Evaluation of Restored Wetlands in the Greater Willamette Valley

By

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ABSTRACT

Wetlands are considered critically important in the delivery of ecosystem services such as water quality improvement, flood protection, and conservation of native biodiversity. A common measure of the effectiveness of these ecosystem services is denitrification, an anaerobic microbial process that converts nitrate (NO_3^-), a common water pollutant, into dinitrogen (N_2) gas. In addition to oxygen, denitrification is controlled by the amount of carbon available (a denitrifying bacterial energy source) in the soil and NO_3^-. My objectives were to determine how different types of sites (agricultural, natural wetland, and restored wetland) cycle nitrogen using denitrification methods.

Potential denitrification rates were determined using denitrification enzyme assays (DEAs) with and without the addition of acetylene gas, which was added to prevent the last step in the denitrification cycle. Other soil characteristics were also measured to compare their relationship to the denitrification potential of each site type: extractable organic carbon (EOC), NO_3^-, and percent moisture (H_2O).

DEA rates were directly related to H_2O, so rates were notably higher during the rainier times of the year. There was no relationship detected between DEA rate and EOC or DEA rate and NO_3^-, although EOC and NO_3^- showed a positive correlation to one another. This positive relationship is consistent with what others have found, and theory would predict. Studies have shown the higher the NO_3^-, generally the lower the N_2O reduction activity because there is ample NO_3^- to accept electrons so there are not as many electrons "left over" to reduce N_2O. By decreasing the levels of NO_3^- in the soil, it may be possible to reduce levels of N_2O production, thereby decreasing its potent effect as a greenhouse gas.

INTRODUCTION

Wetlands comprise approximately 1% of global landmass, containing soils rich in organic matter (Segnini 2010). Soil organic matter plays a crucial role in interrelated functions in terrestrial ecosystems (Lal 2009). Wetlands are often created or restored to mitigate the loss of wetland functions caused by conversion to another land use. Compensatory mitigation is required under Section 404 of the Clean Water Act (Bruland 2004), and may occur onsite or through the purchase of acreage credits in a mitigation bank. Millions of dollars have been spent through governmental programs such as the National Resource Conservation Service's Wetland Reserve Program (WRP) to restore wetlands on marginal croplands (USDA – NRCS 1995). Although structure (e.g. hydrology and vegetation) is restored in these wetlands, very few studies have been conducted to determine if functions have been restored as well (Hunter 1999).

Wetlands are considered critically important in the delivery of ecosystem services such as water quality improvement, flood protection, and conservation of native biodiversity (Mitsch and Gosselink 2007); and wetland restoration is considered to be a critical tool for providing these services (Willamette Partnership 2008). Several organizations – such as Metro in Portland, OR – have discussed the importance of collecting analytical data to assess the productivity of ecosystem services as a way to guide land use policy creation (Kentula 2007). My research aim was to understand the relationship between natural wetland vegetation and their soil microbial communities, and to compare this to the same relationships in agricultural sites and sites undergoing restoration methods.

The methods of evaluating the functionality of wetland production rely on characteristics assumed to be associated with particular functions or properties (i.e. denitrification, extractable organic carbon) due to the absence of reliable methods of data collection concerning the level to which a particular wetland site actually performs such a function. Research is needed on how wetland vegetation, interacting with microbial communities in particular soil types, influences the biogeochemical processes of wetlands. Investigating how different restoration methods influence these processes is a crucial method of evaluating overall success. The elected regional government for the Portland metropolitan area (Metro) works with communities, businesses and residents to create a vibrant and sustainable region for all. Metro, and companies like it, want to evaluate a restored wetland's ability to provide ecosystem services such as conservation of native plant diversity, plant nitrogen uptake, N_2O production, and denitrification. Therefore, I investigated potential relationships among wetland restoration methods, establishment of native vegetation, and soil characteristics (such as soil organic matter content and water content) in evaluating wetland restoration success. To create a basis of comparison, three restored wetland sites were selected, in addition to three natural wetland sites, and three sites that were currently cropped. The cropped sites were meant to represent sites prior to their restoration. This last group was referred to as the "agricultural sites". The research goal was to address the concerns associated with long-term watershed management and focus on creating sustainable (and economically feasible) solutions for maintaining valuable wetland areas.

In order to evaluate the efficiency of restoration methods at the different sites, I performed denitrification enzyme assays (DEAs). Denitrification is the biological reduction of

nitrate (NO_3^-) and nitrite (NO_2^-) ions into gaseous nitrogen in the form of nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) gases (Drury et al. 2007). In agricultural soils, dissolved inorganic nitrogen is an essential nutrient taken up by crop roots, whereas gaseous nitrogen is largely lost from the root zone.

Measuring denitrification in agricultural and wetland soils is important because the gases produced – especially NO and N_2O – are known greenhouse gases that are believed to contribute significantly to global warming (Tallec et al. 2008). Loss of inorganic nitrogen from the root zone reduces both crop productivity and the efficacy of expensive nitrogen fertilizers (Drury et al. 2007). Denitrification of various soils is an essential part of determining nitrogen budgets on plot, field, regional, and national scales so that the most beneficial management practices can be developed; these maximize the efficacy of nitrogen amendments, such as fertilizers and manure, and minimize the off-gassing of nitrogen-based greenhouse gases (Drury et al. 2007).

When faced with a shortage of oxygen, many bacterial species use NO_3^- to support respiration via the process of denitrification. This takes place extensively in nitrogen-rich soils and generates the gaseous products NO, N_2O, and N_2. The denitrifying bacteria protect themselves from the endogenous cytotoxic NO produced by converting it to N_2O, which can be released into the atmosphere. However, N_2O is a potent greenhouse gas and the activity of the enzyme that breaks down N_2O has a crucial role in restricting its atmospheric levels.

Measuring denitrification losses from a soil system involves the use of acetylene in a closed system and measuring the accumulation of N_2O over time. This technique, referred to as the acetylene inhibition technique and initially developed by Yoshinari et al. (1977), utilizes the

ability of acetylene to inhibit the conversion of N_2O to N_2. It thereby allows the investigator to obtain the potential amount of N_2O evolved from the soil system. This is advantageous because it is a relatively easy procedure to do, and also easy to measure increases in N_2O concentrations following the addition of acetylene, as the atmospheric concentrations of N_2O are comparatively low (less than 315 ppb). However, acetylene use is limited because the inhibition can be reversed when residues containing high sulfide contents are present (de Catanzaro et al. 1987), or when incubations are carried out for too long (Yeomans and Beauchamp 1978).

In the last 40 years, annual inputs of biologically available nitrogen have approximately doubled, mostly due to the increased production and use of synthetic-nitrogen fertilizers (Smil 2001). Undesirable runoff and leaching of nitrogen from agricultural fields can be reduced by naturally vegetated riparian areas – zones of land adjacent to bodies of water – between agricultural fields and aquatic ecosystems (Martin et al. 1999). These riparian zones have received considerable attention because denitrification can be a dominant sink for NO_3^- and because riparian soils tend to be wetter and have more organic C compared to upland soils (conditions which favor denitrification). However, little is known about the community composition of denitrifying bacteria in riparian soils or adjacent agricultural and aquatic ecosystems (Groffman et al. 1991, Martin et al. 1999).

As a result, my objectives were to determine how different types of sites (agricultural, natural wetland, and restored wetland) cycle nitrogen using denitrification methods. I wanted to see how this process varied during different times of the year for each site, and how it compared between each site. I also wanted to see how different types of sites affected the soil's carbon and nitrogen contents and whether this affected a type of site's overall

denitrification rate. This information could help the Natural Resource Conservation Service (NRCS) establish more tracts of land for their Wetland Reserve Program, and make better use of lands undergoing the transition from agricultural land to restored wetland.

MATERIALS AND METHODS

Study Site

Because I was based in Corvallis, I chose the Greater Willamette Valley (Fig. 1) for all of my sites. My partnership with Metro in Portland and the Oregon branch of the NRCS enabled me to use nine different sites along the area of what I deemed the "Greater Willamette Valley" (Fig. 2): a few of the sites were not directly located along the Willamette River, and one was located in Southern Washington. The nine sites allowed me to have three replicate experiments of three sites each: an agricultural site (comprised of the Gotter Prairie Ag, Westbrook, and Zugar sites), a restored wetland site (comprised of the Gotter Prairie South, Hutchinson, and Lovejoy sites), and a natural wetland site (comprised of the Gotter Prairie North, Knez, and Green Mountain sites). The agricultural sites were comprised of land that had either recently or currently been covered in crops. Restored wetland sites were former agricultural sites undergoing wetland restoration techniques on behalf of Metro and the NRCS, and each had a restoration time length between three and eight years. Natural wetlands were intact wetland areas that were being preserved.

Plot Design

Each site was randomly selected for three, 100-foot square plots. These plots were determined by estimating the total square foot area of each site via GPS coordinates. Within

each 10-foot by 10-foot plot, a one-foot square plot was randomly selected. Soil sampling was conducted around the perimeter of the inner, one-foot square plots (Figure 3).

Figure 1. Map of Oregon with the Willamette Valley circled.

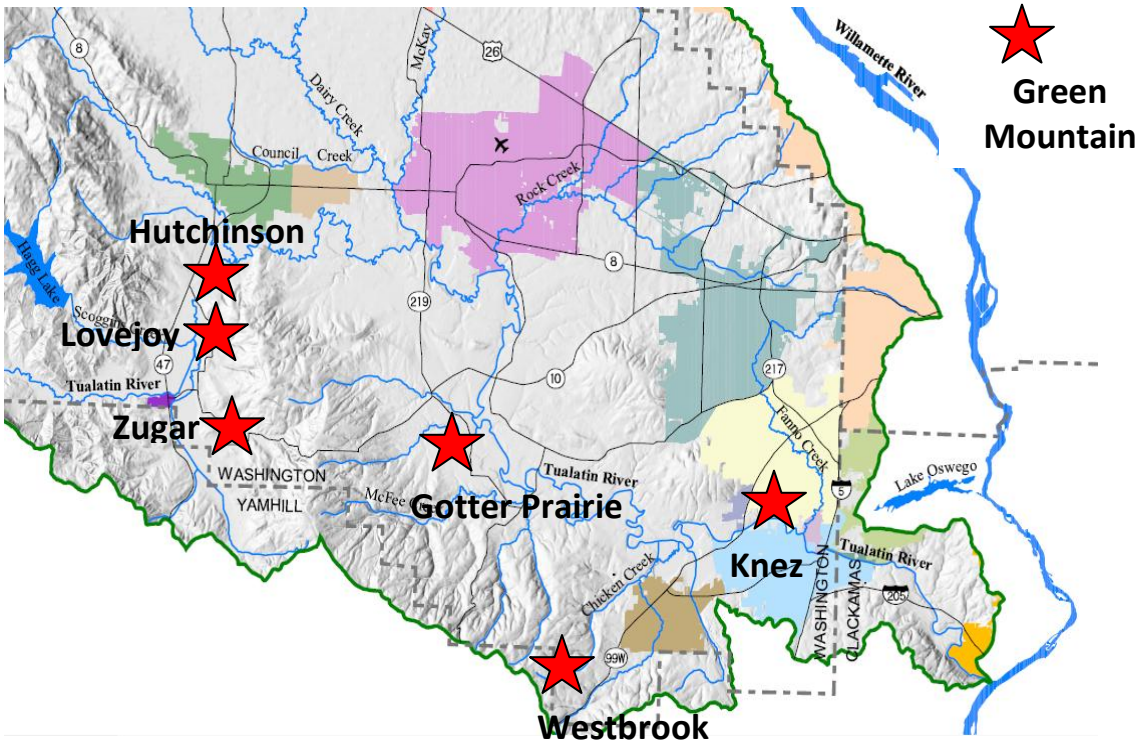

Figure 2. Map of the Greater Willamette Valley showing the site locations.

Soil sampling

Soil sampling occurred once every season (approximately every 2.5 months). The soil was sampled in late November 2009, early February 2010, and late April 2010. Each plot was sampled from its northwest corner: at 10 and 20 paces north of the corner, then 10 and 20 paces west, then 10 paces south. Five samples total were collected from each plot (a total of 15 individual samples per site). Each sample was collected using a metal soil core, collecting the top 15 to 20 cm of soil. The soil was deposited into a plastic bag (gallon size) and refrigerated until processing.

Denitrification Enzyme Assays (DEAs)

Twenty grams of each sample were weighed out in a 125-ml Erlenmeyer flask. 2.5 ml of a mixed solution of 6.00 g/L glucose and 2.88 g/L KNO_3 was combined with 22.5 ml deionized water and added to each flask. Flasks were sealed with rubber stoppers. The flask was

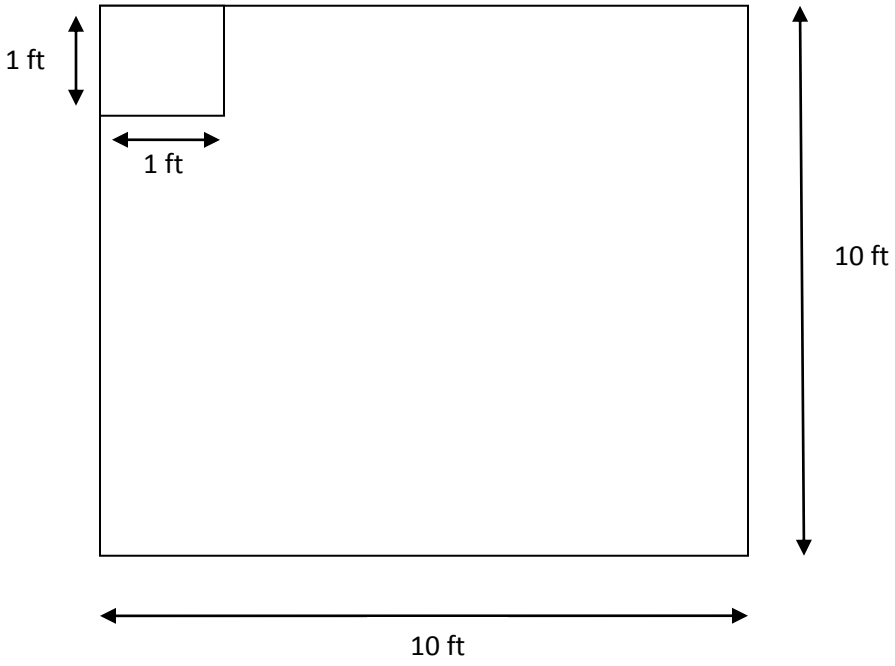

Figure 3. Sample plot showing 10 ft x 10 ft outer perimeter and 1 foot by 1 foot inner perimeter.

connected to an argon gas tank. The headspace of the flask was flushed with argon gas for one minute. All flasks were placed on a shaker table for one hour at 250 RPM to allow the soils to reach room temperature and thoroughly mix with the glucose- NO_3^- solution. After the hour, 500-µl samples were removed from the flask via gas-tight syringes and injected into a gas chromatograph (Drury et al. 2007). Eight 500-µl samples were injected (once every 30 minutes). After the first three injections, 12 ml of acetylene gas was added to each flask (acetylene was added immediately after injection three so each flask had 30 minutes of exposure to acetylene before injection four).

The gas chromatograph data peaks were then used to calculate the rates of denitrification activity in ng of N_2O-N per gram of soil per minute. The calculations used to determine this were taken from Drury et al. (2007). These calculations included finding the headspace volume of the flask to be subtracted out, the sample (syringe) volume, the volume of water present in each soil sample, incubation temperature, and the mass of each dry soil sample.

Extractable Nitrate and Organic Carbon

Ten grams of soil were weighed into screw top containers. 50 ml of 0.05 M K_2SO_4 was added to the containers. These were placed on a shaker table for 60 minutes. The contents were poured into filter-lined funnels and filtered into small screw cap vials and tested for NO_3^- concentration and for extractable organic C (EOC). Nitrate was measured colorimetrically by the Cd-reduction procedure using an autoanalyzer. EOC was determined using a Shimadzu total C analyzer.

Percent Moisture

Twenty grams of soil from each sample were weighed into metal tins. These tins were placed into a drying oven at 100 degrees Celsius for 24 hours. The tins were removed and re-weighed to determine the water content of each soil sample.

Data Analysis

Data were analyzed by finding the mean values of each measured variable for each of the three plots at each site (i.e., mean of GM1, GM2, and GM3 values for denitrification were averaged together to create one value for the Green Mountain site). The site values were averaged by type: Green Mountain, Knez, and Gotter Prairie South sites were averaged to create one value for the Natural Wetland site type; Gotter Prairie Ag, Westbrook, and Zugar were averaged to create one value for Agriculture site type; Gotter Prairie North, Lovejoy, and Hutchinson were averaged to create one value for Restored Wetlands site type. This was done for all variables of analysis: DEA rates (both with and without acetylene), the ratio of rates with and without acetylene, EOC, NO_3^-, and percent moisture. Standard errors of the means were calculated in Microsoft Excel.

To examine relationships between the different site types, I ran a two-way analysis of variance (ANOVA) test with replication, which had a significance level of 0.05. In conjunction with the ANOVA, a Tukey's Test was done to determine which means were significantly different from one another by comparing all possible pairs of means. The test compares the means of every treatment to the means of every other treatment and identifies where the difference between two means is greater than the standard error would be expected to allow (Tukey's Test is essentially a t-test that corrects for experiment-wide error rate). The confidence coefficient is either $1 - \alpha$ for equal sample sizes or greater than $1 - \alpha$ for unequal sample sizes,

meaning the Tukey's method is conservative when there are unequal sample sizes. For the two-way ANOVA and the Tukey's Test, the variables examined for each soil characteristic for significant differences were Treatment (Agriculture, Remnant, or Restored wetland), Time (sampling in November, February, or April), and Treatment by Time interaction. The soil characteristics examined were before acetylene denitrification rates (BA), after acetylene denitrification rates (AA), EOC, percent moisture (H_2O), NO_3^-, and the BA:AA ratio. Log-transformed data was also determined for all of the listed characteristics.

RESULTS

Percent Moisture, EOC, and NO_3^-

There was a consistent pattern among all the sites and site types where the moisture percentage stayed roughly the same between November and February, and decreased between February and April. In November, the percent moisture was 38.3% for the natural wetlands, 36.8% for the restored wetlands, and 25.9% for the agricultural sites. During the February samplings, the percent moisture level was 38.7% for natural wetlands, 32.4% for restored wetlands, and 26.3% for agricultural sites (Table 1). There was negligible difference (0.04% increase) between November and February for the natural wetlands. The restored sites had a much greater difference with a 4.4% decrease in moisture, while the agricultural sites paralleled the natural wetlands with a 0.4% increase in soil moisture for February.

	November	February	April
Natural wetlands	0.383	0.387	0.339
Restored wetlands	0.368	0.324	0.274
Agricultural sites	0.259	0.263	0.220

Table 1. Percent moisture measurements for each site type and sampling time.

The last sampling, which took place in April 2010, determined percent moisture levels of 33.9% for natural wetlands, 27.4% for restored wetlands, and 22.0% for agricultural sites. These values all showed a marked decrease in moisture between February and April with natural wetlands showing a 4.8% decrease in moisture level, restored sites showing a comparable 5.0% decrease in moisture level, and agricultural sites also with a comparable 4.3% decrease in soil moisture (Table 1).

Extractable organic carbon was initially measured in ppm before being converted to mg C per kg soil. In the November samplings, the natural sites had 18.3 mg C/kg soil, restored sites were at 16.8 mg C/kg soil, and agricultural sites were at 16.0 mg C/kg soil. The February samplings measured 30.9 mg C/kg soil for natural wetlands, 21.9 mg C/kg soil for restored wetlands, and 27.8 mg C/kg soil for agricultural sites. While all site types showed a distinctive increase in EOC amounts between November and February, they were at different rates. Natural wetlands increased in EOC by 12.6 mg C/kg soil (a 51.2% increase), restored wetlands increased by 5.1 mg C/kg soil (a 26.4% increase), and agricultural sites increased by 11.8 mg C/kg soil (a 53.9% increase) over the November samples. The April samples measured 24.4 mg C/kg soil for natural wetlands, 28.0 mg C/kg soil for restored sites, and 28.1 mg C/kg soil for agricultural sites. The natural wetland sites showed a decrease in carbon by 6.5 mg C/kg soil (a 23.5% decrease), the restored wetlands sites had an increase in carbon by 6.1 mg C/kg soil (a 24.4% increase), and agricultural sites had a comparatively negligible 0.3 mg C/kg soil increase in carbon (a 1.1% increase) between February and April (Fig. 4).

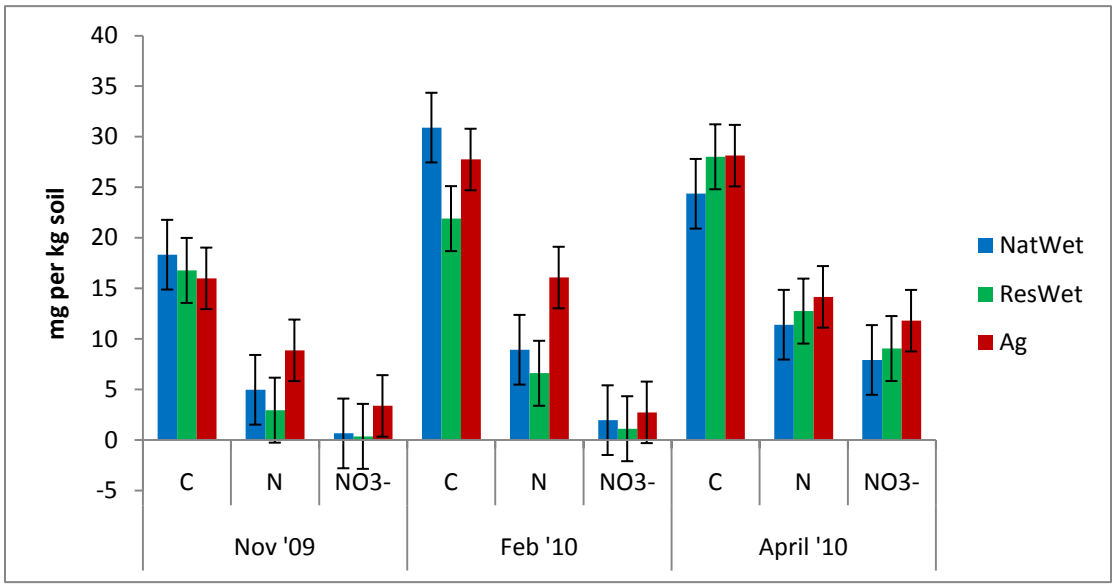

Figure 4. Total organic carbon, total extractable nitrogen, and nitrate amounts (in mg (C/N/NO_3^-) per kg soil) in natural wetlands, restored wetlands, and agricultural sites by season showing standard error.

Nitrate values were reported in mg NO_3^--N per kg soil. The November samplings found a NO_3^- value of 0.65 mg NO_3^--N per kg soil for natural wetlands, 0.36 mg NO_3^--N per kg soil for restored wetlands, and 3.4 mg NO_3^--N per kg soil for agricultural sites. In February, natural and restored sites both underwent considerable increases. Natural sites had a NO_3^- level of 2.0 mg NO_3^--N per kg soil (a 1.35 mg NO_3^--N per kg soil increase and a percent increase of 101.9%), while restored sites had a nitrate level of 1.1 mg NO_3^--N per kg soil (a 0.74 mg NO_3^--N per kg soil increase and a percent increase of 101.3%). Agricultural sites represented a NO_3^- level of 2.7 mg NO_3^--N per kg soil, a decrease of 0.7 mg NO_3^--N per kg soil and a percent decrease of 23.0%. The April samples for natural, restored, and agricultural sites reported NO_3^- values of 7.9 mg NO_3^--N per kg soil mg NO_3^--N per kg soil, 9.1 mg NO_3^--N per kg soil, and 11.8 mg NO_3^--N per kg soil, respectively. All site types showed considerable increases over the February samplings. Natural sites had an increase of 5.9 mg NO_3^- per kg soil (an increase of 119.0%), restored sites had an

increase of 8.0 mg NO_3^--N per kg soil (an increase of 157.0%), and agricultural sites had an increase of 9.1 mg NO_3^--N per kg soil (an increase of 126.0%). These relationships are noted in Figure 4.

Denitrification Enzyme Assays

The rates of denitrification were measured in ng N_2O-N per gram of soil per minute. Since I used an acetylene-inhibition technique, I measured denitrification rates both before and after the addition of acetylene as a basis for comparison. I predicted that acetylene would increase the rate of the N_2O-N concentration, but I wanted to know by how much. I also examined how denitrification rates differed between site type and sampling time.

The before acetylene (BA) denitrification rates for November were 0.0109 ng N_2O-N per gram of soil per minute, 0.00469 ng N_2O-N per gram of soil per minute, and 0.00365 ng N_2O-N per gram of soil per minute for natural, restored, and agricultural sites respectively. In February, all the BA DEA rates declined. Natural sites had a DEA rate of 0.00595 ng N_2O-N per gram of soil per minute, a decrease of 0.00495 ng N_2O-N per gram of soil per minute (a 58.8% decrease). Restored sites had a DEA rate of 0.00405, a decrease of 0.00064 ng (a percentage decrease of 14.6%). Agricultural sites had a DEA rate of 0.00256 ng N_2O-N per gram of soil per minute, a decrease of 0.00109 ng N_2O-N per gram of soil per minute (a decrease of 35.1%). For April, natural sites had a DEA rate of 0.00870 ng N_2O-N per gram of soil per minute. This was a 0.00275 ng N_2O-N per gram of soil per minute increase and a 37.5% increase in rate from February. Restored sites were measured at 0.0104 ng N_2O-N per gram of soil per minute, a notable increase from February by 0.00632 ng N_2O-N per gram of soil per minute (an 87.6% increase). Agricultural sites were measured at 0.00407 ng N_2O-N per gram of soil per minute, an

increase of 0.00152 ng N_2O-N per gram of soil per minute, and a percent increase of 45.7% (Figure 5).

The after acetylene (AA) denitrification rates for November were 0.0100 ng N_2O-N per gram of soil per minute, 0.0110 ng N_2O-N per gram of soil per minute, and 0.00747 ng N_2O-N per gram of soil per minute for natural, restored, and agricultural sites respectively. In February, the DEA rates were 0.0192 ng N_2O-N per gram of soil per minute for natural sites (an increase of 0.0092 ng N_2O-N per gram of soil per minute and 63.0%). Restored sites had a DEA rate of 0.00893 ng N_2O-N per gram of soil per minute, a decrease of 0.00207 ng N_2O-N per gram of soil per minute and 20.8%. Agricultural sites had a rate of 0.00443 ng N_2O-N per gram of soil per minute for agricultural sites, a decrease of 0.00304 ng N_2O-N per gram of soil per minute and 51.1%. For April, the DEA rates were 0.0130 ng N_2O-N per gram of soil per minute for natural sites, a decrease of 0.0062 ng N_2O-N per gram of soil per minute and 38.5% from February rates. Restored sites had an AA DEA rate of 0.0109 ng N_2O-N per gram of soil per minute, an increase of 0.00101 ng N_2O-N per gram of soil per minute and 10.2%. Agricultural sites had a DEA rate of 0.00555 ng N_2O-N per gram of soil per minute, an increase of 0.00112 ng N_2O-N per gram of soil per minute and 22.4% from February (Figure 5).

The BA and AA rates were compared by ratios with BA as the numerator and AA as the denominator. The November samplings showed BA:AA ratios of 0.489, 0.488, and 0.467 for natural, restored, and agricultural sites respectively. February displayed ratios of 0.377, 0.652, and 0.619 respectively. April's ratios were 0.718, 0.958, and 0.734 respectively. The smaller the ratio, the more the DEA rate increased after the addition of acetylene. Only the November sampling of the natural sites showed a decrease in rate after the addition of acetylene. All the

other sites showed varying degrees of DEA rate increase. The highest rate increase occurred for the natural sites in February. With the exception of the odd natural wetland ratio in November, all of the ratios calculated increased throughout the sampling times. While restored sites showed a small increase between November and February, they had a tremendous increase between February and April, appearing exponential. This pattern was also exhibited in the natural sites, with the exception of the odd point in November. The agricultural sites displayed a linear relationship with a very gradual, steady increase in the BA:AA ratio over time.

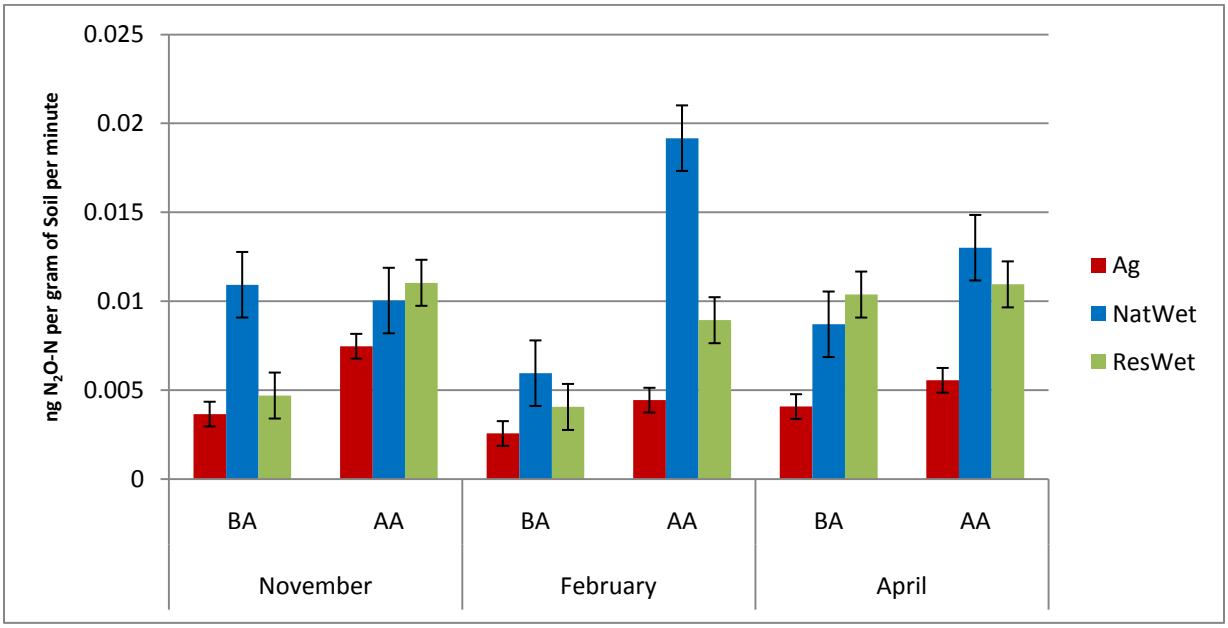

Figure 5. Before (BA) and After Acetylene (AA) Denitrification rates between Agricultural, Natural Wetland, and Restored Wetland sites sampled in November 2009, February 2010, and April 2010.

Tukey's Test and Correlations

P-values with a value less than 0.05 were considered to be statistically significant. Of all of the combinations in the Tukey's Test, the following were found to have statistically significant p-values: BA and treatment, BA:AA ratio and treatment, BA:AA ratio and time, AA and treatment, percent moisture and treatment, EOC and time, NO_3^- and treatment, and NO_3^- and time (Table 2). While all statistically significant values had relationships with either

treatment or time, NO_3^- and the BA:AA ratio had statistically significant relationships with treatment by time. Five of the six soil characteristics were affected by treatment (the sample being from a natural, restored, or agricultural site) and three of the six soil characteristics were affected by when the samples were taken (November, February, or April).

I used Tukey's Test to compare means of the natural, restored, and agricultural sites. For the BA and AA DEA rates, the natural and restored wetlands were not significantly different; in addition, the restored and agricultural sites were not significantly different. This showed that while restored sites were not very different from natural or agricultural sites, the natural and agricultural sites were significantly different from each other. This same relationship was also found in the percent moisture variable.

For the BA:AA ratio, the agricultural sites were not significantly different from the other two site types, but the restored sites were different from the natural sites. A significant difference also existed between the April samples and the other two samples times, but the November and February samples were not significantly different from each other.

EOC showed an interesting Tukey grouping for time. February and April were statistically similar to one another, while November was different than both. Nitrate had Tukey groupings for treatment and time. For treatment, the Agriculture sites were statistically different than the other two site types (these sites were similar to each other); for time, April was statistically different than the November and February samplings (which were similar to one another).

I ran correlations on the following soil characteristics to see how they compared to each other over all treatments (natural, restored, and agricultural) and over all times (November, February, and April). These numbers are summarized in Table 2. Any values above 0.381 and

below -0.381 represent a high positive or negative correlation respectively between the two values being compared. This was determined by the significance level set in the Tukey's Test ($P < 0.05$).

	Treatment	Time	Treatment by time
Before Acetylene	0.0456	0.1583	0.4952
After Acetylene	0.0453	0.9012	0.5031
BA:AA ratio	0.0449	0.0006	0.6060
Percent Moisture	0.0033	0.1565	0.9316
Total Organic Carbon	0.7258	0.0048	0.5081
Nitrate	0.0132	<0.0001	0.6357

Table 2. Tukey's Test p-values for soil characteristics based on treatment, time, and treatment by time effects, with statistically significant p-values highlighted.

	BA	AA	BA:AA	% H_2O	EOC	NO_3^-
BA	1	-	-	-	-	-
AA	0.425876	1	-	-	-	-
BA:AA	0.372848	-0.22712	1	-	-	-
% H_2O	0.409928	0.745827	-0.48914	1	-	-
EOC	0.015959	0.22664	0.332972	-0.22119	1	-
NO_3^-	0.164751	-0.19945	0.75738	-0.6583	0.500112	1

Table 3. Correlation values for all site types and times with statistically significant correlations highlighted.

DISCUSSION

Summary

There was a positive correlation between percent moisture and DEA rates. Since acetylene merely prevents N_2O being converted into N_2, and has no effect on how much N_2O is

produced in the first place, it is understandable that this relationship would exist both before and after the addition of acetylene. We would also expect BA and AA to correlate to each other for this same reason. The acetylene is not acting as a catalyst to the denitrification process, overcoming the limiting reactant to produce more available amounts of N_2O; it is instead allowing more N_2O to be detected because it is preventing its final transformation into dinitrogen (N_2) gas.

I expected the DEA rates to have a positive correlation with percent moisture, EOC, and NO_3^- concentration; however, this relationship was only present in the percent moisture. This relationship is perhaps the most expected as denitrification is an anaerobic process and an increase in water cover will increase the amount of denitrifying activity present.

In addition, I expected additional relationships between denitrification rates and the amount of carbon in the soil and the amount of available NO_3^-. The denitrification process starts with NO_3^-, so an increased amount of available NO_3^- in the soil would seem to predict a higher amount of N_2O; yet there was no correlation between NO_3^- and either of the DEA rates, either before or after acetylene addition. However, these are potential assays, so the in situ rates could be much lower with NO_3^- higher at times. It seems that NO_3^- concentrations in all the soils were high enough that they did not limit denitrification. While I would expect there to be a higher level of activity in the wetland areas due to the increased water levels, the addition of nitrogen-based fertilizers on agricultural sites may have allowed for greater enzymatic activity in those areas instead. I predicted a positive relationship between EOC and DEA because denitrifiers use carbon as their energy source. This did not have any correlation to the DEA data.

While the EOC and NO_3^- data did not relate to either of the DEA categories, they did positively correlate to each other. This could mean that higher organic matter has higher levels of nitrogen, which favors the production of NO_3^-. The relationship of note is the one between percent moisture and NO_3^-. These are negatively correlated, which may be because nitrification (the process that produces NO_3^-) is aerobic. Less NO_3^- is produced in wetter soils (or possibly more is denitrified), although the lack of correlation between the NO_3^- and DEA data was not strongly supportive of this. The DEA rates (AA and BA) were positively correlated, as were BA and percent moisture, AA and percent moisture, BA:AA ratio and NO_3^-, and EOC and NO_3^-. The BA:AA ratio's relationship with NO_3^- is consistent with what others have found, and theory would predict. Generally, the higher the NO_3^-, the lower the N_2O reduction activity, because there is ample NO_3^- to accept electrons, so there are not as many electrons left over to reduce N_2O. BA:AA ratio and percent moisture and percent moisture and NO_3^- were negatively correlated.

The DEA rates showed similar trends over time. The highest activity for all site types was in February, which was the wettest time point measured. The next highest rates were found in November and then April. The highest DEA rates were found in the natural wetland sites, with restored wetlands next and agricultural sites the lowest. However, there was a point where the restored and agricultural sites measured about the same, and another point in November where the before acetylene natural wetland rate was lower than the other two.

The addition of acetylene had an effect on the overall DEA rates, as we expected. All showed a consistent decrease in DEA rate during the April samplings, while February's DEA rates had the most reaction to the addition of acetylene. Natural wetlands, with the exception

of November, had the highest reaction to the addition of acetylene. Restored wetlands stayed about the same until the noted drop in April. Agricultural sites showed a consistent decrease in how much the acetylene boosted DEA rate, which may have been the result of the addition of fertilizers manipulating the soil microbe composition.

Related Studies

Many papers have discussed the relationship between N_2O emission and the amount of organic carbon or nitrogen present in the soil (Wu et al. 2009). They argue that controlling the amount of carbon and nitrogen in the soil will prevent the greenhouse gas effect created by the emission of N_2O into the atmosphere. However, this applied to studies that examined the denitrification rates against other soil characteristics. Studies that compared different denitrification rates among natural and created wetlands (Bruland 2006) showed that restored wetland areas were significantly more homogenous in their NO_3^- concentrations than natural wetlands. According to Bruland (2006), "denitrification potential and related soil properties in CW/RWs and NWs appeared to be influenced by a complex interplay of factors including prior land-use (agriculture, upland, type of mitigation (restoration versus creation), and hydrogeomorphic setting" (Bruland 2006, P. 1054). However, he also stated that "without suitable soil properties, CWs may never functionally replace the natural wetlands that were destroyed" (Bruland 2004, P. 2076).

Since denitrification is an anaerobic process, other studies have looked at how the presence of O_2 affects the denitrification process in wetland areas (Burgin 2010). With an increase in moisture content, there is a greater potential for denitrification to occur. Therefore

Burgin (2010) compared wet and dry soils. Her conclusions confirmed that DEA rates increase as the moisture level increases.

A study by DeSimone and colleagues (2010) concluded that N_2O is an indicator of microbial activity at any particular moment. The authors further explained that the collection of N_2O with NO_3^- was to elucidate the soil profile processes that drive the production and movement of N_2O released. However, since each sampling is referred to as a "snapshot" of subsurface activities, a depleted supply of NO_3^- could imply rapid turnover of N, causing low NO_3^- levels; it was determined that this depletion was the result of rapid denitrification and loss of NO_3^- via N_2O release, as could be the same case in my study.

In McGill's (2010) research, DEA rates were compared with available N and C. As in my study, the authors expected a positive correlation between the DEA rate and N and C. However, like our study, this was true at given time points as opposed to a general overall trend in DEA rates.

Sources of Error

I was extremely limited in terms of site selection. All of the sites selected were either under jurisdiction of the Portland Metro, belonged to private land owners, or were not considered "ideal" wetland areas to work with. A prime example of the latter was the Knez site (one of the three replicate natural sites). This site was a small swatch of land located behind an industrial park next to I-5. Its soil composition was very dissimilar to the other two wetland areas used to determine overall natural wetland statistics.

The three natural wetland sites (Green Mountain, Gotter Prairie North, and Knez) were just one example of the soil variability at the three supposedly replicated sites. According to the

USDA and NRCS soil reports on the sites, the Knez site was comprised of 67.5% Verboort silty clay loam and 32.5% Huberly silt loam; Green Mountain was comprised entirely of Cove silty clay loam. Gotter Prairie North was comprised of several different kinds of soil: Wapato silty clay loam (60.8%), McBee silty clay loam (4.8%), Laurelwood silt loam (7.3%), Labish mucky clay (13.2%), Cove clay (13.7%), and Chehalis silty clay loam (0.3%). While all of these sites contain silty clay loam, it is in varying degrees and concentrations, and some sites contain other soil types. While averaging the three sites together helped to present a clearer picture, they did not represent replicates.

The restored wetland sites were a little more complicated. Gotter Prairie South was comprised of the following soil types: Wapato silty clay loam (79.2%), McBee silty clay loam (9.4%), Cove clay (0.1%), and Chehalis silty clay loam (11.3%). Hutchinson was comprised of Wapato silty clay loam (51.0%) and McBee silty clay loam (49.0%). Lovejoy was comprised of the following soil types: Aloha silt loam, Cove Clay, McBee silty clay loam, Quatama loam, and Wapato silty clay loam. The similarity of the three restored sites as compared to the more dissimilar natural sites could account for why the soil characteristics and other data were more reliable among each of the restored sites.

Of the three agricultural sites, the Zugar property was comprised of Willakenzie silty clay loam (99.0%) and Waldo silty clay loam (1.0%). Westbrook and GPA are both privately owned lands, so information on their soil composition was not readily available. Instead, the owners provided some basic background information on tilling practices and crop rotations. Zugar had some herbicide and fertilizer use, including the addition of N, P, K, and lime. Westbrook was fertilized every year, but had no herbicide use. GPA had fertilizer use as well: triple 16 and 45%

Nitrogen. Weeds were spot-sprayed with 2,4-D and Roundup. The addition of nitrogen and other nitrogen-based fertilizers is likely to have caused the higher rates of nitrate and nitrogen measured for the agricultural sites as opposed to the other site types.

Future Research

This research was a part of a pilot project crafted by Metro and the NRCS as a way to evaluate current wetland management practices and how useful they will be in converting restored wetland sites to a site comparable to natural wetlands. The information collected here should help not only researchers to better understand the microbial communities in the wetland areas they are trying to conserve, but also help farmers who use fertilizers and herbicides to tend to their soil. If farmers want to reduce their greenhouse gas emissions, perhaps new research could provide them with opportunities to increase their farming productivity without contributing to the production of N_2O. Furthermore, this will help conservationists learn the perfect cocktail of nutrients and conditions to expedite the transformation of restored sites to what is expected from a natural wetland area. It may also contribute to more sites being donated as restored sites, with people recognizing the benefit of increased number of natural wetland areas.

Impact (local, global, greenhouse gas emissions)

Although N_2O is a non-toxic gas (it has been used for years as a mild anesthetic and fuel additive), it is a powerful greenhouse gas that can persist for up to 150 years while it is slowly broken down in the stratosphere. Although N_2O only accounts for around 0.03% of total greenhouse gas emissions, it has a 300-fold greater potential for global warming effects, based on its radiative capacity, compared to that of carbon dioxide (CO_2). More than two-thirds of

N_2O emissions come from bacterial and fungal denitrification in soil; this has been exacerbated through the increased intensity of agriculture (the "green revolution"), which has increased the presence of nitrogen in soil through the application of synthetic nitrogen-based fertilizers.

Many scientists believe that reducing the amount of N_2O produced will reduce overall greenhouse gas emissions. One method under consideration is mitigating N_2O emissions by enhancing the transformation of N_2O to N_2. Some research looks at the specific enzymes involved in converting nitrate to N_2O, while other research would like to improve farming practices, moving away from the fertilizer and herbicide-rich practices. Considering that a large amount of N_2O can be produced by inhibiting its production to N_2 (as demonstrated by the addition of acetylene), researchers have looked at two main enzymatic pathways that control the denitrification cycle (Richardson 2009).

Either way, it is important to examine this research when thinking about the global impact of restoring a former agricultural site into a wetland. Through the proper manipulation of soil characteristics and moisture level, a site that was formerly used for high-intensity agricultural could be transformed, or returned in some cases, to a functioning wetland area. The restored sites in this study prove that some of the techniques provided by Metro and the NRCS are working; however, if they want to amplify their efforts, they will need to alter some of their current management practices. Furthermore, they will need to decide if transforming all of these former agricultural sites into wetlands will benefit the community on a global scale, as the natural process of denitrification will create additional N_2O, a strong greenhouse gas.

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