Effect of increasing nitrogen deposition on soil microbial communities

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ABSTRACT

Increasing nitrogen deposition, increasing atmospheric CO_2, and decreasing biodiversity are three main environmental changes occurring on a global scale. The BioCON (Biodiversity, CO_2, and Nitrogen) ecological experiment site at the University of Minnesota's Cedar Creek Ecosystem Science Reserve started in 1997, to better understand how these changes would affect soil systems. To understand how increasing nitrogen deposition affects the microbial community diversity, heterogeneity, and functional structure impact soil microbial communities, 12 samples were collected from the BioCON plots in which nitrogenous fertilizer was added to simulate the effect of increasing nitrogen deposition and 12 samples from without added fertilizer. DNA from the 24 samples was extracted using a freeze-grind protocol, amplified, labeled with a fluorescent dye, and then hybridized to GeoChip, a functional gene array containing probes for genes involved in N, S and C cycling, metal resistance and organic contaminant degradation. Detrended correspondence analysis (DCA) of all genes detected was performed to analyze microbial community patterns. The first two axes accounted for 23.5% of the total variation. The samples fell into two major groups: fertilized and non-fertilized, suggesting that nitrogenous fertilizer had a significant impact on soil microbial community structure and diversity. The functional gene numbers detected in fertilized samples was less that detected in non-fertilizer samples. Functional genes involving in the N cycling were mainly discussed.

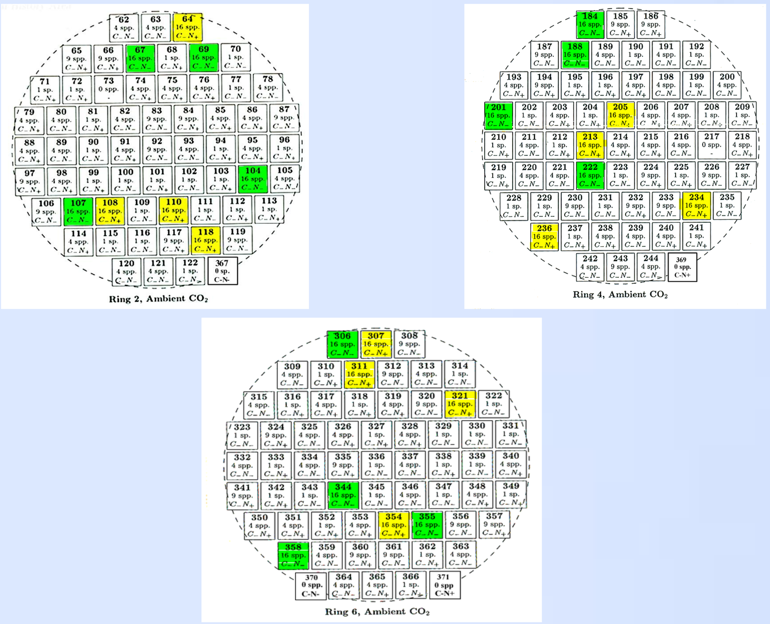
INTRODUCTION

Increasing nitrogen deposition, increasing atmospheric CO_2, and decreasing biodiversity are three main environmental changes occurring on a global scale [1- 4]. Human activity has doubled the amount of nitrogen (N) entering terrestrial ecosystems throughout eastern North America and Europe, a trend that is likely to increase over the next several decades [5].

Increasing nitrogen deposition may be the main cause of forest degradation in some regions of Europe and America [6]. N deposition clearly affects ecological processes such as soil respiration, soil microbial activity, soil pH, soil nutrient elements and litter decomposition [7] and may have a greater impact on plant species diversity than previously thought [8].

To date, most research on N deposition has focused on the aggregate effects on soil chemistry and N cycling [9-13]. However, very little is known about the response of the microbial communities responsible for soil nutrient cycling and decomposition. In this study, we examine changes to microbial communities as a result of increased N deposition.

STUDY SITE



BioCON (Biodiversity, CO_2 and Nitrogen) is an experiment site located at the Cedar Creek Ecosystem Science Reserve in Minnesota, USA (lat. 45° N, Long. 93° W). Plots were established in 1997 on a secondary successional grassland on a sandy outwash soil after removing the previous vegetation. There are 4 levels of plant biodiversity (1, 4, 9 and 16 species) and 2 levels each of CO_2 through FACE technology and nitrogen addition.

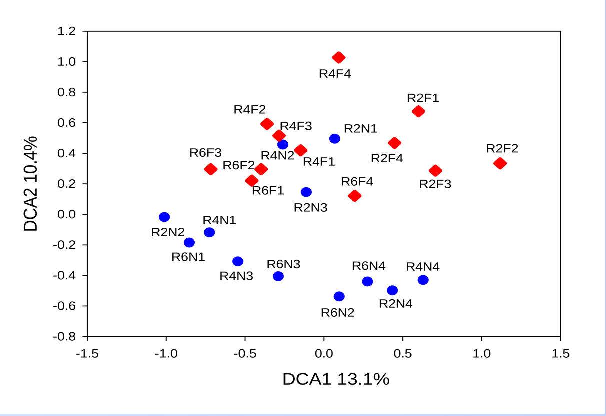
METHODS

▪**Soil collection:** Soil samples were collected from 24 plots, 12 from plots in which nitrogenous fertilizer was added to simulate the effect of increasing nitrogen deposition (4 g NH_4NO_3/ M^2/year and 0.474g $^{15}NH_4{}^{15}NO_3$/plot/year), 12 from plots without added fertilizer.

▪**DNA extraction, amplification and labeling**: High molecular weight DNA was extracted by freeze-grinding and phenol/chloroform extraction [14]. DNA was then amplified by whole community rolling circle amplification using a Templiphi kit (GE Healthcare, Piscataway, NJ). Amplification products were labeled with Cy-5 fluorescent dye by random priming.

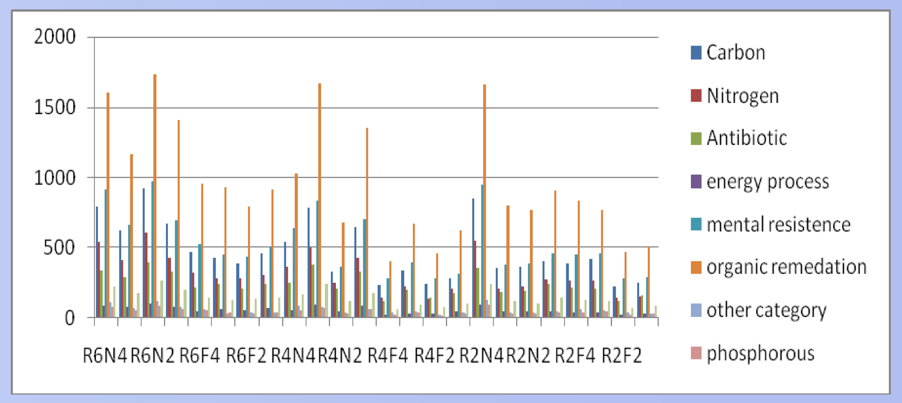
▪**Microarray hybridization, scanning and data processing**: Labeled DNA was hybridized to a comprehensive functional gene array (GeoChip 3.0) to examine changes in the microbial community structure. Hybridizations were carried out at 45 °C for 12 hours. Arrays were scanned and the signal intensity for each probe was digitally determined by ImaGene version 6.0 (Biodiscovery, El Segundo, CA).

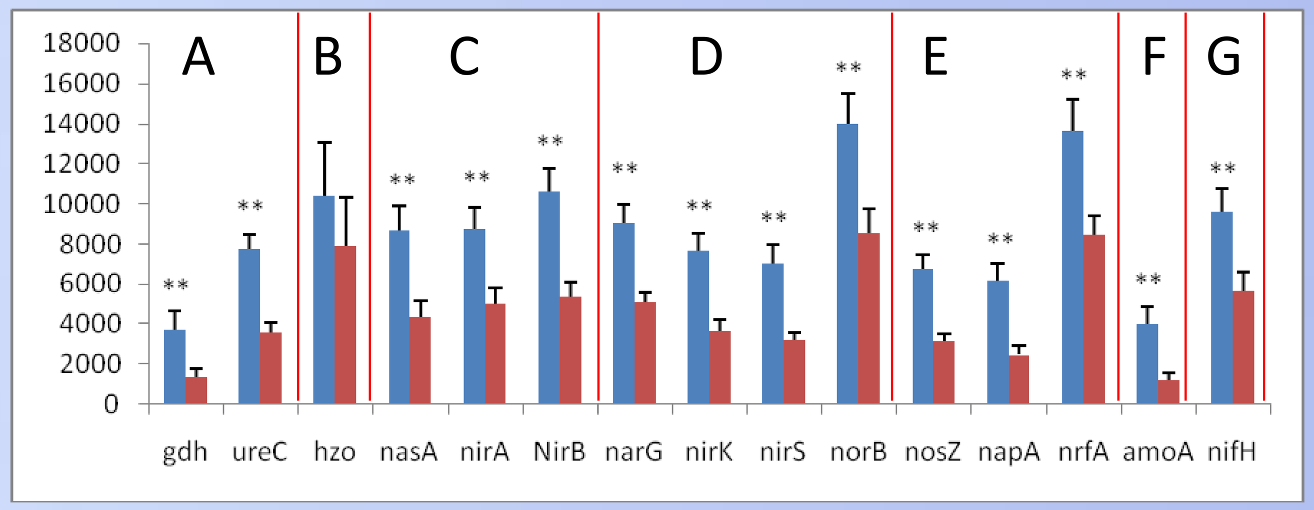
RESULTS



Detrended correspondence analysis (DCA) of all detected genes was used to examine the overall functional structure changes in the microbial communities (left). The 24 samples clustered into two main groups: one with nitrogenous fertilizer (red) and the other without nitrogenous fertilizer (blue) . These results indicate that the microbial communities were significantly altered after nitrogenous fertilizer addition.

The number of functional genes detected from the 9 main gene categories. Fewer genes were detected in samples with nitrogenous fertilizer addition, suggesting that nitrogenous fertilizer addition may decrease microbial diversity.





The normalized average signal intensity of detected key gene families involved in the N cycling. Blue bars represent samples without fertilizer, red bars represent samples with fertilizer. (A) Ammonification; (B) Anammox; (C) Assimilatory N reduction; (D) Denitrification; (E) Dissimilatory N reduction; (F) Nitrification; (G) Nitrogen fixation. All data are presented as the mean ± SE (error bars). **p < 0.05, *p < 0.10.

Gene abundance was significantly lower in samples with added fertilizer.

SUMMARY

- Adding nitrogenous fertilizer influences the structure of microbial communities in the soil samples
- After adding nitrogenous fertilizer, microbial community diversity decreased
- Abundance of several main functional genes involving in the N cycling deceased after adding nitrogenous fertilizer

REFERENCES

[1] Barabasz W., Albińska D., Jaśkowska M., Lipiec J. (2002). Biological Effects of Mineral Nitrogen Fertilization on Soil Microorganisms, Polish Journal of Environmental Studies, 11, 3:193-198.
[2] Magill A.H., Aber J.D., Hendricks J.J., Bowden R.D., Mëlillo J.M., Paul A., Steudler A. (1997). Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecol Appl, 7: 402-415.
[3] Reich P.B., Hobbie S.E., Lee T., Ellsworth D.S., West J.B., Tilman D., Knops J.M.H., Naeem S. and Trost J. (2006). Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature, 440, 922-925.
[4] Reay D.S., Dentener F., Smith P., Grace J. and Feely R.A. (2008). Global nitrogen deposition and carbon sinks, Nature, doi: 10.1038/ngeo230.
[5] Bouwman, A.F., Van Vuuren, D.P., Derwent, R.G., Posch, M., (2002) A global analysis of acidification and eutrophication of terrestrial ecosystems. Water Air Soil Pollut. 141, 349–382.
[6] Boxman A.W., Blanck K., Brandrud T.E. (1998). Vegetation and soil biota response to experimentally-changed nitrogen inputs in coniferous forest ecosystems of the NITREX project. For Ecol Manag, 101: 65-79.
[7] Mo J.M., Brown S., Xue J.H., Fang Y.T., Li Z.A. (2006). Response of litter decomposition to simulated nitrogen deposition in disturbed, rehabilitated and mature forests in subtropical China. Plant Soil, 285: 135-151.
[8] Clark C.M., Tilman D. (2007) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature, 451, 712-715.
[9] Vitousek P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H., Tilman G.D. (1997). Human alteration of the global nitrogen cycle: Sources and consequences. Ecol Appl, 7(3): 737-750.
[10] Lamarque, J.F., Kiehl J.T., Brasseur G.P., Butler T. (2005). Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: Analysis of nitrogen deposition. *J. Geophys. Res.* 110, doi:10.1029/2005JD005825.
[11] Dentener F., Drevet J., Lamarque J.F., Bey I., and Eickhout B. (2006). Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. *Global Biogeochem. Cycles* 20, doi:10.1029/2005GB002672.
[12] Suding K.N., Collins S.L., Gough L. (2005). Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences of the United States of America, 102, 4387-4392.
[13] Berg B. (1988). Dynamics of nitrogen (^{15}N) in decomposing Scots pine (Pinus sylvestris) needle litter: Long-term decomposition in a Scots pine forest. Can J Bot, 66: 1539-1546.
[14] Zhou J.Z., Bruns M.A., Tiedje J. M. (1996). DNA recovery from soils of diverse composition. Appl. Environ. Microbiol., 62: 316-322.

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