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Drought-induced nitrous oxide flux dynamics in an enclosed tropical forest

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Abstract

El Niño–La Niña cycles strongly influence dry and wet seasons in the tropics and consequently nitrous oxide (N_2O) emissions from tropical rainforest soils. We monitored whole-system and soil chamber N_2O fluxes during 5-month-long droughts in the Biosphere 2 tropical forest to determine how rainfall changes N_2O production. A consistent pattern of N_2O flux changes during drought and subsequent wetting emerged from our experiments. Soil surface drying during the first days of drought, presumably increased gas transport out of the soil, which increased N_2O fluxes. Subsequent drying caused an exponential decrease in whole-system ($4.0 \pm 0.1\%$ day^{-1}) and soil chamber N_2O flux ($8.9 \pm 0.8\%$ day^{-1}; south chamber; and $13.7 \pm 1.1\%$ day^{-1}; north chamber), which was highly correlated with soil moisture content. Soil air N_2O concentration ([N_2O]) and flux measurements revealed that surface N_2O production persisted during drought. The first rainfall after drought triggered a N_2O pulse, which amounted to 25% of drought-associated reduction in N_2O flux and $1.3 \pm 0.4\%$ of annual N_2O emissions. Physical displacement of soil air by water and soil chemistry changes during drought could not account for the observed N_2O pulse. We contend that osmotic stress on the microbial biomass must have supplied the N source for pulse N_2O, which was produced at the litter–soil interface. After the pulse, N_2O fluxes were consistently 90% of predrought values. Nitrate change rate, nutrient, [N_2O], and flux analyses suggested that nitrifiers dominated N_2O production during the pulse and denitrifiers during wet conditions. N_2O flux measurements in Biosphere 2, especially during the N_2O pulse, demonstrate that large-scale integration methods, such as flux towers, are essential for improving ecosystem N_2O flux estimates.

Keywords: closed system, drought, N_2O, pulse, soil emission, soil profile, tropical forest

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Introduction

Nitrous oxide (N_2O) is the fourth most important greenhouse gas (IPCC, 2001). Its long atmospheric residence time, combined with its low waveband separation, yields a global warming potential ~ 300 times that of CO_2. The global significance of N_2O is further increased as the source for stratospheric NO, which removes nonpolar ozone, through the reaction with electronically excited oxygen (Crutzen, 1970). Mainly because of agricultural fertilizer use, tropospheric N_2O concentration ([N_2O]) has increased to ~ 315 ppb from the preindustrial level of ~ 270 ppb (Fluckiger *et al*., 1999) and is increasing at 0.25% year^{-1} (IPCC, 2001).

Despite recent progress, the N_2O budget contains major uncertainties (Matson & Vitousek, 1990; Kim & Craig, 1993; Kroeze *et al*., 1999; Rahn & Wahlen, 2000). The uncertainties result from local variations

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(Bouwman *et al.*, 1993) and rapidly changing conditions, such as precipitation (Kiese *et al.*, 2003) and land use (Garcia-Montiel *et al.*, 2001), all of which influence extrapolation of soil measurements to ecosystem fluxes.

Approximately 20% of natural N_2O emissions originate in wet rainforest ecosystems, which represent the largest natural source (Matson & Vitousek, 1990). Many tropical forests, such as the Amazon, are alternately wet and dry (Borchert, 1998; Jipp *et al.*, 1998), and the average N_2O flux in these forests is much greater in the wet than dry season (Verchot *et al.*, 1999; Perez *et al.*, 2000; Garcia-Montiel *et al.*, 2001; Kiese *et al.*, 2003). Current global warming models predict that El Niño events will intensify and increase in frequency, which cause more intense dry periods in tropical forests (Hulme & Viner, 1998; Timmerman *et al.*, 1999). Hence, the predicted decrease of N_2O source strength in tropical forests will strongly impact the global N_2O budget.

Drought can lead to significant changes in soil nitrogen chemistry as above- and belowground organisms cope with water stress. Depending on rooting depth, trees can shed leaves as a response to drought (Borchert, 1998; Rascher *et al.*, 2004), adding litter to the soil surface and thus increasing N input into the soil. Soil bacteria and fungi respond to drought by reducing activity, although net mineralization appears unaffected by soil moisture changes in tropical forests (Neill *et al.*, 1995). Nitrification rates are at a maximum at 60% water-filled pore space (WFPS) and decline rapidly towards higher soil moisture contents, when denitrification rates increase (Linn & Doran, 1984; Neill *et al.*, 1995; Breuer *et al.*, 2002). N_2O is produced as a byproduct in nitrification and dentrification. Reduced N_2O production in the late dry season has been attributed to reduced microbial activity during drought (Davidson *et al.*, 1993). Following the first wet season rains, nitrification is expected to become the most dominant microbial process because of a rapid response of nitrifiers to water addition (Azam *et al.*, 2002; Breuer *et al.*, 2002). Furthermore, bacteria can add significantly to soil C and N when they respond to rapid wetting of soil by releasing cytoplasmic solutes to prevent lysis (Kieft *et al.*, 1987). Mineralizers and nitrifiers can rapidly consume these solutes and, in the process, convert $\sim 0.1\%$ of nitrogen to N_2O (Jiang & Bakken, 1999).

Here, we present daily whole-system and soil N_2O fluxes measured during five 1-month-long droughts (between January 2000 and May 2003) in the tropical forest at Biosphere 2 Laboratory (B2L). The enclosure allows for rainfall control and its atmosphere-to-biomass ratio is relatively small so that plants and soils affect atmospheric trace gas composition orders of magnitude faster than in the real world. The tropical forest can be likened to a very large soil chamber with trees up to 25 m in height. The system, thus, integrates soil and plant interactions that potentially cause variability in small soil chambers. Our experiments were designed to measure whole-system and $1\,m^2$ soil chamber N_2O flux changes caused by drought and rainfall. Additionally, soil N_2O and nutrient concentration and N_2O fluxes from chambers were used to ascertain (1) whether substantial N_2O production occurred during drought, (2) which types of soil organisms were responsible for N_2O production, and (3) the cause for, and location of, the observed exponential increase in whole-system $[N_2O]$ immediately following the first rain after drought.

Methods

Site description

The B2L in southeastern Arizona included a semiclosed $26\,700\,m^3$ tropical forest (Lin *et al.*, 1998; Walter & Lambrecht, 2004), which contained over 500 plants, comprising about 110 species (Leigh *et al.*, 1999). Soils were constructed from local sources (Leigh *et al.*, 1999; Scott, 1999) and had matured over 12 years. Soils in B2L's tropical forest were slightly alkaline (pH 7–8) and nutrient-rich with total N and C/N ratio averaging $0.2 \pm 0.02\%$ and 8.75 ± 0.35, respectively. Day and night-time temperatures were maintained at 27 °C and 23 °C, respectively, and high-speed fans insured adequate gas mixing (J. L. M. van Haren, unpublished data).

Atmospheric composition was monitored at two canopy heights and at two locations for soil N_2O fluxes and concentrations (Fig. 1). Soil $[N_2O]$ analyses from 33 locations (J. L. M. van Haren, manuscript in preparation) confirmed that the soil chamber locations represented extremes of N_2O production in the B2L tropical forest. Air and soil temperature, pressure, relative humidity, and soil moisture data were collected as 15 min averages (Lin *et al.*, 1998). Soil moisture was measured at 0–30 and 30–60 cm depths in five locations with time domain reflectrometry probes (CS615, Campbell Scientific, Logan, UT, USA) (Fig. 1b). Bulk density and gravimetric soil moisture were measured at two locations to check installed soil moisture probes (Rascher *et al.*, 2004). Soil moisture was recalculated as %WFPS according to Linn & Doran (1984) using bulk density data from this study and Scott (1999), combined with the daytime (08:00–18:00 hours) soil moisture averages. Rain was administered via overhead sprinklers and monitored with in line flow meters (Marino & Odum, 1999). Average annual rainfall was 1500 mm between 1999 and 2003. Drought was induced by withholding rain for a preplanned period.

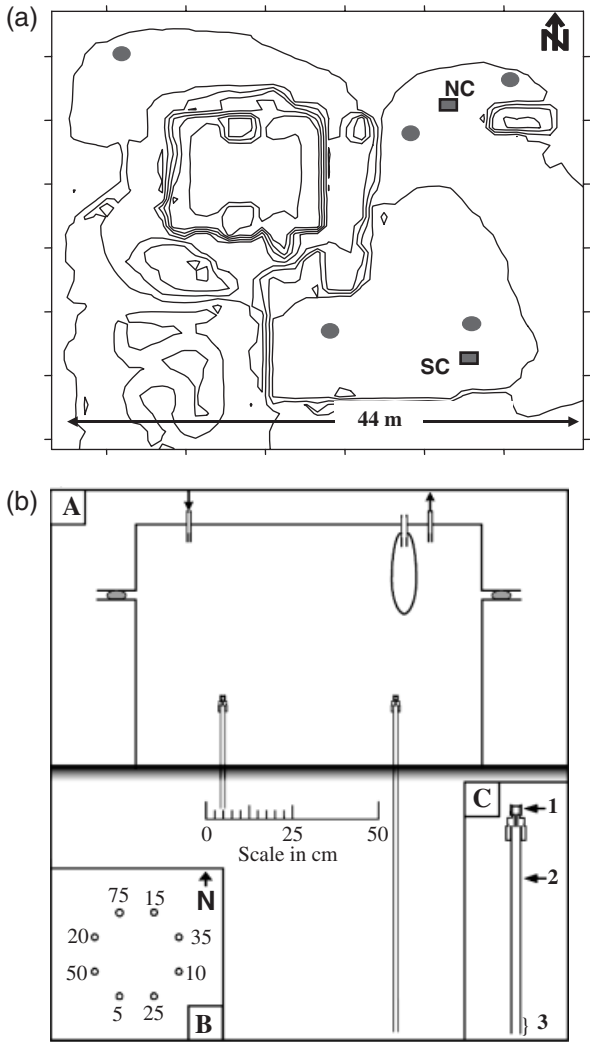

Fig. 1 (a) Rainforest contour (thin lines) map with soil moisture probe and chamber locations and (b) diagram of soil chamber and probe sampling setup. (a) Chamber and probes in cross-section are shown; (b) contains a map outlining probe locations (borders represent the large chamber dimensions); and (C) a detailed drawing of probe design.

Soil nutrient sampling

Soil samples were taken at five locations (Fig. 1a) before and at the end of the 2002 droughts. Samples were stored in a freezer and analyzed at Institute of Physiochemical and Biological problems in Soil Science, Pushchino, Russia. Ammonia ($[NH_4^+]$), nitrite ($[NO_2^-]$), and nitrate concentration ($[NO_3^-]$) were determined by extraction with 0.5 M K_2SO_4 according to Bochkariov & Kudeyarov (1982).

We sampled for dissolved organic carbon (DOC) and total amino acid (TAA) before the drought in April of 2002 and at the end of the October 2002 drought. To measure DOC, we combined soil and distilled water at 1:10 into 125 mL Nalgene® (Cole Palmer, Vernan Hills, IL, USA) bottles, which were shaken for 12 h, centrifuged at 2500 rpm and filtered through a 0.2 mm filter. The solution was freeze-dried and analyzed with a Europa GEO 20/20 (Sercon Ltd, Cheshire, UK). TAA analyses were conducted according to Martens & Loeffelmann (2003).

N_2O analyses

The $[N_2O]$ of the tropical forest atmosphere and soil chambers was measured to $\pm 0.3\%$ SD with an automated gas chromatograph system (AGCS). Air Cadet® pumps (Cole-Palmer) circulated air through a single piece of 3/8″ Dekabon® tubing (Goodrich Sales Inc., Naperville, IL, USA) from six sampling locations to the AGCS and back. Analyses were conducted as described by Weiss (1981). In short, every 5 min sampling loops were flushed for 90 s at 100 mL min^{-1} and then 2 mL of air was let onto a Molesieve 5A column kept at 240 °C for N_2O separation from air. N_2O was analyzed on an electron capture detector (ECD) kept at 340 °C. The instrument also analyzed carbon dioxide, methane, and sulfur hexafluoride on separate sample loops, columns, and detectors. Two standards ($\sim$ 310 and $\sim$ 1200 ppb N_2O, with all gases in ultra-clean air; Scott-Marrin, Riverside, CA, USA) were analyzed every hour. The tropical forest atmosphere was sampled at least once every 40 min (Fig. 2).

We measured soil gas fluxes at two locations in 720 L soil chambers covering 1 m^2 of soil (Fig. 1b; courtesy of W. Reeburgh). Chamber lids were fitted with a Mylar® balloon to facilitate pressure equilibration during

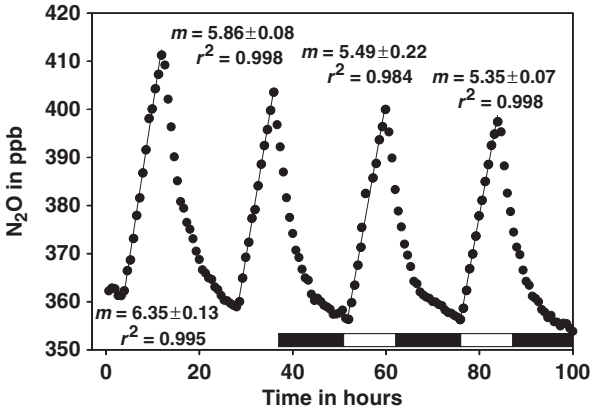

Fig. 2 A few days of nitrous oxide concentration ($[N_2O]$) measured in the rainforest ecosystem (the *x*-axis represents time in hours from an arbitrary zero). The trend lines represent the linear regression of the data when the rainforest is closed. *m* denotes the slope of the line. The black and white bar in the bottom of the graph indicates night and day.

closure. Small fans Gamma 26, (Nidel American Corp., Norwood, MA, USA) were used to mix chamber air without causing advection in the soil. We installed two Swagelock® male connectors (Arizona Valve and Fitting, Phoenix, AZ, USA) in the lid to connect the AGCS to the chamber in a closed loop. Soil chambers were sampled at least five times by the AGCS during closures.

Soil air compositions were measured at eight different depths with 1/2″ stainless-steel soil probes (Fig. 1b). 10 cc B&D® plastic syringes with stopcocks (Cole-Palmer) were used to first remove existing air and draw gas samples one minute later. During the fall of 2002, we analyzed soil air samples on a Shimadzu GC14-A GC (Shimadzu Scientific Instruments, Columbia, MD, USA) fitted with an ECD at 250 °C (McLain & Martens, 2005). A 80/100 HayeSep-Q column, 2 m × 3 mm ID at 45 °C separated CO_2 and N_2O with N_2 as a carrier gas (Thomson *et al.*, 1997). Certified N_2O standards (Praxair Technology, San Ramon, CA, USA) were used for calibration and calibrated with the AGCS gas standards. In April and May of 2003 we analyzed the soil air samples on an Agilent 5890 (Agilent, Palo Alto, CA, USA) with an ECD detector at 280 °C. CO_2 and N_2O were separated with a 3 m × 3 mm Hayesep D® column (Alltech, Deerfield, IL, USA) kept at 80 °C. The AGCS gas standards were used for the soil gas samples and we routinely obtained a standard deviation better than ± 2%. All samples were analyzed within 24 h of collection.

Data analyses

$[CO_2]$ in the tropical forest was maintained at 400 ± 10 ppm during the day and <550 ppm at night. Each day the tropical forest was closed at 07:30 hours and $[N_2O]$ increased during the following 11 h (Fig. 2). Nightly flushing with outside air reduced $[N_2O]$ back to baseline values before 07:30 hours the next day. Daily whole-system N_2O fluxes, reported in $\mu g\, N_2O\text{-}N\, m^{-2}\, h^{-1}$, were calculated from linear regression of daytime leak-corrected $[N_2O]$, air temperature, air pressure and area and volume of the tropical forest. An average leak rate of $1.8 \pm 0.2\%\, h^{-1}$ between the rainforest and the rest of the structure was measured with the AGCS by weekly injection of sulfur hexafluoride (SF_6). Soil fluxes ($\mu g\, N_2O\text{-}N\, m^{-2}\, h^{-1}$) were calculated from air temperature and pressure and ppb per hour change obtained from linear regression of N_2O concentration increase in the chamber. We determined the intrinsic chamber leak rate by sealing a base with a sheet of PVC plastic and then injecting SF_6 into the chamber after closure. The resulting leak rate ($1.0 \pm 0.1\%\, h^{-1}$) was used to correct all soil chamber analyses for exchange with the rainforest atmosphere.

We used SigmaPlot 2000 (SPSS Inc., Chicago, IL, USA) to calculate the N_2O flux deceleration rate during drought by means of an exponential fit to the data. The same program was used to estimate the total N_2O flux during the postdrought pulse by integrating the measured fluxes using a four parameter Lognormal fit.

To compare the soil N_2O flux with the soil nitrogen components, we calculated all nitrogen mineral pools, N_2O flux and $[N_2O]$ profile data in same units ($mg\, N\, m^{-2}$). Average soil $[N_2O]$ from 0–30 to 30–60 cm was combined with soil air temperatures, pressures, bulk density, and soil moisture contents to recalculate soil N_2O in mg N_2O-N m^{-2}.

Results and discussion

After the last predrought rain, soil moisture content (Fig. 3) decreased rapidly in the top 30 cm and at a much slower rate between 30 and 60 cm depth. At the start of all droughts WFPS was between 50% and 60% in the top 30 cm, and between 60% and 70% in 30–60 cm depth. Detailed soil measurements (Rascher *et al.*, 2004) showed that the majority of drying occurred in the top

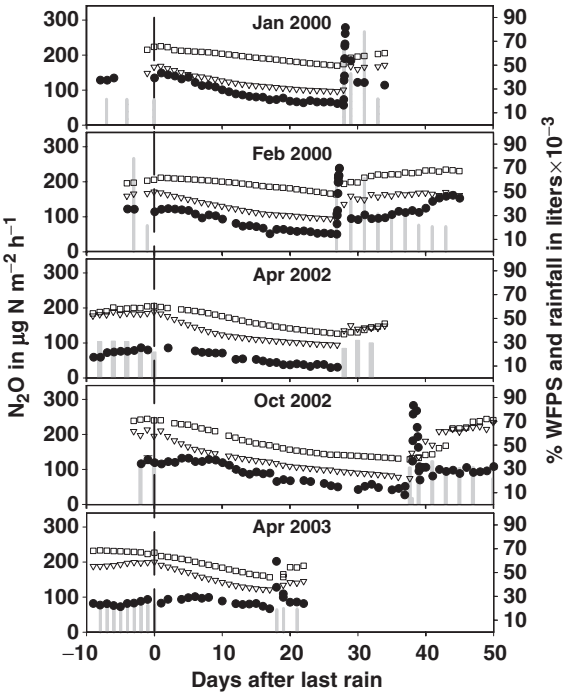

Fig. 3 Daytime averages of soil moisture (% water-filled pore space (WFPS), 0–30 cm (▽) and 30–60 cm (□)), daily whole-system N_2O flux (• , in $\mu g\, N\, m^{-2}\, h^{-1}$), and rainfall amount in liter × 10^{-3} indicated with the gray bars. Last rain day at the start of a drought is taken as day zero.

of the soil column. At 0–5 cm depth, soil moisture content was reduced from 70% to 20% WFPS during the October 2002 drought. However, soil matrix water potentials (ψ_{soil}) never decreased below −2 mPa (Rascher *et al*., 2004). Below 50 cm in the soil column, we found no discernible change in the soil moisture content during this drought.

Soil $[NH_4^+]$ and $[NO_3^-]$ (Table 1) taken from the north chamber (NC) and south chamber (SC) locations and as an average of all five sampling locations did not change significantly during the April 2002 and October 2002 drought. Soil $[NO_2^-]$ remained below the detection limit of our method (0.01 mg kg^{-1} dry soil) for all samples, which implied that nitrite oxidation was more rapid than ammonium oxidation (Smith *et al*., 1997). Minimal nutrient concentration variability with time in the soil suggested that nutrients were cycled rapidly through the soil and that the system was in balance. Soil NH_4^+ and $[NO_3^-]$ dynamics at the soil chamber locations during the droughts agreed well with the $[NO_3^-]$ change rate results (van Haren *et al*., 2001). During the fall of 2000, they measured $[NO_3^-]$ change rate in plastic bags at both locations and found that below the SC soils were net producers of $[NO_3^-]$ and below the NC net

Table 1 (a) Soil $[NH_4^+]$, (b) $[NO_3^-]$ (both in mg kg^{-1} dry soil), (c) [TAA], and (d) [DOC] (both g kg^{-1} dry soil) during the April and October 2002 droughts

	Average		North		South	
Depth (cm)	Start	End	Start	End	Start	End
(a) $[NH_4^+]$			April 2002			
0–5	10.8	6.3	8.9	4.6	20.1	5.5
0–30	8.4 ± 2.2	6.3 ± 0.6	7.0 ± 1.9	7.1 ± 2.2	12.4 ± 7.0	5.3 ± 0.2
30–60	6.4 ± 1.2	6.0 ± 1.0	4.0 ± 1.0	6.3 ± 2.1	5.8 ± 2.9	7.4 ± 2.6
60–90	5.3 ± 1.3	3.9 ± 0.7	3.6	3.4	3.9 ± 0.4	3.9 ± 0.6
			October 2002			
0–5	3.6	4.2	3.5	5.2	4.0	4.4
0–30	3.6 ± 0.5	3.4 ± 0.3	3.8 ± 0.3	3.9 ± 1.1	3.0 ± 0.7	2.8 ± 1.1
30–60	2.8 ± 0.4	2.7 ± 0.2	3.0 ± 0.2	3.4 ± 0.5	2.4 ± 0.1	2.3 ± 0.2
60–90	2.1 ± 0.3	1.8 ± 0.1	1.7 ± 0.5	1.7 ± 0.5	2.4 ± 0.5	1.6
(b) $[NO_3^-]$			April 2002			
0–5	23.0	20.7	37.5	20.5	48.4	24.6
0–30	21.5 ± 10.6	21.4 ± 6.4	17.3 ± 17.7	14.8 ± 5.4	43.9 ± 6.1	36.0 ± 10.8
30–60	34.9 ± 33.3	33.1 ± 28.7	11.9 ± 7.9	9.7 ± 6.3	60.2 ± 21.8	69.8 ± 22.4
60–90	34.1 ± 34.0	33.6 ± 31.2	16.5	10.2	83.1 ± 0.4	91.8 ± 27.2
			October 2002			
0–5	56.3	32.2	33.3	18.1	80.1	63.6
0–30	41.3 ± 22.3	28.7 ± 15.0	23.1 ± 10.3	15.8 ± 2.1	76.3 ± 4.4	65.7 ± 9.5
30–60	38.7 ± 24.0	32.0 ± 22.0	13.7 ± 1.6	15.3 ± 0.2	90.8 ± 17.7	86.9 ± 13.2
60–90	49.8 ± 30.6	40.6 ± 32.0	5.3 ± 2.1	6.3 ± 4.2	99.5 ± 44.0	120.6
(c) [TAA]			April 2002			
0–5	5.1 ± 1.0	N/A	5.3 ± 1.0	N/A	5.8 ± 0.5	N/A
10–15	2.8 ± 0.1	N/A	3.1 ± 0.9	N/A	2.9 ± 0.7	N/A
20–25	2.8 ± 0.3	N/A	2.8 ± 0.3	N/A	2.3 ± 1.1	N/A
			October 2002			
0–5	N/A	7.1 ± 1.3	N/A	6.0 ± 2.1	N/A	8.1 ± 2.7
10–15	N/A	5.1 ± 0.7	N/A	4.1 ± 1.0	N/A	6.3 ± 1.1
20–25	N/A	4.1 ± 0.3	N/A	4.1 ± 1.2	N/A	4.7 ± 1.4
(c) [DOC]			April 2002			
0–5	0.23 ± 0.03	N/A	0.26	N/A	0.20	N/A
10–15	0.25 ± 0.01	N/A	0.26	N/A	0.23	N/A
20–25	0.26 ± 0.05	N/A	0.22	N/A	0.24	N/A
			October 2002			
0–5	N/A	0.26 ± 0.05	N/A	0.22	N/A	0.34
10–15	N/A	0.20 ± 0.04	N/A	0.24	N/A	0.16
20–25	N/A	0.24 ± 0.06	N/A	0.18	N/A	0.26

TAA, total amino acid; DOC, dissolved organic carbon.

consumers of $[NO_3^-]$. Thus, suggesting that nitrification was more dominant below SC and denitrification was more dominant below NC.

Soil $[NH_4^+]$ (Table 1a) ranged from 2.8 to 20 mg kg^{-1} during the April 2002 drought and from 1.0 to 5.2 mg kg^{-1} during the fall 2002 drought. Soil $[NH_4^+]$ at NC and SC locations was not significantly different and decreased with soil depth from 4.2 ± 0.8 to 1.8 ± 0.4 mg kg^{-1}. The low $[NH_4^+]$ implied that nitrification was relatively faster than ammonification. Soil $[NO_3^-]$ (Table 1b) ranged from 3.3 to 130.7 and 0.3 to 120.6 mg kg^{-1} during the April and October 2002 droughts, respectively. Soil $[NO_3^-]$ below NC decreased with depth from 18.1 to 3.3 mg kg^{-1}, whereas, below SC $[NO_3^-]$ increased from 63.6 to 120.6 mg kg^{-1} with depth. $[NO_3^-]$ buildup in soils below SC was consistent with nitrification being dominant at this location (van Haren *et al.*, 2001).

Soil [TAA] ranged from 2.1 to 6.31 g kg^{-1} during the wet season to 3.4–8.7 g kg^{-1} during the dry season. A significant difference between wet and dry sampling was only observed from the SC (Table 1c). The change in [TAA] was mainly caused by an increase in Valine. [DOC] changed significantly from wet to dry conditions only in the top 0–5 cm at the SC location (0.22–0.34 g kg^{-1}). No difference was observed in the NC [DOC] and whole-system average [DOC].

N_2O fluxes measured during five drought experiments in the tropical forest at B2L were comparable with field measurements made during annual wet and dry cycles in tropical rainforests (Table 2). The analyses of whole-system and soil N_2O fluxes as well as soil profiles during the drought and following rainfall allowed for detailed analyses of the mechanisms causing the observed flux changes. In our discussion of the N_2O results, we separated our experiment into four distinct phases: (1) start of drought, (2) drought, (3) N_2O pulse after wetting, and (4) recovery after drought.

Start of drought

Whole-system, wet-soil N_2O-N fluxes (Fig. 3) were larger in autumn/winter (121.0 ± 9.1 μg N m^{-2} h^{-1} before droughts starting in January, February, and October) than in spring (83.2 ± 5.5 μg N m^{-2} h^{-1} before April droughts). Likewise, soil chamber wet-soil N_2O-N fluxes (Fig. 4) were larger in autumn (632.8 (NC) and 75.2 (SC) μg N m^{-2} h^{-1} before the October 2002 drought) than in spring (123.4 (NC) and 21.1 (SC) μg N m^{-2} h^{-1} before the April 2003 drought). With the lower flux, we expected a lower $[N_2O]$ below NC in April 2003, but it was five times higher than the October 2002 maximum (Figs 5b and d). Soil $[N_2O]$ below SC was also significantly higher at the start of the April 2003 drought (Figs 5a and c). The discrepancy between the N_2O fluxes and profile concentrations implied that surface N_2O production must have been lower during spring droughts. Surface (0–5 cm) $[NH_4^+]$ concentrations were consistently higher in the April 2002 than October 2002 drought (Table 1), suggesting that mineralization rates were increased relatively to nitrification rates during this drought, or that nitrification rates were reduced. Seasonal changes in nutrient uptake, root

Table 2 Comparison of wet and dry N_2O flux measurements within B_2C and from tropical rain forests around the world

Location	Wet (μg N m^{-2} h^{-1})	Dry (μg N m^{-2} h^{-1})
B2C October 2002 whole system	131 ± 5.2	36.2 ± 9.2
B2C October 2002 north soil chamber	708.1 ± 108	19.1 ± 5.7
B2C October 2002 south soil chamber	71.5 ± 5.1	5.0 ± 0.7
Lake Eacham, Australia*	103.2	0.9
Bellenden Ker, Australia†	501.6	23.9
Pin Gin Hill, Australia†	570.8	5.4
La Selva, Costa Rica‡	205.7	6.6
East Amazon, Brazil§	93.8	4.1
Fazenda Vitoria, Para Brazil¶	180	12.2
Mayombo, Kongo‖	545.8	2.6
WSI Forest, Chamela, Mexico**	120	2.4

*Breuer *et al*. (2000).
†Kiese & Butterbach-bahl, (2002).
‡Keller & Reiners (1994).
§Verchot *et al*. (1999).
¶Cattannio *et al*. (2002).
‖Serca *et al*. (1994).
**Davidson *et al*. (1993).

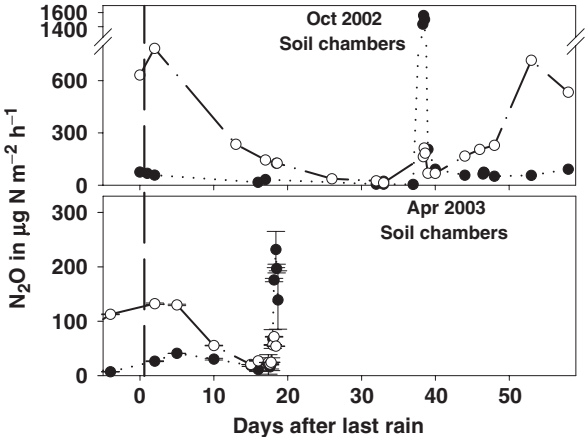

Fig. 4 Soil chamber nitrous oxide (N_2O) fluxes during the October 2002 and April 2003 droughts. The open circles denote north chamber, whereas the solid circles represent south chamber. The error bars on the symbols represent the 95% confidence interval of the slope of the [N_2O] increase in the chamber with time.

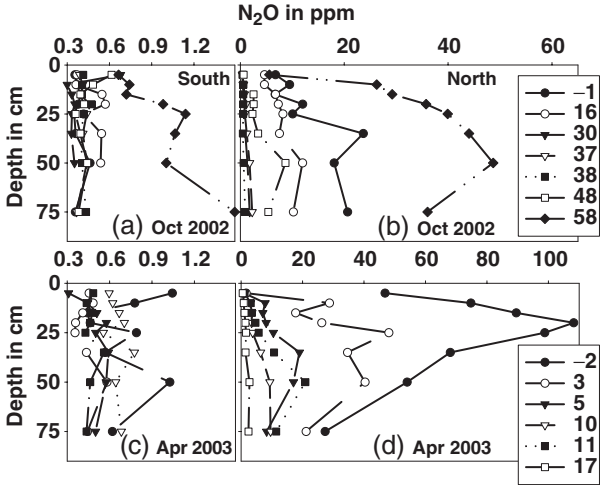

Fig. 5 Soil nitrous oxide concentration ([N_2O]) profiles for south chamber (SC) (a) and north chamber (NC) (b) during the October 2002 drought and SC (c) and NC (d) during the April 2003 drought. The legend numbers denote days from the last rain. Note the different scales in (a) and (c) vs. (b) and (d).

growth and mortality, and litter deposition by plants could have contributed to changes in N_2O production.

During all droughts, soil moisture content decreased from day 1, but whole-system N_2O fluxes (Fig. 3) increased $\sim$ 10% during the first days without rain. At NC N_2O fluxes (Fig. 4) increased $\sim$ 40% (October 2002) and $\sim$ 8% (April 2003). At SC N_2O flux decreased from day 1 of the October 2002 drought and increased $\sim$ 30% during the April 2003 drought. The whole-system increase could have been caused by increased diffusion caused by drying of soil pore-space or increased production at the soil surface. During wet conditions, the observed 70% WFPS in the top of the soil could have restricted gas diffusion from deeper soil layers to the surface. A rapid reduction in moisture content in the top few centimeters of soil through evaporation or consumption of water by the plants could have opened soil pore space for gas diffusion. The chamber data were in general consistent with increased diffusion except for the relatively low increase in flux at NC during the April 2003 drought.

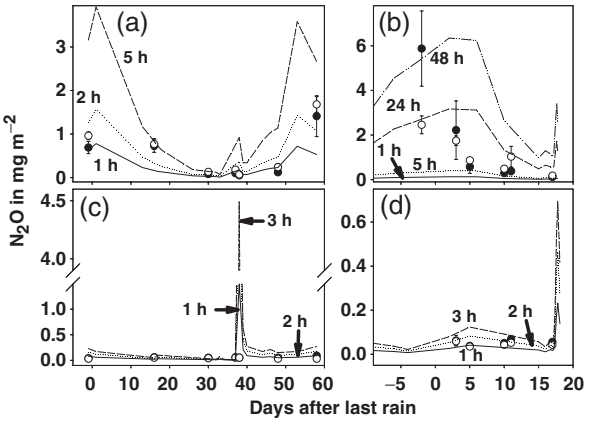

Fig. 6 Soil nitrous oxide concentration ([N_2O]) and N_2O flux comparison for north chamber (NC) (a) and south chamber (SC) (c) during the October 2002 and NC (b) and SC (d) during the April 2003 drought. The lines represent N_2O flux for the duration indicated in the figures. Open circles denote the [N_2O] at 0–30 cm and filled circles indicate the [N_2O] at 30–60 cm depth.

Drought

In concurrence with the soil moisture data, whole-system and chamber N_2O fluxes decreased with time during the droughts (Fig. 3). A similar trend was observed in the soil [N_2O] (Fig. 5). Maximum [N_2O] in soil profiles at NC were located a greater depth with progression of drought, suggesting that N_2O production decreased more rapidly in surface soils. We compared the soil fluxes and profiles (Fig. 6) to determine whether N_2O production was likely to persist during the drought. Soil [N_2O] at NC (Fig. 6a) accounted for less than 2 h of flux, during the last drought days. Early during the April 2003 drought, the soil [N_2O] at NC (Fig. 6b) could have sustained the observed flux for up to 48 h. However, at the end of the drought soil [N_2O] could only have supplied 5 h of surface flux. At all times during the experiments, soil [N_2O] below SC (Figs 6c and d) accounted for no more

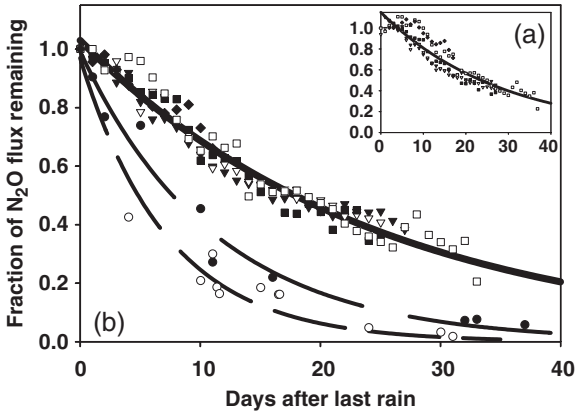

Fig. 7 Exponential fit to whole-system nitrous oxide (N_2O) flux reduction during drought calculated from (a) first day of drought and (b) maximum N_2O flux (January 2000 (▼), February 2000 (▽), April 2002 (■), October 2002 (□), and April 2003 (◆), SC (•), and NC (○). The chamber data from the October 2002 and April 2003 droughts were pooled for the exponential fit.

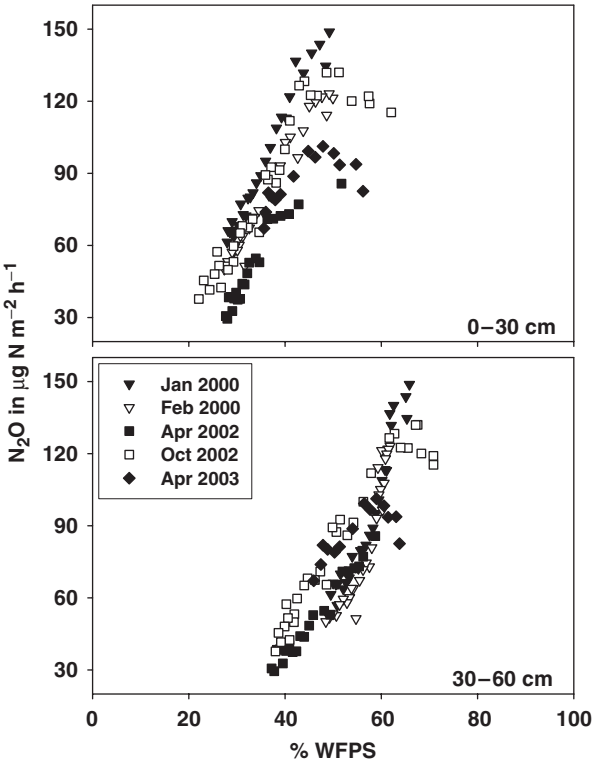

Fig. 8 N_2O flux correlation with % water-filled pore space (WFPS) in 0–30 cm (a) and 30–60 cm (b) soil depth. The symbols for the different droughts are the same as in Fig. 7.

than 3 h of surface flux. Thus N_2O production must have persisted throughout the droughts.

Daily measurement of whole-system fluxes (Fig. 3) and control over rainfall enabled us to model the N_2O flux reduction during drought. Exponential decay gave the best fit to the data and was used to calculate the flux reduction rate (Fig. 7). When the last rain day was used as drought day 0 the dataset appeared quite noisy (inset Fig. 7), because of the increased N_2O flux during the first days of some droughts. We decided to recalculate the rate with the N_2O flux maximum as starting point. The calculated deceleration of the whole-system N_2O flux was $3.5 \pm 0.2\%\,day^{-1}$ and $4.0 \pm 1\%\,day^{-1}$ with the first drought day and N_2O flux maximum as starting day, respectively. To calculate the decay rate for each soil chamber, we pooled the October 2002 and April 2003 data because of the limited number of observations during drought (Fig. 7). The reduction rates, calculated from the maximum N_2O flux only, were $8.9 \pm 0.8\%\,day^{-1}$ and $13.7 \pm 1.1\%\,day^{-1}$ for the SC and NC, respectively. Both soil chamber reduction rates were much higher than the whole-system rate. We suspect that N_2O fluxes in area of the rainforest with dense ground coverage were less affected by drought.

We observed a strong and reproducible correlation between the whole-system N_2O flux and the average WFPS at both depths (Fig. 8). WFPS from 0 to 30 cm depth and N_2O are linearly correlated ($r^2 > 0.92$) below 42–50% WFPS. This correlation only existed during wet periods after the rewetted system had stabilized, post-N_2O pulse (not shown in Fig. 8). Our results confirm the influence of soil moisture on N_2O-N flux reduction during drought (Linn & Doran, 1984).

N_2O pulse after wetting

Within hours following the drought-ending rain, we measured an exponential increase in the rainforest atmosphere [N_2O] (Fig. 3). This pulse may have been caused by: (1) outgassing because of the physical displacement of soil atmosphere by water, (2) change in nutrient concentrations in the soil during drought, and (3) a rapid increase of bacterial activity during the drought-terminating rain. Clough *et al*. (2000) measured N_2O production from soils enclosed in a glove box after wetting of the soil and attributed the increase in N_2O after water addition to physical displacement of soil air. The B2L tropical forest has a similar configuration to the extent that the rainforest contains a soil basin within an enclosed atmosphere.

We combined total rainfall with soil [N_2O] measured just before the rain from the October 2002 drought, to determine whether physical displacement caused the N_2O pulse. The quantity of water supplied (35 thousand liters) on October 28, 2002 replaced soil air in less than 10 cm of the soil column. Eight hours before

the rain, average soil [N_2O] below 10 cm was 420 (SC) and 611 ppb (NC) (Figs 5a and b, day 37). These values were only slightly elevated from atmospheric [N_2O], which was 410 ppb during sampling. If 10 cm of air-filled pore space ($\sim$ 45%) was degassed to produce the observed 61 ppb increase in atmospheric N_2O on October 28, 2002, soil [N_2O] had to be $\sim$ 20 000 ppb in the top 10 cm of all rainforest soil. Only below NC, at the start of this drought (Fig. 5b, day -1), did we observe similar concentrations. Even if a 1 m column of soil air was replaced, a soil [N_2O] of $\sim$ 2000 ppb would have been required to explain the measured emissions. Because neither soil profile contained [N_2O] this high, we conclude that displacement of N_2O rich soil air by water could not have caused the observed N_2O pulse.

Davidson *et al*. (1993) found that irrigation at the end of the drought season in Jalisco, Mexico resulted in a N_2O pulse 100 times larger than one caused by irrigation during the wet season. Kiese & Butterbach-Bahl (2002) measured a larger N_2O pulse in a control plot during an uncontrolled rain event after planned irrigations in a tropical forest in Queensland, Australia. In the same area, Butterbach-bahl *et al*. (2004) found that N_2O fluxes rapidly fluctuated during a 2-week period of small rain events, suggesting that length of drought did not affect pulse size. The latter measurements were conducted under much drier conditions, therefore small rainfall events would probably only wet the surface and be subject to because of rapid drying. In B2L, the maximum whole-system N_2O flux during the pulse was 269.5 ± 8.8, 237.8 ± 0.7, 362.2 ± 6.2, and $202.0 \pm 39\ \mu g\,N\,m^{-2}\,h^{-1}$, for the January 2000, February 2000, October 2002, and April 2003 droughts, respectively. Because of instrument failure, the April 2002 rain event was not recorded. The postdrought pulse size (Table 3) in the enclosed rainforest was correlated with the length of the drought ($r^2 = 0.89$). The correlation was further improved when we defined drought length from the N_2O flux maximum ($r^2 = 0.93$). There was only a weak correlation with the amount of rainfall ($r^2 = 0.10$), which suggested that N_2O production was located at the litter–surface interface. Lack of increase in [N_2O] in soil profiles measured during the pulse (Figs 5a and b, day 38) further indicated that the observed N_2O production must have occurred at the soil–litter interface.

We propose that microbial conversion of readily available nitrogen to N_2O caused the N_2O pulse. Davidson *et al*. (1993), Breuer *et al*. (2000), Garcia-Montiel (2003b) observed a buildup of NH_4^+ and NO_2^- during drought, which was rapidly converted to NO_3^- and N_2O after rewetting. We observed no buildup of NO_2^-, or $[NH_4^+]$ in the B2L soils during our drought experiments. At SC the change from wet to dry conditions was accompanied by a small increase in TAA concentration, mainly because of an increase in Valine, and a 70% increase in DOC. At NC neither component increased significantly. We did not take soil samples during the pulse and cannot confirm whether soil microbes caused a rapid increase in solutes immediately following rain as proposed by Kieft *et al*. (1987). However, our finding that N_2O production was predominantly in the top of the soil, where soils dried the most, would be consistent with this hypothesis.

Using the year 2001 and the wet periods during 2000 and 2002 for the annual tropical forest flux, we calculated that the pulse corresponds to $1.3 \pm 0.4\%$ of the annual N_2O flux. This value is similar to pulses observed in tropical forest sites (Davidson *et al*., 1993; Garcia-Montiel *et al*., 2003b). Using data from all except April 2003 droughts, we calculated that the pulse compensated for $27.8 \pm 4.8\%$ and $23.2 \pm 1.1\%$ of the flux reduction caused by drought when calculated from the day following the last rain and the maximum flux day, respectively (Table 3). Data from the April 2003 drought was omitted because of its short duration (17 days). The strongest pulse signal was measured at SC, where the N_2O flux increased from 5.1 and 11.1 to 1558 and $231.8\ \mu g\,N\,m^{-2}\,h^{-1}$ during the October 2002 and April 2003 droughts, respectively. We found a much smaller increase (from 24.6 and 21.2 to 212.7 and $71.4\ \mu g\,N\,m^{-2}\,h^{-1}$, respectively) at NC. The SC soils produced more N_2O in 1 day than the total N_2O flux reduction during drought (Table 3). At NC the pulse compensated for only 0.5–5% of drought N_2O flux reduction caused by drought.

Table 3 N_2O flux totals during drought and following pulse

	Whole rainforest					North chamber		South chamber	
	Jan '00	Feb '00	Apr '02	Oct '02	Apr '03	Oct '02	Apr '03	Oct '02	Apr '03
Length of drought (days)	27	26	27	37	17	37	17	37	17
Days after max flux (days)	26	24	25	33	11	35	15	37	12
Drought reduction ($mg\,N\,m^{-2}$.)	28.6	26.5	15.2	35.9	1.3	362.8	34.5	N/A	N/A
Maximum drought reduction ($mg\,N\,m^{-2}$)	38.2	27.3	19.2	44.8	4.5	640	38.3	40.8	4.0
Pulse size ($mg\,N\,m^{-2}$)	8.9	5.9	N/A	10.7	2.7	3.7	1.5	45.4	7.1

As the pulse was much larger at SC, where nitrification dominated (van Haren *et al.*, 2001), we infer that nitrification was the mainly responsible for N_2O production during the pulse. An immediate response to wetting by nitrification has also been observed in laboratory incubation experiments (Azam *et al.*, 2002).

Recovery

In the first days after the pulse, daily N_2O fluxes were approximately 10% lower than the N_2O flux before drought. Then, slowly, N_2O fluxes increased to either predrought rates or higher. During the February 2000 and October 2002 droughts, the N_2O flux reached 157.4 ± 3.3 and $103.3 \pm 3.4\,\mu g\,N\,m^{-2}\,h^{-1}$ after 3 weeks of heavy rainfall. Only during the October 2002 drought, we measured the recovery period after drought and pulse in the soil chambers. One week after the pulse, the N_2O flux was 228.3 and $63.5\,\mu g\,N\,m^{-2}\,h^{-1}$ for NC and SC, respectively. After 3 weeks of intensive rains (150 mm total), the N_2O fluxes had returned to predrought rates (716.6 (NC) and 91.5 (SC) $\mu g\,N\,m^{-2}\,h^{-1}$). At that time soil air [N_2O] below 30 cm in the SC profile increased to more than 1 ppm, while [N_2O] below 30 cm in the NC increased to ~ 43 ppm. A slow N_2O increase deep in the soil (>1 m) was also observed in eastern Amazonia soils (Verchot *et al.*, 1999). The slow flux and soil [N_2O] increase suggested that denitrification did not respond quickly to water addition, probably because of low available C and slow reestablishment of anoxic conditions in the soil. Experiments in Costa Rica (Nobre *et al.*, 2001) and Brazil (Garcia-Montiel *et al.*, 2003a) showed that only when glucose was added to the soil would water and NO_3^- addition cause N_2O fluxes and soil [N_2O] increase at depth within 24 h, i.e. at rates similar to the pulse we observed after drought in B2L. Therefore, bacteria at depth required a readily available source of carbon to rapidly produce N_2O in response to water and [NO_3^-] addition.

Conclusions

Soil moisture changes caused by rainfall had a profound effect on the B2L tropical forest N_2O fluxes. Initial soil surface drying during the first days of drought presumably increased gas transport out of the soil, which increased N_2O fluxes. During drought, we measured an exponential reduction in N_2O flux, which was highly correlated with soil moisture content. Soil wetting after drought caused a rapid response of soil organisms, which lead to a transient pulse of N_2O. The combination of soil fluxes and profiles allowed us to locate the pulse immediately at the soil–litter interface, which was subject to the largest water potential change and thus most likely to have caused microbes to release available N in response to rainfall. Based on the soil chamber locations we concluded that N_2O was mainly produced through nitrification during the pulse and that during wet periods denitrification was the dominant source of N_2O. N_2O flux patterns from the two soil–gas sampling chambers and the whole system were consistent. However, the magnitude of response to changes in soil moisture was highly variable.

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