Soil Microbial Composition and Nitrogen Cycling in a Disturbed Wet Prairie Restoration (Wisconsin)

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Replanted almost entirely by the botanist Henry Greene in the 1940s and 1950s, Lower Greene Prairie is among the oldest wet-mesic prairie restoration sites in the United States. Subsequent urbanization and highway construction since the 1970s have led to an influx of stormwater, sediments, nutrient inputs, and invasive plants. Approximately one-third of the prairie (> 3 ha) is now dominated by reed canarygrass (*Phalaris arundinacea*). The historical significance of the site now extends to its present use as an adaptive restoration project that integrates research with restoration (Werner and Zedler 2002, Zedler 2005), as researchers at the University of Wisconsin (under the supervision of Joy Zedler) plan to divert stormwater around the restored prairie.

Our objective was to determine differences in soil function within the native restored prairie in comparison to the highly disturbed section dominated by reed canarygrass. Such measurements can provide a useful index to assess how ecosystem function changes in relation to the different adaptive phases used in restoration projects. We can then assess whether the diversion of stormwater runoff away from the site will alter soil function to resemble the undisturbed native plant community. Here we report the impacts of the multiple disturbances on soil function by measuring gross nitrogen (N) mineralization rates and soil microbial community structure.

Lower Greene Prairie is a restored wet-mesic prairie located in Madison, Wisconsin (43°1'40" N, 89°26'15" E), and managed by the University of Wisconsin Arboretum (Figure 1). In July 2004, we sampled from replicated plots (n = 11) located within three zones of the restoration site: 1) sites receiving heavy stormwater inputs and dominated by a monoculture of reed canarygrass (Invaded community); 2) sites not receiving stormwater inputs and dominated by restored native wet prairie plants (Native community); and 3) sites in the transitional zone between reed canarygrass and the native wet prairie community (Mixed community).

One of the key functional indices was gross nitrogen mineralization rate, determined using the ^{15}N isotope dilution method (Hart et al. 1994). We used this process-based index to assess nitrogen cycling in relation to microbial breakdown of organic matter into ammonium. In each plot, we pounded four PVC cores (9 cm long and 5 cm diameter) into the soil and removed these to label the soil with 99% ^{15}N as $(^{15}NH_4)_2SO_4$. The stable isotope ^{15}N occurs at very low levels in soil (less than half a percent),

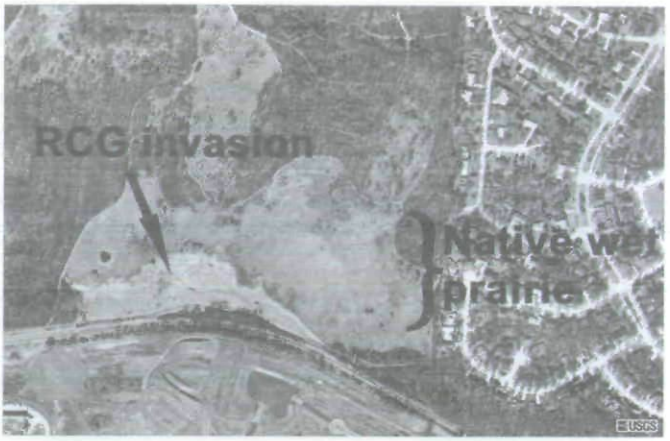

Figure 1. Lower Greene Prairie in Madison, Wisconsin. The wet-mesic prairie restoration (approximately 9 ha) was planted with a diverse community of native plants starting in the 1940s (top, *foreground*). The southern portion (> 3 ha) of the site has received stormwater inputs since the 1970s and is now dominated by reed canarygrass (top, *background*). Plots (bottom) were located in the zones of reed canarygrass (RCG), the transitional zone (between reed canarygrass and restored prairie), and native restored prairie. Photos by J. Kao-Kniffin (top) and courtesy of TerraServer (bottom)

which makes it a useful tracer of nitrogen transformation processes. We placed two of the cores back into the ground for 24 hours, while the other two cores were processed separately in the lab for initial nitrogen readings then averaged together. Gross mineralization rates are determined by measuring the changes in the ^{15}N:^{14}N ratio when microorganisms mineralize the more abundant form of organic ^{14}N into $^{14}NH_4$ after ^{15}N enrichment. The second pair of cores was retrieved the next day and processed the same as the initial samples. Dilution of the ^{15}N label in the recovered soil (24 h later) indicates nitrogen consumptive processes, which include microbial immobilization or assimilation into plants. Thus gross nitrogen mineralization rates differ from net nitrogen mineralization rates by accounting for the various nitrogen consumptive processes.

Another important functional index was change in soil microbial community structure, determined using a combination of phospholipid fatty acid analysis and fatty acid methyl ester analysis (PLFA/FAME). Individual fatty acids are found in the membranes of nearly all microorganisms, but because the relative amount of each fatty acid

varies among organism groups, lipid profiles are useful in determining the presence and relative abundance of general groups of organisms in soil. We collected three (5 cm diameter) soil cores from each plot, and pooled these as a composite sample after plant debris was removed. We kept the soil frozen at –20°C until the cores were freeze-dried, then we extracted lipids from 3 g of soil using the methods described by Kao-Kniffin and Balser (2007).

For multivariate analysis of microbial lipid data, we performed nonmetric multidimensional scaling (NMS) on the arcsine-transformed mole fractions of individual lipids using PC-ORD (vers. 5, MJM Software, Gleneden OR). We calculated a matrix of Sorensen (Bray-Curtis) dissimilarities of the mole fractions and subjected these to NMS. Starting from random configurations, 50 runs were performed with the real data and checked against the runs carried out on randomized data. The model suggested a two-dimensional solution as optimal, and NMS was rerun with two dimensions and the best starting configuration. The final stress value for this solution was 3.3. We analyzed the NMS scores and nitrogen data using a randomized complete block one-way ANOVA to evaluate the effects of vegetation/disturbance zone on the dependent variables using JMP software (vers. 5, SAS Institute, Cary NC). We performed Fisher's LSD post hoc means comparisons test on data with significant probability values of $p < 0.05$. Microbial relative abundance refers to the proportion of an indicator lipid relative to the sum of all microbial lipids in a sample.

Nonmetric multidimensional scaling of microbial lipids revealed that the overall soil community structure under the native plant community zone differed significantly (axis 1: $p < 0.05$) from the invaded and mixed plant zones (Figure 2a). Axes 1 and 2 explained 98% of the variation in the dataset. Axis 1 was largely influenced by saturated, branched chain, and fungal lipids. In other words, microorganisms indicative of fungi, gram-negative bacteria, and gram-positive bacteria drove much of the separation among the plant community zones. Axis 2 showed no significant differences in microbial community structure among plant community zones. Further analysis of lipid biomarkers indicated that the ratio of gram-positive to gram-negative bacterial indicators increased with greater levels of disturbance ($p < 0.01$) (Figure 2b). However, total microbial biomass did not differ significantly among the plant community and disturbance zones.

In this study, stormwater runoff combined with the invasion of reed canarygrass into the native wet prairie restoration site decreased gross nitrogen mineralization rates in comparison to the less disturbed sites in the restored native plant community and transitional zones ($p < 0.05$) (Figure 2c). If slower rates of nitrogen mineralization in disturbed areas are sustained over time, the amount of extractable nitrogen could decline in the reed canarygrass and stormwater plots. The lower rate of gross nitrogen mineralization found in reed canarygrass plots was consistent with

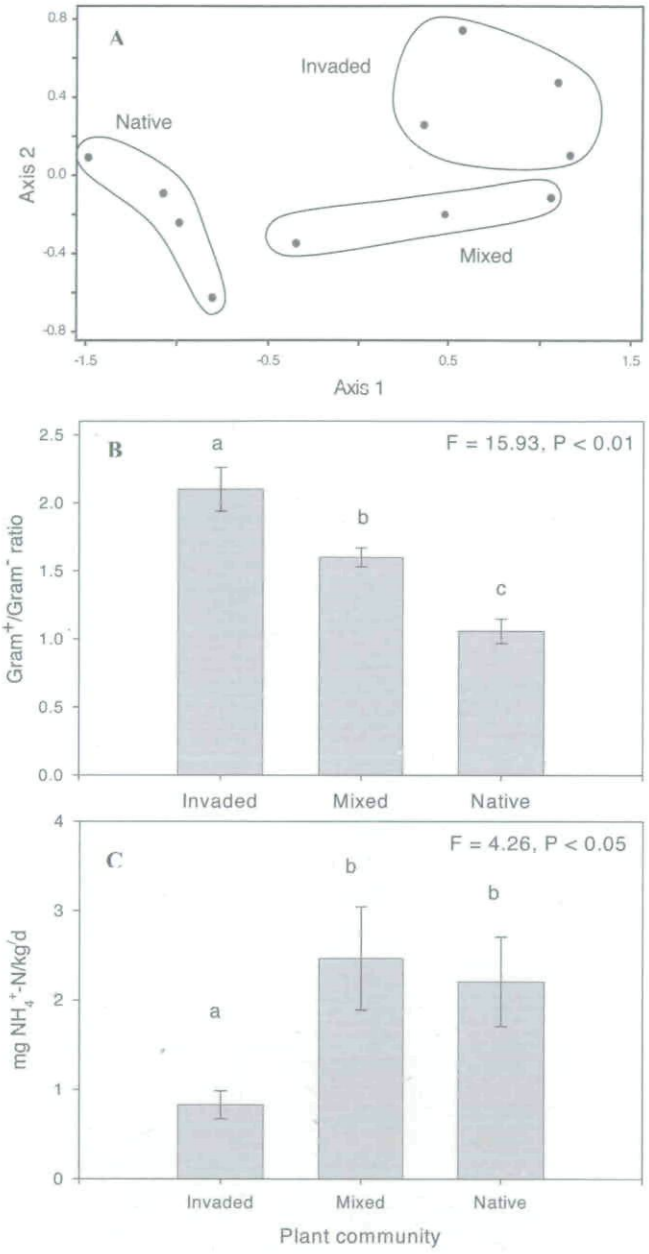

Figure 2. Soil microbial community structure and nitrogen (N) cycling: A) nonmetric multidimensional scaling of microbial lipid mole fractions; B) mean (± SE) relative abundance of gram-positive to gram-negative bacterial lipid indicators; and C) mean (± SE) gross nitrogen mineralization in soils collected from Invaded, Mixed, and Native plant communities using ^{15}N isotope dilution. Bars showing different letters indicate significantly different means at $p < 0.05$ (Fisher's LSD).

the shifts in the relative abundance of gram-positive and gram-negative bacterial indicators (Figures 2b, 2c). This is in agreement with other work showing that gram-negative bacterial abundance is positively associated with high rates of nitrogen mineralization (Fraterrigo et al. 2006). Gram-negative bacteria tend to be "colonizers" and rhizosphere specialists capable of rapid growth and turnover (Schlegel 1992). In contrast, gram-positive bacteria tend to be stress tolerant, slowly growing, and potentially associated with lower rates of nitrogen cycling (Balser 2005).

The ecological significance of this shift toward an increase in gram-positive bacteria and decrease in gram-negatives may lead to slower nitrogen and carbon cycling in sites receiving stormwater inputs and dominated by reed canarygrass. In practical terms, slower rates of gross nitrogen mineralization indicate lower microbial activity and possibly lower quality substrates for microbial use. Over time, the shift towards slower nitrogen and carbon cycling could lead to greater carbon accretion in the wetlands receiving stormwater inputs. It also suggests that nitrogen availability may eventually decline in the sites receiving stormwater and dominated by reed canarygrass.

Initial extractable NH_4^+-N and NO_3^--N indicated that soils under the different plant community and disturbance zones had similar levels of available N. Soils with identical levels of available nitrogen can have differing rates of nitrogen mineralization because nitrogen transformation processes can vary significantly across biological and environmental parameters. Extractable NH_4^+-N and NO_3^--N do not indicate biological- or physicochemical-based rate changes in nitrogen availability. Extractable nitrogen is simply a measure of inorganic nitrogen available for plant and microbial uptake, whereas gross nitrogen mineralization indicates the total amount of nitrogen released from the microbial conversion of organic nitrogen to NH_4^+. Research projects that document shifts in soil function, therefore, need process-based measurements as a biological index of change. However, repeated sampling of the plots over several years or after subsequent phases of restoration are implemented could show changes in the levels of extractable nitrogen across the plant community zones. Seasonal or multiple sampling points within a year may also reveal differences in extractable nitrogen levels that our single time point method did not find. As many restoration projects are limited by the financial costs of replicated and repeated sampling, we chose to use a single time point (summer) of soil functional measurements to keep lab analysis costs to a minimum. Sampling in the summer captures high levels of microbial activity. Consistent resampling at the same time points should provide an adequate measurement of functional changes as a basic index.

However, nitrogen and microbial community measurements alone do not give a sufficient understanding of the trajectory of ecological processes at a restoration site. Based on our results, we suggest measuring litter turnover, soil total organic carbon, and (physical) carbon fractions as additional indices of soil function that highlight carbon dynamics. These inexpensive techniques could provide a more comprehensive view of both nitrogen and carbon cycling changes at wetland restoration sites in the context of global change. Taken together, these soil functional indices can help elucidate how different restoration or land management techniques modify biogeochemical components of a wetland throughout the site and over time.

Our initial measurements indicate that multiple disturbances brought about by stormwater runoff can alter ecosystem function by slowing down nitrogen cycling rates, with concurrent changes in soil microbial community structure. New sets of hypotheses can be tested after the planned stormwater diversions to determine if and when soil function can revert to predisturbance levels. Thus continued measurements of biogeochemical parameters throughout adaptive phases could be useful in evaluating how restoration efforts influence ecosystem functioning across space and time.

Acknowledgments

We thank Filip Litwinuik and Katherine Faust with help in the field and laboratory. This study was supported by grants from the Department of Energy's National Institute for Climate Change Research and the University of Wisconsin–Madison Graduate School.

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