Isotopic evidence for large gaseous nitrogen losses from tropical rainforests

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The nitrogen isotopic composition ($^{15}$N/$^{14}$N) of forested ecosystems varies systematically worldwide. In tropical forests, which are elevated in $^{15}$N relative to temperate biomes, a decrease in ecosystem $^{15}$N/$^{14}$N with increasing rainfall has been reported. This trend is seen in a set of well characterized Hawaiian rainforests, across which we have measured the $^{15}$N/$^{14}$N of inputs and hydrologic losses. We report that the two most widely purported mechanisms, an isotopic shift in N inputs or isotopic discrimination by leaching, fail to explain this climate-dependent trend in $^{15}$N/$^{14}$N. Rather, isotopic discrimination by microbial denitrification appears to be the major determinant of N isotopic variations across differences in rainfall. In the driest climates, the $^{15}$N/$^{14}$N of total dissolved outputs is higher than that of inputs, which can only be explained by a $^{14}$N-rich gas loss. In contrast, in the wettest climates, denitrification completely consumes nitrate in local soil environments, thus preventing the expression of its isotopic effect at the ecosystem scale. Under these conditions, the $^{15}$N/$^{14}$N of bulk soils and stream outputs decrease to converge on the low $^{15}$N/$^{14}$N of N inputs. N isotope budgets that account for such local isotopic underexpression suggest that denitrification is responsible for a large fraction (24–53%) of total ecosystem N loss across the sampled range in rainfall. Climate change | isotope | tropics | ecosystem | global change

Coherent patterns in N isotope composition across forests and soils suggest that natural abundance isotopes can provide critical information on the N cycle across broad geographic areas. Tropical forests, which are highly productive and play an important role in the Earth’s climate system (1), are among the most $^{15}$N-enriched of the terrestrial biomes (2); however, the $^{15}$N/$^{14}$N of plant and soil pools in tropical forests decrease systematically with increasing rainfall across tropical forests (3–8). This latter trend has garnered considerable attention because it implies a coupling between climate and the N cycle; however, the underlying cause remains unclear. The decline in forest soil $^{15}$N/$^{14}$N with increasing rainfall has been attributed to: (i) changes in the $^{15}$N/$^{14}$N of atmospheric N inputs (9, 10) such as biological $N_2$ fixation or rainfall and cloud deposition, (ii) preferential leaching of isotopically light N to stream waters (3–8, 11), or (iii) gaseous losses of isotopically light N to the atmosphere (3–8, 11).

Here, we examine these hypotheses in a series of tropical montane rainforests on Mt. Haleakala, Maui, HI; we also identify the mechanism(s) responsible for the elevation of soil $^{15}$N/$^{14}$N relative to atmospheric $N_2$. At this location, Schuur and Matson (6) characterized a sequence of six tropical forests, across which mean annual precipitation (MAP) varies from 2,200 to 5,050 mm, thus spanning the majority of tropical rainforest MAP worldwide (12). Ecosystem state factors (13) such as bedrock ($\approx 400,000$-year-old basaltic tephra), plant composition (trees dominated by Metrosideros polymorpha), mean annual temperature (16°C), topographic relief (slope of <5%), and elevation (1,270–1,370 m) are relatively constant across the gradient (6). The forests have never been cleared for timber or agriculture (6), are composed of species native to Hawaii, and have thus far escaped modern increases in anthropogenic N deposition. Because N pools equilibrate within 20,000 years of development in Hawaii (14, 15), the internal N pools in these $\geq 20,000$-year-old forests should be at steady state with respect to N inputs and losses.

In the soil N of most of these forests, Schuur and Matson (6) have observed the high $^{15}$N/$^{14}$N characteristic of the mean of the tropical forest biome (2). Moreover, they observed that the integrated $^{15}$N/$^{14}$N of the top 50 cm of soils decreased with MAP, with the steepest change occurring between the sites with 3,350 and 4,050 mm of MAP (Fig. 3a). Together with a similar decrease in plant $^{15}$N/$^{14}$N with rainfall along this gradient (6), these trends mimic the patterns observed for forests worldwide (4, 7).

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Abbreviations: TDN, total dissolved N; MAP, mean annual precipitation.

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Results and Discussion

We measured over 4 years (14 events distributed across seasons and hydrological conditions) the $^{15}$N/$^{14}$N of NO$_3^-$ and of total dissolved N (TDN) in precipitation and cloud water inputs, in stream water losses, and in soil waters and extracts. In Fig. 1 we show that the assumption of steady state N pools permits us to exclude effects of plant N uptake, because this process only impacts bulk soil N isotopes under transient conditions such as net N accumulation (7). On the basis of previous work (11), we assume that the N contributed by biological N$_2$ fixation has a $^{15}$N/$^{14}$N close to that of air [i.e., its $^{15}$N is $0\%e$, where $^{15}$N (per mil, $\%e$, vs. air) = ($^{15}$N/$^{14}$N$_{sample}$/$^{15}$N/$^{14}$N$_{air}$ - 1)$\times$1,000]. Combining these constraints on N isotope budgets with the isotope data for bulk soils and plants from Schuur and Matson (6), we test the competing hypotheses for the elevation of the $^{15}$N/$^{14}$N in these forests relative to atmospheric N$_2$ and for the observed $^{15}$N/$^{14}$N decrease in soil and plant N with increasing MAP (Fig. 1).

Elimination of Two Competing Hypotheses. Neither the forms of N in bulk deposition nor its $^{15}$N/$^{14}$N changed systematically with rainfall (Fig. 2a and b). Concentrations of all dissolved N forms were exceedingly low (<3 μmol per liter) in the rainfall but did not change in relative contribution across the sequence (Fig. 2d). The $^{15}$N/$^{14}$N of TDN in deposition was statistically indistinguishable from 0‰ across the sequence (one-tailed $t$ test, $P > 0.15$; $n = 42$) and thus similar to the isotopic ratio of N$_2$ fixation (indicated by the dashed line in Fig. 2d). In addition, cloud water $^{15}$N/$^{14}$N did not differ significantly from either bulk deposition or N$_2$ fixation (one-tailed $t$ test, $P > 0.10$; $n = 6$) (Fig. 2f). Thus, we found no evidence for our first hypothesis: that a trend in the $^{15}$N/$^{14}$N of N inputs explains the decline in soil $^{15}$N/$^{14}$N with rainfall.
The $^{15}\text{N}/^{14}\text{N}$ of bulk soil (and thus of forest-integrated N) was substantially elevated relative to inputs across all but the two wettest forests (Fig. 3a). Such a difference between inputs and internal pools requires a pathway of preferential $^{14}\text{N}$ loss from forests receiving $>4,050$ mm of MAP, which acts to elevate forest $^{15}\text{N}/^{14}\text{N}$ above the $^{15}\text{N}/^{14}\text{N}$ of the inputs to these forests (Fig. 1). However, we found no evidence for our second hypothesis: that the trend in soil $^{15}\text{N}/^{14}\text{N}$ was caused by decreasing isotopic fractionation of N leaching losses with increasing rainfall (3–8, 11). The forms of dissolved N in stream water losses changed sharply with rainfall: $\text{NO}_3^-$ dominated in streams draining drier forests ($<2,750$ mm of MAP), whereas DON (TDN minus inorganic N) was the most important form of N in streams draining wet forests (Fig. 2c). This pattern indicates that $\text{NO}_3^-$ accumulates in the drier forests to the point where $\text{NO}_3^-$ leaching occurs, whereas only DON leaks from the wet forest ecosystems. Despite this shift in N forms, the $^{15}\text{N}/^{14}\text{N}$ of stream water TDN changed in a manner similar to the trend in bulk soils (Figs. 2a and 3a). In fact, we found only slight differences in $^{15}\text{N}/^{14}\text{N}$ between stream losses and soils, most notably in forests with $>2,500$ mm of MAP, where the $^{15}\text{N}$ of stream water TDN was 1–2‰ lower than soils. This isotopic difference between streams and soils, if anything, should cause an increase (rather than the observed decrease) in $^{15}\text{N}/^{14}\text{N}$ forest soils with increasing MAP.

Given that these forests can be assumed to be in steady state with respect to total N inputs and losses (14, 15), the $^{15}\text{N}/^{14}\text{N}$ of total losses must balance that of total inputs (Fig. 1). The $^{15}\text{N}/^{14}\text{N}$ ratios of leaching losses were consistently higher...
both $^{15}$N and $^{18}$O during NO$_3^-$ across forests (Fig. 3). The only remaining hydrologic loss for preferential $^{14}$N removal. The only remaining two wettest sites (Fig. 2) isotope effect line was calculated using an approximate form of the Rayleigh equation, corresponding $^{15}$N.

Examination of the isotope ratios of $^{14}$N and 18O in the 15N enrichment in NO$_3^-$ in the 15N-reduced soils (9, 11). Ammonia volatilization is unlikely, owing to the acidic (pH of 3.2–4.2) and NH$_4^+$-poor soil conditions of our forests (6).

**Evidence for Denitrification.** Examination of the isotope ratios of NO$_3^-$ and TDN in local soil solutions and extracts indicates that soil denitrification was an important process in these forest soils. In both soil extracts and lysimeter samples, the $^{15}$N of NO$_3^-$ was substantially elevated above the $\approx 0\%e$ average input (Fig. 3c and Fig. 6), which is published as supporting information on the PNAS website; see also ref. 16). The $^{15}$N/14N of soil water TDN (Fig. 3b) tracked this $^{15}$N enrichment in NO$_3^-$, with highest values and greatest range in the $^{15}$N/14N in the wetter forests. This similarity in $^{15}$N/14N between NO$_3^-$ and TDN indicates that dissimilatory microbial reduction of NO$_3^-$ to NH$_4^+$ (17) or some other aspect of internal cycling could not explain the local $^{15}$N enrichment in NO$_3^-$. Previous work indicates that, under the nutrient conditions that characterize these forests, plant uptake of dissolved inorganic N does not appear to discriminate between the N isotopes (11).

$^{15}$N/14N and $^{18}$O/16O ratios of NO$_3^-$ in soil solutions were highly correlated ($R^2 = 0.76; n = 44; P < 0.0001$) within and across forests (Fig. 3c). Such a correlation has previously been found in terrestrial systems only when denitrification contributes actively to N loss (18, 19); it is caused by discrimination against both $^{15}$N and $^{18}$O during NO$_3^-$ reduction. Moreover, the slope of the relationship (0.66) was similar to that associated with denitrification in groundwater systems (0.49–0.67) (18, 19).

Soil extracts (2 M KCI) indicated that the $^{15}$N/14N ratios of extracted NO$_3^-$ were markedly elevated relative to those of extracted NH$_4^+$ in all but the shallowest forest soils (16). Without denitrification, NO$_3^-$ that accumulates in soils would be lower in $^{15}$N/14N than NH$_4^+$ as a consequence of fractionation during nitrification of NH$_4^+$ to NO$_3^-$ (9). Moreover, the soil extracts revealed decreasing NO$_3^-$ concentrations and sharply increasing $^{15}$N/14N ratios from shallow to deep soils (Fig. 6). The most dramatic trends occurred in the wettest sites, where profiles of soil O$_2$ availability (6) show sharp declines with depth, approaching levels that favor anaerobic metabolism. To our knowledge, our measurement of a $\delta^{15}$N of 180‰ for NO$_3^-$ extracted from soil at a 35-cm depth in the 4,050-mm MAP forest is the highest $\delta^{15}$N ever reported from a natural soil system. This parallel decline in NO$_3^-$ concentration, increase in the $^{15}$N/14N and $^{18}$O/16O of NO$_3^-$, and decrease in O$_2$ availability with soil depth is highly suggestive of active denitrification (16).

Finally, the presence of measurable NO$_3^-$ in soil extracts and soil solutions from wet forests (>2,750 mm; Fig. 3d), coupled with the virtual absence of NO$_3^-$ in streams draining the same forests (Fig. 2c), suggests that denitrification occurred along the path of water flow from local soil solutions to watershed streams.

**Scale Dependence of Isotopic Imprint.** Although denitrification raises the $^{15}$N/14N of soil N in our forests relative to atmospheric inputs, we have not yet resolved why this isotope ratio in soil organic matter decreases systematically with rainfall, especially because the greatest $^{15}$N enrichment of NO$_3^-$ and TDN in soil waters and soil extracts is observed in the wettest forests. We must consider how the isotope effect of denitrification, a local process, is expressed at the largest scales, in the streams and bulk soils. Partial consumption of NO$_3^-$ (“open-system kinetics”) leaves behind $^{15}$N-enriched NO$_3^-$, which can diffuse out of the
zone of ongoing denitrification and enter the larger plant–soil N cycle. Complete NO$_3^-$ consumption (“closed-system kinetics”) would cause underexpression of the isotope effect at larger scales (21, 22), because little or no $^{15}$N-rich NO$_3^-$ would escape the zone of denitrification to elevate the $^{15}$N/$^{14}$N of the larger plant–soil system.

We found evidence of such scale dependence of isotope effect expression in our sites. When we plot $\delta^{15}$N vs. the fraction of NO$_3^-$ consumed locally (see the legend of Fig. 4 for the calculation method), we find that the expressed isotope effect of denitrification decreased with increasing scale: the theoretical organism-level isotope effect (23, 24) (black line in Fig. 4) > local soil solution extracts (circles and squares) > water intercepted below rooting zones (triangles) > first-order watershed streams (inverted triangles). Moreover, the scale-dependent isotope effect underexpression was greatest in the wettest sites. Soil extracts and soil waters showed the greatest (and most variable) $^{15}$N enrichment of NO$_3^-$ and TDN in the wettest sites (Figs. 3b, c, e, and f and 6), but the stream waters and bulk soils, which integrate over the ecosystem-scale N budget (Fig. 1), displayed little to no $^{15}$N enrichment relative to inputs in these sites (Figs. 2a and b and 3a). The absence of detectable NO$_3^-$ in streams from the wettest forest provides a final indication that denitrification consumed NO$_3^-$ locally, preventing isotopic expression at the watershed scale. We conclude that increasingly complete NO$_3^-$ consumption by denitrification causes the observed decline in ecosystem $^{15}$N/$^{14}$N with increasing rainfall.

**An Isotope-Balance Approach for Reconstructing Pathways of N Loss.**

We apply an isotope-balance approach to partition the relative magnitudes of gaseous vs. hydrologic N losses across forests. We present two calculations, which derive from the same mass-balance equation but differ in the degree to which they incorporate the above findings of isotopic underexpression (see the legend of Fig. 5; see also Supporting Materials and Methods, which is published as supporting information on the PNAS web site).

In the most conservative case, we assume no isotopic underexpression of denitrification [i.e., the ecosystem-level isotope effect is taken as 20‰ (23, 24)], which allows us to place a lower bound on the proportion of N loss by means of denitrification. This calculation (Fig. 5a) shows that denitrification constitutes ~20% (and hydrological losses constitute ~80%) of total N losses in sites with <4,050 mm of MAP. However, the fraction of N lost to denitrification drops to 0% in the 4,050-mm MAP site because, as discussed above, the organism-level isotope effect of denitrification is not expressed in streams draining our wettest forests. Given our evidence for underexpression of the denitrification isotope effect (Fig. 4), we believe that this calculation shows the lower limit of the true importance of gaseous N loss in the wettest sites.

In the second calculation, we seek to account for local isotopic underexpression of denitrification by using the $^{15}$N/$^{14}$N of NO$_3^-$ from soil solutions collected from beneath the plant-rooting zone, coupled with an empirically determined denitrification isotope effect to derive the $^{15}$N of gaseous N loss (see the legend of Fig. 5). The field-calibrated isotope effect was intended to match the degree of underexpression observed at scales of soil waters: we used *in situ* cores, removing the effects of plant N uptake (by excluding plant roots) and nitrification (by additions of N source, i.e., nitrapyrin) (Supporting Materials and Methods).

The $^{15}$N/$^{14}$N of NO$_3^-$ in these core incubations displayed a strong relationship of $^{15}$N enrichment with the logarithm of the NO$_3^-$ consumed, consistent with consumption of a closed NO$_3^-$ pool (ref. 20 and Fig. 3f). The slope (13.2‰) of this relationship ($n = 13; R^2 = 0.97; P < 0.0001$) identifies an empirical isotope effect that is lower than the organism-level isotope effect (of ~20‰; refs. 23 and 24); this result offers further evidence of heterogeneity in isotope expression within our soils.

Applying this empirical isotope effect to the soil water TDN-$\delta^{15}$N data (as opposed to the stream TDN-$\delta^{15}$N data in the calculation), we calculate that denitrification accounts for 24–53% of total N losses across our forests (Fig. 5a, circles connected by solid line). The estimates for the three driest sites (~25%) are almost identical to the stream-based approach. However, for the 3,350- and 4,050-mm MAP sites, denitrification increased substantially above the stream-based estimates to ~50% of total N losses.

By combining these calculations with estimates of dissolved N losses, we can derive total fluxes of both gaseous and hydrological N losses. In Fig. 5b we show that total gas N fluxes (~2–9 kg of N per hectare per year) are appreciable when compared with the range of total N inputs (including biological N$_2$ fixation, rain, and cloud water deposition) of <6 kg of N per hectare per year in Hawaiian forests which are not immediately downwind of active volcanoes (25, 15). In the drier forests, our fluxes do not differ.
likely, denitrification is converting almost all of the NO\textsubscript{3}\(^{-}\) to N\(_2\) in the wettest sites, consistent with expectations for denitrification under low-O\(_2\) conditions (27).

### Conclusions

We conclude that the pattern of decreasing ecosystem \(^{15}\text{N}/^{14}\text{N}\) with increasing rainfall cannot be explained by changes in N inputs (9, 10) or preferential leaching of \(^{14}\text{N}\) to streams (3–8, 11). Rather, gaseous N loss is the major influence on forest N\(_2\) relative to atmospheric N\(_2\) and the measured N\(_2\) inputs to the forests. In the wettest sites, complete denitrification prevents its isotopic expression at larger scales, explaining why the \(^{15}\text{N}/^{14}\text{N}\) ratios of bulk soil \(^{15}\text{N}\) relative to atmospheric N\(_2\) and the measured N\(_2\) inputs to the forests. In the drier sites, incomplete nitrate denitrification as a dominant pathway. In the drier sites, incomplete nitrate denitrification to N\(_2\) in

### Methods

We collected bulk deposition in polypropylene funnels connected with silicon tubing to high-density polyethylene bottles. We sampled cloud water by an active collector mounted on a telescoping tower 15 m above the ground (25). We collected soil water from silica gel lysimeters at a 35-cm depth by using slight vacuum pressure (32 cm of Hg) over 72 h (14). Streams were sampled with syringes. Samples were immediately filtered through precleaned glass fiber filters (Gelman A/E, 1.0-\(\mu\)m nominal pore size). Chemical methods for the following were as described in ref. 14: ion chromatography for NO\(_3\)\(^{-}\), colorimetry for NH\(_3\)\(^{+}\), and persulfate oxidation followed by colorimetry or furnace combustion for TDN. The \(^{15}\text{N}/^{14}\text{N}\) and \(^{18}\text{O}/^{16}\text{O}\) ratios of NO\(_3\)\(^{-}\) were analyzed by using the denitrifier method (28, 29). \(^{15}\text{N}/^{14}\text{N}\) of TDN was analyzed by persulfate oxidation followed by the denitrifier method (30). (See Supporting Materials and Methods.)

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