

## Final Report for DOE grant no. DE-FG02-04ER63883

**Title:** Can soil genomics predict the impact of precipitation on nitrous oxide flux from soil

**Institution:** Northern Arizona University, Office of Grant and Contract Services  
Peterson Hall, Building 22, Room 108  
800 South Beaver Street  
Flagstaff, Arizona 86011-4130

**Principal Investigator:** Egbert Schwartz  
P.O. Box 5640, Department of Biological Sciences  
Northern Arizona University  
Flagstaff AZ 86011-5640  
(928) 523-6168  
[Egbert.Schwartz@nau.edu](mailto:Egbert.Schwartz@nau.edu)

**Co-Principal Investigators:**

Bruce Hungate  
Paul Keim  
Michael Cummings

**DOE Program contact:** Jeff Amthor, Program for Ecosystem Research

## **Abstract**

Nitrous oxide is a potent greenhouse gas that is released by microorganisms in soil. However, the production of nitrous oxide in soil is highly variable and difficult to predict. Future climate change may have large impacts on nitrous oxide release through alteration of precipitation patterns. We analyzed DNA extracted from soil in order to uncover relationships between microbial processes, abundance of particular DNA sequences and net nitrous oxide fluxes from soil. Denitrification, a microbial process in which nitrate is used as an electron acceptor, correlated with nitrous oxide flux from soil. The abundance of ammonia oxidizing archaea correlated positively, but weakly, with nitrous oxide production in soil. The abundance of bacterial genes in soil was negatively correlated with gross nitrogen mineralization rates and nitrous oxide release from soil. We suggest that the most important control over nitrous oxide production in soil is the growth and death of microorganisms. When organisms are growing nitrogen is incorporated into their biomass and nitrous oxide flux is low. In contrast, when microorganisms die, due to predation or infection by viruses, inorganic nitrogen is released into the soil resulting in nitrous oxide release. Higher rates of precipitation increase access to microorganisms by predators or viruses through filling large soil pores with water and therefore can lead to large releases of nitrous oxide from soil. We developed a new technique, stable isotope probing with  $^{18}\text{O}$ -water, to study growth and mortality of microorganisms in soil.

## **Project background**

Nitrous oxide is a potent greenhouse gas that is generated by microorganisms in soil and nitrous oxide release from soil is both spatially and temporally highly variable (Mueller et al., 2004). As a result it is, at present, difficult to predict how changes in precipitation patterns will impact the release of nitrous oxide from soil and models of future nitrous oxide release from arid lands in the southwest contain large amounts of uncertainty.

Changes in global climate are predicted to alter precipitation regimes in the southwestern United States where water is a limiting factor for many ecosystem processes including those in the nitrogen cycle. Small changes in soil moisture could cause large changes in microbial community composition which in turn could impact important ecosystem services such as purification of air and water and regeneration of soil fertility. The soil nitrogen cycle is an important player in delivering these services. For instance, nitrous oxide released from soil may migrate to the stratosphere where it is destroyed through photo-dissociation forming nitric oxide which in turn can deplete ozone concentrations (Bates and Hays, 1967). The soil nitrogen cycle also impacts water quality. Denitrification reduces nitrate, a common ground water pollutant, to nitrous oxide or dinitrogen gas thereby avoiding ground water pollution (Maier et al, 2000). Conversely, nitrification generates nitrate, a highly mobile form of nitrogen that is likely to leach into groundwater (Maier et al, 2000). The nitrogen cycle is also an important controller of soil fertility (Paul and Clark, 1996, Hungate et al., 2003). Formation of nitrous oxide leads to nitrogen being released from an ecosystem and hence a decline in soil fertility (Hungate et al., 1997). Thus nitrous oxide flux does not only impact the levels of greenhouse gasses in the atmosphere which impact global climate but also affects several important ecosystem services. Presently, it remains unclear how changes in precipitation will affect the soil nitrogen cycle and the important ecosystem services it impacts.

## **Project Objective**

Most microorganisms in soil can not easily be cultured on artificial medium, and therefore their role in biogeochemical transformations remains unknown (Madigan et al., 2003). In the N cycle we measure process rates (Hart et al., 1994) but have difficulty in identifying the organisms that transform N in the environment. We proposed to analyze DNA in soil in order to uncover links between the abundance of specific DNA sequences and process rates. Several molecular biological techniques were applied to this challenge including quantitative realtime PCR to measure the relative abundance of DNA sequences (Suzuki et al., 2000), microarray analysis of bacterial 16S rRNA genes (Brodie et al., 2006) and stable isotope probing (Schwartz, 2007). All of these experimental approaches have a similar objective:

*To find a soil genomic signature that correlates with nitrous oxide flux from soil.*

Such a genomic signature is of great value because a very large number of flux measurements are presently required to evaluate nitrous oxide flux from soil.

## **The C. Hart Merriam Climate Change Experiment.**

We studied nitrous oxide release from soil in the C. Hart Merriam Climate Change Experiment, which is used as a teaching and research tool for global change

(<http://www.mpcer.nau.edu/gradient/>). This is a gradient which spans 3000 m in elevation, represents a broad range of climatic conditions, and slices through some of the major temperate ecosystems of the world. The study included four life zones along the gradient in which grass-dominated interspaces occur, providing a range of climate conditions, from 3 to 15 °C mean annual temperature and 100 to 700 mean annual precipitation, and allowing a comparison among ecosystems where the major limitation to net primary production shifts from precipitation (grassland, piñon- juniper) to temperature (ponderosa pine, mixed conifer).

Soil	Elevation (m)	MAT (°C)	MAP (mm)	%C	%N	C/N
Grassland	1760	14.4	230	0.86	0.08	11.02
Pinyon- Juniper	2020	12.5	380	1.01	0.09	11.37
Ponderosa Pine	2300	10.2	660	1.11	0.07	15.82
Mixed Conifer	2615	7.3	790	2.63	0.21	12.60

Table 1. Selected environmental parameters of soils used in this study.

**Each site along the elevation gradient has a distinct soil bacterial community.**

We used a variety of techniques to characterize the bacterial communities in soils along the elevation gradient including TRFLP analysis, micro array analysis of the bacterial 16S rRNA gene, and 454 pyrosequencing of the soil meta genome. TRFLP analysis (Liu et al., 1997; Tokunaga et al., 2001) showed that the bacterial communities in the soils are distinct. Figure 1 shows results of non-metric multi dimensional scaling (NMDS) analysis of the TRFLP patterns. Four replicate soil samples (outlined in circles or ovals) from each soil cluster together. The separation of the TRFLP patterns generated from bacterial communities in the Ponderosa Pine (PP) forest soil and Mixed Conifer (MC) forest soil from the patterns generated from the bacterial communities in the Pinjon –Juniper (PJ) and grassland (GL) soils along the first axis coincides with the directions of the two vectors representing mean annual temperature (MAT) and mean annual precipitation (MAP), suggesting that these environmental parameters are important in shaping the soil bacterial communities.

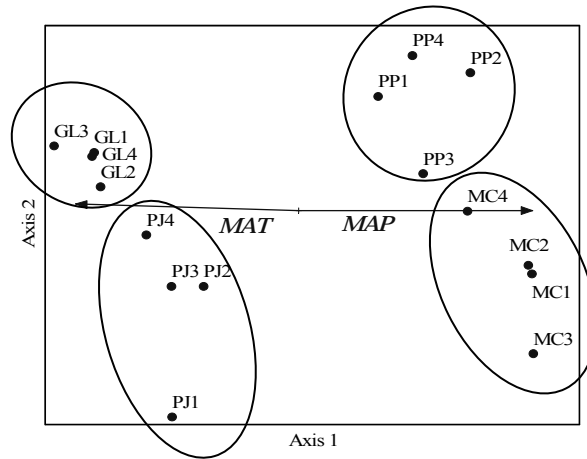


Figure 1. NMS joint biplot of TRFLP patterns produced with bacterial primers from DNA extracted from soils taken from arid Grassland (GL), Pinjon Juniper (PJ), Ponderosa Pine (PP) or Mixed Conifer (MC) forests. The two vectors represent mean annual temperature (MAT) or mean annual precipitation (MAP).

**There were significant differences in the abundance of fungal or bacterial genes between sites along the elevation gradient.**

We used realtime-quantitative polymerase chain reactions (RTQ-PCR) to measure the abundance of specific DNA sequences in soil. Sergey Kachur, a graduate student working in Paul Keim’s group, developed new primers, specific to either bacteria or fungi, which target a larger fraction of published bacterial and fungal sequences than previously described primers.

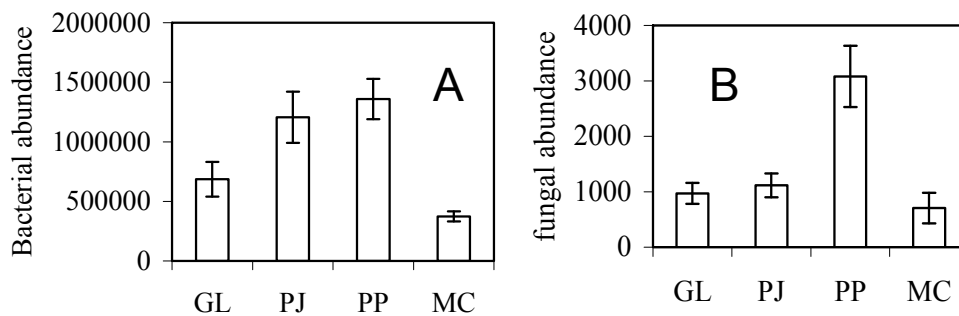


Figure 2. Abundance of bacteria (A) or fungi (B) across four different ecosystems along a northern Arizonan elevation gradient.

Using a Taqman RTQ-PCR approach Sergey was able to quantify the relative abundance of fungal 28S rRNA genes and bacterial 16S rRNA genes in soils along the elevation gradient. He found that there were significant differences in the abundance of fungi or bacteria among the sites (Figure 2). There were significantly more fungal sequences in the PP soil than in soils from the other sites and there were fewer bacterial sequences in the GL and MC sites than in the PJ and PP sites. Interestingly, the soils at the GL and MC sites were wetter, having been harvested

after a rain event, than the PJ and PP soils. There were always far fewer fungal sequences in the soils we studied than bacterial sequences.

**The abundance of bacterial genes in soil significantly correlated with gross nitrogen mineralization rates.**

Graham Hymus, a post doctoral researcher in Bruce Hungate's group, used a pool dilution technique to measure gross nitrogen mineralization rates in the same soil samples in

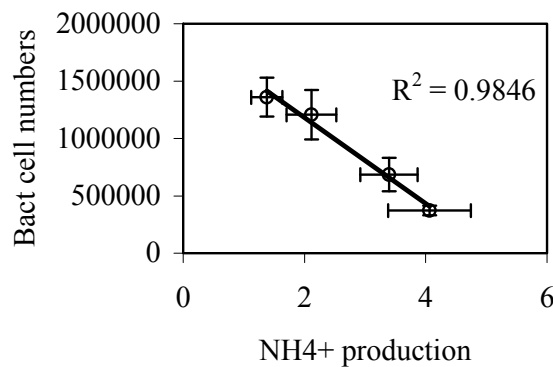


Figure 3. Relationship between gross NH<sub>4</sub><sup>+</sup> production and bacterial abundance across four different ecosystems.

which the abundance of fungal and bacterial genes were determined. There was a very strong negative relationship between the abundance of bacterial genes in soil and gross nitrogen mineralization rates (Figure 3). This relationship suggests that bacteria in the MC and GL soils had died, through predation by nematodes, protozoa and *bdellovibrio* bacteria or infection by phage, resulting in a release of NH<sub>4</sub><sup>+</sup> in the soils. As previously mentioned, the MC and GL soils were wetter when harvested and increased soil moisture can facilitate access by predators or phage to isolated bacterial colonies in soil.

**There was a negative relationship between the abundance of bacterial genes in soil and net nitrous oxide release from soil.**

Net nitrous oxide release from soils was also measured on the same soil samples. Again a strong negative relationship was observed. In soils where fewer bacteria were present more nitrous oxide was released (Figure 4). This suggests that the ammonium released from bacteria fueled microbial processes that resulted in the production of nitrous oxide.

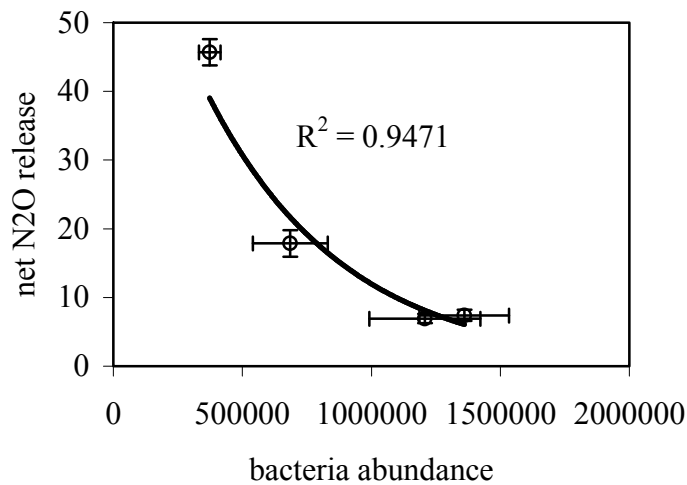


Figure 4. Relationship between bacterial abundance and net N<sub>2</sub>O flux from soil.

### Precipitation manipulation along the elevation gradient

Because of the central role of water in modulating ecosystem biogeochemistry, and because of uncertainty in changes in precipitation that will accompany climate warming, we examined the range of possible changes in precipitation – reduced (Grissino-Mayer et al. 1997), unchanged, or increased (Neilson 1993). From each site, large grass/soil monoliths were placed in 30-cm diameter x 40 cm deep PVC lysimeters, each equipped with leachate collection ports, soil thermocouples, and soil water content probes. At each site, a fenced enclosure contained a weather station and an array of our experimental lysimeters. These were installed in the field in May of 2002 and since then have been subjected to three different precipitation treatments: ambient, increased (+50%), and decreased (-30%), affected by redirecting rainfall into the lysimeters that receive extra precipitation, and by diverting rain from those in the low rainfall treatments. Thus this experiment was well suited for studying the impact of precipitation on nitrous oxide release in a wide variety of soils.

### Impact of precipitation on N<sub>2</sub>O release from soil.

Nitrous oxide fluxes from soil are spatially and temporally highly variable (Azam et al., 2002, Mueller et al., 2004). Therefore it was necessary to make many measurements before the impact of precipitation on nitrous oxide flux from soil could be evaluated. We measured N<sub>2</sub>O fluxes from soils in the precipitation manipulation experiments since 2003 and several patterns have emerged. Higher elevation soils that support ponderosa pine and mixed conifer forests released more nitrous oxide when precipitation was increased (Figure 5). A decrease in precipitation negatively impacted nitrous oxide release from soil. Lower elevation soils did not seem to respond strongly to changes in precipitation.

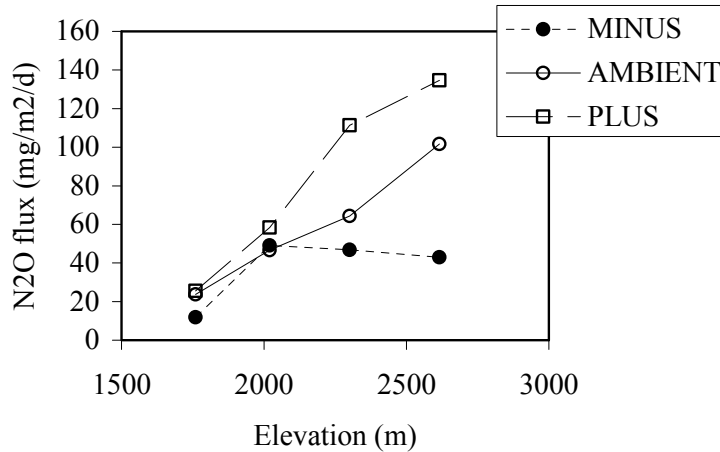


Figure 5. Average N<sub>2</sub>O flux from soils of 24 separate measurements taken between 7/7/2003 and 8/25/2006 with increased (□), ambient (○) or decreased (●) precipitation.

However, when soils were sampled for genomic analysis nitrous oxide release from soil was very different (Figure 6) from the averages shown in Figure 5. The discrepancy occurred because there were large differences in soil moisture. At the time of harvest, the mixed conifer and grassland sites had recently received precipitation while the ponderosa pine and pinion-juniper soils were drier. We compared our genomic analysis to both the long-term averages and to nitrous oxide release of the day of harvest.

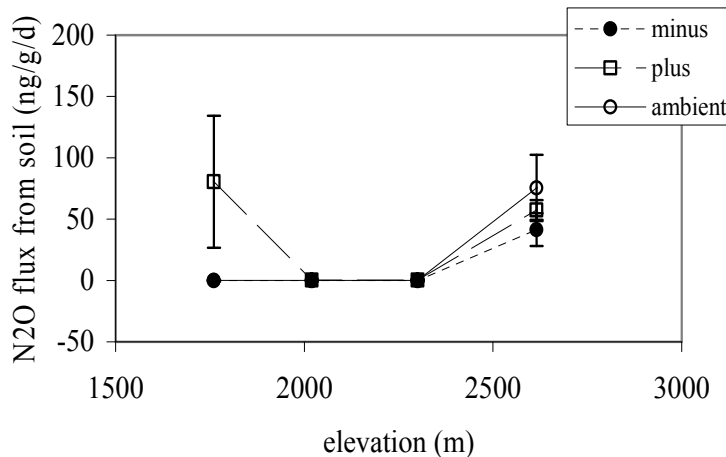


Figure 6. Average N<sub>2</sub>O flux from soil on day samples were harvested for genomic analysis

### Denitrification rates corresponded to net N<sub>2</sub>O flux from soil

The higher elevation soils contain more carbon, a major controller of denitrification rates in soil (Romain et al., 2004, Rich et al., 2003). Denitrification is one of the processes that release nitrous oxide (Azam et al., 2002) and therefore we hypothesized that denitrification rates as measured with an acetylene block was correlated with net nitrous oxide flux from soil. Figure 7



shows that there was a log-linear positive relationship between denitrification rates and nitrous oxide release from soil.

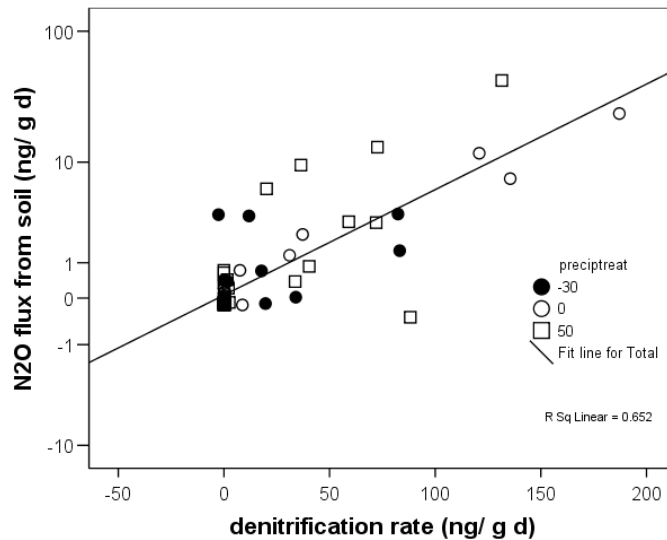


Figure 7. Correlation between denitrification rate and net N<sub>2</sub>O flux from soil.

**We did not obtain reliable quantitative measurements of genes related to denitrification in soil.**

Because we found a positive relationship between denitrification rates and net N<sub>2</sub>O flux from soil we spend considerable effort trying to measure the abundance of nirK, norB and nosZ genes, which encode subunits of the nitrite reductase, nitric oxide reductase and nitrous oxide reductase, respectively. However, we were not able to consistently produce clean PCR products in which all amplified DNA was of the expected size. Obtaining a clean PCR product of one size is a prerequisite for RTQ-PCR analysis. Despite a very large number of PCR attempts in which every possible variable was manipulated, including the use of many other possible primer combinations, we only generated smears for PCR products. Denitrification, is a function that is spread across a wide range of phylogenetic groups and the variation in gene sequences that encode enzymes important in denitrification may be so great that primers presented in the literature can not reliably amplify genes associated with denitrification across all taxa.

**Gross Nitrification rates did not correspond to net N<sub>2</sub>O flux from soil.**

We measured nitrate production rates in soil using a pool dilution technique and did not uncover any relationships with nitrous oxide fluxes from soil. Gross nitrification rates also did not correspond to precipitation treatment. High rates were observed in soils with elevated, ambient or reduced precipitation.

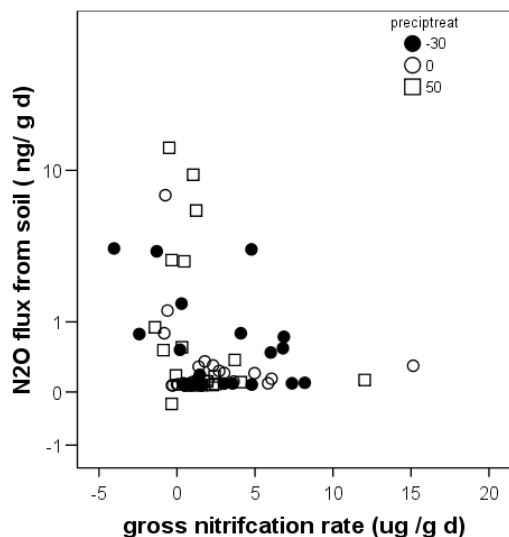


Figure 8. Comparison of net  $\text{N}_2\text{O}$  flux and gross nitrification rates in soil

**There were more archaeal amoA genes in all soils along the elevation gradient than bacterial amoA genes.**

When we submitted our proposal, the dominant ammonia oxidizing microorganisms in soil were thought to be bacteria. Ammonia oxidizers are important in nitrogen cycling because they are thought to control the rate limiting step in nitrification, the oxidation of ammonia into nitrite. However, several new studies published during this project redirected our approach to studying ammonia oxidation. We took advantage of the discovery of ammonia oxidizing archaea (Leininger et al., 2006) and the description of primers to amplify the amoA gene (Francis et al., 2005), encoding a subunit of ammonia monooxygenase. Karen Adair, a graduate student in Egbert Schwartz's group found that genes of ammonia oxidizing archaea were far more abundant in all soils along the elevation gradient than genes of ammonia oxidizing bacteria. However, the abundance of archaeal amoA genes did not correlate with any environmental parameters while the abundance of bacterial amoA corresponded with mean annual day temperature, mean annual precipitation and bulk density in soil (Adair and Schwartz, 2008). Because ammonia oxidizers compete with heterotrophic microorganisms in soil for oxygen it is not surprising that these environmental parameters, which impact oxygen availability in soil, were important for bacterial ammonia oxidizer abundance.

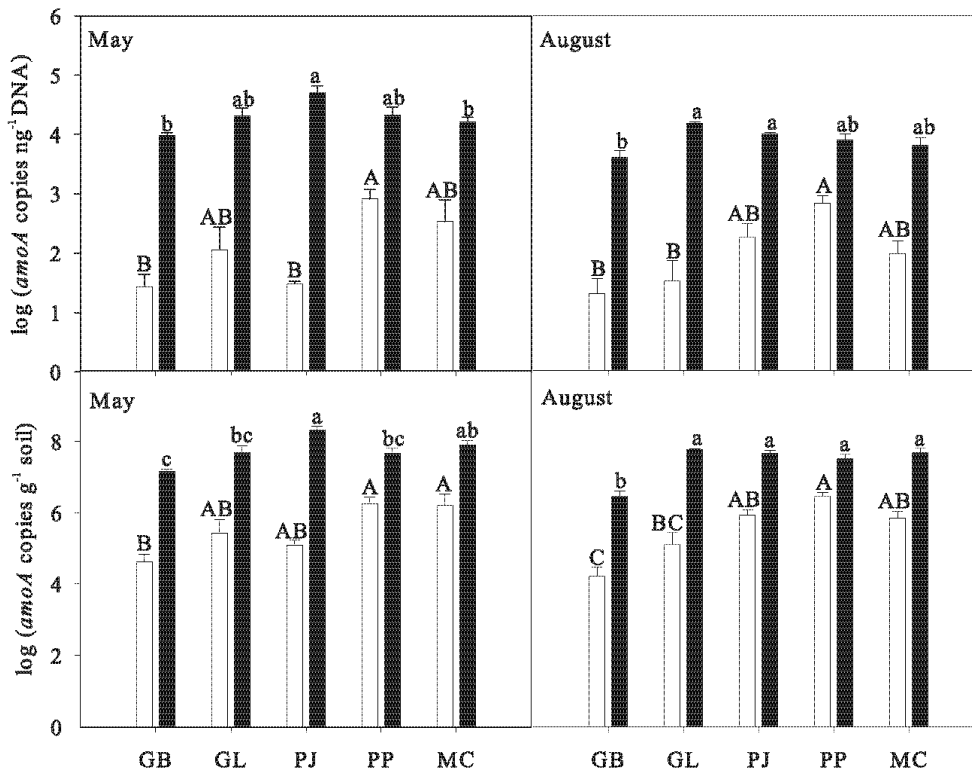


Figure 9. Abundance of bacterial (open bars) and archaeal (closed bars) *amoA* genes in soils from five different northern Arizonan ecosystems taken at two different times during the season

**In Mixed Conifer soils the abundance of archaeal *amoA* genes positively, but weakly, correlated with gross and net nitrous oxide fluxes from soil.**

Because ammonia oxidizing archaea appeared important in nitrogen cycling in our soils, we expended extra efforts to study the relationship between archaeal ammonia oxidizers and nitrogen cycle process rates. We found a positive, but weak, relationships between archaeal *amoA* gene abundances in the mixed conifer soil and gross or net nitrous oxide flux, suggesting these organisms may play a role in nitrous oxide production in soil (Figure 10). Note however, that these relationships are dependent on relatively few samples. If they were omitted from the study the relationships would disappear. Even if this relationship persists, it is not evidence that ammonia oxidizing archaea themselves produce nitrous oxide. Archaeal ammonia oxidizers may influence denitrification rates by producing nitrate which is used as an electron acceptor by denitrifying organisms.

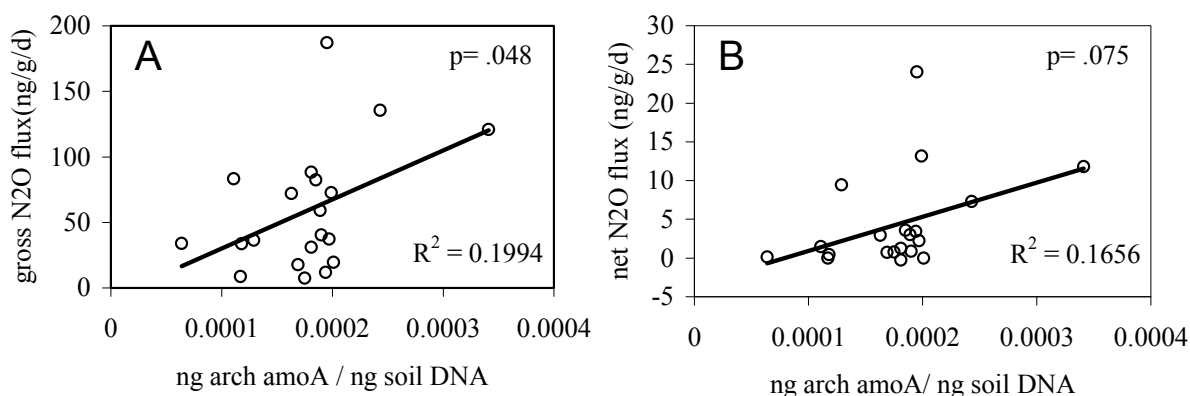


Figure 10. Comparison of archaeal amoA gene abundance with gross N<sub>2</sub>O fluxes (A) and net N<sub>2</sub>O fluxes (B) from mixed conifer soil.

**Microarray analysis of soil bacterial communities correlates with long term but not short term N<sub>2</sub>O flux from soil.**

We analyzed the bacterial communities in the mixed conifer soil samples with a new microarray developed by Gary Anderson's group at Lawrence Berkeley National Lab and produced by Affymetrix, Inc (Wilson et al., 2002, Brodie et al., 2006). This microarray detected the 16S rRNA genes of more than 8,000 different bacterial taxa. We detected more than 2,000 different bacterial groups in the mixed conifer forest soil. This data was used in principal component and regression tree analysis. Principal component 1 which explained 75% of the variation in the data set was regressed against N<sub>2</sub>O flux from soil. There were no significant relationships between the microarray data and N<sub>2</sub>O flux rates on the day the soils were harvested, however, there was a significant relationship between PC1 and average N<sub>2</sub>O flux rate over the last 4 years.

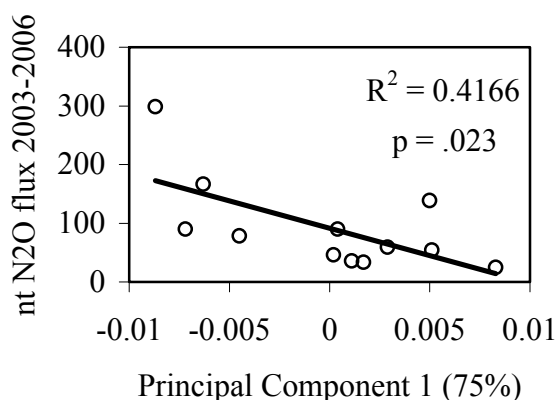


Figure 11. Comparison of principal component 1 generated from microarray analysis of bacterial 16S rRNA genes vs. average N<sub>2</sub>O flux from mixed conifer soil between 2003 and 2006.

### Microbial DNA in soil can be labeled with H<sub>2</sub><sup>18</sup>O.

In our project, we struggled to relate nitrous oxide flux from soil, which changes very rapidly, with genomic signatures in soil that vary much more gradually. Microbial populations may remain in soil, inactive, for years whereas nitrous oxide fluxes can change within hours. Therefore in comparing soil genomic signatures with N<sub>2</sub>O fluxes from soil we were often comparing apples and oranges and could only expect significant relationships between the two data sets when N<sub>2</sub>O flux at the time of sampling was representative of longer term flux rates. It is more likely that links between soil genomic signature and nitrous oxide flux from soil will be uncovered if only growing or dying microbial populations are included in an analysis. During this project we discovered that DNA in soil can be labeled with <sup>18</sup>O derived from H<sub>2</sub><sup>18</sup>O. Because only newly formed DNA, in newly grown cells, becomes labeled, it is possible to quantify the growth rate of microorganisms in soil. We found that in the Ponderosa Pine soil, after 48 hours, microorganisms had grown faster as the soil became more moist until it reached a moisture content of approximately 23% after which the growth rate declined (Figure 12).

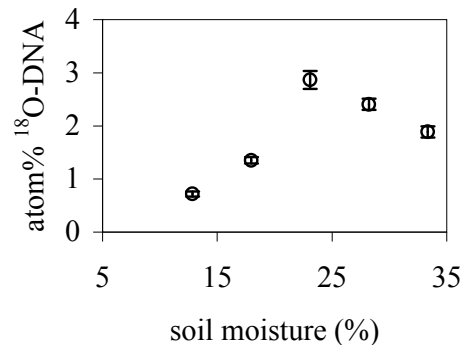


Figure 12. Atom% <sup>18</sup>O of DNA extracted from soil incubated with H<sub>2</sub><sup>18</sup>O versus soil moisture content. Error bars represent standard errors of the mean. Tukey analysis ( $\alpha = 0.05$ ) showed all pairs were significantly different from each other.

### Growth or Mortality rates of microbial populations in soil could be determined through stable isotope probing with H<sub>2</sub><sup>18</sup>O.

We showed that DNA in soil labeled with <sup>18</sup>O could be separated along a cesium chloride gradient from non labeled DNA because it had a higher bouyant density (Schwartz, 2007). This technique is termed stable isotope probing. DNA in the bottom bands in tubes 4, 5 and 6 shown in Figure 13 consist of labeled newly formed DNA while the top band contains non-labeled DNA from organisms that survived but did not grow during the incubation. DNA of microorganisms that died during the incubation is present in tubes 1, 2 and 3, which was extracted prior to the incubation, but is absent from the top band in tubes 4, 5 and 6.

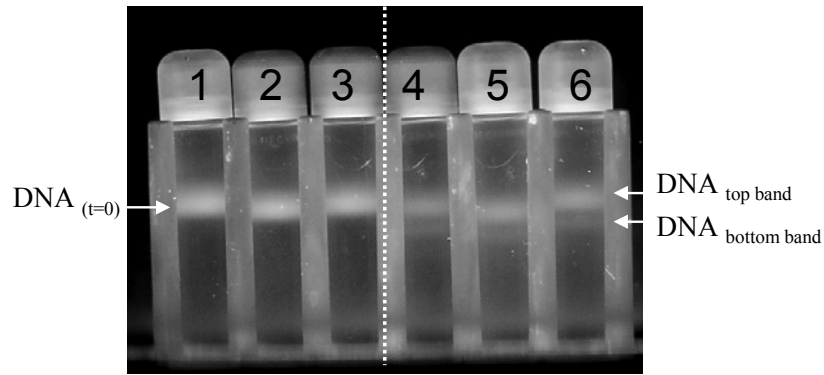


Figure 13. Stable Isotope probing with  $H_2^{18}O$ . Tubes 1,2 and 3 contain DNA from soil prior to the incubation while tubes 4, 5 and 6 contain DNA extracted from soils incubated with  $H_2^{18}O$  for 6 days. DNA in the top band in tubes 4,5 and 6 belongs to organisms that survived but did not grow during the incubation while DNA in the bottom bands is derived from newly grown microorganisms.

By combining RTQ-PCR with stable isotope probing with  $^{18}O$ -water we determined growth and mortality indices of specific microbial populations in soil during the incubation. The Growth index ( $G_{index}$ ) is calculated by dividing the abundance of a gene in the bottom band by the abundance of the same gene in DNA prior to the incubation:

$$G_{index} = \frac{\text{target copies/ng DNA}_{\text{bottom band}}}{\text{target copies/ng DNA}_{(t=0)}}$$

We found that fungi grew faster (i.e. had a higher growth index) in the Ponderosa Pine soil than bacteria (Figure 14).

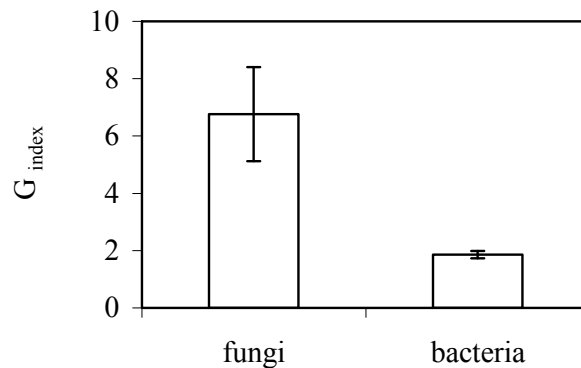


Figure 14. Growth indices of fungi and bacteria in soils wet up with  $H_2^{18}O$  and incubated for 6 days.

The mortality index ( $M_{index}$ ) is determined by dividing the abundance of a gene prior to the incubation with the gene abundance in the top band after the incubation:

$$M_{\text{index}} = \frac{\text{target copies/ng DNA}_{(t=0)}}{\text{target copies/ng DNA}_{(\text{topband})}}$$

We found that in Ponderosa Pine Forest soils bacteria had a higher mortality index (i.e. died faster) than fungi. These results are consistent with observations described in Figure 2. In moist soils, the GL and MC soils were harvested after precipitation events, abundance of bacterial sequences were lower. Mortality indices derived from stable isotope probing with  $\text{H}_2^{18}\text{O}$  indicate that bacteria die rapidly in a moist soil.

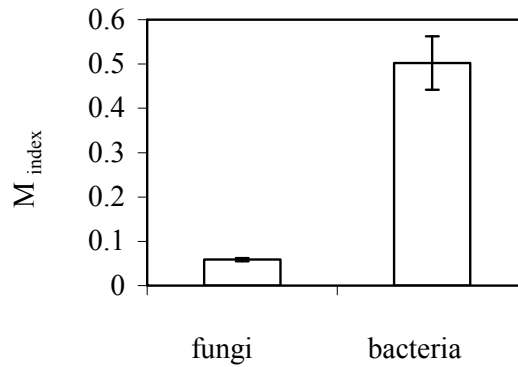


Figure 15. Mortality indices of fungi and bacteria in soils wet up with  $\text{H}_2^{18}\text{O}$  and incubated for 6 days.

**Ammonium stimulated the growth of ammonia oxidizing bacteria in Ponderosa Pine forest soils but not ammonia oxidizing archaea.**

We applied the stable isotope technique to study the impact of  $\text{NH}_4^+$  on the growth of ammonia oxidizing bacteria and archaea in soil. We found that  $\text{NH}_4^+$  increased the growth rates of ammonia oxidizing bacteria (i.e. the bacteria had a higher growth index in soils supplemented with ammonium) while the growth index of ammonia oxidizing archaea in non-amended soil was not significantly different from that in amended soil. Ammonium amendment did not appear to have a significant impact on the mortality indices of either ammonia oxidizing bacteria or archaea.

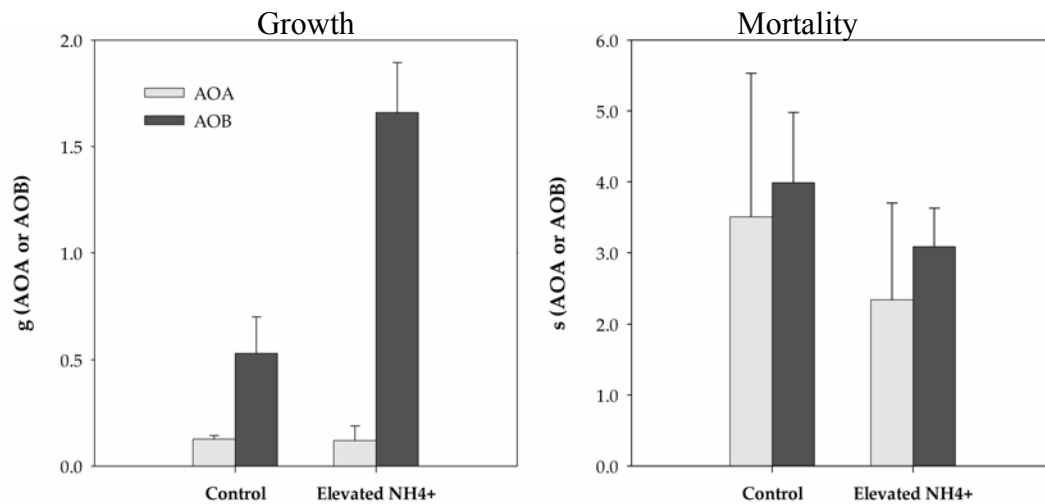


Figure 16. Impact of elevated  $\text{NH}_4^+$  on growth and mortality indices of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) in soil.

**Microbial enrichment in  $^{15}\text{N}$  relative to the soil extract positively correlated with net N mineralization rates.**

Because it appeared that nitrogen mineralization rates were an important factor in nitrous oxide release from soil we focused on developing new assays for nitrogen mineralization. Nitrogen mineralization is notoriously difficult to measure, the pool dilution technique provides only short term rate estimates and net rates are highly susceptible to ammonium assimilation, which can be a rapid process. We discovered that the natural abundance  $^{15}\text{N}$  content of microorganism in soil was strongly correlated with the net nitrogen mineralization rates. Because the microbial biomass turns over more slowly, this measure assesses nitrogen mineralization over a much longer term than previously described techniques.



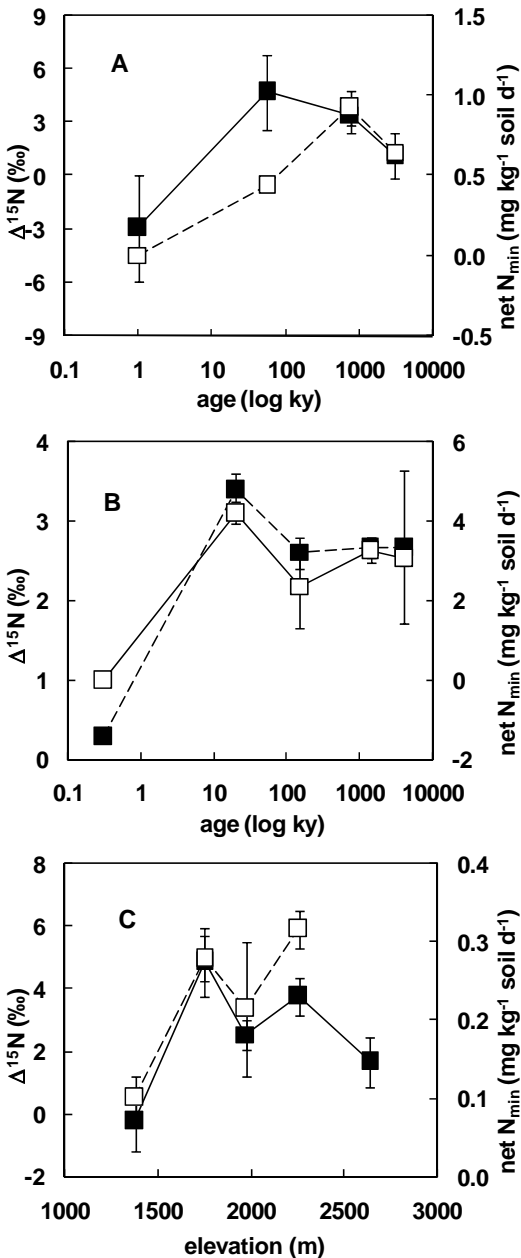


Fig. 17.  $^{15}\text{N}$  enrichment of the microbial biomass ( $\Delta^{15}\text{N} = \delta^{15}\text{N}$  of soil microbial biomass -  $\delta^{15}\text{N}$  of soil soluble N; ‰, closed symbols) and net N mineralization (open symbols, mg N kg<sup>-1</sup> soil d<sup>-1</sup>) with substrate age (A, Substrate Age Gradient of Arizona; B, Long Substrate Age Gradient-Hawaii) and elevation (C, C. Hart Merriam Elevation Gradient-Arizona). Symbols are mean values per site ( $\pm$  se). There was a significant positive correlation between net N mineralization and microbial  $^{15}\text{N}$  enrichment for the substrate age gradient in Hawaii ( $r = 0.98$ ,  $P < 0.01$ ), and a positive association for the substrate age gradient in Arizona ( $r = 0.71$ ,  $P = 0.29$ ) and the elevation gradient ( $r = 0.93$ ,  $P = 0.067$ ).

### The natural abundance $^{15}\text{N}$ and $^{13}\text{C}$ of DNA extracted from soil can be accurately determined.

Because this project focused on uncovering relationships between soil genomic signatures and nitrous oxide flux from soil we developed a new technique to determine the natural abundance  $^{13}\text{C}$  or  $^{15}\text{N}$  content in DNA extracted from soil in order to extend the observations described in Figure 17 to DNA. This would allow the ability to simultaneously measure the isotopic content of DNA in soil and abundances of specific genes. This proved challenging because large quantities of DNA were required to accurately determine the isotopic content and these measurements were highly susceptible to artifacts due to inclusion of nitrogen or carbon from contaminants. However, using the protocol described in Schwartz et al., 2007, it

was feasible to make accurate measurements of the natural abundance  $^{15}\text{N}$  or  $^{13}\text{C}$  content of DNA extracted from soil. DNA is enriched in  $^{13}\text{C}$  but not  $^{15}\text{N}$  relative to soil (Figure 18).

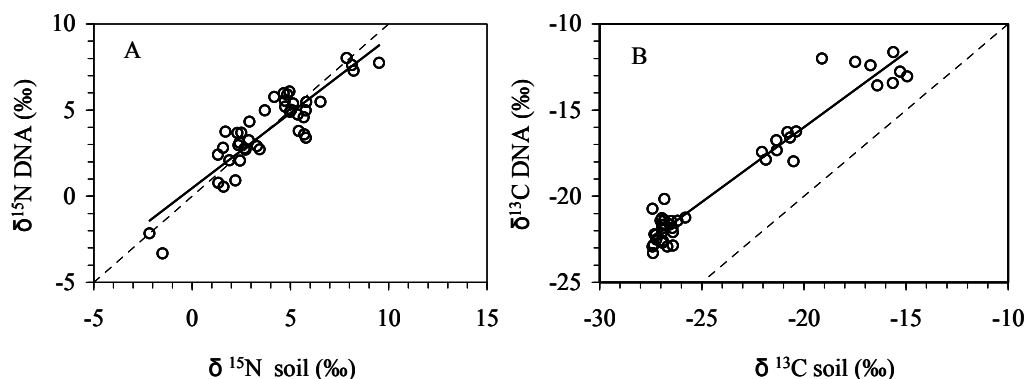


Figure 18. Comparison of isotopic values of DNA extracted from nine different surface mineral soils with those of total elemental pools. Figure shows data taken from field replicates. Panel A shows  $\delta^{15}\text{N}$  of DNA vs.  $\delta^{15}\text{N}$  of soil total N ( $r = 0.892$ ,  $n = 42$ ,  $p < 0.001$ ). Panel B shows  $\delta^{13}\text{C}$  of DNA vs.  $\delta^{13}\text{C}$  of soil total C ( $r = 0.971$ ,  $n = 43$ ,  $p < 0.001$ ). Dashed line in each figure panel shows the 1:1 relationship.

### Summary and Conclusions

In summary, we found that the abundance of specific DNA sequences in soil could be related to nitrous oxide flux from soils. The strongest relationships were found between the abundance of bacterial genes and  $\text{N}_2\text{O}$  flux from soil, indicating that bacterial death leads to nitrogen mineralization which in turn promotes  $\text{N}_2\text{O}$  generation. We summarized this mechanism in a flow chart shown in Figure 19. Microbial death led to increased nitrogen mineralization which in turn elevated the availability of ammonium in soil. As a result the abundance of ammonia oxidizers increased, which in turn produced more nitrate in soil. The nitrate was used as an electron acceptor by denitrifying bacteria. Both nitrification and denitrification are known to result in the release of nitrous oxide. In our studies we found positive correlations between denitrification rates or ammonia oxidizing archaea abundance and nitrous oxide flux from soil. While at first glance these relationships seemed contradictory both denitrification and ammonia oxidation are reliant on the availability of inorganic nitrogen. It appeared that when microbial death rates in soil were low, the nitrogen was bound up in the microbial biomass and nitrous oxide production was low. When microorganisms died, more nitrogen was released as inorganic nitrogen, which stimulated either nitrification or denitrification rates and resulted in higher nitrous oxide flux from soil. Techniques such as stable isotope probing with  $\text{H}_2^{18}\text{O}$  could test these mechanisms and may provide greater insight into the relationships between soil moisture, microbial mortality and nitrous oxide release from soil.

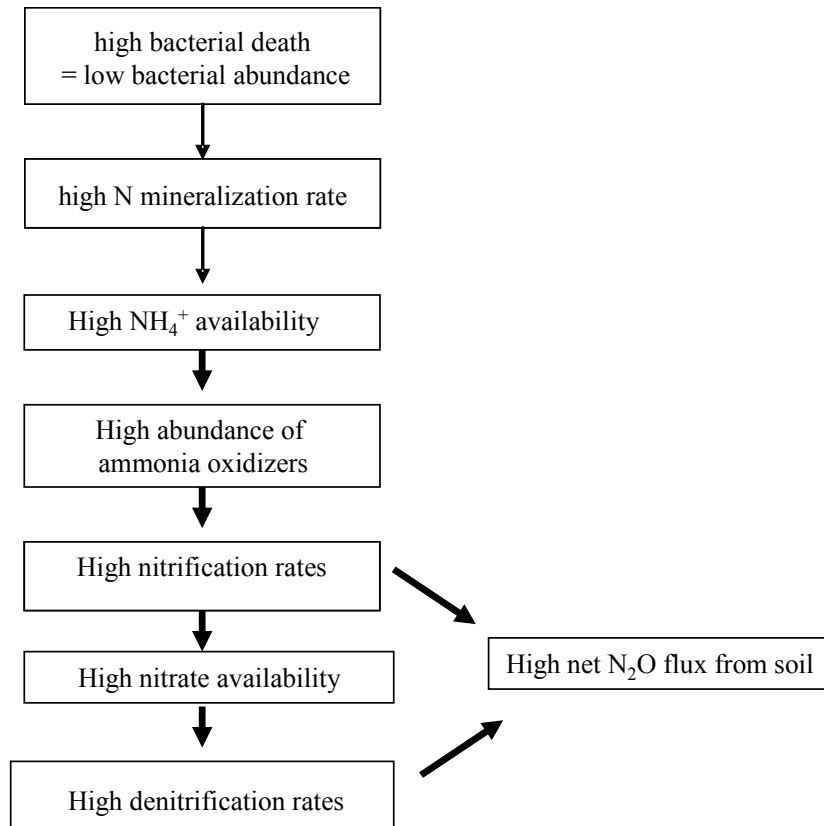


Figure 19. Model to explain relationships between bacterial abundance, ammonia oxidizer abundance, denitrification rates, gross N mineralization and net N<sub>2</sub>O flux from soil.

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Dijkstra, P., E. Schwartz, S.C. Hart, R. Doucette and B. Hungate The  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  natural abundance isotope composition of microbial biomass or DNA in soil. 2005 AGU Fall Meeting, 5-9 December. San Francisco, CA.

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Dijkstra, P, Coyle, JS, Selmants, PC, Schwartz, E, Hart, SC and Hungate, BAH. 2006. <sup>15</sup>N abundance of the soil microbial biomass is related to C and N availability. Poster presentation at the ASA-CSSA-SSSA 2006 international meeting, November 2006, Indianapolis.

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Dijkstra, P., Brown, J, Blankinship, J and Hungate, BAH 2007. The importance of manure accumulation for N<sub>2</sub>O production near water sources in semiarid grasslands. Invited presentation at the 4th USDA Greenhouse Gas Conference, Baltimore, USA Feb 2007

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