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Plant uptake and stream chemistry set global bounds on nitrogen gas emissions from humid tropical forests

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Key points

- Microbial denitrification represents a potentially large loss of N that could limit plant productivity across humid tropical forests
- Ecosystem modeling and stream chemistry data suggests that gas emissions dominate (>45-82%) bioavailable N exports from tropical forests
- Plant uptake of N required to maintain observed net primary production sets an upper bound on gaseous N loss from tropical soils

Abstract

Denitrification and hydrologic leaching are the two major pathways by which nitrogen is lost from the terrestrial biosphere. Humid tropical forests are thought to dominate denitrification from unmanaged lands globally, but there is large uncertainty about the range and key drivers of total N gas emissions across the biome. We combined pantropical measures of small watershed stream chemistry with ecosystem modeling to determine total nitrogen gas losses

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and associated uncertainty across humid tropical forests. Our calculations reveal that denitrification in soils and along hydrologic flowpaths contributes on average >45% of total watershed N losses. However, when denitrification occurs exclusively in shallow soils, simulations indicate that gas emissions would exceed N inputs and render plants severely N-limited, which contradicts observations of widespread N-sufficiency in tropical forests. Our analyses suggest an upper bound on soil denitrification of ~80% of total external N losses beyond which tropical plant growth would be compromised.

1. Introduction

Humid tropical forests cover <10% of the global land surface yet dominate terrestrial exchanges of energy, water and carbon with the atmosphere [*Pan et al*., 2011; *Wohl et al*., 2012; *le Quéré et al*., 2015]. These flows are accompanied by the largest natural fluxes of nitrogen (N) between land and atmosphere globally. Annually, humid tropical forests transform more N_2 via biological N_2 fixation, lose more dissolved inorganic N (DIN) to rivers and return more N gas to the atmosphere via denitrification than any other forest biome [*Hedin et al*., 2009; *Bai et al*., 2012; *Brookshire et al*., 2012a; *Cleveland et al*., 2013]. These large N fluxes influence global climate by fueling plant drawdown of CO_2 and microbial production of nitrous oxide (N_2O), both powerful greenhouse gases. Understanding these processes is essential for climate forecasting [*Houlton et al*., 2015; *Thomas et al*., 2015] and advances hinge on how well we understand how inorganic N losses in tropical soils are partitioned among plant uptake, hydrologic leaching and gaseous emissions [*Houlton and Bai*, 2009]. Unfortunately, all three of these N sinks are poorly constrained in current global models due to a dearth of field data and because considerable uncertainty exists on how best to conceptualize plant uptake and microbial denitrification [*Thomas et al*., 2015], particularly

whether they behave as competitive sinks within the soil or avoid competition by being separated in space and/or time across the landscape.

Global mass-balance [*Bouwman et al*., 2013] and isotope-based models [*Bai et al*., 2012] identify terrestrial soils as the dominant source of N gas emissions, though significant denitrification can also occur along hydrologic flowpaths below the rooting zone and within stream and river corridors [*Houlton et al*., 2006; *Mulholland et al*., 2008; *Groffman et al*., 2009; *Fang et al*., 2015]. At the watershed scale, DIN retention is most commonly attributed to plant uptake [*Brookshire et al*., 2012a; *Gerber and Brookshire*, 2014], but it remains unclear the extent to which microbial denitrifiers compete with plants for a common nitrate pool in the soil rooting zone or whether they rely on residual soil nitrate not accessed by plants. Tropical forests have been shown to be a significant CO_2 sink [*Pan et al*., 2011; *Brienen et al*., 2015]. Maintaining that function requires a large and sustained supply of bioavailable N [*Cleveland et al*., 2013]. Thus, understanding how N losses from tropical soils constrains plant growth and C sequestration is essential for improving the performance of global models of C and N cycling. In contrast to DIN losses which can be directly estimated at the watershed level, total N gaseous emissions cannot, owing to methodological limitations [*Groffman et al*., 2009]. While recent studies using natural abundance isotopes of nitrate offer a way to constrain total N gas emissions at the watershed level [*Houlton et al*., 2006; *Fang et al*., 2015], these studies have been conducted at only a handful of tropical sites worldwide, making generalization difficult. Here, we apply a novel data-driven modeling approach to resolve watershed-level denitrification and the extent to which it may act as a competitive sink for inorganic N with tropical plants.

In this study, we estimate total N gas emissions across the humid tropics using small watershed budgeting. We take advantage of the chemistry of small streams as they reflect the cumulative up-stream signature of N uptake by plants and microbial processing within

watersheds [*Mulholland et al*., 2008; *Gerber and Brookshire*, 2014; *Helton et al*., 2015]. We combine geographically extensive solute chemistry data spanning broad gradients in elevation, topography, geology and net primary production (NPP) with a model [*Gerber and Brookshire*, 2014] of soil N processing that integrates DIN production via net mineralization, plant N uptake, and solute transport in soils to estimate DIN concentrations in soil water that are available for both leaching and denitrification. Discrepancies between modeled DIN in soil water (after plant uptake) and those observed in the stream can be used to constrain denitrification rates at the small watershed scale. Our goals were to (1) compare the relative sink strength for N among plants, hydrologic leaching and denitrification and their association with environmental and biogeochemical factors and (2) quantify minimum and maximum bounds on gaseous N losses from tropical forests.

2. Methods

2.1. Field Measures

From 2007 to 2016 we sampled a total of 126 small watershed (< 5 km^2) streams distributed across ~900 km^2 of remote mature forests in five countries following protocols in *Brookshire et al*. [2012b]; *Brookshire and Thomas* [2013]. Most data from montane and lowland old-growth forests in Costa Rica (n =51) and Trinidad (n =30) have been reported on previously [*Brookshire et al*., 2012b; *Brookshire and Thomas*, 2013]. These watersheds were sampled seasonally (wet/dry season) over 2007-2008 in Costa Rica (n= 115 samples) and on average three times per stream over 2007-2012 in Trinidad (n =110 samples). We sampled montane and lowland streams (n =26) in Erawan National Park, Sai Yok National Park and the Ratchaburi province in western Thailand over the wet season of 2013; lowland streams (n =15) within the Iwokrama Forest reserve in central Guyana during the short transitional dry season of 2014; and lowland streams (n =5) near the Madre Selva Biological Station in

Amazonian Peru during the wet seasons of 2015 and 2016. For streams sampled multiple times, we used site-averages to decrease over-representation in analyses. For all watersheds we recorded altitude using a hand-held GPS or used literature values and used reported mean annual precipitation. All samples were analyzed using a combination of SEAL Quatro pro chromatography or Dionex Ion chromatography for DIN (NO_3^-, NH_4^+ and NO_2^-) and PO_4^-, Shimadzu elemental analysis for dissolved organic C (DOC) and total dissolved N (TDN), and ICP-OES for cations. Dissolved organic N (DON) was calculated as TDN-DIN.

2.2. Literature Values

To expand the geographical scope of our original samples, we included published data from small streams draining closed-canopy mature forests in which stream chemistry had been sampled over time using comparable methods. We included streams from a lowland forest of Rondonia (*n*=1; [*Neill et al*., 2001]) and montane forests of Atlantic Brazil (*n*=6; [*Andrade et al*., 2011]), a lowland forest of the Osa Peninsula in Costa Rica (*n* =1; [*Taylor et al*., 2015]), and montane forests of Puerto Rico (*n*=3; [*McDowell and Asbury*, 1994]), Malaysia (*n* =1; [*Bruijnzeel et al*., 1993] and Rwanda (*n*=1; [*Rütting et al*., 2015]). To our knowledge, our combined data span the range of reported stream chemistry from the global tropics.

2.3. Ecosystem model

We modeled denitrification using previously developed analytical tools [*Brookshire et al*., 2012a; *Gerber and Brookshire*, 2014]. Our basic framework describes the evolution of total inorganic N (ammonium and nitrate) concentrations in soil water as determined by inputs and first order sink terms:

$$\frac{d[N]}{dt} = \frac{M+A+F_A}{h} - k_p N - k_w N - \varepsilon k_D N \quad (1)$$

Where [*N*] is the combined ammonium and nitrate concentration (kg m^{-3}), *M* is N mineralization (kg m^{-2} yr^{-1}), *A* is atmospheric deposition (kg m^{-2} yr^{-1}), F_A is asymbiotic N_2 fixation (kg m^{-2} yr^{-1}), *h* is the rooting zone depth (defined below), k_p the plant uptake rate (yr^{-1}), k_w the drainage rate per unit rooting depth (yr^{-1}), k_D the first order denitrification rate (yr^{-1}) and ε a binary that indicates whether soil denitrification occurs (ε=1) or not (ε=0). We obtained data for atmospheric N deposition for all watersheds using the average of the years 2001 through 2010 [*Lamarque et al*., 2011]. We estimated N inputs from asymbiotic N_2 fixation (F_A) based on the reported mean and global range for tropical forests [*Reed et al*., 2011]. We assume N inputs from symbiotic N_2 fixation are integrated into *M* as this input is fed directly into plant biomass. We estimate k_w using either reported runoff values or based on mean annual precipitation assuming a mean of 0.5 and range of 0.4 -0.625 of precipitation contributes to soil drainage *Q* (m^3 m^{-2}; [*Schlesinger and Jasechko*, 2014]):

$$k_w = \frac{Q}{h} \tag{2}$$

Turnover of available N in soil is fast, such that quasi steady state can be assumed:

$$\frac{d[N]}{dt} \cong 0 \tag{3}$$

Which yields

$$[N] = \frac{(A+M+F_A)/h}{k_p+k_w+\epsilon k_D} \tag{4}$$

2.4. Net N mineralization

To determine net N mineralization for each watershed, we used NPP and plant stoichiometry roughly following the approach of *Cleveland et al*. [2013]. At the heart of this calculation is that mineralized N is approximated by detrital N inputs to soils, assuming a

system's N accumulation is small compared to its throughput. Thus mineralized N (*M*) is calculated as plant N return

$$M = \sum_i \frac{NPP\, f_i\, (1-r_i)}{S_i} \quad (5)$$

Where *i* represent different tissues (*i* = leaves, stem, coarse roots and fine roots), f_i the fraction of NPP allocated to the respective tissue, r_i the fraction of N retranslocated before turnover, and S_i the C:N ratio for the respective tissue. The fraction allocated to the different tissues, C:N ratios and translocation for broadleaf evergreen trees (representing the tropics) was based on *Cleveland et al*. [2013] but we changed the mean C:N ratio of stems to 275 based on *Martin et al*. [2014]. N retranslocation only occurs in leaves.

To determine NPP for each watershed we used the Moderate Resolution Imaging Spectroradiometer (MODIS) subset tool [*ORNL DAAC*, 2014] available through Oak Ridge National Laboratory. We used the long-term average (2000-2010) NPP for each site coordinate positioned mid watershed at a resolution of 1 km^2. As many of our watersheds within individual countries were geographically clustered, NPP estimates were statistically identical. We thus aggregated subsets to a total of 18 geographically independent NPP estimates (Table S1).

2.5. Plant uptake

Plant uptake (k_p) is calculated based on *Gerber and Brookshire* [2014], which takes into account N transport from the site of mineralization to the root surface and the efficiency of N transport across the root surface:

$$k_p = \frac{2\pi\theta m_r \sigma}{h}\left[\frac{1}{2\phi\kappa} * \left(log\left(\frac{h}{\pi m_r \sigma r^2}\right) - \frac{3}{2}\right) + \frac{1}{p_{root} r}\right]^{-1} \quad (6)$$

Where h is the rooting zone depth, θ the volume fraction of water, m_r the root mass (kg m^{-2}), σ the root length per mass, ϕ the impedance characterizing the tortuous pathway of solutes in the soil, r the root's diameter and p_{root} the uptake velocity at the root surface. These parameters are shown in Table S2. We note that the above equation is simplified assuming that the root volume is much smaller than the soil volume. Fine root mass m_r is estimated based on NPP allocated to roots (f_{root}), a parameter that defines root longevity or turnover (τ_{root}; [*Gill and Jackson*, 2000]), and taking into account a factor that determines the amount of biomass per unit carbon β_{root}, which we fixed at 2:

$$m_r = NPP\, f_{root} \tau_{root} \beta_{root} \quad (7)$$

We further note that plant uptake operates on all N in the dissolved phase following mineralization and thus implicitly integrates dissolved organic and mineral N forms.

2.6. Denitrification

We model denitrification based on whether it occurs within the rooting zone competing with plant uptake and leaching ("parallel" denitrification, D_p, $\varepsilon = 1$ in equation 1), or whether it takes place after soil water leaves the rooting zone ("sequential" denitrification, D_s, $\varepsilon = 0$). In the sequential case, we calculated rates based on the reduction in DIN concentrations observed in soils versus streams:

$$D_s = Q * ([N]_m - [N]_s) \quad (8)$$

where $[N]_m$ is the soil water N concentration calculated based on equation 4 and $[N]_s$ is the observed stream concentration. In contrast, "parallel" denitrification (D_p) was assumed to occur only in the rooting zone of soils, obtained by setting ε=1, a first order denitrification rate that yields observed stream DIN. Using equation 4 yields the following relationship for parallel denitrification:

$$D_p = k_D[N]_s h = A + M + F_A - (k_p + k_w)[N]_s h \quad (9)$$

Parallel denitrification assumes that microbial denitrifiers and plants have equal access to a homogenous pool of soil N, whereas in the 'sequential' case, denitrification only occurs along the flowpath that links the rooting zone to the stream sampling location *after* plant uptake sets available N concentrations. The parallel case requires k_D to be on par with the strong plant sink in order to effectively change concentrations. In contrast, the sequential sink only has to compete against advective transport through the watershed that could carry N away before denitrification can occur.

2.7. Simulations and sensitivity analysis

We calculated mean, range, and probability for denitrification based on Monte Carlo sampling (n =10,000) of a broad defined range of parameter values within and across sites (Table S2). We find that ranges in modeled DIN concentrations from parameter uncertainty at each site are much larger than differences across sites (Figure S2). To incorporate this uncertainty, we performed simulations across all individual watersheds (n=139) with equal weight. Random draws were considered as either a factorial uncertainty or drawn from a specific distribution. In the case of factorial uncertainties, we drew from a uniform distribution in the interval r = (-1,1), and the parameter was then calculated as

$$par = par_o x^r \quad (10)$$

Where *par* is the randomly selected parameter, par_0 is the "best guess", and x the factorial uncertainty. This allowed equal amount of sampling above and below the "best guess", and a mean close to par_0. In order to specify the soil depth, we use the exponential distribution (beta factor; to infer rooting depth. We allow rooting depth to vary between 5 cm and infinity, but assign decreasing probabilities to increasing depths, with $f(z) = 1-\beta^z$, where $f(z)$ is the

cumulative probability density function of total root mass and beta is parameter of the function defining the root distribution with depth [*Jackson et al*., 1997]. We note that *f(z)* fits the description of the exponential function where λ = -log (*β*)

$$f(z) = \frac{dF(z)}{dz} = \lambda \exp(-z\lambda) \quad (11)$$

We used mean and standard deviation of root parameters σ and *r* reported for global tropical forests [*Jackson et al*., 1997]. However, they likely covary and thus independent sampling of both of these parameters from their probability density function would unrealistically inflate the uncertainty of k_p. The covariance can be reduced by taking into account that root mass density is approximately the same across different root class sizes. We calculated the probability (*p*) of overestimating DIN concentrations (i.e., resulting from denitrification) for each watershed as the proportion of cases in which modeled DIN (from the Monte Carlo simulations) > observed DIN. We conducted model sensitivity analysis for all parameters using variance-based methods [*Saltelli et al*., 2010] in two ways. First, we determined for each parameter how much model variance would be reduced by fixing that parameter. Second, for each parameter we determined how much model variance would remain if all other parameters were fixed. We analyzed how stream DIN and *p* varies with DOC using mixed effects modeling with MAP/MASL ratio as a random effect (using the nlme package in R). All analyses were conducted in R [*R Development Core Team*, 2013].

3. Results and Discussion

Stream DIN concentrations varied substantially across watersheds (22-889 μg N/L, global mean =207 μg N/L, *n*= 139; Figure 1) but did not vary systematically with altitude (MASL), mean annual precipitation (MAP), NPP, or with concentrations of rock-derived phosphorus and cations (Table S1; Figure S1) thought to limit plant growth across much of the tropics

[*Townsend et al*., 2011; *Wright et al*., 2011]. In contrast, we document a significant ($P < 0.001$) negative relationship between nitrate (NO_3^-) and DOC and significant ($P < 0.001$) positive relationships between ammonium (NH_4^+) and DOC and between nitrite (NO_2^-) and DOC across watersheds globally (Figure 1). There were no watersheds that simultaneously display high DOC and high NO_3^- or total DIN. These patterns are consistent with the expected imprint of dissimilatory nitrate reduction [*Taylor and Townsend*, 2010; *Helton et al*., 2015]. In particular, the inverse relationship between NO_3^- and $NH_4^+$with increasing DOC is consistent with increasing dissimilatory NO_3^- consumption and NH_4^+ accumulation due to decreasing chemoautotrophic nitrification and/or reduction of NO_3^- to NH_4^+ (DNRA) under low oxygen conditions. Simultaneous increases in NO_2^-, which is held at low concentrations in oxic waters, is also consistent with NO_2^- accumulation during DNRA [*Philips et al*., 2002]. Moreover, DOC: NO_3^- ratios were highest in relatively wet lowland landscapes (high MAP: MASL), environments known to be conducive to reducing conditions and high DOC production [*Groffman et al*., 2009]. Further, the decline in total DIN concentrations ($P = 0.035$) with increasing DOC points to denitrification of NO_3^- to N gas as a vector of permanent N loss across watersheds.

The correlations observed between these solutes are broadly consistent with the signature of microbial denitrification at the watershed level but alone cannot constrain the magnitude of gas emissions relative to N inputs, internal plant-soil fluxes and dissolved losses. Our simulations using NPP and plant tissue specific N data suggest large potential N mineralization inputs (which include inputs from symbiotic N_2 fixation) of 134 -403 kg N ha^{-1} yr^{-1} (2.5 and 97.5% quantiles [$q_{2.5\text{-}97.5}$]) across our forests, which greatly exceed external inputs from N deposition ($q_{2.5\text{-}97.5}$ = 1.4 – 8.9 kg N ha^{-1} yr^{-1}) and asymbiotic N_2 fixation ($q_{2.5\text{-}97.5}$ = 1.1 – 54.2 kg N ha^{-1} yr^{-1}; Methods). By comparison, simulations combining water fluxes with observed stream N concentrations, indicate that our forests export on average 2.7 kg N

ha^{-1} yr^{-1} ($q_{2.5\text{-}97.5}$ = 0.3 -7.9 kg N ha^{-1} yr^{-1}) of DIN and 0.9 kg N ha^{-1} yr^{-1} ($q_{2.5\text{-}97.5}$ = 0.04 -3.0 kg N ha^{-1} yr^{-1}) of dissolved organic N (DON), levels similar to reported global means based on long-term monitoring [*Brookshire et al*., 2012a, 2012b].

Combined, these observations suggest that tropical watersheds lose <2% of DIN production via streams, equating to >98% of N retained or lost via gas emissions. Proportionally low hydrologic N losses could imply N accumulation, yet any net accumulation would be small relative to annual internal (uptake-mineralization) fluxes. Alternatively, we may underestimate ecosystem N losses if NO_3^- is removed by denitrification in soils, along hydrologic flowpaths below the rooting zone and within streams.

To further constrain watershed N losses we independently resolved plant-specific N retention [*Gerber and Brookshire*, 2014] using a small set of solute transport and root parameters (Equation 6, Table S2). A central prediction of the model is the DIN concentration in soil solution remaining after plant uptake that is available for leaching or denitrification. Simulations accounting for full parameter uncertainties indicate that our model over-predicts stream DIN relative to observations, and thus underestimates the watershed-level DIN sink if gas losses are not considered ($\varepsilon = 0$ in equation 1), across most (76%) sites (Figure 2). These patterns are robust to uncertainties in plant N allocation, runoff, soil depth and solute transport, with highest uncertainties in root turnover (which affects root mass/surface area and DIN uptake; Table S2 and Figure S2 and S3). Full Monte Carlo sampling of the uncertainty range revealed that if DIN retention was governed by plant uptake only, concentrations would on average be 84% higher than observed. Moreover, the probability of a missing N sink was highest in watersheds with high DOC (Figure 2), further implicating denitrification as a major pathway of N export across these forests.

Where do these gaseous losses occur? While global patterns in stream DOC-DIN (Figure 1) imply significant denitrifier effects at the watershed level, our analyses suggest a large (>30 fold) range in potential gaseous fluxes depending on whether denitrification is modeled as a 'sequential' process occurring 'downstream' of plant uptake or as a 'parallel' process competing with plant uptake in the rooting zone of soils (Figure 3; Methods). When occurring sequentially, our model estimates that plants acquire 98% of soil N inputs before any external losses occur and 'downstream' denitrification removes an average of 47% of the inorganic DIN that is exported ($q_{2.5\text{-}97.5}$=0.4 -4.6 kg N ha^{-1} yr^{-1}). In contrast, when denitrification and plant uptake occur in parallel, gas fluxes constitute a remarkable 97% of N losses ($q_{2.5\text{-}97.5}$ = 5.1-177.4 kg N ha^{-1} yr^{-1}) and plants acquire on average only 64% of inputs. While N mass balance is resolved in both cases, sequential denitrification never depletes soil DIN and plant uptake matches demand across the full range of N export. This is not the case when denitrification occurs in the rooting zone. When DIN concentrations are set within the rooting zone prior to leaching (ε=1), both plant and denitrifier sinks must be sufficiently strong to account for observed stream water concentrations and gas losses would render plant growth severely N limited across most watersheds (Fig. 3).

Such a large N deficit is difficult to reconcile with observations of widespread N sufficiency [*Hedin et al*., 2009] and net C uptake [*Brienen et al*., 2015] across mature tropical forests. For N inputs to roughly balance such losses would also require rates of symbiotic N_2 fixation that are much higher than field estimates indicate [*Batterman et al*., 2013; *Sullivan et al*., 2014]. Together, these observations suggest that perfect homogeneous access to soil nitrate is unlikely, otherwise NPP would be chronically N limited. We explored the possibility that parallel denitrification occurs at lower realized rates resulting from heterogeneity in the soil environment that would still allow for stoichiometrically balanced plant uptake. We calculate that, on average, up to ~20% of soil DIN could be lost via

denitrification (equating to 82% of total external N losses) within the range of uncertainty for balanced plant uptake (Figure 3) and reasonable estimates of symbiotic N_2 fixation. This fractional contribution and resulting average gas flux (17 kg N ha^{-1} yr^{-1} ; $q_{2.5\text{-}97.5}$ = 1 -35 kg N ha^{-1} yr^{-1}) is consistent with high spatial and temporal variability in rooting zone microsite O_2 concentrations conducive to nitrate reduction [*Liptzin and Silver*, 2015] and also corresponds well with global [*Bai et al*., 2012; *Houlton et al*., 2015] and watershed-scale [*Houlton et al*., 2006; *Fang et al*., 2015] isotope-based estimates which place N gas emissions near 6- 30 kg N ha^{-1} yr^{-1}. By comparison, empirical measures of NO and N_2O fluxes from tropical forest soils average ~1-4 kg N ha^{-1} yr^{-1} globally [*van Lent et al*., 2015], suggesting that our sequential model, in which denitrification occurs below the rooting zone, substantially underestimates total watershed N emissions. These results support the idea that soil denitrification tends to dominate mineral N losses from tropical forests.

While our results account for a wide range of uncertainties, our use of mean stream chemistry data and our analytical approach for estimating plant N uptake may have introduced error into the analysis. First, our geographically extensive sampling approach was designed to encompass a broad array of biophysical features. Yet, stream DIN concentrations can vary substantially within years due to changes in discharge and therefore upscaling from samples collected at different times and frequencies could affect DIN flux estimates. While these effects have been accounted for in most of our original data [*Brookshire et al*., 2012b; *Brookshire and Thomas*, 2013] and for all literature data, it is possible that fluxes from sites sampled only once (Guyana and Thailand) are less accurate. However, our simulations do account for considerable uncertainty in discharge, and thus DIN fluxes, within and across watersheds. Further, though temporal variation is critical for precise estimation of watershed-specific fluxes, it is less important for our analysis because even the highest of DIN fluxes are dwarfed by the size of the internal plant-mineralization cycle.

Second, our model does not explicitly address potential plant uptake of organic N or differentiate between NH_4^+ and NO_3^- but rather integrates uptake of all mobile phase N resulting from mineralization [*Gerber and Brookshire*, 2014] and also implicitly includes simple dissolved organic forms. As denitrification acts on NO_3^- only, it is possible that differential N uptake could affect our estimates. For example, exclusive uptake of NH_4^+ would lead to nitrification as the competing (parallel) process and the key factor in determining k_D. Regardless, the stream data suggest that nitrification is not a rate-limiting step, as observed concentrations of DIN are dominated by NO_3^-. We also consider plant and denitrifier fluxes as first-order processes and thus do not explicitly model biological competition. Because microbial denitrification requires anaerobic conditions, the process should not compete directly with plant uptake in upland soils [*Weintraub et al*., 2014]. However, in mass balance terms, any NO_3^- transferred from sites of nitrification to anaerobic microsites that cannot be accounted for in hydrologic leaching represent a loss from the common plant-soil pool and thus a form of parallel denitrification.

Our results have important implications for how N losses and inputs are represented in Earth system and climate models. Currently, models that have coupled C-N cycles vary dramatically in how N losses are partitioned between gaseous and dissolved forms and how losses and inputs via symbiotic N_2 fixation are parameterized [*Thomas et al*., 2015]. Our stream data and simulations indicate that gas emissions tend to account for at least half of total N losses from tropical forests but the magnitude depends critically on the degree to which microbial denitrification represents a plant-controllable N loss. Furthermore, our approach places mass balance constraints on rates of symbiotic N_2 fixation that would be necessary to balance such losses and sustain CO_2 drawdown by plants.

4. Conclusions

Our data-driven modeling framework offers a new way to constrain denitrification across tropical forests at the small watershed scale. We resolve denitrification based on observed hydrologic concentrations, physical consideration of plant uptake, and rates necessary to maintain NPP. These constraints narrow global uncertainties in tropical N gas emissions by establishing an upper limit to denitrification that would allow for sustained plant draw-down of atmospheric CO_2. How plant-denitrifier interactions respond to projected climate and atmospheric changes could have important implications for the trajectory of the tropical forest C sink.

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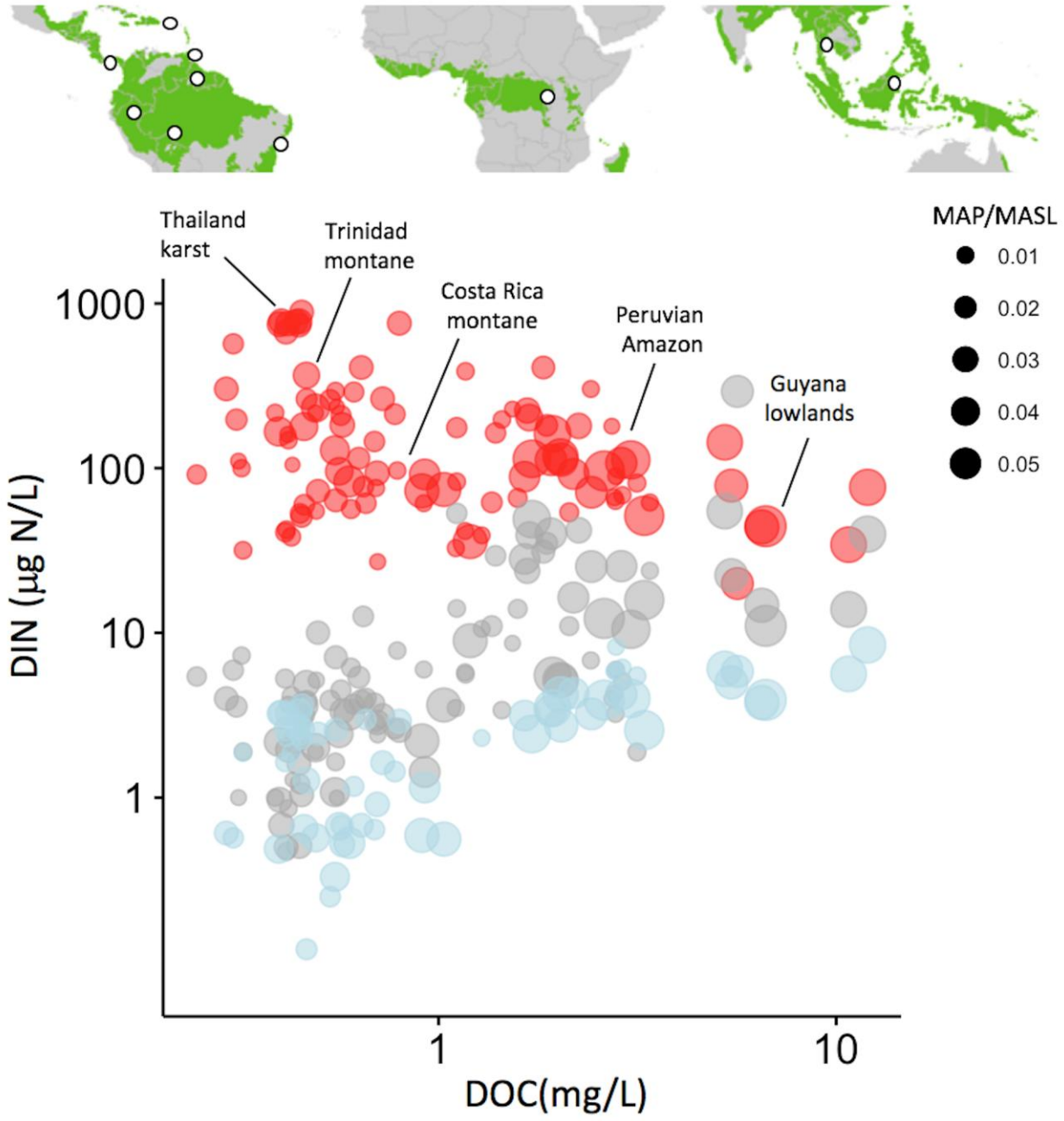

Figure 1. Stream DIN and DOC concentrations across humid tropical forests and their geographic distribution. The map shows countries (white dots) in which small watersheds were sampled. Across watersheds NO_3^- (red symbols) decreased (P<0.001, n=105) and NH_4^+ (grey symbols; P<0.001, n=105) and NO_2^-(light blue symbols; P<0.001, n=65) increased with increasing DOC and landscape MAP/MASL ratios. Shown are geographic identities of data clusters for original sampling campaigns.

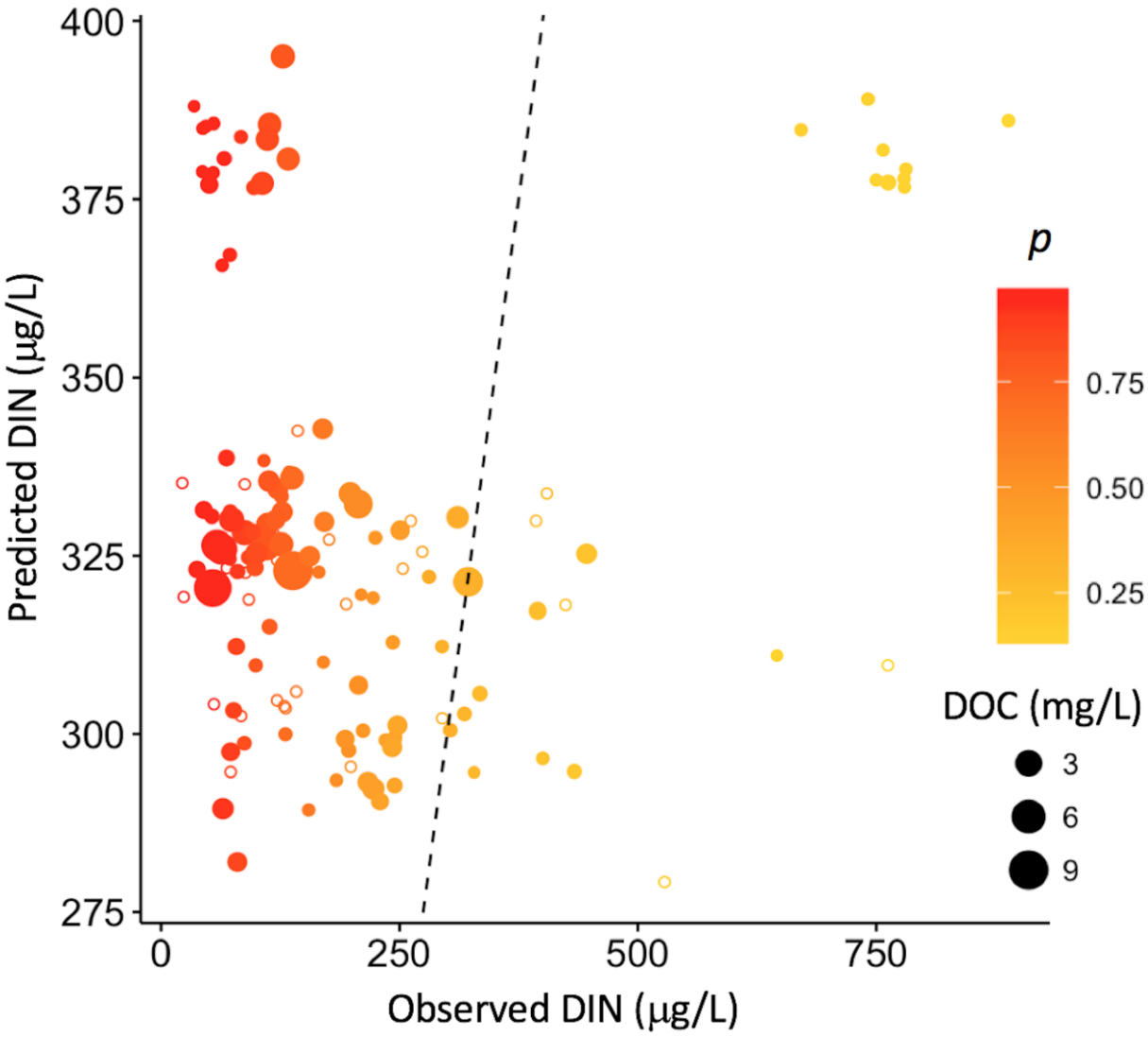

Figure 2. Simulated versus observed stream DIN and relationship with DOC across watersheds. Symbols represent observed and predicted stream DIN concentrations across all watersheds (*n* =139). Symbol colors represent the probability of model over-prediction and symbol sizes represent observed DOC concentrations. Unfilled symbols represent sites for which DOC data were unavailable. Dashed line represents the 1:1 relationship.

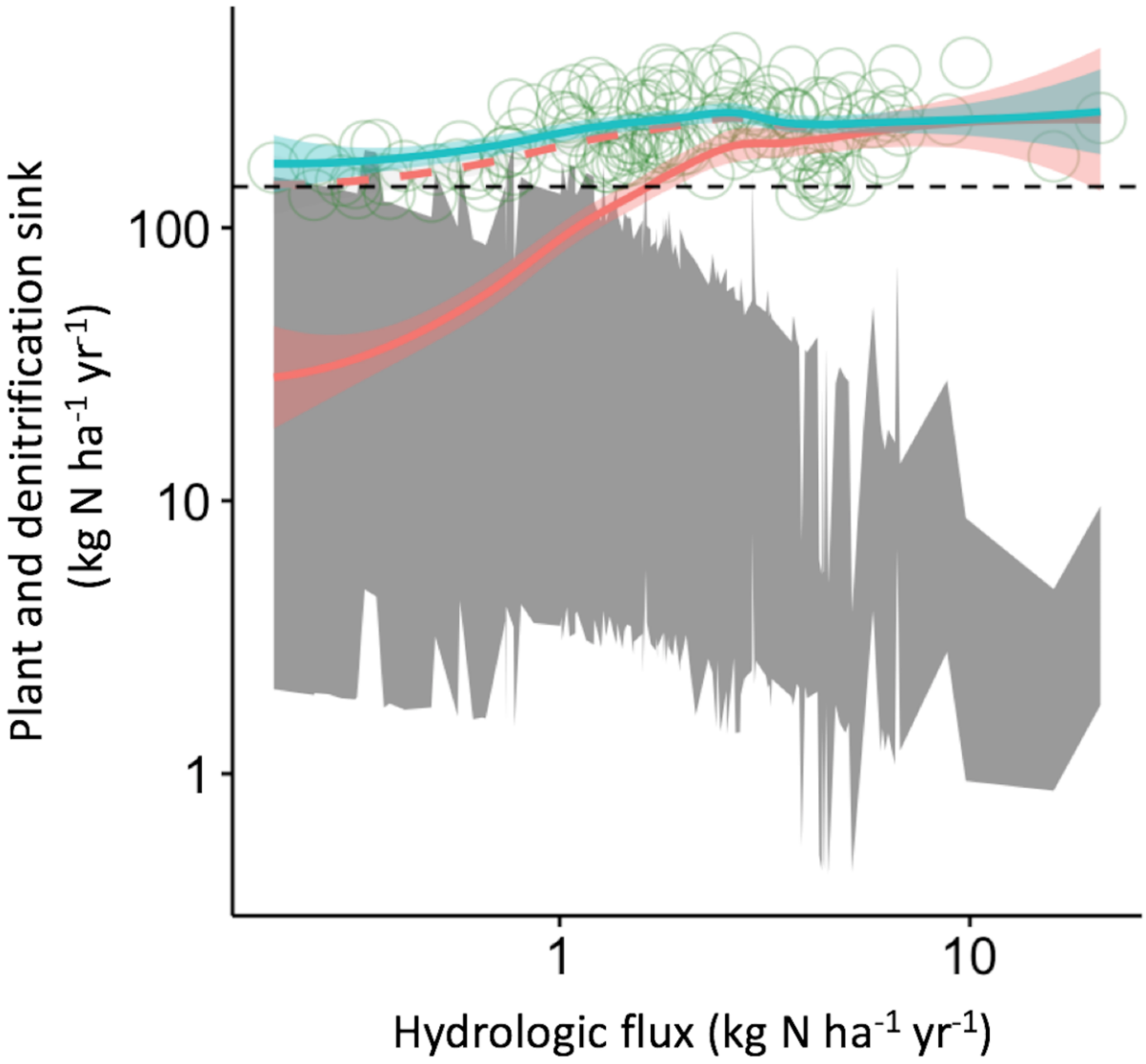

Figure 3. Ecosystem N losses and plant sinks across tropical forests. Green symbols represent watershed-specific plant N turnover (N demand). Blue and red lines and 95% confidence intervals show Loess regressions for calculated plant N uptake constrained by stream DIN concentrations, using 'sequential' and 'parallel' denitrification regimes, respectively. In the sequential scenario, plant demands are satisfied (i.e. calculated uptake rate is within the range of plant demand). If denitrification occurs 100% in soils (parallel denitrification), calculated uptake rates fall well below plant demand. For illustration, the 2.5% quantile for plant N demand across watersheds is indicated by the dashed black line. The total grey shaded area shows the range between average 'parallel' (maximum) and 'sequential' (minimum) denitrification fluxes estimated via 10,000 Monte Carlo simulations. The darker shaded area represents the 'heterogeneous' scenario where 20% of the soil DIN sink occurs as parallel denitrification, which is likely sufficient DIN to sustain plant growth (red dashed line).