Remotely sensed canopy nitrogen correlates with nitrous oxide emissions in a lowland tropical rainforest

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Abstract. Tropical forests exhibit significant heterogeneity in plant functional and chemical traits that may contribute to spatial patterns of key soil biogeochemical processes, such as carbon storage and greenhouse gas emissions. Although tropical forests are the largest ecosystem source of nitrous oxide (N_2O), drivers of spatial patterns within forests are poorly resolved. Here, we show that local variation in canopy foliar N, mapped by remote-sensing image spectroscopy, correlates with patterns of soil N_2O emission from a lowland tropical rainforest. We identified ten 0.25 ha plots (assemblages of 40–70 individual trees) in which average remotely-sensed canopy N fell above or below the regional mean. The plots were located on a single minimally-dissected terrace (<1 km^2) where soil type, vegetation structure and climatic conditions were relatively constant. We measured N_2O fluxes monthly for 1 yr and found that high canopy N species assemblages had on average three-fold higher total mean N_2O fluxes than nearby lower canopy N areas. These differences are consistent with strong differences in litter stoichiometry, nitrification rates and soil nitrate concentrations. Canopy N status was also associated with microbial community characteristics: lower canopy N plots had two-fold greater soil fungal to bacterial ratios and a significantly lower abundance of ammonia-oxidizing archaea, although genes associated with denitrification (*nirS, nirK, nosZ*) showed no relationship with N_2O flux. Overall, landscape emissions from this ecosystem are at the lowest end of the spectrum reported for tropical forests, consist with multiple metrics indicating that these highly productive forests retain N tightly and have low plant-available losses. These data point to connections between canopy and soil processes that have largely been overlooked as a driver of denitrification. Defining relationships between remotely-sensed plant traits and soil processes offers the chance to map these processes at large scales, potentially increasing our ability to predict N_2O emissions in heterogeneous landscapes.

Key words: Costa Rica; denitrification; ecosystem function; imaging spectroscopy; microbial community; nitrogen cycling; plant traits.

Introduction

Tropical forest ecosystems are characterized by extremely high plant phylogenetic, structural and chemical diversity (Townsend et al. 2008, Asner and Martin 2010). Despite increasing recognition that this diversity can drive spatial heterogeneity in local soil nutrient cycling (Binkley and Giardina 1998, van Haren et al. 2010, Keller et al. 2013), the magnitude and mechanisms by which plant traits influence important soil biogeochemical processes remain poorly defined at scales beyond individual trees (van Haren et al. 2010, Waring et al. 2015). Tropical biomes, especially tropical forests, account for over two thirds of global production of greenhouse gas nitrous oxide (N_2O; Bai et al. 2012). N_2O is produced primarily during the soil microbial processes of nitrification and denitrification, the rates of which are influenced by inputs of carbon (C) and nitrogen (N), soil physical conditions and microbial community composition (Firestone and Davidson 1989, Butterbach-Bahl et al. 2013). Plant soil feedbacks can affect each of these factors (Eviner and Chapin 2003, Legay et al. 2014), and the effects of individual species on soil N processing have been documented in both temperate (Lovett et al. 2004) and tropical forests (Ewel 2006, Russell and Raich 2012, Keller et al. 2013). However, it has been difficult to link tropical canopy chemistry to soil process at spatial scales beyond individual trees, since in most tropical forests biodiversity is extremely high (there may be hundreds of species per hectare) and the majority of species are locally rare (Phillips et al. 1994). Thus, while it is possible that spatial patterns of plant chemical and functional diversity influence spatial patterns of N_2O production in tropical forests, robust evidence for such a link has been elusive. Nevertheless, characterizing the patterns and mechanisms underlying spatial heterogeneity in denitrification fluxes is essential to accurately scale fluxes from small chambers to the landscape, as well as to predict changes in emissions driven by altered plant productivity or stoichiometry under global change.

Determining the significance of plant chemistry/soil process relationships at the ecosystem level is complicated by the fact that both of these variables are challenging to sample intensively at large scales, especially in relatively inaccessible areas such as tropical forests (Martin et al. 2018). Recent advances in remote sensing image spectroscopy allow the collection of high spatial-resolution canopy chemistry data at the ecosystem scale, which can be used to identify links between the canopy and the soil below. For example, Asner and Vitousek (2005) used remote sensing to map a doubling of canopy N associated with invasion of *Myrica faya* into Hawaiian montane forest. Combining these maps with spatial field sampling, Hall and Asner (2007) generated predictive relationships to describe how high density invasive stands increase soil N cycling rates and N oxide (N_2O and NO) emissions. In our study area in southern Costa Rica, forests are characterized by hundreds of species per hectare (compared with just a few dominants in Hawai'i) and imaging spectroscopy has identified wide variation in canopy N (Balzotti et al. 2016) that is linked to differences in soil N availability (Osborne et al. 2017). Thus, this site offers potential to test links between canopy N and soil N_2O fluxes in a diverse tropical forest.

Differences in species chemistry might influence denitrification rates on small spatial scales via several demonstrated or hypothesized mechanisms. Tree species differ in the quantity and quality of C and N inputs via litter, which drive differences in soil organic matter mineralization rates that in turn control the supply of inorganic N substrate for denitrification (Binkley and Giardina 1998). Interspecific spatial variation in leaf litter C:N ratios has been shown to drive variability in soil C:N ratios in tropical forests (Uriarte et al. 2015), as well as C mineralization and inorganic N availability beneath individual canopies (Keller et al. 2013). In French Guiana, Fromin et al. (2013) found that denitrification enzyme activity varied most strongly at small (1 m) spatial scales along with soil C and N, interpreted as reflecting localized differences in litter input chemistry. Soil microbial communities are also structured by tree species identity, with Barberán et al. (2015) observing spatial correlations between plant and microbial community composition independent of abiotic conditions at scales of 2–20 m in a Panamanian tropical rainforest. In Amazonia, species identity was by far the strongest predictor of N_2O emissions beneath individual trees (van Haren et al. 2010). Emissions correlated negatively with tree growth rates and increased with C additions, leading the authors to conclude that denitrification may be reduced by strong plant competition for N or increased in species with greater belowground C inputs. This is supported by data from Costa Rica in which forest tree growth rate also correlated with N_2O fluxes (Weintraub et al. 2014), and variation in biomass accumulation between species correlated negatively with total system N losses (Russell and Raich 2012). Finally, tree species have the potential to affect redox conditions - Wieder et al. (2008) observed that large species-specific differences in litter solubility between tropical trees strongly affected rates of respiration when applied to soil, with potential to alter microsite oxygen availability.

Rates of nitrogen cycling and denitrification are also driven in part by the composition of the soil microbial community, including the abundance and expression of specific N-cycling related functional genes. However, our understanding of processes associated with N_2O production continues to expand. In addition to production during nitrification and denitrification, N_2O can also be produced by a suite of microbial processes that include nitrifier denitrification and dissimilatory nitrate reduction to ammonium (Hu et al. 2015). Heterogeneity in plant diversity, soil chemistry and fluctuating soil redox conditions make these associations especially challenging to parse in tropical soils (Pett-Ridge et al. 2006, Pajares and Bohannan 2016). Ammonia oxidation, the first step in autotrophic nitrification, is thought be driven largely by archaea (AOA) in the wet tropics, though studies vary in finding correlations between AOA abundance and process rates (Yao et al. 2011, Pett-Ridge et al. 2013, Wieder et al. 2013). Quantification of molecular markers (for genes encoding enzymes that catalyze steps in the denitrification pathway, such as nitrite reductase; *nirS, nirK*; and nitrous oxide reductase; *nosZ*), has shown that denitrifier abundance varies with pH, C and N availability and nitrate concentrations in tropical forest soils (Stone et al. 2015, Pajares and Bohannan 2016). However, the few studies that have looked for relationships between denitrification gene abundance and gas emission rates within tropical ecosystems have generally not found clear relationships (Liu et al. 2013, Pajares and Bohannan 2016).

On the Osa Peninsula of Costa Rica, lowland tropical rainforests are characterized by high tree diversity and considerable variability in canopy N concentrations, which have been mapped by airborne imaging spectroscopy (Balzotti et al. 2016). These data were used to identify plots in the landscape (0.25 ha) with high or low canopy foliar N concentrations (above or below the regional mean) that consisted of assemblages of between 40 and 70 individual trees and their associated understory community. Compared with lower-foliar N species assemblages, high foliar N patches have substantially lower litter C:N ratios, significantly higher rates of mineralization and nitrification and greater soil nitrate concentrations (Osborne et al. 2017). In this study, we revisited these high and low foliar N plots to test the following hypotheses: (1) high foliar N plots with greater rates of soil N cycling generate elevated N_2O emissions; and (2) elevated N_2O emissions are associated with distinct microbial community characteristics, such as greater abundance of functional genes associated with N_2O production and/or lower abundance of functional genes involved in N_2O reduction. Finally, we discuss potential for remotely-sensed variables to be used to better predict rates of soil processes.

MATERIALS AND METHODS

Sampling site

Field sampling for this project was conducted in mature, primary lowland tropical rainforest on the Osa Peninsula in southwest Costa Rica, adjacent to the Osa Conservation Piro Biological Station (8°24′42″ N, 83°20′00″ W). The study area has no documented history of extensive human land-use or deforestation. The site experiences mean annual

temperature of ~26°C and mean annual rainfall of ~3,400 mm with a dry season typically extending January–March, and frequent heavy rains for the rest of the year (peaking in September and October). In the measurement year (October 2016–September 2017) the site received ~5,000 mm of rain. Soils around Piro are Ultisols (mostly Typic Tropohumults) derived from basaltic and andesitic volcanic debris, and all sampling occurred on a single uplifted and relatively undissected marine terrace, ~100 m above sea level. Air temperature and precipitation were monitored in a clearing adjacent to the study sites at ten-minute intervals using a HOBO Microstation Data Logger (Onset, Bourne, MA).

Sampling was conducted in 10 established 0.25 ha plots within an area of 1 km^2 that represent areas of high (on average, 1.3 standard deviations above the regional mean, $n = 5$) or low (on average 0.4 standard deviations below the

regional mean, $n = 5$) foliar N of emergent canopy trees (Table 1, Fig. 1, Osborne et al. 2017). Remotely-sensed foliar N in high or low foliar N plots averaged 1.9 ($\pm$ 0.2) % or 2.9 ($\pm$ 0.2) %, respectively (Table 1). These plots were initially identified using high-fidelity imaging spectrometer data collected by the Carnegie Airborne Observatory's Airborne Taxonomic Mapping System in February 2012 (Fig. 1). Plot selection is described in detail in Osborne et al. (2017). In addition to foliar chemistry, plots differ significantly in metrics of soil N content and cycling. These characteristics are summarized in Osborne et al. (2017) and in Table 1. An ordination plot generated using NMDS suggested that emergent tree community composition differed between plot types, and all plots contain few putative N fixing species (Osborne et al. 2017). Average litter C:N ratios are greater for low N (42) than high N (32) plots (Osborne et al. *in preparation*).

TABLE 1. Characteristics of ten 0.25 ha high and low canopy nitrogen plots. Values are means $\pm$ 1 SD (brackets) and values in bold differ significantly between cover types ($P < 0.05$). "Relative canopy N" is the number standard deviations by which remotely-sensed foliar N in each plot differs relative to the landscape terrace mean. Soil values are for 0–10 cm.

Plot type	Canopy foliar N (%)	Relative canopy N (SD)	Bulk soil N (g/kg)	Bulk soil C (g/kg)	pH†	[NH$_4^+$] (mg/kg)†	[NO$_3^-$] (mg/kg)†	Net min (mg kg^{-1} d^{-1})†	Net nit (mg kg^{-1} d^{-1})†
High N	**1.9 (0.2)**	**+1.3 (0.3)**	4.8 (0.29)	52 (4.0)	5.8	1.8 (1.1)	**2.1 (1.3)**	**6.4 (0.55)**	**7.8 (0.62)**
Low N	**2.9 (0.2)**	**−0.4 (0.3)**	5.0 (0.37)	59 (6.3)	5.7	2.1 (1.3)	**0.2 (0.2)**	**0.56 (0.34)**	**1.4 (0.50)**

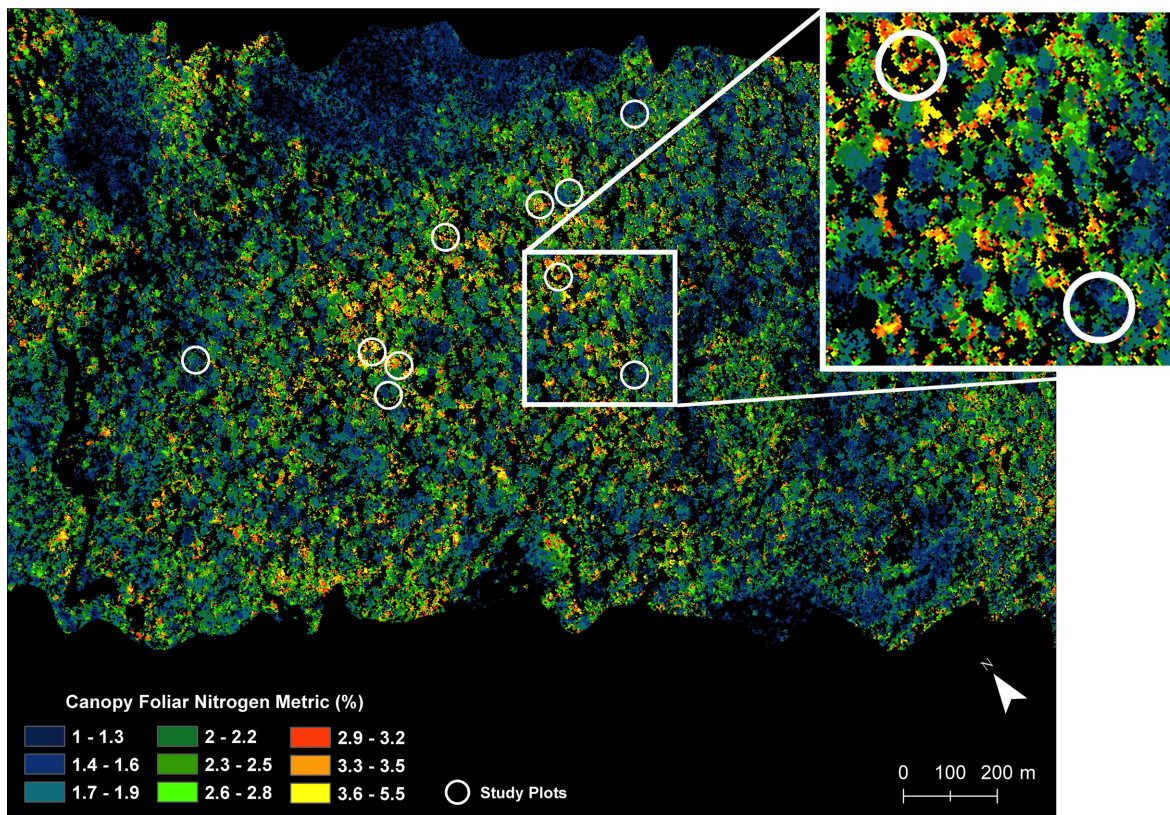*Note:* †Data from Osborne et al. (*in preparation*).

FIG. 1. Canopy foliar nitrogen map for the lowland tropical rainforest study region on the Osa Peninsula of southwest Costa Rica. Image generated by imaging spectroscopy from the Carnegie Airborne Observatory. White circles indicate the locations of 0.25 ha study plots. Inset highlights examples of high and low foliar nitrogen plots.

Gas flux sampling

In situ N$_2$O and CO$_2$ fluxes were measured monthly for 1 yr, beginning October 2016. Thirty gas collars (22 cm diameter, 12 cm high) were installed ~2 cm into the soil in October 2016. In each plot ($n = 5$ high N, $n = 5$ low N), three collars were installed ~5 m from the center of the plot, distributed at approximately equal distance from one another. One high foliar N study plot was extensively colonized by leaf cutter ants during the course of the study and was excluded from trace gas analysis.

Chambers were sealed with lids made from 22 cm PVC sewer end caps with butyl rubber gaskets. Collars were beveled at the top edge to fit within the gaskets and ensure a seal. Chamber lids were fitted with brass bulkhead union fittings (Swagelok, Solon, OH) and 9.5 mm Thermogreen septa (Sigma-Aldrich, St. Louis, MS, USA) and vented with 13 cm (7 mm internal diameter) Tygon tubing vents (Sigma Aldrich) to equilibrate pressure. Headspace samples (17 mL) were withdrawn at 0, 15, 30 and 45 min using a syringe and transferred to evacuated 12 mL glass vials (Labco Limited, UK). Soil moisture was measured gravimetrically for the center of each study plot twice per month for the duration of the experiment. Water filled pore space was calculated using bulk density measurements from Weintraub et al. (2015), sampled on the same terrace.

Gas samples were analyzed for N$_2$O and CO$_2$ using a gas chromatograph fitted with flame ionization and electron capture detectors (Shimadzu GC-2014, Shimadzu Corp. Kyoto, Japan) at the University of Nevada, Reno. Two sets of identical N$_2$O standards were created in the Reno lab prior to the beginning of the project. One set remained at the lab and the second accompanied the vials to Costa Rica and was analyzed with field samples to assess vial integrity.

To determine chamber volume, inside depths of the collar were measured at four points and averaged. Fluxes were calculated based on the linear rate of increase in concentration over the four time points. Fluxes were calculated by multiplying average monthly values for each plot by measurement interval and summing values to arrive at an annual total.

Molecular analysis

In May 2017, five soils cores (0–10 cm) from each plot were combined to form one composite sample per plot that was homogenized by hand to break up large aggregates and remove large roots. Soil was kept on ice for 3 d during transport. Genomic DNA was extracted from ~0.25 g soil using a MoBio DNeasy Powersoil Kit (MoBio, Carlsbad, CA, USA). Extracted DNA, in elution buffer, was stored at −80°C prior to analysis. Duplicate subsamples were dried for 3 d at 90°C to determine soil moisture.

Abundance of microbial genes was measured by real-time quantitative PCR (qPCR) quantification. DNA sample concentrations were quantified using Quant-iT DNA Assay Kit (Invitrogen, Thermo Scientific, Villebon/Yvette, France) and diluted to a target concentration of 1 ng/µl with UP water. qPCR assays were used to measure abundance of the following group markers: bacterial 16S rRNA (as a proxy for total bacterial community; Muyzer et al. 1993), thaumarchaeal and crenarchaeal 16S rRNA (as a proxy for

archaeal community size; Ochsenreiter et al. 2003, Gantner et al. 2011), internal transcribed spacer regions of fungi (as a proxy for fungal community size (White et al. 1990, Schoch et al. 2012), bacterial and thaumarchaeal catalytic subunits of ammonia monooxygenase (*amoA*; Leininger et al. 2006, Tourna et al. 2008), cytochrome *cd$_1$* heme nitrite reductase (*nirS*) or Cu-containing nitrite reductase (*nirK*; Henry et al. 2004, Kandeler et al. 2006) and nitrous oxide reductase (*nosZ*I and *nosZ*II; Henry et al. 2006, Jones et al. 2013).

Reactions were carried out using a ViiA7 (Life Technologies, Villebon/Yvette, France). Quantification was based on the increasing fluorescence intensity of the SYBR Green dye during amplification. The real-time PCR assay was carried out in a 15 µl reaction volume containing the Takyon low ROX SYBR 2X MasterMix blue dTTP (Eurogentec, Angers, France), 1–2 µM of each primer, and 3 ng of DNA. Two independent quantitative PCR assays were performed for each gene. Standard curves were obtained using serial dilutions of linearized plasmids containing the studied genes. PCR efficiency for the different assays ranged between 77–99%. For each qPCR assay, two to three no-template controls were run. The presence of PCR inhibitors in DNA extracted from soil was estimated by mixing a known amount of plasmid DNA with soil DNA extract or water prior to qPCR. The measured cycle threshold (Ct) values obtained when quantifying the added plasmid DNA were not significantly different between the different soil DNA extracts and the controls with water, which indicates that no inhibition occurred.

Statistical analysis

Statistical analyses were conducted in Graphpad Prism 7.0 (GraphPad Software, La Jolla, USA). To analyze differences between high and low N plots over time for moisture and gas fluxes, a repeated measures mixed model was used where cover type and date were main effects and plot and collar were a random effect nested within cover type. Residuals were inspected to assess normality. Where data did not meet assumptions of normality required for parametric tests, values were log transformed. Two-tailed *t*-tests were used to compare soil characteristics, qPCR values and DNA concentrations per unit soil, and annual total fluxes of gases. Significance was set at alpha = 0.05.

Results

Trace gas fluxes

Soil emissions of N$_2$O and CO$_2$ showed strong seasonality, with lowest emissions during the dry season (December-April) when soil moisture was at a minimum (Fig. 2a–c). When grouped categorically, high foliar N plots emitted a total of 0.41 ± 0.13 kg N$_2$O-N ha^{-1} yr^{-1} (mean ± 1 SD), exceeding by three-fold the average emissions from soils in the low foliar N plots (0.13 ± 0.04 kg N$_2$O-N ha^{-1} yr^{-1}, Fig. 2b). Though CO$_2$ emissions were, on average, 1100 kg C ha^{-1} yr^{-1} (~11%) greater in low N plots than in high N plots, this difference was not statistically significant (Fig. 2c). There was no significant temporal correlation

between soil KCl-extractable NO_3^- and NH_4^+ concentrations and N_2O fluxes (data not shown).

Soil characteristics and functional gene abundance

Though pH, total C and N, and NH_4^+ were not significantly different between high and low canopy N plot soils, concentrations of soil NO_3^- and average annual rates of mineralization and nitrification were significantly elevated in high N plots (Table 1). Soil moisture was significantly greater in the low N than high N plots (repeated measures mixed model, $P < 0.02$, $r^2 = 0.84$).

Several measures of soil microbial community abundance differed between high and low foliar N plots (Fig. 3). In particular, low N plots had twice as many fungal internal transcribed spacer (ITS) copies, a proxy for fungal community size (Fig. 3a). Because the abundance of bacteria (as measured by bacterial 16S rRNA gene copies) was comparable between cover types, this led to a substantially lower bacterial to fungal (16S/ITS) ratio in low N plots (7.5, vs. 15.0 in high N plots). Abundance of the *amoA* gene in archaea, but not in bacteria, was almost a factor of ten greater in high foliar N plots ($P < 0.02$, Fig. 3b). Abundance of denitrification genes *nirS*, *nirK*, *nosZ*I and *nosZ*II did not differ significantly between cover types ($P > 0.05$). Extractable DNA per unit dry soil was not significantly different between high and low N plots ($P > 0.05$, data not shown), and results thus did not differ when data were analyzed on a per unit dry soil basis. Finally, abundance of archaeal *amoA* in soil samples showed a significant relationship with average annual soil nitrate concentrations and N_2O emissions on a plot basis (Fig. 3c).

DISCUSSION

We found clear evidence that heterogeneity in canopy foliar N content at the scale of tens of meters strongly influences rates of soil N_2O emissions, correlating with three-fold
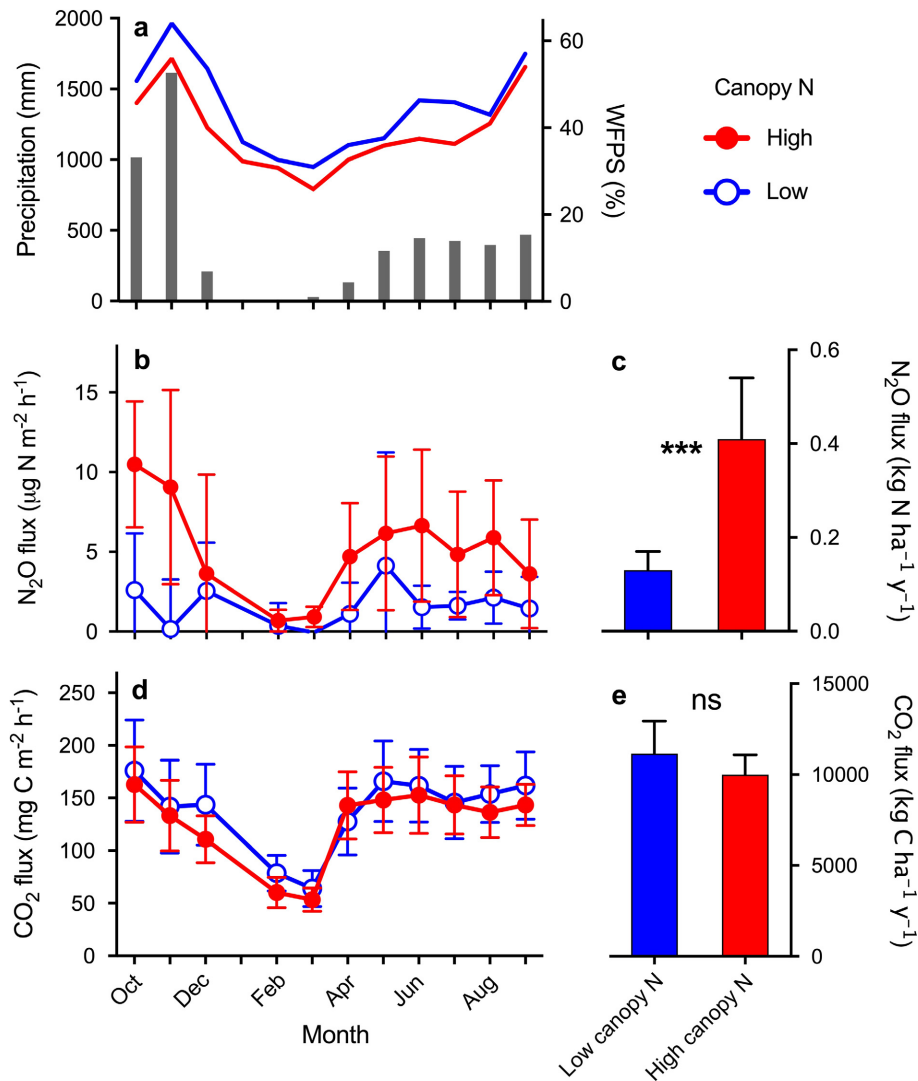

FIG. 2. (a) Monthly precipitation and water filled pore space, (b) monthly; and (c) total annual fluxes of N_2O, (d) monthly; and (e) total annual fluxes of CO_2 from high and low foliar nitrogen plots from October 2016–September 2017. Each point value represents the mean ± 1 SD of 15 collars per cover type.
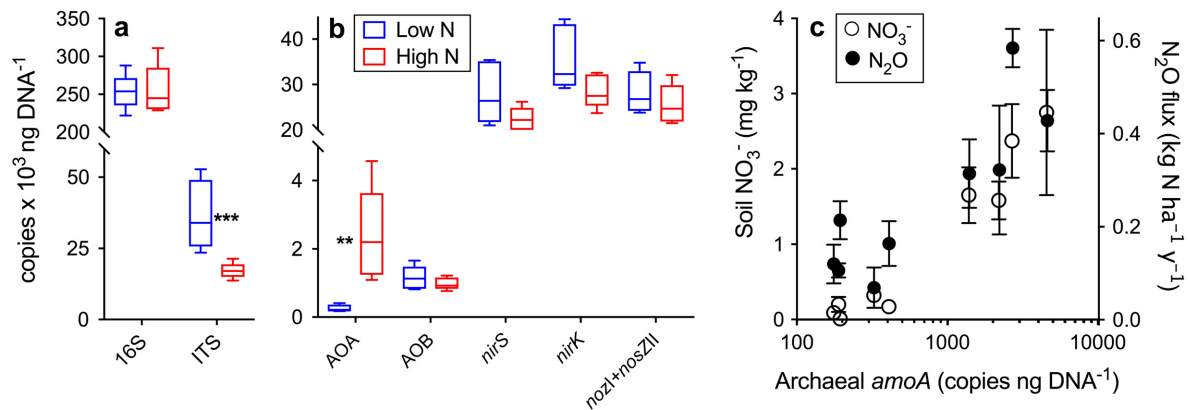
Fig. 3. Abundance and ratios of markers and functional genes between high and low canopy nitrogen plots, and relationship to soil N. (a) 16S: bacterial 16S rRNA, ITS: fungal internal transcribed spacer regions, (b) AOA and AOB: archaeal and bacterial catalytic subunits of ammonia monooxygenase, respectively, *nirS*: cytochrome cd_1 heme nitrite reductase, *nirK:* copper-containing nitrite reductase, *nosZ*I and *nosZ*II: nitrous oxide reductase. (c) Relationship between archaeal *amoA* abundance and average annual soil nitrate concentrations and N₂O emissions. *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$.

differences in average emissions even within a single forest with constant topographic and climatic conditions. In areas with higher canopy N, greater N₂O emissions coincided with greater soil nitrate concentrations and nitrification rates and a 10-fold greater abundance of archaeal ammonia oxidation genes, though not an increased abundance of functional genes associated with denitrification. In addition, the overall very low N₂O emissions observed even in the "high" N plots adds to research showing that tropical forests do not uniformly lose large quantities of inorganic N via gaseous or hydrologic pathways (Soper et al., 2017, Taylor et al. 2015b). Controls over denitrification rates in tropical systems are much more poorly resolved than in temperate systems (Xu et al. 2013). This finding adds to evidence that plant traits may be an underappreciated driver of spatial heterogeneity across a range of scales.

Mechanisms linking canopy N and flux rates

Tree assemblages with high foliar N are characterized by markedly different litter stoichiometry (C:N ratio = 32 in high N vs. 42 in low N plots; Osborne et al. *in preparation*). These differences are reflected broadly in the relative abundances of different taxa within the microbial community. While the size of the bacterial community was similar between plots, there was two-fold greater fungal abundance (as measured by qPCR) in soils of low N plots. Though quantification of fungi using qPCR has inherent limitations, our observations are consistent with broad global relationships between fungal:bacterial ratios and substrate stoichiometry (Waring et al. 2013). Generally, as C:N ratios increase, the decomposer community shifts toward organisms (fungi) with higher biomass C:N ratios (Waring et al. 2013). Concurrently, shifting limitation in the microbial community (from energy to N) drives a pattern where net N mineralization decreases while C mineralization (CO₂ flux) increases, as observed in tropical forests of Puerto Rico (Erickson et al. 2001) and in this study. In Puerto Rico, these links between foliar stoichiometry and mineralization also showed clear continuous relationships with nitrification

rates, soil nitrate concentrations and N₂O emissions (Erickson et al. 2001), while in another Costa Rican study, Russell and Raich (2012) also observed that tree species with greater foliar N promoted faster overall turnover of soil N.

In this study, high foliar N plots had higher rates of nitrification, and greater total soil nitrate concentrations than the low N plots. Mechanistically, this observation is consistent with the ten-fold greater in abundance of ammonia-oxidizing archaea (AOA) in these soils, and correlations between AOA abundance and nitrification rates have been observed at other tropical forest sites (Wieder et al. 2013). While the relative contribution of AOA vs. AOB (ammonia-oxidizing bacteria) to nitrification unclear, these data suggest that AOA may be more related to process rates in this system, and support previous studies suggesting niche differentiation between the two groups (Prosser and Nicol 2012). At these sites, and across many other field studies, the abundance of functional genes associated with denitrification (*nirS*, *nirK*, *nosZ*) did not correlate with N₂O fluxes (e.g. Liu et al. 2013). Liu et al. 2013). After analysis of more than 59,000 N₂O field measurements, Domeignoz-Horta et al. (2017) found that only high emissions rates were explained by variation in abundance and diversity of microbial communities, while rates of N₂O emissions measured in this study fell at the low end of the observed range.

We found no evidence that canopy N divergence between our plots was associated with physical differences that might explain differential gas flux, such as topography, parent material, or soil pH (Osborne et al. 2017). Instead, these differences are consistent with clear variation in plant functional traits, namely canopy (and resulting litter) stoichiometry. Though low N plots were associated with slightly greater water filled pore space, we hypothesize that this may be driven by differences in vegetation transpiration rate (higher foliar N has been associated with greater growth rates in tropical trees; Cernusak et al. 2009) rather than by physical soil properties (clay content, for example, did not differ between an overlapping set of high and low canopy N plots types; Osborne, *unpublished data*). However, without more detailed characterization, we cannot currently rule out that some
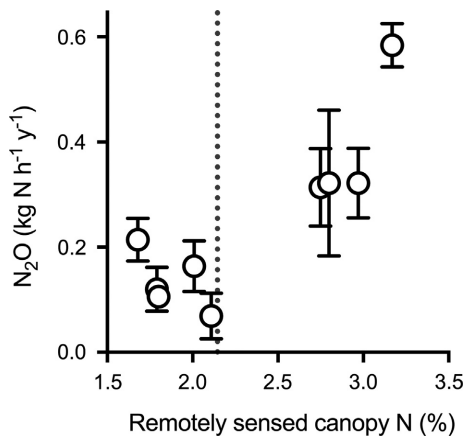
FIG. 4. Regressions between total annual N_2O flux and remotely-sensed canopy foliar nitrogen content on a per-plot basis. Values are means $\pm$ 1 SE. Dotted line is the mean remotely sensed canopy %N value for the study terrace pictured in Fig. 1.

differences in soil texture may contribute to formation of the varied community assemblages that we observe here. Functionally, these moisture differences are unlikely to explain differences in nitrification, as tropical AOA (strongly associated here with variation in nitrification rates) have been shown to be relatively insensitive to soil oxygen conditions (Pett-Ridge et al. 2013). Though relationships are broadly non-linear, higher soil moisture within the observed range of WFPS (30–60%) would tend to increase, rather than decrease the proportion of N_2O emitted from the soil based on observations from a variety of other tropical systems (Keller and Reiners 1994, Breuer et al. 2000, Erickson and Davidson 2002, Werner et al. 2007), and is thus unlikely to explain the observed flux differences. It is also possible that other plant traits associated with species assemblages that differ in foliar N may influence or reinforce the differential soil N cycling we observed. For example, species with high N uptake rates can reduce N availability to microbes via competition (van Haren et al. 2010, Moreau et al. 2015), potentially reducing substrate available for denitrification. In addition, the quality and magnitude of total C inputs to the soil have been shown to not only stimulate denitrification but also affect the N_2O/N_2 denitrification end product ratio (Henry et al. 2008, van Haren et al. 2010). Thus, observed differences in gas flux may not arise wholly from a single plant trait (canopy N), but may be influenced by suites of traits that co-occur.

An obvious question is why some areas of the landscape have elevated foliar N, at the scale of tens of meters. These differences are not readily explained, for example, by differing abundance of N-fixing trees (Osborne et al. 2017), although there is evidence that community composition of canopy emergent trees is distinct (as would be expected given that foliar N is largely a phylogenetically-conserved trait; Asner et al. 2014). Instead, we hypothesize that these differences arise from plant-soil feedbacks that reinforce population- or community-level processes governing species assembly (Osborne et al. 2017). Specifically, once some high foliar N species establish in a small area, litter from these species may influence soil N cycling to the extent that patches with higher soil N availability are created (Keller et al. 2013), potentially favoring ongoing preferential

recruitment or growth of high-N affinity species (John et al. 2007). Generally, wide variation in foliar N between species is characteristic of mature tropical forests, and not unique to this site — "low" N plots examined in this study had average foliar N values (1.7%) equivalent to the global mean, while average values for high N plots in this study (~2.9%) fell toward the higher end (though certainly not the maximum) of the global range (Townsend et al. 2007).

Rates in context

Though tropical forests are broadly considered a strong source of N_2O to the atmosphere (Vitousek and Matson 1992, Bai et al. 2012, Brookshire et al. 2017), emission rates also vary widely by up to two orders of magnitude among sites, from 0.1 to > 9 kg N_2O-N ha^{-1} yr^{-1}, with a reported mean of 2.8 kg N_2O-N ha^{-1} yr^{-1} (Erickson et al. 2001, Castaldi et al. 2013). We measured N_2O flux rates ranging from 0.13 to 0.41 kg N_2O-N ha^{-1} yr^{-1} in low and high canopy N areas, respectively, and we note that the average canopy N of this landscape sits closer to values categorized as "low", suggesting that high foliar N areas are more akin to hot spots on the landscape. Though summing monthly measurements is insufficient to generate an accurate annual flux, due to the potential to miss episodic events, there are very few continuous annual measures of N_2O in any tropical forest (Barton et al., 2015), so that the majority of estimates are generated by methods similar to this one (Castaldi et al. 2013). In that context, these rates fall at the low end of the global range, and are consistent with the lower end of the range measured by Weintraub et al. (2015) (0.4 kg N_2O-N ha^{-1} yr^{-1}) at a site adjacent to our study area.

In considering tropical forests as a source of N_2O, Vitousek and Matson (1992) suggested that these ecosystems should be treated as a continuum rather than category, reflecting their broad gradients in climate, soil fertility and disturbance. This study contributes further evidence that some very wet tropical forests are characterized by low total losses of biologically-available N. However, these forests do not apparently fall on the "low fertility" end of the gradient mentioned by Vitousek and Matson (1992). Instead, they are extremely productive (hosting some of highest biomass C densities in the Neotropics; Taylor et al. 2015a), with rapid decomposition rates and turnover of N (Wanek et al. 2008, Wieder et al. 2009) that may promote retention of N within the system (Soper et al., 2017). Significant hydrologic losses of particulate N associated with the high rainfall and sometimes steep topography of these forests (Taylor et al. 2015b) may also serve to constrain N availability in the system relative to the high demand over time and thus reduce N losses. A complete watershed N budget for the northern end of the Osa Peninsula suggests that the vast majority of N loss from this system (~75%, of a total ~20 kg N ha^{-1} yr^{-1}) occurs as biologically-inaccessible particulate N, rather than soluble forms (Soper et al., 2017). It is currently unclear how common this co-occurring suite of traits may be across other lowland tropical forests, but the fact that low (<1 kg N_2O-N ha^{-1} yr^{-1}) N_2O emissions are not uncommon (Erickson et al. 2001, Wieder et al. 2013, Hassler et al. 2017) suggests that widespread controls may need to be reassessed to come up with a better predictive framework for emissions.

Scaling

The ability to use remotely-sensed data to describe or predict rates of key soil biogeochemical processes on large scales has clear potential to be a transformative tool. Such extrapolations require establishment of robust relationships between parameters. While this study found clear differences in N_2O emissions correlating with canopy chemistry on relatively small scales (<1 km^2), our approach focused on assemblages with relatively divergent canopy chemistry. Therefore, our sampling design did not comprehensively span the middle range of canopy N necessary to generate robust, predictive relationships, and because the data were not fully continuous, we were not able to confidently determine the exact shape of the relationship between canopy N and N_2O emissions in Fig. 4. As the landscape average canopy N falls closer to the "low N" plots in this study, it seems likely that the high N plots represent "hot spots" on the landscape. This suggests that an exponential, or possibly threshold response, best describes the relationship between the variables. Support for this comes from Hall and Asner (2007), who adopted a remote-sensing/field sampling coupled approach analyzing the effect of a high N invasive legume on soil N_2O and NO emissions, and found that changes in soil N_2O emissions responded exponentially to changes in foliar N concentrations.

We conclude that the strong relationships between canopy N and N_2O emissions offer possibilities for using remotely-sensed canopy chemistry data to describe, map and predict patterns of soil processes. Though this study sought specifically to isolate the effect of canopy chemistry as driver of N_2O emissions, including this variable in models along with factors such as landscape topography that are well known to influence emissions (Groffman et al. 2009, Wolf et al. 2011) may improve spatial predictions of N_2O emissions, especially in areas where other data may be limited. This is increasingly true as the broader applicability of spectronomics-based approaches for retrieving foliar chemical trait data is demonstrated for diverse tropical ecosystems (Martin et al. 2018).

Author Contributions

All authors conceived of or designed the study. FMS, MKN, BBO, DB, CB and BWS performed the research and analyzed samples. FMS analyzed the data. All authors interpreted results and contributed to the MS - writing led by FS.

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