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Throughfall exclusion and leaf litter addition drive higher rates of soil nitrous oxide emissions from a lowland wet tropical forest

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Abstract

Tropical forests are a significant global source of the greenhouse gas nitrous oxide (N_2O). Predicted environmental changes for this biome highlight the need to understand how simultaneous changes in precipitation and labile carbon (C) availability may affect soil N_2O production. We conducted a small-scale throughfall and leaf litter manipulation in a lowland tropical forest in southwestern Costa Rica to test how potential changes in both water and litter derived labile C inputs to soils may alter N_2O emissions. Experimentally reducing throughfall in this wet tropical forest significantly increased soil emissions of N_2O, and our data suggest that at least part of this response was driven by an increase in the concentration of dissolved organic carbon [DOC] inputs delivered from litter to soil under the drier conditions. Furthermore, [DOC] was significantly correlated with N_2O emissions across both throughfall and litterfall manipulation plots, despite the fact that native NO_3^- pools in this site were generally small. Our results highlight the importance of understanding not only the potential direct effects of changing precipitation on soil biogeochemistry, but also the indirect effects resulting from interactions between the hydrologic, C and N cycles. Finally, over all sampling events we observed lower mean N_2O emissions (<1 ng N_2O-N cm^{-2} h^{-1}) than reported for many other lowland tropical forests, perhaps reflecting a more general pattern of increasing relative N constraints to biological activity as one moves from drier to wetter portions of the lowland tropical forest biome.

Keywords: climate change, Costa Rica, experimental drought, litter manipulation, nitrogen cycle, Nitrous oxide, Osa Peninsula, precipitation manipulation, tropical rain forest

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Introduction

Human activities have greatly altered the global water, nitrogen (N) and carbon (C) cycles, and additional future changes are virtually certain (e.g., Hungate *et al.*, 1997a, 2003; Schlesinger & Lichter, 2001; Trenberth *et al.*, 2007; Galloway *et al.*, 2008). In turn, human-driven alterations of water, N, and C availability, either alone or in combination, are likely to affect soil emissions of nitrous oxide (N_2O), a globally important greenhouse gas with a radiative forcing potential nearly 300 times greater than that of CO_2 (Forster *et al.*, 2007), largely responsible for the destruction of nonpolar stratospheric ozone (Crutzen, 1970; Ravishankara *et al.*, 2009). Global N_2O budgets show that soils under natural vegetation, particularly in tropical latitudes, emit nearly as much N_2O as all anthropogenic sources combined (Denman *et al.*, 2007). These observations highlight the need to resolve both rates of and controls over soil N_2O fluxes in natural ecosystems, especially in tropical forests.

In soils, N_2O is a chemical by-product of nitrification and an obligatory intermediate by-product of denitrification, with the latter typically providing the dominant source – especially in wet soils (Davidson *et al.*, 1986; Veldkamp *et al.*, 1998; Wrage *et al.*, 2005). Denitrification, N_2O emissions are primarily controlled by the availability of oxygen (O_2), nitrate (NO_3^-), and labile C (Robertson, 1989; also see Nömmik, 1956). Microbial denitrification requires anoxic conditions, thus water-filled pore space (WFPS) – which strongly regulates O_2 diffusion rates in soils –can serve as a robust, first-order predictor of denitrification rates (e.g., Davidson, 1991; Keller & Reiners, 1994; Bouwman, 1998; Davidson *et al.*, 2000; Werner *et al.*, 2007). When anoxic soil conditions do exist, NO_3^- availability appears to exert primary control over N_2O fluxes via denitrification, especially in N-poor systems (Robertson & Tiedje, 1988; Matson & Vitousek, 1990; Parsons *et al.*, 1993; Phillips *et al.*, 2001; Weitz *et al.*, 2001; Barnard *et al.*, 2005). However, denitrification is also a heterotrophic process that requires

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electron donors in the form of reduced organic C molecules. Thus, while fewer data exist to evaluate the importance of labile C as a control over N_2O emissions (Nobre *et al.*, 2001; Garcia-Montiel *et al.*, 2003; Tiemann & Billings, 2008; also see Taylor & Townsend, 2010), the potential for C limitation to occur clearly exists, especially in anoxic systems where N is relatively abundant.

While generalizations about nutrient cycling and limitation in the tropics as a whole should be approached with caution (e.g. Townsend *et al.*, 2008; Hedin *et al.*, 2009), multiple lines of evidence suggest that many lowland tropical rain forests appear to cycle N in relative excess (e.g., Vitousek, 1984; Martinelli *et al.*, 1999). The tendency of such forests to have relatively large soil pools of NO_3^-, combined with warm, often wet conditions, results in some of the largest N_2O emissions from any unmanaged ecosystem (Matson & Vitousek, 1987; Vitousek & Matson, 1988; Davidson *et al.*, 2007). As such, understanding the controls over tropical forest N_2O fluxes is particularly important to predicting future atmospheric N_2O concentrations. Making such predictions, however, is challenging because all of the major controls over soil emissions – including climate, N inputs and C availability – are likely to change markedly in the tropics over the next century (Hungate *et al.*, 1997a; Hall & Matson, 1999; Matson *et al.*, 1999; Schlesinger & Lichter, 2001; Malhi *et al.*, 2009).

For example, climate change models predict changes in precipitation over tropical latitudes – with most models indicating decreases in precipitation for the Amazon Basin, Caribbean, and Central America (Cox *et al.*, 2004; Neelin *et al.*, 2006; Malhi *et al.*, 2009). Although our understanding of how changes in precipitation may affect ecosystem processes in lowland tropical forests remains limited, changes in rainfall may not only alter the prevalence of low O_2 conditions that favor denitrification, but also drive shifts in both C and N availability in soils through rainfall's effects on processes such as decomposition, net primary production (NPP), biological N fixation, and litter production (Wieder *et al.*, 2009;Townsend *et al.* in press). Accordingly, one might expect reduced rainfall in tropical forests would reduce N_2O emissions by promoting relative increases in soil $[O_2]$, as observed following an experimental drought in the eastern Amazon Basin (Davidson *et al.*, 2004); although declines in N_2O emissions with drying are not consistently presented in the literature (Cattanio *et al.*, 2002; Vasconcelos *et al.*, 2004; Holtgrieve *et al.*, 2006). However, the eastern Amazon lies at the dry end of the tropical rain forest precipitation spectrum (Schuur, 2003), and given the links between water, O_2 availability and N_2O production, one might predict biogeochemical processes in wetter portions of the biome would respond much differently to a reduction in rainfall (Cleveland *et al.*, 2010).

Beyond climate, understanding how changes in soil C availability affect N_2O emissions may be important to predicting how tropical forests will respond to other drivers of environmental change. Increasing atmospheric carbon dioxide concentrations $[CO_2]$, for example, may enhance labile C availability in soils via increases in root exudates, root mortality, and/or litterfall inputs (Hungate *et al.*, 1997a; Phillips *et al.*, 2001; Schlesinger & Lichter, 2001). To date, direct experimental CO_2 manipulations have shown little effect on soil N_2O emissions in some N-limited temperate ecosystems (Hungate *et al.*, 1997b; Ambus & Robertson, 1999; Billings *et al.*, 2002; Mosier, 2002; Welzmiller *et al.*, 2008), but similar data from experiments conducted in tropical forests are largely absent from the literature (Vasconcelos *et al.*, 2004). Taken as a whole, the importance of organic C availability in regulating N_2O emissions from tropical forests remains poorly resolved, and results of direct tests of C availability range from no effect on N_2O emissions (Parsons *et al.*, 1993; Vasconcelos *et al.*, 2004) to strongly positive effects (Nobre *et al.*, 2001; Garcia-Montiel *et al.*, 2003). Increasing C availability increases rates of denitrification either directly, by stimulating C-limited denitrifiers, or indirectly, by creating anoxic microsites favorable to denitrification through heterotrophic consumption of O_2.

Here, we used experimental manipulations of both throughfall and litterfall to surface soils to explore how precipitation, soil O_2 availability, and labile C substrate availability may interact to control soil N_2O emissions from a wet, lowland tropical forest in southwest Costa Rica. We hypothesized that seasonal variability in precipitation would concurrently drive natural changes in soil redox conditions so that maximum N_2O efflux would coincide with periods of heavy precipitation (and hence lower soil O_2 concentrations). As such, we also hypothesized that experimentally reducing precipitation (throughfall) would drive declines in N_2O emissions via increased soil aeration. Finally, we hypothesized that increased litterfall would stimulate N_2O production by increasing delivery of dissolved organic matter (DOM) – a source of reduced, C-rich organic matter to soil.

Materials and methods

Study site

The experiment was conducted in a lowland tropical rain forest on the Osa Peninsula in the Golfo Dulce Forest Reserve (8°43′N, 83°37′W), southwest Costa Rica. Soils at the site are classified as Ultisols (Berrange & Thorpe, 1988), soil texture is clay (i.e., >75% clay content; Cleveland *et al.*, 2006), and

Table 1 Soil physical and chemical properties from control plots

Soil texture (% sand/silt/clay)*	5.0/18.8/76.2
bulk density ($g\,cm^{-3}$)	0.58 ± 0.03
pH†	5.2 ± 0.06
C (%)	5.35 ± 0.27
N (%)	0.46 ± 0.01
$d^{15}N$ (‰)	4.04 ± 0.21
NH_4^+ ($mg\,N\,kg^{-1}$)	10.27 ± 1.81
NO_3^- ($mg\,N\,kg^{-1}$)	3.74 ± 0.75
Total P ($mg\,P\,kg^{-1}$)‡	667.9 ± 20.6
$NaHCO_3^-$ extractable P ($mg\,P\,kg^{-1}$)	10.93 ± 0.50

Values are mean $\pm$ 1 SE from surface soils (0–10 cm).
*From Cleveland *et al.* k (2006).
†Measured in DI, mean of all samples reported by Nemergut *et al.* (2010).
‡Total P from hot H_2SO_4 and H_2O_2 digest (S. R. Weintraub *et al.* unpublished results).

surface soil (0–10 cm) bulk density is $0.58\,g\,cm^{-3}$ (see Table 1 for more detailed soil physical and chemical characteristics). Rainfall averages approximately $5000\,mm\,yr^{-1}$ and average annual soil temperature is ~25 °C. Between December and April (the dry season), litterfall and standing litter mass reach annual maxima and rainfall is typically $<100\,mm\,month^{-1}$ (Cleveland & Townsend, 2006; Fig. 1).

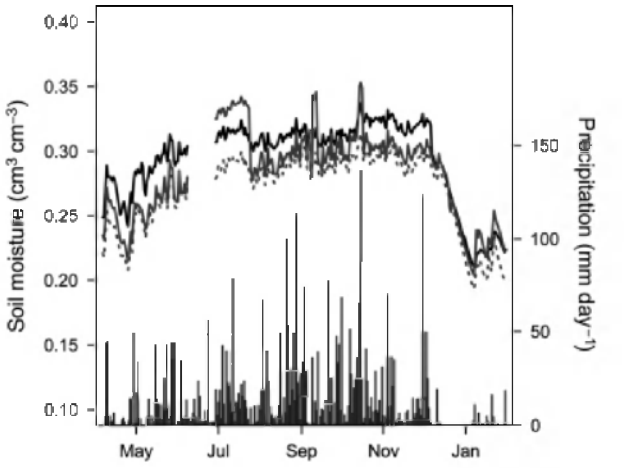


Fig. 1 Mean daily volumetric soil moisture ($cm\,cm^{-3}$, calculated from hourly measurements) from throughfall manipulation and total daily precipitation ($mm\,day^{-1}$) recorded over the experiment. Mean soil moisture data from control plots (solid line) and throughfall manipulation (solid grey line; −25%, dashed grey line; −50%), are not significantly different from each other ($P > 0.1$). We did observe a significant seasonal effect on soil moisture ($F = 52.4$, $P < 0.0001$), with lower soil moisture at the beginning (April–May) and end (January) of the rainy season. We observed no significant temporal variation in soil moisture from June to December ($P > 0.4$), despite a nearly sixfold variation in monthly precipitation over this time period. For clarity, soil moisture error estimates are not shown here.

Experimental design

We constructed and deployed a set of replicated throughfall exclosures to manipulate precipitation and impose an experimental drought throughout the 2008 rainy season. For each exclosure, 5 cm diameter polyvinylchloride (PVC) pipes were cut in half lengthwise and mounted at either 5 or 15 cm intervals (to simulate −50% or −25% reductions in total throughfall) on a 2.4 m × 2.4 m aluminum frame ~1 m above the soil surface. The PVC pipes acted as partial rain sheds, preventing the experimental plots from receiving full incoming throughfall but also allowing ambient light to penetrate to the forest floor and air to circulate freely. Twenty randomly assigned plots received either −50% or −25% throughfall treatments ($n = 10$ per treatment). Control plots from the litter manipulation also served as experimental controls for the throughfall manipulation (also see Wieder *et al.*, 2009).

The throughfall exclosures included a 1 cm wire mesh immediately beneath the PVC gutters that excluded most natural litterfall from reaching the forest floor. Therefore, at the beginning of the experiment all litter under throughfall exclosures was collected, homogenized, weighed, and equally distributed ($750\,g\,litter\,m^{-2}$) to each plot (to match standing litter mass on control plots). Subsequently, litter from an adjacent set of parallel 2.4 m × 2.4 m plots was weighed, divided into 20 equal portions (by mass) and placed under each of the exclosures at monthly intervals to normalize litter inputs. This strategy had two purposes: first, to minimize experimental artifacts imposed by the exclosures on litterfall and second, to standardize the amount of litter between the experimental plots thereby minimizing the chance that treatment responses were driven by factors other than throughfall.

For the litter manipulation, we established ten blocks of three 3 m × 3 m plots in April 2007. Initially, all of the standing litter was removed from each block, homogenized, and weighed. We then redistributed litter so that two-thirds of the standing litter mass was placed on randomly assigned double litter input plots (2 ×), one-third of the standing litter mass was placed on control plots, and litter removal plots (0 ×) received no litter inputs ($n = 10$ per treatment). Subsequently, at monthly intervals all fine litterfall was removed from 0 × plots, weighed, pooled, and evenly distributed on the 2 × plots. Over 2 years of the litter manipulation (April 2007–March 2009) we removed $0.90 \pm 0.05\,kg\,m^{-2}\,yr^{-1}$ of fine litter from litter removal plots (0 ×). Accordingly, we estimate control plots received litter inputs totaling $0.90 \pm 0.05\,kg\,m^{-2}\,yr^{-1}$, while litter addition plots (2 ×), received fine litter inputs totaling $1.79 \pm 0.11\,kg\,m^{-2}\,yr^{-1}$.

Field manipulations were designed to examine the effects of throughfall and leaf litter inputs on the delivery of DOM to the soil surface, thus plots were not trenched (e.g., Davidson *et al.*, 2004). This design allowed us to concentrate on how DOM inputs to surface soils affected surface N_2O production, and minimized any disturbance (via trenching) that could affect soil C or N cycling (Ngao *et al.*, 2007). Soil moisture (0–10 cm) and temperature (5 cm) in the throughfall manipulation plots

were measured using an array of HOBO sensors deployed in the plots (Microdaq Inc., Contoocook, NH, USA), and precipitation was measured using a HOBO data logging rain gauge placed in a clearing ~400 m from the study plots.

Soil characterization

Soil O_2 concentrations [O_2] in the soil were assessed using a method modified from Silver *et al.*, (1999). Here, soil oxygen chambers were constructed from 5 cm × 12 cm PVC tubes that were capped at one end; a brass hose barb connected a 5 cm nylon tube closed with a stopcock allowed for air sampling. In each experimental plot, soil O_2 chambers were installed to ~ 9 cm in the soil and allowed to equilibrate for 1 week with the soil atmosphere. Soil [O_2] were measured weekly by extracting 50 mL of headspace from the chamber and flushing a specially designed 5 mL chamber surrounding a YSI 550A handheld dissolved O_2 probe (YSI Incorporated, Yellow Springs, OH, USA). Between measurements, the probe chamber headspace was flushed with 200–300 mL of atmospheric air to restore instrument readings to ~ 100% of ambient [O_2].

To document the volume and concentration of dissolved organic C (DOC) passing through the litter layer, each plot was instrumented with a zero tension lysimeter constructed by bisecting a 0.5 cm × 50 cm PVC pipe longitudinally and installed at the soil surface. Lysimeters were filled with washed gravel (<2.5 cm diameter) and covered with a 0.5 mm mesh nylon screen to exclude large debris. Throughfall captured in the lysimeters was drained to polyethylene collapsible carboys housed in opaque plastic buckets buried to the soil surface outside the experimental plots. Throughfall/DOC volume was determined gravimetrically every 3–4 days using a hanging scale (Intercomp Inc., Medina, MN, USA), and a subsample from each lysimeter was collected and immediately frozen for subsequent DOC analyses. DOC was determined in all samples using a high temperature combustion total C analyzer (Shimadzu TOCvcpn, Kyoto, Japan; Cleveland *et al.*, 2010).

Surface soil samples were collected from all plots every 2 months using a hand soil corer (6 cm × 10 cm). Within 72 h of collection, soils were returned in a cooler on ice to the laboratory at the University of Colorado, to avoid artifacts incurred during long-term storage, and coarsely sieved (4 mm) to remove plant material. Inorganic N (ammonium [NH_4^+] and nitrate [NO_3^-]) was determined in 40 mL of 2 M KCl solution (18 h extraction, as in Cleveland *et al.*, 2006). Concurrently, rates of net N-mineralization and net nitrification were determined with a 2 M KCl extraction after a 25-day incubation of soils under aerobic conditions at 25 °C with soil moisture maintained at field capacity (Hart *et al.*, 1994). NH_4^+ and NO_3^- in extracts were analyzed colorimetrically on an Alpkem autoanalyzer (OI Analytical, College Station, TX, USA). Subsamples of these soils were also used to determine gravimetric soil moisture from all plots at all sampling times.

N_2O emissions

N_2O emissions were measured at approximately monthly intervals for one complete rainy season (April 2008–January 2009). We did not monitor N_2O emissions in the short dry season because prior test measurement in the region showed negligible fluxes during this time. We recognize limitations in forgoing more frequent gas sampling strategies and observing high temporal variation commonly driven by single precipitation events, wetting and drying cycles in soils, and variable redox conditions (e.g. Li *et al.*, 1992, 2000). The low temporal resolution of this trace gas dataset may limit the sensitivity of our analyses to detecting differences, especially in the throughfall manipulation; although, soil moisture remains surprisingly consistent at our study site (see 'Results'). Furthermore, adaptations of regional biogeochemical models to estimate N_2O production in the tropics require modeled estimates of litter inputs and N-fixation in surface soils (Kiese *et al.*, 2005), which are both strongly seasonally dynamic at the Osa study site (Cleveland *et al.*, 2006, 2010; Reed *et al.*, 2007), thus the aim for this study was to quantify seasonal shifts in redox conditions and resource availability and their affect on soil N_2O emissions.

One week before initial gas sampling, we installed bases for static chambers into each of the 50 experimental plots ($n = 10$ for each treatment: control, −50% throughfall, −25% throughfall, 0× litter, 2× litter). Bases were constructed from 12 cm × 19.5 cm inner diameter (ID) PVC pipe inserted 7 cm deep into surface soils. Static chambers were constructed by drilling brass bulkhead union fittings with 9.5 mm thermogreen septum into commercially available 21.3 cm ID PVC end caps, creating a 3.14 L headspace once installed. This chamber design and its seal on the soil surface receive 'good' ratings based on classification of non-flow-through, non-steady-state chamber by Rochette & Eriksen-Hamel (2008). Samples were taken during the morning (between 08:00 and 12:00 hours) and no temperature differences were recorded between the chamber headspace and ambient atmosphere.

Before flux measurements leaf litter was removed from inside the chamber bases and a small amount of silicon grease was applied to ensure a good seal between each base and static chamber. During each sampling event, four 30 mL headspace samples were removed over 30 min from each chamber, including a time zero sample. Gas samples were stored in 20 mL serum vials with thick butyl rubber stoppers (Bellco Glass, Vineland, NJ, USA), previously purged with ultrahigh purity helium and evacuated before sample injection and storage. Sample vials were over pressurized (~2 atm) and flown to the University of Colorado for analyses; pressurizing air samples for storage and transport allows for detection of leaky vials and avoids contaminating during analysis. For each sampling event reference standards were similarly injected into sample vials and later analyzed to verify the integrity of transporting and storing samples as described above, and all samples were analyzed within 2 weeks of samples collection. This method of air sampling handling and storage has been shown to produce reliable results (Rochette & Eriksen-Hamel, 2008).

N_2O concentrations in the samples were analyzed with a gas chromatograph equipped with an electron capture detector (ECD, Shimadzu Scientific Instruments, Columbia, MD, USA). Oven temperature on the gas chromatograph was maintained at 70 °C and gases were separated using a 3 m long Porapak N column using ultra-high purity helium as the carrier gas and

with N_2 as the make-up gas for the ECD. Rates of N_2O efflux were calculated using a linear model of the change in headspace [N_2O] over time (dC/dt), using at least three of the four field samples. We excluded samples which failed to hold their gas seal (i.e., were not over pressurized) or when their exclusion significantly improved the goodness of fit of our linear model – this resulted in exclusions of ~10% of all samples taken. Nonlinear models for N_2O flux estimation were not considered here because our primary interest was to determine differences caused by the experimental manipulation (Venterea *et al.*, 2009).

Statistical analyses

We used R ver. 2.10.1 (R Corporation, Vienna, Austria) to examine patterns in repeatedly measured variables. Annual means for all variables were calculated by calculating average monthly values for each plot over the entire year and analyzed using linear mixed-effects models with treatment as a fixed factor and plot as the random factor. Similarly, mean N_2O emissions over the study period were analyzed using linear mixed-effects models with treatment as a fixed factor and plot as the random factor, although we only had one set of observations for each sampling event. Our aim here was not to scale up limited measurements of highly variable process into annual flux estimates, but to contextualize our results from the experimental manipulations described above and to compare these findings with other similar studies.

We examined seasonal shifts in soil conditions and N_2O emissions using linear mixed-effects models with treatment and time as fixed factors, and plot as the random factor. Correlations between monthly means of individual variables were made using Pearson's product moment correlation. We used stepwise multiple linear regressions with backwards elimination to determine how well mean variation in soil conditions from each treatment at each time predicted observed N_2O emissions. For all analyses, quantile–quantile plots were used to assess the normality of residuals, and data were tested for homogeneity of variance with fitted vs. residual plots. In most cases, we used log (ln) transformed data to meet the assumptions of parametric statistics.

Results

Throughfall manipulation

The experimental throughfall manipulation was designed to reduce incoming throughfall reaching the soil surface by 25% and 50%. Over the course of the experiment (April 2008–January 2009) precipitation measured in a clearing near the experimental plots was 3740 mm, with heaviest precipitation from August through October (Fig. 1). Under the canopy, intercepted throughfall in control plots was 3555 ± 336 mm (mean $\pm$ SE of untransformed data). Manipulated plots received significantly less throughfall ($F = 12.4$, $P = 0.0002$; 2611 ± 280 and 1583 ± 212 mm in −25% and −50% plots, respectively), a 26% and 55% reduction in total throughfall (Fig. 2a).

Despite reducing the magnitude of throughfall reaching the litter layer, we did not observe significant differences in soil moisture between treatments ($F = 2.85$, $P > 0.1$; Table 2, Fig. 1). Mean volumetric soil moisture across all treatments at all times was $0.28 \pm 0.02\ cm^3\ cm^{-3}$. The lack of response in soil moisture data to the experimental throughfall reduction may have occurred because drying down this wet forest still

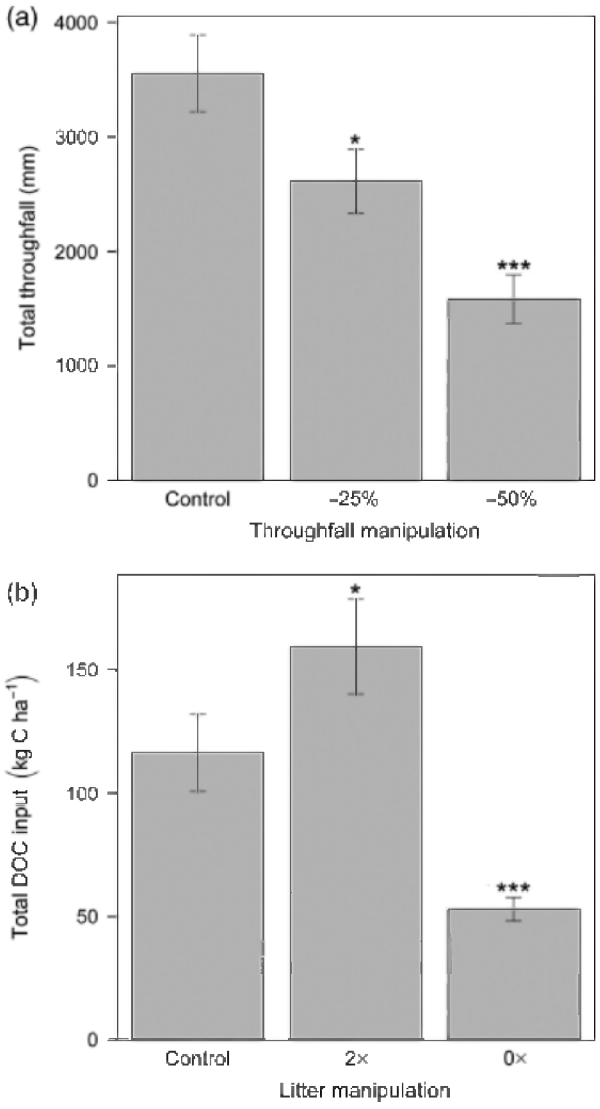


Fig. 2 Experimental treatment effects observed over the course of the 10-month field study for the: (a) throughfall manipulation – total intercepted throughfall (mm); and the (b) litter manipulation – total dissolved organic carbon (DOC) inputs (kg C ha^{-1}) to surface soils. Values represent means $\pm$ 1 SE ($N = 10$ for all treatments. Significant differences between treatments are $^*P < 0.05$ and $^{***}P < 0.001$.

resulted in wet soil conditions, with throughfall between 100 and 350 mm m^{-1} for most of the study period even in the 'driest' plots. Moreover, subsurface lateral water movement, high hydraulic conductivity of soils, and/or large movement of water through macro-pore flow may have reduced the effectiveness of the manipulation at reducing soil moisture in our relatively small experimental plots. In all plots, we observed significant seasonal variation in soil moisture ($F = 52.4$, $P < 0.0001$; Fig. 1), although we observed no significant temporal variation from June through December ($P > 0.4$), while over the same time period monthly precipitation varied by nearly a factor of 6 (Fig. 1). Given variability in the timing and intensity of precipitation events over the course of the experiment, the range of soil moisture observations measured on an hourly time-step over the study period was relatively constrained (0.27–0.36 $cm^3\ cm^{-3}$). Thus, using less frequent measurements of gravimetric soil moisture (0.44 g g^{-1} measured in control plots in April 2008, June 2008, September 2008, and January 2009), bulk density (0.58 g cm^{-3}), and assumed particle density (2.9 g m^{-3}, Hall *et al.*, 2004) we estimate WFPS of 32% for most of the study period.

Mean annual soil O_2 availability did not vary significantly between treatments ($F = 0.09$, $P > 0.9$; Table 2, Fig. 3a). As with soil moisture, we observed significant seasonal variation in soil O_2 availability ($F = 66.6$, $P < 0.0001$). During periods of low precipitation (e.g. April and January) the surface soil [O_2] were roughly equal to atmospheric [O_2]; as precipitation increased through the wet season, soil [O_2] declined, reaching a minimum in November (14.8 ± 0.72% O_2). In control plots, mean soil [O_2] were significantly negatively correlated with precipitation at time intervals from 1 to 31 days, but most strongly so with total precipitation over the 4 weeks before individual O_2 sampling point ($P < 0.0001$; $r = -0.83$). Similarly, we observed a significant negative correlation between mean monthly soil moisture and soil O_2 availability ($P < 0.0001$; $r = -0.92$).

The throughfall manipulations led to significant increases in [DOC] delivered to the soil surface relative to the control plots ($F = 13.7$, $P < 0.0001$; Table 2, Fig. 3b). As expected, we observed significant seasonal variation in [DOC] fluxes ($F = 61.6$, $P < 0.0001$), with highest [DOC] observed early in the rainy season when standing litter mass was at its annual maximum. Averaged over the entire year, the concentration of DOC inputs was more than 1.5 times higher in plots receiving a 25% reduction in throughfall and more than two times higher in plots receiving a 50% reduction in throughfall when compared with control plots (Table 3, Fig. 4a).

Total DOC inputs to surface soils did not change as a result of the throughfall manipulation because decreases in throughfall volume were matched by concurrent increases in [DOC]. The total amount of DOC delivered to surface soils over the 10-month study in control plots was 116 ± 15.6 kg C ha^{-1}, representing ~ 5% of annual fine litter fall over the same time period. This was not significantly different from DOC inputs to plots receiving −25% or −50% throughfall treatments ($F = 0.86$, $P = 0.44$; Tables 2 and 3). In all plots, total DOC inputs were highest in May, with the start of consistent precipitation, and declined throughout the year as standing litter mass decreased.

The throughfall manipulation had no significant effect on inorganic soil N concentrations, or on the net rate of N transformations (Tables 2 and 3). Across all sampling points, soil NH_4^+ pools were 6.32 ± 0.62 mg NH_4^+-N $kg\ soil^{-1}$ and varied seasonally, with significantly less NH_4^+ observed in September and January (4.13 ± 0.50 and 5.39 ± 1.75 mg NH_4^+-N $kg\ soil^{-1}$, respectively; $F = 19.5$, $P < 0.0001$). Mean soil NO_3^- pools observed across all sampling points were 5.46 ± 1.06 mg NO_3^--N $kg\ soil^{-1}$ and varied seasonally, with significantly less NO_3^- observed when precipitation and soil moisture reached their maximum (in October; 3.74 ± 0.75, $F = 14.7$, $P < 0.0001$). Rates of net N-mineralization and net nitrification were 0.83 ± 0.13 and 1.01 ± 0.12 mg N kg^{-1} day^{-1}, respectively. Rates of net N transformation varied seasonally, with significantly higher rates of both net N-mineralization ($F = 25.3$, $P < 0.0001$) and net nitrification ($F = 31.6$, $P < 0.0001$) observed in June.

We estimate that average N_2O emissions were 0.87 ± 0.06 ng N_2O-N $cm^{-2}\ h^{-1}$ in control plots (Table 3). Reducing throughfall significantly increased N_2O emissions ($F = 6.13$, $P = 0.006$; Table 2, Fig. 3c), increasing mean rates of N_2O efflux by 35% (Fig. 4b). Across sampling events we observed significant variation of N_2O emission rates ($F = 22.4$, $P < 0.001$), with maximum rates corresponding with periods of maximum precipitation and minimum soil O_2 availability (October–November; Fig. 3). For each sampling event observed N_2O emissions were significantly higher in plots receiving throughfall reduction during April, May, November, and January ($P < 0.05$, Fig. 3c).

Litter manipulation

The litter manipulation was designed to test the effects of C substrate availability on soil N_2O emission and, therefore, was not instrumented with soil moisture probes to record continuous soil moisture data. However, gravimetric soil moisture was recorded at regular intervals, and displayed a significant decrease at all time points in 0× plots; we observed significantly higher gravimetric soil moisture in 2× plots during September and October ($F = 32.9$, $P < 0.0001$; Tables 1

Table 2 ANOVA results from linear mixed effects model testing treatment and seasonal effects of experimental manipulations on soil variables measured over 10-month field study

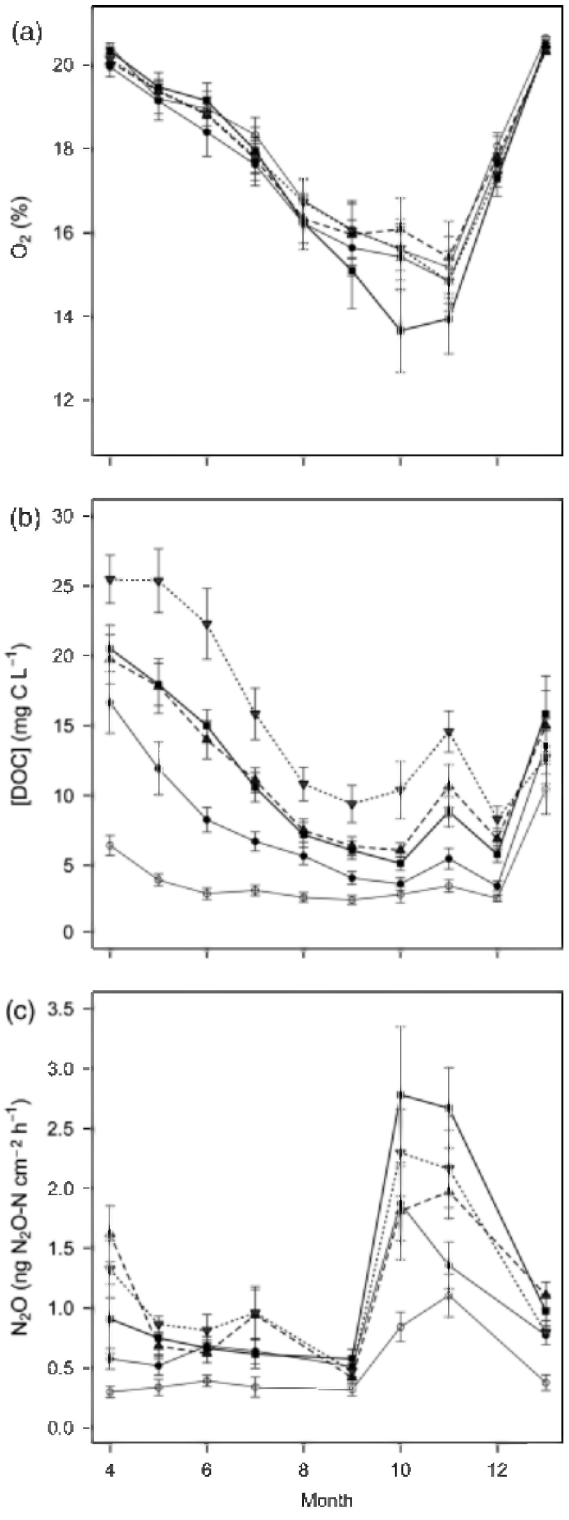
	All plots				Throughfall manipulation				Litter manipulation			
	df	df	*F*	*P*	df	df	*F*	*P*	df	df	*F*	*P*
Soil moisture($g\,g^{-1}$)												
Treatment	4	45	19.43	****	2	27	0.92		2	27	32.94	****
Season	4	180	63.91	****	4	108	29.39	****	4	108	45.26	****
tx:season	16	180	3.18	***	8	108	1.47		8	108	3.76	***
Soil O_2												
Treatment	4	45	0.27		2	27	0.09		2	27	0.47	
Season	9	405	110.95	****	9	243	66.59	****	9	243	63.56	****
tx:season	36	405	1.39		18	243	0.34		18	243	1.38	
[DOC]												
Treatment	4	45	29.09	****	2	27	13.71	***	2	27	26.23	****
Season	9	405	108.59	****	9	243	61.64	****	9	243	80.34	****
tx:season	36	405	4.75	****	18	243	4.47	****	18	243	3.84	****
DOC												
Treatment	4	45	11.89	****	2	27	0.86		2	27	22.72	****
Season	9	405	170.60	****	9	243	144.46	****	9	243	70.30	****
tx:season	36	405	2.59	****	18	243	2.48	***	18	243	1.56	
NH_4^+												
Treatment	4	45	9.22	****	2	27	0.14		2	27	17.72	****
Season	4	180	44.42	****	4	108	19.51	****	4	108	30.36	****
tx:season	16	180	1.40		8	108	0.53		8	108	1.82	
NO_3^-												
Treatment	4	45	10.64	****	2	27	0.60		2	27	8.65	***
Season	4	180	31.39	****	4	108	14.71	****	4	108	18.98	****
tx:season	16	180	4.30	****	8	108	0.32		8	108	6.06	****
Net N mineralization												
Treatment	4	45	2.36		2	27	0.57		2	27	2.53	
Season	4	180	39.07	****	4	108	25.30	****	4	108	28.14	****
tx:season	16	180	1.54		8	108	1.85		8	108	1.10	
Net nitrification												
Treatment	4	45	4.94	**	2	27	0.79		2	27	6.51	**
Season	4	180	47.27	****	4	108	31.55	****	4	108	33.39	****
tx:season	16	180	1.98	*	8	108	2.10	*	8	108	2.07	*
N_2O												
Treatment	4		20.63	****	2		6.13	**	2		20.16	****
Season	7		32.99	****	7		22.45	****	7		19.11	****
tx:season	28		1.50		14		1.79	*	14		0.50	

*$P<0.05$.
**$P<0.01$.
***$P<0.001$.
****$P<0.0001$.
DOC, dissolved organic carbon; N_2O, nitrous oxide.

and 2). As in the throughfall manipulation, we did not observe a litter treatment effect on soil O_2 availability ($F = 0.47$, $P = 0.63$), but we observed significant seasonal variation ($F = 63.6$, $P < 0.0001$; Tables 1 and 2, Fig. 3a).

As intended, the litter manipulation significantly changed the concentration of DOC inputs ($F = 26.3$, $P < 0.0001$; Fig. 3b). Averaged over the entire study period, [DOC] was 53% and 147% of control plots in $0\times$ and $2\times$ plots, respectively (Table 3); notably, reducing throughfall by 25% had roughly the same effect on [DOC] as doubling standing litter pools (Fig. 4a). Observed changes in [DOC] corresponded to a significant change in total DOC inputs to surface soils in the litter manipulation over the course of the experiment ($F = 22.7$, $P < 0.0001$; Fig. 2b). Concurrently, we observed

a significant seasonal effect on [DOC] inputs ($F = 70.3$, $P < 0.0001$; Table 2) – again with the highest [DOC] coinciding with maximum standing litter mass early in the rainy season.



The litter manipulation significantly changed soil inorganic N pools and net rates of N transformations. Specifically, mean soil NH_4^+ pools were significantly higher in 2× plots than control plots ($F = 17.7$, $P < 0.0001$; Tables 1 and 2). Mean soil NO_3^- pools were significantly lower in both 2× and 0× plots than in control plots ($F = 8.65$, $P = 0.001$). Concurrently, mean rates of net nitrification in litter removal plots were significantly lower than in control plots ($F = 33.4$; $P < 0.0001$). Over all sampling points, we observed no significant litter treatment effects on rates of net N-mineralization ($F = 2.53$, $P = 0.10$; Table 2).

The litter manipulation significantly changed N_2O emissions ($F = 20.2$, $P < 0.0001$; Table 2, Fig. 3c). Compared with control plots, average rates of N_2O efflux were −42% and +43% from 0× to 2× plots, respectively (Fig. 4b, Table 3). Overall treatment effects were driven by significant differences from control plots in June, July, October, and January in 0× plots, and November in 2× plots ($P < 0.05$). Again, rates of N_2O efflux showed significant seasonal variation ($F = 19.1$, $P < 0.0001$), with maximum rates of N_2O efflux observed during periods of maximum precipitation and minimum soil O_2 availability (October–November; Fig. 3).

Modeling N_2O production

We conducted stepwise multiple linear regressions with backward elimination using a generalized linear model (GLM) to examine the relationship between mean measured soil variables and observed mean N_2O emissions across all treatments and at all time points. In this model the best predictors of observed N_2O fluxes were soil $[O_2]$ ($t = -7.93$, $P < 0.001$) and [DOC] ($t = 4.06$, $P < 0.001$) with a log-normal error structure (dispersion parameter 0.169; AIC = 50.0; null deviance 17.00, 39 df; residual deviance 6.08, 36 df; Fig. 5). Parameter estimate from this model are given in the following equation:

$$[N_2O] = 0.53 \times \ln[DOC] - 4.35 \times \ln[O_2] + 11.20.$$

GLMs are useful in linear regressions when errors are not normally distributed, but do not provide R^2 estimates. For comparison, a similar analysis using a linear model (which assumes errors are normally distributed) using $\ln[N_2O]$ (to normalize errors) provided slightly

Fig. 3 Mean (a) monthly soil O_2 availability (%); (b) monthly dissolved organic carbon (DOC) inputs (mg C L^{-1}); and (c) nitrous oxide (N_2O) emissions, from a individual sampling events shown (ng N_2O-N cm^{-2} h^{-1}) from control plots (filled circle, solid line) throughfall manipulation (dashed lines; −50%, inverted triangle; −25% filled triangle) and litter manipulation (solid lines; 2×, filled squares; 0×, open circles). Values represent means ± 1 SE.

Table 3 Mean (± SE) soil characterization measured over the 10-month study from control, throughfall, and litter manipulation plots

		Throughfall manipulation		Litter manipulation	
	Control	−25%	−50%	2 ×	0 ×
Soil moisture ($cm^3\,cm^{-3}$)	0.28 ± 0.020	0.27 ± 0.020	0.26 ± 0.020	–	–
Soil moisture ($g\,g^{-1}$)	0.44 ± 0.003	0.45 ± 0.005	0.44 ± 0.005	**0.46* ± 0.007**	**0.40*** ± 0.005**
Soil O_2 (%)	17.40 ± 0.42	17.68 ± 0.45	17.61 ± 0.35	17.13 ± 0.50	17.80 ± 0.41
[DOC] ($mg\,C\,L^{-1}$)	6.69 ± 0.59	**10.35** ± 0.94**	**13.90*** ± 1.20**	**9.84** ± 0.82**	**3.57*** ± 0.39**
DOC ($kg\,C\,ha^{-1}$)	116.33 ± 15.62	136.37 ± 14.80	112.84 ± 13.05	**159.34* ± 19.24**	**52.94*** ± 4.81**
NH_4^+ ($mg\,N\,kg^{-1}$ soil)	6.32 ± 0.62	6.72 ± 0.60	6.66 ± 0.61	**11.39*** ± 0.90**	5.35 ± 0.58
NO_3^- ($mg\,N\,kg^{-1}$ soil)	5.46 ± 1.06	6.77 ± 0.90	5.97 ± 1.04	**1.56*** ± 0.27**	**1.86** ± 0.25**
N mineralization ($mg\,N\,kg^{-1}\,day^{-1}$)	0.83 ± 0.13	0.68 ± 0.06	0.79 ± 0.07	**0.54* ± 0.09**	0.56 ± 0.06
Net nitrification ($mg\,N\,kg^{-1}\,day^{-1}$)	1.01 ± 0.12	0.86 ± 0.06	0.98 ± 0.06	0.92 ± 0.09	**0.59*** ± 0.05**
N_2O ($ng\,N\,cm^{-2}\,h^{-1}$)	0.87 ± 0.06	**1.14* ± 0.09**	**1.21** ± 0.07**	**1.24** ± 0.12**	**0.50** ± 0.04**

Significant differences between control and treatment means from linear mixed effects model are in bold. ($n = 10$ per treatment).
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
DOC, dissolved organic carbon; N_2O, nitrous oxide.

lower model fit (AIC = 52.3) but allows calculation of an R^2 (adjusted $R^2 = 0.52$).

Discussion

Initially, we hypothesized that throughfall reductions would decrease soil N_2O efflux via improved O_2 diffusion and soil aeration, as observed in previous precipitation manipulations in lowland moist tropical forests (Nepstad *et al.*, 2002; Davidson *et al.*, 2004, 2008; Vasconcelos *et al.*, 2004). Instead, reducing throughfall significantly increased N_2O emissions from this tropical wet forest (Fig. 4b). Although the throughfall manipulation did not significantly effect soil moisture, soil [O_2], (which serve as proxies for soil redox conditions, Davidson *et al.*, 2000), or NO_3^- availability (Table 2); it significantly increased the [DOC] reaching the soil surface (Fig. 4a). Similarly, the litter manipulation significantly altered total DOC inputs. Fluxes of DOM provide labile substrates to surface soils that, in turn, stimulate heterotrophic soil respiration (Cleveland *et al.*, 2006, 2010; Wieder *et al.*, 2008). Other studies have also shown direct relationships between glucose inputs and both soil N_2O emissions and denitrifier gene abundance (Nobre *et al.*, 2001; Garcia-Montiel *et al.*, 2003; Bárta *et al.*, 2010). Here, we show via both the throughfall and litter manipulations that increases in [DOC] correlate with significant increases in soil N_2O emissions (Fig. 4); monthly soil O_2 availability and [DOC] inputs explain 52% of the variation in observed N_2O emissions from all treatments (Fig. 5).

Seasonal variations in precipitation and soil O_2 availability were strongly correlated with N_2O production, with maximum N_2O fluxes occurring during periods of heavy precipitation (and low soil O_2; Fig. 3). In many trace gas studies, soil moisture (or WFPS) reasonably predicts seasonal variation in N_2O fluxes (e.g. Keller & Reiners, 1994). Here, we did not observe significant changes in soil moisture (and therefore WFPS) from May to December, however, precipitation and soil O_2 data varied significantly over this time (Figs 1 and 3). Thus, soil O_2 data provided insight into seasonal changes in soil redox conditions that would not have been apparent in the soil moisture data alone (*sensu* Silver *et al.*, 1999). The seasonal synchrony of precipitation, soil O_2 availability, and N_2O production suggests that as abiotic conditions become more conducive for denitrification, the importance of DOC availability in regulating ultimate rates increases. That is, at the onset of the rainy season, [DOC] were highest and declined across all treatments as the rainfall intensified, but N_2O fluxes only rose substantially when soil [O_2] were at annual lows. During this time, the impact of DOC availability on N_2O production was clearly seen, with higher [DOC] in the 2 × litter addition and throughfall manipulation plots driving higher N_2O fluxes.

The effect of DOC availability on N_2O fluxes is noteworthy given some evidence from this site suggesting that N does not cycle in excess of biological demand. Previous research in tropical forests has established a general paradigm suggesting that N accumulates in excess of biological demand, creating N-rich soil conditions with high rates inorganic N loss to both

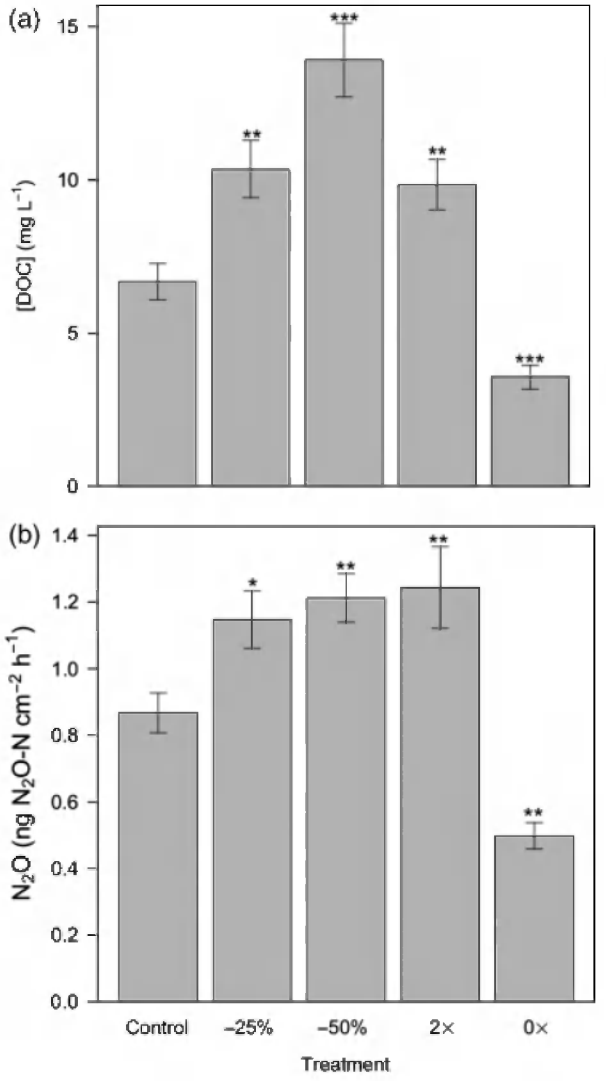


Fig. 4 Mean (a) dissolved organic carbon (DOC) inputs (mg C L^{-1}); and (b) nitrous oxide (N_2O) emissions (ng N_2O-N cm^{-2} h^{-1}) observed in all experimental manipulations and control plots. Values represent mean ± 1 SE. Treatment effects that are significantly different from controls are signified (*P < 0.05, **P < 0.01, ***P < 0.001).

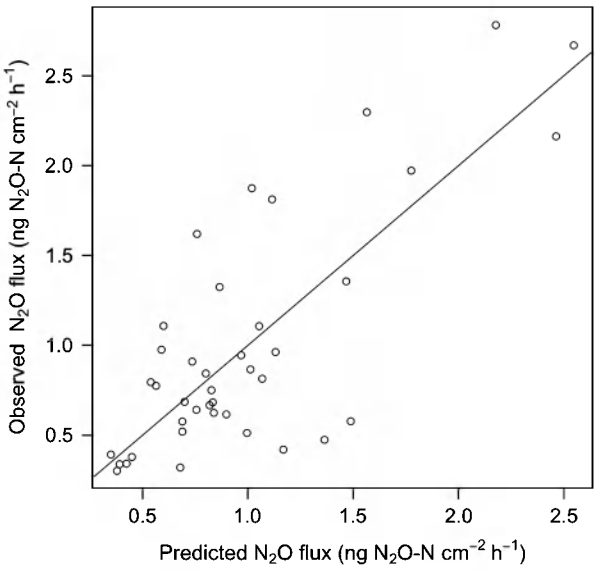


Fig. 5 Observed vs. predicted nitrous oxide (N_2O) flux from multiple linear regression with backward elimination using a generalized linear model (GLM) with log-normal error distribution. The best predictors of observed mean monthly N_2O emissions from all experimental treatments were ln[O_2] and ln[DOC] (P < 0.001; AIC = 50.0). Parameter estimates from the model are [N_2O] = 0.53 × ln[DOC] − 4.35 × ln[O_2] + 11.20.

aquatic and atmospheric realms (Vitousek, 1984; Martinelli *et al.*, 1999; Hedin *et al.*, 2003). However, in our site, extractable nitrate pools (Table 3) are notably lower than those reported for multiple other lowland forest sites (Vitousek & Matson, 1988; Keller & Reiners, 1994; Davidson *et al.*, 2000, 2007). As well, past fertilization experiments at this site showed that root growth responded to N (but not P) additions (Cleveland & Townsend, 2006). Foliar ^{15}N values, soil water and stream nitrate concentrations, and gross nitrification: gross N mineralization ratios are all also comparatively low for this site relative to other lowland regions (W. Wieder *et al.*, unpublished results), supporting an emerging pattern that the wettest of lowland tropical forests display a more conservative N cycle than their drier counterparts (e.g. Nardoto *et al.*, 2008). Furthermore, soil conditions at the site, (reducing soil conditions, low soil [NO_3^-], and large DOC inputs), may favor dissimilatory nitrate reduction to ammonium (DNRA); facilitating rapid turnover of a small NO_3^- pools and limiting the availability of NO_3^- to gaseous and hydrologic losses (Silver *et al.*, 2001). Thus, while NO_3^- availability is often presumed to exert dominant control over N_2O production (Robertson & Tiedje, 1988; Matson & Vitousek, 1990), our results suggest that even in forests where NO_3^- accumulation is limited, DOC availability may still be an important constraint on N_2O production.

Our data also suggest that future changes in precipitation over tropical regions could affect N_2O emissions in multiple ways. For example, in our study, reducing throughfall caused an increase in N_2O emissions. A common explanation for this response might be an increase in the ratio of N_2O: N_2 production resulting from increased soil aeration (Davidson *et al.*, 2004; Houlton *et al.*, 2006). However, we observed no treatment effects of soil moisture or soil [O_2] (Table 2). Instead, our data suggest the increase in N_2O flux was due to higher concentrations of litter-derived DOC that occurred with throughfall reductions (Figs 3 and 5). However, under prolonged changes in precipitation

that are driven by a changing climate, one would expect shifts in both soil oxygen conditions and in the delivery of soluble C. The latter will be a function of both the amount of water passing through the canopy, litter layer and surface soils, and the overall amount of C present under a new climate regime. The combined litter and throughfall manipulations reported here (Figs 3 and 4; Table 3) illustrate the importance of both of these factors.

Finally, data from our site and other lowland tropical forests suggest that N_2O emissions from the wettest of forests are comparatively low. To illustrate this point, we compared studies (including our own) reporting N_2O emissions in moist [mean annual precipitation (MAP) 1000–2500 mm yr^{-1}] and wet (MAP > 2500 mm yr^{-1}) lowland tropical forests (see supplementary information for additional details). On average, moist forests produced significantly more N_2O than wet forests (3.1 ± 0.6 vs. 1.8 ± 0.7 ng N_2O-N cm^{-2} h^{-1}). We recognize that such comparisons with other studies should be interpreted with caution given the limited temporal resolution of data collection and different sampling protocols between studies. Despite these limitations, average N_2O emissions measured in the control plots of the present study were much lower than those measured in moist tropical sites, but similar to fluxes measured in other wet tropical forests. This observation suggests that the response of N_2O production to a given change in precipitation may be notably different, perhaps even in direction, in wet vs. dry-to-moist tropical forests. In drier portions of the biome, increased rainfall may favor N_2O production, reflecting the central importance of soil O_2 levels. By contrast, at the wet end of the spectrum, that relationship between precipitation and N_2O production may be negative. Such a negative relationship is not only a possible outcome of an increased N_2: N_2O ratio in the wettest of systems (*sensu* Houlton *et al.*, 2006), but may also reflect a dilution of labile C inputs to soils under wetter conditions (Fig. 4), and perhaps an increasingly conservative N cycle in wet lowland forests (e.g. Nardoto *et al.*, 2008).

Collectively, our results highlight key uncertainties in our understanding of N cycling in lowland tropical forests, limiting our ability to predict their response to environmental change. First, the concentration of labile C inputs appears to be important in controlling rates of soil N_2O efflux, and presumably denitrification. Second, these data challenge the common generalization that N-cycles in relative excess, at least in wet lowland tropical forests. Third, in contrast to work conducted in dry to mesic tropical rain forests (e.g., those found throughout much of the Amazon Basin), our results suggest that declines in precipitation in wet tropical forests may actually stimulate soil N_2O production. Given the near certainty of simultaneous changes in precipitation, soil C availability, and soil N inputs across much of the tropics over the coming decades, the work presented here highlights the need for prognostic models of N_2O emission (e.g. Daycent, del Grosso *et al.*, 2006; ForestDNDCtropica, Kiese *et al.*, 2005; Werner *et al.*, 2007; PnETDNDC Li *et al.*, 2000), must effectively capture the interactions among N, C, and redox-based controls over soil N_2O production in a diversity of tropical forest types.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. To contextualize our results we compared our findings with other similar studies. We limited this comparison to studies reporting N_2O emissions from lowland moist tropical forests (mean annual precipitation [MAP] between 1000–2500 mm) and wet tropical forests (>2500 mm MAP) with at least 6 sampling points throughout a year (to achieve rudimentary season/temporal representation). As with our data we used linear interpolation of observed fluxes at each sampling time to provide a coarse estimation of mean N_2O efflux from moist and wet forests. On average we found moist forests produce more N_2O than wet forests (3.1 ± 0.6 and 1.8 ± 0.7 ng N_2O-N cm^{-2} h^{-1}, mean ± 1 SE for moist and wet forests, respectively; 1-way ANOVA of ln transformed data $F = 4.1$, $P = 0.057$).

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