Denitrification in the Northern Forest

Principal Investigator: Peter M. Groffman

Cary Institute of Ecosystem, 2801 Sharon Turnpike, Millbrook, NY 12545, groffmanP@caryinstitute.org

Collaborators: Irena Creed, University of Western Ontario and Ivan Fernandez, University of Maine

Completion date: September 14, 2012

Denitrification, the conversion of reactive inorganic nitrogen (N) is returning significant amounts of atmospheric N deposition to the atmosphere in the northeastern region.

Funding support for this project was provided by the Northeastern States Research Cooperative (NSRC), a partnership of Northern Forest states (New Hampshire, Vermont, Maine, and New York), in coordination with the USDA Forest Service.
http://www.nsrcforest.org

Project Summary

Understanding the nitrogen (N) cycle at landscape and regional scales is a great challenge in environmental science. Uncertainties about the N cycle arise from large amounts of "missing N" that dominate N balances at all scales. This uncertainty has led to increased interest in N gas production as an important fate of N. Gas fluxes are perhaps the least well understood component of the N cycle due to problematic measurement techniques, high spatial and temporal variability, and a lack of methods for scaling point measurements to larger areas. We tested the hypothesis that denitrification, the anaerobic reduction of nitrate to nitrous oxide (N_2O) and dinitrogen (N_2) is a significant flux (equal to atmospheric deposition) in ecosystem, landscape and regional scale N budgets in northeastern North America. We measured N_2O and N_2 fluxes at three sites across northeastern North America that differ in N richness, soil and vegetation properties (Turkey Lakes (Ontario), Bear Brook (Maine), Hubbard Brook (New Hampshire)). We characterized soil O_2 dynamics and N gas fluxes at each of these sites and used relationships between soil, vegetation, O_2 and flux to produce preliminary estimates of the importance of denitrification at each site. Our results suggest that denitrification returns a significant portion of reactive atmospheric N deposition back to the atmosphere as unreactive N_2. At Hubbard Brook and Bear Brook, denitrification accounts for approximately 40% of deposition. These ecosystem-scale estimates are also relevant at the landscape scale as there was little variation among sampling locations at these sites. At Turkey Lakes, rates at the ecosystem scale were lower and require accounting for the variation among wetland and upland locations that were sampled to make evaluations at the landscape scale. Still, none of the ecosystems at Turkey Lakes were producing significant amounts of N gas so the landscape-scale implications of these fluxes will be minor. These are preliminary estimates that will be refined and expanded to the regional scale with further analyses. N_2 fluxes were much greater (10x) than N_2O fluxes suggesting that N_2 is the dominant end-product of denitrification in the region and that this process is returning reactive N to the atmosphere with a relatively low yield of radiatively active N_2O.

Background and Justification - 1

Understanding the nitrogen (N) cycle at landscape and regional scales is a great challenge in environmental science. Humans have doubled the annual global production of "reactive" N, leading to degradation of air and water quality and coastal ecosystems in many areas. The development of management strategies and policies to address N pollution problems has been hindered by uncertainties about the fate and transport of different N forms.

Uncertainties about the N cycle arise from large amounts of "missing N" that dominate N balances at all scales. Inputs of N in fertilizer, atmospheric deposition and sewage have been found to be substantially higher than hydrologic outputs of N in many studies, at many scales. There is much uncertainty about the fate of this excess N – is it stored in soils or vegetation? Is it converted to gas, and if so, in which forms? This uncertainty is particularly compelling in the northeastern U.S. In this region, atmospheric N deposition is high, ^{15}N tracer studies indicate that little of this N ends up in vegetation, and changes in soil N stocks are difficult to detect. At the same time, N concentrations in streams in many forested watersheds are declining, counter to expectations based on prevailing theories of N saturation and ecosystem development.

Uncertainty about N balances has led to increased interest in N gas production as an important fate of N. The gases nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) are produced by microbial processes (primarily nitrification and denitrification) in soils and sediments. These processes can convert reactive N to unreactive N_2 or to more reactive gases (NO, N_2O) that contribute to the greenhouse effect and/or to the destruction of ozone in the stratosphere, or the creation of ozone in the troposphere. Gas fluxes are perhaps the least well understood component of the N cycle. They are difficult to quantify because of problematic measurement techniques (especially for N_2), high spatial and temporal variability, and a lack of methods for spatial and temporal scalin. A particular challenge is the fact that that small areas (hotspots) and brief periods (hot moments) often make up a high percentage of N gas flux activity.

The uncertainty about N gas fluxes, and N balances, in the northeastern U.S. is relevant to both air and water quality issues in this region. There are active legislative and management efforts to address the effects of N pollution on tropospheric ozone levels, coastal eutrophication and drinking water quality, and to determine "critical loads" for atmospheric N deposition. Gas fluxes are central to all of these questions, and therefore influence the integrity of water bodies such as the Gulf of Maine and Long Island Sound and the drinking water and air quality of the most densely populated regions of the U.S.

Background and Justification - 2

In this project, we tested the hypothesis that denitrification, the anaerobic reduction of nitrate to NO, N_2O and N_2 is a significant flux (equal to atmospheric deposition) in ecosystem, landscape and regional scale N budgets in northeastern North America. We further hypothesized that variation in denitrification is driven by 1) ecosystem N richness and 2) soil oxygen (O_2) dynamics, which are controlled by rainfall, soil texture and forest floor structure. Our specific objectives were to answer the following questions:

Do rainfall events cause soils to go anaerobic for biogeochemically significant periods of time, e.g., 8 – 48 hours? Are anaerobic events restricted to certain soil types, e.g. fine texture, thick or dense forest floors?

Do rainfall-driven periods of anaerobiosis create "hot moments" of denitrification such that a series of rainfall events can lead to significant, e.g. 5 – 10 kg N ha^{-1} y^{-1} amounts of denitrification. Do hot moments only occur in N rich sites?

Are there enough hotspots and hot moments in forested landscapes to make denitrification a significant controller of the fate of atmospheric N deposition, ecosystem N retention and N export to coastal waters at the regional scale?

We addressed these questions by measuring N_2 fluxes at three sites across northeastern North America that differ in N richness, soil and vegetation properties; Turkey Lakes in Ontario (TLW, sugar maple dominated, very high N richness), Bear Brook in Maine (BBWM, contrast between N rich fertilized and N poor reference watersheds with both hardwoods and conifers) and the Hubbard Brook Experimental Forest in New Hampshire (HBEF, moderate N richness). There is past or ongoing work on N gas fluxes at all of these sites. We characterized relationships between soil moisture, soil O_2 dynamics and denitrification at each of these sites. We then used relationships between soil, vegetation, O_2 and denitrification to produce preliminary estimates of the importance of denitrification to ecosystem, landscape and regional scale N budgets for northeastern North America.

Methods

At BBWM we established a comparison of deciduous (hardwood) versus conifer (softwood) plots in with two oxygen sensors and two soil moisture/temperature sensors in each vegetation type in the reference (unfertilized) watershed. Denitrification measurements were made on cores from both fertilized and unfertilized watersheds (4 sites x 2 replicates per site).

At TLW, we installed two oxygen sensors and two soil moisture/temperature loggers in replicate upland (toeslope), outer wetland and inner wetland sites along two transects (six sites total). Extra oxygen probes (8) were purchased and installed by collaborator Irena Creed. Denitrification measurements were made on two replicate cores from each of these six sites.

At HBEF, we installed two oxygen sensors and two soil moisture/temperature loggers in three low elevation (~ 400 m) and three high (~700 m) elevation sites. Denitrification measurements were made on two replicate cores from each of these six sites.

Oxygen measurements were from Apogee diffusion-head soil O_2 sensors (SO-100 series, Apogee Instruments, Logan UT) buried with the diffusion heads at 6-cm. Soil temperature and moisture measurements were from Decagon 5TM combination probes. The sensors were controlled by Campbell Scientific Dataloggers (CR-800; Campbell Scientific, Logan UT).

Denitrification was measured using a Nitrogen-Free Atmospheric Recirculation Method (N-FARM). In this method, soil cores are encased in glass bottles connected to a gas-tight flow injection system built from Swagelok connections (Swagelok, Crawford Fitting Co., Solon, OH) inline with two Shimadzu GC8A gas chromatographs (GC; Kyoto, Japan); one with a thermal conductivity detector (TCD) to measure N_2, CO_2, and O_2 and one with an electron capture detector (ECD) to measure N_2O. The glass bottles are submerged in water and housed in a plexiglass box that is continuously flushed with Argon gas at 100 mL min.

The atmosphere within the soil core incubation system consisted of mixtures of helium and oxygen (HelOx), and incubations were carried out at different O_2 concentrations of (10%, 5%, 20%). The incubation gas was injected into the cores and then removed by very low vacuum, which was switched with a slight over-pressurization (860 torr) at 90-sec intervals. Methods development tests showed that 14 hours of this procedure was enough to ensure that the background N_2 concentrations were removed. The 14 hour vacuum/flush cycle on 90 second intervals results in 560 switches between the two, creating a long and effective serial dilution and evacuation of the headspace which removes all traces of atmospheric N_2. After the 14 hour flush/vacuum cycle, the HelOx gas was flushed through the cores for an additional 2 hours to ensure the cores were at equilibrium.

To ensure that measured N_2 production was coming from denitrification and not from leakage or degassing from small soil pores, we used the rate of N_2 production in “killed” cores to correct for any background N_2 that may not have been flushed from the soil porespace. Cores were killed by autoclaving three times over two days at 134°C for 60 mins. Killed cores were incubated at 0% O_2 (anoxic conditions) to maximize any denitrification.

Our GC-TCD can detect a change as small as 7.5 ppmv N_2 over the course of a single incubation. This translates to detection limits for N_2 on the flow-through system of 0.438 μg N $hour^{-1}$, or in areal terms 87μg N m^{-2} $hour^{-1}$.

Once cores were done flushing, the system was set to “incubate” mode wherein gases that are produced by the cores accumulate in the glass bottles. Gases were sampled at time intervals dependent on the rates of production, but were generally 5-6 hours. To sample the headspace of the bottles, 40-mL (approximately $1/10^{th}$ the volume of one core) was released from a pressurized chamber into the bottle, slightly over-pressurizing the headspace. After mixing, this over-pressurization was released and allowed to flush the system lines and loops, which contain a total volume of approximately 10 mL. The sample from the loop was then transferred onto the GC columns. Flux rates were calculated by regression of N_2 and N_2O versus time of incubation and are expressed in areal terms by dividing by the area of the core cylinder.

In addition to oxygen and soil moisture, denitrification is controlled by carbon and nitrate availability. We therefore made measurements of pools of KCL extractable inorganic N (NH_4^+ and NO_3^-) and mineralizable C and N. Mineralizable C and N are measured as the production of CO_2 and inorganic N over a 10 day laboratory incubation.

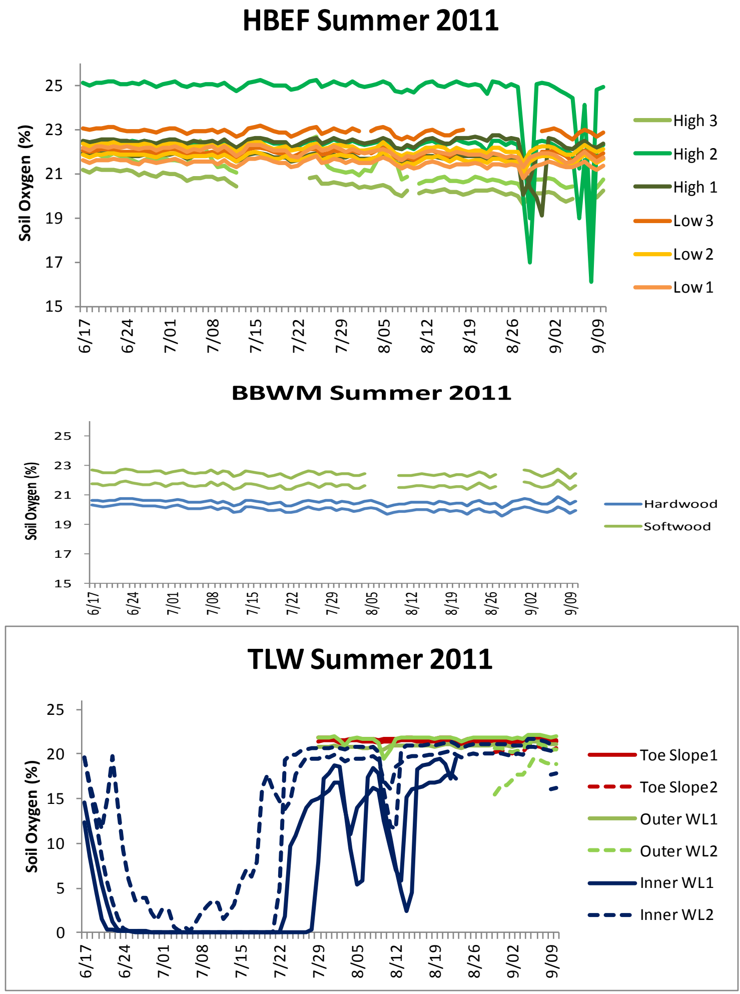
Results/Project outcomes - 1

Soil oxygen dynamics

Contrary to expectations, anaerobic conditions are not common in forest soils in the northeastern U.S.. We expected that rainfall events would cause significant periods of low (< 5%) O_2, especially in soils with fine texture or thick and/or dense forest floors. Yet, we saw little evidence of this. Rainfall events caused transient increases in soil moisture and small drops in O_2 in the forest floor, but never below 15% O_2 at any of our upland sites. It is important to note that the soil O_2 probes that we use are large enough that they provide estimates of O_2 levels in soil macropores, which are likely well connected to the atmosphere. They do not provide estimates of O_2 levels in micropores within the soil, which may have much lower O_2, especially during the periods when macropore O_2 drops in response to rainfall or snowmelt.

The absence of anaerobic conditions in our soils was likely due to the high drainage capacity of forest floor and surface horizons at our sites. Even large rainfall events drain away quickly leading to rapid declines in soil moisture that prevent the development of anaerobic conditions. The other potential driver of anaerobiosis in surface horizons would be high soil respiration. In other work, we have found that stimulating respiration by adding glucose can cause marked reductions in O_2 following wetting. Such conditions (high respiration) might occur following marked drying and rewetting, but we did not observe any such events during our study.

As expected, low O_2 was common in the wetland soils at the TLW site. These areas support conditions suitable for denitrification during nearly the entire growing season, i.e. they are potential “hotspots” of denitrification in the landscape. Overall, our O_2 data support the idea that hotspots are important drivers of denitrification but does not support the idea that rainfall and/or snowmelt driven “hot moments” are important for denitrification.

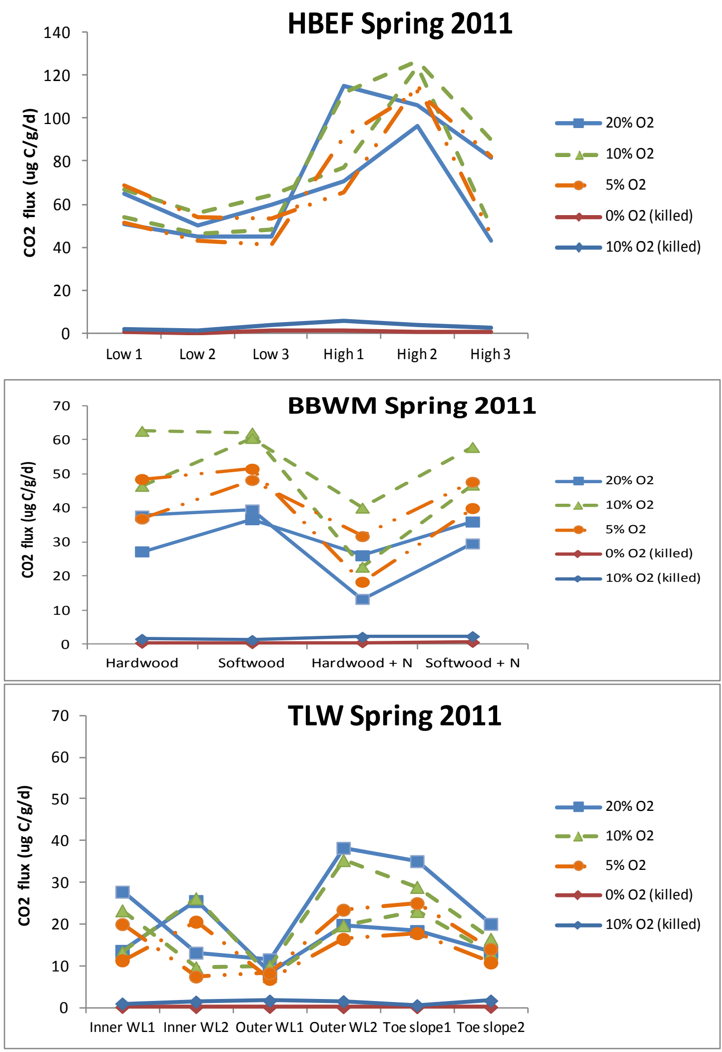


Soil oxygen at 5 cm depth at the Hubbard Brook (HBEF), Bear Brooks Watershed Maine (BBWM) and Turkley Lakes (TLW) sites during the growing season of 2011. Values greater than 22% are due to calibration uncertainty.

Results/Project outcomes - 2

Soil respiration (CO_2)

The N-FARM system provides estimates of CO_2 and N_2O production as well as N_2. The CO_2 measurements (Figure 2) provide particularly valuable context for the more variable and uncertain N gas fluxes. CO_2 data from the “killed core” incubations provide strong evidence that autoclaving eliminates detectable microbial activity in the cores and ensures that these incubations provide an excellent control for leakage and/or degassing of N_2. The CO_2 data are also useful for evaluating the effects of the prolonged use of extracted cores (up to three weeks) in the N-FARM system. For example, in the BBWM cores, respiration was highest in the first series of incubations, at 10% O_2 and then decreased in the subsequent incubations at 5% and then 20% O_2. While the decline at 5% would be expected given that respiration should be lower at this O_2 level, the decline in the third incubation, at 20% O_2 was unexpected and suggests that soils were becoming depleted of the labile carbon that fuels respiration in these cores. This depletion was only observed at the BBWM sites. Rates of respiration were highest at HBEF, followed by BBWM and then TLW. There was a marked elevation effect at HBEF, with higher rates at high elevation, consistent with previous measurements at these sites. At BBWM respiration was lower in fertilized than in unfertilized soils and at TLW the inner wetland sites had lowest respiration than the toe-slope sites.

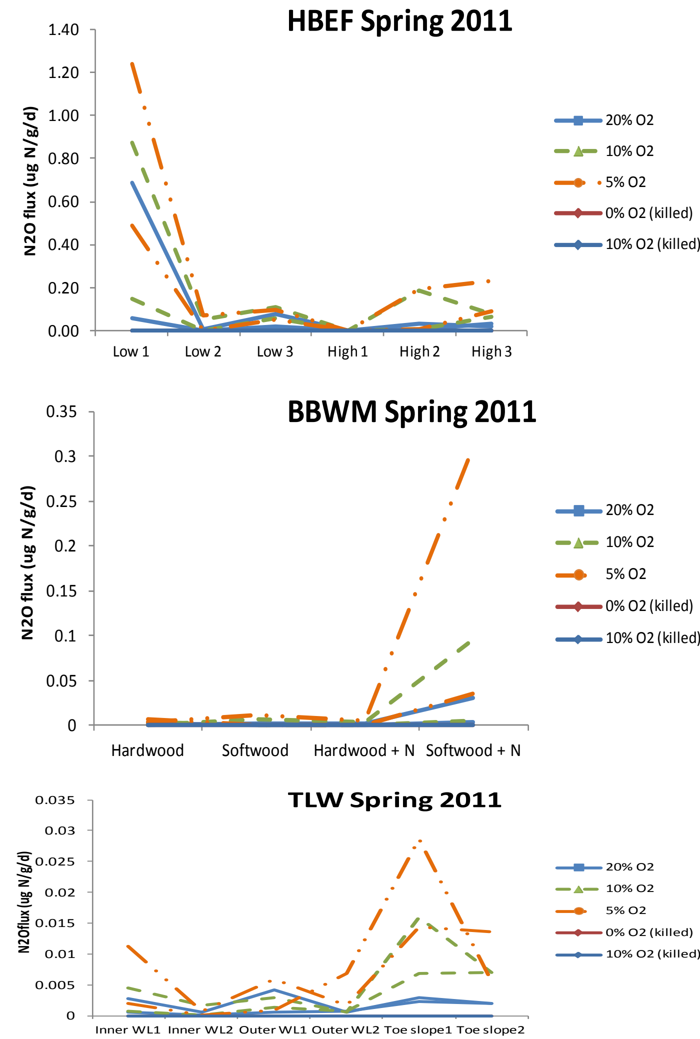


Soil respiration measured at 10%, 5% and 20% O_2 in soil cores taken from the forest floor at the Hubbard Brook (HBEF), Bear Brooks Watershed Maine (BBWM) and Turkey Lakes (TLW) sites during the growing season of 2011.

Results/Project outcomes - 3

Nitrous oxide (N_2O)

Fluxes of N_2O exhibited marked spatial patterns, with particular locations at each site having markedly higher fluxes than other locations, at all O_2 levels. At HBEF, one of the low elevation sites had the highest fluxes measured in the study, and the other locations at HBEFl had much lower fluxes. At BBWM, the fertilized softwood site was the only site with high fluxes. At TLW, both toe-slope sites had higher fluxes than the other sites. At all sites, the highest N_2O fluxes were measured at 5% O_2, which is difficult to interpret. One hypothesis is that the N_2O was being produced by denitrification, which would be expected to be highest at 5% O_2 (but see below). An alternative hypothesis is that while nitrification rates might not be highest at 5% O_2, the N_2O yield of nitrification, via nitrifier denitrification, might be highest at this O_2 level.

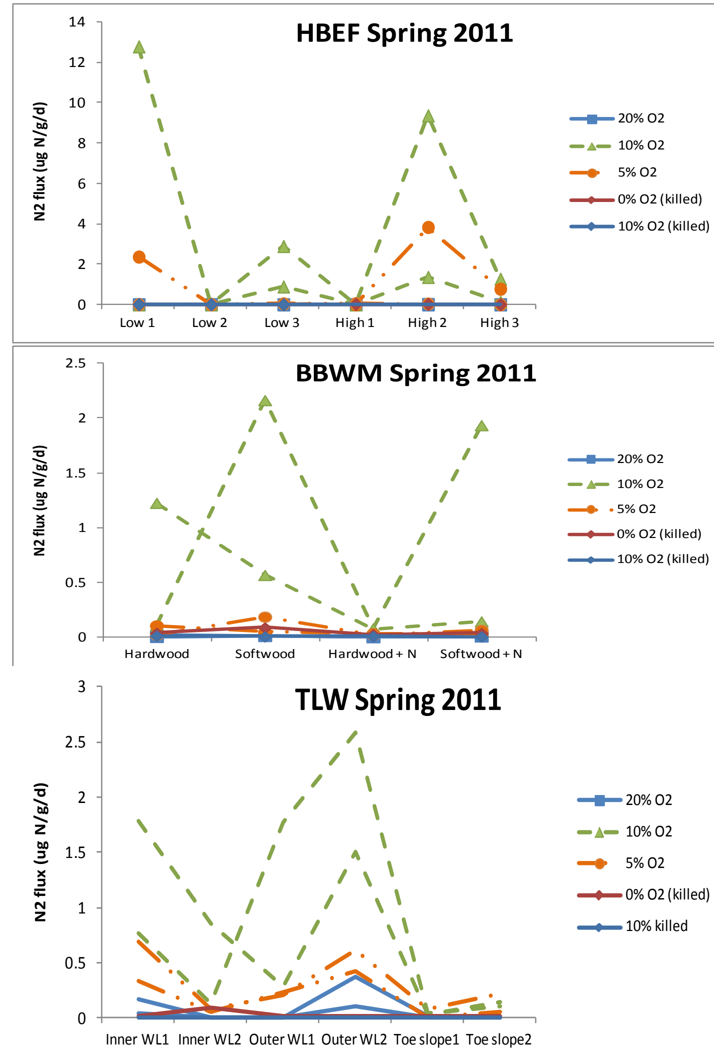


N_2O production measured at 10%, 5% and 20% O_2 in soil cores taken from the forest floor at the Hubbard Brook (HBEF), Bear Brooks Watershed Maine (BBWM) and Turkey Lakes (TLW) sites during the growing season of 2011.

Results/Project outcomes - 4

Dintrogen (N_2)

At all sites, the highest fluxes of N_2 were measured at 10% O_2. This strongly suggests that this flux was originating from a coupled nitrification-denitrification process rather than from denitrification of an existing NO_3^- pool. The highest N_2 fluxes were measured at the HBEF, with no pattern with elevation which was somewhat surprising given that the high elevation sites were wetter and had higher soil NO_3^- concentrations and net nitrification than the low elevation sites. At BBWM, there was no difference between hardwoods and softwoods or between fertilized and unfertilized watersheds. This was also surprising as we expected higher fluxes in the fertilized and softwood sites. At TLW, the toe-slope sites had lower N_2 flux than the wetland sites. This was as expected, as the wetland sites had much lower O_2 than the upland sites and likely supported higher populations of denitrifiers. The fluxes at the TLW wetland sites were not particularly high however which is surprising given the high litter quality (sugar maple) and high soil NO_3^- concentrations and nitrification rates that have been observed at this site. N_2 fluxes were much greater (10x) than N_2O fluxes suggesting that N_2 is the dominant end-product of denitrification in the region and that this process is returning reactive N to the atmosphere with a relatively low yield of radiatively active N_2O.



N_2 production measured at 10%, 5% and 20% O_2 in soil cores taken from the forest floor at the Hubbard Brook (HBEF), Bear Brooks Watershed Maine (BBWM) and Turkey Lakes (TLW) sites during the growing season of 2011.

Results/Project outcomes - 5

Ecosystem and Landscape Fluxes

Estimates of ecosystem scale N_2 flux can be produced from the measured N_2 fluxes, estimates of forest floor mass and assumptions about the number of days of relatively low O_2 conditions at the different sites. Analysis of these data are still ongoing, but preliminary estimates suggest that denitrification returns a significant portion of reactive atmospheric N deposition back to the atmosphere as unreactive N_2. At HBEF and BBWM , denitrification accounts for approximately 40% of deposition. These ecosystem-scale estimates are also relevant at the landscape scale as there was little variation among sampling locations at these sites. At TLW, rates at the ecosystem scale were lower and require accounting for the variation among wetland and upland locations that were sampled to make evaluations at the landscape scale. Still, none of the ecosystems at TLW were producing significant amounts of N gas so the landscape-scale implications of these fluxes will be minor. It is important to note that these are preliminary estimates that will be refined and expanded to the regional scale with further analyses over the next few months.

Site	Forest type	Forest floor mass (kg/m^2)[1]	Denitrification rate ($mg\ N/kg/d^{-1}$)[2]	Annual denitrification flux ($g\ N/m^2/y$)[3]
Hubbard Brook, NH	Low elevation	12.5	2.76	0.35
	High elevation		2.02	0.25
Bear Brook, ME	Hardwood control	8.4	0.67	0.06
	Softwood control	14.9	1.36	0.20
	Hardwood fertilized	8.4	0.08	0.01
	Softwood fertilized	14.9	1.04	0.16
Turkey Lakes, ON	Toe-slope	4.0	0.08	0.003
	Outer wetland	4.0	1.54	0.06
	Inner wetland	4.0	0.88	0.04

Implications and applications in the Northern Forest region

- Our results suggest that denitrification returns a significant portion of reactive atmospheric N deposition back to the atmosphere as unreactive N_2 at least at some sites. At Hubbard Brook and Bear Brook, denitrification accounts for approximately 40% of deposition. At Turkey Lakes, rates were much lower.

- N_2 fluxes were much greater (10x) than N_2O fluxes suggesting that N_2 is the dominant end-product of denitrification in the region and that this process is returning reactive N to the atmosphere with a relatively low yield of radiatively active N_2O.

Future directions

- Further research is needed to determine why denitrification rates were much lower at the Turkey Lakes site compared to the Hubbard Brook and Bear Brook sites. We hypothesize that the thin forest floors at Turkey Lakes do not support the anaerobic microsites that drive denitrification, but this needs to be tested with detailed studies.Describe possible future work in this arena, in the context of the Northern Forest region.

- Further analysis is necessary to produce landscape and regional scale estimates of flux. At Hubbard Brook and Bear Brook, our ecosystem-scale estimates are also relevant at the landscape scale as there was little variation among sampling locations at these sites. At TLW, rates at the ecosystem scale were lower and require accounting for the variation among wetland and upland locations that were sampled to make evaluations at the landscape scale. Regional scale analysis will require development of an approach to map and scale forest floor characteristics.

List of products

- Peer reviewed publications:
  - Morse, J.L., P.M. Groffman, I.Creed and I. Fernandez. Denitrification in northern forest soils. Manuscript in preparation. Expected June 2013.
  - Fernandez, I., J.L. Morse et al., Soil oxygen and nitrogen gas fluxes at Bear Brook watersheds, Maine. Manuscript in preparation. Expected June 2013.
  - Creed, I., J.L. Morse, et al., Soil oxygen and nitrogen gas fluxes at Turkey Lakes watersheds, Ontario. Manusript in preparation. Expected June 2013.

- Leveraged grants:
  - NSF DEB0919047 – Landscape and regional scale studies of denitrification.