## PROJECT DESCRIPTION

### Anaerobic Ammonium Oxidation: New Pathways for $N_2$ Production in Humid Tropical Forest Soils?

The flux of dinitrogen ($N_2$) to the atmosphere is one of the most poorly understood fluxes of the terrestrial nitrogen (N) cycle, limiting our ability to constrain N exports from ecosystems and construct accurate N budgets (Galloway et al. 2004; Davidson and Seitzinger 2006). In upland soils, $N_2$ is thought to be produced mainly by denitrification, an anaerobic microbial process by which nitrate ($NO_3^-$) is reduced to nitrous oxide ($N_2O$) and subsequently to $N_2$. Nitrous oxide is a potent greenhouse gas and catalyst for stratospheric ozone depletion (Prather et al. 1995). Thus, there is concern about global changes that could alter denitrification dynamics to shift the relative amounts of $N_2O$ and $N_2$ produced from denitrification (Galloway et al. 2003). In particular, industrial development and agricultural activity is expected to dramatically increase N deposition rates in tropical regions (Matson et al. 1999). Tropical forest soils are already the largest natural terrestrial source of $N_2O$ (Stein and Yung 2003). The goal of my dissertation research is to better understand controls on the $N_2/N_2O$ ratio of denitrification end-products in humid tropical forest soils.

In the course of my dissertation research, I have found evidence of new pathways for $N_2$ production in humid tropical forest soils (Figure 1). One pathway is anaerobic ammonium ($NH_4^+$) oxidation (anammox), the microbially mediated process by which nitrite ($NO_2^-$) and $NH_4^+$ react to create $N_2$ under anoxic conditions (Jetten et al. 1998; Strous and Jetten 2004). It was originally not thought to occur in nature but now reportedly occurs in a variety of aquatic systems and accounts for 30-70% of the marine $N_2$ flux, previously attributed entirely to denitrification (Thamdrup and Dalsgaard 2002; Kuypers et al. 2003; Francis et al. 2007). Despite its importance in these systems, anammox has not yet been found in soils. However, there is molecular evidence for anammox bacteria in a wide range of natural systems, including soils (Brodie et al. in prep., Jetten 2001; Francis et al. 2007). The other novel pathway for $N_2$ production is related to iron (Fe) mediated anaerobic $NH_4^+$ oxidation. Dinitrogen can be produced directly from abiotic $Fe^{3+}$ reduction coupled to anaerobic $NH_4^+$ oxidation. It can also be produced indirectly from microbially mediated ferric iron ($Fe^{3+}$) reduction coupled to anaerobic $NH_4^+$ oxidation.The latter process, termed Feammox, produces $N_2$ through biotic or abiotic denitrification of $NO_2^-$ produced from Fe reducing bacteria. Anaerobic $NH_4^+$ oxidation coupled with the reduction of metallic cations to abiotically produce $N_2$ is thermodynamically favorable at acidic pHs (Luther et al. 1997) and has been reported to take place in sediments with manganese but not with $Fe^{3+}$ (Hulth et al. 1999; Anschutz et al. 2000). Similarly, Feammox has been detected in a fluid bed reactor (Sawayama 2006) and has been speculated, but never experimentally shown, to occur in wetland soils (Clement et al. 2005; Weber et al. 2006). Many soils, particularly highly weathered soils such as those found in humid tropical forests, are rich in reactive Fe minerals. These systems are also characterized by low and fluctuating redox (Silver et al. 1999) and available $NH_4^+$. The occurrence of anammox, Feammox, and/or abiotic Fe-coupled $NH_4^+$ oxidation in humid tropical forest soils could significantly change our view of $N_2$ sources from soils. Furthermore, new $N_2$ production pathways could impact my efforts to characterize the controls on the $N_2/N_2O$ ratio of denitrification end-products. Thus, I propose to conduct a study to determine the significance of these pathways. Specifically, I will test the following hypothesis:

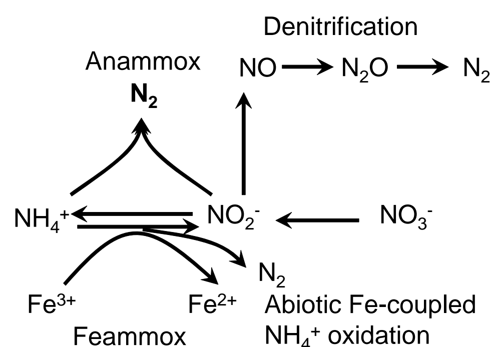



Figure 1. Potential pathways for $N_2$ production.

**Microbially mediated ferric iron reduction coupled to anaerobic ammonium oxidation (Feammox) leads to significant production of $N_2$ in humid tropical forest soils.**

## PROGRESS TO DATE

### *Developing the $N_2$/Ar Method for Measuring Soil $N_2$ Fluxes*

Greater understanding of denitrification in soils has been hampered by the lack of reliable methods of measuring $N_2$ fluxes (Davidson and Seitzinger 2006; Groffman et al. 2006). The measurement of $N_2$ fluxes from upland soils is difficult due to the high atmospheric $N_2$ background. A variety of methods are used to quantify denitrification to $N_2$ including acetylene ($C_2H_2$) inhibition, $^{15}N$ tracer additions, and direct measurement in a $N_2$-free headspace. These methods provide comparable estimates of $N_2$ fluxes based on the assumption that denitrification is the sole or dominant process responsible for $N_2$ production. Because these methods require removing soil from its natural environment or have well-recognized shortcomings (Knowles 1990; Groffman et al. 2006), the first step of my dissertation has been to develop a novel method for measuring $N_2$ fluxes from upland soils. The $N_2$ to argon (Ar) elemental ratio ($N_2$/Ar) method has been successfully used for *in situ* measurements of $N_2$ production in sediments and aquatic ecosystems (e.g., Laursen and Seitzinger 2002) but has not been tested in upland soils. Argon is a biologically inert gas found in relatively low atmospheric concentrations; thus it can serve as a conservative tracer for measuring $N_2$ production. The barrier to the development of the $N_2$/Ar method for upland soils has been the ability of gas chromatographs and mass spectrometers to detect small differences in the $N_2$/Ar ratio of gas samples.

I am collaborating with Dr. Jeffrey Severinghaus (UC-San Diego) to measure surface $N_2$ fluxes using a closed dynamic chamber and a dual inlet isotope ratio mass spectrometer (IRMS) equipped with multicollectors that can measure the $N_2$/Ar ratio with extremely high precision. The chamber headspace is sampled using a pump that circulates air between an aluminum chamber and a glass flask (Figure 2). An aliquot of the gas sample is quantitatively transferred from the flask to a secondary storage container on a vacuum line using liquid helium. During the transfer, the aliquot is passed through ethanol-liquid $N_2$ and liquid $N_2$ traps, and a copper furnace heated to 500 °C to remove water vapor, carbon dioxide, hydrocarbons, and oxygen ($O_2$), which can interfere with the analysis of $N_2$/Ar on the IRMS. Temperature gradients between and within storage containers (including the IRMS bellows) and the speed of closing the valves on the glass flasks introduce the most variability in repeated $N_2$/Ar measurements from the same flask. I have refined the gas handling protocol to achieve a 10 per meg standard deviation on 3 to 8 replicate aliquots from the same flask (where per meg is three orders of magnitude less than the delta notation unit, per mil). This translates to a detection limit of 30 ng-N/cm$^2$/h measured over one hour (i.e., from the difference in $N_2$/Ar between samples taken at two time points). While the magnitude of *in situ* $N_2$ fluxes is unknown, this low detection limit, which could be lowered even further, suggests that the $N_2$/Ar method will be able to capture surface $N_2$ fluxes from soils.

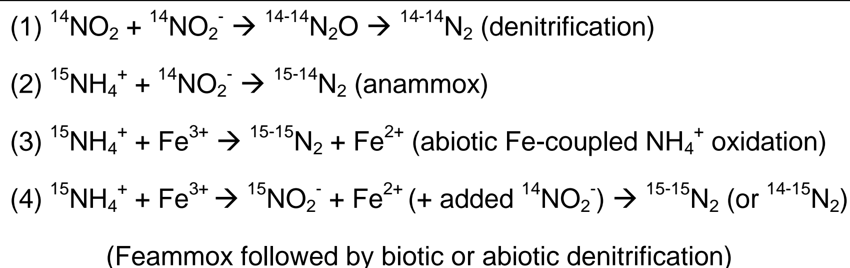
Figure 2. Surface $N_2$ flux chamber, pumping module, and flask (in bubble wrap).

I am currently using a soil diffusion box in a controlled laboratory environment to test for potential physical effects on $N_2$/Ar such as water vapor flux and thermal diffusion. Preliminary results suggest that these physical effects will not be important under conditions found in humid tropical forests (e.g., high humidity and stable temperature under closed canopy). The $N_2$/Ar method shows promise as a new way to measure $N_2$ fluxes from upland soils; however, this method cannot distinguish $N_2$ produced by denitrification from other potential sources of $N_2$ from soils.

### *Investigating New $N_2$ Production Pathways*

Humid tropical forest soils are often characterized by high concentrations of poorly crystalline reactive Fe and fluctuating redox conditions which are likely to support anammox, Feammox, and/or abiotic Fe-coupled $NH_4^+$ oxidation. Surface soils (0-10 cm depth) in humid tropical forests can have dynamic soil $O_2$ concentrations and become depleted in $O_2$ in the bulk soil for extended periods (Silver et al. 1999, Schuur et al. 2001). Anammox is generally considered an obligately anaerobic process (Jetten et al. 1998; Strous and Jetten 2004), so it is possible that this process could occur in soils during anoxic periods or in anoxic microsites, such as within soil aggregates (Sexstone et al. 1985). Highly weathered soils, such as ultisols and oxisols found in humid tropical forests, are rich in Fe oxides and tend to be acidic. High soil Fe concentrations and soil pH below 6.8 provide conditions under which Feammox and abiotic Fe-coupled $NH_4^+$ oxidation to produce $N_2$ could take place (Luther et al. 1997). However, production of $NO_2^-$ from abiotic Fe-coupled $NH_4^+$ oxidation is only thermodynamically favorable at pH < 1 (Luther et al. 1997), and thus, probably does not occur.

I recently performed a preliminary experiment to determine if anammox, Feammox, or abiotic Fe-coupled $NH_4^+$ oxidation could occur in highly weathered tropical soils. Soils were collected from eight humid tropical forest sites in the Luquillo Experimental Forest, Puerto Rico and included soils from different topographical positions (e.g., valley and ridge) and forests dominated by different plant communities (e.g., lower montane forest, cloud forest, and palm forest). The soils were pre-incubated in an $N_2$ (i.e., $O_2$-free) headspace for five days to create anoxic conditions and inhibit nitrification, which supplies $NO_3^-$ for denitrification and would confound the results. We analyzed a separate set of soils to measure gross nitrification rates and determine the absence of $NO_3^-$ during the incubation. We used a $^{15}N$ tracer technique adding $^{15}NH_4Cl$ and $Na^{14}NO_2$ in a 1:1 molar ratio to increase the soil $NH_4^+$ pool to upwards of 90 atom % $^{15}N$. Denitrification of $NO_3^-$ or $NO_2^-$ not produced by Feammox would produce mass 28 $N_2$ ($^{14\text{-}14}N_2$) only. Mass 29 $N_2$ ($^{14\text{-}15}N_2$ or $^{15\text{-}14}N_2$) production would result from any of the three alternative $N_2$ production pathways whereas mass 30 $N_2$ ($^{15\text{-}15}N_2$) production would only occur in association with Fe reduction (Figure 3).



(1) $^{14}NO_2 + {}^{14}NO_2^- \rightarrow {}^{14\text{-}14}N_2O \rightarrow {}^{14\text{-}14}N_2$ (denitrification)

(2) $^{15}NH_4^+ + {}^{14}NO_2^- \rightarrow {}^{15\text{-}14}N_2$ (anammox)

(3) $^{15}NH_4^+ + Fe^{3+} \rightarrow {}^{15\text{-}15}N_2 + Fe^{2+}$ (abiotic Fe-coupled $NH_4^+$ oxidation)

(4) $^{15}NH_4^+ + Fe^{3+} \rightarrow {}^{15}NO_2^- + Fe^{2+}$ (+ added $^{14}NO_2^-$) $\rightarrow {}^{15\text{-}15}N_2$ (or $^{14\text{-}15}N_2$)

(Feammox followed by biotic or abiotic denitrification)

Figure 3. The four major potential pathways for the added $^{15}NH_4^+$ and $^{14}NO_2^-$ to create $N_2$. Other pathways may exist but all would involve $NO_2^-$ produced via Feammox.

The $^{15}N_2$ data showed that anammox, Feammox, and/or Fe-coupled $NH_4^+$ oxidation occurred across all eight humid tropical forest sites. No $O_2$ or $NO_3^-$ was detected during the experiment suggesting that nitrification followed by denitrification could not be responsible for $^{15}N_2$ production. Over the 96 hour incubation, 9 -24 % of added $^{15}NH_4^+$ was converted to mass 30 $N_2$ while 0-1.3 % had been converted to mass 29 $N_2$. Of $NH_4^+$ lost during the incubation, mass 30 $N_2$ production accounted for 27-100% while mass 29 $N_2$ production accounted for up to 5 %. Mass 29 $N_2$ production implies that anammox could have been active in the soils. An experiment to isolate the activity of anammox is difficult to perform, so at this time, we cannot conclusively show that anammox does or does not

occur in humid tropical forest soils. The large proportion of $^{15}NH_4^+$ converted to mass 30 $N_2$ suggests that Feammox coupled denitrification and/or abiotic Fe-coupled $NH_4^+$ oxidation are important $N_2$ production pathways in these soils.

A follow-up experiment using slurried soils from one site and including an $Fe^{3+}$ addition treatment (in the soluble HFO form) showed that Feammox does indeed exist in humid tropical forest soils. Corresponding increases in $Fe^{2+}$, $NO_2^-$, and mass 30 $N_2$ for the $Fe^{3+}$ addition treatment relative to the control demonstrate that Feammox was leading to $N_2$ production (Figure 4a-c). However, this experiment was unable to provide insight into the contribution of Fe-coupled $NH_4^+$ oxidation to $N_2$ production or provide strong evidence for biotic denitrification or abiotic chemodenitrification of $NO_2^-$ produced by Feammox. Abiotic processes can occur immediately after substrate is added while time lags may be observed for biotic processes as microbes respond to the added substrate. Mass 30 $N_2$ production was not detected until the third sampling time point at 24 hours, suggesting that denitrification, and not chemodenitrification, was responsible for the $N_2$ production following Feammox.

These experiments were designed to stimulate these new $N_2$ production pathways to increase the probability of detecting them. Clearly, additional and larger scale experiments are necessary to reveal the significance of these pathways under conditions more similar to those found in the field.

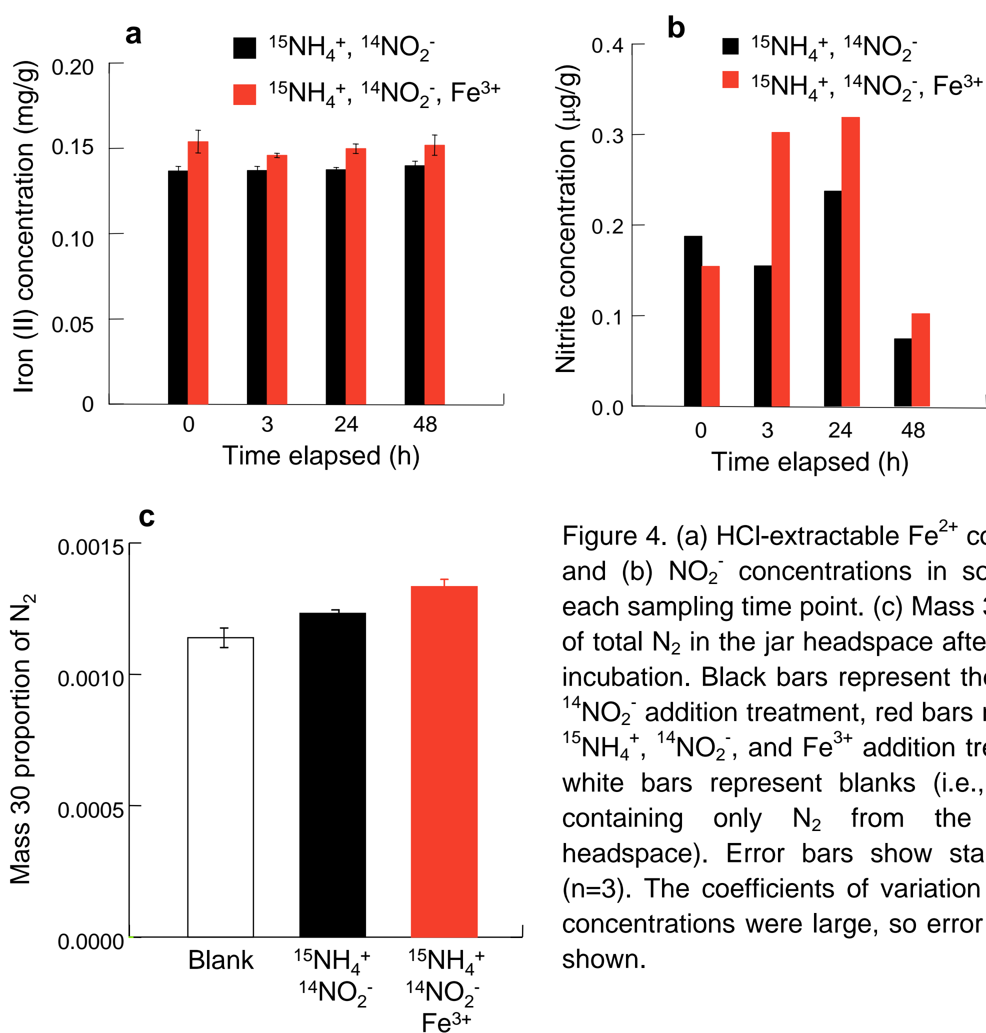



Figure 4. (a) HCl-extractable $Fe^{2+}$ concentrations and (b) $NO_2^-$ concentrations in soil slurries at each sampling time point. (c) Mass 30 proportion of total $N_2$ in the jar headspace after 24 hours of incubation. Black bars represent the $^{15}NH_4^+$ and $^{14}NO_2^-$ addition treatment, red bars represent the $^{15}NH_4^+$, $^{14}NO_2^-$, and $Fe^{3+}$ addition treatment, and white bars represent blanks (i.e., sealed jars containing only $N_2$ from the glove bag headspace). Error bars show standard errors (n=3). The coefficients of variation for the $NO_2^-$ concentrations were large, so error bars are not shown.

## PROPOSED RESEARCH

I propose two laboratory experiments to test the following hypothesis:

**Microbially mediated ferric iron reduction coupled to anaerobic ammonium oxidation (Feammox) leads to significant production of $N_2$ in humid tropical forest soils.**

*Study soil:* I will use soils from a mid-elevation montane forest in the Luquillo Experiment Forest (LEF), a NSF Long-Term Ecological Research (LTER) site in Puerto Rico and a site used in both preliminary experiments. The soils are clay-rich ultisols with low $NO_3^-$ concentrations and which experience frequent and high amplitude fluctuations in soil $O_2$ concentrations (Silver et al. 1999; Silver et al. 2001). Soil pH measured in 1M KCl is 4.0 ± 0.1 (Cusack et al., in prep). Biological N fixation rates in these soils are low (Cusack et al., in prep) so that $N_2$ consumption is not important. Members of Dr. Whendee Silver's lab based at LEF will collect soil from 0-10 cm depth and store it in gas permeable bags. The soil will be shipped overnight to the University of California, Berkeley (UCB) at ambient temperature so that fresh soil will be available for each experiment.

***Rationale for Experiment 1:***
***The significance of the new $N_2$ production pathways relative to one another***
There are multiple new $N_2$ production pathways that may occur in iron-rich and moderately acidic humid tropical forest soils, but biotic denitrification of $NO_2^-$ produced from Feammox (hereafter, Feammox-denitrification) is likely the major pathway. My preliminary data show that, if anammox occurs, it is minor compared to Feammox or Fe-coupled $NH_4^+$ oxidation. While it is thermodynamically feasible for Fe-coupled $NH_4^+$ oxidation to abiotically produce $N_2$ directly at pH below 6.8 (Luther et al. 1997), this has not been reported. However, there is reported evidence of $NO_2^-$ production due to Feammox (Clement et al. 2005; Sawayama 2006). My preliminary data also suggest increased $NO_2^-$ concentrations after the $NH_4^+$ and $NO_2^-$ addition and greater increases with $Fe^{3+}$ addition compared to without (Figure 4b). While this does not preclude direct $N_2$ production from Fe-coupled $NH_4^+$ oxidation, it does show that $NO_2^-$ is produced to allow $N_2$ production via denitrification.

Chemodenitrification can occur in soils, reducing $NO_2^-$ to NO, $N_2O$, or $N_2$ through self-decomposition of nitrous acid or in reaction with metallic cations such as $Fe^{2+}$ (Stuven et al. 1992; Van Cleemput and Samater 1996). Chemodenitrification involving organic matter results in formation of organic N from $NO_2^-$ but no gaseous N (Van Cleemput and Samater 1996). Nitrous acid formation from $NO_2^-$ is only important at pH below 3.3, so self-decomposition to NO and $N_2O$ is likely not important in soils from my proposed study site where soil pH is 4.0. In my preliminary experiment, most of the $NO_2^-$ was consumed by the end of the incubation while $Fe^{2+}$ concentrations remained stable throughout the incubation. This suggests that chemodenitrification using $Fe^{2+}$ as an electron donor is not the major pathway responsible for the consumption $NO_2^-$ produced from Feammox.

Humid tropical forest soils are thought to have high biotic denitrification rates (Seitzinger et al. 2006; Houlton et al. 2006), leaving low background soil $NO_3^-$ concentrations. Nitrite is an intermediate in denitrification so that it can act as a substrate to stimulate denitrification rates when it is produced by Feammox. Due to low background $NO_3^-$ and frequent anaerobic events, soil denitrifiers may be primed with $N_2O$ reductase enzymes to reduce any $N_2O$ that may be produced from the reduction of $NO_2^-$. While theory suggests that $N_2O$ would not be reduced when more energetically favorable electron acceptors (such as $NO_3^-$ and $NO_2^-$) are available, studies have shown stimulation of $N_2$ production with $NO_3^-$ addition to low $NO_3^-$ soils previously exposed to anaerobic conditions (Yang et al., in prep; Blackmer and Bremner 1979). Thus, biotic denitrification of $NO_2^-$ could be the dominant, if not sole, source of $N_2$ from Feammox.

**Approach**
The biotic and abiotic processes contributing to $N_2$ production can be distinguished by comparing activity in live and sterilized soils under anoxic conditions (when nitrification is inhibited). When $^{15}NO_2^-$ is added to sterilized soil, $^{15}N_2$ should be produced by chemodenitrification only. When $^{15}NH_4^+$ is added to sterilized soil, $^{15}N_2$ should be produced by abiotic Fe-coupled $NH_4^+$ oxidation only. The activity of abiotic Fe-reduction in any of these cases can be confirmed by measurement of $Fe^{3+}$ and $Fe^{2+}$ concentrations. In live soils with $^{15}NH_4^+$ added, both biotic and abiotic processes can produce $^{15}N_2$. Thus, comparing $^{15}N_2$ production rates between live and sterilized soils can reveal whether or not Feammox, a microbially mediated process, is contributing to $N_2$ production.

***Methods:*** Soil will be sterilized at the Lawrence Livermore National Laboratory (located near UCB) using a 25 Gy dose of gamma irradiation over 16 h, which will minimize effects on soil N concentrations while ensuring sterilization (McNamara et al. 2003). The soils will then be slurried using autoclaved deionized water and Mason jars, and pre-incubated for five days in an $O_2$-free glove bag (i.e., $N_2$ headspace) inside a sterile ultraviolet hood at UCB. Soils will receive one of two label additions: $^{15}NH_4Cl$ or $Na^{15}NO_2$. One day prior to the experiment, five replicate subsamples of soil will be extracted in 2M KCl and analyzed colorimetrically on a Lachat autoanalyzer at UCB to determine background $NH_4^+$ and $NO_2^-$ concentrations. $^{15}NH_4Cl$ and $Na^{15}NO_2$ solutions in autoclaved deionized water will be prepared in the sterile hood so that adding 1 mL of each solution will raise the soil $NH_4^+$ or $NO_2^-$ pool in each jar to at least 90 atom % $^{15}N$. Immediately after the $^{15}N$ solution is added, the jar will be sealed with a viton gasket and custom-made stainless steel lid to ensure a leak tight seal. Five replicate jars of each of the four treatments will be destructively sampled at four time points (15 min, 1 h, 3 h, and 24 h). A live soil treatment receiving $^{15}NH_4Cl$ additions will be prepared similarly to the sterile soil treatment described above except sterile procedures will not be used.

The jar headspace will be sampled using a gas tight syringe, and a 60 mL sample will be stored in a 40 mL serum bottle sealed with a butyl rubber septum and aluminum crimp. An additional 60 mL gas samples will be immediately analyzed on an $O_2$ meter. The stored gas samples will be analyzed on an IRMS for $^{15}N_2$ and $^{15}N_2O$, and on a gas chromatograph equipped with an electron capture detector (Shimadzu GC-14A) for $N_2O$ concentration at UCB. A subsample of the slurry will be centrifuged; the supernatant will be analyzed for $NO_2^-$ colorimetrically on a spectrophotometer (Wetzel and Likens 1991) and for soluble $Fe^{3+}$ and $Fe^{2+}$ using hydroxylamine and ferrozine assays (Van Bodegom et al 2003). A second subsample of the slurry will be extracted in 0.5M HCl for $Fe^{2+}$ analysis (Roden and Zacchara 1996). A third subsample will be used to streak plates to ensure the soils remained sterile (Trevors 1996). The remaining soil will be extracted in 2M KCl for colorimetric analysis of $NH_4^+$ and $NO_3^+$ on a Lachat autoanalyzer at UCB.

***Data analysis:*** Dinitrogen and $N_2O$ production rates will be calculated from $^{15}N_2$ and $^{15}N_2O$. Mass balance will be used to determine the proportion of added $^{15}NH_4^+$ or $^{15}NO_2^-$ that was lost as $N_2$ and $N_2O$. Multivariate analysis of variance will be used to test for differences between live and sterile soils in the production of $N_2$ and $N_2O$, and concentrations of $NO_2^-$, $NO_3^-$, $NH_4^+$, water soluble $Fe^{3+}$, and HCl-extractable $Fe^{2+}$ at the different time points.

**Expected Results**
Fe-coupled $NH_4^+$ oxidation and chemodenitrification are expected to be minor $N_2$ production pathways, so $^{15}N_2$ production from the sterilized soils is expected to be low. Based on results from the preliminary experiments, $^{15}N_2$ production in the live soils should be high. Thus, this experiment will show that biotic denitrification of $NO_2^-$ produced from Feammox is the dominant pathway responsible for $N_2$ production from $NH_4^+$. I will also measure $^{15}N_2O$ because there is the potential for $N_2O$ production via these pathways, which is also relevant to my dissertation goal to understand controls the $N_2/N_2O$ ratio emitted from soils. In the preliminary experiments, $N_2$ production accounted for 27-100% of $NH_4^+$ lost, suggesting that a large proportion of $NO_2^-$ produced by Feammox could be denitrified only to $N_2O$. Accordingly, I anticipate that $N_2O$ production in the live soils will be great.

***Rationale for Experiment 2:***
***The significance of the new $N_2$ production pathways relative to the denitrification of nitrate***
The significance of anaerobic $NH_4^+$ oxidation pathways to $N_2$ production relative to denitrification of $NO_3^-$ depends on the controls on the processes producing the initial substrate, most significant of which is $O_2$ availability. Mineralization of organic N to $NH_4^+$ is often not strongly redox sensitive (Myrold 1987; Pett-Ridge et al. 2006). Thus, at low $O_2$ concentrations, the supply of $NH_4^+$ to Feammox can continue. The $NO_2^-$ produced from Feammox can then be denitrified to $N_2O$ and $N_2$. However, nitrification is an obligately aerobic microbial process, so that the supply of $NO_3^-$ to denitrification is low or ceases at low $O_2$ concentrations. Furthermore, dissimilatory $NO_3^-$ reduction to $NH_4^+$, an anaerobic process, can be high in tropical soils (Silver et al. 2001), decreasing the $NO_3^-$ pool while increasing $NH_4^+$.Thus, the new $N_2$ production pathways are likely to be important relative to the denitrification of $NO_3^-$ at low $O_2$ concentrations. At higher $O_2$ concentrations, nitrification can compete for $NH_4^+$ with these other pathways so that denitrification of $NO_3^-$ may dominate.

**Approach**
Rates of $N_2$ production will be determined via the $^{15}N$ tracer method. Anaerobic $NH_4^+$ oxidation pathways to $N_2$ can be measured using $^{15}NH_4^+$ addition, and denitrification of $NO_3^-$ to $N_2$ can be measured using $^{15}NO_3^-$ addition. Nitrapyrin, a nitrification inhibitor, will be used in the $^{15}NH_4^+$ addition treatments so that anaerobic $NH_4^+$ oxidation pathways can be isolated. While this removes competition for $NH_4^+$ by nitrification, there is no other way to isolate these pathways. Since the $N_2/N_2O$ ratio of denitrification end-products is sensitive to redox and the total amount of N moving through these pathways is also important, $N_2O$ production rates will also be measured using the $^{15}N$ tracer method. Production rates of $N_2$ and $N_2O$ will be compared between the two $^{15}N$ label treatments at five different $O_2$ levels (0, 1, 5, 10, 15 %) to determine if the anaerobic $NH_4^+$ oxidation pathways to $N_2$ production are important relative to the denitrification of $NO_3^-$.

***Methods:*** Live soils will be pre-incubated for 24 hours in a glove bag filled with the desired $O_2$-$N_2$ mixture. A third of the soils will receive $^{15}NH_4Cl$ as described for the previous experiment except 15 atom % $^{15}N$ of the $NH_4^+$ pool will be targeted to minimize changes in the source pool size while maximizing the signal. Nitrapyrin will also be added to these soils. Another third of the soils will receive $K^{15}NO_3$ as just described. Five replicate jars of each treatment will be destructively sampled at each of four time points as described in the previous experiment. The remaining soils will receive nitrapyrin and $K^{15}NO_3$ to measure gross nitrification rates and check if nitrification was indeed inhibited completely by nitrapyrin. Five replicate jars will be destructively sampled at each time point for 2 M KCl extraction of $NO_3^-$. All KCl extracts from $^{15}NO_3^-$ treated soils will be analyzed for $^{15}N$-$NO_3^-$ using the diffusion method (Herman et al. 1995) and an IRMS at UCB.

***Data Analysis:*** Dinitrogen and $N_2O$ production rates will be calculated from $^{15}N_2$ and $^{15}N_2O$. Mass balance will be used to determine the proportion of added $^{15}NH_4^+$ or $^{15}NO_2^-$ that was lost as $N_2$ and $N_2O$. Gross nitrification rates will be calculated from $^{15}NO_3$ pool dilution (Kirkham and Bartholomew 1954). Multivariate analysis of variance will be used to compare $N_2$ and $N_2O$ production rates between the two treatments at the five different $O_2$ levels. ANOVA and Tukey tests will be used to determine differences in gross nitrification rates for the $^{15}NO_3$ only treatment between $O_2$ levels.

**Expected Results**
Anaerobic $NH_4^+$ oxidation is expected to lead to more $N_2$ production at low $O_2$ concentrations where gross nitrification rates are too low to support significant denitrification of $NO_3^-$. Gross nitrification rates are expected to increase with $O_2$ concentration so that $N_2$ production from denitrification of $NO_3^-$ will increase. However, because the ratio of $N_2/N_2O$ produced by denitrification decreases with increasing $O_2$ concentrations, $N_2O$ production rates will also be an important measure of the significance of the alternative $N_2$ production pathways. Nitrous oxide production with $NH_4^+$ as the initial substrate is expected to be significant relative to that with $NO_3^-$ as the initial substrate at lower $O_2$ concentrations.

## SIGNIFICANCE AND BROADER IMPACTS

The proposed research will contribute to the fund of knowledge needed to create accurate ecosystem N budgets and denitrification models. It will also improve predictions of future emissions of $N_2O$ from humid tropical soils. Existing denitrification models to predict $N_2O$ and $N_2$ soil emissions are based on $NO_3^-$ concentrations in addition to other parameters that do not include $NH_4^+$ concentrations (Boyer et al. 2006). Anthropogenic deposition of both $NO_3^-$ and $NH_4^+$ are expected to increase in the future, potentially impacting anaerobic $NH_4^+$ oxidation in addition to the denitrification of $NO_3^-$. Thus, understanding the significance of these alternative $N_2$ production pathways is necessary to improve these denitrification models and make better projections of future climate change and stratospheric ozone depletion. Moreover, the determination of significant new $N_2$ production pathways can lead to new avenues of biogeochemical research.

My overall dissertation will also improve greatly as a result of this proposed research. I aim to elucidate controls on the $N_2/N_2O$ ratio of denitrification of $NO_3^-$, so $N_2$ and $N_2O$ production resulting from anaerobic $NH_4^+$ oxidation would impact my planned experiments. The knowledge I will gain from the proposed research will inform the methods I choose to measure $N_2$ production from denitrification and alert me to potential confounding factors.

The proposed research will also have broader impacts beyond the field of terrestrial biogeochemistry. Soils and methods from the proposed experiments will be used in interactive classroom science projects through the TechBridge Girls and Community Resources for Science programs. These programs operate in elementary and middle schools mostly located in low-income and minority neighborhoods near UCB, so this is an opportunity to broaden the participation of underrepresented groups in science. Undergraduates at UCB will also be engaged with the proposed research through Ecosystem Ecology lectures on N cycling and through the Undergraduate Graduate Mentorship Program. Research results will also be disseminated broadly through national scientific conferences and through a video that will be available on a UCB website for public viewing.