N-131 Diversity of Microbial Genes Involved in Nitrogen Cycling, Methanogenesis and Methane Consumption at Two Forested Free Air CO2 Enrichment (FACE) Sites in the Southeastern United States

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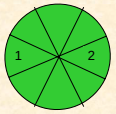
Abstract

Increases in global CO2 levels have the potential to change ecosystems and their functions. Such changes may affect microbial communities either directly or indirectly through changes in temperature or plant communities. Soil microbial communities that drive processes such as those involved in nitrogen and methane cycling are of particular interest to ecosystem ecologists. We have begun a broad study of microbial functional gene diversity at two Free Air CO2 Enrichment (FACE) sites. Thus far we have focused on denitrification (nitrite reductase, nirK) and nitrogen fixation (nitrogenase, nifH) genes, completing sequencing of ambient and elevated libraries from each site. Diversity of the clone libraries for both genes was high, but much higher in nirK than in nifH. Libraries also contained numerous gene-types (nirK 35/255; nifH 16/250) significantly different on a similarity basis (<85%) than any reported sequences. Interestingly, neither gene showed significant changes in composition between libraries originating from ambient and elevated CO2 levels, as most dominant gene types were shared between treatments. This corresponds with recent surveys of soil enzyme activities that showed no significant differences between treatments. There were however marked differences in nirK genes found between the two sites, but this was less apparent in nifH where one sequence group related to *Bradyrhizobium* dominated both at ~20% (52/250) of all sequences. Currently, we are sequencing libraries containing ammonium and methane monooxygenase (amoA & pmoA) genes involved in nitrification and methane consumption. We are also initiating libraries for nitrate reductase, nitrous oxide reductase (denitrification), methyl M reductase (methanogenesis) and Rubisco (carbon fixation) using existing primers. Additionally we are testing new primers for chitinase, cellulase and laccase genes to study carbon polymer turnover. These data provide a first glimpse of how elevated CO2 levels may affect important microbial functional diversity. An additional goal of these studies is development of microarrays to be used to conduct finer resolution analyses.

DUKE FOREST SITE
4 (sectors of each ring)
6 (3 experimental and 3 control rings)
2 (seasonal samplings)
TOTAL = 48 samples

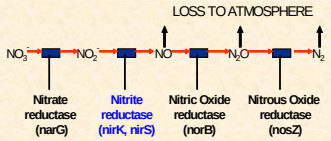


ORNL FOREST SITE
2 (sectors of ea. ring)
5 (depth samples from 2 sectors ea. ring)
4 (2 experimental and control rings)
2 (seasonal samplings)
TOTAL = 80 samples

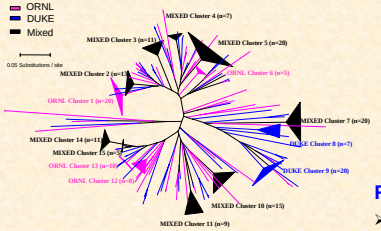
Methods

Samples were obtained from the Duke and ORNL Free Air CO_2 Enrichment (FACE) facilities in Spring 2003. The samples used in this study were composited first by ring and then by treatment. DNA samples from each treatment and site was extracted (Zhou et al., AEM, 1996). Clone libraries were constructed and sequenced using previously described methods (Liu et al., AEM, 2003). These methods were designed specifically to minimize biases associated with PCR amplification. DNA and AA sequences were aligned in clustal and adjusted by hand were required. Phylogenetic analyses were performed in Mega 3.0 using the Kimura-Nei or PAM site substitution corrections Neighbor-Joining methods. All analyses were bootstrapped 500 times. Average pairwise distance between any two taxa was calculated on the partitioned and full data set and used an approximation of sample diversity. Survey results are being used in microarray design (Poster K-070) and the additional samples outlined above will be used in eventual fine scale microarray analyses.

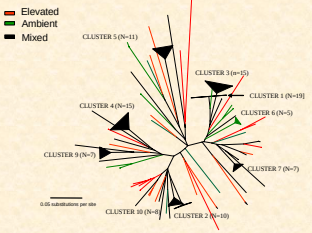
Microbial Denitrification Pathway



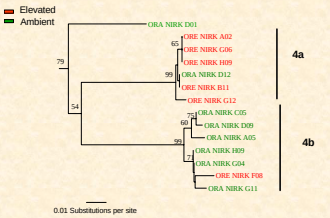
FACE *nirK* (nitrite reductase)



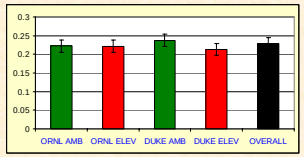
ORNL-FACE *nirK* (nitrite reductase)



ORNL-FACE *nirK* Cluster 4 (nitrite reductase)



Mean phylogenetic distance (*d*) between *nirK* clones from ambient and elevated CO_2 soils.



Results for denitrification genes (*nirK*)

➢Changes in microbial denitrification processes have the potential to alter ecosystem responses via nitrogen availability, and global warming via NO and N_2O production. Nitrite reductase enzymes catalyze the first denitrification step that can result in loss of N to the atmosphere.

➢Over 255 *nirK* clones have been sequenced from each site. Approximately 15 clones appear to be highly novel types with less than 85% homology to other sequenced *nirK*-types.

➢On the scale surveyed there is little evidence for changes in the overall diversity or distribution of nitrite reducing microbial communities between treatments, however very significant changes in the composition and distribution of *nirK*-types are observed between sites (Duke and ORNL).

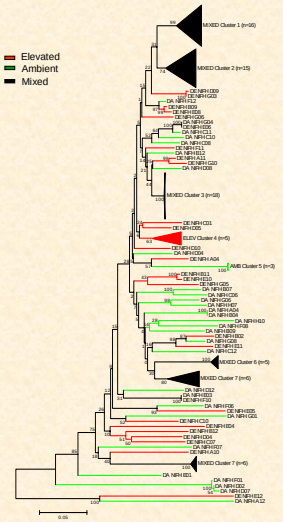
Principal Project Objectives

- Survey of enriched and control ring soils for diversity of genes related to C & N cycling.
- Collaboration, with Duke researchers doing rDNA microbial diversity analyses
- Sample collection and archiving for fine scale microarray analyses.

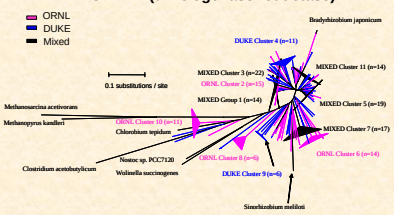
- Carbon Cycle Genes
  - Methane oxidation / reduction
    - Methane monooxygenase (pmoA)*
    - Methyl M reductase (mrr)
  - Polymer Degradation
    - Chitinase (chiA)
    - Cellulase (cellM)
    - Laccase (lcc1)
  - Carbon Fixation
    - Rubisco (rbcL)
- Nitrogen Cycle Genes
  - Denitrification
    - Nitrate reductase (narG)
    - Nitrite reductase (nirK, nirS) *
    - Nitric Oxide reductase (norB)
    - Nitrous Oxide reductase (nosZ)
  - Nitrification
    - Ammonium monooxygenase (amoA)*
  - Nitrogen Fixation
    - Dinitrogenese reductase (nifH) *

* Sequencing completed
* Sequencing in progress

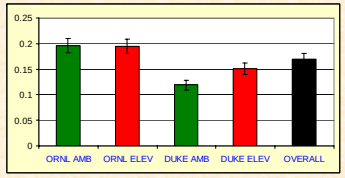
Duke-FACE *nifH* (dinitrogenase reductase)



FACE *nifH* (dinitrogenase reductase)



Mean phylogenetic distance (*d*) between *nifH* clones from ambient and elevated CO_2 soils.



Results for N fixation genes (*nifH*)

➢Changes in microbial nitrogen fixation processes have the potential to alter overall ecosystem responses to increased CO_2 via nitrogen availability to plants. Dinitrogenase reductase *(nifH)* enzymes catalyze the second essential step required for both free-living and symbiotic N fixation.

➢Over 250 *nifH* clones have been sequenced from each site. Sequences closely related to *Bradyrhizobium japonicum* (a well studied free-living N fixer) dominated both sites, contributing approx 20% of the genes recovered.

➢On the scale surveyed there is some evidence for changes in the overall diversity of Nitrogen fixing microbial communities between treatments at the DUKE forest site. It appears the diversity of N-fixing organisms slightly increased with CO_2 treatment.

➢As with *nirK* above, significant changes in the composition and distribution of *nifH*-types are observed between sites (Duke and ORNL).

Conclusions and Future Directions

➢On the scale surveyed there is some evidence for changes in the overall diversity of Nitrogen fixing microbial communities between treatments at the DUKE forest site. It appears the diversity of N-fixing organisms slightly increased with CO2 treatment.

➢While these surveys suggest high diversity in these microbial functional genes, their utility in accessing the overall effects of CO2 enrichment is limited do to issues of sample temporal and spatial scale, experimental design, etc…

➢We will continue to survey and analyze clone libraries for 8 other microbial genes and processes important in N and C cycling in these systems.

➢Clone sequences are being used in the design of a new Functional Gene Microarray that will be used to address (poster K070) issues at more experimentally relevant scales

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