The impact of denitrification of alter vegetations and soils under precipitations treatments

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Abstract Nitrous oxide is a product of nitrification and denitrification. It can act as a powerful greenhouse gas to cause stratospheric ozone destruction in the stratosphere. This study examines the methods and patterns of nitrous oxide accumulation and the effects of disturbance, precipitation patterns, and vegetation on denitrification. The objective of this study is to understand how the denitrification of different precipitation levels and soil contents affect the nitrification and denitrification cycle, and to better understand the characteristics of nitrous oxide, especially in the process of denitrification of microbial communities. This experiment was conducted at Richmond Field Station's glasshouse. Soil cores were used to collect samples. We measured levels of nitrous oxide and denitrifying enzyme activity (DEA) using the acetylene block assay. We also measured levels of nitrate, mineralization, and water moisture to understand how soil properties influence DEA.

Introduction

Denitrification proceeds through a combination of the following steps: nitrate –nitrite----- nitric oxide----nitrous oxide----dinitrogen gas (Madigan and Martinko, 2006). The process of denitrification reduces nitrate and nitrite. Nitrate and nitrite are high oxidized forms of nitrogen gas, this transformation represents a very important step in Nitrogen cycling. Denitrification is the mechanism of anaerobic respiration in which $NO3^-$ is reduced to gaseous nitrogen compounds, primarily N2 (Madigan and Martinko, 2006). The rapid microbial response to the changes in soil moisture on nitrous oxide production has an important implication on Nitrogen-cycling processes (Rudaz et al. 1990). This study is part of a larger project to study the effects of soil variance on microbial communities and their soil processes. Also this study evaluates the genomic basis of plant and soil microbial controls of terrestrial ecosystem in response to climatic change. Whether the structure of soil microbial communities control Nitrogen or Carbon transformation, production and consumption of reactive traces gas in microbial community ecology is important process to the Nitrogen and Carbon cycle.

Previous studies have examined known rates of denitrification and N-cycling processes (Oquist et al.., 2004, Parson et. al., 1991). But those studies can not yet link these rates to the microbial communities Using molecular methods, scientists have discovered functional genes that control denitrification, and that denitrification varies between cultivated and uncultivated, riparian and agricultural soils, wetland and forested uplands, forest and meadow soils (Rich and Myrold, 2004, Rich et. al. 2003, and Mergel et al., 2001). Recent studies have also assisted in the development of models on n-cycling that involve the microbial communities and its environmental processes (Boyle et.al., 876).

The amount of nitrous oxide released to the atmosphere is thought to be low, and estimated at 0.2 kg N ha^{-1} $year^{-1}$ (Boyle, 2006). Based on the global production of nitrogen fertilizer from 1920 to 1985, biogeochemists estimate the global budget of nitrous oxide, N_2O, to have a mean concentration of 331 ppb, 2.4 x 10^15 g of N_2O or 1.5 x 10^15 g N in the atmosphere which increases at an annual rate of 0.3% (Prinn et al 1990, Khalil and Rasmussen 1992). Based on the statistical analysis of Cycle of Soil , N_2O in stratospheric destruction is a significant sink and consumes about 12x 10^12 g N as N_2O per year. Each molecule of N_2O has the potential to contribute to the greenhouse effect at three hundreds times relative to potential of impact of the

global climate in 21st century. Furthermore, some soils also to consume N_2O, but the global sink in soils is unknown and perhaps very small (Stevenson and Cole 2001).

Determining the potential for ecosystems to respond to changing of environments is a major concern nowadays due to the global warming issue. Scientists connect the rapidly increasing rates of global warming with increases in nitrous oxide production. Global warming has resulted in rising sea levels, extreme weather changes, hurricanes, and swift-changing precipitation patterns.

The hypothesis of this study is that where soil and root interact with each other, as well as where soil interactions with plant *Avena barbata,* will give a higher gas trace than in the bare soil alone. It also focuses on the denitrifying patterns of plant root activity and the interaction between plant microbial communities within the rhizosphere, which will help us have better understand plant and microbial communities overall. Furthermore, the objective of this research could lead to deeper understandings on biophysical interactions of bacteria with the soil environment. This further understanding on physical characteristics of the soil matrix will help us to understand the growth and activity of soil microbes and the characteristic of the microhabitats in soil. Lastly, it can help to figure out the mechanical understanding of how terrestrial ecosystems could impact on global environment change and on the denitrification process.

Methods

Site and soil descriptions The study was conducted at the glasshouses located in Richmond Field Station, Richmond CA. The eight mesocosms were roots in 50 x 75 schedule 40 PVC cylinders. These mesocosms were exposed to patterns of precipitation: low, ambient high rainfall. Four bare soil cylinders and four cylinders contained *Avena barbata* communities. California annual grassland mesocosms being used in the project were from Hopland and Sedgwick Field Stations. These soils were used for this study because they represent northern and southern California, respectively, under treatments of low and high precipitation in the greenhouse. Characteristics of these soils are described in the table 1.

Site	Location	Mean Annual Rainfall	Mean Annual Temperature	Soils	Plant community
Hopland	39° N 123° 4' W	940 mm (37")	15° C (59°)	Sandstone-derived Alfisol and Mollisols	Annual grassland
	Soil characteristics, Laughlin Series: fine sandy loam pH 5.6 – 6.0; ~2% original C in upper 15 cm. Most abundant grassland species: Avena barbata, Bromus diandrus, Bromus hondeaceus, Erodium botrys, Hordeum marinum, Taeniatherum caput – meduasae, Vulpia myuros. Also Geranium and Lolium.				
Sedgwick	34° N 36N 124° 04' W	380 mm (15")	15° C (59°)	Sandstone-derived Alfisol and Mollisols	Annual grassland
	Soil characteristics, Salinas Series: pH 8.0 Silty Clay loam 1.4% original C in upper 20 cm. Dominant grassland species: Bromus hordeaceus, B. diandrus. Nassella plulchra. Avena barbata. Sub-shrubs and .herbs include Lessignta filaginifolia. Charkia cylindrical. Erodium cicutarium (a geranium) (http://nrs.ucop.edu)				

Table 1. Site characteristics of Hopland Field Station (seed and soil source), and Sedgwick Field Station (soil source)

Collected soils and nitrous oxide samples To obtain gas and soil samples we clipped vegetation from within a 4 inch diameter circle and placed a PVC collar in the soil within the clipped circle, with 1cm remaining above the soil surface. After taking gas flux trace samples, we took out the collars and chambers to core the soils from each mesocosm. Then, equally replaced the amount of soils from the spared soils under the same treatments. We then transported all the samples to Hilgard Hall at UC Berkeley.

For the soil samples, we weighed 20g of soil collected and put it in a jelly jar, weighed the jar and soil, and recorded the weights. Then we added 50ml DEA solution and shook vigorously. We then flushed each jar with nitrogen 3 times, drew 20ml of air from the jar, and added 20ml of acetylene. Before injecting samples into the gas chromatography (GC), we injected room air samples or CO_2 samples into the GC until stable results were achieved. Using a plastic syringe, we immediately draw 5ml of air from the sample jar and injected it into the GC. We ran each sample in the GC again after 45 minute intervals from the 1st run. Finally, we analyzed all the GC sample results from each mesocosm.

Denitrifying enzyme potential The basic procedure for the assay is to add 20 g soil amended with 30 ml of solution containing glucose and KNO_3 into the jell jar. Seal the jar with a

fresh lid and rubber stopper, evacuate the headspace, and flush the jar with N_2 three times. Equilibrate with atmospheric pressure after the last N2 flush using a glass syringe. The soil slurries were mixed on the rotary shaker for 45 minutes after adding acetylene. The assay was made anaerobic and acetylene was added to block N2O reductase activity. Two gas samples were removed and injected into the gas chromatography from the headspace for N_2O analysis. After analyzing the N_2O, we performed rock content determinations by passing the soil through a 2mm mesh wet sieve and recorded the rock weights after placing the rock in pre-weighed tins in the oven overnight to dry at 105° C.

Statistical analysis DEA and N2O production were analyzed using repeated measures of analysis of variance (ANOVA) by using the Statistix 7.0 package. The sum of square, mean square and F-test are approximated in order to complete analyses with missing values.

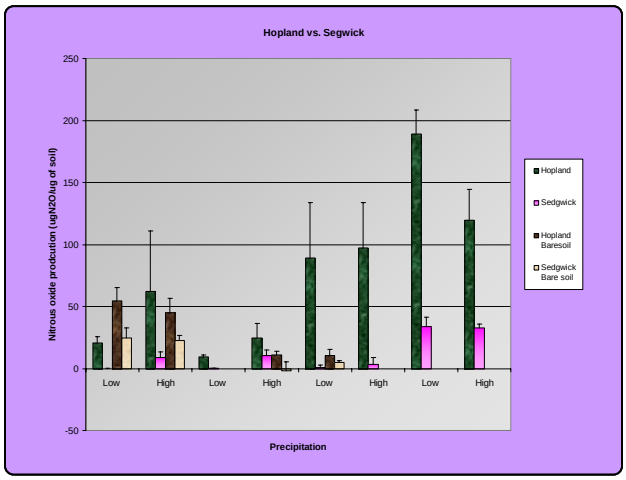
Result

The community composition of denitrifying microbes is very diverse in the different soil environments (Boyle 2006). In this study, over several consecutive periods of time throughout the year, there were no significant effects in the levels of precipitation and vegetation on denitrification ($p>0.05$). Based on my data, the monoculture community has a great impact compared to the mixed community even though the data turned out not significant.

Hopland soils had significantly higher nitrous oxide than Sedgwick soils ($p=0.003$). The comparison between bare soils and vegetation soils was statistically significant ($p=0.001$). The data demonstrates that vegetation soils produced more nitrous oxide production than bare soil; this could be a result of the specific kinds of interactions between the soil and the roots of the plants.

Figure A and B. Comparison between Hopland and Sedgwick soils (bare soils and vegetation soils)

A B



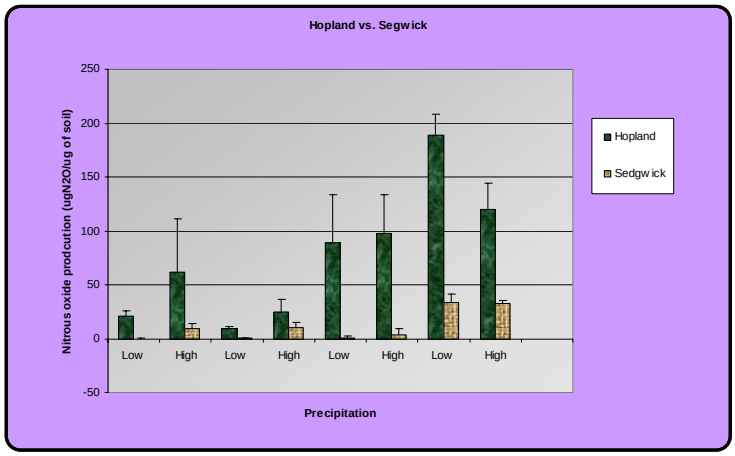
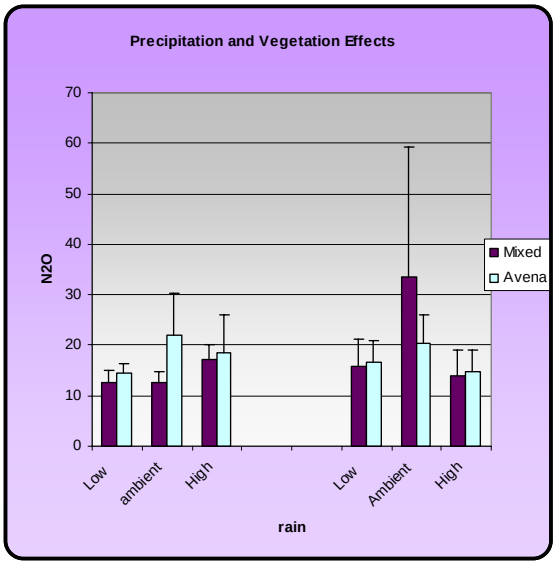
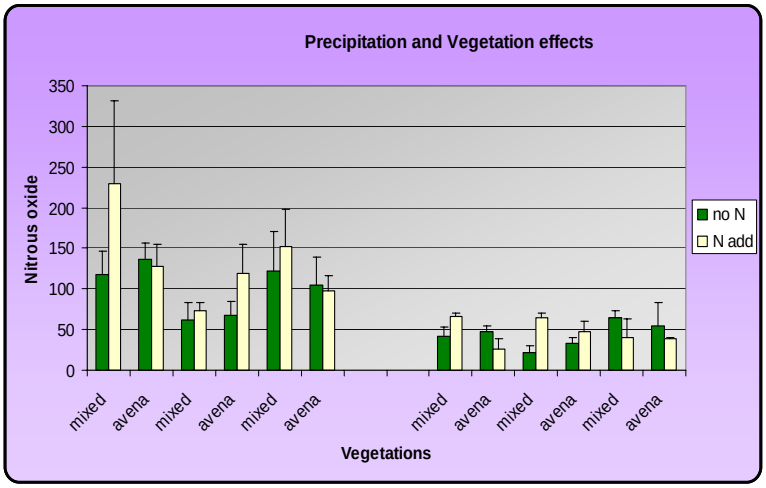


Figure B and C: The production of nitrous oxide under various treatments of precipitation among the mixed and monoculture vegetation.

B C





Discussion

DEA and soil properties were analyzed using repeated measure analysis of variance (ANOVA) (Ramsey and Schafer, 2002). Significant interactions between time of sampling, site, and treatment required that the data be analyzed separately for treatment. Treatment effects were considered significant if the p value was less than 0.05 However, the results were inconclusive.

Although disturbance effects were observed in specific stage of the experiment, the results were not statistically significant.

The lack of significant changes in denitrifying community composition as a whole suggest that these communities may be well enhanced DEA in most vegetation soils and also suggests that a rhizoshpere effect may have favored a larger more active denitrifying population. The influence of roots on denitrification is a balance between positive and negative effects. Respiration by roots and enhanced microbial respiration from root-derived C may decrease O_2 availability, thereby favoring denitrifying bacteria (Hart 1988). Plant uptake of water and NO_3^- could have a negative impact on denitrifying bacteria, the former by increasing aeration and the latter by direct competition (Boyle, 2006). We measured the soil water content as a proxy for soil aeration to calculate nitrous oxide production.

We hypothesize that a longer test time would have resulted in significant changes in our results. Future studies should examine patterns of denitrifying activity, denitrifier community dynamics, and plant-root activity in order to better understand plant and microbe interactions.

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References

Boyle, S. A., J. J. Rich, P. J. Bottomley, J. K. Cromack, and D. D. Myrold. 2006. Reciprocal transfer effects on denitrifying community composition and activity at forest and meadow sites in the Cascade Mountains of Oregon. *Soil Biology and Biochemistry* 38: 870–878.

Firestone, M. K., M. S. Smith, R. B. Firestone, and J. M. Tiedje. 1979. The influence of nitrate, nitrite and oxygen on the composition of the gaseous products of denitrification in soil. *Soil Science Society of America Journal* 43: 1140–1144.

Hart, J. Consider a Spherical Cow 1988 University Science Book Sausalito, California

Hart, S., Firestone, M., Paul, E., and Smith, J. 1992 Flow and fate of soil nitrogen in an annual grassland and a young mixed-conifer forest. *Soil Biology and Biochemistry* 25 (4), 431-442.

Madigan, M and Martinko, J. 2006. Brock Biology of Microorganisms, 11th ed. Pearson Prentice Hall.

Mendocino County, California, Western Part. Table 16. Classification of the Soils http://www.ca.nrcs.usda.gov/mlra02/wmendo/tables/table16.txt

Mergel, A., Schnitz, O., Mallmann, T., Bothe, H., 2001. Relative abundance of denitrifying and dinitrogen fixing bacteria in layers of a forest soil. FEMS Microbiology Ecology 36, 33-42.

Myrold, D.D. 1998. Transformations of nitrogen. *IN*: Sylvia, D.M. et al (eds*). Principles and applications of Soil Microbiology*. Prentice Hall, Upper Saddle River, N.J.

National Cooperative Soil Survey http://ortho.ftw.nrcs.usda.gov/osd/dat/S/SANTA_YNEZ.html.

Oquist, M.G., Nilsson, M., Sorensson, F., Kasimir-Klemedtson, A., Persson, T., Weslien, P., Klemedtsson, L., 2004. Nitrous oxide production in a forest soil at a low temperatures processes and environmental controls. *FEMS Mircobiology Ecology* 49, 371-378.

Parson, L.L., Murray, R.E., Smith, S.M., 1991. Soil denitrification dynamics spatial and temporal variations on enzyme activity populations, and nitrogen gas loss. *Soil Science Society of America Journal* 55, 90-95.

Rich, J.J., Heichen, R.S., Bottomley, P.J., Cromack Jr. , K., Myrold, D.D., 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied & Environmental Microbiology 69, 5974-5982.

Rich, J.J., Myrold, D.D., 2004. Community composition and activities of denitrifying bacteria from adjacent agricultural soil, riparian soil, and creek sediment in Oregon, USA. Soil Biology & Biochemistry 36, 1431-1441.

Rudaz, A., Davidson E., and Firestone, M. 1990 Sources of nitrous oxide production following wetting of dry soil FEMS Microbiology Ecology 85, 117-124.

Schaller, J. Royer, T., David, M., and Tank, J. Deinitrification associated with plants and sediments in an agricultural stream. Journal of North American Benthological Society 23 (40), 667-676.

Stevenson, F.J. and Cole, M.A. 2001 Cycle of Soil 2nd ed. JohnWiley & Son Ins, USA

Tiedje, J.M. 1982. Denitrification. pp 1011-1026. *IN:* Page, A.L. et al (eds). Methods of soil analysis. Part 2. Second edition. American Society of Agronomy, Madison, WI, USA.

Tiedje. J.M. 1995. Denitrifiers. pp 245-267. *IN*: Weaver, R.W. et al (eds). Methods of Soil Analysis. Part 2. American Society of Agronomy, Madison, WI, USA.

Wood, P.M (1986) Nitrification as a bacterial energy source, *IN* Nitrification Proseer, J.I., Ed pp. 39-62. IRL Press, Oxford.