



Towards an ecological understanding of biological nitrogen fixation

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Abstract. N limitation to primary production and other ecosystem processes is widespread. To understand the causes and distribution of N limitation, we must understand the controls of biological N fixation. The physiology of this process is reasonably well characterized, but our understanding of ecological controls is sparse, except in a few cultivated ecosystems. We review information on the ecological controls of N fixation in free-living cyanobacteria, vascular plant symbioses, and heterotrophic bacteria, with a view toward developing improved conceptual and simulation models of ecological controls of biological N fixation.

A model (Howarth et al. 1999) of cyanobacterial fixation in lakes (where N fixation generally increases substantially when N:P ratios are low) versus estuaries (where planktonic N fixation is rare regardless of N:P ratios) concludes that an interaction of trace-element limitation and zooplankton grazing could constrain cyanobacteria in estuaries and so sustain N limitation. Similarly, a model of symbiotic N fixation on land (Vitousek & Field 1999) suggests that shade intolerance, P limitation, and grazing on N-rich plant tissues could suppress symbiotic N fixers in late-successional forest ecosystems. This congruence of results raises the question – why do late-successional tropical forests often contain many potentially N-fixing canopy legumes, while N fixers are absent from most late-successional temperate and boreal forests? We suggest that relatively high N availability in lowland tropical forests permits legumes to maintain an N-demanding lifestyle (McKey 1994) without always being required to pay the costs of fixing N.

Overall, both the few simulation models and the more-numerous conceptual models of ecological controls of biological N fixation suggest that there are substantial common features across N-fixing organisms and ecosystems. Despite the many groups of organisms capable

of fixing N, and the very different ecosystems in which the process is important, we suggest that these common controls provide a foundation for the development of regional and global models that incorporate ecological controls of biological N fixation.

Introduction

As this volume illustrates, the nitrogen cycle poses many challenges. None of these is more fundamental than the question “Why is the supply of N so important to the functioning of many terrestrial and aquatic ecosystems?” This challenge can be framed as follows:

- The supply of fixed N demonstrably limits the productivity, composition, dynamics, and diversity of many ecosystems, in that all of these change when N is added.
- Organisms with the capacity to fix N_2 from the vast quantity in the atmosphere are widespread and diverse.
- How can limitation by fixed N, an abundant supply of N_2 in the atmosphere, and the biological capacity to make use of N_2 coexist for long in any ecosystem?

A number of processes tend to reduce the biological availability of N in ecosystems, notably the strong link between organic N and recalcitrant C compounds in soils and sediments, and the mobility of N out of ecosystems by hydrologic and atmospheric pathways (especially leaching and denitrification). However, the capacity of biological nitrogen fixers to convert N_2 to organic N is substantial, often exceeding $100 \text{ kg ha}^{-1} \text{ y}^{-1}$ where symbiotic N fixers are abundant – more than enough to maintain N pools in ecosystems and to replenish N losses. Why then are N fixers not a dominant species in all systems where N limits primary productivity? And as a byproduct of their activity, why is N limitation not alleviated? These questions are fundamental to understanding why N limitation is prevalent in terrestrial and aquatic ecosystems.

In many systems, N fixers do drive the accumulation of fixed N on long time scales, bringing N supply close to equilibrium with other potentially limiting resources; in some systems, this equilibration is relatively rapid. For example, in most freshwater lakes, N-fixing cyanobacteria respond to N deficiency (when other resources are available) with increased growth and activity, and the supply of fixed N equilibrates stoichiometrically with that of another resource (normally P) (Schindler 1977). This rapid response to N deficiency is easy to understand in lakes; why is the response much slower (or nonexistent) in many other ecosystems, even allowing for differences in generation times of the dominant organisms? Or, asked another way,

what constrains the presence, growth and/or activity of N fixers in N-limited ecosystems?

The answer to these questions is necessary (though not sufficient) to explaining why N limits productivity (and other processes) in many ecosystems; thus it represents a fundamental question about the biogeochemistry of ecosystems. At the same time, it has substantial implications for understanding components of human-caused global change. The extent of human alteration of the global N cycle is well documented (Galloway et al. 1995; Vitousek et al. 1997). It is clear that systems in which N is limiting can be altered substantially by anthropogenic N (e.g., Aber et al. 1998). Where N is not limiting, N deposition may have smaller effects on within-system processes but greater effects on N losses than it does where N is limiting (Hall & Matson 1999; Matson et al. 1999). Accordingly, the mechanisms that control ecosystem-level N limitation strongly determine the consequences of increased N deposition.

For another example, the consequences of increasing levels of atmospheric carbon dioxide depend on what controls the supply of N and other nutrients. Short-term experiments show that plant growth increases substantially under elevated CO_2 , averaging a 30–40 percent increase for a doubling of CO_2 , more or less independently of nutrient status. However, longer-term simulations of the effect of elevated CO_2 suggest that ultimately the enhancement will be much less, perhaps 5–10 percent, due to a reduction in N availability caused by immobilization of N in plant biomass and decomposing litter (Rastetter et al. 1997; McKane et al. 1997; Schimel et al. 1997). These simulations capture much of our understanding of N supply and ecosystem response – but generally they do not include the possibility that N fixation could be enhanced under elevated CO_2 . A number of field experiments do show a positive response of symbiotic N fixers to elevated CO_2 (Arnold & Gordon 1990; Lüscher et al. 1998; Hungate et al. 1999). However, to predict the responses of N fixers to elevated CO_2 , we should know what constrains their activity in N-limited systems today, and how any constraints will be altered by elevated CO_2 . In the same way, we should understand how N fixation will be affected by altered hydrologic regimes, land use change, and other global changes.

Despite its importance in understanding ecosystems and in predicting how they will respond to change, biological N fixation is either under-represented or not represented in most ecosystem models. For example, the Century ecosystem model (Schimel et al. 1997) now calculates N fixation as a linear function of actual evapo-transpiration (AET); most other models do less. Similarly, the one data-based effort to extrapolate global rates of N fixation in terrestrial ecosystems correlated point estimates with AET, and extrapol-

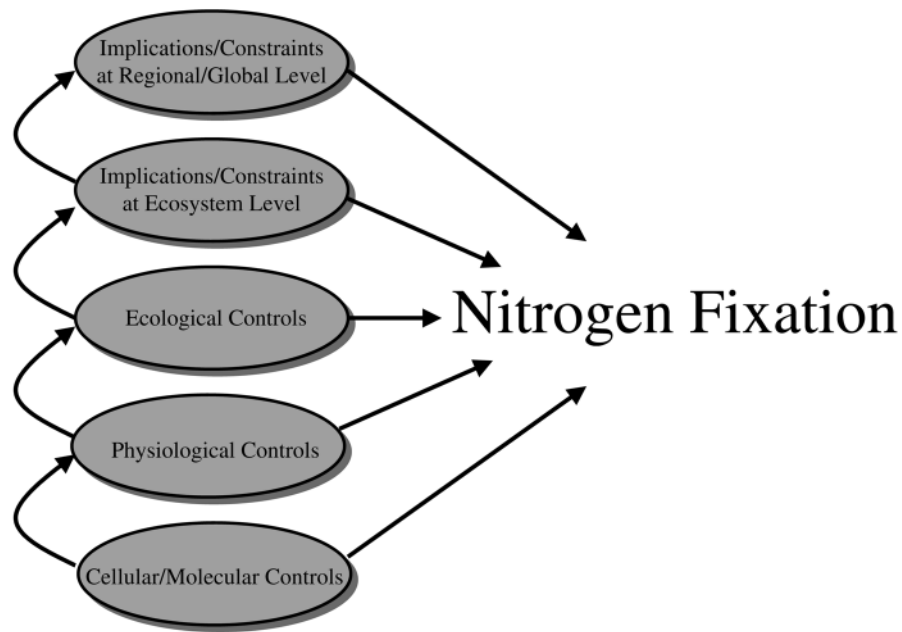


Figure 1. A hierarchy of explanation for patterns of nitrogen fixation. The focal level considered in this paper is the ecosystem level. Mechanistic explanation can be sought at cellular/molecular, physiological (whole organism), and sub-ecosystem levels (i.e., ecological controls). At the lowest level are controls at the sub-organismal level, including genetic control, enzyme synthesis, and other mechanisms. At the whole organism level, nitrogen fixers are subject to physiological controls that determine whether nitrogen fixation can occur; for example, oxygen concentrations or the ability to acquire molybdenum. In addition, the ability of nitrogen-fixing organisms to colonize or persist in a given environment is a function of competitive interactions, predation pressure, and availability of limiting nutrients. The third hierarchical level comprises this suite of ecological controls. At the ecosystem level, the patterns and balance of nitrogen inputs and outputs set constraints on the rates of nitrogen fixation, while at the final and highest level regional and global patterns of nitrogen fixation are controlled by patterns of land cover and use, biome distribution, global climatic patterns, and patterns of N deposition.

ated globally on that basis (Cleveland et al. 1999). While the correlation is useful for the latter purpose, it conveys little understanding of what controls N fixation, and how it is likely to change in the future.

We believe that a lack of information on the ecological regulation of N fixation is the foremost reason why understanding and modeling of N fixation at the ecosystem level lags behind our recognition of its importance. For any process that is important at a number of levels of organization, we need to identify a focal level, and seek mechanistic explanations at hierarchical levels below that focal level. Constraints to the process develop at higher levels in

the hierarchy (Figure 1). For example, Hartwig (1998) considered the regulation of symbiotic N fixation at five levels – gene expression, biochemical, nodule, whole plant, and ecosystem. There is a wealth of information on the molecular biology of N fixation and its regulation and about N fixation on the whole-plant level, at least for a few well-studied (mostly crop) legumes. In contrast, our information on ecological controls on symbiotic N fixation in ecosystems other than crop production systems is substantially weaker. This lack of ecological information extends to free-living and heterotrophic N-fixing systems as well.

There are a number of good reasons why the understanding of ecological controls has lagged behind other levels of explanation. A lack of resources (to investigators) certainly contributes, but more importantly:

- Accurate measurements of N fixation rates are essential to testing hypotheses about controls of the process. However, it is extremely difficult to measure rates of N fixation accurately – especially in the field, especially working with long-lived perennial organisms such as N-fixing trees. It is possible to measure rates of N accumulation in ecosystems over relatively long periods of time, or to use acetylene reduction to detect the nitrogenase enzyme – but measuring fixation itself, in natural systems, is very difficult.
- There are many classes of biological N fixers that need to be considered in any thorough analysis. While understanding of the symbiotic system in a few legume crop plants is relatively advanced, much less is known about N fixation in non-agricultural legumes or in other N-fixing organisms, such as symbiotic cyanobacteria or free-living heterotrophic bacteria. In many ecosystems, the contribution of such organisms is significant and needs to be accounted for in any full understanding of N fixation.
- N fixation will need to be understood in a very wide variety of ecosystems, from estuaries to boreal forests. While there may be parallels in the ecological controls of N fixation, by different classes of organisms in different ecosystems, we cannot assume that the same controls are important everywhere.
- Ecologists have not studied or modeled the regulation of N fixation in any systematic way, with relatively few exceptions (e.g., Parrotta et al. 1996; Hartwig 1998; Howarth et al. 1999, and examples discussed below).

Our goal in this paper is to encourage the development of ecologically based understanding of N fixation in natural and managed ecosystems, and to work towards incorporating this understanding into ecosystem models. We review the nature of ecological controls of N fixation, summarize informa-

tion on those controls for some of the better-understood classes of N-fixing organisms, and synthesize that information into relatively simple process-based models wherever possible. We also discuss other potentially important classes of fixers for which less is known of their capabilities and controls. Finally, we discuss a number of outstanding questions and uncertainties that we believe would reward concentrated effort to resolve them, and that would contribute substantially to understanding the regulation of N fixation.

Ecological controls

By ecological controls, we mean controls over the rate of N fixation that are or can be influenced by interactions between the N fixer and other organisms (excluding symbiotic partners, if any) and/or the N fixer and its environment. N fixers, like all other organisms, are subject to a very wide variety of biotic and abiotic controls; it can be too hot or too cold, too dry or too wet, too acid or too alkaline; there can be too many competitors for crucial resources, or too many grazers that restrict N fixers' distribution or abundance. We are particularly interested in such controls where they influence N fixers (or their activity) to a greater extent than they affect non-fixing organisms, because only in those circumstances will N fixers be constrained relative to other organisms.

Differential suppression of N fixers can occur where N fixers require a resource that other organisms do not need, or where they require more of a resource or less of another environmental factor than do non-fixers. It can also occur when N fixers experience systematically higher mortality than non-fixers, or when environmental conditions are outside the limits of adaptation for all N fixers. While requiring a resource that other organisms need much less of (e.g., molybdenum) could be considered a physiological rather than an ecological control, and conditions outside the bounds of all organisms might be considered an environmental constraint, we will treat these together. Also, the ecological distribution of N-fixing organisms may be wider than that of their ability to fix N (Hartwig 1998) – and we focus on the ability to fix N.

What are the general features of the N-fixation process that could lead to differential suppression of N fixers in some environments?

- N fixation is relatively energy-intensive.
- Nitrogenase enzymes are inactivated by O₂. Organisms maintain a delicate balance between the efficiency of using O₂ as an electron acceptor and the inactivation of nitrogenase, and free-living photosynthetic N fixers must segregate the O₂ they produce from their nitrogenase system.

- Most nitrogenases require molybdenum in order to function; many non-fixers require much less molybdenum. As discussed below, N fixers may also need more P, Fe, and/or other nutrients than other organisms.
- In most N-fixing organisms, the synthesis and/or activity of nitrogenase is inhibited by high levels of combined N.
- Many N-fixing organisms are rich in N compared to non-fixers and so may be grazed preferentially.

How can these overall differences between N fixers and other organisms translate into ecological controls of N fixation? In this analysis, we will consider three major groups of N fixers – free-living cyanobacteria, bacteria and cyanobacteria in symbiotic associations with plants, and heterotrophic bacteria. There are many other N fixers, including lichens with cyanobacterial phycobionts, bacteria in animal digestive systems, and many minerotrophic bacteria. These fixers are important to the metabolism of particular organisms and to the N budgets of particular ecosystems; lichens especially have been evaluated in a number of ecosystems (Fritz-Sheridan & Coxson 1988; Kurina & Vitousek 1999). However, symbiotic N fixers, free-living cyanobacteria, and heterotrophs are by far the most important contributors of fixed N in most ecosystems, and if we can understand and model what controls their rates of fixation, that will contribute substantially to explaining the interactions between N limitation and N fixation in most ecosystems globally.

Free-living cyanobacteria

Aquatic cyanobacteria

Lakes and estuaries. N-fixing cyanobacteria are among the most widespread and important N fixers on Earth. They are the major N fixers in freshwater and marine systems, and they also grow and fix N in many terrestrial environments, from rainforests to deserts.

What regulates N fixation by cyanobacteria? What constrains it? These questions have been addressed most clearly in comparisons of the planktonic portions of lake and estuarine ecosystems. In temperate lakes of moderate to high productivity, the regulation of nitrogen fixation is relatively well understood (see review by Howarth et al. 1988b). When the ratio of nitrogen to phosphorus is high, little or no nitrogen fixation by planktonic cyanobacteria occurs as there is no competitive advantage to this energetically expensive process. However, when the ratio of nitrogen to phosphorus is low, certain species of cyanobacteria often dominate the planktonic community and fix nitrogen (e.g., Smith & Bennett 1999). While N may briefly limit phytoplankton production, cyanobacteria quickly add enough to bring N

availability into alignment with P availability – and hence P is the ultimate limiting nutrient in most freshwater lakes of moderate to high productivity (Schindler 1977). In addition to the ratio of N to P, other factors can regulate N fixation by plankton in lakes, including light and depth of the mixing zone, and perhaps grazing (Schaffner et al. 1994). Interestingly, N fixation by heterotrophic bacteria has never been observed as an important process in the water column of lakes (Howarth et al. 1988b), perhaps because heterotrophic bacteria cannot adequately protect the nitrogenase enzyme from the poisoning effect of O₂. Also, N fixation by planktonic cyanobacteria tends to be low in the water column of extremely oligotrophic lakes, many of which may therefore be N limited. The reasons for this require further study.

In contrast, most temperate estuaries are limited by N. Added N stimulates production, and can drive estuarine eutrophication. Added P does not stimulate N fixation in most estuaries; N-fixing cyanobacteria rarely are observed following loading by P. Why do N fixers respond to N limitation in productive lakes but (generally) not estuaries, with enormous consequences for the functioning of those systems? Many hypotheses have been proposed to account for this difference (Howarth et al. 1988b), but most attention has focused on two: (1) that the greater turbulence in estuaries breaks up cyanobacterial filaments and other aggregations, depriving nitrogenase of protection against O₂ (Paerl 1985); and (2) that molybdenum limitation caused by a stereochemical interference of molybdate uptake by sulfate suppresses N fixation (Howarth & Cole 1985; Marino et al. 1990).

Recently, Howarth et al. (1999) developed a simple simulation model for the growth of cyanobacteria in estuaries and lakes. The model (Figure 2) includes phytoplankton growth rates that can be decreased by a low supply of dissolved inorganic N and P (DIN, DIP), the potential for molybdenum limitation of cyanobacterial growth rate, and grazing by zooplankton. Rates of N fixation are modeled by simulating the particular characteristics of N-fixing filamentous cyanobacteria; N fixation takes place only in specialized cells called heterocysts that do not carry out photosynthesis, and so avoid the attendant inactivation of nitrogenase by oxygen. The energy needs for N fixation in each heterocyst are supported by many, in free-living cyanobacteria often 12–50, photosynthetic cells.

The model enables one to evaluate conditions that permit or inhibit the development of a bloom of N fixers; it does not calculate a mass balance of N, so it cannot yet evaluate the point at which enough N has been fixed to suppress further fixation. When the model is run for conditions representing the surface water of lakes, beginning with N concentrations that could limit the growth of phytoplankton, cyanobacteria rapidly respond to N deficiency with growth and substantial N fixation – just as is observed in most lakes

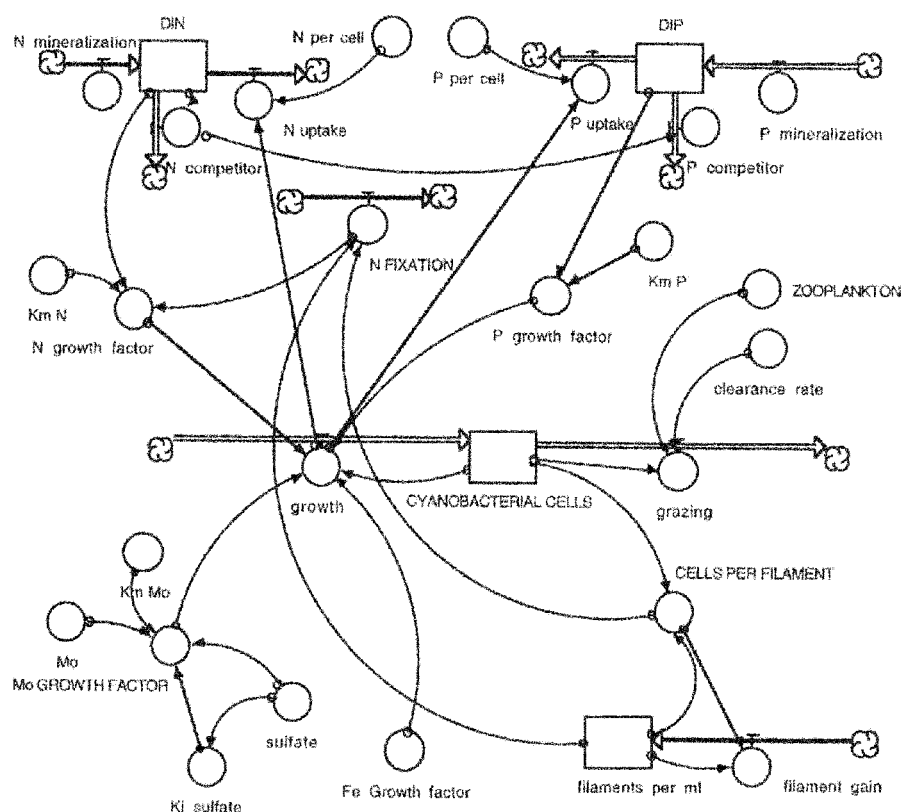


Figure 2. A graphical representation of the Howarth et al. (1999) model for controls on planktonic N fixation in lakes and estuaries.

(Figure 3A). When it is run for estuaries, however, no bloom occurs – again as is observed in most estuaries (Figure 3B). However, if grazing is turned off in the estuarine model, N-fixing cyanobacteria bloom. The growth of a bloom of N-fixing cyanobacteria is delayed relative to lakes, as a consequence of trace element limitation, but the delay is insufficient to prevent a bloom from developing within a season (Figure 3C). The effect of grazing is to cleave the growing cyanobacterial filament, and prevent the accumulation of enough photosynthetic cells to support the energetic requirements of N fixation in heterocysts.

The model describes a situation in which bottom-up (low availability of trace metals leading to slow growth rates) and top-down (grazing) controls interact to suppress the development of N-fixing blooms in estuaries. Neither alone suffices to explain the lack of N fixation (and so the pervasiveness of N limitation) in estuaries, but together they are sufficient.

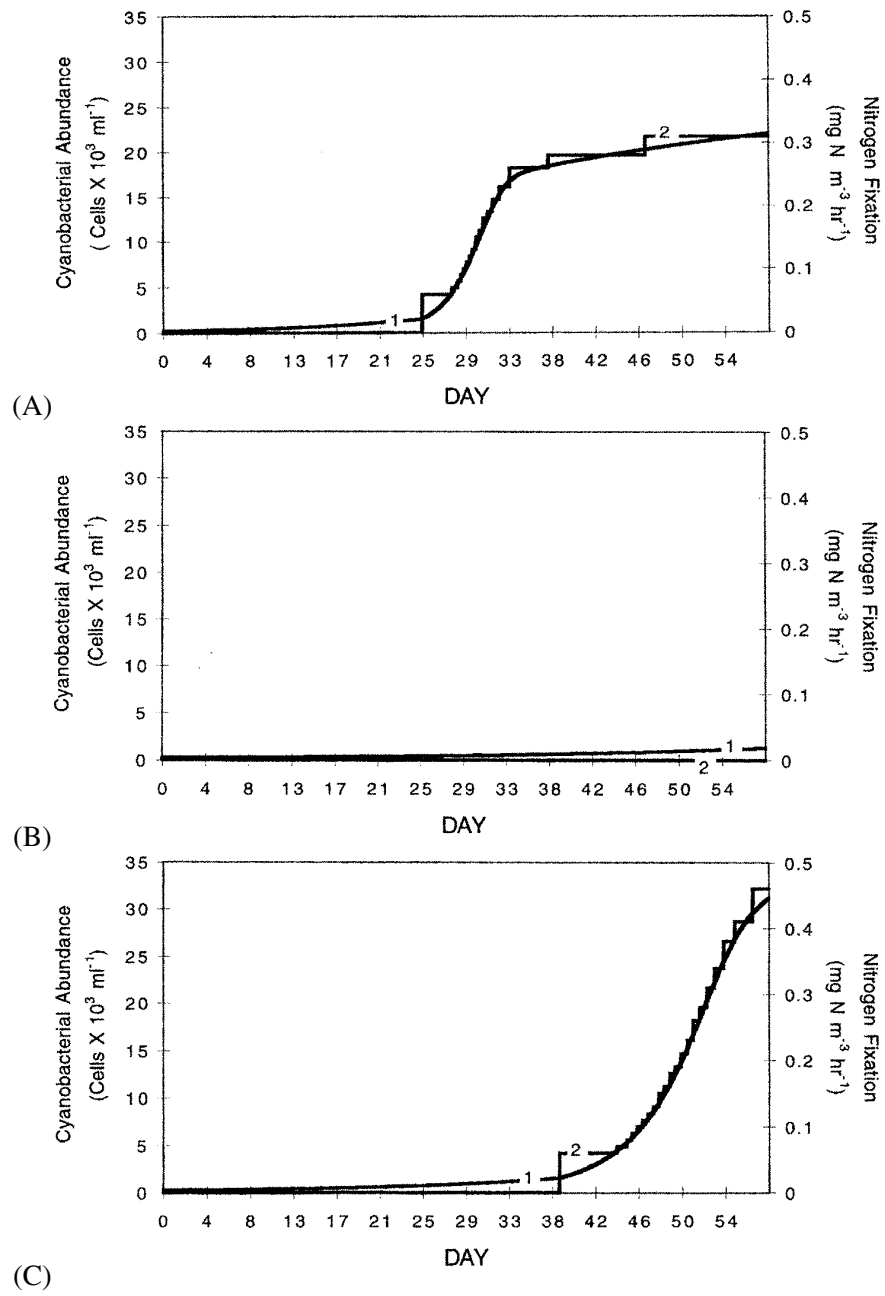


Figure 3. The abundance of N-fixing, heterocystous cyanobacteria and rates of nitrogen fixation under three different simulated conditions.

A. Average freshwater condition of Mo availability and zooplankton grazing.

B. Average seawater condition of Mo availability and zooplankton grazing as in A.

C. Average seawater condition of Mo availability, but zooplankton grazing set to zero.

Mesocosm experiments in estuarine water have validated portions of the model. When zooplankton populations were kept low by the activities of zooplanktivorous fish, planktonic cyanobacteria grew and fixed N, although their growth rate was slower than would typically be observed in freshwaters, probably because of the lower availabilities of trace metals. When zooplankton populations were more representative of estuaries, or when benthic filter feeding animals were added to the mesocosms, their grazing suppressed planktonic cyanobacteria (Marino et al, in preparation).

The model could be expanded to include feedbacks from N fixation to N availability, and from DIN concentrations to N fixation. There are two potential effects of increasing DIN availability on N fixation: (1) suppression of heterocyst formation and nitrogenase synthesis; and (2) loss of competitive advantage for the cyanobacteria. As DIN concentrations increase, other phytoplankton species can grow rapidly. To the extent these other phytoplankton are better competitors for DIP than are the N-fixing cyanobacteria, DIP is drawn down, potentially decreasing cyanobacterial growth.

To what extent can this or other models be extended to N fixation by other cyanobacteria in aquatic systems, or to cyanobacteria in other environments? Heterocystous cyanobacteria are widespread, but numerous non-filamentous cyanobacteria are known – and the model as it presently stands would not identify any constraints to them (over and above factors that influence all phytoplankton), other than trace metal limitation.

Benthic cyanobacteria. Both heterocystous and non-heterocystous free-living cyanobacteria colonize benthic sediments, forming extensive mats, turfs, or felts. These fixers are particularly successful in coastal marine ecosystems such as salt marshes, coral reefs, and intertidal zones. The constraints of high turbulence and control by grazing that operate in estuaries are less important in these types of marine habitats, and the availability of trace metals may be greater because the mats are underlain by reducing sediments (Howarth et al. 1988b). Consequently, rates of nitrogen fixation are among the highest reported for any assemblage of N fixers, from 100 to over 150 mg N₂ m⁻² d⁻¹ in some reports (Wiebe et al. 1975; Jones 1992). Indeed, water flow and grazing are believed to stimulate N fixation in coral reefs by reducing diffusion gradients and pruning turfs to actively growing cell layers (Wiebe et al. 1975; Carpenter et al. 1991; Williams & Carpenter 1997). Genera common in benthic marine assemblages include *Calothrix*, *Microcoleus*, *Lyngbya* and *Oscillatoria*. The last three genera are non-heterocystous and must rely on diel partitioning of N fixation and C fixation, and to some extent spatial partitioning of these processes, to avoid oxygen inhibition (Bebout et al. 1987; 1993; Villbrandt et al. 1991; Joye & Paerl 1993, 1994; Stal 1995).

Benthic cyanobacteria also exploit freshwater ecosystems, where they have received less attention than their planktonic counterparts. Mat-forming genera include *Anabaena* and *Oscillatoria*. Cyanobacteria also occur as epilithic periphyton; common genera include *Calothrix*, *Amphithrix*, *Dichothrix*, *Schizothrix*, *Rivularia*, *Nostoc*, and many others. Phosphorus is often the limiting nutrient in lakes, and fixers may become abundant when P loading increases, in shallow lake margins as well as for the plankton. In one study, N fixation by a periphyton community dominated by *Calothrix* was measured in three lakes receiving different whole-system enrichment treatments (P only, N+P, no addition); highest rates were found in the P-only lake (Bergmann & Welch 1990).

Control of cyanobacterial biomass by benthic grazers is evident in some lakes (Tuchman & Stevenson 1991; Schultze et al. 1996; McCollum et al. 1998), but these studies did not report effects of grazing on rates of N fixation. In one study, a snail (*Elimia* sp.) differentially cropped cyanobacteria, appearing to select food on the basis of growth form rather than by taxon (Tuchman & Stevenson 1991). If grazers select filamentous forms, this preference could control heterocystous-cyanobacterial N fixation in a way similar to that postulated by Howarth et al. (1999) for estuaries. Rates of N fixation by lake periphyton and benthic mats may therefore be subject to some of the same controls as for plankton (e.g., DIN and DIP availability and grazing, and perhaps trace metal availability), but may also be affected by physical factors such as wave action and light availability.

Flowing-water ecosystems. The few studies of nitrogen fixation in flowing-water systems suggest that cyanobacteria and N fixation are limited by some of the same factors limiting other lotic algae: low light in small, heavily shaded streams (Horne & Carmiggelt 1975) and high current velocity (small to mid-sized streams) or high turbidity (large rivers). Indeed, planktonic cyanobacteria do not occur at all in most streams. In desert streams, where shading is minimal and current velocity low, filamentous cyanobacteria often form extensive mats, and very high rates of N fixation have been observed therein (Grimm & Petrone 1997). These systems are characterized by very low DIN availability and abundant DIP, warm water temperatures, slow currents, and high light – all conditions conducive to N fixation. As with marine microbial mats, grazing and turbulence are not important controlling factors, and N fixation rates in *Anabaena* mats approach $150 \text{ mg N}_2 \text{ m}^{-2} \text{ d}^{-1}$. Mats can become very thick, and steep microgradients in the availability of dissolved gases and nutrients may form within them (Stal 1995). In contrast to the *Lyngbya* or *Microcoleus* mats typical of marine benthic habitats, N fixation in heterocystous mat-forming fixers like *Anabaena* peaks during daylight

hours. Cyanobacterial mats may shift from being sources to sinks for fixed N when conditions conducive to denitrification occur deep within mats or during times of low oxygen availability (Joye & Paerl 1993, 1994). Thus one control on the importance of N fixation in aquatic, mat-forming cyanobacteria may be the competing process of denitrification, which can result in loss of up to 20 percent of the nitrogen fixed (Joye & Paerl 1994).

Another constraint to N fixation operates at a larger scale in streams: disturbance of assemblages by infrequent spates, to which cyanobacterial mats are particularly susceptible (Grimm & Fisher 1989). Littoral zones of lakes and marine intertidal zones also experience hydrodynamic disturbance. Export of cyanobacteria due to disturbance affects fixation through its impact on the standing biomass of fixers; it represents a larger-scale sporadic constraint on nitrogen fixation in frequently disturbed ecosystems.

Terrestrial cyanobacteria

The occurrence of cyanobacteria on land is related to their ability to colonize extreme environments that are not fully occupied by vascular plants. Free living cyanobacteria are most abundant relative to other vegetation in dry and/or cold regions such as deserts, grasslands and tundra. Cyanobacteria also regularly occur and fix nitrogen in wetter climates such as temperate or tropical forests, where they occur epiphytically on tree trunks and leaves, in bryophytes or on decomposing logs (Goosem & Lamb 1986, Crews et al. in press). However, their spatial extent and importance as nitrogen fixers appears to be greater in more extreme environments.

Habitat. In arid ecosystems, cyanobacteria typically occur in microphytic soil crusts that can include algae, fungi, bacteria as well as mosses, lichens and liverworts (Evans & Johansen 1999). In general, the extent to which surfaces of arid and semi-arid soils are occupied by these crusts is inversely related to the stone-free ground covered by vascular plants and their litter (Knapp & Seastedt 1986; West 1990; Eldridge & Greene 1994), but some soils are more conducive to colonization by microphytes than others. Soil crusts are generally more extensive in fine textured soils, presumably due to greater moisture holding capacity (Shield & Durell 1964; Graetz & Tongway 1986), and in soils with neutral to slightly alkaline pH (Stewart 1974; Hoffmann 1989).

Soil climate. The niche of terrestrial cyanobacteria is largely defined by their ability to endure (often in a dormant state) conditions of low moisture and/or extreme temperatures. Under these environmental conditions the growth of vascular plants is limited, which in turn allows light to reach the ground

and support the growth of cyanobacteria. However, cyanobacteria grow best under conditions of moderately high temperatures and adequate moisture; thus the success of cyanobacteria is not only attributable to their ability to endure climatic extremes, but also to their rapid physiological response to episodic and often brief favorable growing conditions. Moisture availability in particular has been shown to regulate rates of nitrogen fixation by cyanobacteria in tundra (Liengen & Olsen 1997), prairie (Kapustka & DuBois 1987), semi-arid grassland (Coxson & Kershaw 1983), and desert ecosystems (Rychert & Skujins 1974). Optimal moisture conditions for nitrogen fixation by cyanobacteria are near zero kPa (saturation), and fixation declines linearly with declines in soil water potential (Rychert & Skujins 1974; DuBois & Kapustka 1983; Kapustka & duBois 1987). Desiccated cyanobacteria have been shown to attain maximum rates of nitrogenase activity within 4–14 hours of rehydration (Coxson & Kershaw 1983; Kapustka & DuBois 1987). Temperature also influences rates of N fixation by terrestrial cyanobacteria, with maximum rates occurring between 19–35 °C depending on species and ecosystem (Rychert & Skujins 1974; Coxson & Kershaw 1983; Johansen & Rushforth 1985; Chapin et al. 1991; Liengen & Olsen 1997).

Available N, P and other elements. The extent to which combined N controls cyanobacterial N fixation in natural terrestrial ecosystems is not clear. Liengen and Olsen (1997) found N fixing activities by cyanobacteria to positively correlate with C:N ratios of the cyanobacterial layer in an Arctic tundra site. Work by Eisele et al. (1989), however, suggests that the inhibition of N fixation by combined N may be tied to the ratio of available N: available P, paralleling the behavior of cyanobacteria in many freshwater ecosystems and managed terrestrial ecosystems (Schindler 1977; Smith 1992). In a tallgrass prairie, Eisele et al. (1989) found that fire effectively lowered the ratio of available N:P, stimulating N fixation. The development of a stoichiometric approach to understanding elemental controls on fixation rates by terrestrial cyanobacteria (Eisele et al. 1989) provides a powerful framework for reconsidering other reports demonstrating the regulation of N fixation by combined N, available P or other nutrients (e.g., Wilson & Alexander 1979; Paul & Clark 1989; Chapin et al. 1991; Liengen & Olsen 1997; Crews et al., in press).

Disturbance by trampling and fire. The abundance and diversity of cyanobacteria in soils is strongly influenced by the frequency and intensity of two types of disturbance: fire and trampling. In semi-arid ecosystems that receive sufficient precipitation to accumulate a fuel load, fire has been shown to reduce populations of cyanobacteria (West 1990) (in contrast to the wetter tallgrass prairie discussed above). For example, Johansen et al. (1993) reported

the disappearance of a common N-fixing species, *Nostoc commune*, from recently burned rangelands in the lower Columbia Basin. Trampling of soil microphytic communities by humans, vehicles and ungulates also has been shown to reduce the diversity and/or abundance of soil cyanobacteria in semi-arid ecosystems (Beymer & Klopatek 1992; Belnap et al. 1994). Within a year following a disturbance, free-living cyanobacteria re-colonize sites and dominate for years or decades until slower growing lichens, liverworts and other microphytes reestablish (Anderson et al. 1982; Evans & Belnap 1999).

The importance of N fixation by terrestrial cyanobacteria. Cyanobacteria have the potential to supply an appreciable amount of combined N to terrestrial ecosystems. Reviews report N fixation rates to range between 1–41 kg ha⁻¹ yr⁻¹ with the majority of studies ranging between 1–10 kg ha⁻¹ yr⁻¹ (Boring et al. 1988; West 1990; Warren 1995; Cleveland et al. 1999). Since the vascular plant communities in arid to semi-arid regions generally do not achieve canopy closure due to water limitation, cyanobacteria on soils have the potential to persist indefinitely. Even relatively low rates of N fixation could therefore have the potential to reduce or eliminate N limitation in arid ecosystems. Nitrogen limitation, however, has been well documented in most arid ecosystems studied (see Peterjohn & Schlesinger 1990; Hooper & Johnson 1999).

N limitation in arid ecosystems could be driven by N outputs that offset the relatively small but steady inputs from N fixation. With the exception of vertical leaching of nitrate through the soil profile, which is negligible, arid ecosystems experience the same avenues of N loss as more mesic systems, including erosion, ammonia volatilization and denitrification (Peterjohn & Schlesinger 1990; Crawford & Gosz 1982). The high pH of alkaline soils in arid regions can lead to high rates of N loss through ammonia volatilization. Evans and Boyd (as cited in Evans & Johansen 1999) measured high rates of NH₃ volatilization from microphytic crusts compared to soil where crusts had been removed. A number of studies suggest that episodic denitrification is the most important avenue of N loss in many arid ecosystems; indeed, relatively high rates of N loss associated with fluctuating soil moisture may contribute to the enriched $\delta^{15}\text{N}$ observed in dryland ecosystems (Austin & Vitousek 1998; Handley et al. 1999). Peterjohn and Schlesinger (1991) estimated annual N losses via denitrification to be greater than 7 kg ha⁻¹ at the La Jornada LTER site in the Chihuahuan Desert. High rates of denitrification reported in arid ecosystems led Skujins (1981) to suggest that over 99 percent of the nitrogen fixed by cryptogamic crusts may be lost through this process,

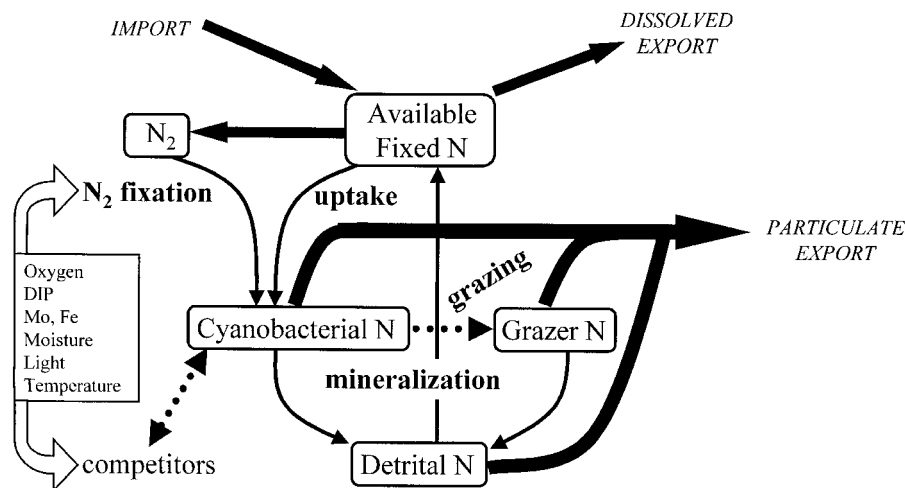


Figure 4. A generic model of the fluxes of nitrogen associated with free-living cyanobacterial nitrogen fixing systems. The relative importance of controls on these fluxes varies among the main system types considered: planktonic fixers, aquatic cyanobacterial mats, and terrestrial cyanobacterial crusts. Factors that control the rates of nitrogen transformations (thin black arrows), including oxygen, P, Fe, and Mo availability, light, temperature, and water availability, are shown as a box (ecophysiological controls) linked to N fixation (open arrows). At least some of these controls affect competitors as well as fixers. Grazing and competitive interactions (dotted arrows) are ecological controls that determine the presence/absence of nitrogen fixers and whether N fixation or uptake of fixed N is the predominant route of N acquisition. Export and denitrification are outputs (thick, black arrows) that affect the mass balance of N at the ecosystem level.

although there is evidence that vascular plants can acquire much of the N fixed by soil microphytic crusts (Evans & Ehleringer 1993).

Cyanobacteria in differing environments

Are there sufficient commonalities among the free-living cyanobacteria in oceans, estuaries, lakes, streams, and soils to develop a generic conceptual model of controls on their fixation of N? A start towards such a model is summarized in Figure 4. It includes both physiological and ecological controls, as well as higher level constraints, that apply to varying degrees across ecosystems. It suggests that we could use N as a currency, allowing us to consider other N transformations (e.g., assimilation of DIN and denitrification) as factors potentially controlling N fixation. In general, availability of DIN, DIP, and micronutrients, and, for non-heterocystous cyanobacterial mats, the ability to spatially or temporally segregate oxygen production and N fixation, are the most important controls on rates of N

fixation in free-living cyanobacteria. Competitors and grazers change the availability of nutrients; they may also exclude cyanobacteria altogether in some environments (e.g., shading of terrestrial free-living cyanobacteria by vascular plants).

Vascular plant-bacteria symbioses

The most important N fixers in terrestrial ecosystems are symbioses involving legumes and rhizobia, and the similar symbioses of a number of plants (*Alnus*, *Myrica*, *Rosaceae*, and others) with the actinomycete *Frankia*. These symbioses are well-studied on the biochemical and organismal levels. Areas dominated by symbiotic N fixers, whether agricultural or natural, can have very high rates of N fixation – often exceeding $100 \text{ kg N ha}^{-1} \text{ y}^{-1}$. Moreover, while woody legumes are most abundant in tropical regions, both herbaceous legumes and actinorhizal (*Frankia*-based) symbioses are widespread in temperate and even boreal regions. Many terrestrial ecosystems, especially at higher latitudes, are limited by N supply. Again, we ask why don't symbiotic N fixers respond to this N deficiency, and (ultimately) reverse it?

Vitousek and Field (1999) recently described a simple model for the ecosystem-level control of symbiotic N fixation. This model was designed for the same purpose as the estuarine model discussed above (Howarth et al. 1999) – to explore mechanisms that could keep N fixers from responding to N deficiency in ecosystems, and so could maintain limitation by N. Unlike the estuarine model, the terrestrial symbiotic fixer model is not individual-based; rather, it evaluates productivity and nitrogen acquisition by a generic non-fixer and a generic symbiotic N fixer. Also unlike the estuarine model, the terrestrial model is mass-balanced in N, including outputs as well as inputs, and it is possible for fixation to change the N status of an ecosystem with consequent effects on the relative abundance of fixers and non-fixers.

The model includes two pathways of N input: precipitation, which is controlled externally, and N fixation. It also includes two types of outputs: leaching and denitrification of the “excess available N” that remains within the soil after plants and microbes have taken up what they can, and losses of N by pathways that could continue even where and when N strongly limits biological processes in the ecosystem. Dissolved organic N loss may be an example of such a loss (Hedin et al. 1995; Vitousek et al. 1998; Campbell et al. 2000).

Initially, the model is based on a greater cost for N acquisition via fixation as opposed to uptake from inorganic N pools in soil. This cost has been evaluated biochemically; it conforms to the inverse relationship between the amount of available inorganic N supply and the proportion of plant N derived

from N fixation by legumes (Allos & Bartholomew 1959), and to models of biological N fixation in terrestrial (Pastor & Binkley 1998) and marine (Tyrell 1999) ecosystems. The cost of N acquisition by the non-fixer is assumed to be dependent on N availability, being relatively low when N availability is high, but increasing once most of the available N in the soil has been taken up. In contrast, the cost of N fixation is assumed to be independent of N availability in the soil; it is higher than the cost of N acquisition from the soil as long as any appreciable available N remains in the soil. The model gives non-fixers priority for any available N in the soil; only if nearly all the inorganic N in the soil is exhausted and other resources remain available do N fixers grow and fix N. This last assumption represents too strong a constraint on N fixers, because they are known to be able to compete for available soil N. However, the point of the model is to ask why N fixers fail to respond to N deficiency – and if they do respond despite this rigid constraint, to identify other kinds of constraints that could keep N fixers out of N-limited ecosystems.

The model has been run for systems that start with no N or biomass, and accumulate N from either precipitation or the atmosphere. When the model is run without N fixation, N can only be accumulated slowly from very dilute concentrations in precipitation (prior to widespread alteration of the N cycle by humanity), and biomass accumulation is slow (Figure 5A). Adding the potential for N fixation causes a much more rapid accumulation of biomass and N, to the same equilibrium point (Figure 5C). These results are based on simulations of a system in which N can only be lost from an excess available N pool. If an additional pathway of loss is added that is independent of available N, then it is possible to get sustained N limitation in the absence of N fixation (Figure 5B). However, where N fixation can occur (under the conditions of this model), it has the capacity to overwhelm any losses of N, and maintain the system at the same equilibrium as occurs with losses of only excess available N (Figure 5D).

Constraining N fixation simply by giving non-fixers absolute priority for fixed N does not work to sustain N limitation, under the conditions in this model. What else could constrain symbiotic N fixation? A number of other pathways have been suggested (Vitousek & Howarth 1991; Vitousek & Field 1999), including:

- (1) Reduced shade tolerance of symbiotic fixers, such that they are unable to enter closed canopy systems, even though if they would reach the canopy they could fix actively. (Disturbance will open the canopy, but disturbance also enhances N availability temporarily, thereby denying fixers an advantage at the one time in succession when sufficient light is available (Vitousek & Howarth 1991).)

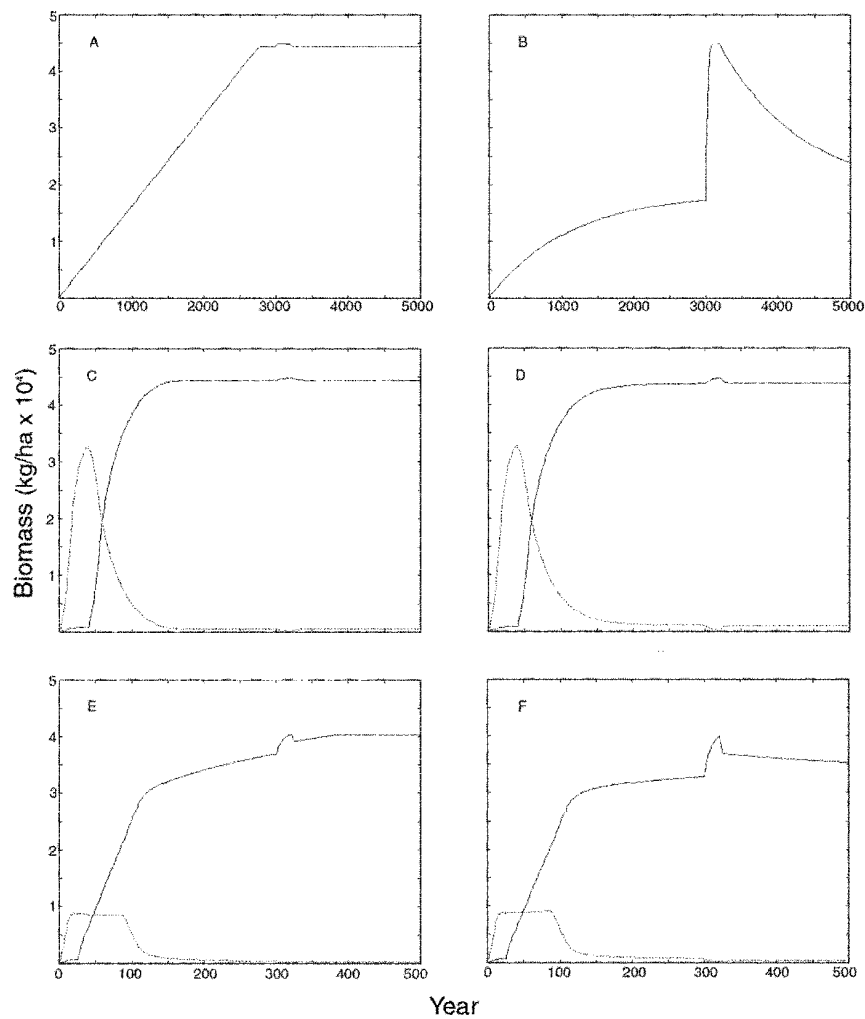


Figure 5. Simulated biomass of a symbiotic N-fixing plant (dashed line) and a non-fixer (solid line) over time, starting with no organic C or N in soil. In each, N fertilization was simulated for 20 years (after 3000 years in A and B, 300 years in C–F) to show the extent of N limitation. Revised from Vitousek and Field (1999).

A. No N fixation (inputs of N from atmospheric deposition only), and losses of N from an excess available N pool only (N remaining in available form after plants take up what they can).

B. No N fixation, an uncontrollable N loss in addition to excess available N.

C. Symbiotic N fixation can occur; it is controlled by a higher cost of N acquisition via fixation; losses of excess available N only.

D. Symbiotic N fixation, both pathways of loss.

E. Symbiotic N fixation can occur, but it is further constrained by shade intolerance, a greater P requirement, and preferential grazing; losses of excess available N only.

F. Symbiotic N fixation with the additional constraints; both pathways of N loss.

- (2) A greater sensitivity of N-fixing organisms to limitations caused by a deficient supply of P or another nutrient (Smith 1992; Cassman et al. 1993).
- (3) Preferential grazing on the protein-rich tissues of N fixers, preventing them from responding to N deficiency (e.g., Ritchie & Tilman 1995; Ritchie et al. 1998). Many N fixers are chemically defended against grazing, but that defense comes at the energetic cost of synthesizing and maintaining defensive compounds.

The model was modified to include each of these possible controls; each is capable of constraining N fixation substantially, and thereby delaying the accumulation of biomass and N in simulations of developing ecosystems (Vitousek & Field 1999). However, even when all three were combined, there was relatively little effect on the equilibrium state of the system – as long as N could only be lost from excess available N pools (Figure 5E). Adding an additional leak of N yielded a simulated system that is substantially and persistently limited by N at equilibrium, and in which N fixers are not able to grow enough to offset that N deficiency (Figure 5F). This result is interesting because most conceptual models of ecosystems, in which N is lost from excess available N pools and symbiotic N fixation is constrained by its energetic cost, take us to Figure 5C – while much of the world (especially in the temperate and boreal zones) appears to work more like Figure 5F. It would be rewarding to understand the processes that take us from the scenario illustrated in Figure 5C to that in Figure 5F.

Issues in symbiotic N fixation

Discussions among the authors of this chapter identified several ways that our understanding of – and ability to model – symbiotic N fixation might be improved. These include:

- (1) A more realistic characterization of the energetics of N acquisition from the soil versus by N fixation, and their consequences.
- (2) A clear determination as to whether symbiotic N fixers in fact have a systematically greater requirement for P, or less competitive ability to acquire it, than do non-fixing plants.
- (3) An explanation for the abundance of woody legumes in the canopy of late-successional tropical forests, in contrast to the near absence of symbiotic N fixers from closed-canopy forests elsewhere.

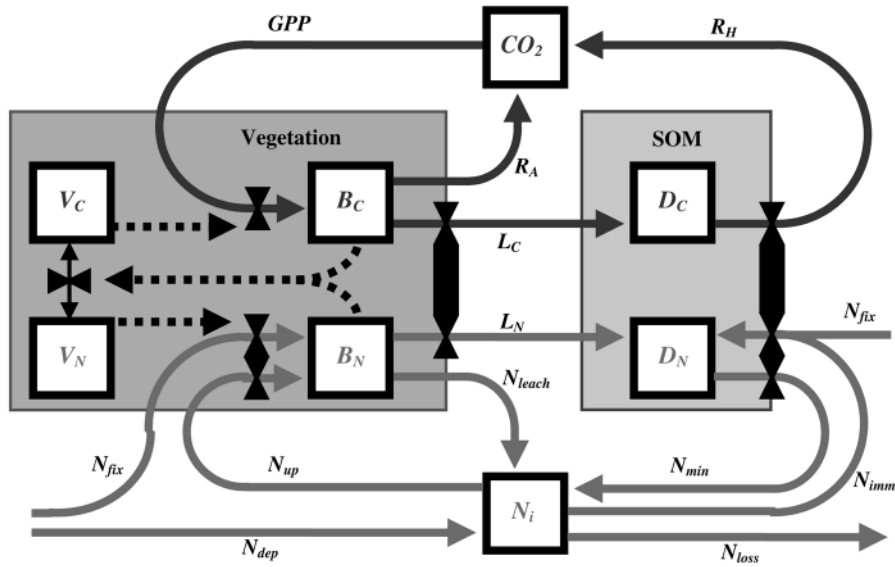


Figure 6. Structure for the Multiple Element Limitation (MEL) model (Rastetter et al. 1997) with inputs of N via biological fixation included. N fixation can occur when the energetic cost of acquiring N by investing in roots/mycorrhizae exceeds the cost of acquiring N via fixation.

Modeling costs of N-fixation

The costs and benefits of N fixation were assessed within the framework of the Multiple Element Limitation (MEL) model (Rastetter & Shaver 1992; Rastetter et al. 1997; Herbert et al. 1999), allowing the development of a theoretical model of symbiotic N fixation based on a resource optimization paradigm (Mooney & Gulmon 1979; Bloom et al. 1985; Chapin et al. 1987; Field et al. 1992; Rastetter & Shaver 1992; Rastetter et al. 1997; Herbert et al. 1999). Under this paradigm, resources within plants should be allocated so that growth is co-limited by all external resources; otherwise internal resources would be wasted by taking up some external resources in excess at the expense of not acquiring resources that are more limiting to growth. An implication of this paradigm is that N fixation should occur when the resource cost of N fixation is less than that of N uptake from other sources. We therefore develop our model by assessing the relative costs of N fixation versus N uptake. The structure of this model is outlined in Figure 6.

We assume that the only cost of N fixation to the plant is through the supply of C to the N-fixing symbiont and denote this cost as r_{Nfix} ($g\ C\ g^{-1}\ N$). This cost has been estimated at about $8\ g\ C\ g^{-1}\ N$ for symbiotic N fixation in terrestrial ecosystems (Gutschick 1981). However, the value of r_{Nfix} might not be fixed. For example, if N fixation is limited by the availability of P or

of cofactors like Mo and Fe, then r_{Nfix} would have to be adjusted to reflect the cost of acquiring these cofactors.

We assess the cost of N uptake in terms of the C gain that would be realized if the resources expended on N uptake were reallocated toward photosynthesis. To make this assessment, we use a modified version of the coupled C and N uptake equations from the MEL model:

$$U_C = g_C \left(\frac{C_a}{k_C + C_a} \right) (1 - e^{-b_C R_I V_C}) \quad (1)$$

$$U_N = g_N \left(\frac{N_I}{k_N + C_I} \right) (1 - e^{-b_N R_I V_N}) \quad (2)$$

where U_C and U_N are the C and N uptake rates, C_a and N_I are the environmental concentrations of available inorganic C and N (i.e., CO_2 and $\text{NH}_4 + \text{NO}_3$), R_I is a measure of the total amount of internal resources that can be allocated toward the uptake of external resources, and V_C and V_N are the fractions of R_I allocated toward C and N uptake, respectively. g_C and g_N are maximum uptake rates under saturating substrate concentrations and fully exploited canopy and soil volume; k_C and k_N are half-saturation constants for the Monod kinetics relating uptake to substrate concentration; and b_C and b_N are parameters controlling the diminishing return on uptake effort as the canopy and soil volumes become fully exploited by leaves and fine roots, respectively. For C, this diminishing return on uptake effort can be interpreted as a Beer's Law formulation of light extinction. For N, an analogous diminishing return on uptake effort is associated with the interference among fine roots as they become densely packed in the soil.

Under the optimization paradigm described above, V_C and V_N should adjust so that growth is equally limited by C and N. In the MEL model, this adjustment is achieved by comparing the C:N ratio of the simulated vegetation to an allometrically determined optimum C:N ratio. V_C and V_N are adjusted incrementally to drive the C:N ratio toward the optimum. For the present purposes, we must also consider uptake effort expended toward the acquisition of other resources (e.g., P). We represent the fraction of R_I allocated toward the acquisition of all these other resources as V_O and define a variable f such that $V_O = (f - 1)V_C$ (f will be a function of the availability of these other resources and C). Because the V_i represent fractions of the internal resources that can be allocated toward acquisition of external resources, we impose the restriction that $fV_C + V_N = V_O + V_C + V_N \equiv 1$.

With this relationship, a C cost of N uptake can be assessed in terms of the reallocation of uptake effort between N and C; under unchanged environmental conditions, the rate of C uptake must decrease as the rate of

N uptake increases when effort is reallocated. Thus, the cost of N uptake (gCg^{-1}N) can be assessed as the incremental decrease in C uptake per incremental increase in N uptake,

$$r_{Nup} = -\frac{dU_C}{dU_N} = \frac{dU_C}{dV_C} \frac{dV_C}{dV_N} \left(\frac{dU_N}{dV_N} \right)^{-1} \quad (3)$$

Because $fV_C + V_N \equiv 1$ and assuming that f does not change appreciably with changes in V_N ,

$$\frac{dV_C}{dV_N} = -\frac{1}{f}.$$

From the uptake equations,

$$\frac{dU_C}{dV_C} = G_C b_C R_I e^{-b_C R_I V_C}$$

$$\frac{dU_N}{dV_N} = G_N b_N R_I e^{-b_N R_I V_N}$$

where $G_C = g_C \left(\frac{C_a}{k_C + C_a} \right)$ and $G_N = g_N \left(\frac{N_I}{k_N + N_I} \right)$. Thus, the cost of N uptake can be calculated as

$$r_{Nup} = \frac{G_C b_C}{f G_N b_N} e^{R_I (b_N V_N - b_C V_C)}. \quad (4)$$

If $r_{Nup} > r_{Nfix}$ then it is more economical to expend C fixing N than taking N up from inorganic sources in the soil. This equation implies that there should be a tendency towards fixing N if:

- (1) the CO_2 concentration is high (G_C large) so that there is a high return on resource allocation towards the canopy,
- (2) inorganic N concentrations in the soil are low (G_N small) so there is a low return on the allocation of resources towards N uptake from soil,
- (3) the canopy is open (small $b_C R_I V_C$) so there is a high return on resource allocation to the canopy,
- (4) the soil is well exploited by roots (large $b_N R_I V_N$) so there is a low return on allocation of resources towards N uptake from soil, and
- (5) other resources like P are readily available so that the allocation of effort toward the uptake of these other resources is small relative to that allocated toward C acquisition (f is small).

Equation 4 automatically scales the relative costs and benefits of these factors.

The optimization paradigm implies that N fixation will occur if a further reallocation of uptake effort toward N acquisition will result in $r_{Nup} > r_{Nfix}$. The critical N uptake effort (V_{N*}) above which it becomes more economical to fix N rather than take it up can be calculated by setting Eq. 4 equal to r_{Nfix} and solving for V_N :

$$V_{N*} = \frac{f \ln \left(\frac{r_{Nfix} f_{G_N} b_N}{G_C b_C} \right) + R_I b_C}{R_I (f b_N + b_C)} \quad (5)$$

Thus, in the adjustment scheme for uptake effort, V_N should never increase above V_{N*} . If the C:N ratio of the vegetation is still too high with $V_N = V_{N*}$, then an increase in N fixation (U_{Nfix}) will be more cost effective than an increase in V_N . The increase in U_{Nfix} can be formulated in much the same way as the incremental increases in V_N described above, except that the C cost of this increase is reflected in an increase in respiration ($R_{Nfix} = r_{Nfix} U_{Nfix}$) rather than in a decrease in V_C .

This approach to modeling N fixation is being implemented in MEL, for a range of ecosystems (Rastetter et al., in preparation). It offers a more mechanistic way of evaluating the energetic costs of N fixation than has been available to date. As such, it should be useful for analyzing the consequences of anthropogenic or natural changes in CO₂, N availability, and other resources.

Phosphorus requirement and acquisition

The importance of P supply and of N:P supply ratios in controlling rates of symbiotic N fixation is discussed widely (e.g., Smith 1992; Cassman et al. 1993; Crews 1993). What is the basis of the effect of P on N, and how general is it? There are at least two possible mechanisms, with rather different implications for the control of N fixation. One is that most systems are limited (ultimately) by P supply, with the supply of N adjusting to that of P in the long run (e.g., Walker & Syers 1976; Schindler 1977; Vitousek & Farrington 1997; Tyrrell 1999). If we add P to systems (or lose N without losing P), then N becomes limiting to organisms other than N fixers, and N fixers could have an advantage. The other alternative is that symbiotic N fixers systematically require more P or are less competitive in acquiring P than are non-fixers. In this case there will be a level of P availability at which N fixers are limited by P, but non-fixers are limited by N. The greater the disparity in P requirements between fixers and non-fixers, the greater the disequilibrium in supply of N versus P.

We see two reasons why symbiotic N fixers might have a greater requirement for P than non-fixers. The first is that building and/or maintaining the symbiosis, and/or the fixation process itself, requires more P than is needed by non-fixing organisms. Alternatively, fixers could have a P-demanding lifestyle, to go with, indeed to support, the N-demanding lifestyle (discussed below) that McKey (1994) proposes to be driving the legume-rhizobium symbiosis. To the extent that N is used metabolically (as opposed to its use in defensive compounds), a commitment to high N levels in organisms may entrain a commitment to high P levels as well.

Does N fixation per se involve a higher demand for P than other forms of N acquisition? This question can be considered in two parts, capital and running costs. The bacteroid component of infected cells has a high P content, partly for the adenosine nucleotides needed to provide energy used in the nitrogenase reaction (Sprent & Raven 1985). Bacteroids are enclosed in membranes that also have a high P content. In annual herbaceous species reliant on atmospheric N₂, the plant maintains about 5 percent of its dry weight as nodules, but in woody perennials the proportion decreases as more nutrients are recycled within the plant. The P concentration of nodules can range from 0.2–0.6% of DW depending on species and age (Allen et al. 1988), similar or slightly higher than that of leaves, which normally are a much higher fraction of total plant DW. Thus the P investment in nodules is a relatively small part of total plant P. Although fixation of N is energy intensive, P is derived from ATP recycling once the necessary ATP has been synthesized.

In order to show that legumes fixing N require more P than those reliant on mineral N, comparisons must be made on an appropriate basis. These can be done by examining the N × P interaction when plants are grown on N₂ or mineral N with increasing levels of P supply (Robson 1983 and other publications from Robson's lab). If the interaction is negative, it suggests that plants fixing N₂ have a higher requirement for P than those growing on mineral N. A zero interaction suggests that the two forms of N are equally demanding of P and a positive interaction that high P levels may be inhibitory to N₂ fixation. Most of the interactions reported are negative. However, most studies have been of agricultural species, which are not adapted to low-nutrient soils. A zero interaction may be more common in natural environments and/or when woody plants are studied. For example Sanginga et al. (1995) found that nodulated *Gliricidia sepium*, a species widely used in agroforestry, only showed a response to P at the lowest level applied, which probably reflected the basic requirement for plant growth. *Acacia mangium* was found by Ribet and Drevon (1996) to have similar P requirements for growth on either N₂ or urea.

Another indicator of P requirement could be the P content of tissues other than nodules; if leaves of nitrogen fixing plants have higher P than those of non-fixing plants, this could reflect a greater requirement for P. Values in the literature fall into two groups, those from agricultural experiments and those from natural or unfertilised soils. Within these two groups legumes and non-legumes overlap. Although these data should be regarded with caution, since they were not gathered to address this particular question, they do suggest that not all legumes have an inherently higher requirement for P than do non-legumes.

The indisputable fact that many grasslands will only support nodulated legumes when they have sufficient available P may be a reflection, not only of the legumes' requirement for P, but also of inherent differences between grasses and legumes. These differences may influence P acquisition and concentrations. Simulation of P uptake from soil identifies root length density as the most sensitive parameter governing the rate of uptake (Barber 1984). While grasses have fibrous root systems that thoroughly exploit the top soil layer where P availability is greatest, many legumes have a tap root system with relatively less root length development in top soil. In addition, legume root nodules can represent a significant fraction of total root system biomass during early vegetative growth stages in low N supply environments. Hence, competition between nodule and root growth for assimilate supplied by the shoot can result in a reduction in the number and length of roots (Nutman 1948; Dart & Pate 1959; Cassman et al. 1980). Taken together, root system architecture and the effects of nodule growth on early root development may cause legumes to be less competitive for P against grasses that have dense, fibrous root systems, which may in turn help explain the need for P fertilizer applications to sustain legume components in grazed pastures (Smith 1992).

Sprent (1999) has summarized some of the ways in which legumes endemic to infertile soils can release P from normally unavailable sources. These include a greater overall allocation to roots (e.g., Binkley & Ryan 1998), possession of either or both of ecto- and arbuscular mycorrhizas, cluster (proteoid) roots and secretion of chelating agents which can separate P from inorganic complexes. All of these impose a carbon cost, and may be most suited to plants growing in a high light environment. These strategies are no different from those of other plants living in the same environments.

Overall, our understanding of the nature of the relationship between P supply and symbiotic N fixers remains incomplete. There is no question that some legume crops require large quantities of P, more when they acquire N by fixation than when they acquire it from soil. However, whether this greater P requirement extends to symbiotic N fixers that are adapted to infertile soils is unclear, as is the question of whether a greater requirement (where it exists)

represents a P cost of N fixation per se or competitive disadvantages in P acquisition compared to non-fixers. Alternatively, legumes may have evolved a commitment to a more P- demanding lifestyle. Information on P concentrations in symbiotic N fixers and non-fixers in high diversity ecosystems, such as the patterns for N concentrations in potentially N-fixing legumes, non-fixing legumes, and non-legumes discussed in the next section, would be a useful first step towards addressing this last possibility.

Legumes in the canopy of tropical forests

Trees with the capacity to fix atmospheric N are very sparse or absent in the canopy of most late-successional temperate forests. However, legumes represent one of the most diverse and abundant families of higher plants in the canopy of many lowland tropical forests (e.g., Prance et al. 1976; Allen & Allen 1981; Moreira et al. 1992). A number of lines of evidence, including N concentrations in leaves and litterfall, rates of N mineralization, N trace gas emissions, and patterns of ^{15}N enrichment, suggest that soil N supply is relatively high in many lowland tropical forests – indeed that it may function as an excess nutrient there (Vitousek & Sanford 1986; Matson & Vitousek 1987; Keller & Reiners 1994; Martinelli et al. 1999; Matson et al. 1999). It is not hard to see why abundant canopy legumes might lead to high N availability – but it is more difficult to understand why potential N fixers persist (in abundance) in the biome in which N availability appears to be the greatest.

A possible explanation for the abundance of legumes in tropical forests can be developed based on McKey's (1994) suggestion that legumes in general have an N-demanding lifestyle – that they require higher concentrations of N than do plants in other families. He suggests that this greater requirement for N should be observed whether or not an individual plant is acquiring its N by fixation, and whether or not an individual species of legume even has the capacity to fix N.

McKey (1994) reviewed results of a number of studies; all reported higher concentrations of N in legumes than non-legumes. Moreover, while legumes in the subfamilies *Mimosoideae* and *Papilionoideae* (which most often support N-fixing symbioses) had greater N concentrations than those in the *Caesalpinioideae* (which generally do not), the *Caesalpinioideae* had higher N concentrations than did non-legumes. McKey noted that relatively few species were included in these comparisons, but suggested that the results are consistent with a greater demand for N by legumes – independent of N fixation.

Additional data on N concentrations in tropical forests can be applied to this question. In the lowland primary forest of the Samuel Reserve in Brazil,

the average foliar N concentration of *Mimosoideae* species ($2.73 \pm 0.90\%$, $n = 9$) was significantly higher than that of *Caesalpinioideae* species ($2.20 \pm 0.60\%$, $n = 44$) which, in turn, was significantly higher than that of non-legume species ($1.79 \pm 0.50\%$, $n = 254$). Analyses of foliar N in Brazilian cerrado show that mimosoid legumes averaged $1.76 \pm 0.14\%$ ($n = 39$), papilionoid legumes averaged $1.93 \pm 0.08\%$ ($n = 86$), nodulated caesalpinoid legumes of the genus *Chamaecrista* $1.61 \pm 0.07\%$ ($n = 287$), non-nodulated caesalpinoids $1.75 \pm 0.12\%$ ($n = 71$), and non-legumes $1.28 \pm 0.07\%$ ($n = 57$) (Sprent et al. 1996). In an Amazonian inundation forest (*várzea*), the average foliar concentration of *Papilionoideae* species was $3.28 \pm 0.19\%$ ($n = 11$), *Mimosoideae* averaged $2.62 \pm 0.45\%$ ($n = 7$), *Caesalpinioideae* species averaged $2.45 \pm 0.85\%$ ($n = 5$), and non-legume species $1.96 \pm 0.61\%$ ($n = 59$). Although in the latter case N concentrations in *Caesalpinioideae* did not differ significantly from non-legumes, the pattern is consistent with McKey's explanation. Yoneyama et al. (1993) also found higher foliar concentrations of N in legume species in comparison to non-legumes in a study site near Manaus (central Amazon), regardless of nodulation capacity.

To what extent are the potentially N-fixing legumes present in the canopy of late-successional tropical forest deriving their abundant N from fixation? As noted earlier, it is difficult to measure rates of fixation in the field, especially in perennial vegetation. However, ^{15}N natural abundance can provide a qualitative means for identifying trees that derive a substantial fraction of their N via fixation. The rationale for this is that N fixers acquire some of their nitrogen from the air, which has a nitrogen isotopic composition ($\delta^{15}\text{N}$) of 0‰. Where the N derived from soil has a very different isotopic composition, the isotopic composition of a fixing plant differs from that of a non-fixing plant. Relatively large differences between atmospheric and soil $\delta^{15}\text{N}$ are necessary for the success of this approach (Hogberg 1997; Handley & Scrimgeour 1997).

Fortunately, the $\delta^{15}\text{N}$ of soil, and of trees that derive their N from soil, is highly enriched in many lowland tropical forests (Martinelli et al. 1999), so it should be possible to identify individual legumes that derive much of their N from fixation with some confidence. The comparison of average foliar $\delta^{15}\text{N}$ content in leguminous trees and non-fixing species from two areas of primary forest in the Amazon region indicated that only a few trees in "terra-firme" forests were fixing N. In the Samuel forest, only four individuals out of 34 had $\delta^{15}\text{N}$ significantly lower than the average foliar $\delta^{15}\text{N}$ value of non-fixers, while Yoneyama et al. (1993) found only one individual out of 18 with $\delta^{15}\text{N}$ significantly below the foliar $\delta^{15}\text{N}$ of non-fixers in a site near Manaus.

The facts that legume trees are abundant in tropical forests, support high N concentrations, and are not regularly fixing N suggests that symbiotic N fixa-

tion might occur only during temporary N shortage (McKey 1994). Bonnier and Brakel (1969), cited by Sylvester-Bradley (1980), suggested a similar explanation for the lack of nodulation in African primary forests. Moreover, measurements of nitrogen fixation in several areas of tropical forests in Brazil, carried out by Sylvester-Bradley et al. (1980), support this working hypothesis. The occurrence of nodules was rare in “terra- firme” primary forest in the central Amazon where N is highly available, sporadic in disturbed primary forests (roadside and small clearings) and secondary forests, and frequent in cultivated soils where N losses generally are substantial. Acetylene-reducing activity also showed a similar trend, being greater in perturbed than in pristine areas. Clearing, fire, and cultivation cause a temporary shortage of N that, in turn, could trigger N fixation by legumes.

With few exceptions, legumes are absent from the canopy of late- successional temperate forests, although herbaceous legumes fix N actively all the way to high arctic and alpine ecosystems. The absence of woody legumes could represent a phylogenetic constraint (Crews 1999), in that legumes originated and radiated in the tropics. However, woody actinorhizal species are widespread in temperate and boreal forests, if generally confined to early successional ecosystems. The extent to which actinorhizal species may have committed to an N-demanding lifestyle is worth investigating; recent phylogenetic work demonstrates that actinorhizal and rhizobial species share a clade (Soltis et al. 1995).

The Vitousek and Field (1999) model discussed above addressed a number of reasons why N fixers are absent from the canopy of late-successional temperate forests, where N is in short supply and an N-fixer might be expected to have a substantial advantage. Another reason that could contribute to this pattern is the lower overall availability of N in temperate forests – the very factor that it seems should give fixers an advantage. The striking difference in N availability between most lowland tropical forests and most temperate forests could be due, at least in part, to processes in addition to the abundance of legumes. One possibility is the more rapid rates of decomposition in the tropics; these cause rapid cycling of N through litter, while colder systems immobilize N in litter for much longer periods (Vitousek & Howarth 1991). Another possibility is that periods of high N availability in spring/early summer in temperate regions are congruent with periods of adequate soil moisture, while later-season N deficiency is congruent with low water availability. In contrast, water availability is more continuous in the lowland tropics. On a larger time scale, another factor could be the more advanced stage of soil development in many tropical ecosystems, whereas temperate and boreal soils are reset frequently by glaciation (Chadwick et al. 1999). Whatever the reason, the relatively low N availability in temperate forests

could mean that N-demanding legumes can *only* persist there if they fix N, and therefore they can be excluded by the mechanisms discussed above. In contrast, the relatively high availability of soil N in lowland tropical forests could mean that legumes can grow there without fixing N, most of the time.

Other symbiotic systems

In addition to the nodulated rhizobial and actinorhizal symbioses considered above, there are a number of other N-fixing symbioses that include higher plants. In general, these are not known as well as rhizobial systems; as far as we know, there are no models describing ecosystem-level controls of fixation in these symbioses. Some of the major symbioses and what we know of them are summarized below. Unless otherwise referenced, this listing draws heavily upon Sprent and Sprent (1990).

- All species of the genus *Gunnera* are symbiotic with *Nostoc*. These may be important as a source of N in some environments, mainly in the montane tropics and the southern hemisphere, e.g., New Zealand, southern part of South America (Chile down to Tierra del Fuego), Falklands (Malvinas). These plants may be more-or-less obligate for N fixation; they are always observed with endophytes in nature. Estimates of N fixation in the field are almost lacking, but some approximations could be made on a biomass basis. We suspect that these systems are constrained similarly to other higher plant-based symbioses, although moisture stress may be relatively more important.
- Cycads have coralloid roots with endophytic cyanobacteria (*Nostoc* and some other genera) which can apparently fix enough N for the plant's needs. We know of no studies on environmental constraints, except that some cycads (e.g., *Macrozamia reidlii*) are fire resistant. They are likely to be relatively drought tolerant, and, in view of the time when they evolved, they are likely to thrive as atmospheric carbon dioxide levels rise. Cycads effectively replace legumes (such as species of *Acacia* and some endemic Australian genera) in some open forest ecosystems in Australia and southern Africa.
- The aquatic fern genus *Azolla*, with six species native to tropical and warm temperate freshwaters, is the only pteridophyte known to have a nitrogen fixing symbiosis. Its cyanobacterial endophyte is usually called *Anabaena azollae*, although it is almost certainly a species of *Nostoc*, in common with most cyanobacterial symbioses. It has not yet been cultured axenically, making detailed analysis of its genes difficult. In natural conditions *Azolla* usually grows symbiotically, although when grown in the laboratory with high levels of added nitrate or ammonium

it excludes the cyanobacteria. The endosymbiont inhabits pouches on the underside of the dorsal lobe of the leaf. *Azolla* is capable of rapid growth, doubling its weight in seven days; it has been exploited for many years as a green manure in wetland rice production, but it can also be a weed. Agriculturally it is particularly satisfactory in areas where it is killed by high summer temperatures; otherwise its use is labor intensive.

There are also looser associations between plants and N fixers. For all of these, there is little good evidence of direct transfer of combined N from the fixing component to the plant. The alternative, that the plants receive N after bacterial cell death, may reduce the total quantity of N fixed, because there is no continuous export of ammonium that can be assimilated by plant cells (as in rhizobia and *Frankia*).

- Associative symbioses. This is taken here to include organisms on the outer cells of root cortices and on root surfaces, whose carbon sources vary from dead cells to root exudates. The latter could support significant fixation, but will be competed for by other soil microbes. Measuring N fixation in this environment is methodologically difficult, but long-term mass balance studies in a number of cases reveal a gap which might well be filled by N-fixing associative bacteria.
- Endophytic bacteria. These are species that are found inside the plant. There is much evidence, especially from land races of sugar cane, that significant nitrogen fixation may occur, and nitrogen-fixing organisms have been isolated (James & Olivares 1998; James 2000). In global terms, the significance of this source of fixation is unknown, but one cannot ignore the fact that some grasslands in Brazil (*Brachiaria* spp, *Paspalum notatum*) can grow well continuously without added N or associated legumes. Similarly, sugar cane in Brazil is generally grown with substantially less fertilizer N than the crop's N requirement. After much skepticism, the Brazilian work is now being extended to other countries. It is impossible to put meaningful values upon rates of fixation at present, much less on controls, but this source may well turn out to be significant in ecological terms.

Heterotrophic N fixation

The third major pathway of N fixation is by heterotrophic bacteria in soils and sediments. Heterotrophic fixation during the decomposition of plant litter might be expected to be important in terrestrial ecosystems, because most plants produce litter with ratios of C:N substantially greater than those required by most microorganisms. A C:N ratio of 20:1 would be low for a terrestrial plant, while 8:1 is more typical of heterotrophic bacteria. Often the ratio in plants is very much wider; leaf litter in low-N systems not infre-

quently has a C:N ratio of 150:1, and the ratio in wood is wider yet. These wide ratios suggest that N might ordinarily be in short supply for microorganisms, while energy (reduced C) should be abundant. Consequently, microbial growth (and decomposition) could be limited by N, giving heterotrophs that can fix N an advantage (as long as other resources are abundant).

How important is heterotrophic N fixation? The capacity to fix N is widespread in anaerobic and microaerophilic bacteria (Sprent & Sprent 1990), and most freshwater and marine sediments support measurable rates of fixation. However, the ecosystem-level contribution of N by this pathway is constrained by (1) the inefficiency of anaerobic metabolism, which reduces the quantity of N that can be fixed; and (2) the importance of denitrification in anaerobic environments. Rates of denitrification exceed rates of N fixation in those sediments where both processes have been measured (Howarth et al. 1988a). Nevertheless, the relatively high rates of fixation that often occur in anaerobic sediments even where ammonium concentrations are high (Howarth et al. 1988b) suggest that the energetic cost of fixation is relatively low where there is no need to protect nitrogenase against O₂.

The net contribution of heterotrophic N fixation to ecosystem N budgets may be greater in wetland soils. N budgets of flooded rice suggest that 50–100 kg N ha⁻¹ y⁻¹ may be added by N fixation (Cassman et al. 1995), and heterotrophic fixers contribute a substantial proportion of this total (Eskew et al. 1981). Similarly, high rates of heterotrophic fixation may support plant production in some natural wetlands, for example the annually-burned papyrus marshes along the margins of the Amazon River in Para, Brazil.

The capacity to fix N is more restricted taxonomically among heterotrophic bacteria in aerobic environments. Such organisms must be able to protect their nitrogenase against O₂, often by barriers to diffusion or by high rates of respiration or some combination thereof. This protection against O₂ can be energetically costly. In addition, the activity of heterotrophic N fixers may be restricted to a narrower range of environmental conditions than is decomposition; low soil pH in particular is associated with lower rates of N fixation in aerobic soils (Sprent & Sprent 1990). Nevertheless, rates of N fixation in the range of 1–5 kg N ha⁻¹ y⁻¹ have been reported for decomposing litter in a range of environments. These rates are far below what symbiotic N fixers or aquatic cyanobacteria can achieve, but in the long term they could be significant to the N budget of unpolluted sites.

Overall, where N supply limits rates of decomposition (and the growth of microbial populations), then N-fixing heterotrophs should have an advantage, and N fixation should be relatively high. A fundamental question is then – when and where does N supply limit rates of decomposition? There is a great deal of indirect evidence for N limitation to decomposition, including positive

correlations between N concentration in litter and rates of decomposition, and the fact that a substantial quantity of N is immobilized by decomposers in many sites. However, experimental studies which add N to decomposing litter yield mixed results. N limitation is generally observed in agricultural systems that produce relatively decomposable litter. However, in natural systems N additions sometimes stimulate decomposition, more often have no effect, and occasionally even slow decomposition (Prescott 1995; Downs et al. 1996; Hobbie & Vitousek 2000).

Alternatively, the activity of decomposers could be controlled by carbon quality, more than by the supply of N or other nutrients. The litter of plants growing in low nutrient sites often contains much of its C in lignin, soluble polyphenols, and other recalcitrant compounds. To the extent that the growth of microbes and the decomposition of litter are controlled by the abundance of such compounds, we would expect that N supply would not limit microbial growth or decomposition, and so heterotrophic N fixation would be very slow or absent. Where microbial activity is not proximally constrained by N supply, then N fixation represents an energetically costly activity without a substantial benefit.

A recent study evaluated the control of decomposition rate, and of associated heterotrophic N fixation, by C quality, N supply, and the supply of other nutrients in Hawaiian montane forests (Hobbie & Vitousek 2000; Vitousek & Hobbie, 2000). It made use of the litter of a single tree species that occupies a wide range of sites that differ in nutrient supply, and that has a wide range of litter chemistry (N concentrations from 0.19 to 0.90 percent, P from 0.013 to 0.27 percent, lignin from 11 to 28 percent). Moreover, long-term fertilization experiments with N, P, and all other essential elements, alone and in factorial combinations, were underway on several of the sites, allowing the effects of tissue chemistry and rates of external nutrient supply to be considered separately. Results of the study included:

- Decomposition of low-lignin litter was stimulated by additions of N, while the stimulation was small or absent for high-lignin litter.
- Rates of heterotrophic N fixation were several-fold higher in low-lignin than in high-lignin litter, with a strong positive correlation between the integrated quantity of N fixed during decomposition of unamended litter and the extent to which decomposition of that litter was stimulated by additions of N (Figure 7).
- P supply did not affect N fixation directly. While litter produced in some P-fertilized plots supported high tissue P concentrations and enhanced rates of N fixation, the increase in N fixation could be explained by decreased lignin concentrations in that litter.

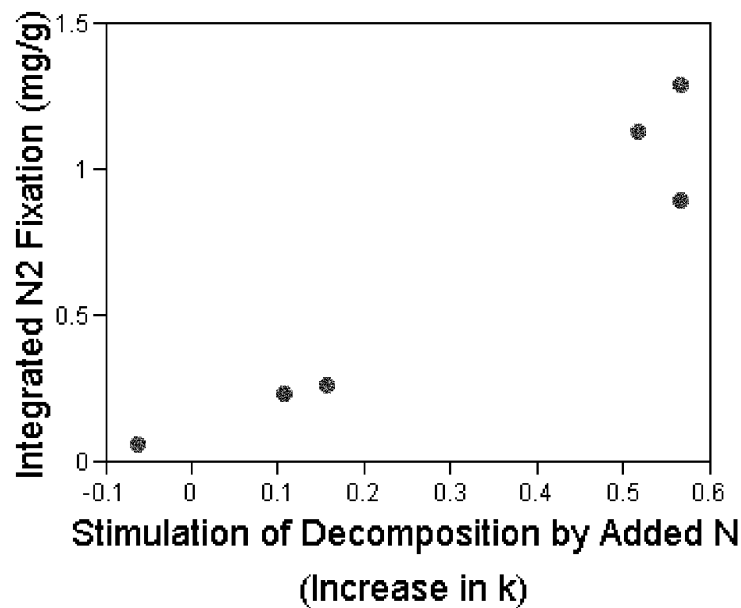


Figure 7. The relationship between N limitation to litter decomposition and heterotrophic N fixation in leaf litter from Hawaiian montane forests. The x-axis is the extent to which rates of decomposition (k , per year) are increased by additions of N fertilizer in a range of litter types, while the y-axis is the integrated N fixation during the course of decomposition for each litter type, in the absence of fertilization. From Vitousek and Hobbie (2000).

- Sites in which N supply demonstrably limited rates of forest growth had low C quality (high lignin) litter, with low rates of heterotrophic N fixation and rates of litter decomposition that responded little to added N. This disconnection between proximate N limitation to forest growth versus proximate C-quality limitation to decomposition and N fixation can keep heterotrophic N fixers from responding to N limitation, in effect sustaining N limitation to forest growth.

We have begun to summarize possible controls of heterotrophic N fixation in a simple model analogous to those for estuarine cyanobacteria and symbiotic higher plants; however, this model is at a more preliminary stage than the others. We consider three populations of decomposers. One population (#1) makes use of available C and N from litter (or the turnover of other microorganisms) at a C:N ratio of 16 (respiring half of the C for a cellular C:N ratio of 8). The second population is an N fixer. When C from litter plus microbial turnover is available at a ratio of C:N exceeding 16:1, this population (#2) can use C to fix N_2 . The third population is a lignin-degrader; it can invest C available above the 16:1 ratio in the breakdown of lignin or polyphenol-

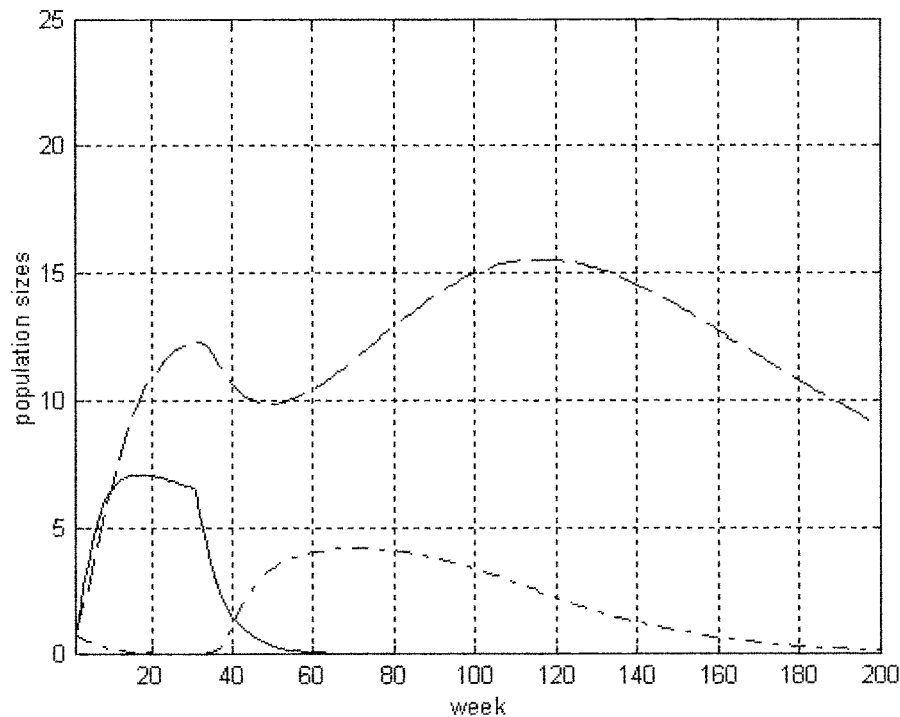


Figure 8. Simulated microbial populations during litter decomposition. The dashed line represents a microbial population that acquires N from labile pools in the substrate; the solid line represents a N-fixing population, and the dash-dot combination represents a lignin-degrading population.

protein complexes, thereby obtaining N. We assume that the relative success of populations 2 and 3 depend on the N yield from investing C in N fixation versus investing it in the breakdown of recalcitrant polyphenol-protein complexes.

For the model, litter consists of available C and N that can be used by microbes in a first-order decay process, and lignin C and lignin-associated N. A fraction of the available N associates with lignin as it becomes accessible to decomposers. All decomposers have the same stoichiometry and are grazed at the same rate.

This simple model yields an intuitively reasonable pattern of N fixation and lignin degradation over time. Population 1 dominates the decomposer community initially, followed by a pulse of the N fixer (#2), with the lignin-degrader growing once the concentration of N-associated lignin makes that N source rewarding (Figure 8). In practice, a peak in heterotrophic N_2 fixation some weeks to months following the initiation of decomposition has

been observed in longitudinal studies of heterotrophic N_2 fixation in the field (Thompson & Vitousek 1997; Vitousek & Hobbie, in press). However, varying the initial lignin concentration in the model yields a pattern different from field observations – the more lignin that is incorporated in the simulated litter, the lower the quantity of lignin-associated N per unit of lignin C, and so the less rewarding (in terms of N yield) is lignin degradation. Consequently, more N is fixed in the model when more lignin is present – the opposite of field observations.

If our other assumptions are reasonable, then the effect of C quality (lignin concentration) on N fixation cannot be captured simply by evaluating the cost of N acquisition by fixation versus lignin degradation. Alternatively, if we assume that lignin (or compounds correlated with it) act directly to suppress N fixers in particular, we can generate the pattern observed in the field. However, we do not have a mechanistic basis for such an assumption. More information on the physiology and biochemistry of these functional groups of soil microbes will be required in order to carry this modeling approach further.

Conclusions

Overall, the ecological regulation of N fixation has a number of features in common, across the diverse N-fixing systems considered here. There is good evidence that non-N nutrients (P, in some cases Mo or Fe) can control the growth of cyanobacteria in lakes, estuaries, and terrestrial ecosystems, and also the growth of rhizobial and actinorhizal symbiotic systems. There is evidence that grazing disproportionately reduces the growth and activity of N fixers, in estuaries and rhizobial symbioses. Moreover, there is a suggestion that energetic constraints to N fixation may be more usefully considered qualitatively rather than quantitatively. For rhizobial and actinorhizal symbioses, the possibly greater shade intolerance of N fixers could have a larger effect on the N status of ecosystems in the long term than can their greater cost for N acquisition. For free-living heterotrophic fixers, the carbon (= energy) quality of their substrate is more important than its quantity in controlling rates of fixation. However, the treatment of N fixation in the multiple element limitation (MEL) model (Figure 6) is quantitative rather than qualitative, and Tyrrell's (1999) marine model similarly evaluates proximate N limitation to marine primary production in terms of relative costs of N acquisition by fixers and non-fixers.

More broadly, as Hartwig (1998) pointed out, N fixers may grow or actively fix N across a narrower range of environmental conditions than do non-fixers. To the extent that fixers are constrained by pH, drought, temperature, salinity, or other conditions, other organisms might be more likely to be

limited by N under those conditions. However, our knowledge of the biology of N fixers is limited to a few relatively well-studied systems, and we are uncertain about the environmental tolerances of many fixers, particularly in natural systems.

Towards regional and global models

The similarities in controls of N fixation across very different groups of organisms suggest that ultimately it may be possible to incorporate physiological and ecosystem controls of N fixation into regional and global ecosystem models – an improvement that would allow such models to deal more realistically with the long-term consequences of global environmental change. However, a number of steps will be required before mechanistic controls of N fixation can be incorporated into regional and global models.

We believe that modeling the energetic controls of N fixation is closest to application. A framework for evaluating physiological components of these controls exists in marine, freshwater, and terrestrial ecosystems (Howarth et al. 1999; Tyrrell 1999, discussion of the multiple element limitation model [MEL] in this chapter). Moreover, light absorption by algal communities and plant canopies is calculated in existing ecosystem models, and also is accessible to direct remote sensing (Field et al. 1998). This information could be used to identify where N fixation by terrestrial cyanobacteria could be supported, and where shade-intolerant rhizobial or actinorhizal symbioses could colonize.

Other components of ecological controls over N fixation will be more difficult to incorporate in models. First, models will need to identify areas where N supply proximately limits NPP and other ecosystem processes – something that is now simply assumed to be true everywhere in many terrestrial ecosystem models. Second, the availability and dynamics of non-N nutrients, especially P, will need to be dealt with more realistically. P dynamics are incorporated in some but not all regional and global models (they are in Century but not TEM, for example), but even where they are included their treatment is sketchy in natural ecosystems, particularly forests. Other elements that could control the distribution and activity of N fixation, from major cations to trace elements like Mo, are not well represented in any terrestrial model. Finally, to the extent that herbivory represents an important control on the distribution and abundance of N fixers (Ritchie et al. 1998; Howarth et al. 1999), we are far from realistic models that can be applied on regional to global scales.

Modifying our models to include these controls will be difficult, but it should not be impossible. Moreover, these improvements to regional/global models are needed for reasons beyond understanding controls of N fixation,

particularly to deal effectively with the large portions of Earth where N appears *not* to be a proximate limiting resource.

While the effort to develop more mechanistic and realistic regional/global models continues, are there useful steps we can take to scale up our analysis of rates/patterns of N fixation in the meantime? We have such measures for NPP – correlations between climatic parameters and NPP are well worked out regionally and globally, and more directly we can use satellite remote sensing to measure light absorption by plants, and to drive models of photosynthesis and NPP (Sellers et al. 1997; Field et al. 1998). Are there comparable correlates/controls for N fixation?

Cleveland et al.'s (1999) synthesis of empirical studies of N fixation demonstrated that rates of fixation are correlated with calculated actual evapotranspiration (AET), across a range of biomes. Schimel et al. (1997) earlier estimated N fixation globally within the Century model using an assumed relationship between fixation and AET, although the slope of the relationship assumed by Schimel et al. is shallower than that suggested by the data in Cleveland et al.

This correlation of N fixation with climate may be the best that can be done globally, for now, but the empirical correlation is crude, not particularly strong, and there is no clear mechanism underlying the pattern. It is intriguing that the empirical analysis of Cleveland et al. (1999) identifies several biomes in which N fixation appears to be greater than expected based on AET, including deserts, arid shrublands, tropical savannas, and xenomorphic forest and woodland. All of these are open-canopied systems in which light availability at the soil surface could support cyanobacterial fixation and allow colonization by shade-intolerant symbiotic systems; all have fluctuating precipitation that could drive high levels of N losses. We think it likely that a hybrid between the empirical approach of Cleveland et al. (1999) and Schimel et al. (1997) on the one hand and the conceptual model approach outlined in this paper may provide useful interim predictions of N fixation, regionally and globally, while we work to improve the mechanistic basis of regional and global models.

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