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# Dinitrogen Emissions Dominate Nitrogen Gas Emissions From Soils With Low Oxygen Availability in a Moist Tropical Forest

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**Key Points:**

- Dinitrogen dominated nitrogen gas emissions from tropical forest soils
- Valleys were hot spots of nitrogen emissions in this tropical landscape
- Dinitrogen fluxes may change our understanding of nitrogen budgets in this forest

**Supporting Information:**

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

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**Abstract** Lowland tropical forest soils are relatively N rich and are the largest global source of $N_2O$ (a powerful greenhouse gas) to the atmosphere. Despite the importance of tropical N cycling, there have been few direct measurements of $N_2$ (an inert gas that can serve as an alternate fate for $N_2O$) in tropical soils, limiting our ability to characterize N budgets, manage soils to reduce $N_2O$ production, or predict the future role that N limitation to primary productivity will play in buffering against climate change. We collected soils from across macro- and micro-topographic gradients that have previously been shown to differ in $O_2$ availability and trace gas emissions. We then incubated these soils under oxic and anoxic headspaces to explore the relative effect of soil location versus transient redox conditions. No matter where the soils came from, or what headspace $O_2$ was used in the incubation, $N_2$ emissions dominated the flux of N gas losses. In the macrotopography plots, production of $N_2$ and $N_2O$ were higher in low $O_2$ valleys than on more aerated ridges and slopes. In the microtopography plots, $N_2$ emissions from plots with lower mean soil $O_2$ (5%–10%) were greater than in plots with higher mean soil $O_2$ (10%–20%). We estimate an N gas flux of ~37 kg N/ha/yr from this forest, 99% as $N_2$. These results suggest that $N_2$ fluxes may have been systematically underestimated in these landscapes, and that the measurements we present call for a reevaluation of the N budgets in lowland tropical forest ecosystems.

**Plain Language Summary** Nitrogen is a macronutrient that limits plant productivity in much of the world. Tropical forests have a relatively large number of nitrogen fixing species, they tend to cycle nitrogen in excess of plant demand. This excess nitrogen is released into the environment in either dissolved or gaseous forms. Wet, warm, and organic matter-rich soils of tropical forests result in a hot spot for denitrification; a process that removes nitrogen from soils and converts in into either nitric oxide, nitrous oxide, or dinitrogen gas. Because dinitrogen makes up 80% of earth's atmosphere, it's difficult to measure a small flux coming off the soil. This knowledge gap has resulted in a limited understanding of soil nitrogen cycling in tropical ecosystems. Because soil moisture can influence denitrification, we tested how wet points in the landscape influence emissions of dinitrogen gas. We found that wetter low points in the landscape result in the highest dinitrogen fluxes, and that dinitrogen dominated nitrogen gas emissions from these soils. We used these data to estimate dinitrogen emissions for this forest and generated a larger value than previously calculated. Such results suggest a need to integrate dinitrogen measurements into studies of nitrogen cycling in tropical forest soils.

## 1. Introduction

Nitrogen (N) is the nutrient that most frequently limits primary productivity. The nutrient status of tropical forest soils are a key determinate in whether they will continue to act as a carbon (C) sink in the face of rising temperatures and atmospheric $CO_2$ concentrations (Oren et al., 2001; Y. P. Wang & Houlton, 2009). Lowland tropical soils are also an important source of greenhouse emissions to the atmosphere. As a result of circulating large amounts of N annually, tropical soils experience high emission rates in the form of nitric oxide (NO), nitrous oxide ($N_2O$—a powerful greenhouse gas i.e., responsible for ~10% of current anthropogenic warming), and dinitrogen ($N_2$—an inert and environmentally benign gas; Cleveland et al., 1999; Firestone & Davidson, 1989;

Vitousek & Matson, 1988; Vitousek & Sanford, 1986; Templer et al., 2008). As such, tropical forest soils are the largest source of $N_2O$ to the atmosphere (Zhuang et al., 2012). Determining the current and future N status of soils requires an understanding of how inputs balance with outputs to produce net accumulation or loss. A major impediment to improving this understanding is our ability to measure $N_2$ emissions from soils. Dinitrogen is not only the largest pool of N on earth, but it's flux from soils is incredibly difficult to measure given its high atmospheric background. Despite the importance of $N_2$ to understanding soil nutrient status and its influence on the future climate, few direct measurements of $N_2$ have been made from tropical forest soils (Almaraz et al., 2020), limiting our ability to understand the spatial distribution and magnitude of this flux.

Dinitrogen is typically produced through the process of microbial denitrification, which converts soil nitrate ($NO_3^-$) to $N_2$ under anaerobic conditions. This process requires C to act as an electron donor, soil $NO_3^-$ as a substrate, and redox conditions that allow for complete denitrification, with the latter typically driven by low soil $O_2$ conditions, high soil moisture, or soil aggregates that result in anaerobic microsites. In tropical soils where soil $O_2$ is depleted, soil redox conditions may favor the reduction of $N_2O$ to $N_2$ before the gas escapes to the atmosphere. However, without adequate redox conditions, incomplete denitrification can result in the production of either NO or, more commonly in moist tropical forest soils, $N_2O$. Thus, both $N_2O$ and $N_2$ can be produced during anaerobic denitrification (Stein, 2019). Nitrous oxide can also be produced during aerobic nitrification as well as during other processes, such as anaerobic dissimilatory nitrate reduction to ammonium (Butterbach-Bahl et al., 2013; Silver et al., 2001) and iron-coupled ammonium oxidation (Feammox; Yang et al., 2012). The relative amounts of $N_2O$ and $N_2$ emitted from soils are thus also thought to be influenced by controls on these processes such as soil redox potential, N substrate, and C availability (Davidson et al., 2000; Groffman & Tiedje, 1989; Weier et al., 1993).

While redox is undoubtedly an important driver of the $N_2O$ yield from tropical forests soils, testing this control in the field has been difficult (Butterbach-Bahl et al., 2013; Firestone & Davidson, 1989; Firestone et al., 1980; Groffman & Tiedje, 1989; Weier et al., 1993). Given the high spatial and temporal variability of soil redox in natural ecosystems, it is perhaps not surprising that our understanding of the ratio of $N_2O$:$N_2$ emissions is poorly constrained. This gap in understanding is particularly relevant in tropical forests, where N gas losses are thought to be high (Houlton et al., 2006), but where factors that influence soil N gas emissions may also be highly variable (Townsend et al., 2008). Quantifying N gas fluxes from tropical forests is a key step in understanding N cycling and availability in tropical forests.

A recent review of denitrification studies found an overreliance on laboratory manipulation studies (e.g., studies that add C, N, or water to soils) and suggested that measurements made across natural gradients could help to overcome knowledge gaps (Almaraz et al., 2020). While many studies have measured N gas emissions in response to laboratory manipulated $O_2$ (Almaraz et al., 2020), few have observed how N gas emissions vary across natural soil redox gradients. Because $O_2$ diffuses 10,000 times more slowly through water than through air, soil moisture is a primary control on redox (Ponnamperuma, 1972). Soil water content is largely a function of rainfall, evapotranspiration, soil texture and aggregation. Soils microsites that remain saturated for longer periods are more likely to support complete denitrification to $N_2$, rather than having $N_2O$ escape to the atmosphere prior to complete reduction (Bremner & Blackmer, 1981; Goreau et al., 1980; Poth & Focht, 1985). However, even in well-aerated soils, denitrification can occur in anaerobic microsites where biological activity depletes $O_2$ supply. Anaerobic microsites in well aerated soils are more common in well-aggregated or clay rich soils and those with high rates of decomposition (Parkin, 1987). The prevalence of such textural and microsite characteristics varies somewhat predictably with topography, as a result of erosion, deposition, illuviation and eluviation.

Topographic gradients provide an excellent way to explore the link between N gas emissions and soil redox. In the Luquillo Experimental Forest in Puerto Rico, soil $O_2$ varies predictably with topographic position and affects rates of N cycling and loss (Hall et al., 2013; O'Connell et al., 2018; Pett-Ridge et al., 2006; Silver et al., 1999, 2013). The Luquillo landscape is characterized by highly dissected topography, where "knife-edge" ridge tops drain into steep gradient ephemeral streams and form small first- and zero-order valleys (Scatena, 1989). Extreme precipitation events in these riparian valleys can cause preferential removal of clay-size particles, increasing sand content in valleys and causing topographic texture patterns to be reversed from that of typically clay rich valleys (Hall et al., 2015; Hook & Burke, 2000; O'Connell *personal communication*). Despite high annual precipitation, high soil drainage rates result in soils that are rarely inundated, making saturated microsites within aggregates particularly important for N gas production (Almaraz et al., 2019; Hall et al., 2013). High biological activity also plays an important role in these soils, as $O_2$ consumption can often exceed diffusive resupply (Silver et al., 1999).

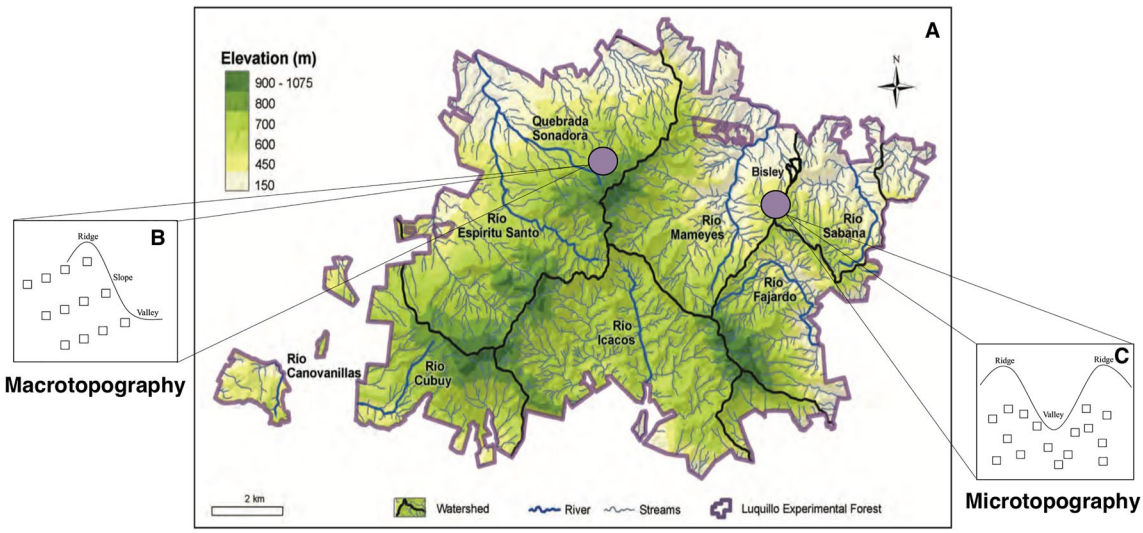


**Figure 1.** (a) The Luquillo Experimental Forest, located in northeastern Puerto Rico. (b) Macrotopography (ridge, slope, valley) plots near the El Verde field station in the Sonadora watershed, and (c) microtopography (slope plots that differ in soil oxygen availability) plots located in the Bisley 3 Watershed. Adapted from Harris et al. (2012).

Methodological challenges associated with measuring soil $N_2$ flux into an atmosphere that is 78% $N_2$ make it difficult to partition N gas emissions between their various forms. Many $N_2$ measurement techniques involve the use of inhibitors that have ancillary effects on controlling processes, alteration of substrate levels, or disruption of soil physical conditions (Almaraz et al., 2020; Groffman et al., 2006). Because N gas emissions are dependent on soil aggregation, anaerobic microsites, biological activity, and moisture and drainage, understanding the relative emissions of different N gas species will require measuring them with methods that maintain conditions that are as similar as possible to those in the field.

In this study, we used a laboratory-based gas flow incubation system that allows direct measurement of $N_2$ and $N_2O$ emissions from intact soil cores under controlled headspace $O_2$ conditions in the laboratory. We asked whether N gas emissions vary predictably from soils collected in different topographic positions and whether this variation also occurs with short-term manipulations of headspace $O_2$ during laboratory incubations. We analyzed all soils under a range of headspace $O_2$ concentrations (0%, 5%, 10%, 20%), and hypothesized that, (a) the $N_2$:$N_2O$ ratio would be inversely related to headspace $O_2$ concentration, (b) valleys and low points in the landscape (i.e., low soil $O_2$ plots) would be hot-spots for N gas production, and (c) both $N_2$ and $N_2O$ emissions would be negatively correlated with soil $O_2$ availability, which co-varies with soil moisture, topography and soil texture in this landscape (Silver et al., 1999, 2013).

## 2. Methods

### 2.1. Study Location

This study was conducted in the Luquillo Experimental Forest (LEF), a U.S. National Science Foundation funded Critical Zone Observatory and Long Term Ecological Research site in the USDA Forest Service managed El Yunque National Forest in northeastern Puerto Rico (Figure 1a). The LEF is a premontane tropical forest landscape that receives 3–4 m of rain annually with a mean annual temperature of 23°C (Garcia-Montino et al., 1996; Scatena, 1989). Rainfall at the time of sampling was typical of this location and preceded the 2015 drought, which began in May of 2015 (O'Connell et al., 2018). We sampled in forests dominated by Tabonuco (*Dacryodes excelsa* Vahl) and underlain by volcaniclastic parent material in the Bisley 3 watershed (400 m asl; Silver et al., 1999) and near the El Verde field station (500 m asl, Crow, 1980).

### 2.2. Experimental Design

We collected intact soil cores from two sites to examine how topographic controls on soil $O_2$ influence N gas emissions. First, we measured how N emissions varied across a macrotopographic gradient by collecting soils

from ridge, slope and valley positions (4 replicates per topographic position) from sites near the El Verde field station (Figure 1b) that are described in O'Connell et al. (2018). Macrotopography intact soil cores were collected and analyzed for N gas emissions at 0%, 10%, and 20% headspace $O_2$ in April of 2015. Second, we measured how N gas emissions varied across a microtopographic gradient, which consisted of 16 plots (1 $m^2$) that differed in soil moisture and soil $O_2$ dynamics, as described by Hall et al. (2013). Because we did not measure soil $O_2$ availability at the time of this soil collection, the microtopography sites were binned by historic soil $O_2$ status into three categories: low, medium, and high soil $O_2$ based on Hall et al. (2013) (see Section 2.5 below). Microtopography intact soil cores were collected from Bisley watershed 3 (Figure 1c) and were analyzed for N gas emissions at 0%, 5%, 10%, and 20% headspace $O_2$ in January of 2015.

### 2.3. Soil Sampling

Mineral soils (0–10 cm) were collected using a split core sampler (3.4 cm width) and hammer auger in order to avoid compaction and to keep soil core structure intact. Intact soil cores were collected in triplicate (one for gas incubation measurements, one for soil incubation measurements, and one extra). Soils were collected in plastic sleeves, stored at room temperature in Ziploc bags and transported to the Cary Institute of Ecosystem Studies in Millbrook, NY USA the same day they were sampled. All laboratory analyses occurred within 3 days of soil sampling. Soil chemical analyses and $N_2$, $N_2O$, and $CO_2$ emissions were measured at the Cary Institute, while soil $O_2$ and soil moisture data were collected in the field (see Section 2.5 below).

### 2.4. $N_2$, $N_2O$, and $CO_2$ Emissions

The Nitrogen Free Air Recirculation Method (N-FARM) laboratory-based gas flow incubation system was used to directly measure $N_2$, $N_2O$, and $CO_2$ emissions from intact soil cores (Burgin et al., 2010). Soil cores were incubated at multiple headspace $O_2$ concentrations, and each incubation took place on a separate day using fresh soil cores in order to avoid soil nitrate depletion. Intact soil cores were removed from Ziploc bags prior to incubation and carefully placed upright into incubation jars to maintain the soil structure (Figure S1 in Supporting Information S1). Soil cores were sealed in jars and cyclically flushed and evacuated with a given helium-oxygen mixture (0%, 5%, 10%, or 20% headspace $O_2$) for ~16 hr to completely replace the $N_2$ atmosphere. After this flush, headspace samples were directly transferred via sample lines to Shimadzu GC 14 gas chromatographs equipped with thermal conductivity (for quantification of $N_2$ and $CO_2$) and electron capture (for $N_2O$) detectors (Burgin et al., 2010). To calculate an emissions flux, headspace samples were collected 0, 3, and 6 hr after headspace flushing concluded. Gas incubations were conducted on field moist soils (±1%). As a quality control measure, empty jars and autoclaved soil cores were run to ensure that the system was leak-free and that $N_2$ was not leaking from soil pores that were not adequately flushed prior to measurement. The method detection limit for the system was 3.98 mg $N_2$ $L^{-1}$, 0.0678 mg $N_2O$ $L^{-1}$, and 10.5 mg $CO_2$ $L^{-1}$, based on the standard deviation of six replicate standards (MDL = $T_{(df,0.01)} \times$ SD; Morse et al., 2015).

### 2.5. Soil Oxygen and Soil Moisture

We relied on previous year-long continuous soil $O_2$ measurements at the microtopography plots (Figure S2 in Supporting Information S1; Hall et al., 2013) to classify these sites into three categories: low, medium, and high soil $O_2$. At the macrotopography plots, we measured soil moisture and soil $O_2$ for ~1 year using time-domain reflectometry (Campbell Scientific, Logan, Utah) and galvanic cell sensors (Apogee Instruments, Logan, Utah) housed in polyvinyl-chloride tubes installed at a depth of 10 cm. Soil $O_2$ levels and precipitation during the time of soil $O_2$ data collection (2010) and soil core collection (2015) were typical of this forest under non-drought conditions (Figure S2 in Supporting Information S1; O'Connell et al., 2018; Silver et al., 1999, 2013). In situ soil $O_2$ concentrations measured in the field are henceforth referred to as "soil $O_2$" in order to differentiate them from incubation headspace $O_2$ manipulations, which are henceforth referred to as "headspace $O_2$." Gravimetric soil moisture was also determined on a subsample of all soil incubation cores at the time of sampling by calculating mass loss after oven drying soils at 105°C for 48 hr. Soil cores were stored in Ziploc bags at the time of collection until the time of analysis to maintain field moisture.

In situ soil $O_2$ data collected across the microtopography gradient several years prior to N measurements were used to bin the microtopography plots into groups that were comparable with headspace manipulated $O_2$ levels.

Plots with mean in situ soil $O_2$ concentrations from 5% to 10% are referred to as "Low," 10%–15% are referred to as "Medium," and 15%–20% are referred to as "High." Since no plot had a mean soil $O_2$ concentration <5%, a fourth $O_2$ category was omitted from our analyses (Figure 3). Given that soil $O_2$ patterns across the landscape remain relatively consistent over time, we assumed that soil $O_2$ emissions measured by Hall et al. (2013) were similar to those at the time of soil collection.

### 2.6. Statistical Analysis

Nitrogen gas emission data were not normally distributed, therefore we assessed differences between macro- and micro-topographic positions using a non-parametric Wilcoxon Ranked sum test and examined relationships between variables using Spearman's Rho correlation coefficients. Where N emissions were undetectable, a value of 0.0001 was used to calculate $N_2$:$N_2O$ ratios for individual samples (Figure 4). Data are reported as means plus or minus the standard error.

## 3. Results

Dinitrogen dominated the N gas fluxes across all conditions (mean $N_2$:$N_2O$ = 275). On average, $N_2$ emissions from all of our plots were 4.0 ± 0.7 μg N $g^{-1}$ $d^{-1}$, compared to $N_2O$ emissions of 0.02 ± 0.003 μg N $g^{-1}$ $d^{-1}$. While laboratory incubation headspace $O_2$ concentrations had no significant effect on $N_2$ emissions, in situ soil $O_2$ concentrations, mediated by differences in macro- and micro-topographic position, had a significant influence on $N_2$ dynamics and variation in the $N_2$:$N_2O$ ratio (see Tables S1 and S2 in Supporting Information S1).

Emissions of $N_2$ were much greater than $N_2O$ emissions in incubated cores sampled from macrotopography plots (mean $N_2$:$N_2O$ = 70). Dinitrogen and $N_2O$ emissions were higher from valleys compared to ridges and slopes ($p < 0.05$) at all headspace $O_2$ concentrations, with the exception of $N_2O$ at 0% headspace $O_2$, which showed no difference between topographic positions ($p = 0.6$; Figure 2). Emissions of $N_2O$ at 0% headspace $O_2$ were all zero, with the exception of a single outlier (0.14 μg N $g^{-1}$ $d^{-1}$). Although $N_2$ emission rates were highest in valleys, the $N_2$:$N_2O$ was highest on aerated ridges, in part because $N_2O$ emission rates were especially low there (Table 1). Soil moisture, which is related to in situ soil $O_2$ availability, was significantly higher in valleys ($p < 0.0001$; Table 1).

As with the macrotopography plots, $N_2$ emissions were much greater than $N_2O$ emissions in incubated cores sampled from microtopography plots (mean $N_2$:$N_2O$ = 410). The influence of microtopography on $N_2$ and $N_2O$ emissions was more nuanced than that of macrotopography, with more consistent effects on $N_2$ than $N_2O$ emissions (Figure 3). Microtopography plots were categorized by their mean in situ soil $O_2$ content, and plots categorized as having low mean soil $O_2$ had marginally higher soil moisture ($p = 0.1$; Table 2). Dinitrogen emissions were marginally higher ($p < 0.1$) from plots with low in situ soil $O_2$ concentrations at all incubation headspace $O_2$ concentrations, with the exception of the 5% headspace $O_2$ treatment (Figure 3). Microtopography had little effect on $N_2O$ emissions. The only marginally significant difference in $N_2O$ emissions was observed in the 20% headspace $O_2$ treatment where the plots with low soil $O_2$ had the highest emissions (0.02 ± 0.01 μg N $g^{-1}$ $d^{-1}$, $p = 0.06$) compared to those with high or medium soil $O_2$ (0.007 ± 0.002 and 0.002 ± 0.001 μg N $g^{-1}$ $d^{-1}$, respectively). The $N_2$:$N_2O$ ratio was high from microtopography plots with low mean in situ soil $O_2$ (Table 2); where $N_2$ (12.1 ± 3.06 μg N $g^{-1}$ $d^{-1}$) was nearly three orders of magnitude higher than $N_2O$ (0.02 ± 0.004 μg N $g^{-1}$ $d^{-1}$).

Soils were incubated under multiple headspace $O_2$ concentrations, which affected $N_2O$ but not $N_2$ emissions. Soil collected from macrotopography sites had significantly higher $N_2O$ emissions when incubated at 20% headspace $O_2$ ($p = 0.01$), compared to 0% and 10% headspace $O_2$. Nitrous oxide emissions from microtopography sites were significantly higher when incubated at 5%, 10%, and 20% headspace $O_2$ ($p < 0.0001$) than when incubated with 0% headspace $O_2$. The $N_2$:$N_2O$ ratio was significantly higher from 0% headspace $O_2$ incubations at both the micro- and macro-topography plots ($p < 0.0001$ and $p = 0.009$, respectively), as little $N_2O$ was emitted during those incubations (Figure 4).

## 4. Discussion

### 4.1. The $N_2$ Emissions and the $N_2$:$N_2O$ Ratio

We found that $N_2$ emissions dominated the N gas fluxes across all conditions. Mean $N_2$ emissions were three orders of magnitude higher than $N_2O$ emissions and were highly variable across the landscape at both macro and

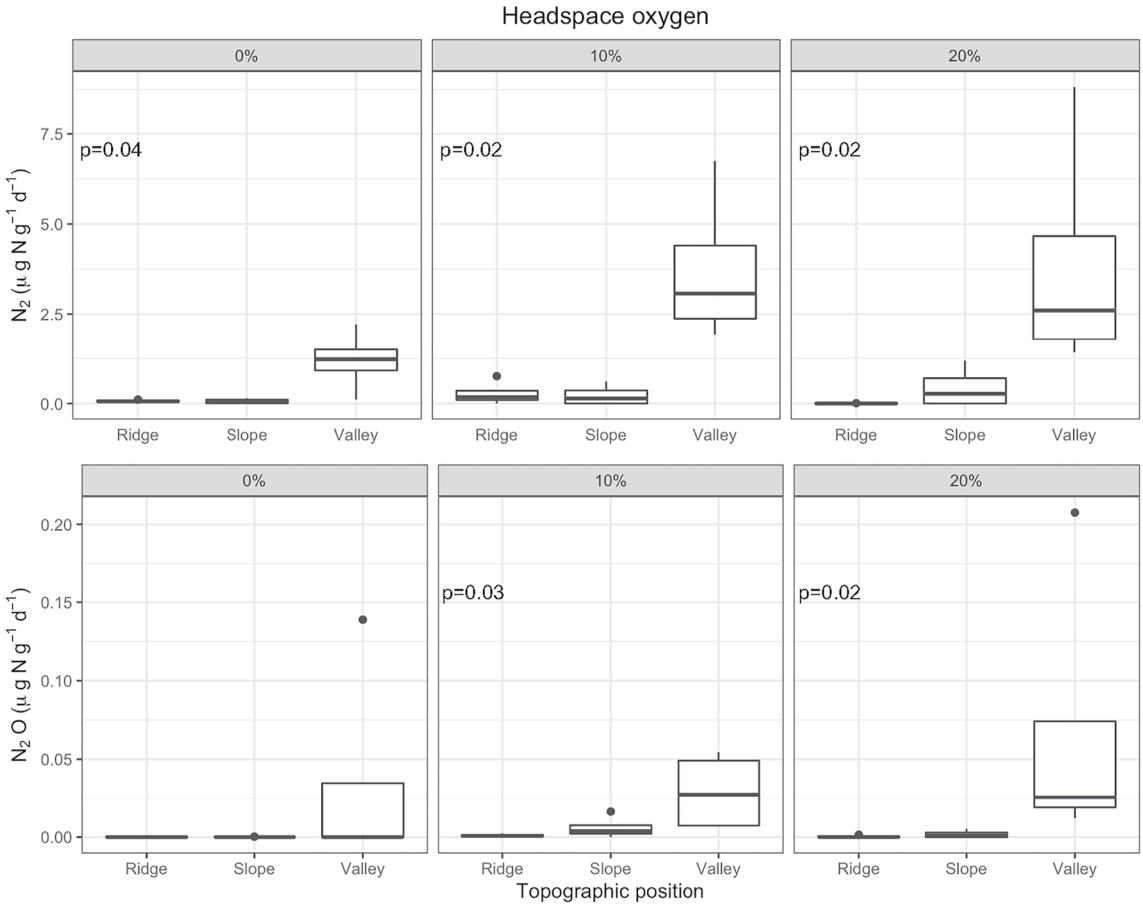


**Figure 2.** Dinitrogen and nitrous oxide emissions from ridge, slope, and valley positions (macrotopography) near the El Verde Field Station measured at 0%, 10%, and 20% headspace $O_2$.

micro scales. The magnitude of the $N_2$ flux make the inclusion of this measurement critical for accurate estimates of N budgets in lowland tropical forest. The three greatest uncertainties in most N budgets are N fixation, N storage in soil, and denitrification (Galloway et al., 2004); without accurate estimates of $N_2$ losses it is difficult to constrain inputs and storage. Furthermore, denitrification's variability speaks to the inadequacy of mass balance or static ratio approaches for estimating the $N_2$ emissions. Since methodological difficulties often preclude $N_2$ measurements in N budget studies, we show that soil $O_2$ concentrations co-vary with $N_2$ emissions, making this a useful parameter for estimating $N_2$ hot spots across the landscape, despite inadequate field measurement techniques.

The complex controls on $N_2O$ emissions make it difficult to discern landscape-scale patterns and controls on the $N_2$:$N_2O$ ratio. A more nuanced understanding of controls on the $N_2$:$N_2O$ ratio can help inform soil management to reduce fluxes of $N_2O$ to the atmosphere. Even while variable, the $N_2$:$N_2O$ ratio was dominated by a high numerator. Trends in this ratio were driven by $N_2$ in incubated cores sampled from microtopography plots, since $N_2O$ showed little variability while $N_2$ varied greatly between plots (Table 2). However, in incubated cores sampled from macrotopography plots we saw opposing trends between $N_2$:$N_2O$ and $N_2$, in part because $N_2O$ production decreased more dramatically than $N_2$ on ridges (Table 1). In situ soil $O_2$ exerted stronger control on $N_2$ than on $N_2O$, the latter of which can be driven primarily by N availability particularly when produced by nitrification (Bremner & Blackmer, 1978; Firestone & Davidson, 1989) or affected by disturbances such as root mortality (Keller et al., 2000). While we report differences observed for field moist soils, soil saturation and subsequent entrapment of gases can affect observed emissions in gas incubations, particularly for $N_2O$, which is more likely to be reduced to $N_2$ when entrapped and thus underestimated (Letey et al., 1980). Broad scale patterns and controls of $N_2$:$N_2O$ will likely remain difficult to quantify due to these differential controls on the two gases and methodological limitations. These findings suggest scale-dependent controls on the $N_2$:$N_2O$ ratio, which provide

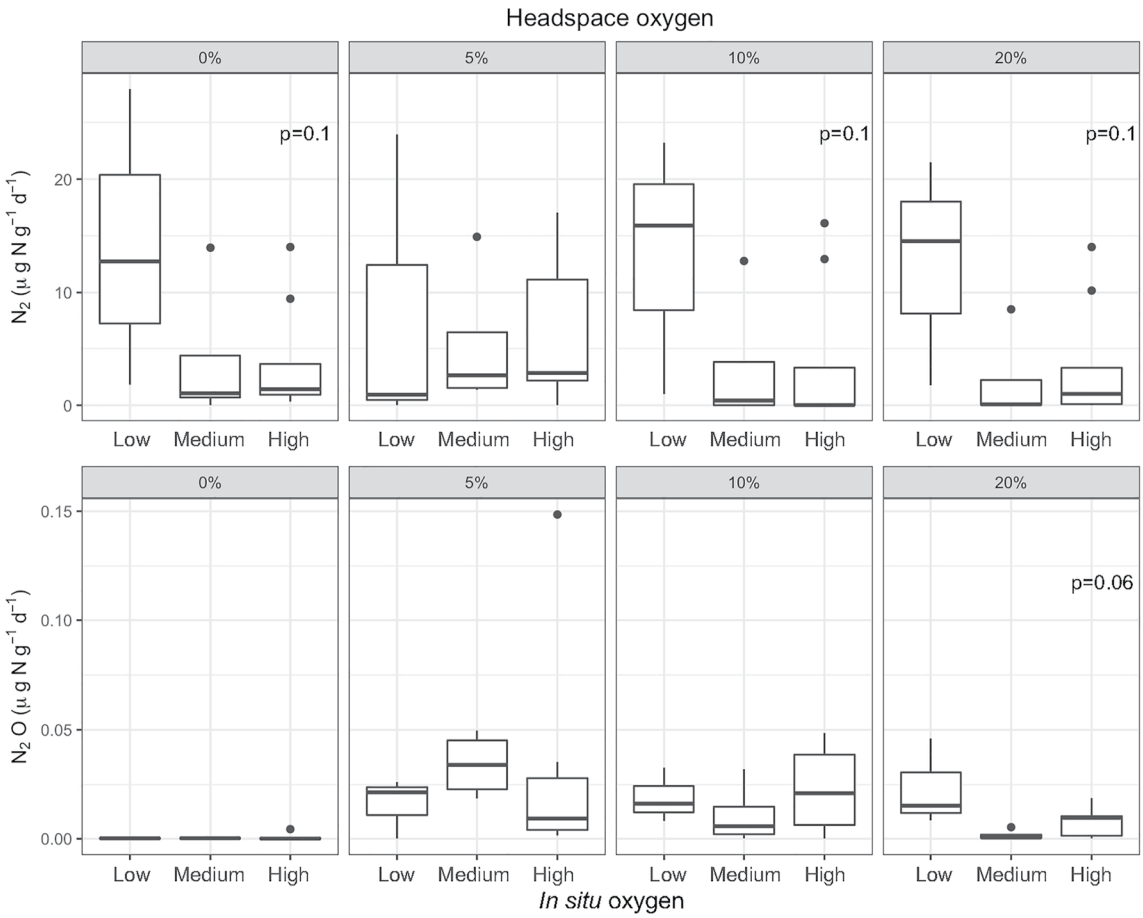


**Figure 3.** Dinitrogen and nitrous oxide emissions from slope plots that differed in their in situ mean soil $O_2$ (categorized as 5%, 10%, or 20%; microtopography) that were measured at 0%, 5%, 10% and 20% headspace $O_2$ in Bisley Watershed 3.

a framework for upscaling from variation within a given topographic position to the landscape scale (see section on Section 4.6).

### 4.2. Macrotopography and N Gas Emissions

Macrotopography was a very important control on N gas emissions. Both $N_2O$ and $N_2$ emissions were highest in the valley samples regardless of incubation headspace $O_2$ concentration. Valleys are typically zones of high moisture, high soil $O_2$ consumption rates leading to low redox conditions (Liptzin et al., 2011; Silver et al., 1999), and can simultaneously have relatively high C and N concentrations that stimulate denitrification (McClain et al., 2003). While low points in the landscape often have finer soil texture than upland soils, valleys in the Bisley 3 and El Verde watersheds were found to be lower in both labile C and clay content than slopes and ridges (Hall et al., 2015; Hook & Burke, 2000; O'Connell et al., 2018; Pachepsky et al., 2001), thus it is unlikely that soil texture or carbon availability explain high N gas fluxes in valleys. Valleys at this site have been found to have higher soil moisture (O'Connell et al., 2018), which limits $O_2$ diffusion through the soil profile, than upland soils, suggesting that soil moisture and subsequent soil $O_2$ availability may be a key driving factor. Although valleys make up a small portion of the landscape in the forest, they are important hot spots for N gas production.

While valleys are important hot spots for N gas production, ridges can still produce N gases via anaerobic pathways in soil microsites. Although valleys produced the largest $N_2$ fluxes, the $N_2$:$N_2O$ ratio was highest on aerated ridges. While ridges are indeed "aerated" at the bulk soil scale, Hall and Silver (2015) found that they actually had a greater mean Fe(II) content at the landscape scale (even relative to valleys), indicative of anaerobic microsites that could be driving $N_2$ production on ridges. This is consistent with the observation that ridges in this forest have been found to have greater aboveground biomass, root biomass, soil organic matter, and decomposition

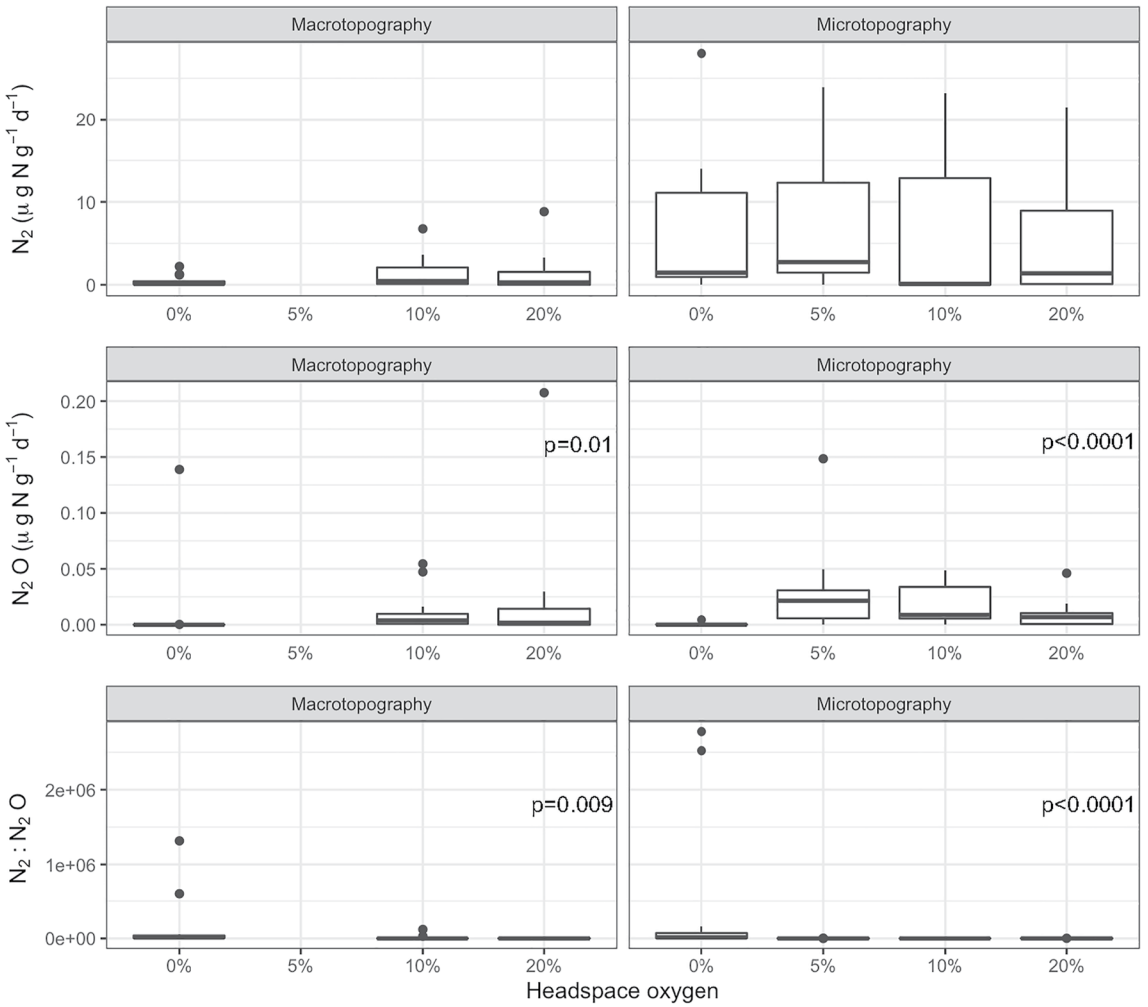


**Figure 4.** Mean dinitrogen and nitrous oxide emissions from (a) macrotopography plots measured at 5%, 10%, or 20% headspace $O_2$, and (b) microtopography plots measured at 0%, 5%, 10%, and 20% headspace $O_2$.

activity (as indicated by extracellular enzymes) compared to other topographic positions, biological activity that would result in $O_2$ consumption in soil micropores (Hall et al., 2015; Scatena et al., 1993; Silver et al., 1994). The importance of these hot spots to ecosystem and landscape fluxes will depend on their areal extent and the magnitude of the denitrification rates that they support.

### 4.3. Microtopography and N Gas Emissions

The influence of microtopography (defined here as differences in mean soil $O_2$ availability among nearby plots) on $N_2$ and $N_2O$ emissions was more nuanced than that of macrotopography. The microtopography effect was

**Table 1**
*Overall Mean $N_2$ and $N_2O$ Emissions (With Standard Deviation in Parentheses) and $N_2$:$N_2O$ Measured in Incubations at 0%, 10%, and 20% Headspace $O_2$, and Mean In Situ Soil Oxygen and Gravimetric Soil Moisture for Macrotopographic Position Plots Near the El Verde Field Station*

| Topographic position | $N_2$ (ug N $g^{-1}$ $d^{-1}$) | $N_2O$ (ug N $g^{-1}$ $d^{-1}$) | $N_2$:$N_2O$ | Mean in situ soil $O_2$ (%) | Gravimetric soil moisture (%) |
|---|---|---|---|---|---|
| Ridge | 0.12 (0.21) | 0.0005 (0.0008) | 240 | 18.47 | 41 |
| Slope | 0.24 (0.37) | 0.003 (0.005) | 80 | 16.07 | 43 |
| Valley | 2.9 (2.5) | 0.04 (0.02) | 73 | 0.04 | 50 |

**Table 2**
*Overall Mean $N_2$ and $N_2O$ Emissions (With Standard Deviation in Parentheses) and $N_2$:$N_2O$ Measured in Incubations at 0%, 5%, 10%, and 20% Headspace $O_2$, and Mean In Situ Soil Oxygen and Gravimetric Soil Moisture for Microtopography Plots in Bisley Watershed 3 That Were Categorized Based on Their Mean In Situ Soil Oxygen Class (i.e., 5%, 10%, or 20%—No Plot had Mean Soil $O_2$ Below 5%)*

| Oxygen class | $N_2$ (ug N $g^{-1}$ $d^{-1}$) | $N_2O$ (ug N $g^{-1}$ $d^{-1}$) | $N_2$:$N_2O$ | Mean in situ soil $O_2$ (%) | Gravimetric soil moisture (%) |
|---|---|---|---|---|---|
| High | 4.34 (5.78) | 0.015 (0.028) | 289 | 18.17 | 45 |
| Medium | 3.74 (5.48) | 0.012 (0.017) | 312 | 12.36 | 44 |
| Low | 12.1 (10.6) | 0.015 (0.015) | 808 | 7.16 | 47 |

smaller than the macrotopography effect for both $N_2$ and $N_2O$ emissions, suggesting that other factors such as C and mineral N availability may be important drivers here. Still, despite variability in N gas emissions and tropical forest heterogeneity (Townsend et al., 2008), we observed a relationship between long-term mean soil $O_2$ concentrations and one-time measurements of N gas emissions among soils that were otherwise very similar. While other factors are likely also important, these findings suggest that soil $O_2$ can serve as a useful integrating variable for predicting N fluxes at fine spatial scales (meters).

Dinitrogen emissions were greater from microtopography plots with low soil $O_2$, and while $N_2O$ emissions were also greater from plots with low soil $O_2$ when incubated at 20% headspace $O_2$, $N_2O$ emissions were more variable across soils when incubated under different headspace $O_2$ concentrations (Figure 3). These data suggest that soil $O_2$ is a primary driver of $N_2$ emissions, but that $N_2O$ may have other drivers that dictate production. In other tropical soils, $N_2O$ emissions were correlated with nitrification rates (Matson & Vitousek, 1987), a process whose drivers may differ from that of denitrification. The decoupling of $N_2O$ production from $O_2$ that we observed implicates roles for both nitrification and denitrification in $N_2O$ production from this system.

### 4.4. Headspace Oxygen Availability and N Gas Emissions

While $N_2$ production was observed at all incubation headspace $O_2$ concentrations, reducing headspace $O_2$ to 0% stopped the production of $N_2O$ completely at both types of sites (with the exception of a single outlier; Figure 4). This suggests that either $N_2O$ from these sites is almost entirely produced from an aerobic pathway (i.e., nitrification) or that $N_2O$ was produced by any number of pathways but was completely reduced to $N_2$ in an anaerobic environment. While $N_2O$ emissions in riparian wetland soils from the temperate zone were found to be produced via aerobic pathways (Burgin & Groffman, 2012), those of temperate upland soils were found to decrease with increasing headspace $O_2$ (Kulkarni et al., 2014; Morse et al., 2015), implying that anaerobic processes were responsible for $N_2O$ production. In agricultural, natural ecosystem, and culture studies, aerobic processes are often considered to be a primary source of $N_2O$, whereas denitrification is responsible only when soil moisture is high enough to create anaerobic microsites (Anderson & Levine, 1986; Bremner & Blackmer, 1978; Wrage et al., 2001). In riparian wetland and wet tropical forest soils, we expect the presence of such anaerobic microsites where $N_2O$ is likely to be reduced to $N_2$. These complexities highlight the need for more comparisons between temperate upland soils, where most denitrification studies have taken place, and wet environments, such as riparian wetlands and wet tropical forests, where N gas production likely differs as a result of high moisture and differences in soil aggregation/structure. Labile C and nitrate substrate are other potentially important mediating factors on N gas emissions (Weier et al., 1993); however, we did not explore those factors in this study.

The fact that $N_2O$, but not $N_2$ emissions, responded to changes in incubation headspace $O_2$ concentrations may reflect the location where production processes occur in the soil environment. Previous studies have found that intact soil core gas flow incubation system methods may overestimate $N_2O$ emissions relative to $N_2$ (Morse et al., 2015), likely as a result of increased surface to volume ratios in the cores that reduce the residence time and potential for $N_2O$ reduction. If $N_2O$ production is occurring in macropores, which are exposed to $O_2$ during the soil core incubation, this can increase $N_2O$ production. In contrast, if $N_2$ production is occurring in anaerobic microsites, this process is likely less vulnerable to short term changes in headspace $O_2$ caused by the core treatments and would thus be less responsive to induced headspace $O_2$ differences, as was observed in this study. This is consistent with our observation that $N_2$ emissions were sensitive to in situ $O_2$ concentrations (or topography) but not incubation headspace $O_2$ concentrations.

### 4.5. Tropical N Gas Emissions

Dinitrogen emissions were much greater than $N_2O$ emissions (mean $N_2$:$N_2O$ = 275). High $N_2$ emissions suggest high denitrification fluxes from this forest, likely resulting from soil aggregation of clays that allow for anaerobic microsites, moisture frequency in or above field capacity, high N mineralization rates, and some of the highest

decomposition rates on Earth (Cusack et al., 2010; Matson & Vitousek, 1987; Parton et al., 2007). Coupled nitrification-denitrification might explain why sites at Luquillo have been found to have high rates of net nitrification, yet available N pools dominated by $NH_4^+$. Alternatively, this occurrence could be explained by dissimilatory nitrate reduction to ammonium (Silver et al., 2001). Feammox is another process that might lead to high $N_2$ emissions, as this process has been documented to emit $N_2$ at a rate similar to denitrification in this forest (Yang et al., 2012).

We report higher $N_2$ fluxes than have been detected using other methods in this forest. Our median and mean $N_2$ emissions were 0.14 and 1.09 μg N $g^{-1}$ $d^{-1}$, respectively, but ranged from 0 to 8.8 μg N $g^{-1}$ $d^{-1}$. Other estimates from this forest have been considerably lower. Using $^{15}N$ tracer technique, Yang et al. (2014) estimated $N_2$ emission rates of 0.02 μg N $g^{-1}$ $d^{-1}$, while Silver et al. (2001) reported that they were unable to detect $N_2$ fluxes from ridge soils. Estimates by Yang et al. (2014) are similar to those we measured from ridges, but much lower than those we measured from valleys, consistent with our result that topography influences $N_2$ emissions. Our highest rates were from valleys and at low soil $O_2$ plots, where low redox potential and evidence of elevated N substrate availability (Scatena et al., 1993) likely created optimum conditions to produce $N_2$. Differences in flux magnitude may result from either spatial/temporal heterogeneity or improved detection of $N_2$ using the NFARM.

Few measurements of denitrification have been made in other tropical forests and results vary widely. Kachenchart et al. (2012) found similar rates of $N_2O$ (0.01 μg N $g^{-1}$ $d^{-1}$) from forest soils in Thailand, but lower rates of $N_2$ (0.05 μg N $g^{-1}$ $d^{-1}$) than we found in the LEF. Parsons et al. (1993) made measurements in Costa Rican soils and found $N_2O$ (0.1 μg N $g^{-1}$ $d^{-1}$) emission rates that were similar in magnitude to $N_2$ emissions (0.09 μg N $g^{-1}$ $d^{-1}$). Cheng et al. (2014) and Zhu et al. (2013) found high rates of denitrification from subtropical forest soils in China (12.2 μg N $g^{-1}$ $d^{-1}$ and 5.38 μg N $g^{-1}$ $d^{-1}$, respectively), however, the former found $N_2O$ dominated the N emissions pathway, whereas the latter found that $N_2$ was the dominant product. All measurements reported here used the acetylene inhibition method, which might explain the low $N_2$ estimates, due to inhibition of coupled nitrification-denitrification and incomplete diffusion of acetylene throughout the soil core (Groffman et al., 2006). More recently, Soper et al. (2018) reported low N gas emissions from a lowland tropical forest in Costa Rica using a mass balance approach. There is a clear need for more measurements of total N gas emissions from tropical soils, using improved methods that directly quantify $N_2$ emissions.

### 4.6. Scaling Up N Gas Emissions

There are two main challenges to producing ecosystem level N fluxes: (a) getting good estimates of instantaneous fluxes and (b) scaling up over space and time. The NFARM approach allowed us to overcome the first challenge, but our instantaneous fluxes range over several orders of magnitude depending on topographic position and sampling date (Tables 1 and 2). Thus, ecosystem N flux estimates are widely variable depending on the scaling approach taken.

Here we present topographically scaled values of $N_2$ production that are plausible from the perspective of mass balance and in the context of previous measurements. If we estimate the annual $N_2$ flux based on the mean of emissions measured near the El Verde Field Station, we get a flux in the hundreds of kg N $ha^{-1}$ $yr^{-1}$, which is obviously too high given that N inputs to this ecosystem are in the tens of kg N $ha^{-1}$ $yr^{-1}$ (Chestnut et al., 1999; Cusack et al., 2009). Since we found macrotopography to be a good indicator of denitrification, we used a spatially weighted mean based on the spatial extent of topographic positions across the landscape (i.e., 85% slope, 10% ridges, and only 5% valleys; Yang Lin, *personal communication*) and generate an annual $N_2$ flux of 93 kg N $ha^{-1}$ $yr^{-1}$. While accounting for topography cuts the annual flux estimate by more than half of that using the mean, it is likely still too high as average N fixation rates in tropical forests are 15–36 kg N $ha^{-1}$ $yr^{-1}$ (Cleveland et al., 1999). Since $N_2$ production rates are not constant over time, a better way to generate a flux might be to couple the topographic approach with soil $O_2$ dynamics, based on findings from headspace manipulation experiments. If we assume a threshold for $N_2$ production of <10% headspace $O_2$ ($N_2$ was significantly greater from plots with <10% in situ soil $O_2$; Figure 3), and that soil $O_2$ falls beneath this threshold 95% of the time in valleys, 5% on slopes and 0% on ridges (O'Connell et al., 2018), then we generate an $N_2$ flux of 33 kg N $ha^{-1}$ $yr^{-1}$. If we use our overall median rather than mean $N_2$ flux, our estimate based on soil $O_2$ dynamics increases to 32 kg N $ha^{-1}$ $yr^{-1}$ (see Table S3 in Supporting Information S1).

The large $N_2$ flux estimates we calculated are consistent with those of other tropical forests but have yet to be identified in the LEF. A recent study found that humid tropical forest soils average 17 kg N $ha^{-1}$ $yr^{-1}$ via

denitrification (Brookshire et al., 2017), while others have estimated 6–30 kg N $ha^{-1}$ $yr^{-1}$ (Bai et al., 2012; Houlton et al., 2006). These estimates are on par with measurements made from subtropical forests in China using a dual isotope technique, where they found denitrification rates of 2.4–21.7 kg N $ha^{-1}$ $yr^{-1}$ (Yu et al., 2019). Chestnut et al. (1999) estimated an $N_2$ flux of 1.3–3.75 kg N $ha^{-1}$ $yr^{-1}$ for the LEF, however, these measurements were made on sandier quartz diorite derived soils which have been found to display higher $N_2O$ emission rates than that of clayey volcaniclastic soils (Bowden et al., 1992). Furthermore, Chestnut et al. (1999) calculated total N emissions by applying an $N_2$:$N_2O$ (1:1) ratio to flux chamber measurements of $N_2O$, which based on variability in the $N_2$:$N_2O$ ratio, is likely to generate estimates with a high degree of error.

For $N_2O$, if we use the overall mean flux, we generate an annual flux that is an order of magnitude higher than our spatially and temporally weighted estimates. When we use a spatially and temporally weighted mean, we estimate an $N_2O$ flux of 0.45 kg N $ha^{-1}$ $yr^{-1}$. If we use the overall median flux, we generate a $N_2O$ flux of 0.18 kg N $ha^{-1}$ $yr^{-1}$ (see Table S3 in Supporting Information S1). In comparison, Chestnut et al. (1999) estimated and $N_2O$ flux of 0.6–1.7 kg N $ha^{-1}$ $yr^{-1}$ and Erickson et al. (2001) estimated an N oxide (NO and $N_2O$) flux ranging from 0.9 to 9 kg N $ha^{-1}$ $yr^{-1}$ in this forest.

Together these estimates highlight the importance of quantifying $N_2$ emissions in order to adequately estimate the N flux from tropical forest soils. These findings emphasize how the proportion of these gases emitted from soils varies across both space and time. Despite variability in N gas fluxes and heterogeneity across tropical landscapes (Townsend et al., 2008), we show that field measurements across naturally occurring ecosystem gradients can help to build a more robust understanding of tropical N cycling.

## 5. Conclusions

These are the first unamended direct measurements of $N_2$ fluxes from tropical forest soils. We report some of the highest $N_2$ flux rates for tropical forests and found that in situ soil $O_2$ dynamics have a strong influence on $N_2$ emissions, which dominate the N gas loss pathway. Soil $O_2$ dynamics have a greater effect on N gas emissions at the macro-than at the micro-scale. Valleys appear to be a hot spot for both $N_2O$ and $N_2$ production, while smaller scale microtopographic variation in soil $O_2$ across the rest of the landscape influences $N_2$ fluxes much more than $N_2O$. Incubation headspace $O_2$ influenced $N_2O$ but not $N_2$ production along both macro and micro-topographic gradients, suggesting a potential difference in either the physical location in which each gas is being produced or the process that is producing them. It was difficult to discern controls on the $N_2$:$N_2O$ ratio, likely due to differential controls on the two gases. Dinitrogen emissions appear to be driven by characteristic differences in soil $O_2$ dynamics, whereas $N_2O$ emissions may have alternate or more complex drivers. These results suggest that it is not possible to model the emissions of one gas based on measurements of the other (Chestnut et al., 1999), which complicates our ability to assess and model N transformations across tropical forest landscapes. More sophisticated modeling efforts should be employed to accurately capture the effect of hot spots and hot moments on annual N gas emission fluxes. Furthermore, the distribution and intensity of these hot spots are likely sensitive to multiple components of global environmental change.

These findings confirm the theoretical conclusion that N gas emissions from tropical forests may in fact be large (Davidson et al., 2000; C. Wang et al., 2017) and support the idea that there is wide variation in the nature of N cycling and losses in lowlands tropical forests (Soper et al., 2018). The magnitude of emissions estimated here are higher than inputs, this finding may speak to a need for better estimates of tropical forest inputs, which currently rely mainly on a single analysis (Cleveland et al., 1999). Increasing direct measurement of N inputs and outputs from tropical forests are still necessary to understanding N budgets in these understudied ecosystems.

## Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

## Data Availability Statement

Summarized data for this publication are available in Tables S1 and S2 of Supporting Information S1. Non-aggregated data can be made available by authors upon request or is publicly available on the Zenodo data repository (https://doi.org/10.5281/zenodo.7411198).

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