Problem and research objectives

Wetlands are an important ecosystem for the biological removal of nitrate by denitrification. Wetland mitigation is a common practice used to satisfy the "no net loss" requirements of the Clean Water Act, but monitoring is rarely carried out to determine whether function is adequately replaced by restored wetlands [1]. Restored wetlands often do not achieve similar rates of denitrification as compared to more pristine wetlands [2, 3]. Fluctuating anaerobic hydrologic conditions are known to favor denitrification activity because it is an anaerobic process, but simply restoring the hydrology does not always restore denitrification capacity as expected [4]. Since this is a process performed by microbial communities, it is possible that there is an overlooked biological component. It is essential that we investigate how microbial communities differ between restored and natural wetlands and whether this influences function in order to better understand the ability of restored wetlands to perform desired functions. The objectives of this project aim (1) to show how denitrifying communities in restored wetlands can change over time, (2) to compare denitrifier composition and denitrification capacity across a chronosequence of restored wetlands, and (3) to identify environmental factors that may facilitate the restoration of denitrifying services by affecting the microbial organisms involved.

Methods

Soil was collected from 45 wetlands located across the state of Illinois, including 30 restored wetlands, 15 of which were paired with adjacent reference wetlands. Samples were collected both during a drought in 2012 and again in 2013, which was a much wetter year. Four paired restored and reference wetlands were also analyzed during 2007, so a historical comparison could be made for a subset of the sites, and three of these paired sites were revisited in 2014 to continue the time series. Potential denitrification rates were determined in the lab by an acetylene reduction assay. Changes in denitrifier composition were determined by performing terminal restriction fragment length polymorphism (T-RFLP) of the nitrous oxide reductase (*nosZ*) gene in bacteria [5]. Redundancy analysis (RDA) was used to identify a core set of denitrifier populations that represent natural reference wetlands. A suite of environmental data was collected simultaneously, including total soil N and C, available N and P, pH, and moisture content, which was combined with information about plant community characteristics determined by our collaborators.

Principle findings

In order to determine whether denitrifying microbial communities are becoming more similar to a reference wetland community, denitrifer communities from four different restored wetlands were compared to a core reference community that was identified using redundancy analysis (RDA). These four sites were selected because they were sampled in 2007, 2012, and 2013, and three of them were sampled again in 2014. In general, there is a non-significant trend showing decreasing dissimilarity of denitrifier assemblages in restored wetlands to this core reference community (as determined by the Bray Curtis dissimilarity index) as the restored wetlands age, which indicates that the communities are becoming more similar to the core reference community over time (Fig. 1). Two of the sites showed a significant decrease in dissimilarity: Site 028 in Stephenson County ($p < 0.05$) and Site 029 in Sangamon County ($p<0.01$).

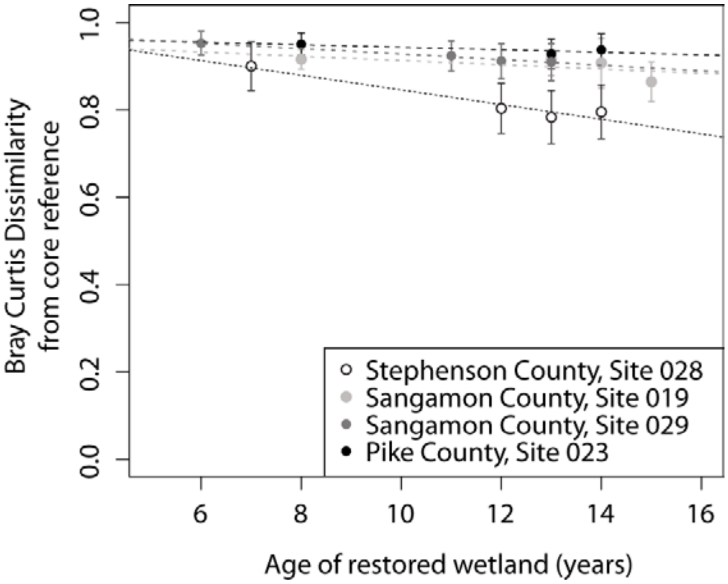

Figure 1. Denitrifier community dissimilarity between restored sites and a core reference community determined by RDA. Significant trends (regression significance at $p < 0.05$; indicating increasing similarity) were seen for Site 028 in Stephenson County (white) and for Site 029 in Sangamon County (dark grey), but not for the other two sites. Error bars represent the 95% confidence interval.

Contrary to what has been found previously, there was no significant difference between the denitrification capacities of restored and reference wetlands in either 2012 or 2013 (Fig. 2), though the drought in 2012 significantly limited denitrification capacity for both. Thus, chronosequence analysis was performed only for the wetlands sampled in 2013 to avoid confounding factors related to the drought. The denitrifier communities in the restored sites were very different from the core reference community (Bray Curtis dissimilarity = 0.87 ± 0.02), and communities in older restoration projects were no more similar to the reference than those in younger restored wetlands (Fig. 3a). However, denitrification showed a non-significant increasing trend as the age of the restoration increased (Fig. 3b), which may indicate a rapid response to the development of wetland environmental factors, while the microbial community shows a lag in development.

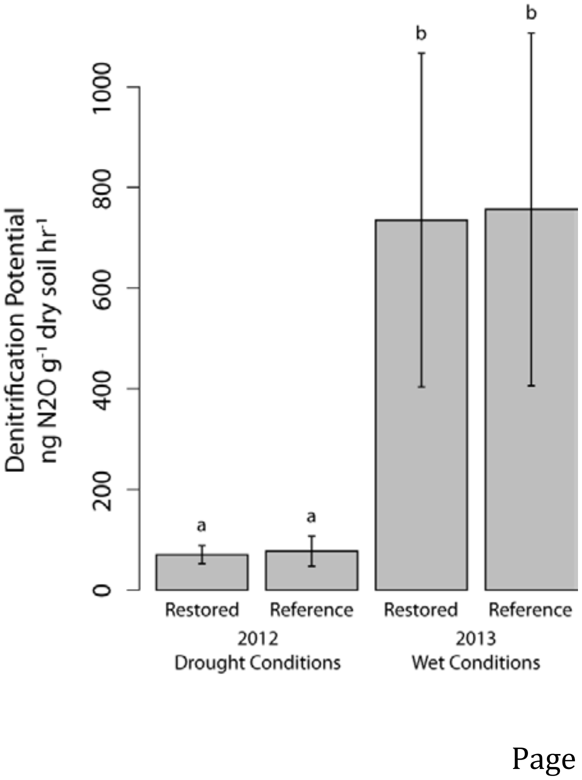

Figure 2. Average denitrification capacity for restored and reference wetlands under drought conditions and under more normal conditions. Error bars represent the 95% confidence interval.

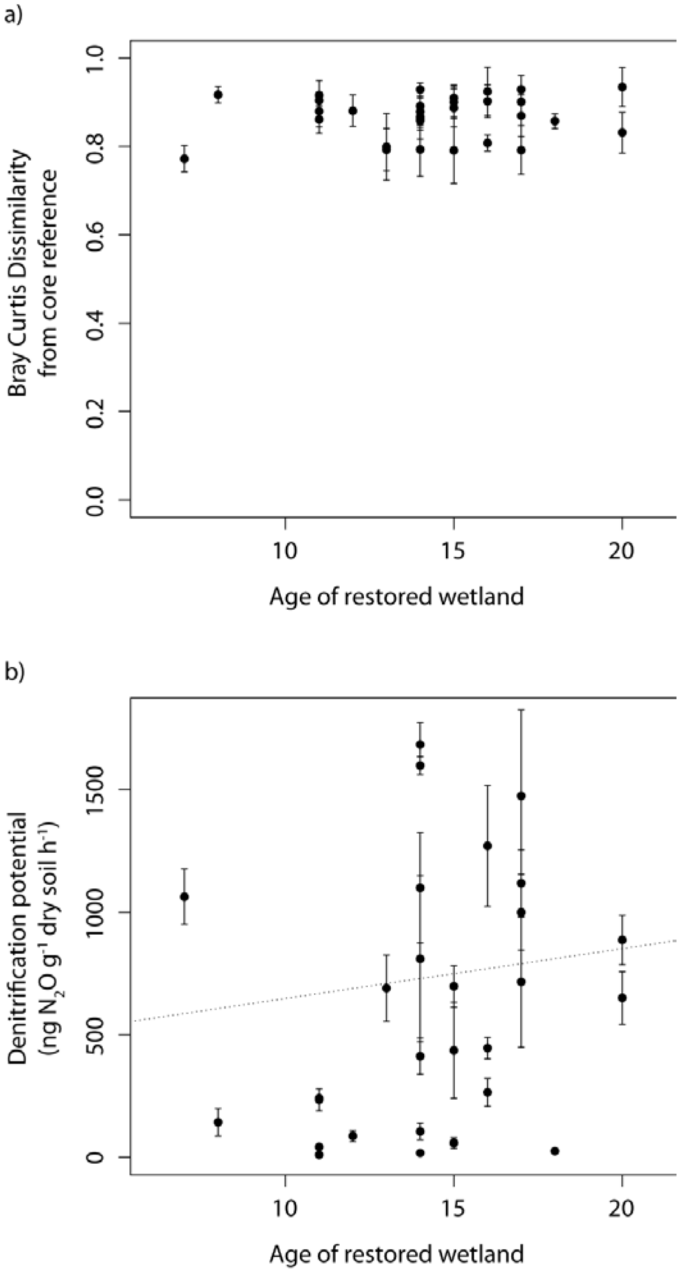

Figure 3. (a) Dissimilarity between denitrifier communities in a chronosequence of restored wetlands compared to a reference core, and (b) denitrification potential of each restored site plotted by the age of the restoration in 2013 only. There was no trend in denitrifier community over time, and the dotted line in panel b shows a non-significant upward trend in denitrification rates as the restored sites get older.

In order to determine which factors influence denitrifier composition, permutational analysis of variance (PERMANOVA) models were built using soil factors and plant factors separately, which was visualized by correspondence analysis (CA) plots (Fig. 4).

The factors influencing communities in restored and reference wetlands were different, indicating that the denitrifiers are responding to different drivers in these systems. The soil factors with the most influence on denitrifier communities in the restored sites were moisture ($R^2 = 0.09$, $p < 0.001$), pH ($R^2 = 0.07$, $p < 0.001$), and total N ($R^2 = 0.02$, $p < 0.01$) and the plant factors with the most influence were mean coefficient of conservatisms (mean C; $R^2 = 0.06$, $p < 0.001$), floristic quality index (FQI; $R^2 = 0.03$, $p < 0.001$), and the percent of perennial species present ($R^2 = 0.03$, $p < 0.01$). Soil factors with the greatest influence on the communities in the reference sites were pH ($R^2 = 0.12$, $p < 0.001$), nitrate ($R^2 = 0.03$, $p < 0.001$), and moisture ($R^2 = 0.03$, $p < 0.001$), while the plant factors with the greatest influence were the percent native species present ($R^2 = 0.10$, $p < 0.001$), mean C ($R^2 = 0.03$, $p < 0.01$), and FQI ($R^2 = 0.03$, $p < 0.01$).

The factors that show up as important most often seem to be moisture and pH, as well as FQI and mean C, most of which were correlated to denitrification (Fig. 5). However, moisture was not a significant explanatory variable for potential denitrification, presumably because the laboratory assays are provided with adequate water regardless of the moisture content of the soil, while available phosphate was the most important factor, even though it did not appear to affect the denitrifier composition at all. The mismatch between factors that influence denitrification rates and those that influence community composition further support the idea that denitrifier communities experience a lag in development following restoration. Put together, our results show that denitrifying services might not always attain restoration goals if factors influencing the development of microbial communities, like soil pH and moisture, are not addressed.

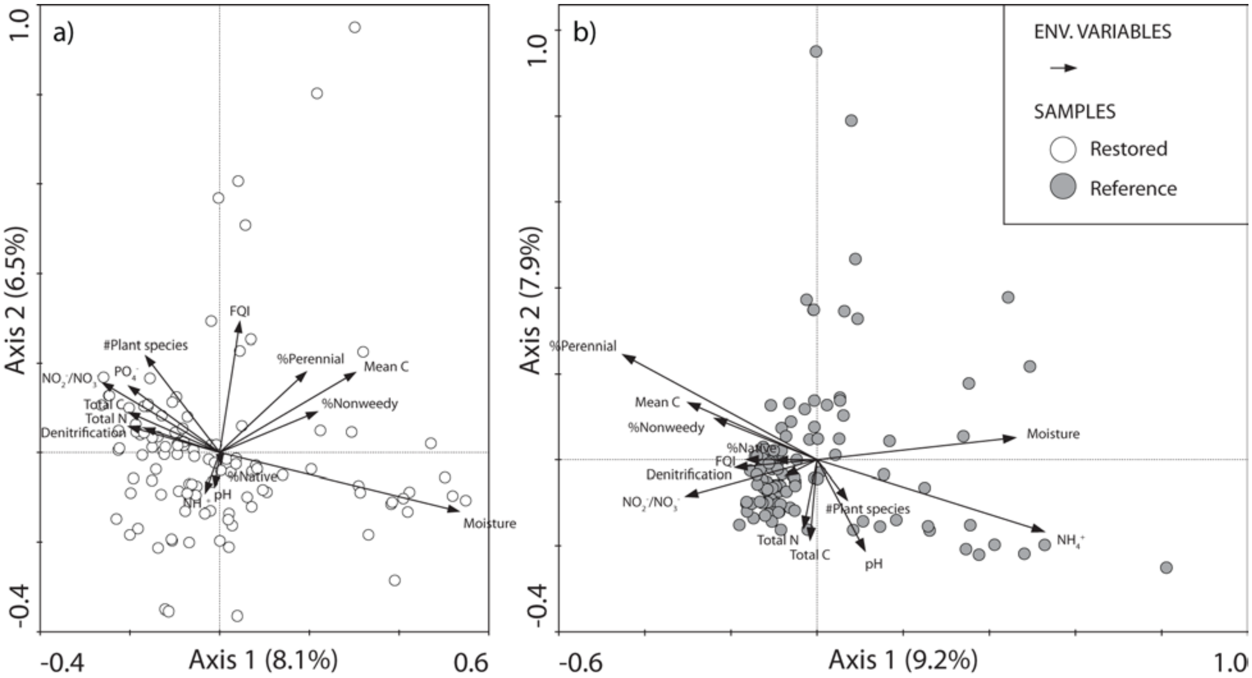

Figure 4. Correspondence analysis (CA) plots showing factors influencing denitrifier communities in restored and reference wetlands separately. Separate permutational analysis of variance (PERMANOVA) models showed that the three most influential factors for the restored sites were moisture, pH, and total N, as well as mean C, FQI, and the percent perennial plant species (% perennial). The most influential factors for the reference sites were pH, nitrate, and moisture, as well as the percent native species present (% native), mean C, and FQI.

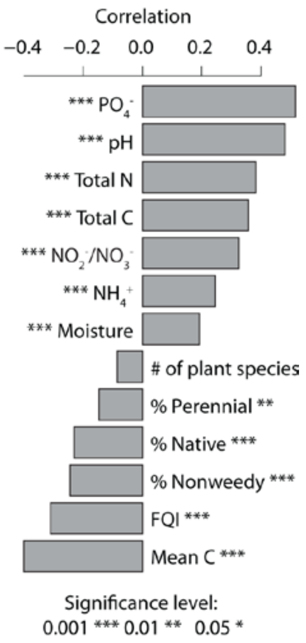

Figure 5. Correlation of soil and plant factors with potential denitrification rates in 2013. In general soil factors were positively correlated, while plant factors were negatively correlated with denitrification. Asterisks (*) represent the level of significance for each correlated term as determined by a simple linear regression model

Students involved in this project

There are two graduate students who contributed work on this project, including field collection and laboratory analyses. Dora Cohen is a third year Ph.D. student in the program for Ecology, Evolution, and Conservation Biology, while Natalie Stevenson is a second year masters student in Natural Resources and Environmental Science. Two undergraduate students also assisted with this project. Jonathan Bressler majored in Natural Resources and Environmental Sciences and was an intern in the summer internship program offered by the National Great Rivers Research and Education Center (NGRREC) during the summer of 2012. Emily Mackley majored in Integrative Biology and was an intern in the NGRREC summer internship during 2013.

Publications or pending publications

Parts of this work have been presented in the form of posters at conferences. Dora Cohen presented the poster "Microbial community composition has ramifications for denitrification capacity in restored wetlands" at the Joint Aquatic Sciences Meeting in Portland, OR on May 22, 2014. Natalie Stevenson presented a poster at the Illinois Water Conference in Urbana, IL on Oct. 14, 2014. Dora Cohen is currently using the data to write a manuscript entitled "Drought limitation and resiliency of denitrifiers in restored Illinois wetlands".

References

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