

Experimentally induced root mortality increased nitrous oxide emission from tropical forest soils

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[1] We conducted an experiment on sand and clay tropical forest soils to test the short-term effect of root mortality on the soil-atmosphere flux of nitrous oxide, nitric oxide, methane, and carbon dioxide. We induced root mortality by isolating blocks of land to 1 m using trenching and root exclusion screening. Gas fluxes were measured weekly for ten weeks following the trenching treatment. For nitrous oxide there was a highly significant increase in soil-atmosphere flux over the ten weeks following treatment for trenched plots compared to control plots. N₂O flux averaged 37.5 and 18.5 ng N cm⁻² h⁻¹ from clay trenched and control plots and 4.7 and 1.5 ng N cm⁻² h⁻¹ from sand trenched and control plots. In contrast, there was no effect for soil-atmosphere flux of nitric oxide, carbon dioxide, or methane.

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1. Introduction

[2] Soil-atmosphere exchange of carbon dioxide (CO₂), nitric oxide (NO), nitrous oxide (N₂O) and methane (CH₄), is controlled by complex biogeochemical processes [Conrad, 1996]. The soils of tropical forests are globally important sources of N₂O and NO [Matson *et al.*, 1990; Davidson and Kinglerlee, 1997]. Tropical forest soils can change from a sink to a source of CH₄ depending upon soil moisture conditions and land use [Keller and Reiners, 1994; Steudler *et al.*, 1996].

[3] Previous studies in tropical forests soils in Brazil, Costa Rica, and Puerto Rico suggested that root mortality increases emissions of NO and N₂O from the soil

surface [Matson *et al.*, 1990; Keller *et al.*, 2000]. In these studies, the effect of root mortality was inferred from observations of increased trace gas emissions following the emplacement of bases for soil enclosures to depths of 1 to 2 cm into the soil surface. The emplacement procedure cut roots. In Brazil, Matson *et al.* [1990] found that N₂O fluxes measured from permanently installed chamber bases showed significant increases compared to fluxes from control bases emplaced immediately before measurement after only 5 hours of insertion of the first set of bases. In Costa Rica and Puerto Rico, Keller *et al.* [2000] observed increased fluxes of NO and N₂O from several hours up to 6 weeks after base emplacement.

[4] In order to provide a more direct test of the effect of root mortality on the soil-atmosphere exchange of trace gases (CO₂, NO, N₂O and CH₄), we established a series of plots, intended to kill all roots within the plots, in the Tapajos National Forest, Brazil. We evaluate the effect of the experimentally induced root mortality on trace gas exchange in clay and sand soil in tropical forest.

2. Methods

2.1. Study Area

[5] The study site in the Tapajos National Forest (TNF) is near km 83 on the Santarem-Cuiaba Highway south of Santarem, Para, Brazil. The region receives approximately 2000 mm of precipitation per year and has an annual mean temperature of 25°C [Silver *et al.*, 2000]. Vegetation at the site is evergreen, mature tropical forest with a total biomass of about 372 Mg ha⁻¹ [Keller *et al.*, 2001]. We located experiments on contrasting soils, a clay textured Oxisol (80% clay, 18% sand, 2% silt) and a sand textured Ultisol (60% sand, 38% clay, 2% silt) [Silver *et al.*, 2000].

2.2. Experimental Design

[6] The experiment was a randomized complete block design. For each soil type, 5 pairs of 2.5 × 2.5 m plots were located so that there were no trees greater than 10 cm diameter at breast height (DBH; 1.3 m) on the plots. One plot in each pair was randomly selected for trenching. In the trenched plots, trenches were dug to 1 m depth and were lined with a fine stainless steel mesh (<0.5 mm) to prevent the penetration of roots while allowing the movement of water and gases. All vegetation was clipped from the trenched plots at the time of trenching and every two weeks thereafter to prevent colonization of the plot by live roots. We completed the trenching operations in the period from Julian day 147

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through 156 in 2000 (May 27 through June 4). For all plots, we made measurements in an interior square region, 2×2 m that was surrounded by a 0.5 m wide buffer strip.

2.3. Trace Gas Flux Measurement

[7] We measured the soil-atmosphere fluxes of CO_2 , NO, N_2O and CH_4 weekly for approximately 10 weeks following the trenching treatment. Two chamber bases were inserted approximately 2 cm depth in the soil at randomly selected points in the sampled plots within 30 minutes of the weekly flux measurement. These chamber bases were removed immediately after flux measurements were completed. Dynamic flow-through chambers were used for measurement of NO and CO_2 and static vented chambers were used for measurements of N_2O and CH_4 Keller and Reinert [1994]. The measurement of these two pairs of gases was sequential after lifting the chamber top to equilibrate the headspace with ambient air.

[8] We used an integrated backpack system to measure NO and CO_2 over 3 to 10 minutes from enclosures. The flow through the chamber was regulated to about $300 \text{ cm}^3 \text{ min}^{-1}$. Air entered the chamber through a chimney-like air-gap that was specifically designed to minimize exchange with the outside air and to avoid pressure fluctuations within the chamber. Rayment and Jarvis [1997] found that the pressure differential between the chamber and the outside air was less than 0.004 Pa in laboratory tests.

[9] Air flowed from the soil enclosure through a Teflon-lined polyethylene sample line 30 m in length and then it entered an infrared gas analyzer (Li-Cor 6262) for CO_2 measurement. From the Li-6262, the sampled air then passed through a flow control manifold where it was mixed with a make-up air flow of about $1200 \text{ cm}^3 \text{ min}^{-1}$ and a flow of NO (1 ppm) standard gas that varied from 3 to $10 \text{ cm}^3 \text{ min}^{-1}$ as measured on an electronic mass flowmeter (Sierra Top-Trak). The make-up air and standard addition maintain optimum and linear performance of the NO_2 chemiluminescent analyzer (Scintrex LMA-3). The mixed sample stream passed through a Cr_2O_3 catalyst for conversion of NO to NO_2 [Levaggi et al., 1974]. The NO_2 chemiluminescent analyzer was standardized by a two-point calibration approximately hourly. We checked the intra-day stability of the calibration on each sampling date by comparison of each standard run to a linear interpolation between the standards runs at the beginning and end of the daily measurement period. We also compared the concentration of the field NO standard periodically with laboratory standards to assure that they did not drift [Veldkamp and Keller, 1997]. Signals from the CO_2 and NO_2 analyzers and the mass flow meter for the NO standard gas were recorded on a datalogger (Campbell CR10). Fluxes were calculated from the linear increase of concentration versus time.

[10] Static enclosure measurements were made for CH_4 and N_2O fluxes using the same bases and vented caps [Keller and Reinert, 1994]. Four enclosure headspace samples were taken over a 30-minute sampling period with 20 ml nylon syringes. Analysis of grab samples for CH_4 and N_2O were completed within 36 hours by FID

and ECD gas chromatography. Gas concentrations were calculated by comparing peak areas for samples to those for standards.

2.4. Additional Measurements

[11] Soil samples were taken to 10 cm depth in each plot on each date for determination of soil moisture (oven dried at 105°C). Soil moisture was expressed as water-filled-pore-space (WFPS) using soil bulk densities of 1.34 and 1.02 for sand and clay soils respectively [Silver et al., 2000].

2.5. Fine Root Sampling and Analysis

[12] Roots were sampled using a root corer with a 6 cm internal diameter [Vogt and Persson, 1991]. Cores were removed to 10 cm depth on 2 dates (June 4 (day 156) and 30 (day 182)) following trenching. Roots were sorted and dried at 65°C and weighed.

2.6. Data Analysis

[13] To analyze differences in a randomized complete block design, we reduced the flux, moisture and root biomass data to plot means for the entire experimental period. We compared plot means using a two-way ANOVA within each soil type testing for differences for treatment and plot with no interactions. For N_2O and root biomass, we log transformed the means prior to analysis to homogenize the variances. For the cases of NO, CO_2 , and CH_4 we also checked for treatment differences within each soil type by running a two-way ANOVA for each gas on each date.

3. Results

3.1. Stability of NO Standardization

[14] NO standards were run in the field at the beginning and end of 8 enclosure flux samples or approximately every hour. NO standard response calculated using a linear fit of the two standards encompassing the measurement period was compared to the frequent (generally hourly) standardization. A given hourly standard run varied by as much as 60% from the standard response calculated from the linear fit (Figure 1). On two dates of eight tested, at least 50% of the standards fall outside of the predicted standard response based on the starting and ending standards by at least 20%. On two other dates at least 10% of the standard runs fall outside of this $\pm 20\%$ window.

3.2. Trace Gas Fluxes

[15] Trace gas fluxes and soil WFPS over the ten weeks of the trenching experiment are shown in Figures 2 and 3. Because of equipment malfunction, we did not measure NO and CO_2 flux on the final study date, day 227 (August 14). Analysis of variance of data reduced to plot mean within each soil type showed no significant differences for fluxes of CO_2 , CH_4 , NO or soil WFPS for treatment or plot effect on both soil types. Additional tests with ANOVA on each individual treatment date showed no significant effects.

[16] For both sand and clay soils N_2O fluxes from the trenched plots significantly exceeded fluxes from the controls over the experimental period ($p < 0.001$) (Figure 3).

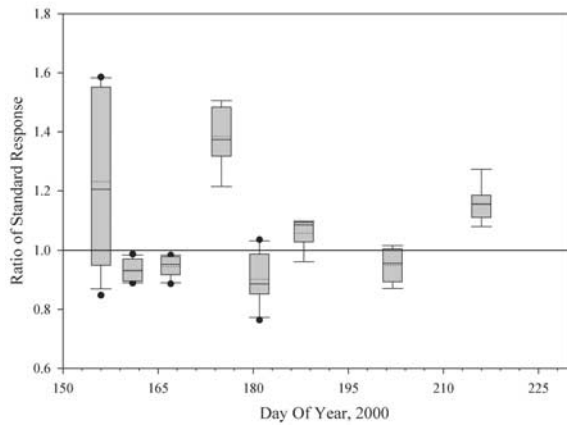


Figure 1. Ratio of NO standard response at a given time compared to response calculated from a linear fit of the first and final standards versus time on a given day. Closed circles represent all data that lie outside the 10th and 90th percentiles, error bars represent the 10th and 90th percentiles, the 25th and 75th percentiles are the ends of the shaded box with the 50th percentile represented by the solid line. The dashed line is the arithmetic mean. Data from Julian day 195 (July 13) has been omitted because there were few samples that day and only two standards run.

N_2O flux from the **trenched plots averaged 37.5 and $4.7 \text{ ng N cm}^{-2} \text{ h}^{-1}$ from clay and sand soils respectively.** These fluxes **exceeded average control fluxes by more than a factor of 2 (18.5 and $1.5 \text{ ng N cm}^{-2} \text{ h}^{-1}$ from clay and**

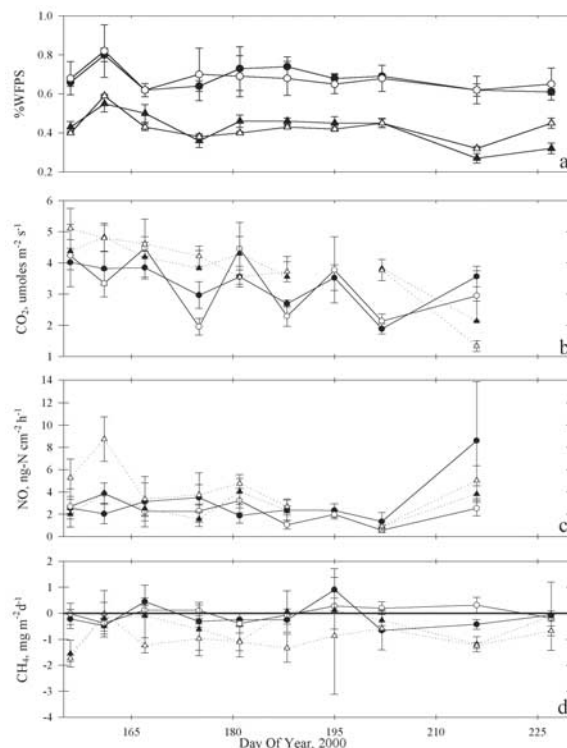


Figure 2. Measurements of (a) percent water-filled pore space (%WFPS), (b) CO_2 , (c) NO, and (d) CH_4 fluxes from day 158 (June 6) through 227 (August 14) 2000 for clay (●), clay trench (○), sand (▲), and sand trench (△) sites. Error bars are the standard error for plots ($n = 5$).

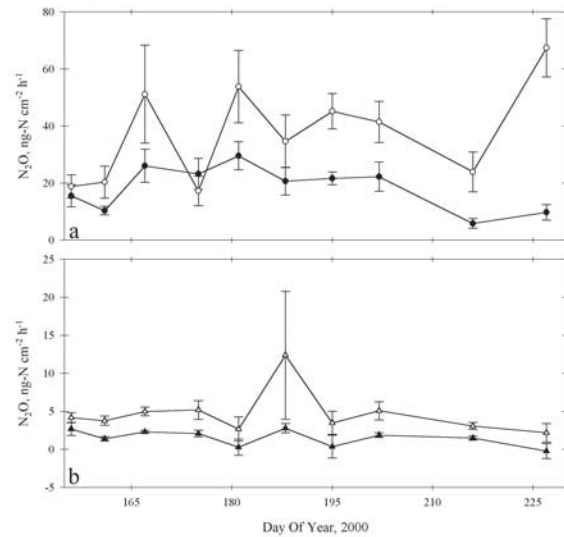


Figure 3. N_2O flux measurements for the (a) clay and (b) sand plots. Symbols and error bars as in Figure 2.

sand soils respectively) over the 10 weeks following the trenching treatment.

3.3. Root Biomass

[17] Root biomass in the trenched plots averaged $222 (\pm 25) \text{ g m}^{-2}$ in the clays and $260 (\pm 25) \text{ g m}^{-2}$ in the sands. Root biomass decreased slightly over the first four weeks following trenching in the clay soils to $173 (\pm 21) \text{ g m}^{-2}$. In contrast, root biomass increased slightly in the sand trench plots following root mortality to $277 (\pm 30) \text{ g m}^{-2}$, possibly resulting from colonization by decomposers [Silver and Vogt, 1993]. In both cases, the differences in root biomass were not statistically significant.

4. Discussion

4.1. Data Quality for NO Fluxes

[18] It has been common practice to standardize the Scintrex LMA-3 instrument when used for measurement of soil-atmosphere NO flux, only at the beginning and the end of the sampling period in a laboratory near the field site [e.g., Verchot et al., 1999; Garcia-Montiel et al., 2001]. This practice avoids the need to transport a compressed gas cylinder to the field. Often 4 to 8 hours or more pass between standardizations. Using field standards at nearly hourly intervals, we found that on 2 of 8 sampling dates, that standardization based on a linear interpolation of standard response from standards taken prior to and following sampling would have caused an error greater than 20% in the measured NO fluxes more than half the time (Figure 1). Moreover, on those dates, individual field standards could vary by as much as 60% from the linear interpolation of the first and last standardization.

[19] We have taken precautions to minimize the variability inherent in our operation of the Scintrex LMA-3. In particular, we maintain the system within its designed linear range (3 to 50 ppbv) by use of a standard addition. We also maintain a relatively constant relative humidity over the CrO_3 catalyst. Tests using CrO_3 catalysts for conversion of NO to NO_2 show that the catalysts are sensitive to relative

humidity changes and that they function most efficiently in the range 35% to 80% relative humidity. Oxidation efficiency drops for higher relative humidity levels [Levaggi *et al.*, 1974; Hutchinson *et al.*, 1999]. Frequent field standardization of the LMA-3 is necessary.

4.2. Effects of the Trenching Treatment on N₂O Fluxes

[20] Root mortality may have caused a decrease in oxygen in the rhizosphere because of the oxygen demand from increased decomposition. Lack of oxygen tends to increase the production of N₂O whether through nitrifier-denitrification or through denitrification. So-called “hotspots” of organic decomposition, have been shown to support rapid rates of N₂O production in experiments with soil cores [Parkin, 1987]. Additionally, root decay and lack of plant nutrient uptake can lead to nitrate concentration buildup [Silver and Vogt, 1993] and therefore more N available for N₂O production. Root mortality and the initial stages of decay are likely to result in the release of soluble C that can stimulate N₂O production.

[21] Soil N₂O emissions from plots treated by trenching significantly exceeded emissions from control plots by a factor of 2 for 10 weeks following trenching. The trenching treatment did not increase soil moisture content. Root mortality and the complex of biogeochemical changes in the rhizosphere surrounding dead roots is the likely cause of the doubled N₂O emissions. Root mortality and turnover is an ongoing process in soils. Fine roots (≤ 2 mm) have a lifetime of months to a few years depending upon the environment [Gill and Jackson, 2000; Silver and Miya, 2001]. The possible effect of root mortality on the production and flux of N₂O from soils has not been considered in recent simulation models of soil N₂O production and emission such as DNDC [Li *et al.*, 2000], CASA [Potter *et al.*, 1996] or DAYCENT [Parton *et al.*, 2001]. Given that models of N₂O emissions still fail to reliably simulate a wide range of field conditions [Frolking *et al.*, 1998] this cause of N₂O production merits further investigation.

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