Conditions Under Which Nitrogen Can Limit Steady-State Net Primary Production in a General Class of Ecosystem Models

Duncan N. L. Menge[1,2]*

[1]National Center for Ecological Analysis and Synthesis, Santa Barbara, California 93101, USA; [2]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, USA

ABSTRACT

Human activity is drastically altering global nitrogen (N) availability. The extent to which ecosystems absorb additional N—and with it, additional CO_2—depends on whether net primary production (NPP) is N-limited, so it is important to understand conditions under which N can limit NPP. Here I use a general dynamical model to show that N limitation at steady-state—such as in old-growth forests—depends on the balance of biotically controllable versus uncontrollable N inputs and losses. Steady-state N limitation is only possible when uncontrollable inputs (for example, atmospheric deposition) exceed controllable losses (for example, leaching of plant-available soil N), which is the same as when uncontrollable losses (for example, leaching of plant-unavailable soil N) exceed controllable inputs (biological N fixation). These basic results are robust to many model details, such as the number of plant-unavailable soil N pools and the number and type of N fixers. Empirical data from old-growth tropical (Hawai'i) and temperate (Oregon, Washington, Chile) forests support the model insights. Practically, this means that any N fixer—symbiotic or not—could overcome ecosystem N limitation, so understanding N limitation requires understanding controls on all N fixers. Further, comparing losses of plant-available N to abiotic inputs could offer a rapid diagnosis of whether ecosystems can be N-limited, although the applicability of this result is constrained to ecosystems with a steady-state N cycle such as old-growth forests largely devoid of disturbance.

Key words: nitrogen fixation; lichen; ecosystem theory; dissolved organic nitrogen; nitrogen deposition; biogeochemical theory; nitrogen loss.

INTRODUCTION

Many ecosystems are receiving increasing amounts of nitrogen (N) deposition due to fossil fuel burning (Galloway and others 2004), and ecosystem responses to this global change depend on whether N limits net primary production (NPP). Ecosystems in which N limits NPP, which are relatively common (LeBauer and Treseder 2008), will respond to additional N deposition by taking up additional CO_2 from the atmosphere, whereas those that are not

N-limited will not take up additional CO_2. Moreover, additional N deposition to non-N-limited ecosystems facilitates the release of the greenhouse gas nitrous oxide (N_2O) (Aber and others 1989; Hall and Matson 1999, 2003) and exacerbates other environmental issues such as eutrophication and acidification (Aber and others 1989). For these and other reasons, it is critically important to understand the conditions under which N can limit NPP.

Nitrogen availability to plants is ultimately constrained by N inputs to and N losses from ecosystems. In terrestrial ecosystems, N inputs include atmospheric deposition of fixed N (that is, not N_2), biological N fixation (BNF), and in rare cases rock weathering (Holloway and Dahlgren 2002). Nitrogen losses include leaching into waterways, gas losses, and erosion. Each of these inputs or losses includes many separate processes or sources. For example, BNF comes from symbioses between plants and bacteria, lichens, and a host of free-living prokaryotes; and gas losses come from denitrification, nitrification, ammonia volatilization, fire, and other processes.

Despite these complexities, grouping N inputs and losses by the degree to which they are under biotic control has advanced our understanding of N limitation. Vitousek and Reiners (1975) argued that N-limited plants should take up most or all plant-available N in the soil, drastically limiting N losses. Because abiotic inputs such as wet and dry deposition are omnipresent and essentially independent of biotic demand, they argued that plant control over N losses should render N limitation a transient phenomenon. Noting that N losses also come from plant-unavailable N pools such as recalcitrant organic molecules (Binkley and others 1992; Currie and others 1996; Hedin and others 1995; Sollins and others 1980), Hedin and others (1995) suggested that sufficiently large losses of plant-unavailable N could perpetuate N limitation.

Ecosystem models have formalized these arguments by assuming N limitation and determining conditions that prevent infinite N accumulation, typically in the context of old-growth forests largely devoid of disturbance. This approach works because we know N does not accumulate without bound in reality. If a model that assumes N limitation leads to an equilibrium, it is a decent reflection of reality. On the other hand, if it leads to infinite N accumulation, it is not a decent reflection of reality, so we conclude that the assumption of N limitation is incorrect under those conditions. DeAngelis (1992) showed in an analytical model of autotrophs and available nutrients that N accumulated infinitely when losses originated from only the available pool, whereas N accumulation was constrained when losses originated from the autotroph pool, with or without losses from the available pool. Using simulation models with plant, detritus, and available nutrient pools, Vitousek and others (1998) showed that losses via episodic disturbance such as fire or losses of plant-unavailable forms also constrained N accumulation. Menge and others (2009b) used an analytical model with plant, detritus, and available nutrient pools and a wide range of functional forms for ecosystem processes to show that losses of plant-unavailable N were necessary to prevent infinite N accumulation, but this effect only became apparent at centurial time scales.

These examples illustrate one of the challenges with classifying losses as ''controllable'' versus ''uncontrollable'' and pools as ''plant-available'' versus ''plant-unavailable.'' Autotrophs likely have some degree of control over losses from themselves, yet according to these studies these losses fit in the ''uncontrollable'' category. Similarly, N in a plant is certainly available to that individual, yet it falls into the ''plant-unavailable'' category. Here I retain the labels for ease of discussion and because I do not include any empirical data on losses from plants, but I note that ''control'' and ''availability'' are not absolute, and that the terms are poor descriptions for N within autotrophs.

Because symbiotic BNF—a biotically controlled input that uses the inexhaustible pool of atmospheric dinitrogen gas—can bring large quantities of N into ecosystems when symbiotic N fixers are abundant, it should have the capacity to overcome any N limitation imposed by losses of plant-unavailable N (Vitousek and Howarth 1991). Vitousek and Field (1999) extended an earlier simulation model to include symbiotic BNF, and found that symbiotic BNF could overcome N limitation unless it was constrained by other resources or processes. More complex simulation models (Jenerette and Wu 2004; Rastetter and others 2001) have yielded similar results. Menge and others (2008) included symbiotic BNF in an analytical model of plant biomass and available N and found that the exact amount of symbiotic BNF necessary to overcome N limitation at steady-state was the amount lost from plant-unavailable N pools.

All of these ecosystem models were developed to address certain questions and issues—not necessarily those discussed here—so they exclude a number of issues pertinent to the current study. Most of these models use at most a single detritus pool with a single turnover time, but detailed

empirical and modeling studies show that decomposition of litter alone is best described by three pools with different turnover times (Adair and others 2008), to say nothing of additional detrital pools. Although some models cited above incorporate BNF by plant–microbe symbioses, they do not include BNF by other N fixers such as lichens, cyanobacteria in bryophyte mats, or free-living heterotrophs. Plant–microbe symbioses can fix tens to hundreds of kg N ha^{-1} y^{-1} when abundant (Cleveland and others 1999), but they are rare or absent from many ecosystems (Menge and others 2010; Vitousek and Howarth 1991). On the other hand, other N fixers are ubiquitous and often important N sources, accounting for ones to occasionally tens of kg N ha^{-1} y^{-1} (for example, Antoine 2004; Cleveland and others 1999; DeLuca and others 2008; Matzek and Vitousek 2003; Menge and Hedin 2009; Reed and others 2008).

In addition to omitting some key ecosystem features that are relevant to understanding N limitation, most of the models cited above are mathematically specific. Simulation models require parameter values and specific functional forms for each process, and most analytical models also specify functional forms. The insights about biotic control over inputs and losses have greatly facilitated our understanding of N limitation, but the utility of these insights depends on their generality. For example, if the insights depend on whether plant N uptake is described by a Type I (proportional) versus a Type II (saturating, for example, Michaelis–Menten) functional response, they are less generally applicable than if both functional responses produce the same results.

In this study I investigate the generality of insights about plant-controllable versus uncontrollable inputs and losses in light of some known ecosystem complexities. Using a model with generalized functional forms for most processes, many different types of N fixers, and multiple soil organic N pools, I ask the following question: What are the conditions under which N limitation can be maintained at equilibrium? To find conditions under which N limitation can be maintained, I follow a similar approach to previous models (Menge and others 2008, 2009b; Vitousek and others 1998). First, I specify a general class of models that assumes N limitation to NPP and a few other details such as ''N uptake increases with available N.'' Resources aside from N—such as phosphorus, water, light, and space—and other potentially limiting factors such as herbivory or disease are not included in the model. This omission does not imply that these other factors are unimportant;

indeed, there is a large body of literature demonstrating the importance of each for many questions. Rather, the focus on N alone is meant to isolate conditions that could produce sustained N limitation, a puzzling phenomenon that has received substantial attention (Jenerette and Wu 2004; Menge and others 2008; Rastetter and others 2001; Vitousek and Field 1999; Vitousek and Howarth 1991).

To analyze the model I assume that plants can become established in an ecosystem without any facilitation and that after they become established, the amounts of N in plants, other N fixers, and soils will eventually equilibrate (common dynamics in myriad specific ecosystem models). I then derive conditions—call them X—that must be true if these assumptions are met. Because they lead to equilibration, conditions X can constrain N accumulation and thus perpetuate N limitation.

After deriving these conditions I use data from a number of well-studied temperate and tropical forests that are at or near equilibrium (here I use ''equilibrium'' as a synonym for ''steady-state'') to test the model and make predictions about whether the forests can be N-limited. If the data satisfy conditions X, they are consistent with the assumptions I made in constructing and analyzing the model, and thus consistent with N limitation. It is important to note that they do not guarantee N limitation because X may also be consistent with other models assuming that NPP is limited by other factors. However, if the data disagree with X, at least one of the assumptions I made in constructing the model must be wrong. Because I chose forests at or near equilibrium of the N cycle, the assumption most likely to be wrong is N limitation, so I interpret data that disagree with X to mean that the forests cannot be N-limited. This study builds on previous work in this area by generalizing ecosystem theory to a much broader set of conditions and offering a preliminary test of the theory with data from well-established field sites.

METHODS

Model Description

The model framework includes potentially N-fixing plants (B for biomass), heterotrophic N fixers (H), autotrophic N fixers such as cyanobacteria living in lichens and bryophytes (C for 'cyanobacteria,' which I will hereafter use as shorthand for these pools), an arbitrary number (m) of plant-unavailable N pools in the soil (D_i for 'detritus'), and plant-available N (A). All variables are in units of [mass N area^{-1}]. Within-system fluxes of the model include

turnover of biotic N, decomposition and mineralization of detrital N, and plant uptake of available N, but it is not necessary to specify mathematical forms for most of these fluxes.

There are abiotic inputs of plant-available N forms (I) such as wet and dry deposition that do not depend on any variable in the system, and thus are not under biotic control. I consider losses of plant-available N ($k(A)$) from the rooting zone—such as denitrification or leaching of nitrate, ammonium, or small organic N—"controllable" because they come from a pool plants can access. I assume that there are no losses when there is no available N ($k(0) = 0$) and that losses increase monotonically as available N increases ($dk(A)/dA > 0$). The increase could be linear, saturating (for example, Michaelis–Menten), sigmoidal (for example, logistic), or any other curve with a positive first derivative. Unless otherwise stated, these are the assumptions I make about all functions of single variables. An important point about "controllable" losses in this type of model is that plants never acquire all available N, so there are losses from that pool ($k(A) > 0$) despite plant control. I consider losses from plants ($\varphi_B B$, where φ_B is a constant rather than a function), heterotrophic N fixers ($\varphi_H(H)$), cyanobacteria ($\varphi_C(C)$), and all plant-unavailable soil N pools ($\varphi_{D_i}(D_i)$) "uncontrollable" because plants cannot take up N directly from any of these pools. These losses include leaching of plant-unavailable dissolved organic N.

Finally, biotically controllable inputs are BNF from plants (BF_B), heterotrophs ($F_H()$), and cyanobacteria ($F_C()$). There is evidence that some N-fixing plants down-regulate BNF when they cease to be N-limited (for example, Barron and others 2011; Pearson and Vitousek 2001), whereas others maintain a relatively constant BNF rate per biomass despite changing soil N and other resource conditions (Binkley and others 1992; Menge and Hedin 2009). However, because the model in the current work assumes that plants are solely N-limited, they should fix at the maximum rate regardless of whether they can down-regulate BNF, so F_B is a constant. Although much is known about controls on heterotrophic and cyanobacterial BNF (for example, Barron and others 2009; Benner and others 2007; Crews and others 2000; Eisele and others 1989; Silvester 1989), I need not assume anything about the arguments of the functions $F_H()$ and $F_C()$ (that is, for this analysis it does not matter whether they depend on any combination of variables or none of them) or their forms, although I do assume they cannot be negative.

Net change of total N (T) in the ecosystem is described by

$$dT/dt = I + \sum F - \left(k(A) + \sum \varphi\right), \quad (1)$$

where $\sum \varphi$ is the sum of all losses other than $k(A)$ ($\varphi_B B + \varphi_H(H) + \varphi_C(C) + \sum_i \varphi_{D_i}(D_i)$) and $\sum F$ is the sum of all BNF inputs ($BF_B + F_H() + F_C()$). The change in plant N is given by

$$dB/dt = B^q(g(A) + F_B - \mu - \varphi_B), \quad (2)$$

where the new terms are $g(A)$, the N uptake function (which follows the standard assumptions listed above), μ, the turnover rate of plant N, which transfers N into one or more detritus pools, and q, a proportionality constant. The parameter q is typically 1 in models examining mechanistic nutrient-limited plant growth (DeAngelis 1992; Tilman 1982) because uptake, fixation, turnover, and loss are likely to be close to proportional to plant biomass (or in this case, plant biomass N). However, because some processes might be proportional to plant surface area rather than biomass (in which case $q = 2/3$) (Rastetter and Shaver 1992), I include q and allow it to be any real number. Notably, however, I do restrict this model to cases where $1/B^q$ dB/dt (that is, the term in parentheses in equation (2)) is independent of B. Doing otherwise would implicitly introduce limitations other than nitrogen, and I am interested in understanding when limitation by nitrogen alone can be maintained. Rastetter and Agren (2002) have argued that changes in allometry should make $1/B^q$ dB/dt depend on B, but this implicitly introduces limitations other than N (allometric changes typically stem from constraints associated with resource acquisition, such as building structural wood to compete for light). Furthermore, allometric effects are less of an issue when the accounting is done with biomass N rather than biomass due to the small concentrations of N in wood.

The change in available N is given by

$$dA/dt = I - k(A) + M() - B^q g(A) - U(), \quad (3)$$

where the arguments and mathematical form of net mineralization ($M()$) and uptake of available N by other N fixers ($U()$) need not be specified. These three equations are all that need be specified for the analyses in this work. Other equations describing the flow of nitrogen through plant-unavailable soil pools (D_i) or other N fixers (C or H) could be specified, but including them here would lose generality.

In reality each of these processes likely includes pulses and seasonal variation, which may be

important for vegetation dynamics (Scheffer and others 2008). As in many simplified modeling studies, I make the assumption that these stochastic processes average across time and space and only examine mean-field dynamics.

Empirical Forest Data Used to Evaluate the Model

To test the predictions of this model it would be necessary to find data from old growth terrestrial ecosystems (that is, with the N cycle at or near steady-state) that had been fertilized with N and in which all N inputs and losses had been measured. To my knowledge there are few sites that fit all these criteria, so I also included sites that meet subsets of these criteria. The sites I included are montane tropical rainforests on the Long-term Substrate Age Gradient (LSAG) (Vitousek 2004) and the Maui rainfall gradient (Schuur and Matson 2001) in Hawai'i, conifer-dominated temperate forests (without alders) in the Wind River and Cascade Head Experimental Forests in Washington and Oregon (Binkley and others 1992), and montane temperate rainforests in Parque Nacional Chiloé, Chile (Hedin and others 1995) (Appendix A in Supplemental Material). Some sites in these areas such as the young Hawaiian sites are excluded because they are far from steady-state.

Atmospheric N deposition and BNF measurements likely capture the majority of N inputs to these forests, although not all potential BNF sources were measured at each site. Except for Wind River and Cascade Head, fire is unlikely to be an important N loss vector at these sites, so hydrologic and gaseous N losses from the soil likely capture most N losses. All major inputs and losses except fire are represented in at least some sites (Appendix A in Supplemental Material), so these measurements should give decent estimates of the possibility of N limitation given the model assumptions.

In many cases there are single or aggregated measures of the fluxes in these sites, but the exceptions are worth noting. At Wind River a local measurement suggested abiotic inputs of about 6.1 kg N ha^{-1} y^{-1} but nearby measurements are 2.0–2.5 kg N ha^{-1} y^{-1} (Klopatek and others 2006), so I use high (6.1) and low (2.0) estimates. To my knowledge there are no N deposition data from Cascade Head, so I used the regional estimate from a nation-wide map (Holland and others 2004). For some Maui sites two different approaches have been used to measure N gas losses. Houlton and others (2006) used isotopic measurements whereas Holtgrieve and others (2006) directly measured gas

losses. I present both datasets, with Houlton's estimates of gas to hydrologic N loss ratios for both. I also present two different estimates of abiotic N inputs on the Maui sites. The data in Houlton and others (2006) yield bulk deposition fluxes around 2 kg N ha^{-1} y^{-1}, but estimates of nearby Hawaiian forests suggest that including cloud N inputs may more than triple abiotic N inputs (Carrillo and others 2002; Vitousek 2004), so I present low (2) and high (6) estimates. Hydrologic N losses are from streams except at the LSAG sites, where only lysimeter measures are available (Hedin and others 2003), and Chiloé, where both lysimeter and stream estimates are available (Perakis and others 2005; Perakis and Hedin 2002). More site and methods details are in Appendix A (Supplemental Material).

In the model soil N is divided into plant-available (A) and unavailable (D_1, …, D_m) forms, which poses a minor problem for linking with data. It is generally agreed that inorganic N forms such as nitrate and ammonium are plant-available, whereas many forms of organic N such as complex molecules of humus or lignin are not. However, some organic N molecules such as amino acids are directly plant-available (for example, Näsholm and others 1998), that is, plants can acquire and use them before mineralization (Schimel and Bennett 2004). Unfortunately, to my knowledge no studies have distinguished organic N losses that are directly plant-available from those that are not. For lack of a way to divide organic N data into available and unavailable, here I initially assume that all organic N is plant-unavailable, which might underestimate plant-available N and overestimate plant-unavailable N. To examine the sensitivity of this assumption I then derive the percentages of organic N in losses that would need to be plant-available to qualitatively change the results for each site.

RESULTS
Model Analysis

For an N-limited equilibrium to exist, N inputs must equal N losses under the assumption of N limitation. If "*" denotes an equilibrium value of a variable (for A) or flux (for F and φ, which depend on variables), the statement that inputs equal losses from equation (1) can be rearranged to

$$I - k(A^*) = \sum \varphi^* - \sum F^*. \qquad (4)$$

In many ecosystems primary succession begins with N fixers like lichens, and plants invade later. Although other N fixers might facilitate plant

growth, I assume that plants do not *require* facilitation to become established because abiotic inputs will eventually supply enough N for non-fixing plants to establish. Although current evidence does not suggest that facilitation by other N fixers is obligatory for primary succession (Walker and del Moral 2003), and I think the assumption is reasonable, it is difficult to evaluate empirically due to the ubiquity of N-fixing microbes. In the example models I have examined, assuming that facilitation is required for plant establishment leads to an unstable system that either crashes or grows without bound, and thus is unrealistic. I show the implications of requiring facilitation in Supplemental Material Appendix B (Supplemental Material).

The assumption that plants can become established without facilitation means that the growth rate (dB/dt from equation (2)) of a very small population—say a small individual—is positive when no other plants, cyanobacteria, heterotrophic N fixers, or detritus are present. The equilibrium amount of available N in an ecosystem with no plants, other N fixers, or detritus, which I denote A_0, is defined by the relationship

$$I = k(A_0). \tag{5}$$

A very small plant population has a negligible effect on the amount of available N, so the amount of available N immediately after the introduction of a very small population is still approximately A_0. Therefore, the assumption that plants can establish (dB/dt evaluated for very small $B > 0$) is equivalent to

$$g(A_0) > \mu_B + \varphi_B - F_B. \tag{6}$$

Assuming that plants can establish in the empty equilibrium does not necessarily mean that the ecosystem had to be *at* the empty equilibrium before plants were introduced—only that they could establish at the empty equilibrium if they could not before. From setting equation (2) equal to zero, the equilibrium of A when plants are present (A^*) is defined by

$$g(A^*) = \mu_B + \varphi_B - F_B. \tag{7}$$

From conditions 6 and 7, $g(A_0) > g(A^*)$. The function $g(A)$ increases monotonically, so

$$A_0 > A^*. \tag{8}$$

This makes sense from the perspective of standard plant-resource models (for example, Tilman 1982). If plants can survive in an ecosystem and are limited by a resource, they will eventually draw that resource down to a lower level than it had reached without plants.

Because $k(A)$ is also monotonically increasing, and because of conditions 5 and 8,

$$I > k(A^*) \tag{9}$$

Biologically, this means that if plants do not require facilitation to become established, N limitation at equilibrium is only possible if abiotic inputs exceed losses from plant-available N pools. Continuing this reasoning, equation (4) and condition 9 show that an N-limited equilibrium is only possible when biotically uncontrollable losses exceed all BNF inputs:

$$\sum \varphi^* > \sum F^*. \tag{10}$$

What happens when condition 9 or 10 is violated, that is, when losses from plant-available N pools exceed abiotic inputs or BNF inputs exceed losses from plant-unavailable N pools? In a real ecosystem at or near equilibrium, a measurement of losses from plant-available N pools exceeding abiotic inputs or a measurement of BNF exceeding losses of plant-unavailable N would indicate that at least one assumption in this model is wrong. Provided that facilitation is not needed, the assumption most likely to be incorrect is N limitation, in which case a measurement of $k(A^*) > I$ or $\sum F^* > \sum \varphi^*$ would indicate that the ecosystem cannot be solely N-limited.

In the model, an N-limited equilibrium is impossible when condition 9 or 10 is violated, as the following contradiction proves. At equilibrium, if $\sum F^* \geq \sum \varphi^*$, then from equation (4), $k(A^*) \geq I$. If $k(A^*) \geq I = k(A_0)$, then $A^* \geq A_0$, so $g(A^*) \geq g(A_0)$. If $g(A^*) \geq g(A_0)$, then assuming plants can establish in the empty ecosystem (condition 6), $g(A^*) > \mu_B + \varphi_B - F_B$. However, the equilibrium value A^* is defined by $g(A^*) = \mu_B + \varphi_B - F_B$ (equation (7)), and $g(A^*)$ cannot be greater than itself, revealing the contradiction. In fact, when plant-available N remains at or above its base level ($A \geq A_0$), as implied by a measurement of $k(A^*) > I$, plants in the model grow without bound ($g(A) \geq g(A_0) > \mu_B + \varphi_B - F_B$; $dB/dt > 0$). Perpetual growth is impossible in reality, so this model result suggests that N cannot constrain plant growth when $k(A^*) \geq I$ or $\sum F^* \geq \sum \varphi^*$.

Successional Changes in Species and Parameters

This analysis concerns late-successional (equilibrium) conditions, yet makes the assumption that

late-successional plants could invade a plant-less environment given sufficient time and lack of competition from early-successional plants. This is not quite the same as assuming that late-successional plants could invade an early-successional *environment*, because the ''plant-less environment'' is set by parameters (loss rates and atmospheric deposition fluxes) measured during late succession. Even so, it is difficult to evaluate this assumption in nature because early-successional plants would colonize such environments before late-successional plants. Hence, it is useful to ask whether these assumptions can be derived from more defensible assumptions. Using different subscripts for parameter values measured when plants are absent (''0''), when early-successional plants are present (''early''), and when late-successional plants are present (''late''), I now ask whether more standard assumptions lead to the same conclusion that late-successional plants could invade a plant-less, late-successional environment, that is, that $I_{\text{late}} > k_{\text{late}}(A^*_{\text{late}})$.

Almost by definition, early-successional plants can invade early-successional environments and late-successional plants can out-compete early-successional plants. From the same logic as above, early-successional plants can invade early-successional environments when $I_0 > k_{\text{early}}(A^*_{\text{early}})$, which is equivalent to when $A_0 > A^*_{\text{early}}$. In this type of model, species replacement can only happen if late-successional species survive on a lower A^* than early-successional species (Tilman 1982), so assuming that late-successional plants can out-compete early-successional plants is the same as assuming that $A^*_{\text{early}} > A^*_{\text{late}}$. Do these assumptions imply that $I_{\text{late}} > k_{\text{late}}(A^*_{\text{late}})$?

From the inequalities in the previous paragraph it is clear that these assumptions imply that $A_0 > A^*_{\text{early}} > A^*_{\text{late}}$, and therefore that $I = k(A_0) > k(A^*_{\text{early}}) > k(A^*_{\text{late}})$ for given I and function k. However, the abiotic N input parameter I and the loss function $k(A)$ may also change during succession. Although I know of no evidence, it is conceivable that atmospheric N deposition could increase through succession because increased surface area from plants is more likely to trap N molecules from the atmosphere, so I examine the case of $I_{\text{late}} > I_{\text{early}} > I_0$. Hydrologic losses per unit N molecule might decrease through succession because soils are increasingly deep and clayey, which would mean that $k_0(A) > k_{\text{early}}(A) > k_{\text{late}}(A)$ for a given A. Conversely, denitrification losses per unit N molecule might increase through succession because of the increase in organic matter and the increased proportion of anaerobic sites.

If the successional change in hydrologic N losses outweighs the change in denitrification or they are similar,

$$I_{\text{late}} > I_{\text{early}} > I_0 = k_0(A_0) > k_{\text{early}}(A^*_{\text{early}}) > k_{\text{late}}(A^*_{\text{late}}),$$

$$(11)$$

which would guarantee that $I_{\text{late}} > k_{\text{late}}(A^*_{\text{late}})$. Even if the successional change in denitrification is larger than the change in hydrologic N losses, it is still likely that the other inequalities in I and A^* outweigh it. So, the initial assumption and therefore the main results can be derived directly from the assumptions that early-successional plants can establish in early-successional habitats, late-successional plants can out-compete early-successional plants, abiotic N inputs do not decrease through succession, and the loss strength of available N decreases or does not increase much through succession. Even though other parameters and functions aside from I and $k(A)$ might change through succession, they would not affect this conclusion except through effects of $g(A)$, μ_B, φ_B, and F_B on A^*, which are already covered under the assumptions of early-successional plants colonizing the empty habitat and late-successional plants out-competing early-successional plants.

Model Evaluation with Data

Along the Hawaiian age (LSAG) gradient, abiotic N inputs (I) exceeded losses from plant-available N pools ($k(A^*)$) in all but the oldest site (Figure 1; gray indicates N limitation is impossible). However, losses of DON ($\sum \varphi^*$) exceeded BNF inputs ($\sum F^*$) at all sites, including the oldest. Total measured losses exceeded inputs by 4.2 kg N ha^{-1} y^{-1} at the oldest site, accounting for the discrepancy. In temperate forests at Wind River and Chiloé, I exceeded $k(A^*)$ and $\sum \varphi^*$ exceeded $\sum F^*$ for all combinations of high and low estimates (Figure 1). Nitrogen losses from fire would only increase $\sum \varphi^*$, so they would enhance the observed inequality in these sites. In contrast, $k(A^*)$ exceeded I by over 11 kg N ha^{-1} y^{-1} in the temperate forest at Cascade Head. Losses of DON at Cascade Head were 7.3 kg N ha^{-1} y^{-1} and including fire would increase $\sum \varphi^*$, but BNF was not measured. On Maui, isotopic estimates of losses from plant-available N pools suggested that $k(A^*)$ exceeded low and high I estimates (I_{low} and I_{high}) except at the 2750 mm y^{-1} site where $k(A)$ fell between I_{low} and I_{high} (Figure 1). On the contrary, direct estimates of gas losses suggested that $k(A^*)$ exceeded both I_{low} and I_{high} at the 2200 mm y^{-1} site but neither at the
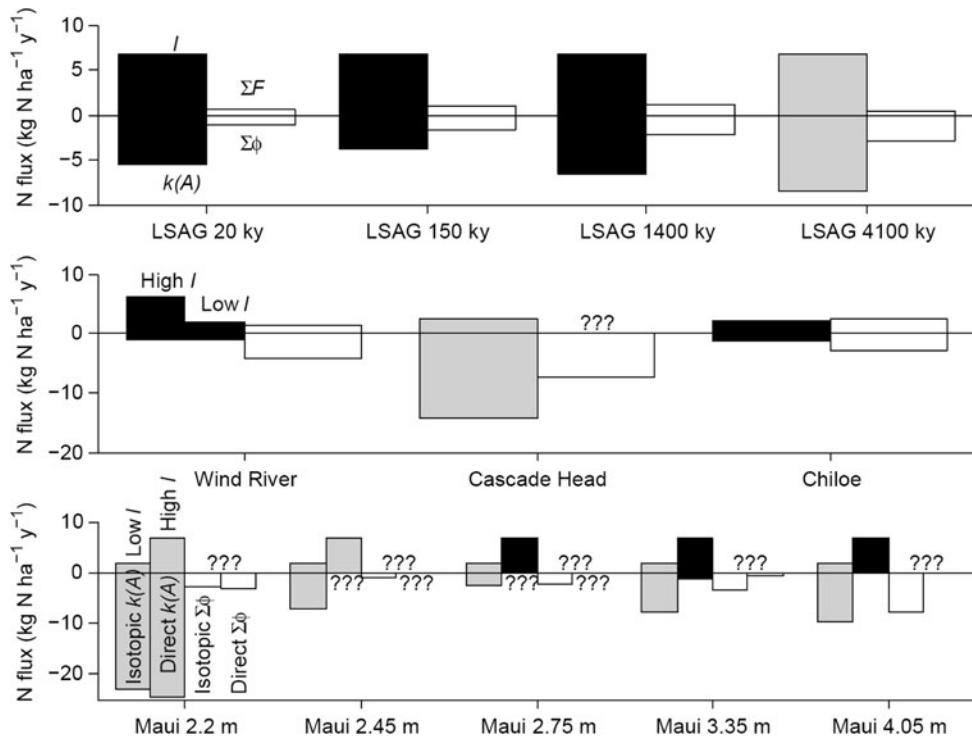
Figure 1. Model predictions about whether nitrogen (N) can limit net primary production in different forest ecosystems. According to the model, N limitation is only possible when abiotic N inputs (I, which include wet deposition, dry deposition, and cloud deposition) exceed losses originating from plant-available N pools ($k(A^*)$, which include hydrologic and gaseous losses from the nitrate and ammonium soil pools), which should be equivalent to when losses originating from plant-unavailable N pools ($\sum\varphi^*$, which include leaching of dissolved organic N) exceed biological N fixation (BNF) inputs ($\sum F^*$, from all types of N fixers). *Gray bars* indicate that $k(A^*) > I$, implying that N limitation is not possible in these sites. Not all of these fluxes are included at each site (see Appendix A in Supplemental Material). Abiotic inputs (I) and losses from plant-available N pools ($k(A^*)$) are on the left of each site, in *black* if $I > k(A^*)$ or *gray* if $k(A^*) > I$. BNF inputs ($\sum F^*$) and losses from plant-unavailable N pools ($\sum\varphi^*$) are on the *right* of each site in *white*. Missing flux data are indicated with '???.' In some cases different flux estimates are available; these are shown as *split bars*.

3350 or 4050 mm y^{-1} site. Estimates of DON loss ranged from 1.1 to 7.9 kg N ha^{-1} y^{-1} for the isotopic measurements and 0.14 to 3.1 kg N ha^{-1} y^{-1} for the direct measurements. BNF was not measured for any of the Maui sites.

Figure 2 shows the effects of relaxing the assumption that all organic N lost from the ecosystem is unavailable to plants. If the original comparisons already suggested that N limitation is impossible, as at the LSAG 4100 ky, Cascade Head, and some Maui sites/comparisons (all based on I vs. $k(A^*)$), increasing the magnitude of losses from plant-available N pools does not alter the prediction. For a number of other sites (LSAG 150 ky, Wind River with high I, and all other Maui sites/comparisons, all based on I vs. $k(A^*)$), the prediction that N limitation is possible cannot be altered even if all organic N losses were in plant-available form. At all other sites, assuming that some fraction of organic N loss is from plant-available pools alters

the prediction, depending on the exact fraction. For example, at the LSAG 20 ky site, if half (based on $\sum F^*$ vs. $\sum\varphi^*$) to most (based on I vs. $k(A^*)$) of organic N losses are in plant-available form, N limitation is impossible at this site. At the LSAG 1400 ky site, assuming that 3% (based on I vs. $k(A^*)$) to about half (based on $\sum F^*$ vs. $\sum\varphi^*$) of organic N losses are in plant-available form renders N limitation impossible. At Wind River, the cutoffs are 22% based on low I versus $k(A^*)$ and 37% based on $\sum F^*$ versus $\sum\varphi^*$. At Chiloé the cutoffs are between 36% and 89%.

DISCUSSION

Model Comparison to Data

In general, my model accurately predicted N limitation in real ecosystems. Empirical tests of nitrogen limitation—that is, fertilization studies—would
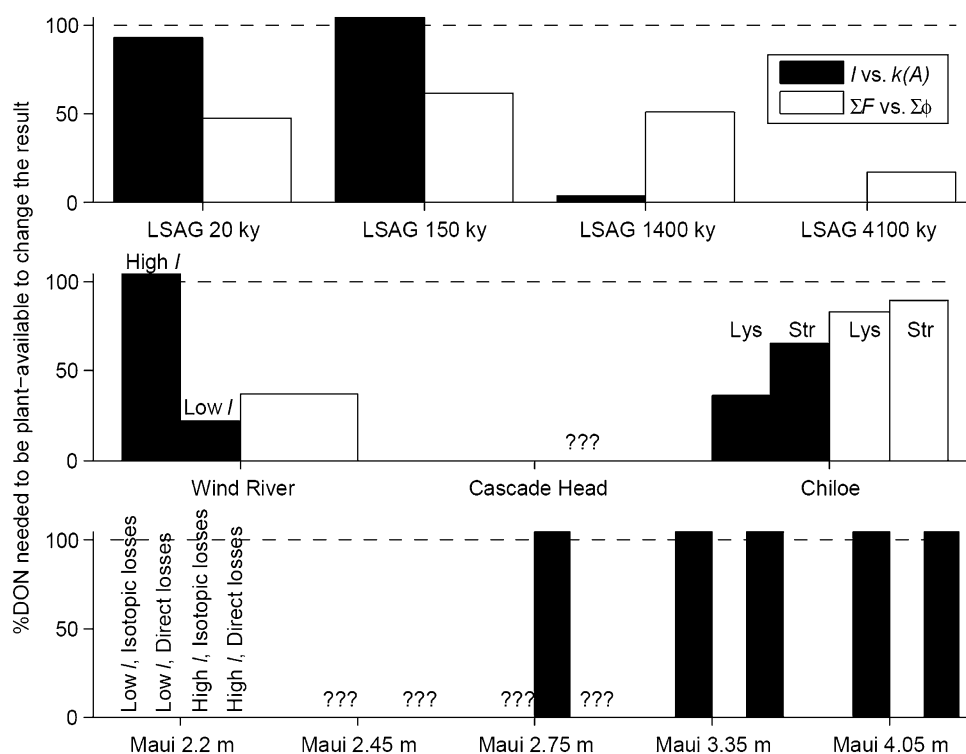
Figure 2. Sensitivity analyses of results in Figure 1 to organic N availability. Values presented are percents of organic N losses that would need to be in plant-available form to alter the result shown in Figure 1, for the *I* versus *k(A*)* (*black*) and the $\sum F^*$ versus $\sum \varphi^*$ comparisons (*white*). For example, based on the *I* versus *k(A*)* comparison at the LSAG 20 ky site, if more than 93% of the organic N being lost were in plant-available form, the model would indicate that N limitation at this site would be impossible. Cases where the results in Figure 1 indicate that N limitation is impossible cannot change and are presented as ''0'' values (without '???'). Values above the *dashed line* (100%) also could not change; many of these exceed 110% but the figure is truncated at 110% for ease of data presentation. Comparisons with missing fluxes are indicated with '???.' Sites and scenarios are generally laid out as in Figure 1, except that at the Maui sites all combinations of high and low inputs with isotopic-based and direct measurements of losses are presented.

invalidate the model by showing N limitation to NPP when (i) losses from plant-available N pools exceed abiotic N inputs or (ii) BNF inputs exceed losses from plant-unavailable N pools. Among the sites included in this study, only the 20 and 4100 ky Hawaiian LSAG sites have been fertilized. The 20 ky site is co-limited by N and P, whereas the 4100 ky site is limited by P alone (Vitousek and Farrington 1997). These results match the model predictions, which are that the 20 ky site can be N-limited unless a majority of organic N losses are in plant-available form but the 4100 ky site cannot, regardless of organic N availability.

To my knowledge there have been no measurements of the proportion of organic N in hydrologic losses that is in plant-available form. However, the proportion in hydrologic losses is likely to be similar to the proportion in soil solution, which is thought to be small, corresponding to rapid turnover rates of amino acids (Schimel and Bennett 2004). A recent analysis of N forms dissolved in soil solution

below four different soil types in Sweden found that free amino acids were 50 times less abundant than amino acids bound in dissolved proteins (Jämtgård and others 2010). If the proportion of plant-available organic N is similarly small (2%, or even less than 10%) in hydrologic losses, the only model prediction that might change would be that the 1400 ky LSAG site could not be N-limited, which would still match with the speculation that this site is P limited (Parton and others 2005; Vitousek 2004). Studies elucidating the availability of organic N in hydrologic losses and other media are much needed.

Other models have also reproduced the observed patterns of nutrient limitation along the LSAG sites (Parton and others 2005; Wang and others 2007), and do so in a far more comprehensive way than does the present work, predicting quantitative pools and fluxes as well as nutrient limitation. However, these more complex models require well-constrained information on a multitude of parameters,

whereas the simple framework in the present work requires fairly crude knowledge of only a few fluxes.

Researchers working on some of the other forests included in Figure 1 have speculated about their limitation status, although the forests have not been fertilized. Wind River (Binkley and others 1992) and Chiloé (Hedin and others 1995) are thought to be N-limited. These speculations agree with my model predictions as long as the proportions of organic N losses that are in plant-available form are not large. Cascade Head is thought not to be N-limited (Binkley and others 1992), matching the prediction of my model. Measurements of BNF at Cascade Head, which would be near 20 kg N ha^{-1} y^{-1} if the N cycle is near equilibrium, would further elucidate N cycle dynamics.

The Maui sites highlight the importance of accurate N flux measurements because different estimates yield conflicting results. All estimates of abiotic inputs and losses from plant-available N pools suggest that the 2200 and 2450 mm Maui sites cannot be N-limited, whereas for the wetter sites the possibility of N limitation is unclear. There are some suggestions that the wettest sites are N-limited (Schuur and Matson 2001), but if losses from plant-available N pools are as high as the isotopic measurements suggest, the model or the limitation status would come into question, regardless of the form of organic N losses. Measurements of BNF at these sites would help address these issues, and are likely to yield interesting patterns in their own right. If the N cycle is near equilibrium in these forests, BNF could range from negligible at the wettest site to more than 20 kg N ha^{-1} y^{-1} at the least wet site.

Ecosystem Theory Insights

The theoretical result that equilibrium N limitation is impossible when symbiotic BNF inputs exceed losses from plant-unavailable N pools (Menge and others 2008) is robust to the generalizations tested here. Whereas Menge and others (2008) used linear forms of N uptake and loss with specific within-system N cycling, the current study shows that any monotonically increasing uptake and loss functions—such as Michaelis–Menten or logistic—and myriad combinations of within-system fluxes yield the same results. Similarly, including multiple detrital pools, as seems to be important for describing litter decomposition (Adair and others 2008), has no effect on the basic result, so more complicated models are subject to the same constraint. Whereas some previous studies included N losses from either autotrophs (DeAngelis 1992) or detritus (Menge and others 2009b; Rastetter and others 2005; Vitousek and others 1998), this model shows that all N losses from pools other than plant-available N have a similar qualitative effect on maintaining N limitation, as suggested by Vitousek and Field (1999). Additionally, the model yields similar results if there are successional changes in a variety of parameters.

This model adds new insight by showing that understanding the maintenance of N limitation requires knowledge of controls on all types of N fixers rather than just symbiotic N fixers. Because plant–microbe symbioses can fix tens to hundreds of kg N ha^{-1} y^{-1} (Binkley and others 1992; Cleveland and others 1999), which greatly exceeds typical losses from plant-unavailable N pools (Figure 1), it is clearly essential to understand what controls their abundance and activity (Vitousek and Howarth 1991), and progress has been made in this area (Jenerette and Wu 2004; Menge and others 2008, 2009a; Rastetter and others 2001; Uliassi and Ruess 2002; Vitousek and Field 1999). However, N-fixing plant–microbe symbioses are rare or absent in many ecosystems (Menge and others 2010; Vitousek and Howarth 1991), yet BNF from other N fixers such as lichens, cyanobacteria in bryophytes, free-living cyanobacteria, and heterotrophic bacteria is ubiquitous. The current work shows that BNF by these other N fixers can prevent N limitation to NPP if it exceeds losses from plant-unavailable N pools. These other N fixers often exist in N-poor biogeochemical niches within a forest (Menge and Hedin 2009; Reed and others 2008), so they often fix N even when soil N availability is high. In addition to responding to their local N availability (Barron and others 2009; Crews and others 2000; Cusack and others 2009; DeLuca and others 2007; Liengen 1999; Zackrisson and others 2004), BNF by lichens, cyanobacteria in bryophytes, and heterotrophs in leaf litter, wood, and soil has been shown to respond to temperature (Antoine 2004; Kurina and Vitousek 2001; Liengen 1999), water (Antoine 2004; Freiberg 1998; Kurina and Vitousek 2001; Vitousek 1994), light (Freiberg 1998; Kurina and Vitousek 2001; Liengen 1999; Reed and others 2008), phosphorus (Benner and others 2007; Crews and others 2000; Eisele and others 1989; Liengen 1999; Reed and others 2007a, b; Vitousek 1999), molybdenum (Barron and others 2009; Silvester 1989), and combinations of nutrients (Crews and others 2000, 2001; Vitousek 1999). Although much is known about potential controls, further studies elucidating which of these controls is important in different environments—and particularly why—would improve our understanding of ecosystem-level N limitation.

Although it is intuitive that sufficiently large BNF rates can overcome N limitation, it seems counterintuitive that sufficiently low abiotic N inputs $(k(A^*) > I)$ render N limitation impossible. For example, the model states that N cannot limit NPP at Cascade Head, where losses of plant-available N forms exceed abiotic inputs. Would increasing abiotic inputs at Cascade Head make N limitation possible? The primary explanation for this apparent paradox is that large losses from plant-available N pools indicate a relaxation of plant control on available N in the soil (Vitousek and Reiners 1975). When purely N-limited plants can inhabit an ecosystem without any facilitation from other N fixers, they draw down available N as they grow, which yields lower losses from plant-available N pools. On the contrary, when N does not limit NPP, plants do not absorb excess N, so increases in I would be matched by increases in $k(A^*)$ to maintain steady-state (I would not exceed $k(A^*)$). A secondary explanation concerns the model assumption of N limitation: it shows when N limitation is *possible* rather than when it is expected because no other resources are included in the model. Increasing abiotic N inputs in real forests eventually saturates N demand (Aber and others 1989), and the model would reflect this if other potentially limiting resources were included.

The steady-state conditions that form the main results of this paper are indicators of whether N can limit NPP, but because the N cycle is a cycle, the causal links go both ways. For example, low losses of plant-available N result from plants depleting available N levels, but low available N levels are what cause N limitation in the first place.

Applying this Theory to Real Ecosystems

Practically, the result that $k(A^*) > I$ renders N limitation impossible could offer a crude but rapid diagnosis of whether old-growth forests can be N-limited. The conditions under which this model applies—chiefly, a steady-state N cycle—restrict its applicability, but using $k(A^*)$ versus I rather than $\sum F^*$ versus $\sum \varphi^*$ is much less restrictive. Available N in the soil (A) approaches its long-term equilibrium on the timescale of plant biomass N (seen from equation (2)), which is typically much sooner (decades to centuries) than the entire ecosystem (millennia or longer) (Menge and others 2009b). This means that comparing losses of plant-available N to abiotic N inputs measured at any time beginning when plant biomass N (rather than the entire N cycle) is near equilibrium indicates what the equilibrium relationship will be. On the contrary,

comparing measurements of BNF and losses of plant-unavailable N only indicates the equilibrium relationship when the entire N cycle is at equilibrium because plant-unavailable soil N pools are typically the last ecosystem component to equilibrate (Menge and others 2009b). In addition to being more applicable, comparing $k(A^*)$ to I is also likely to be more accurate than comparing $\sum F^*$ and $\sum \varphi^*$ given the relatively greater ease of measuring these fluxes. However, none of these fluxes is easy to measure, and the difficulties associated with these measurements—such as determining which N forms are plant-available—can make it hard to use these results operationally. Large losses of plant-available N forms are already used to infer N saturation or N richness in many real ecosystems (for example, Aber and others 1989; Hedin and others 2009), though, and in that sense the current work puts a threshold on what constitutes "large" losses—greater than abiotic inputs. Because abiotic deposition maps are widely available (for example, Holland and others 2004), this work could provide theoretical guidance to a practice already in use.

One benefit of this method is that it does not depend on a full accounting of losses of plant-unavailable N. Gas losses such as denitrification can be difficult to quantify accurately (Groffman and others 2006), and as discussed above, partitioning organic N into plant-available versus plant-unavailable forms is similarly tricky. Hydrologic losses of inorganic N (leaching of ammonium and nitrate), though, are relatively easy to measure, and it is possible to make headway with these alone. A measurement of ammonium and nitrate leaching exceeding N deposition in a forest near steady-state implies a lack of N limitation to NPP. Even if ammonium and nitrate leaching comprised all losses of plant-available N, the opposite result—N deposition exceeding ammonium and nitrate leaching—would not imply N limitation because other potentially limiting resources and factors are not included in the model.

More extensive empirical testing of this theory is necessary, and of course the biogeochemical details of individual ecosystems need to be considered (for example, weathering of bedrock with significant N would need to be included in I), but it might be feasible to use this technique as a rapid diagnosis of whether a steady-state ecosystem can be N-limited. This information, in turn, can help understand broader issues of CO_2 uptake, eutrophication from nitrate leaching, and N_2O emissions that contribute to greenhouse warming, because these issues hinge on whether N limits NPP.

The present work applies to ecosystems in which the N cycle is at or near equilibrium, that is, old-growth forests. Although the mathematics are likely to be less elegant, similar results likely hold for systems farther from equilibrium, and future work in this area could help extend these insights to a larger portion of the globe. Additionally, future work examining some of the other assumptions I make here would help pin down controls on N limitation. An important example is the assumption in the current work of sole limitation by N. Co-limitation is thought to be common (Bloom and others 1985), and some models that incorporate co-limitation with a non-recycled resource such as light allow for stable equilibria even when plant-controlled nutrient inputs exceed uncontrolled nutrient losses (for example, Ju and DeAngelis 2010).

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