The Effects of Inorganic Nitrogen Deposition on Methane Effluxes from Tropical Forest Soils.

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Abstract Anthropogenic nitrogen (N) inputs to tropical forest ecosystems have increased and will likely continue to increase due to industrialization, urbanization, and development. Increased presence of N in the soil can alter the efflux of methane (CH_4) across the soil-atmosphere interface. Molecular similarities between NH_3 and CH_4 allow NH_3 to compete for sites on the methane monooxygenase (MMO) enzyme in methanotrophic bacteria, reducing CH_4 oxidation rates in the soil and increasing diffusion rates of CH_4 to the atmosphere. There is a need to quantify biological sources and sinks of CH_4, particularly in tropical forest soils, as 25-35% of current climate forcing can be attributed to atmospheric CH_4. I examined the effects of N fertilization in a lower montane rain forest and a lower montane wet forest, both part of the Luquillo Experiment Forest, Puerto Rico. Samples of soil gas efflux were collected using the static flux method for eight weeks in the summer of 2006. Between the second and third weeks, three of six plots in each forest type received 50 kg N/ha. High rates of nitrification could explain the absence of long-term effects. A short-term positive response in CH_4 effluxes may have occurred in the higher elevation forest type, likely due to site differences in soil redox dynamics. Variable soil redox dynamics make quantifying the effect across an entire ecosystem difficult.

Introduction

The magnitude of global environmental change is becoming increasingly well documented. One important aspect of the anthropogenic impact on environmental change is the alteration of the global nitrogen (N) cycle (Vitousek 1994). Nitrogen fixation caused by human activity has increased enormously as a result of N fertilization, fossil fuel combustion, and the biological N fixation by leguminous crops. These activities have had such an impact on rates of N fixation that anthropogenic N fixation rates currently exceed rates due to all other natural causes combined (Vitousek et al. 1997; Smil 1997). This has resulted in increasing rates of global N deposition, ultimately affecting stocks of trace gases in the atmosphere, the transfer of N from soils to marine ecosystems, and species composition and diversity in a number of ecosystems worldwide (Vitousek et al. 1997).

Deposition of N is expected to continue increasing with global population. This global increase will occur disproportionately in South America, where rates of N deposition are expected to triple in magnitude from 1980 rates by the year 2020 (Galloway 1994). In the tropics, industrialization and development should result in increasing rates of both fertilizer application and N deposition will continue to increase rapidly. By the year 2020, the proportion of global N fertilizer application occurring in the tropics and subtropics will likely increase from 40% to 75% of global fertilizer application will occur in the tropics and subtropics (Ortiz-Zayas et al. 2006). The percentage of global fuel-related N emissions occurring in these regions is also expected to increase to 75% (Galloway 1994). In Puerto Rico specifically, both wet and dry N deposition are increasing and will likely continue to increase with urbanization of the island (Ortiz-Zayas et al. 2006)

Deposition of inorganic N may affect soil-atmosphere fluxes of methane (CH_4) gas (Sylvia et al. 1999, Bodelier and Laanbroek, 2004). Methane is an effective greenhouse gas, with an atmospheric warming potential 20 times greater than that of carbon dioxide (CO_2) (Hansen et al. 2000). Climate forcing due to atmospheric CH_4 concentrations is approximately half that of CO_2, and is responsible for 25-35% of the current forcing (Hansen et al. 2000). Small changes in atmospheric concentrations of CH_4 can have significant effects on this forcing.

Concentration of CH_4 in the atmosphere has increased from pre-industrial level of 715 ppb to approximately 1774 ppb in 2005 (IPCC 2007). As with rates of N deposition, estimated

anthropogenic emissions of CH_4 (375 Tg/yr) to the atmosphere exceed natural emissions, more than doubling estimated global fluxes to the atmosphere (535 Tg/yr). The total net flux of CH_4 into the atmosphere is estimated at 30 Tg/yr. Atmospheric oxidation of CH_4 is a primary CH_4 sink, estimated at 334 Tg/yr; soil oxidation of CH_4 is also a significant sink, at 30 Tg/yr (Houghton et al. 1995, Chapin et al. 2002).

The atmospheric budget of CH_4 is estimated to be significantly affected by both methanogenic (CH_4-producing) and methanotrophic (CH_4-oxidizing) soil microbes (Sylvia et al. 1999). The balance between methanogenesis and methanotrophy through CH_4 oxidation varies tremendously across ecosystems (Bodelier and Laanbroeck, 2004); submerged wetland soils are thought to make up 55% of current global CH_4 emissions, whereas many upland soils are assumed to act as biological sinks, accounting for 6% of annual global CH_4 consumption (Le Mer and Roger, 2001).

Because this balance is a crucial component of terrestrial CH_4 emissions, recent research has focused specifically on the inhibition of CH_4 oxidation by additions of ammonium ($NH_4^{+)}$ fertilizers (Le mer and Roger 2001, Bodelier and Laanbroeck, 2004). This inhibition is thought to occur because of the structural similarities of CH_4 and ammonia (NH_3), resulting in NH_3's ability to compete with CH_4 for binding at the active site of the enzyme CH_4 monooxygenase (MMO). High concentrations of NH_3 thus inhibit CH_4 oxidation and increase diffusion of CH_4 into the atmosphere (Sylvia et al. 1999, Bodelier and Laanbroeck 2004). Additionally, two intermediates in the oxidation of CH_4 by MMO, nitrite and hydroxlamine, may have much greater inhibitory effects on CH_4 oxidizers than ammonia alone (Schnell 1994). Potential stimulatory effects of inorganic N additions on CH_4 emissions have also been reported. The mechanism of such effects is not currently known, but tend to occur only in soils limited by mineral N (Bodelier and Laanbroeck 2004). It is likely that such stimulatory effects are simply due to a general nutrient limitation, rather than a specific stimulatory mechanism.

Steudler (1989) first documented an inhibitory effect on CH_4 oxidation due to inorganic N deposition in temperate forest soils. Since that time, numerous studies have estimated the magnitude of this inhibitory effect primarily in wetlands, rice paddies, and upland forest soils,

with varying results. Little work has been done on tropical soils, though inhibitory effects of N deposition on methanotrophy have been shown to exceed those of methanogenesis in tropical forest plantation soils (Veldkamp 2001).

Tropical forest soils have generally been characterized as CH_4 sinks (Le Mer and Roger 2001), but recent work suggests otherwise. Humid tropical forests are often characterized by high net primary productivity and variable soil redox dynamics (Silver et al. 1999). These factors can stimulate methanogenic activity during low redox cycles and high CH_4 oxidation during well-aerated periods (Teh et al. 2005, Fletcher et al. 2004). This work suggests that upland tropical forests may actually be a net source of CH_4 to the atmosphere. The need to quantify the strength of tropical forest soils as either a sink or source, and the potential effects of increased N deposition, is thus high (Teh et al. 2005).

I used field experiments to evaluate the effects of inorganic N fertilizer additions on CH_4 efflux across the soil-atmosphere interface. I examined the short term and long term effects of NH_4-NO_3 fertilizer additions in two distinct forest types of the Luquillo Experimental Forest, Puerto Rico (n=3). I expected that the fertilizer additions would inhibit CH_4 oxidation, resulting in increased soil-atmosphere fluxes of CH_4 in the fertilized plots relative to control plots.

Methods

Study Site Field experiments for the study were conducted in the Luquillo Experimental Forest, a NSF Long-Term Ecological Research Site in Northeastern Puerto Rico (18°3'N, 65°8'W). Descriptions of the soils, vegetation and climate can be found in Brown et al. (1983). The forest at this site is classified as subtropical moist, wet, lower montane wet, lower montane rain, and rain forest according to the Holdridge Life Zone System (McDowell et al. 1992). Elevation ranges from 200masl to 1000masl. Precipitation varies from 2500mm to 4500mm/yr, and increases with elevation. Mean maximum temperature decrease with elevation, with total variation estimated at 7ºC (Brown et al. 1983). Vegetation types vary with elevation. The Tabonuco forest type ranges from 300 to 600 masl, the Colorado forest type from 600 to 900 masl (Wadsworth and Bonnet 1951).

In 2002, a N fertilizer experiment began in the Bisley and Icacos watersheds. The Bisley site is part of the Tabonuco forest type at an elevation of 240 masl. The Icacos site is in the Colorado

forest type at 620 masl (McDowell et al. 1992). Rainfall averages 3500-5000 mm/yr at Bisley (McDowell 1992) and 4800 mm/yr at Icacos (Brown et al. 1983). Bisley soils are Los Guineos clays (Ultisol) and Icacos soils are Utuado clays (Inceptisol) (Boccheciamp 1977). Further descriptions of the watershed sites can be found in Mcdowell et al. (1992).

In each watershed, six 20 by 20 m plots were established in paired blocks to account for differences in geomorphology and hydrology; one plot of each pair was treated, and one was established as a control. Treatment plots receive two doses of ammonium nitrate fertilizer each year, totaling 50 kg(N)/ha-year.

Data Collection Soil-atmosphere CH_4 exchange was measured over an eight week period during June-Aug 2006 using the static flux method (Keller et al. 1988). Two pre-fertilization samplings were taken weekly prior to the semi-annual fertilizer application. Four post-fertilization samplings began immediately after application, and were taken every other day for 8 days. An additional three samplings were taken at increasing intervals for the remaining three weeks. A total of nine samplings were taken.

Five cylindrical gas chambers were installed in each plot. Each chamber consisted of a collar and a lid. The collars were installed 1-3 cm into the soil (litter layer removed) to create a sufficient seal. This required using a rubber mallet, often breaking small roots. Thirty minutes after collars were installed, chamber lids were attached. Five gas samples from each chamber were extracted by inserting a needle and syringe into rubber septa located on top of each chamber. The syringe was filled twice to sufficiently mix the gas in the chamber prior to sample extraction. Samples were then injected into evacuated 20ml glass vials, sealed with septa and aluminum crimps. Samples were collected as close to 0, 5, 15, 25, and 40 minutes after lid installation as possible. Exact time points were recorded. Following collection, vials were sealed with silicone and stored for shipment to the UC Berkeley Silver Lab for analysis.

Locations for the collars were chosen in a semi-randomized fashion, avoiding large roots and heavy vegetation. Water drainages and heavily trampled sites in the plot were generally avoided. Microsites between fertilized and unfertilized plots were matched for canopy cover, litter layer thickness, and slope.

During the 40-minute collection time, other relevant data were collected. Two gas samples were taken six feet above the ground in each plot to estimate ambient atmospheric concentrations. A digital soil thermometer was used to measure soil temperature, surface

temperature, and temperature inside the chamber (equilibrated for 3-5 minutes). Two measurements were taken for chamber height from the soil on opposite sides of the chamber (plastic ruler). Observational weather data was also recorded.

To measure soil moisture, a 10cm soil core was extracted 5-10 feet from the plot's edge. Sites of soil extraction were kept the same throughout samplings. Soils were stored in Ziploc bags until they could be handled in the lab (within 18 hours). Soil cores were homogenized, and samples from each were weighed in soil tins. Soil samples were placed in a 125ºC oven for 48 hours and weighed a second time to calculate soil moisture percentage by weight.

Data Analysis Gas samples were measured for concentrations of CH_4, CO_2, and nitrous oxide (N_2O) using gas chromatography in the Silver Lab.

Fluxes of CH_4 were calculated using the following model:

$$F = (D / L) * (S - A)$$

where F is the estimated flux rate of CH_4 (ng C cm^{-2} s^{-1}), D is the diffusivity of the soil (cm^2 s^{-1}), L is the average path length in the soil (cm), S is the concentration of CH_4 in the soil (ng C cm^{-3}), and A is the concentration of CH_4 in the atmosphere (ng C cm^{-3}). The following model estimates values for D and L using simple linear regression:

$$Ln ((S - C) / (S - A)) = (D / L) * t$$

where C is the measured concentration of CH_4 (ng C cm^{-3}), and t is the amount of time passed between lid installation and sample extraction (s). The slope of this regression line is (D/L). S and A are varied to minimize the sum of the squared residuals in the equation. Collected air samples are used as starting values for A. If the regression model has a slope parameter with p-value of less than 0.05, the flux rate is recorded as zero. Final flux rates (F) were reported in ng C cm^{-2} hr^{-1}.

Statistical Analysis Average flux rates are grouped by sampling (before or after semi-annual fertilizer application), treatment, and forest type. Variability in individual flux rates is used to determine variance and standard errors. Lack of homogeneity of variances requires non-parametric rank-sum tests to test for significance between controlled and fertilized plots in each forest type.

Table 1 – Means and (standard error) for CH_4 flux rates (ug C cm^{-2} hr^{-1}) by site and fertilization category. P-values are for Kruskall-Wallis test between treatment groups.

	Icacos			Bisley		
	Fertilized	Control	p-value	Fertilized	Control	p-value
Pre-Fertilization	12.838±(12.666)	3.641±(3.446)	0.314	-0.363±(0.491)	-0.389±(0.578)	0.619
Post-Fertilization	1.919±(0.768)	-0.033±(0.545)	0.449	2.955±(2.720)	2.161±(2.091)	0.99

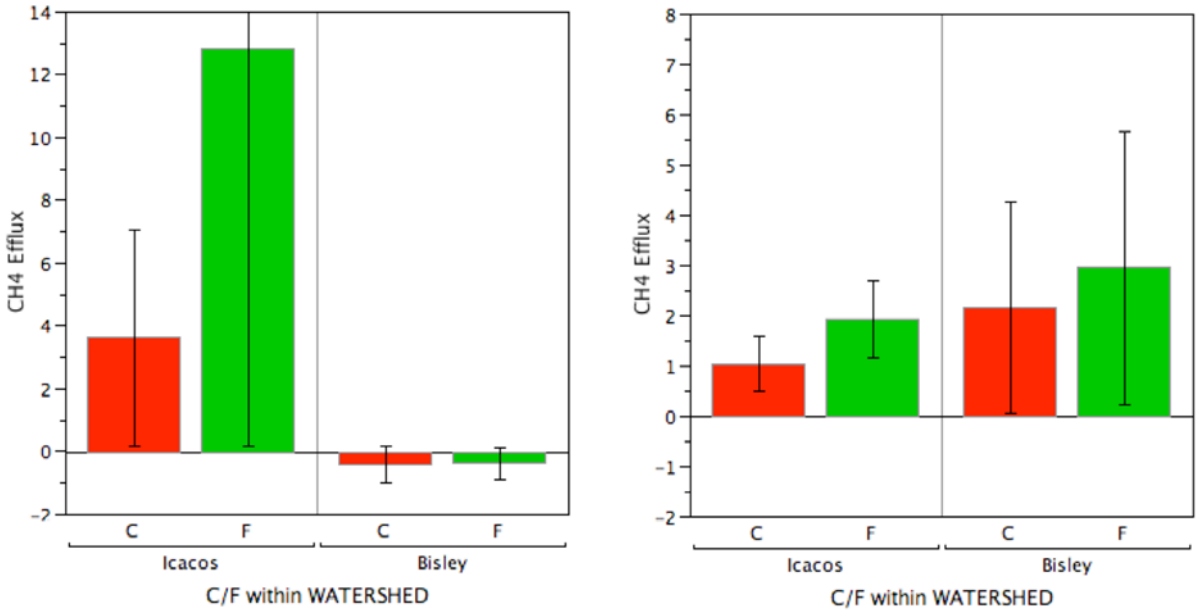

Figure 1 – Methane effluxes (means with std. error bars in ug C cm^{-2} hr^{-1}) grouped by treatment and watershed. 'C' is control 'F,' is fertilized. The left graph depicts pre-fertilization fluxes, the right, post-fertilization fluxes.

Results

I used the flux rate model to calculate effluxes for two pre-fertilization samplings and six post-fertilization samplings. Non-normal distribution of CH_4 effluxes prevented the use of parametric statistics. Over 90% of the CH_4 efflux regressions were nonsignificant and counted as net zero effluxes. Effluxes ranged from -26.4 to 379.7 ug C cm^{-2} hr^{-1}. Data transformation did not correct for heterogeneity of variance. Nonparametric rank sum tests were used.

In the pre-fertilization sampling, there were no significant differences in mean efflux between treatments (Table 1, Figure 1). In Icacos, effluxes in the fertilized plots were greater

Table 2 – Means and (standard error) for CH_4 flux rates (ug C cm^{-2} hr^{-1}) by site and sampling day. P-values are for Kruskall-Wallis test between treatment groups.

		Icacos				Bisley			
Sampling Number	Date	Days after Fertiliation	Fertilized	Control	p-value	Days after Fertiliation	Fertilized	Control	p-value
1	6/26/06	-13	0.702±(1.100)	0.435±(0.700)	0.243	-11	-.0718±(0.645)	0.101±(0.610)	0.906
2	6/30/06	-9	-0.363±(0.631)	0.000±(0.000)	0.655	-8	0.226±(0.751)	-1.124±(1.124)	0.331
3	7/10/06	1	4.477±(2.469)	0.062±(0.424)	0.160	2	13.722±(15.123)	-0.154±(1.208)	0.749
4	7/12/06	3	1.999±(1.644)	-0.138±(0.617)	0.208	4	1.446±(1.446)	1.171±(1.171)	0.860
5	7/14/06	5	0.659±(1.721)	1.729±(1.215)	0.380	6	0.000±(0.000)	-1.888±(1.888)	0.334
6	7/16/06	7	0.000±(0.000)	0.000±(0.000)	n/a	8	1.504±(1.504)	1.716±(1.716)	1
7	7/21/06	12	0.997±(0.997)	1.734±(1.183)	0.633	13	0.000±(0.000)	10.880±(11.364)	1
8	7/28/06	19	2.000±(2.000)	3.785±(3.785)	1	20	0.457±(1.951)	0.374±(0.374)	0.965
9	8/3/06	25							

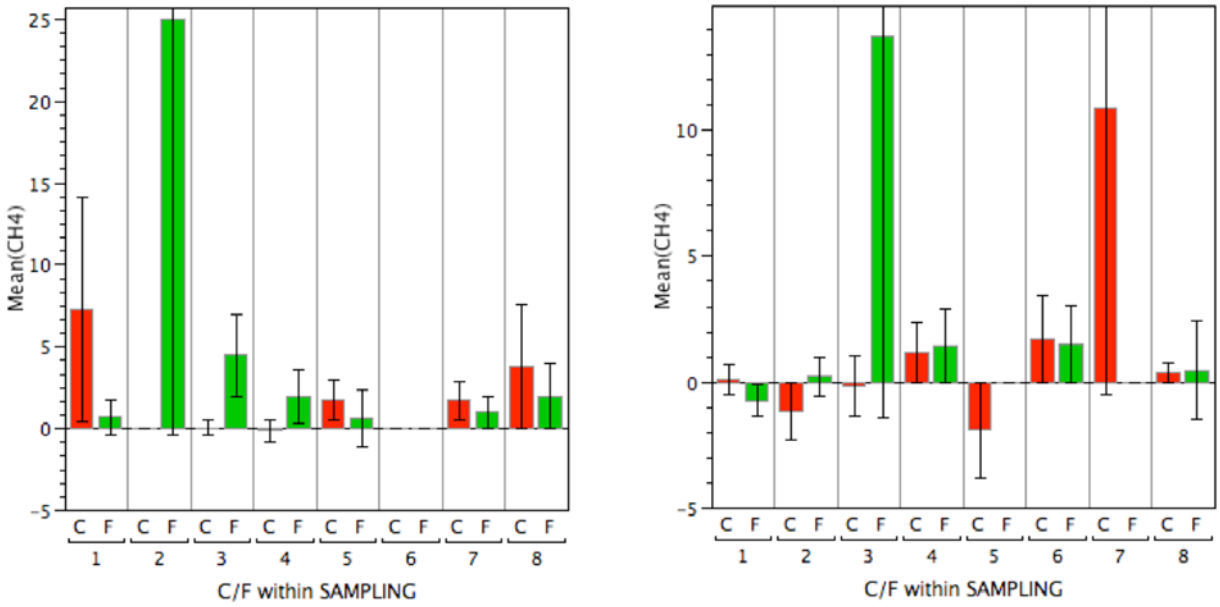

Figure 2 – Methane effluxes (means with std. error bars in ug C cm^{-2} hr^{-1}) grouped by treatment and sampling. 'C' is control 'F,' is fertilized. Fertilization occurred between samplings 2 and 3. The left graph depicts Icacos fluxes, the right, Bisley.

than in the control plots, but in Bisley, both treatment groups had approximately equal mean effluxes. The post-fertilization samplings had higher CH_4 effluxes in the fertilized plots as compared to the control plots, but these differences were also not significant. In the Icacos watershed, mean CH_4 effluxes in the fertilized plots (1.919±0.768 ug C cm^{-2} hr^{-1} (mean std. ±

error)) were greater than those in the control plots (-0.033±0.545 ug C cm^{-2} hr^{-1}) (Kruskall Wallis, p=0.449). In the Bisley watershed, CH_4 effluxes in the fertilized plots (2.955±2.720 ug C cm^{-2} hr^{-1}) were higher, than in the control plots (2.161±2.091 ug C cm^{-2} hr^{-1}), but not significantly so (Kruskall-Wallis p=0.99).

In Icacos, mean CH_4 effluxes in the fertilized plots spike directly after fertilization, reaching 4.477 ug C cm^{-2} hr^{-1}, and decreasing gradually after that (Table 2, Figure 2). Mean CH_4 effluxes in the control plots show no particular pattern. Differences in effluxes approach significance one day after fertilization, where effluxes in the fertilized plots are much greater than in the control plots. This difference is not as pronounced, however, three days after fertilization, and is negligible seven days after sampling. In Bisley, post-fertilization effluxes fluctuate randomly in both treatment groups.

In the pre-fertilization samplings, CH_4 effluxes were much higher in the Icacos plots (8.240±6.535 ug C cm^{-2} hr^{-1}) than in the Bisley plots (-0.377±0.377 ug C cm^{-2} hr^{-1}), but this difference was not significant (Kruskall Wallis p=0.554). In the post-fertilization samplings, differences between forest types were negligible.

Mean CH_4 effluxes across all samplings were positive, and higher in Icacos (3.312±1.822 ug C cm^{-2} hr^{-1}) than at Bisley (1.895±1.329 ug C cm^{-2} hr^{-1}) (Kruskall-Wallis, p=0.168).

Air temperature data averaged 3°C higher in Bisley than in Icacos. In Icacos, soil moisture percentage of soil cores did not change significantly across samplings. In Bisley, soil moisture percentage did not change with the exception of sampling eight, which had significantly lower moisture. Soil moisture did not correlate with mean CH_4 effluxes, nor did it account for any variation when treated as a covariate with treatment type.

Discussion

No long-term responses in CH_4 effluxes are apparent for this dataset, but the data suggest a possible short-term surge in CH_4 effluxes in Icacos. Variability in soil redox dynamics could account for not only site difference in response, but also the high degree of spatial variability evident in this dataset.

The high occurrences of zero fluxes could be explained by the relatively high accuracy

required to determine changes in CH_4 concentrations as opposed to CO_2 (average concentration is three orders of magnitude less). Effluxes closer to zero, then, may have been obscured by error due to instrument precision. Insufficient vial evacuation, poorly sealed vials, poorly insulated chambers, and inadequate chamber mixing prior to sample extraction may have also prevented the detection of smaller effluxes. The high degree of spatial variation in effluxes could be explained by variations in soil-redox dynamics (Teh et al. 2005, Fletcher et al. 2004). Abundant rainfall, high rates of root and microbial activity, and clayey soils result in a dynamic spatial and temporal mosaic of microsites. (Silver 1999). Mean CH_4 effluxes, however, were positive in both sites, suggesting that both sites act as a net source of CH_4, rather than a net sink. This supports recent speculation that tropical forest ecosystems may act as CH_4 sources (Silver et al. 1999, Teh et al. 2005).

Absence of a statistically significant short-term effect could be caused by several factors. Increasing the number of datapoints could substantially increase the robustness of the dataset. The ability to increase the number of datapoints is severely limited, however, by the static flux method. Repeated chamber installments at the same location disturb the soil and often cut through roots. The size of the plots, therefore, limited the number of samplings that could be taken. Time and labor required also limits sampling frequency to every two days for a signal field worker.

The magnitude of the effect could also be masked by variations in soil moisture among plots. Differences in soil moisture between treatment groups suggest higher background rates of methanogenesis in the control plots. This could result in higher effluxes from the control plots, masking the effect of fertilization. Soil moisture, however, does not account for the variations in CH_4 fluxes, probably because the soil was extracted outside of the plots, rather than the microsites where the chambers were installed.

Ecosystem properties may also limit the magnitude of the true effect. High rainfall directly following fertilization might have resulted in high runoff rates, ultimately limiting how much inorganic N entered the soil. Additionally, availability of NH_4^+ is generally the primary determinant of nitrification rates (Robertson 1989). Nitrate (NO_3^-) pools in humid tropical forests are generally larger than NH_4^+ pools (Silver et al. 2005). It is likely that the additional

NH_4^+ is quickly nitrified or taken up by plants, ultimately affecting the available pool of NO_3^- more than NH_4^+. This could explain the short-term surge in CH_4 efflux at Icacos, along with the absence of any long-term effects.

Ammonium has also been shown to stimulate methanotrophic acitvity in rice paddies (Bodelier et al. 1999, Bodelier and Laanbroeck, 2001). This unexpected trend is thought to occur simply due to a shortage of NH_4^+ in the soil environment. High rates of nitrification and plant N uptake in humid tropical forests suggest that methanotrophs would benefit from additional access to NH_4^+. Understanding and quantifying the microbial mechanisms involved are crucial to quantifying these inhibitory and stimulatory effects across an entire ecosystem.

The difference in response between forest types suggests that site factors may alter the magnitude of the effect of inorganic N deposition. Soil O_2 concentration decreases with increasing elevation (Teh et al. 2005). It is therefore likely that the Icacos site experiences higher rates of methanogenesis due to these anaerobic conditions (Sylvia et al. 1999, Teh et al. 2005). This difference in rates of methanogenesis may only become apparent when CH_4 oxidation is significantly inhibited, though pre-fertilization fluxes in Icacos are higher than in Bisley. Because soil-redox dynamics are a main determinant of CH_4 effluxes, further studies should sample for soil moisture by extracting soil cores beneath directly beneath flux chambers.

Root density in Bisley is also significantly higher than in Icacos. High root density can affect quality of insulation of the gas chambers. Chamber equilibration time may also have been insufficient to allow changes in root respiration. Root mortality may also affect emissions rates of trace gases, but the exact effect on CH_4 emissions is still unclear (Varner et al. 2002).

This study suggests that there may indeed be an increase in CH_4 effluxes across the soil-atmosphere interface in response to increase inorganic N deposition. This effect, however, is probably short-lived due to high rates of runoff and high nitrification rates and plant uptake of NH_4^+. Stimulatory effects on CH_4 oxidation may also play a role. The magnitude of the overall effect could be better quantified with similar experiments using more plot area and data points to account for extreme variability in CH_4 effluxes. Microsite soil moisture data should also be used to account for variations in soil-redox dynamics. Studies examining the effects of inorganic N

deposition at the microbial level are also necessary to determine the mechanisms for the overall effect on mean effluxes. As N deposition across the tropics is continually increasing (Galloway 1994), the need to quantify its effects on greenhouse gas emissions, particularly in tropical ecosystems, becomes increasingly necessary.

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