forested site wood blocks had significantly higher biofilm masses than artificial stones blocks, whereas the agricultural site had similar biofilm masses on both substrates. Biofilm was only measured on wood blocks and artificial stones, not on extant large wood. Significant differences within a site are noted by superscripts, p ≤0.05 using a Student’s t-test.

| Site | Substrate   | Mean Biofilm Mass (g) | Standard Deviation | n value | Color  | Description |
|------|-------------|-----------------------|--------------------|---------|--------|-------------|
| a Forest | wood block | 0.530<sup>a</sup> | 0.32               | 8       | dark brown | matted     |
|       | artificial stone | 0.068<sup>b</sup> | 0.05               | 5       | dark brown | matted     |
| b Agric | wood block | 0.304                 | 0.20               | 8       | bright green | filamentous |
|       | artificial stone | 0.132                 | 0.17               | 5       | bright green | filamentous |

*Note: The n-values for the wood block biofilm mass do not match the n-values for denitrification rates. This is because 6 blocks from the site were inadvertently not sampled for biofilm mass.
FIGURE 1. Denitrification capacity of wood block and artificial stone substrates without biofilms (controls) pooled across sites. Different letters above bars indicate significant differences, $p \leq 0.05$ using a Student’s $t$-test.
FIGURE 2. Denitrification rates of in-stream wood and artificial stone substrates in the two study sites. Treatments with different letters above bars are significantly different at p ≤ 0.05 using an ANOVA, Tukey’s post hoc comparison test.
FIGURE 3. Net rate of nitrate-N removal (represented by the value equivalent to the total height of each vertical bar) and the denitrification rate for each mesocosm type.
CHAPTER 2

Beaver Ponds: A resurgent nitrogen sink for rural watersheds in the Northeast U.S.A.

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ABSTRACT

We used $^{15}$N tracer additions in soil core mesocosm incubations with a mass-balance approach to address the fate of nitrate in beaver ponds and understand the capacity of beaver ponds to serve as long-term watershed N sinks. We evaluated and quantified different nitrate transformation pathways: denitrification, assimilation into soil microbial biomass and organic N, and net generation of ammonium N. Denitrification constituted between 52 and 86 percent of total N transformations under enriched levels of nitrate; approximately 3 to 5 fold higher than the rates ascribed to nitrate assimilation in soil organic N, which constituted the next highest mechanism of nitrate transformation. On average, 0.2% of the nitrogen gases from denitrification was released as N$_2$O under low nitrate-N concentrations in the three beaver ponds, while under N-enriched conditions, the average was 7%. Our data suggest that under enriched conditions beaver ponds have greater N$_2$O production than streams, but are similar to wetland soils. Assuming a density of 0.7 beaver ponds per km$^2$ of catchment area we estimate that beaver pond denitrification can remove approximately 50 to 450 kg nitrate-N km$^{-2}$ of catchment area. We estimate that beaver ponds in southern New England can remove 5-45% of watershed nitrate loading from rural watersheds with high N loading (i.e., 1000 kg km$^{-2}$). Thus, beaver ponds represent a proportionally significant sink for watershed N if current beaver populations persist.

INTRODUCTION

Anthropogenic nitrogen (N) inputs into watersheds have increased N in riverine systems (Howarth et al., 1996; Galloway et al., 2004) thereby accelerating rates of eutrophication in coastal waters (Turner and Rabalais, 1994). Much effort has
been made to understand and manage N loads to these aquatic systems in order to improve water quality and reduce habitat degradation (Galloway et al., 2003). These efforts involve a wide range of approaches including controlling and reducing N sources such as fertilizer and sewage and preserving, managing and restoring “N sinks” driven by plant, soil and microbial processes (Davidson et al. 2012).

Recent research has demonstrated that ponds, lakes and reservoirs can function as significant N sinks in watersheds (David et al. 2006, Harrison et al. 2009). These water bodies can support reducing conditions that alter the oxidation state of constituents, such as nitrate (NO$_3^-$) and carbon dioxide (CO$_2$), influencing nutrient transformations throughout the fluvial network (McClain et al., 2003; Groffman et al., 2005). Reduced conditions are favorable for the removal of water-borne NO$_3^-$-N through denitrification, the microbial transformation of NO$_3^-$ to N gases that is perhaps the most important NO$_3^-$ removal mechanism (Galloway, et al., 2003; Seitzinger et al., 2006, Burgin and Hamilton 2007). Similar to other studies, in this paper, denitrification is considered a “sink” for watershed N, even though the nitrate is transformed rather than trapped within the soil or plant biomass (Brezonik & Lee, G. F. 1968; Seitzinger, 1988; Mitch et al., 2001).

North American beavers (Castor canadensis), were functionally extinct from the Northeast U.S. in 1900 due to primarily to trapping, but in the latter half of the 20th century they rebounded at remarkable rates due to trapping regulations, lack of predators, and an abundance of forage (Naiman et al., 1988). Subsequently, beaver-created ponds and dams are reshaping headwater stream networks from extensive, free-flowing reaches to complexes of ponds, wetlands, and connecting streams. These
networks slow the flow of stream water and may increase the amount of N retained at the watershed scale (Jansson et al., 1996; Saunders and Kalff, 2001, Kellogg et al., 2010). The mechanisms responsible for this N retention include plant uptake, sedimentation, and the creation of reducing conditions that may promote denitrification (Devito and Dillon 1993; Naiman et al., 1994; Hill and Duval, 2009). Beaver ponds raise local water tables, increasing interaction of groundwater with near-surface soils, thus promoting higher rates of plant uptake of N and denitrification (Hammerson, 1994; Gold et al. 2001, Hill and Duvall, 2009). Beaver ponds also create patches of open water with minimal shade that encourages aquatic plant growth and nutrient uptake and increases the flow of labile organic matter which serves as fuel to denitrifying bacteria in soils (Hammerson, 1994). Published sedimentation rates in beaver ponds range from less than one to 40 cm per year (Butler and Malanson, 2005). The soil in beaver ponds contains higher carbon (C) and N content, ameliorates stream acidity, and fosters increased anaerobic biogeochemical cycling, compared to adjacent fluvial systems (Hammerson, 1994).

In the Northeast U.S. beavers are moving into mixed-use watersheds with elevated nitrate-N levels due to inputs from un-sewered residential developments and agriculture (Gold et al., 1990). The density of beaver ponds in Northeast is not likely to approach historic levels. The dams and ponds are often considered a nuisance and beaver are trapped or moved. Therefore, the establishment of long-term ponds is often found in conservation lands, as beaver ponds on private lands are likely to occur for briefer periods before the beavers are trapped and the dams destroyed. Even long-term ponds tend to be abandoned within several decades, and N trapped in organic soil
materials can be released back to the fluvial network where it can be transformed and transported to coastal waters. Thus, quantifying the extent of N removal due to denitrification versus storage in soil in these beaver ponds provides insight into the long-term fate of N in this relatively recent watershed feature.

We quantified a number of different nitrate transformation pathways, including denitrification, assimilation into soil microbial biomass and organic N, and net generation of ammonium N. Due to increased residence times, organic matter deposition, and anaerobic biogeochemical cycles in beaver ponds, we hypothesized that 1) beaver pond soil would be a significant sink for NO$_3^-$ and 2) that denitrification would be the dominant N sink process in these soils. The use of $^{15}$N mesocosms also allowed us to assess the production of nitrous oxide, a denitrification intermediate that is a potent greenhouse gas.

**METHODS**

*Study Sites*

We selected three beaver ponds for study based on accessibility and our desire for a range in pond sizes (0.05-8.00 ha; Table 1). All sites were located in Washington County, Rhode Island, USA: two were located on the Chipuxet River (Ponds A and B) and one was located on Roaring Brook (Pond C). Aerial photos taken every 4 years from 1976 to 2012 (RIGIS, 2009) showed that the dams and their associated ponds were first constructed in 1988, 1992 and 2008 at ponds C, A, and B, respectively.

*Sample Collection*

We collected subaqueous (below the water) soil cores from each pond with a soil corer from a canoe during Fall 2011, Spring 2012, and Summer 2012. Each season
we collected 16 cores (6 cm diameter and 13 cm depth) at random locations within each of the three beaver ponds. We stored six cores at 4° C until analysis of “initial” soil conditions. The remaining ten cores were stored in a climate chamber at ambient stream temperature with mesocosm incubations beginning the next day.

Thickness of organic matter was evaluated at a minimum of 7 locations at each pond using a 3 m tile probe, and reported as an average of depth to mineral soil throughout the pond. The entire thickness of the organic soils is not necessarily a result of the beaver ponds. Portions of the ponds may have flooded marshes or swamps; however, the upper 15 cm of the subaqueous soils in the ponds displayed similar characteristics that suggest recent deposition. Dissolved oxygen (DO) and temperature of the pond water were measured at each pond when samples were taken. Additionally, we collected 7 L of pond water on each coring date which was stored in the ambient climate chamber for use in the mesocosm incubations. We filtered a small subsample of pond water from each site and stored it at 4° C until analysis of dissolved inorganic nitrogen (DIN), pH, and dissolved organic carbon (DOC).

*Mesocosm Methods*

Our mesocosm chambers, similar to those used in experiments by Seitzinger et al. (1980) and Nowicki (1994), were constructed of two sections of glass-walled pipe (height=23.5 cm, i.d.=7.6 cm) joined at the center with an O-ring seal and a metal clamp (Figure 1). Three glass stopcocks in the upper half of the mesocosms served as ports – one to add or sample mesocosm water and two to add or sample mesocosm headspace gases. We placed cores, sized to fit the mesocosm chambers, into the lower half of the chambers. Immediately after placing the cores in the lower half of the glass
mesocosms, we added 100 ml of ambient stream water to each mesocosm to ensure saturation and filling of any void space between the core and mesocosm container.

Each season we assayed a total of 11 mesocosms per pond: nine with soil cores amended with $^{15}$N-nitrate ($^{15}$N cores), one with a soil core without amendments (control), and one without a soil core that contained only $^{15}$N-nitrate enriched stream water (blank). During a 48 hour incubation period we subjected each mesocosm to a two-step amendment sequence: near-ambient N condition and enriched N condition. Percent $^{15}$N enrichments ranged from 33-49% depending on background nitrate concentrations in the soil and water column.

Near-ambient N condition mesocosm amendment

We added a 5 ml solution to containing 0.05 mg $^{15}$N-Nitrate-N (99 atom %) to 350 ml of stream water to the top of the nine $^{15}$N cores. For the blank mesocosms, which were filled with approximately 600 ml of stream water, we added a 10 ml solution containing 0.1 mg $^{15}$N-Nitrate-N (99 atom %) to yield a similar near ambient N concentration. All mesocosms remained uncapped overnight to allow degassing and for the $^{15}$N to disperse into the soil. Approximately 12 hours later, we clamped the caps onto the mesocosms. Using a peristaltic pump, we added an additional 350 ml of ambient stream water through a chamber stopcock to fill the mesocosm leaving only a 2 cm headspace at the top of the chamber, to be accessed by the top sampling port to sample headspace gases. To obtain initial NO$_3^-$-N and NH$_4^+$-N (ammonium) concentrations, 15 ml of water was removed from each mesocosm via the sampling port. At this point, all glass stopcocks were closed marking the start of the mesocosm incubation experiment. At this initial time, the headspace volume was 90 ml within
each mesocosm. At the beginning of the incubation period, 15 ml of headspace gas was extracted from each mesocosm. We replaced this headspace with a mix of 80% helium and 20% oxygen via a Tedlar bag that was attached to the opposite port. Fifteen ml of this headspace sample was injected into a 12 ml pre-evacuated Exetainer for later analysis of $^{15}$N-N$_2$ and $^{15}$N-N$_2$O.

During the duration of the four hour incubation, the climate chamber remained darkened. We stirred each mesocosm hourly with a magnetic stir-bar located at the top of the mesocosm chamber. The stir bar was at the interface between the water surface and headspace; stirring prevented a stagnant boundary layer at the soil-water interface and facilitated equilibration of gases at this interface (Seitzinger et al., 1980). The stirrers were rotated by air-driven magnets mounted on top of each mesocosm (Nowicki, 1994). Both the control and blank mesocosms were stirred and sampled exactly as the $^{15}$N core mesocosms. At the end of the incubation, we collected final water and headspace samples from each mesocosm, as described above.

**Enriched N mesocosm amendment**

At the completion of the near-ambient N mesocosm phase of the experiment, we opened the mesocosms to the air and drained water from the top half of each mesocosm. We added a second amendment of a 15 ml solution containing 1.5 mg $^{15}$N-Nitrate-N (50 atom %) to the top of each of the nine $^{15}$N near-ambient soil core mesocosms; this amendment was intended to create an approximate solution of 3 mg NO$_3^-$-N/L after the full volume of stream water was added to the soil cores before incubation; the blank was treated to yield a similar elevated N concentration. Ambient stream water was added to the lower chamber of each mesocosm to maintain
saturation. Mesocosms were left in the dark environmental chamber overnight at ambient stream temperatures. In the morning, 350 ml of ambient stream water was added to each mesocosm and all mesocosms were prepared, sampled, incubated for four hours, and sampled as described above for the near ambient N mesocosm amendments.

Soil Sample Processing

Percent soil moisture was determined on "initial" condition soil core samples within 2 hours of field collection. Percent soil moisture was determined by comparing the wet mass of a soil sample with its dry mass after 72 hours in a 60°C drying oven. Dry bulk density was determined using standard methods (Blake and Hartge, 1986).

The post-incubation mesocosm soil \(^{15}\)N cores and the spare six “initial” soil cores were processed for: soil organic matter, soil microbial biomass, and exchangeable dissolved inorganic N (\(\text{NO}_3^-\) -N and \(\text{NH}_4^+\) -N; DIN) in porewater within 48 hours of the completed mesocosm incubation or initial collection. Each individual soil core was broken apart to remove rocks and coarse wood. The remaining soil was mixed to homogenize the sample. The soil was partitioned into subsamples for analysis of: 1) total C and N, 2) exchangeable inorganic N (\(\text{NO}_3^-\) -N and \(\text{NH}_4^+\) -N; DIN), and 3) microbial biomass C and N. The mean porewater nitrate-N concentration of the spare cores (those not subjected to the mesocosm incubations) was used as the initial nitrate-N to calculate the component of pore-water nitrate-N recovered from the mesocosms (post incubation pore-water nitrate-N minus pre-incubation pore-water nitrate-N concentration).
For analysis of organic soil C and N, soil was dried and ground through a size 10 sieve with material not passing through the sieve being discarded. A small subsample (5-8 mg) of each initial and post-mesocosm $^{15}$N core was weighed into a tin capsule and stored in a desiccator until analysis. Exchangeable inorganic N in the porewater was extracted with 0.5 mol/L K$_2$SO$_4$ (Keeney and Nelson, 1982). After samples were shaken and settled, the supernatant liquid from each replicate was filtered through Whatman filter paper into clean Nalgene bottles. Liquid samples were frozen until analysis for NO$_3$-N and NH$_4^+$-N concentration or diffused onto acidified filter disks in preparation for $^{15}$N determination, as described below.

Soil microbial biomass was determined using a rapid chloroform-fumigation extraction technique (Witt et al., 2000). Microbial C and N were calculated as the difference in extractable fractions between the fumigated and unfumigated soil (Witt et al., 2000). The N extracts were frozen until analysis for NO$_3$-N and NH$_4^+$-N or diffusion onto acidified filter discs for N isotope ratio determination via mass spectrometry. Soil and microbial-biomass N extracts were prepared for $^{15}$N analysis using the six-day polytetrafluorethlyene (PTFE) tape diffusion method as described by Stark and Hart (1996) where NH$_4^+$ in the supernatant liquid is converted to NH$_3$ gas which diffuses onto the filter traps between two pieces of PTFE Teflon tape. Following the 6 day NH$_4^+$ diffusion, Devarda’s alloy was added to each diffusion container and incubated for another 6 days to convert NO$_3^-$ to NH$_4^+$ which was then converted to NH$_3$ gas. This method allowed us to identify $^{15}$N in both the NO$_3^-$ and NH$_3^+$ pools separately. Following diffusion, filters were dried in a desiccator, wrapped in tin capsules, and stored in a desiccator until analysis of N isotope ratios.
Denitrification rates were determined through the comparison of initial versus final headspace samples that quantified the amount of $^{15}\text{N}_2$ and $^{15}\text{N}$-$\text{N}_2\text{O}$ generated over the four hour incubation time in both near-ambient and enriched N mesocosm conditions. Denitrification masses of $^{15}\text{N}_2\text{O}$-$\text{N}$ and $^{15}\text{N}_2$ gases (µmol) in headspace samples were calculated using the headspace equilibration method (Tiedje, 1982) and then divided by the respective $^{15}\text{N}$ sample enrichment. The mass of $^{15}\text{N}_2\text{O}$-$\text{N}$ or $^{15}\text{N}_2$ generated during the incubation period was calculated as the mass present in the final samples minus the mass present in the initial samples. The total masses of $\text{N}_2\text{O}$-$\text{N}$ and $\text{N}_2$-$\text{N}$ produced were calculated by dividing the masses of $^{15}\text{N}_2\text{O}$-$\text{N}$ and $^{15}\text{N}_2$ by the $^{15}\text{N}$ isotope enrichment of the mesocosm. The mass of $\text{N}_2\text{O}$-$\text{N}$ and $\text{N}_2$ generated was dividing by the surface area of the mesocosm and the four hour incubation period to yield gas production rates ($\text{N}_2\text{O}$-$\text{N}$ and $\text{N}_2$) of mg N m$^{-2}$ of soil surface hr$^{-1}$. The computed rates represent denitrification capacity where nitrate is abundant and other factors, such as electron donors or redox conditions control the observed rates (Addy et al., 2005).

We use the phrase “net ammonium-N generation” to refer to the pool of $^{15}\text{NH}_4$-$\text{N}$ that was created during the incubation period based on the $^{15}\text{N}$ enrichment method. The mass of nitrate-$\text{N}$ that was assimilated into organic soil materials was calculated by multiplying the total N mass found in core by the % $^{15}\text{N}$ found, based on $\delta^{15}\text{N}$ values, after subtracting out background levels of $^{15}\text{N}$. We then divide by % $^{15}\text{N}$ enrichment.
Nitrate-N Recoveries

Although we measured the nitrate-N concentrations and volumes of the overlying water that was poured off from the mesocosms, we did not measure the $^{15}$N enrichment of that poured-off water. So, we report the % of nitrate recovered using two approaches -- by summing up the mass of nitrate-N in the poured off overlying water (which was a large mass) and using the $^{15}$N method to obtain the "nitrate-N mass" that was converted or remained in other sources of nitrate -- such as in the porewater, denitrification gases, and in the soil. Total recoveries of applied nitrate-N were computed by dividing the sum of the mass of i) nitrate-N that was transformed (via denitrification, assimilation into soil organic N, and $^{15}$NH$_4$-N methods), ii) nitrate that remained in the pore water, and iii) nitrate that was poured off with the overlying water during the incubation period by the initial mass of nitrate-N at the start of the incubation period, including the nitrate-N that we added. The initial mass of nitrate-N was computed from pore-water nitrate-N, plus the total mass of nitrate-N additions, plus the ambient nitrate-N in the two additions of stream water.

Analytical Methods

The University of California Davis Stable Isotope Facility analyzed the mesocosm headspace samples for concentrations and isotope ratios of N$_2$ and N$_2$O using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany).

We analyzed soil samples for N and C isotope composition using continuous flow isotope ratio mass spectrometry (CF-IRMS) employing a Vario Micro Elemental Analyzer interfaced to a Elementar Isoprime 100 Mass Spectrometer (Elementar...
Americas, Mt. Laurel, NJ). The N isotopic composition is expressed as a part per thousand (permil) difference from the composition of a recognized reference material, which by convention, is N\textsubscript{2} in air (Mariotti, 1983). All samples were analyzed in duplicate with a typical difference of about 0.1 ‰.

We measured NO\textsubscript{3}-N and NH\textsubscript{4}+-N concentrations in soil extracts and water samples using an Astoria Pacific Model 303A Segmented Continuous Flow Autoanalyzer (Astoria-Pacific Inc., Clackamas, OR). On this instrument, the open tubular cadmium reduction method (APHA et al., 1995) was used for NO\textsubscript{3}-N and the alkaline phenol and hypochlorite methods (APHA et al., 1995) were used for NH\textsubscript{4}+-N. Laboratory accuracy was determined by the analysis of reference material and comparison of the resulting value to that of the accepted value. The difference between the accepted and reference value is the percent difference (%D). The %D had to be less than 20 to accept analyses (Green et al., 2009). Precision was assessed through the measurement of duplicate samples and subsequent calculation of the relative percent difference (%RPD) as described below (Green et al., 2009)

\[
\text{%RPD} = \left( \frac{\text{Result of Replicate 1} - \text{Result of Replicate 2}}{\text{Average of Result of Replicate 1 and Result of Replicate 2}} \right) \times 100
\]

The RPD had to be 15% or less to for data acceptance.

Fumigated and unfumigated soil extracts were analyzed for DOC using a Shimadzu Total Organic Carbon Analyzer (Kyoto, Japan). DO and temperature were measured in the field using a YSI DO-temperature meter, model 55 (YSI, Yellow Springs, Ohio). At the end of the incubation DO was measured using the Winkler
titration method (Eaton and Franson, 2005). pH was measured on Accument Research
AR20 pH/conductivity meter.

Statistical Analyses

We tested for differences between site and season using two-way Analysis of
Variance (ANOVA) with a Tukey's post hoc test for the following variables:
denitrification, net generation of NH$_4$-N, nitrate assimilation into soil organic N, and
soil microbial biomass C and N. Because all soil $^{15}$N recoveries were only obtained
from analyses performed following two days of mesocosm incubations that included
24 hours of near-ambient followed by 24 hours of enriched conditions, we cannot
report nitrate-N transformations in soil for the near-ambient conditions that occurred
for the first 24 hours. For the mass balance and estimates of watershed denitrification
capacity, we focused on the denitrification rates associated with the enriched
conditions – which were the conditions in the mesocosms for the final 24 hours of the
incubation.

We evaluated correlations between denitrification rates and log transformed
N$_2$O:N$_2$ data using Pearson product-moment correlation coefficients. Statistical
significance was set at $\alpha <0.05$ for all analyses. Two-way ANOVA statistics were
performed using SAS Software version 9.2, all other statistical analyses were
performed with Analyse-it version 3.0.

RESULTS

Carbon, Oxygen, pH

Soil microbial biomass C did not vary significantly between ponds. Biomass C
across all ponds was significantly different between seasons, with spring displaying
the highest values (Table 2). DOC ranged from 3.9-5.2 mg/L between sites and seasons (Table 2). DO and temperature changed with the seasons, with summer being the warmest and having the lowest DO (Table 2). DO concentrations in the mesocosms ranged from 5-8 mg/L throughout all mesocosm incubations. Percent soil moisture within the soil cores ranged from 70-90%, with an average of 79%. Across all sites and seasons average dry bulk density was 0.33 g cm$^{-3}$. These soils are considered organic soils based on % C (Table 1) (Fanning & Fanning, 1989). Soil pH was similar to water pH (Pond A: 6.3, Pond B: 6.0), Pond C had slightly more acidic soil with a pH of 5.5.

**Nitrate Recovery during the Mesocosm Experiment**

The nitrate-N recovery, based on the fate of $^{15}$N labeled nitrate, changes in porewater nitrate and the nitrate in the water overlying the soil averaged 93.2% (SD:13.8). 29.3-60.6% of the nitrate-N that we added was transformed during the course of the incubation. The water overlying the soil cores contained a sizeable percentage of the added nitrate-N throughout the experiment.

**Denitrification Rates under Enriched Conditions**

Denitrification rates under enriched conditions were high during all seasons and constituted the dominant nitrate-N transformation in the mesocosms. There was significant (p < 0.05) variation in denitrification with season and site (Table 3). Spring had significantly lower denitrification rates than summer or fall across all ponds (Table 3). Pond C, the oldest and largest beaver pond which also had the lowest ambient NO$_3$-N concentrations had significantly lower denitrification rates than the other ponds (Table 3).
Tracing \( ^{15}N \) in Soil

Net N uptake by soil microbial biomass (based on the \( ^{15}N \) enrichment method) was markedly lower than transformations associated with denitrification, ranging between 2-10\% of denitrification rates. Site and season were found to be significant (\( p < 0.05 \)) for net \( ^{15}N \) uptake by microbial biomass based on the results of the two-way ANOVA (Table 4). Net N uptake by microbial biomass was significantly higher in Pond C, the pond with the lowest ambient NO\(_3\)-N concentrations. The spring season had significantly higher net \( ^{15}N \) uptake by microbial biomass than the fall season (Table 4).

Nitrate assimilation into soil organic N was also always lower than denitrification rates. Based on the two-way ANOVA only site factors were significant (\( p < 0.05 \)) (Table 5). Although net \( ^{15}N \) uptake by soil microbial biomass constitutes a portion of measured nitrate assimilation into soil organic N, the patterns and differences across sites and seasons did not coincide. Of note, Site C was found to have significantly lower nitrate assimilation into soil organic N than Site A.

\( ^{15}NH_4^+ \)-N generation rates (mg N m\(^{-2}\) day\(^{-1}\)) were substantially lower than transformations associated with denitrification and assimilation into soil organic N. Rates were not significantly different when comparing sites or season (Table 6).

Denitrification constituted between 52 and 86 percent of total N transformations under enriched levels of nitrate. Pond B had a significantly higher proportion of total N transformation attributed to denitrification compared with the other ponds (Figure 2).
To examine the effects of nitrate enrichment on denitrification, we compared rates of denitrification on day 1 (near-ambient conditions; 0.1 mg N L\(^{-1}\)) to rates of denitrification on day 2 (enriched-N conditions; 3.0 mg N L\(^{-1}\)). There were no differences in denitrification rates between the near-ambient and enriched conditions for all ponds during summer and fall. However, during the spring season, Ponds A and B had significantly lower denitrification rates in the enriched-N mesocosm incubation than in the near-ambient mesocosm incubation (Table 7). 

\(N_2O:N_2\)

\(N_2O:N_2\) ratios displayed a significant exponential decline with increasing denitrification rates (\(p<0.02\)) (Figure 3). \(N_2O:N_2\) ratios were significantly different between near-ambient and enriched-N mesocosm conditions. Under near-ambient mesocosm conditions, \(N_2O:N_2\) ratios averaged 0.002, while under enriched-N mesocosm conditions, \(N_2O:N_2\) averaged: 0.07.

**DISCUSSION**

We used a mass-balance approach based on \(^{15}\)N tracer additions to soil core mesocosm incubations to understand the fate of nitrate in beaver ponds and the capacity of these systems to serve as long-term watershed N sinks. Our mesocosms have been used in the past by Seitzinger et al. (1980) and Nowicki (1994) to assess nitrogen transformations in subaqueous soils. The mesocosm approach enables a suite of processes in both water and soil to be examined simultaneously in replicated samples (Oviatt and Gold, 2005; Fulweiler et al., 2007). Past \(^{15}\)N experiments have studied the effects of N inputs on N retention and mobility, addressing questions such
as microbial uptake, plant-microbial competition for N, and links to C cycling (Tietema et al., 1998; Currie et al. 1999; Nadelhoffer et al., 1999). The use of the stable isotope $^{15}$N as a tracer has provided important insights into the fluxes and transformations of N in soils and at the ecosystem level (Stark and Hart 1997; Tietema et al., 1998, respectively).

We were able to account for a high proportion of the nitrate-N added to the mesocosms. Deviations from complete recovery of added nitrate-N may have partially resulted from nitrification within the cores, or from intra-core variations between the cores.

*Factors Controlling Soil Nitrate Transformation*

Subaqueous beaver pond soils displayed high rates of nitrate transformations from all sites and all seasons, suggesting that these ecosystems can serve as substantial sinks for watershed nitrate. Denitrification rates were much higher than rates found from the other transformation processes; approximately 3 to 5 fold higher than the rates ascribed to nitrate assimilation in soil organic N, which constituted the next highest mechanism of nitrate transformation (Figure 2). Our denitrification rates were comparable to those noted from a number of other studies in freshwater ponds and greater than those reported for streams by Mulholland et al. (2008) (Table 8). Our values exceed those observed in some studies of freshwater ponds and wetlands; however, those rates in those ecosystems may have been limited by low concentrations of nitrate.

We observed significant seasonal patterns, with lower denitrification rates in spring. Soil microbial biomass C and microbial biomass uptake of $^{15}$N were also
higher in the spring, suggesting that high rates of immobilization may have been competing with denitrification during this season. The beaver pond soil had levels of microbial biomass C comparable to other wetlands (Nguyen, 2000 Tietz et al., 2007), while microbial biomass N values were lower than most published values (Truu et al., 2009), but were similar to those reported by Nguyen (2000) at 100-400 mm soil depths. Microbial biomass has been shown to decrease with depth (Nguyen, 2000). Soils used for the biomass experiments were a subsample from the entire core, and therefore from a variety of depths.

In comparing near-ambient and enriched-N mesocosm conditions, there were no significant differences during most of the incubations. However, two ponds in the spring had higher denitrification rates at near-ambient conditions in the first 24 hours of incubation when compared to the nitrate enriched conditions that occurred during the following 24 hours (Table 7). The ambient nitrate-N levels in those two ponds, while not comparable to the high levels found in agricultural watersheds are still much higher than concentrations found in pristine watersheds. In these two instances, denitrification of the near-ambient nitrate during the first 24 hours may have consumed a small pool of highly labile C, resulting in lower denitrification rates under the enriched nitrate conditions that occurred on the following day. The fact that soil microbial biomass C was higher during the spring season supports this idea as the large microbial biomass may have consumed the pool of labile C, leaving little to support denitrification during these incubations. Pond C, which had substantially lower ambient nitrate-N concentrations (about 1/12th of the levels of the other ponds), did not display elevated denitrification during the first 24 hours when near-ambient
nitrate levels were maintained, suggesting the possibility that this pond was nitrate limited at near-ambient conditions. Additionally, Pond C, which is the oldest pond and is dominated by deeper, open water, had lower denitrification rates at both ambient and enriched nitrate-N conditions. This follows previous studies showing young wetlands with emergent macrophyte vegetation have higher denitrification potential than open water wetlands (Anderson et al., 2005 and Mitch and Hernandez, 2007).

Although nitrate assimilation into soil organic N was the second largest ecosystem sink for added nitrate, the large discrepancy between the rates of nitrate assimilation in soil organic N and the rates of net N uptake by microbial biomass may be an abiotic artifact of the addition of nitrate (Davidson et al., 1991; Colman et al., 2008) and are not considered to be biological immobilization. In any case, none of this assimilation into microbial biomass and/or soil organic N may be a long-term sink, increasing the importance of the measured denitrification rates as a more permanent nitrate removal mechanism.

Net NH$_4^+$-N generation (Table 6) may result from rapid immobilization followed by mineralization, or from dissimilatory nitrate reduction to ammonium (DNRA), a microbially mediated pathway involving the transformation of nitrate to ammonium. There are two types of DNRA: fermentive and chemolithoautotrophic. Fermentive DNRA is thought to be favored in nitrate limited environments rich in labile carbon (Burgin and Hamilton, 2007) while chemolithoautotrophic DNRA, which couples the reduction of nitrate to the oxidation of sulfide to sulfate is favored in soils high in sulfur. While we did not measure sulfate over the course of our
mesocosm incubations, freshwater systems in Michigan showed simultaneous nitrate reduction and sulfate production (Burgin and Hamilton, 2008). Measuring sulfate production or identifying the microbial population responsible for generating the ammonium would be useful next steps in documenting DNRA in these beaver pond systems. The eventual fate of the nitrate converted to ammonium is unknown but it is not thought to be a permanent sink for N as it may be converted back to nitrate via nitrification or assimilated into biomass (Burgin and Hamilton, 2007).

*Are beaver pond soils a source of N₂O?*

The denitrification rates were negatively correlated to N₂O:N₂ ratios, but increased with nitrate concentrations. This ratio has been shown to be controlled by a number of factors, including pH, soil moisture and nitrate loading, but there remains considerable uncertainty in these relationships (Seitzinger, 1998, Beaulieu et al., 2011). All of our soils were ponded and had similar pH levels. Several studies have shown that the N₂O:N₂ ratio is positively correlated with nitrate-N concentrations in water (Zaman et al., 2008; Baulch et al., 2011; Clough et al., 2011), although Beaulieu et al. (2011) did not see increased N₂O:N₂ with increased NO₃⁻ loading to rivers. Beaulieu et al. (2011) report the percentage of denitrification released as N₂O ranging from 0.04-5.6% in 53 streams. On average, 0.2% of denitrification is being released as N₂O under low nitrate-N concentrations in the three beaver ponds, while under N-enriched conditions, the average was 7%. Our data suggest that under enriched conditions beaver ponds have greater N₂O production than streams, but are similar to wetland soils which have an average N₂O yield of 8.2% (Schlesinger, 2009). Further fieldwork which measures N₂O flux from beaver ponds should be considered.
Estimating Watershed Sink Capabilities of Beaver Ponds

To provide insight into the potential role of beaver ponds on the export of nitrate-N from small catchments, we linked annual estimates of beaver pond denitrification rates derived from our mesocosm study with estimates of watershed nitrate-N inputs and the ratio of catchment area to beaver pond area in the study region. We did not include N removal due to immobilization, since beaver ponds are transient and the stored organic deposits can be released and mineralized when the pond is destroyed. We used the range of annual beaver pond denitrification rates obtained when the mesocosms were enriched to 3 mg/l NO$_3$-N rather than at the lower ambient levels (0.5 mg/l) to reflect nutrient conditions expected in rural catchments with agricultural or un-sewered residential developments. The annual rate was computed by extrapolating measured seasonal rates over 273 days to represent the Fall, Spring and Summer seasons when we obtained measurements, assuming that denitrification would be negligible during winter due to low temperatures and reduced inflows. Given the likelihood that some denitrification will occur over the winter months, this assumption generated a conservative estimate of annual denitrification.

We assumed 0.7 beaver ponds per km$^2$ of catchment area based on studies conducted in southern New England (DeStefano et al., 2006). Beaver pond area can be quite variable (our three pond areas displayed a range of more than two orders of magnitude) due to factors such as physiography and age of pond. We used both the median beaver pond area (0.26 ha) from our three sites and a pond area of 1.0 ha, which represents a minimum size from many other studies (Weyhenmeyer, 1999 and Pollock et al., 2003).
We estimate that beaver pond denitrification can remove approximately 50 to 450 kg nitrate-N km\(^{-2}\) of catchment area (Table 9). Moore et al. (2004) using the SPARROW model predicted total N catchment yields between 200 and 1000 kg km\(^{-2}\) for undeveloped land uses (i.e., rural) in southern New England. Crumpton et al. (2008) found nitrate mass removal by wetlands in tile-drained agricultural lands to range between 25-78% for wetland/watershed area ratios of 0.57-2.25. Based on the beaver pond/watershed area ratios (0.18-0.7%), and inter-pond variability in denitrification we estimate that beaver ponds in southern New England can remove 5-45% of watershed nitrate loading from rural watersheds with high N loading (i.e., 1000 kg km\(^{-2}\)). Thus, beaver ponds represent a proportionally significant sink for watershed N if current beaver populations persist.

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TABLE 1. Site characteristics. Values for water depth and depth of organic soil materials are mean (SD).

| Beaver Pond | A               | B               | C               |
|-------------|-----------------|-----------------|-----------------|
| Lat/Long    | 41.486175/71.548384 | 41.503464/71.533608 | 41.565725/71.677929 |
| Surface Area (ha) | 0.26             | 0.05             | 8.00            |
| Drainage Area (ha) | 2450             | 2093             | 976             |
| Tributary (name and stream order) | Chipuxet, 2 | Chipuxet, 2 | Roaring Brook, 1 |
| Water Depth (m) mean (SD) | 0.93 (0.48) | 0.59 (0.24) | 0.75 (0.22) |
| Thickness of Organic Soil Materials (m) mean (SD) | 0.29 (0.28) | 0.66 (0.23) | 0.45 (0.18) |
| First documented evidence (yr) | 1992             | 2008             | 1988            |
| Average Pond Nitrate Concentration (mg/L) † | 0.57             | 0.35             | 0.04            |
| Mean % Carbon in Soil Cores | 18.3             | 15.0             | 29.8            |

†Means represent average nitrate-N concentration during soil sampling days.

TABLE 2. Dissolved oxygen in water column, water temperature, dissolved organic carbon (DOC) in water column, pond pH data during each sampling visit, and microbial biomass C data by season.

| Spring | DO (mg/L) | Water Temperature (°C) | DOC (ppm) | Pond pH | Microbial Biomass C, mean (SD) (mg C kg⁻¹ dry soil) |
|--------|-----------|------------------------|-----------|---------|---------------------------------------------------|
| Pond A | 8.1       | 16.4                   | 4.7       | 6.4     | 188.4 (84.0)                                      |
| Pond B | 8.9       | 15.1                   | 5.2       | 6.3     |                                                   |
| Pond C | 7.2       | 16.4                   | 5.0       | 6.0     |                                                   |
|        |           |                        |           |         |                                                   |
| Summer |           |                        |           |         |                                                   |
| Pond A | 3.1       | 26.4                   | 5.6       | 6.2     | 95.7 (68.0)                                       |
| Pond B | 3.7       | 25.5                   | 4.0       | 6.2     |                                                   |
| Pond C | 4.8       | 25.2                   | 4.6       | 6.1     |                                                   |
|        |           |                        |           |         |                                                   |
| Fall   |           |                        |           |         |                                                   |
| Pond A | 4.8       | 8.8                    | 3.9       | 6.3     | 16.9 (18.8)                                       |
| Pond B | 6.3       | 9.3                    | 4.5       | 6.2     |                                                   |
| Pond C | 4.6       | 10.6                   | 5.0       | 5.9     |                                                   |
TABLE 3. Beaver pond denitrification rates (mg N m⁻² day⁻¹) (based on recovery of ¹⁵N) at enriched-N mesocosm conditions. Values within each cell are mean (SD).

| Site     | Spring‡ | Summer‡ | Fall‡ | Site Grand Means† |
|----------|----------|---------|-------|-------------------|
| Site A‡  | 46.6 (71.2) | 248.8 (144.8) | 249.8 (83.9) | 181.7ᵃ |
| Site B‡  | 101.2 (64.1) | 371.0 (105.4) | 236.1 (185.8) | 236.1ᵃ |
| Site C‡  | 40.3 (44.4) | 117.4 (68.3) | 134.7 (64.2) | 97.5ᵇ |

Grand means within a row or column with distinct superscript letters are significantly different (p< 0.05) as determined by a Tukey’s post hoc mean separation test following a 2 way ANOVA (site and season). ‡Cells represent homogeneous subset sets with sample size per cell of n = 9.

TABLE 4. Beaver pond net ¹⁵N uptake by microbial biomass (mg N m⁻² day⁻¹) at enriched-N mesocosm conditions (rates based on recovery of ¹⁵N). Values within each cell are mean (SD). Grand means are the average of the entire sample of interest, not the average of the means. Note that n values were not equal. Spring Pond B data and all of Fall data have an n value of 5 per site. Ponds A and C in the Spring and all three sites during the Summer have an n value of 9.

| Site     | Spring | Summer | Fall | Site Grand Means† |
|----------|--------|--------|------|-------------------|
| Site A   | 2.7 (3.4) | 1.8 (1.9) | 2.1 (2.2) | 2.2ᵃ |
| Site B   | 6.3 (6.6) | 0.2 (0.3) | 5.1 (3.4) | 3.1ᵃ |
| Site C   | 9.5 (6.3) | 8.8 (5.4) | 0.8 (0.8) | 7.3ᵇ |

Grand means within a row or column with distinct superscript letters are significantly different (p< 0.05) as determined by a Tukey’s post hoc mean separation test following a 2 way ANOVA (site and season).
TABLE 5. Rates of nitrate assimilation into soil organic N (mg N m\(^{-2}\) day\(^{-1}\)) in beaver ponds at enriched-N mesocosm conditions (rates based on recovery of \(^{15}\)N). Values within each cell are mean (SD). Grand means are the average of the entire sample of interest, not the average of the means. Note that n values were not equal. Spring Pond B data and all of Fall data have an n value of 5 per site. Ponds A and C in the Spring and all three sites during the Summer have an n value of 9.

|              | Spring    | Summer    | Fall      | Site Grand Means† |
|--------------|-----------|-----------|-----------|-------------------|
| Site A       | 39.5 (22.4) | 48.8 (14.3) | 55.1 (20.1) | 46.5\(^{a}\)       |
| Site B       | 26.4 (9.4)  | 14.3 (5.5)  | 27.5 (9.1)  | 21.0\(^{c}\)       |
| Site C       | 32.3 (27.5) | 47.3 (23.9) | 22.1 (6.8)  | 36.0\(^{b}\)       |
| **Seasonal Grand Means†** | **33.8**   | **36.8**   | **34.9**   |                   |

†Grand means within a row or column with distinct superscript letters are significantly different (p< 0.05) as determined by a Tukey’s post hoc mean separation test following a 2 way ANOVA (site and season).

TABLE 6. Beaver pond net \(^{15}\)NH\(_4\)-N generation rates (mg N m\(^{-2}\) day\(^{-1}\)) at enriched-N mesocosm conditions (rates based on recovery of \(^{15}\)N). Values within each cell are mean (SD). Grand means are the average of the entire sample of interest, not the average of the means. Note that n values were not equal. Spring Pond B data and all of Fall data have an n value of 5 per site. Ponds A and C in the Spring and all three sites during the Summer have an n value of 9.

|              | Spring    | Summer    | Fall      | Site Grand Means† |
|--------------|-----------|-----------|-----------|-------------------|
| Site A       | 2.8 (2.7)  | 8.1 (5.9)  | 6.2 (2.6)  | 5.6\(^{a}\)       |
| Site B       | 10.3 (9.9) | 3.2 (2.5)  | 9.6 (4.3)  | 6.7\(^{a}\)       |
| Site C       | 6.7 (3.6)  | 6.6 (2.7)  | 7.8 (5.9)  | 6.9\(^{a}\)       |
| **Seasonal Grand Means†** | **6.0\(^{a}\)** | **6.0\(^{a}\)** | **7.9\(^{a}\)** |                   |

†Grand means within a row or column with distinct superscript letters are significantly different (p< 0.05) as determined by a Tukey’s post hoc mean separation test following a 2 way ANOVA (site and season).
TABLE 7. Mean denitrification rates (mg N m$^{-2}$ day$^{-1}$) (based on recovery of $^{15}$N) at near-ambient (~0.1 mg N l$^{-1}$) and enriched-N (~3.0 mg N l$^{-1}$) mesocosm incubations.

|       | Near-Ambient Mesocosm Incubation†‡ | Enriched-N Mesocosm Incubation†‡ |
|-------|-----------------------------------|----------------------------------|
|       |                                   |                                  |
| Pond A‡ | 1205.6$^a$                        | 46.6$^b$                         |
| Pond B‡ | 1156.3$^a$                        | 101.2$^b$                        |
| Pond C‡ | 23.9                              | 40.3                             |

**Summer**

|       | Near-Ambient Mesocosm Incubation†‡ | Enriched-N Mesocosm Incubation†‡ |
|-------|-----------------------------------|----------------------------------|
|       |                                   |                                  |
| Pond A‡ | 605.9                             | 248.8                            |
| Pond B‡ | 488.3                             | 371.0                            |
| Pond C‡ | 79.6                              | 117.4                            |

**Fall**

|       | Near-Ambient Mesocosm Incubation†‡ | Enriched-N Mesocosm Incubation†‡ |
|-------|-----------------------------------|----------------------------------|
|       |                                   |                                  |
| Pond A‡ | 189.6                             | 249.8                            |
| Pond B‡ | 102.8                             | 236.1                            |
| Pond C‡ | 118.2                             | 134.7                            |

†Means within a row with distinct superscript letters are significantly different (p<0.05) as determined by a one-way ANOVA analysis between enrichment levels by site. ‡Cells represent homogeneous subset sets with sample size per cell of n = 9.
TABLE 8. Denitrification rates in comparison to other studies of shallow ponds, meadows and wetlands.

| Study                  | Setting            | Denitrification mg N₂-N m⁻² day⁻¹ (method) |
|------------------------|--------------------|--------------------------------------------|
| Naiman et al., 1994    | beaver pond        | 2.0 (Acetylene Block Technique)            |
| Naiman et al., 1994    | wet meadow         | 2.6 (Acetylene Block Technique)            |
| Batson, et al., 2012   | constructed wetland| 3.4 (Acetylene Block Technique)            |
| Song et al., 2012      | constructed wetland| 0.82-15.8 (Acetylene Block Technique)      |
| Bonnett et al., 2013   | wetland            | 17.90 (Acetylene Block Technique)          |
| Scott et al 2008       | constructed wetland| 16.8 (Net N₂ flux)                         |
| Lazar et al., (this study) | beaver ponds    | 96-236 (15N tracer technique)              |
| Xue et al., 1999       | constructed wetland| 48.0-283.2 (Acetylene Block Technique)     |
| Xue et al., 1999       | constructed wetland| 48.0-223.2 (¹⁵N technique)                 |
| Vecherskiy et al., 2011| beaver pond       | 266 (Acetylene Block Technique)            |
| David et al., 2006     | reservoir in ag landscape | 169.9-616.4 (Acetylene Block Technique) |

TABLE 9. Annual catchment scale denitrification capacity of beaver ponds.

| Pond Area (ha)         | Annual catchment scale denitrification capacity of beaver ponds in kg km⁻² yr⁻¹ (% of catchment loading) |
|------------------------|---------------------------------------------------------------------------------------------------------|
| Median from this study (0.26 ha) | 49-118 (4.9-11.8%)                                                                                     |
| Minimum from other studies (1 ha) | 187-454 (18.7-45.4%)                                                                                   |

Assumptions include: 0.7 beaver ponds km⁻² of catchment area; removal processes only occur during spring, summer, and fall; range in rates are due to scaling up the range in rates observed in our study; total N catchment yields up to 1000 kg km⁻² for rural areas in Southern New England (Moore et al., 2004).
FIGURE 1. Soil core incubation mesocosm shown in an illustration (edited from Nowicki, 1994). The mesocosm consists of two pieces of glass pipe held together with an O-ring and metal clamp. Three glass stopcocks are in the top section, one rubber septa is added for sampling the gas phase. Air-driven stirrer is placed on top of the chamber to drive a magnetic stir bar floating in the chamber.

FIGURE 2. Mean N transformations per site at enriched-N mesocosm conditions. Measured nitrate-N transformations include denitrification, soil immobilization (measured in total soil organic N) and net ammonium-N generated.
FIGURE 3. Denitrification rate vs. log transformed N₂O:N₂, Pearson correlation p<0.02. These data do not include observations when denitrification rates were less than 0.1 mg N m⁻² day⁻¹ (32 out of 153).
CHAPTER 3

Resurgent Beaver Ponds in the Northeastern U.S.: Implications for Greenhouse Gas Emissions

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ABSTRACT

We determined the diffusive flux of greenhouse gases (GHGs) — methane (CH₄), carbon dioxide (CO₂), and nitrous oxide (N₂O) — from the air-water interface of three beaver ponds in Rhode Island, USA. We launched five floating static gas chambers on each beaver pond during spring, summer, and fall seasons, and sampled at 15-minute intervals over one hour. Emission rates were derived for each gas from the linear regression of the change in concentration of the gas over time. Fall had significantly higher CO₂ emission than other seasons, mean 9.298 g CO₂ m⁻² day⁻¹ versus 3.305 g CO₂ m⁻² day⁻¹ in spring and 3.188 g CO₂ m⁻² day⁻¹ in summer. CH₄ and N₂O emissions did not show seasonal differences: annual means were 174 mg CH₄ m⁻² day⁻¹ and 1 mg N₂O m⁻² day⁻¹, respectively. When flux was expressed in CO₂ global warming equivalents, CH₄ emissions comprised the majority of the GHG emissions, at 67.5% across all sites and seasons. Significant correlation was found between CO₂ emission rates and pond water DOC, while CH₄ emissions were significantly correlated to air or water temperature. Our results show that beaver ponds generate high fluxes of CH₄ and CO₂ emissions per surface area of the pond. However, the relatively small areal footprint of beaver ponds at the watershed scale greatly diminishes their net effect. Thus, at a catchment scale we estimate that the global warming potential of the GHG emissions from the beaver ponds expressed as CO₂ equivalents range from 3-26 Mg km⁻² yr⁻¹. Assessment of the net effect of beaver ponds on the greenhouse gas budget of the Northeast U.S. must consider more than the GHG emissions from the ponded areas of the beaver ponds. Studies are warranted on the extent of changes in water
tables, and associated changes in GHG emissions, in the lands surrounding the ponds and the fate of the organic soils in abandoned beaver ponds.

INTRODUCTION

Climate change is due to anthropogenic alterations of the atmosphere’s composition, with additional contributions from natural biochemical processes (IPCC, 2007). In particular, the rapid increase in the concentrations of greenhouse gases (GHGs) in the atmosphere trigger atmospheric warming as these gases absorb the heat radiated from the earth and re-emit it into the atmosphere. Research has been directed at understanding the sources of GHGs to better assess how to reduce GHG emission rates. The study of biogeochemical cycling, particularly the cycling of carbon (C) and nitrogen (N), underlies our ability to predict GHG generation from natural environments. Research is necessary to derive accurate estimates of GHG emission rates from different landscapes around the globe and to attempt to correlate these rates with various parameters, such as temperature, to identify potential interaction of emission rates with probable alterations resulting from climate change. These estimates will help inform decisions about GHG management.

Research to date has indicated inland waters play a substantial role in the global C cycle and that certain landscape features, such as wetlands, may function as “hotspots” for GHG emissions (Cole et al., 2007; Reddy and DeLaune, 2008). Because natural wetlands are estimated to account for nearly 30% of total global methane emissions (Reddy and DeLaune, 2008), it is important to better quantify the fluxes of methane and other GHGs from these ecosystems into the atmosphere. Studies (Naiman et al., 1994, Soumis et al., 2004) have shown that wetland
environments, such as beaver ponds, may be sources of atmospheric carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄) – all GHGs. Due to the resurgence of the North American beaver (Castor canadensis), there is increasing interest in investigating beaver ponds as potential hotspots of GHG emission.

North American beaver populations, once decimated due to over-trapping by European settlers in the 1500-1800s, have been rebounding since the 1900s due to hunting restrictions and improved habitat conditions (Butler and Malanson, 2005). Beaver influence stream hydrology and morphology through the construction of dams – blocking the stream channel with trees, sticks and mud. These dams obstruct normal stream flow, causing water to pool, thereby forming ponds with high retention times. These ponds create wetland environments that accumulate organic matter, foster anaerobic conditions, and trap sediments and nutrients. The organic matter trapped within beaver ponds can serve as a C source for enhanced microbial activity, facilitating biogeochemical cycling. Compared to free-flowing riverine systems, beaver ponds alter the cycling of C and N, potentially increasing the rate and areal extent of methanogenesis (producing CH₄), respiration (producing CO₂), and denitrification, (producing N₂O) (Naiman et al., 1994). Methane and nitrous oxide fluxes from these locations are important since the climate-forcing potential of CH₄ is 25 times more than that of CO₂ and N₂O is 298 times more potent than CO₂ on a 100-year time frame (IPCC, 2007).

Beaver ponds accumulate organic C through both allochthonous (import from other sources) and autochthonous (created within the pond) sources. Beaver ponds capture organic sediments flowing downstream and flood terrestrial plants, which then
die and contribute to C accumulation. Within the ponds, primary production from rooted aquatics and plankton also add to the pool of organic C. Bacteria decompose these C sources into CO₂ and CH₄ (St. Louis et al., 2000). Often, wetlands have an aerobic zone at the surface of subaqueous sediments, underlain by an anaerobic zone, which facilitates various reduction-oxidation reactions. In the aerobic zone, heterotrophic bacteria decompose organic matter and respire CO₂. Decomposition of this available organic matter leads to oxygen depletion in the water column and sediments (Huttunen et al., 2003). Methanogens can then use CO₂ and acetate, both of which are produced during degradation of organic matter (Conrad, 2007), as electron acceptors, producing CH₄ (Huttunen et al., 2003). This CH₄ can become oxidized into CO₂ as it travels upwards through the aerobic zone and the water column. CO₂ and CH₄ can then diffuse from the water into the air. CH₄ can also be converted to CO₂ in the oxidized rhizosphere of emergent vegetation (Gerard and Chanton, 1993). Additionally, CH₄ can be transported and emitted via the vascular system of plants (Chanton and Whiting, 1995).

Oxygen gradients can also stimulate N₂O generation due to aerobic nitrification and/or anaerobic denitrification (Huttunen et al., 2003). Rivers in many locations transmit considerable loads of NO₃⁻ that can be transformed to N₂O through denitrification in anaerobic zones. Also, organic N and ammonium (NH₄⁺) are converted to nitrate (NO₃⁻) in the aerobic water column and sediments – which can directly yield N₂O or result in N₂O during subsequent denitrification (Khalil et al., 2004).
In a review of GHG emissions from reservoirs, St. Louis et al. (2000) state that the potential for GHG emission is related to the amount of organic matter present, the age of the reservoir, and water temperature. However, there are few data on emission from beaver ponds, and the spatial and temporal dynamics of these ecosystems create high uncertainty about their importance in landscape and regional scale GHG budgets. We hypothesized that 1) beaver ponds would be significant sources of GHG, 2) that emission rates are highest in summer when temperatures are most elevated and when the rate of \( \text{CH}_4 \) transport from plant roots is most pronounced and 3) that emissions are highest in ponds with long water retention times and high depths of sediment organic matter. We tested these hypotheses by measuring the diffusive flux of \( \text{CH}_4, \text{CO}_2 \) and \( \text{N}_2\text{O} \) from the air-water interface of three beaver ponds in Rhode Island, USA using floating static gas chambers during spring, summer, and fall seasons.

**METHODS**

*Study Sites*

We selected three beaver ponds that varied in age and size in Washington County, Rhode Island, USA: two were located on the Chipuxet River (Ponds A and B) and one was located on Roaring Brook (Pond C). Based on digital aerial photos available in four year intervals from 1976 to 2012 (RIGIS, 2009), the dams were first observed in 1988, 1992 and 2008, respectively, at ponds C, A, and B. We coupled the digital imagery with geographical information system (GIS) to determine the current pond area at each site, which ranged from 0.05-8.00 ha (Table 1).

*Static Chambers*

We used static floating chambers (not equipped with an air circulation system) to measure diffusive fluxes from the ponds to the atmosphere (Moore and Roulet,
Gas samples were collected and then analyzed remotely. Following protocols from many other studies gas flux was calculated based from the linear rate of gas accumulation in the chamber over time (St. Louis et al., 2000).

Five 27-L floating static chambers were launched in sequence at each pond from a canoe and were positioned in open water at least three meters away from the canoe, pond edges, and emergent vegetation (Figure 1). Efforts were made to keep the chambers from accumulating together or drifting closer to the canoe by gently pushing them away with a long pole. Five 10-mL gas samples were drawn from each chamber over the course of one hour via air-tight syringes and placed into 10 ml pre-evacuated vials. We collected headspace samples from each chamber at 0, 15, 30, 45, and 60 minutes after chambers were deployed.

After headspace sampling was completed, time of day, air temperature, water temperature, and dissolved oxygen (DO) were recorded. A 20-mL water sample for dissolved organic carbon (DOC) was filtered through a muffled filter disc into an amber glass vial. Water samples were collected for pH, NO$_3^-$, and NH$_4^+$ analysis, transported in a cooler, and stored at 4°C until analysis. Chambers were deployed and sampled at each of the three sites on a total of 18-19 days; 3-4 days in fall, 6-8 in the spring, and 8-9 in the summer.

**Diffusive Fluxes of GHGs.**

Gas fluxes were determined via linear least squared regression of changes in gas concentration over time, as described in Huttunen et al. (2002). Acceptability criteria for each gas were developed in accordance with published values (Duchemin et al., 1995; Duchemin et al., 1999; Huttunen et al., 2002; Soumis et al., 2004; and
Tremblay et al., 2005). The $R^2$ value for acceptance was 0.90 for CO$_2$ data and 0.85 for N$_2$O and CH$_4$. The $n$ value (number of chambers per site) for each sampling date was $\leq 5$, depending on the number of chambers that met the acceptance criteria. Slope of each regression was expressed in uLL$^{-1}$min$^{-1}$. Because more than half of all the N$_2$O flux measurements did not display significant changes in slope over time, we were concerned that following published acceptability criteria could bias the data by ignoring actual observations with negligible flux. Accordingly, we also analyzed the N$_2$O results from chamber measurements where the slope derived from the linear least squared regression did not meet the acceptance criteria. This primarily resulted in the inclusion of data that generated small positive (or negative) rates which lowered our estimates of N$_2$O flux.

The flux of all measurements used for statistical analyses was then scaled up to a daily rate and multiplied by the density of the gas and headspace volume of the chamber to obtain mass of gas over time ($\mu$gd$^{-1}$). This rate was divided by surface area of pond covered by the chamber to express rates in mg m$^{-2}$ d$^{-1}$ for comparison with other studies.

Additional Analyses

Pond retention times were determined from the ratio of pond volume to average seasonal flow rates into each pond. Average seasonal flow rates were obtained by multiplying watershed area of each pond (calculated from USGS Streamstats; National Land Cover Dataset, 2006, accessed February 12, 2013; http://streamstats09.cr.usgs.gov/) by the normalized seasonal flow data in (flow per unit area) from USGS datasets developed for the study region (Armstrong et al.,
Pond volumes were calculated from field measurements of depth and cross sectional areas.

We collected subaqueous (below the water) sediment cores from each pond with a soil coring device from a canoe during fall 2011, spring 2012, and summer 2012 (Lazar et al., 2013). Each season we collected 16 cores (6 cm diameter and 13 cm depth) from the inundated sediment at random locations within each of the three beaver ponds. Subsamples of the cores were analyzed for microbial biomass using a rapid chloroform-fumigation extraction technique (Witt et al., 2000). For analysis of organic soil C, sediments were dried and ground through a 2 mm (size 10) sieve with material not passing through the sieve being discarded. A small subsample (5-8 mg) was weighed into a tin capsule and stored in a desiccator until analysis. Depth of organic matter was evaluated at a minimum of 7 locations at each pond using a 3 m tile probe, and reported as an average of depth to mineral soil throughout the pond.

Sample Analyses

Gas samples were analyzed on a Shimadzu GC-2014 Greenhouse Gas Analyzer (Kyoto, Japan), with a flame ionization detector for CO₂ and CH₄, and an electron capture detector for N₂O. We measured NO₃⁻-N and NH₄⁺ concentrations in water samples using Astoria Pacific Model 303A Segmented Continuous Flow Autoanalyzer (Astoria-Pacific Inc., Clackamas, OR). On this instrument, the open tubular cadmium reduction method (APHA et al., 1995) was used for NO₃⁻-N and the alkaline phenol and hypochlorite methods (APHA et al., 1995) were used for NH₄⁺-N.

Fumigated and unfumigated sediment extracts were analyzed for DOC using a Shimadzu Total Organic Carbon Analyzer (Kyoto, Japan). Total C was analyzed with
a Vario Micro Elemental Analyzer (Elementar Americas, Mt. Laurel, NJ). DO and temperature were measured in the field using a YSI DO-temperature meter, model 55 (YSI, Yellow Springs, Ohio). pH was measured on Accument Research AR20 pH/conductivity meter.

**Statistical Analyses**

We tested for differences between site and season using two-way Analysis of Variance (ANOVA) with a Tukey’s post hoc test for CH$_4$, CO$_2$, and N$_2$O emissions and soil microbial biomass C. We evaluated correlation between GHG generation rates and pH, DOC, DO, and air and water temperature data using Pearson product-moment correlation coefficients. Statistical significance was set at $\alpha <0.05$ for all analyses. Two-way ANOVA analyses were performed using SAS Software version 9.2 and all other statistical analyses were performed with Analyse-it version 3.0.

**RESULTS**

*Site and Seasonal Characteristics*

Sediment pH was similar to water pH (Pond A: 6.3, Pond B: 6.0), Pond C had slightly more acidic sediment with a pH of 5.5 (Table 1). Nitrate concentrations were highest at Pond B and lowest at Pond C (0.90 mg L$^{-1}$ and below detection limits of 0.02 mg L$^{-1}$, respectively) (Table 1). As expected, air and water temperatures followed seasonal patterns with summer being the warmest and having the lowest water column DO (Table 2). Spring DO was found to be significantly higher than the other two seasons (Table 2). DO was never found to be below 2.0 mg L$^{-1}$.

DOC did not vary significantly between ponds or seasons. Seasonal means ranged from 4.5-5.7 mg L$^{-1}$ with spring DOC being highest (Table 3). Sediment microbial biomass C did not vary significantly between ponds; however, biomass C
across all ponds was significantly different between seasons, with spring displaying the highest values (Table 3).

*Greenhouse Gas Emission Rates*

CH\textsubscript{4} emission rates showed no significant seasonal differences and ranged from 154.9-208.4 mg CH\textsubscript{4} m\textsuperscript{-2} day\textsuperscript{-1}. There were significant (p<0.0001) differences between sites with Pond B generating 2.5 to 9 fold higher CH\textsubscript{4} emission rates than Ponds A or C (Table 4).

Site and season were found to be significant for CO\textsubscript{2} emission rates based on a two-way ANOVA (Table 5). Fall generated CO\textsubscript{2} emission rates were markedly and significantly higher (e.g., 3 fold difference; p<0.0001) than CO\textsubscript{2} emissions during spring or summer. Pond A had significantly higher (p<0.05) CO\textsubscript{2} emission than the other ponds, but mean differences were less than 20%.

When using the acceptance criteria there were no significant differences between site or season for N\textsubscript{2}O emission rates (Table 6). Mean emission rates ranged from 0.96-1.09 mg N\textsubscript{2}O m\textsuperscript{-2} day\textsuperscript{-1} throughout the three seasons. Since 14 of the 55 total sampling days did not generate significant trends in N\textsubscript{2}O emissions over the one hour sampling period, considerable data were excluded from Table 6. When we analyzed the N\textsubscript{2}O results including all observations the mean emission rates ranged from 0.14-0.51 mg N\textsubscript{2}O m\textsuperscript{-2} day\textsuperscript{-1} throughout the three seasons (Table 7). “Site” was found to be significant for N\textsubscript{2}O emissions based on a two-way ANOVA, with Pond C having significantly lower emissions than the other two ponds (Table 7).

CH\textsubscript{4} emission rates were significantly correlated with both air and water temperature (p<0.01, n=258, r=0.199 and 0.199, respectively). CO\textsubscript{2} emission rates
were significantly correlated with the DOC concentration of pond water \((p<0.05, n=223, r=0.143)\). No other significant correlations were found.

*Greenhouse Gas Emissions in CO\(_2\) Equivalents*

To assess the relative greenhouse forcing strength of the cumulative GHG emissions, CO\(_2\), CH\(_4\), and N\(_2\)O fluxes were converted into common units \((\text{mmol m}^2 \text{d}^{-1})\) and multiplied by their respective global warming potentials—CO\(_2\) by 1, CH\(_4\) by 25, and N\(_2\)O by 298 (Figure 2). Overall our sites and seasons, CH\(_4\) comprised 67.5% of the global warming potential of GHG emissions, while CO\(_2\) and N\(_2\)O (with acceptance criteria) constituted the remaining 30.9% and 1.7%, respectively.

**DISCUSSION**

This study examined GHG emissions from the water-air interface of beaver ponds, which have been increasing steadily in southern Rhode Island and across Northeastern North America over the past several decades. Our results show that these beaver ponds have significant CH\(_4\) and CO\(_2\) emissions, as others have shown in the past (Naiman et al., 1991; Yavitt et al., 1992). In addition twenty-one reservoirs were found to be net sources of CO\(_2\) and CH\(_4\) (St. Louis et al., 2000).

*Beaver Pond Emissions*

Mean CH\(_4\) emissions were within the upper range of other previous beaver pond studies (Table 8). The beaver ponds with the lowest CH\(_4\) emissions tend to be in colder climates or in ponds with more aerobic bottom sediments. Pond B had significantly higher CH\(_4\) emissions than the other ponds, with very high variance (Table 4). Although Pond B was the youngest pond, it had the greatest depth of organic matter. It was also substantially smaller than the other ponds (average
diameter of just 25 meters) and largely protected from the wind by a surrounding tree canopy. These characteristics suggest conditions of limited mixing of oxygenated waters with the bottom sediments, potentially creating anaerobic conditions at the water-sediment interface. Bubbles from ebullition may account for some of the variability observed in the CH$_4$ rates (Husted, 1994). Wagner et al. (2003) noted that extensive oxidation of CH$_4$ can occur if only 5 cm of oxidized sediment overlies the zone of methane production. In addition, shallow water columns may limit the time for microorganisms to oxidize the CH$_4$ into CO$_2$ before release into the atmosphere (Keller and Stallard, 1994).

We observed significantly higher CO$_2$ emissions during the fall. Although temperatures were highest during the summer, the increased CO$_2$ emission rate may have been due to high rates of respiration associated with the degradation of fresh input of allochthonous C (fresh leaves) and from plant senescence in the fall (Ford and Naiman, 1988; Gessner, 1991). Bosetta and Agren (1985) suggest that fresh organic matter is highly decomposable and becomes increasing more recalcitrant through the decay process.

When using acceptance criteria (Table 6) N$_2$O emissions were high relative to more aerobic, terrestrial ecosystems and comparable to many aquatic systems (Table 9). Many incubations did not have significant N$_2$O production and therefore without using acceptance criteria the fluxes of N$_2$O lowered. Bodaly et al. (2004) found reservoirs to be sinks of N$_2$O, and N$_2$O fluxes from boreal ponds were found to be negligible (Huttunen et al., 2002).
Pond C, which has the lowest nitrate concentration and is the largest of the sites (Table 1), was a small sink for N\textsubscript{2}O (Table 7). In a related study by Lazar (2013), Pond C had significantly lower denitrification rates than the other sites, which follows previous research showing open water wetlands had lower denitrification potential than emergent macrophyte wetlands (Anderson et al., 2005 and Hernandez and Mitsch, 2007). A recent study in a eutrophic pond, found low N\textsubscript{2}O production (<0.01 mg m\textsuperscript{-2} day\textsuperscript{-1}) due to denitrification going to completion, i.e. any N\textsubscript{2}O was reduced to N\textsubscript{2} (Gao et al., 2013). The high CH\textsubscript{4} emissions that we observed suggest that sediments in our ponds are anaerobic, which fosters complete denitrification. Song et al. (2009) reported N\textsubscript{2}O emissions from wetlands ranging from 0.47-1.2 mg m\textsuperscript{-2} day\textsuperscript{-1} which are comparable to our beaver pond N\textsubscript{2}O emissions (Table 9). The beaver pond sediments are fully saturated with water and several studies suggest that N\textsubscript{2}O emissions tend to peak when sediments are partially saturated, declining markedly at full saturation (Davidson et al., 2000; Jungkunst et al., 2008). Our low N\textsubscript{2}O emissions may be also be due to low nitrate concentrations limiting rates of denitrification (Table 1). These beaver ponds are located in forested watersheds, with low agricultural activity and high capacity for nitrate removal by riparian zones.

*Environmental Drivers of GHG Emissions*

CH\textsubscript{4} emissions were positively correlated with air and water temperature. Increasing temperatures increase rates of organic matter decomposition and microbial activity, and under anaerobic sediment conditions, lead to increased CH\textsubscript{4} emissions. The significant correlation between temperature and CH\textsubscript{4} production is consistent with previously published results (Roulet et al., 1997; Conrad, 2007). As temperatures
increase with climate change, more methane is likely to be released, creating a positive feedback (IPCC, 2007).

CO₂ emissions were positively correlated to DOC, which is consistent with results reported by Hope et al., (1996) for Wisconsin lakes. Increased DOC concentrations in ponds may be caused by increased decomposition of organic matter, and CO₂ is a byproduct. A long-term regional study by Laudon et al. (2012) concluded that average stream DOC is related to mean annual temperature. Optimum conditions for DOC production and export is 0-3°C, beyond that temperature, high mineralization rates reduce production of DOC. For this reason DOC is expected to decrease with increasing temperatures (Laudon et al., 2012). Conversely, decreased atmospheric deposition has increased DOC concentrations in streams (De Wit et al., 2007).

There were no patterns of younger ponds having more or less global warming potential (in CO₂ equivalents) of GHGs than older ponds. Pond B, the youngest pond, had significantly higher CH₄ than the other sites, but not significantly higher CO₂ or total global warming potential. Previously published work shows GHG fluxes both decreasing (St. Louis et al., 2000 and Duchemin et al., 2002) and increasing (Soumis et al., 2004) with age of pond. The oxidative state of the sediment/water interface and the mass and quality of labile carbon inputs – two properties that we were not able to measure – are likely confounding factors.

*Estimating Watershed Scale Emissions from Beaver Ponds*

We hypothesized that beaver ponds would generate high emissions of GHG. To compare the observed beaver pond emissions to studies of other ecosystems, we extrapolated the measured seasonal rates over 273 days to represent the Fall,
Spring and Summer seasons when we obtained measurements. Given that emissions may occur over the winter months, this assumption is likely an underestimate of annual GHG generation. On a per unit area basis, the beaver ponds in our study yielded much higher annual GHG emissions, expressed as global warming potential (Figure 3) than upland land uses in temperate settings. Comparing median values, the annual global warming potential of beaver ponds per unit area were generally more than 20 times higher than fertilized grasslands (Freibauer, 2003) and 2 fold higher than upland forests (Bowden et al., 2000).

However, beaver ponds have a limited areal footprint within the landscape. In southern New England, we estimate that beaver ponds may constitute only 0.3 -0.7% of catchment area. Our estimate of is based on the work DeStefano et al. (2006) who found 0.7 beaver ponds per km² of catchment area, coupled with two estimates of beaver pond area – the median beaver pond area (0.26 ha) from our three sites and a pond area of 1.0 ha, which represents a minimum size from many other studies (Weyhenmeyer, 1999 and Pollock et al., 2003). Given this limited footprint, the recent return of beaver ponds are not likely to dramatically increase GHG emission from the rural landscapes of the northeast. We estimate that beaver ponds in this study are contributing 11 Mg km⁻² of catchment area yr⁻¹ of global warming potential expressed as CO₂ equivalents. In comparison, the median global warming potential of emissions from upland temperate deciduous forests are estimated at approximately 1,700 Mg km⁻² of catchment area yr⁻¹ while emissions from fertilized grasslands have been documented at approximately 175 Mg km⁻² yr⁻¹. These rates are based solely on gaseous emissions, not taking into account sequestration in plants and soils.
The net effect of beaver ponds on the global warming potential of GHG emissions of the Northeast U.S. will depend not only on the ponded areas of the beaver ponds, but also upon changes in the areal extent of riparian lands with elevated water tables. In most riparian sites of the Northeast U.S. water tables display seasonal patterns, rising during the wet season and falling during summer and early fall. A beaver dam can “reset” the boundary conditions that govern groundwater drainage of the riparian land adjacent to the ponds – potentially increasing the temporal areal extent of saturated or partially saturated forest soils and switching these areas from upland GHG sinks to partially wet GHG sources. In a series of microcosm studies on three different wetland soils, Jungkunst et al. (2008) showed that rising water tables (from -40 to -5 cm from the ground surface) exerted control over greenhouse gas emissions as soils switched from aerobic to anaerobic metabolism. CO₂ emissions from the soils decreased with rising water tables, but consistent CH₄ emissions were not observed until water tables were close to the surface (-5 cm). They found the highest global warming potential (expressed as CO₂ equivalents) from forested wetlands when water tables were -20 cm, and both CO₂ and N₂O were the major components of the total emissions (Figure 3). These water table levels may reflect optimum moisture conditions for N₂O generation (Davidson et al., 2000).

A complete examination of the greenhouse gas budget (i.e., net greenhouse gas exchange expressed as the global warming potential in CO₂ equivalents) of beaver ponds requires consideration of both GHG emissions and the long-term fate of carbon that is sequestered in beaver pond sediments. Indeed, both temperate forests and grasslands serve as net C sinks when both emissions and sequestration are quantified
(Valentini et al., 2000; Follett, 2001; Gilmanov et al., 2007). Naiman et al., (1988) found that abandoned beaver ponds in boreal areas may persist for centuries as marshes, bogs and forested wetlands, rather than reverting back to the pre-ponded landscape. Some of the organic material in the beaver ponds might pre-date the establishment of the beaver ponds if they developed in riverine marshes or riparian wetland forests that were subsequently inundated by the pond. Pollen analyses and other dating methods would be required to characterize the pedogenesis throughout the organic horizons of the ponds (Ricker et al., 2012). The carbon stocks accumulated in beaver ponds may be stored under anaerobic conditions for extended periods. This storage might be offset however, by high CH$_4$ and N$_2$O emissions under anaerobic conditions. Alternatively, if beaver ponds are subject to more intensive disturbance and drainage, the carbon rich sediments may undergo more rapid aerobic decomposition to CO$_2$, either in situ or as it is transported through the fluvial ecosystem. Further studies that track the pattern and conditions of abandoned beaver ponds over time will be essential to understanding their role as greenhouse gas sources or sinks.

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TABLE 1. Site characteristics on sampling dates. Values for water depth, depth of organic sediment, water and sediment pH, sediment carbon content and water nitrate concentration are mean, standard deviation (n value).

|                  | A                             | B                             | C                             |
|------------------|-------------------------------|-------------------------------|-------------------------------|
| Lat/Long         | 41.486175/71.548384           | 41.503464/71.533608           | 41.565725/71.677929          |
| Surface Area (ha)| 0.26                          | 0.05                          | 8.00                          |
| Drainage Area (ha)| 2450                        | 2093                          | 976                           |
| Retention Time (hours)| 1.8                       | 0.3                           | 111.4                         |
| Tributary (name and stream order) | Chipuxet, 2 | Chipuxet, 2 | Roaring Brook, 1 |
| Water Depth (m)† | 0.93, 0.49 (23)a             | 0.59, 0.25 (16)b             | 0.75, 0.23 (38)ab            |
| Depth of Organic Sediment (m)† | 0.29, 0.29 (15)b | 0.66, 0.25 (7)b | 0.45, 0.19 (8)ab            |
| Pond Water pH†  | 6.3, 0.1 (19)a               | 6.3, 0.2 (20)a               | 6.0, 0.2 (18)b               |
| Sediment pH†    | 6.3, 0.2 (6)a                | 6.0, 0.4 (7)a                | 5.5, 0.1 (5)b                |
| First documented evidence (yr) | 1992                       | 2008                          | 1988                          |
| Sediment carbon (%)† | 18.3, 5.99 (42)b | 15.0, 2.97 (46)b | 29.8, 13.46 (43)a            |
| Nitrate (mg N L⁻¹)† | 0.30, 0.22 (17)b | 0.89, 0.21 (19)a | Below detection <0.02 (16)c |
| Oxygen (mg L⁻¹)† | 5.1, 2.6 (18)b               | 6.2, 3.4 (19)a               | 5.4, 1.9 (18)ab              |

†Means within a row with distinct superscript letters are significantly different (p<0.05) as determined by a Tukey’s post hoc mean separation test following a 1 way ANOVA.

TABLE 2. Seasonal characteristics on sampling dates.

|                         | Spring                  | Summer                | Fall                  |
|-------------------------|-------------------------|-----------------------|-----------------------|
| Air Temperature (°C)†   | 19.5, 7.0 (23)b         | 29.7, 3.9 (25)a       | 16.5, 3.3 (9)b        |
| Water Temperature (°C)† | 16.5, 5.3 (23)b         | 24.5, 3.4 (25)a       | 13.8, 3.2 (9)b        |
| Dissolved Oxygen (mg L⁻¹)† | 8.0, 2.4 (21)a | 4.1, 1.1 (25)b       | 4.9, 1.8 (9)b         |

†Means within a row with distinct superscript letters are significantly different (p<0.05) as determined by a Tukey’s post hoc mean separation test following a 1 way ANOVA.
### TABLE 3. Dissolved organic carbon and microbial biomass C throughout the seasons. Values are mean, standard deviation, (n value).

|                        | Spring       | Summer       | Fall          |
|------------------------|--------------|--------------|---------------|
| Dissolved Organic Carbon (mg C L\(^{-1}\)) mean | 5.7, 2.1 (16) | 4.6, 1.4 (24) | 4.5, 0.7 (9) |
| Microbial Biomass Carbon (mg C kg\(^{-1}\) soil dry soil) mean (SD) † | 188.4, 84.0 (27)\(^a\) | 95.7, 68.0 (27)\(^b\) | 16.9, 18.8 (27)\(^c\) |

†Means within a row with distinct superscript letters are significantly different (p<0.05) as determined by a Tukey’s post hoc mean separation test following a 1 way ANOVA.

### TABLE 4. CH\(_4\) emission rates (mg CH\(_4\) m\(^{-2}\) day\(^{-1}\)) from beaver ponds. Values within each cell are means, standard error (n value). "Grand Means" cells are mean, standard error. Grand means are weighted averages of the entire sample of interest, not the average of the means. Note that n values (each n representing one chamber on one date) were not equal.

|                        | Spring       | Summer       | Fall          | Grand Means† |
|------------------------|--------------|--------------|---------------|--------------|
| Pond A                 | 32.0, 17.0 (33) | 51.8, 16.1 (40) | 29.3, 7.0 (15) | 40.5, 9.8\(^b\) |
| Pond B                 | 276.1, 77.4 (32) | 487.7, 112.9 (31) | 303.2, 120.8 (17) | 363.9, 57.1\(^a\) |
| Pond C                 | 100.9, 13.3 (30) | 155.2, 18.3 (35) | 139.5, 19.8 (15) | 134.5, 11.0\(^b\) |
| Grand Means            | 136.0, 23.7 | 208.4, 35.4 | 163.5, 47.2 |

†Grand means within a row or column with distinct superscript letters are significantly different (p<0.05) as determined by a Tukey’s post hoc mean separation test following a 2 way ANOVA (site and season).
TABLE 5. CO$_2$ emission rates (mg CO$_2$ m$^{-2}$ day$^{-1}$) from beaver ponds. Values within each cell are mean, standard error (n value). "Grand Means" cells are mean, standard error. Grand means are weighted averages of the entire sample of interest, not the average of the means. Note that n values (each n representing one chamber on one date) were not equal.

|       | Spring          | Summer         | Fall             | Grand Means† |
|-------|-----------------|----------------|------------------|--------------|
| Pond A | 3245.5, 173.7 (34) | 4282.1, 251.2 (38) | 7898.3, 407.1 (15) | 4500.5, 227.5$^a$ |
| Pond B | 3474.1, 436.9 (35) | 3216.9, 223.9 (33) | 5473.6, 419.2 (20) | 3832.1, 235.5$^b$ |
| Pond C | 3160.2, 242.3 (27) | 2243.9, 135.6 (45) | 15795.9, 1348.0 (15) | 4018.1, 593.8$^{ab}$ |
| Grand Means$^†$ | 3304.9, 184.3$^b$ | 3188.4, 141.3$^b$ | 9297.7, 767.2$^a$ |              |

†Grand means within a row or column with distinct superscript letters are significantly different (p< 0.05) as determined by a Tukey’s post hoc mean separation test following a 2 way ANOVA (site and season).

TABLE 6. N$_2$O emission rates (mg N$_2$O m$^{-2}$ day$^{-1}$) from beaver ponds. Values within each cell are mean, standard error (n value). "Grand Means" cells are mean, standard error. Grand means are weighted averages of the entire sample of interest, not the average of the means. Note that n values (each n representing one chamber on one date) were not equal.

|       | Spring    | Summer    | Fall‡   | Grand Means |
|-------|-----------|-----------|---------|-------------|
| Pond A | 0.96, 0.1 (18) | 1.52, 0.4 (14) | 0, no data (0) | 1.20, 0.2 |
| Pond B | 0.98, 0.1 (16) | 1.00, 0.2 (23) | 1.09, 0.4 (12) | 1.02, 0.1 |
| Pond C | 0.90, 0.2 (4) | 0.38, 0.0 (6) | 0, no data (0) | 0.59, 0.2 |
| Grand Means | 0.96, 0.1 | 1.08, 0.2 | 1.09, 0.4 |

‡No significant differences were found between "Grand Means" within a row or column (p< 0.05) as determined by a Tukey’s post hoc mean separation test following a 2 way ANOVA (site and season). †In the fall two ponds did not generate N$_2$O.
TABLE 7. N\textsubscript{2}O emission rates (mg N\textsubscript{2}O m\textsuperscript{-2} day\textsuperscript{-1}), without using acceptance criteria, from beaver ponds. Values within each cell are mean, standard error (n value). "Grand Means" cells are mean, standard error. Grand means are weighted averages of the entire sample of interest, not the average of the means. Note that n values (each n representing one chamber on one date) were not equal.

|                | Spring       | Summer     | Fall\textsuperscript{2} | Grand Means |
|----------------|--------------|------------|--------------------------|-------------|
| Pond A         | 0.69, 0.08 (35) | 1.03, 0.65 (40) | -0.03, 0.08 (15)       | 0.72, 0.29 \textsuperscript{a} |
| Pond B         | 0.80, 0.08 (36) | 0.54, 0.11 (44) | 0.51, 0.09 (20)       | 0.63, 0.06 \textsuperscript{a} |
| Pond C         | -0.07, 0.1 (30) | 0.01, 0.08 (45) | -0.17, 0.08 (15)       | -0.05, 0.05 \textsuperscript{b} |
| **Grand Means** | **0.50, 0.06** | **0.51, 0.21** | **0.14, 0.07**         |             |

\textsuperscript{†}No significant differences were found between "Grand Means" within a row or column (p< 0.05) as determined by a Tukey’s post hoc mean separation test following a 2 way ANOVA (site and season). \textsuperscript{‡}In the fall two ponds did not generate N\textsubscript{2}O.

TABLE 8. CH\textsubscript{4} emission comparison to other static-chamber studies.

| Study                   | Setting                      | CH\textsubscript{4} mg m\textsuperscript{-2} day\textsuperscript{-1} |
|-------------------------|------------------------------|---------------------------------------------------------------------|
| Bowden et al., 2000     | Pennsylvania forest          | 0                                                                   |
| Groffman et al., 2006   | Northern Hardwood forest     | 1                                                                   |
| Soumis et al., 2004     | Sierra Nevada region, reservoir | 3-10                                                               |
| Duchemin et al., 1995   | 1500 km north of Montreal, reservoirs | 15                                                               |
| Ford and Naiman, 1988   | Quebec, beaver ponds         | 27                                                                  |
| Naiman et al., 1991     | Minnesota, beaver ponds      | 78                                                                  |
| Roulet et al., 1997     | Boreal region, Canada, beaver ponds | 109                                                               |
| Yavitt et al., 1992     | Adirondack, beaver ponds     | 150                                                                 |
| **Lazar et al., 2013 (this study)** | **Rhode Island, beaver ponds** | **174**                                                            |
| Yavitt et al., 1990     | West Virginia, beaver pond   | 250                                                                 |
| Keller and Stallard, 1994 | Panama, lake formed by dam | 725                                                                 |
| Hlavacova et al., 2006  | Czech Republic, stream emissions | 6500                                                            |
| Study                      | Setting                           | $\text{N}_2\text{O} \text{ mg m}^{-2} \text{ day}^{-1}$ |
|----------------------------|-----------------------------------|-------------------------------------------------------|
| Brumme et al., 1999        | temperate forest, Germany         | 0.17                                                  |
| Hlavacova et al., 2006     | stream emissions                  | 0.31                                                  |
| Bowden et al., 2000        | forest, Midwest USA               | 0.31                                                  |
| Song et al., 2009          | wetlands, Northeast China         | 0.47-1.2                                              |
| Groffman et al., 2006      | forest, Northeast USA             | <1                                                    |
| **Lazar et al., 2013**     | **beaver pond, Northeast USA**    | **0.14-1.09**                                         |
| Clough et al., 2006        | spring fed river, New Zealand     | 4.1                                                   |
| Jungkunst et al, 2008      | hydric temperate forest, Germany  | 16.9-36.3                                             |
FIGURE 1. Floating static chambers on a beaver pond.

FIGURE 2. Total greenhouse gas emissions expressed in CO$_2$ equivalents. These rates do not account for sequestration from plants and soils.
FIGURE 3. Average emission ranges in CO$_2$-equivalents per hectare from fertilized grassland (Freibauer, 2003), conventional and conservation tillage of a corn/soybean site in Iowa and a corn/wheat site in Hebie, China (Changsheng et al., 2008), upland temperate deciduous forests (Bowden et al., 2000), hydric temperate deciduous forests* (Jungkunst et al., 2008) and the three beaver ponds in this study. Beaver pond emissions represent the range of the three sites in this study based on 270 days of emissions per year, assuming no emissions during winter when ice cover is likely. *Water table position is at -20cm (Jungkunst et al., 2008). These rates do not account for sequestration from plants and soils.