Application of whole-ecosystem $^{15}$N tracer approaches to investigate nitrogen removal by wetlands: Pilot experiments and methods development

Abstract

As the impacts of anthropogenic nitrogen (N) pollution of the biosphere become increasingly evident, the fate of excess N in landscapes is recognized as one of the most pressing environmental research needs. Most of this N disappears somewhere in route from the sites of application (mainly on land) to coastal and offshore marine waters; however the precise regions and mechanisms of removal remains elusive. Wetlands along landscape hydrologic flow paths are likely to be important sites for removal of this nitrogen. Consequently, future wetland management, restoration and establishment decisions will likely be heavily impacted by understanding the role of wetlands as nitrogen sinks. The research proposed here involves the development and testing of new techniques for whole-ecosystem stable-isotope ($^{15}$N) tracer studies in wetlands. This venture grant was only partially spent when we obtained a CWS Postdoctoral Grant to further pursue similar research, and therefore the remaining funds were rolled into the new grant. The proposed experiments are designed to work out methods in a small groundwater discharge wetland and then apply the refined methods in a larger stream/beaver pond system. These kinds of experiments elucidate N cycle processes that transform and remove N in these complex ecosystems. This work builds on our extensive experience with such studies in streams, particularly through the LINX project, and dovetails with more in-depth research that we have recently initiated on the microbial processes responsible for N removal in aquatic sediments. This project is enabling us to establish a strong foundation with which to seek funding for a larger project to systematically examine the role of wetlands as N transformers and sinks.

Background

Wetlands have been described as the “kidneys of the landscape” because they function as sinks for excess nutrients and other contaminants, and this sink function can be significant at the landscape level in situations where large quantities of water moves through wetlands. “Throughflow wetlands” are often situated at points of ground water recharge and discharge or along streams and rivers. Biogeochemical processes in throughflow wetlands affect downstream water quality, as for example by removing nitrogen and phosphorus (Hedin et al. 1998; Tobias et al., 2001; Zedler, 2003). Nitrate (NO$_3^-$) removal by wetlands has received particular attention because of the escalating problem of NO$_3^-$ contamination of drinking water supplies, and the growing recognition that nitrogen (N) pollution of rivers causes eutrophication of marine coastal waters, leading to problems such as harmful algal blooms and O$_2$ depletion (Howarth et al., 1996; Mitsch et al., 2001). Most of the NO$_3^-$ removal is attributed to denitrification, a form of anaerobic bacterial respiration and a major source of atmospheric nitrous oxide (N$_2$O), a potent greenhouse gas (Groffman et al., 2000).

Yet despite a plethora of studies on denitrification, new studies employing isotope tracer techniques are providing evidence that alternative NO$_3^-$ uptake processes in addition to respiratory denitrification can predominate in many aquatic environments (Megonigal et al. 2004; Burgin and Hamilton, in press). These alternative processes include dissimilatory NO$_3^-$ reduction to ammonium (DNRA; Silver et al. 2001), abiotic reduction of NO$_3^-$ (Davidson et al. 2004).
Biogeochemical transformations in wetland waters and sediments are complex and occur at high rates because of the high biological productivity of these ecosystems, combined with the abundance of anoxic environments that foster anaerobic microbial processes, and the tendency for redox conditions to change over time. Of particular biogeochemical importance are the terminal steps of anaerobic microbial decomposition, which can occur via several alternative processes, including denitrification, sulfate \((\text{SO}_4^{2-})\) reduction, and methanogenesis (Fenchel et al., 1998; Megonigal et al., 2004). Along a landscape flow path, anaerobic respiration is potentially stimulated when elevated loads of \(\text{NO}_3^-\) and \(\text{SO}_4^{2-}\) as a result of air and water pollution pass through wetlands and the resultant removal of \(\text{NO}_3^-\) and \(\text{SO}_4^{2-}\) can help ameliorate this pollution (Kelly and Rudd, 1984; Mitsch et al., 2001; Hey, 2002). Ground water in agricultural landscapes is often enriched in \(\text{NO}_3^-\) and \(\text{SO}_4^{2-}\) due largely to fertilizer and animal waste inputs. In addition to ground water inputs, rates of loading of \(\text{NO}_3^-\) and \(\text{SO}_4^{2-}\) to wetlands via atmospheric deposition are greatly enhanced due particularly to fossil fuel combustion (Boyer et al., 2002; Mayer et al., 2002).

These increased N and S loadings represent a biogeochemical perturbation with interesting consequences for ecosystems, including greater N availability for plant growth, albeit sometimes to the point of toxicity (Fenn et al., 1998), as well as acidification of poorly buffered waters and soils and enhancement of redox transformations, all with multifarious impacts on elemental cycling. Greenhouse gas emissions are also potentially affected by increased \(\text{N}_2\text{O}\) production, as well as inhibition of methanogenesis by competitively superior denitrifiers and \(\text{SO}_4^{2-}\) reducers (Conrad, 1996). Sulfide, the product of anaerobic respiration of \(\text{SO}_4^{2-}\), is a particularly active player in biogeochemical reactions that has scarcely been studied in freshwaters, yet may interfere with N cycling in several ways that remain poorly understood.

**Methods Development**

A critical question for wetland management and protection is the role of throughflow wetlands in reducing N and S pollution and thereby providing improved water quality at the landscape level, but our understanding has been limited by the difficulty of measuring in situ process rates and scaling them up to entire flow paths. Many methods have been used to estimate the nature and rates of anaerobic metabolism in sediments, including analysis of dissolved \(\text{H}_2\) concentrations (Lovley et al., 1994), molecular methods (Muyzer and Smalla, 1998), lab assays in microcosms (Groffman et al., 1996; Roden and Wetzel, 1996), natural abundance stable isotope techniques (Ostrom et al., 2002), and whole-ecosystem isotope tracer studies (Peterson et al. 2001; Tobias et al., 2001; Mulholland et al., 2004). The whole-ecosystem approaches are ideal in many respects because they are integrative, and because lab assays can be affected by disturbance of the natural environment, contamination by oxygen, and by introduction of high concentrations of substrate.

Recent advances in field experimental methods have stimulated new insights into how wetlands can reduce \(\text{NO}_3^-\) and \(\text{SO}_4^{2-}\) loads. Most work has focused on \(\text{NO}_3^-\) and very few studies have examined \(\text{NO}_3^-\) and \(\text{SO}_4^{2-}\) together. We have had success with several of these approaches in recent work on headwater streams and wetlands in southwestern Michigan. Whole-ecosystem stable isotope tracer studies are being employed in headwater streams in the Lotic Intersite Nitrogen Experiment (LINX), on which Hamilton is a PI and the Ostroms' lab has played a key role, to examine the fate of \(\text{NO}_3^-\) in streams draining contrasting watershed land cover. In addition, Hamilton’s group has been employing smaller scale, in-situ tracer methods in diverse kinds of aquatic sediments to estimate microbial process rates, both in conjunction with LINX experiments and independently.

The current generation of LINX studies entails coordinated experiments in which \(^{15}\text{N}\)-labelled nitrate is added to small streams and the fluxes of the tracer through the various N cycling compartments are tracked as the water flows downstream. This intersite comparison involves 8 biomes with 9 experiments per biome, and 2005 is the last of 3 years of field work.
This work has been funded under a $3.4M grant from the former NSF program known as Integrated Research Challenges in Environmental Biology. The LINX group has worked with NSF funding since 1996 and has produced over 32 peer-reviewed publications and 7 theses and dissertations, even though the current generation of experiments await completion and synthesis before publication (full list is at http://www.biol.vt.edu/faculty/webster/linx/). Hamilton serves on the steering committee for the overall project and also works with Jennifer Tank of the University of Notre Dame on the Michigan experiments, which have been done in the Kalamazoo River watershed. Nathaniel Ostrom’s lab analyzes samples from all sites.

In addition to the LINX whole-stream tracer addition experiments, we have been conducting small-scale “push–pull” tracer experiments, in which a solution containing reactants and amended with a conservative solute tracer is injected into the sediments, then subsequently withdrawn to measure rates of disappearance of the reactant relative to the conservative solute. This approach was developed for groundwater (Istok et al., 1997; McGuire et al., 2002; Addy et al., 2002) and we have miniaturized it to examine near-surface sediments. The experiments can entail additions of NO$_3^-$ or other reactants together with conservative tracers (e.g., Br$^-$), and can employ isotopic tracers (we have been using $^{15}$N-labelled nitrate and ammonium). In recent work (Whitmire and Hamilton, 2005), we employed push–pull tracer experiments to evaluate rates of removal of NO$_3^-$ and SO$_4^{2-}$ from small wetlands of variable hydrology in the glacial landscape of southwestern Michigan. The results demonstrated the capacity of wetland sediments to rapidly deplete added NO$_3^-$ and SO$_4^{2-}$ within hours of addition. The short time and space scales over which removal was observed underscore the potential importance of narrow riparian wetlands along headwater streams and small isolated depressions in providing this ecosystem service; protection and management of such ecosystems may therefore deserve more attention because they are often excluded from legal protection.

The work of Whitmire and Hamilton (2005) also revealed evidence for heretofore unknown interactions between N and S cycling in these sediment environments, pointing to substantial NO$_3^-$ uptake by sulfur-oxidizing bacteria. Hamilton currently has NSF support to examine relative rates of various potentially important microbial N transformations, and the controls on those rates, using push-pull experiments and other techniques. That project does not include the pilot whole-ecosystem tracer experiments described here, but the two complementary lines of investigation are mutually reinforcing.

**Results**

Our pilot studies to refine the methods for whole-ecosystem tracer additions to throughflow wetlands remain in development. Initial attempts to conduct tracer additions in a small fen wetland on the Kellogg Forest property in September 2005 underscored the challenges of working in these kinds of environments. Addition of conservative tracers (bromide first, then a fluorescent dye) in an amount based on a crude approximation of hydraulic residence time revealed that the system was much more complex than we had assumed. This has led us to reconsider whether we should be working at the scale of the entire water body, or in subunits partitioned by habitat, and we have been rethinking alternative experimental designs.

One option that we are exploring is a non-isotopic tracer approach, in which we add nitrate at varying levels and estimate ambient uptake rates from the differential responses, as has been pioneered in stream studies. This could be done as “patches” of tracer added to different parts of the wetland, or on a whole-ecosystem basis.

Testing of these different options is being conducted in an experimental flume set up in the lakeside boathouse laboratory at KBS. Here we can maintain a 3-m long aquarium filled with wetland sediments and continuously flushed at a low rate with Gull Lake water, which contains nitrate at around 300 µg N/L.

In addition to the methods development, we are conducting surveys of over a dozen throughflow wetlands across a wide range of sizes. Our objective with these surveys is to understand changes in water chemistry as a function of water residence time and other physical features, and how these changes vary over the seasons.
Major Conclusions

Given the complexity of this work and the fact that the Venture Grant work has been rolled into the postdoctoral research, it is premature to declare that we have reached major conclusions, except to say that we are optimistic that this work will lead to methods breakthroughs and make us well poised to pursue future research funding from extramural sources. Our synoptic surveys have clearly shown that throughflow wetlands are hot spots for N removal and other biogeochemical transformations during the warm months, but we do not yet have data from the cooler part of the year.

Publications and Presentations

Following is a list of publications and presentations that were at least partially supported by the Venture Grant and the ensuing postdoctoral grant:

Presentations:


Publications:


Grant Proposals

The pilot experiments are designed to demonstrate the feasibility of whole-ecosystem tracer studies in throughflow wetlands and thus pave the way to future proposals that would expand on this work. The CWS Postdoctoral Fellow (Jon O’Brien) has been at MSU since September and has been taking over the leadership on developing conceptual models and experimental methods. A larger proposal would also seek to involve several other MSU faculty, including people with expertise in microbial ecology and biogeochemistry, as well as collaborators at other institutions. We are engaged in discussions with Dr. Emily Stanley of the University of Wisconsin and Dr. Maury Valett of Virginia Tech about a collaborative proposal to NSF in June 2006 that would focus on nutrient processing in throughflow wetlands. In addition, the topic of throughflow wetlands remains one of several ideas for an eventual LINX project focus (further discussions are planned for May 2007).
References


