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Effects of nitrogen: phosphorus supply ratios on nitrogen fixation in agricultural and pastoral ecosystems

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Abstract. An analysis of data compiled from the literature confirms a strong inverse relationship between annual rates of nitrogen fixation and the soil nitrogen content in agricultural and pastoral ecosystems. However, this inverse relationship is strongly modified by the rate of application of phosphorus fertilizer, which strongly influences the activities of both symbiotic and non-symbiotic nitrogen fixing organisms. In the case of symbiotic legumes, the response of N-fixation to N and P is in part a result of changes in legume dominance within the plant community. These results, as well as supporting data presented from a review of experiments on nitrogen fixation in a variety of other terrestrial and aquatic ecosystems, provide important support for the hypothesis that phosphorus availability is a key regulator of nitrogen biogeochemistry.

Introduction

Nitrogen limitation is generally believed to occur in many ecosystems, and the evidence for nitrogen (N) limitation of net primary production in terrestrial and marine ecosystems was the subject of a recent review by Vitousek & Howarth (1991). They noted that N-limitation in both terrestrial and marine ecosystems is something of a puzzle, since dinitrogen-fixing organisms should in principle have a strong competitive advantage over non-fixers under conditions of nitrogen deficiency, and their production of new nitrogen should help prevent or ameliorate N-limiting conditions.

Indeed, biological nitrogen fixation provides a significant source of new inorganic nitrogen for plant growth in both natural and agricultural ecosystems worldwide. The fixation of molecular nitrogen by free-living organotrophs, free-living phototrophs, symbiotic legume bacteria, and symbiotic actinomycetes contributes up to 139 Tg of new nitrogen globally each year (Burns & Hardy 1975). Of this annual total, more than one third (50 Tg) is derived from associative and free-living nitrogen fixation; the balance is derived from symbiotic associations such as terrestrial legumes, which are economically important both in pastures and in croplands (Paul & Clark 1989). In the global nitrogen budget, biological N-fixation represents the largest single input to terrestrial ecosystems (Tamm 1991).

Despite the importance of this process, the physiological and ecological controls of nitrogen fixation remain relatively poorly understood. Vitousek & Howarth (1991) considered three principal mechanisms that could potentially regulate the response by dinitrogen-fixing organisms to N-limitation, including energetic constraints, the supply of other nutrients, and other physical and ecological constraints. Unfortunately, the relative roles of all three mechanisms are still unclear. In this paper, I examine the constraints imposed upon the biomass and activity of symbiotic and asymbiotic terrestrial nitrogen fixers by their external nutrient supply, using a comparative analysis of data compiled from experimental studies carried out during the past several decades. In addition, I present experimental evidence from a variety of other terrestrial and aquatic ecosystems that supports a developing consensus regarding the importance of N:P ratios in nitrogen biogeochemistry.

Predicted effects of the soil nutrient supply on legume biomass

Global nitrogen fixation by symbiotic legume bacteria accounts for up to 35 Tg N per year (Burns & Hardy 1975, as cited in Paul & Clark 1989). Numerous environmental factors potentially influence the annual rate of nitrogen fixation by the legume-*Rhizobium* association, including the availability of light, water, and soil nutrients; the type and intensity of grazing; and climate (cf. Nutman 1986; Broughton 1981; Hendzell 1990; Vitousek & Howarth 1991).

Among these factors, the availabilities of both soil nitrogen and phosphorus have particularly profound effects. For example, the growth of legumes is strongly inhibited by increasing soil nitrogen (Hoglund & Brock 1987), and legume growth is typically phosphorus-limited (Ae et al. 1990). Because of the inhibitory effects of high inorganic nitrogen concentrations on nitrogenase activity, the rate of nitrogen fixation by legumes is also strongly regulated by the soil nitrogen content (Butler & Ladd 1985; Mallarino & Wedin 1990). Thus, the supply of nitrogen and phosphorus in terrestrial ecosystems can regulate rates of symbiotic N-fixation both by altering the success of legumes, and by influencing the activity of nitrogenase.

The effects of nutrient supplies and nutrient supply ratios on the composition of vegetation are currently of great interest in terrestrial ecology (Tilman 1982, 1988; see also Grace 1991). Because of their ability to fix gaseous N_2, legumes are considered to be good competitors under conditions of low soil nitrogen availability (Tilman 1982). However, when grown in association with grasses, legumes are poor competitors for phosphorus (Williams & Hayes 1990), and herbaceous pasture legumes do not long persist in association with grasses if given a large supply of fertilizer N (Goh & Haynes 1986; Mallarino & Wedin 1990).

These responses of plant community structure to the relative supplies of N and P are consistent with recent resource-ratio theory (Tilman 1982), which predicts that additions of phosphorus alone to legume/grass mixtures growing on soils of similar nitrogen content should result in a consistent reduction in the effective supply ratio of nitrogen and phosphorus. Such a decrease in the N:P ratio should act to shift the vegetation towards nitrogen limitation, and should competitively favor the superior nitrogen competitors, legumes. However, additions of nitrogen alone to the same mixtures would be predicted to favor the competitive exclusion of legumes by the grasses.

Such trends in vegetational composition have been observed in the literature. For example, in his analysis of data from the Rothamsted Park Grass Experiments, Tilman (1982) noted that the maximum relative biomass of legumes occurred in plots receiving fertilization with phosphorus, potassium, and magnesium, but no nitrogen. In contrast, control plots receiving no fertilizer had fewer legumes, and plots receiving complete fertilizer plus nitrogen had trivial proportions of legumes ($<0.3\%$). Moreover, the relative abundance of the legume *Lathyrus* was inversely correlated with the soil N:P ratio. Similarly, the levels of N and P in karri forest soils affected the growth of the understory legume *Bossiaea laidlawiana* in southwestern Australia (Grove & Malajczuk 1992). Where the nitrogen supply in the soils was high, growth of non-legumes was stimulated, suppressing the growth of the legumes. However, where N was limiting, increasing the supply of P increased nitrogen fixation rates and gave *B. laidlawiana* a competitive advantage over the non-legume species. Both sets of results are generally consistent with resource-ratio theory.

Observed effects of N:P supply ratios on legume biomass

Resource-ratio theory makes two important predictions regarding the growth and ecological success of legumes in plant communities composed of both legume and non-legume species. It predicts that a decrease in the N:P supply ratio to these systems (1) should result in significant increases in annual legume production (LPROD, kg ha^{-1} yr^{-1}), and (2) should cause an increase in the *proportion* of total plant production contributed by legumes in the community (%LPROD).

These two predictions were tested using data from New Zealand legume/grass pastures. These pastures require periodic fertilizer applications to maintain their productivity, and symbiotic nitrogen fixation by the legumes (primarily *Trifolium* spp. and *Lotus pedunculatus*) provides an essential source of new nitrogen to support both primary and secondary production (Williams & Hayes 1990). As a result, extensive experimental studies of pasture vegetation and of nitrogen fixation have been made by New Zealand researchers using comparable methods during the past two decades.

Lambert et al. (1982) monitored the responses of legume productivity to differing levels of phosphorus fertilizer in a series of plots of similar initial soil nitrogen content. When I plotted their data, strong positive correlations were observed between both LPROD and %LPROD and the rate of phosphorus fertilization (Fig. 1).

In pastures received continuous P inputs in New Zealand and elsewhere, however, the biomass of legumes often ultimately declines (Lambert et al. 1982; Nguyen et al. 1980), and this lack of persistence of legumes is a major constraint on the use of legumes for animal production (Campbell 1989). This loss of legume biomass is consistent with the successional patterns that would be expected due to the buildup of soil nitrogen reserves by N-fixation. Resource-ratio theory (Tilman 1982) predicts that such an accumulation of soil nitrogen would tend to increase the effective N:P supply ratio for plant growth, and would result in a continuous decline in the competitive superiority of the legumes.

Just as an increase in the phosphorus supply to lakes results in a predictable increase in the biomass of previously rare dinitrogen-fixing planktonic cyanobacteria (Schindler 1977; Flett et al. 1980; Smith 1985), the addition of phosphorus fertilizer to terrestrial ecosystems can lead to the appearance of dense populations of native legumes that were formerly uncommon (Tilman 1982; Friere 1984). Similarly, nitrogen-poor but phosphorus-rich volcanic soils can be successfully invaded by exotic dinitrogenfixing trees (*Myrica faya*: cf. Vitousek et al. 1987). Despite these and other observations of directional vegetational shifts noted above, quantitative evidence for a corresponding effect of the N:P supply ratio on *rates* of nitrogen fixation is much less common.

Effects of N:P ratios on rates of symbiotic N-fixation

Because symbiotic nitrogen fixation is proportional to legume production (Hoglund & Brock 1987; Ledgard et al. 1987), I hypothesized that

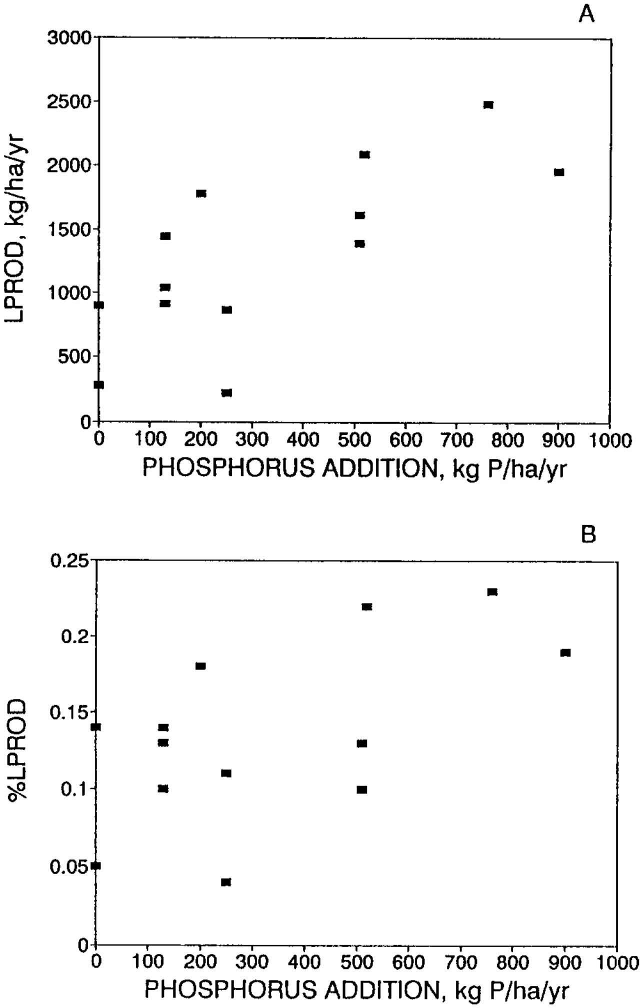

Fig. 1. Effects of phosphorus fertilization (P, kg P ha^{-1} yr^{-1}) on (A) annual legume production (LPROD, kg ha^{-1} yr^{-1}); and on (B) the proportion of total annual plant production attributable to legumes (%LPROD) in New Zealand pastures. The simple regression models for these relationships are (A) LPROD = 1.78 P + 718, $r^2 = 0.55$, $p < 0.001$; and (B) %LPROD = 0.00012 P + 0.095, $r^2 = 0.35$, $p = 0.033$.

changes in the N:P supply ratios also should have significant effects on measured rates of legume N-fixation (NFIX, kg N ha^{-1} yr^{-r}). This hypothesis was tested directly using measurements of nitrogen fixation and soil chemistry pooled from experimental studies of New Zealand pastures (Sears et al. 1965; Ledgard et al. 1987; Hogland and Brock 1978; Grant and Lambert 1979; Hoglund et al. 1979; Crush et al. 1982, 1983). Prior to multiple regression analysis (Wilkinson 1998), the nitrogen fixation and soil nitrogen data were transformed to their common logarithms. These data were then coded into two categories with a dummy variable according to the level of phosphorus fertilization (P, kg P ha^{-1} yr^{-1}): DUMMY = 0 for $P > 350$, and DUMMY = −1 for $P < 350$.

As can be seen in Fig. 2, the annual rate of N-fixation was significantly dependent both on the initial soil nitrogen content (%N) and on a dummy variable accounting for the rate of P fertilization:

$$\text{LOG NFIX} = -0.93\ \text{LOG}\ \%\text{N} + 0.16\ \text{DUMMY} + 1.73,\ R^2 = 0.69,\ p < 0.001. \tag{1}$$

As expected, the highest rates of N-fixation were observed in plots having the lowest effective N:P supply ratios (nitrogen-poor soils receiving levels of phosphorus fertilizer > 350 kg P ha^{-1} yr^{-1}). In contrast, the lowest observed value was obtained in the field having the greatest soil nitrogen content, despite relatively high phosphorus additions.

Although N:P ratio effects were apparently not detected by the original investigators, I found an identical response of nitrogen fixation in legume/non-legume mixtures to changes in the relative supply rates of N and P in a study by Bell & Nutman (1971) in Great Britain. In both years of their study, stimulation of nitrogen fixation by phosphorus fertilization of lucerne/ryegrass fields was observed in all experimental plots. In Year 1, fields at four low N sites (0.06—0.18% initial soil N) consistently exhibited higher rates of N-fixation than did the fifth site, which had a soil nitrogen content of 0.64% N. Phosphorus fertilization resulted in large increases in N-fixation at the first four sites, but no such stimulation was observed at

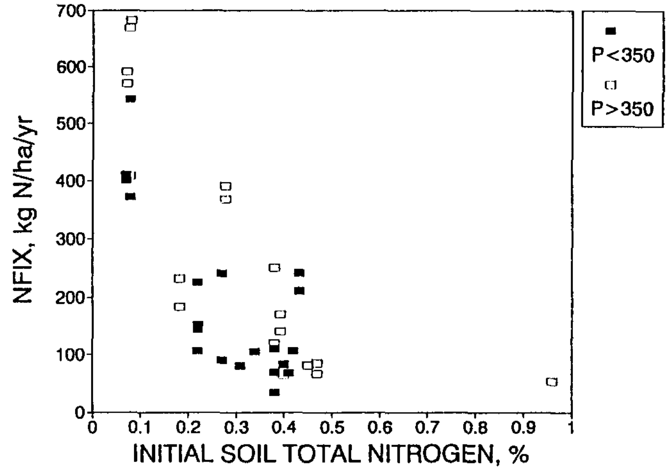

Fig. 2. Effects of initial soil total nitrogen (%N) and rates of phosphorus fertilization (P, kg P ha^{-1} yr^{-1}) on annual nitrogen fixation (NFIX, kg N ha^{-1} yr^{-1}) by New Zealand pasture legumes.

the fifth site (Fig. 3A). By year 2, P stimulation of nitrogen fixation was highest at the site with the lowest initial soil N, and the P fertilization effect diminished rapidly as the soil nitrogen content increased (Fig. 3B).

Additional evidence for phosphorus effects on N-fixation by other symbiotic systems derives from recent studies of an actinorhizal shrub (Weinhold & Klemmedson 1991), and of the black locust (Guofan & Tingxiu 1991). Phosphorus availability has been implicated as a limiting factor for both nodulation and N-fixation in actinorhizal plants, and Weinhold & Klemmedson (1991) have demonstrated strong stimulatory effects of P fertilization on nitrogenase activity in *Cercocarpus betuloides*

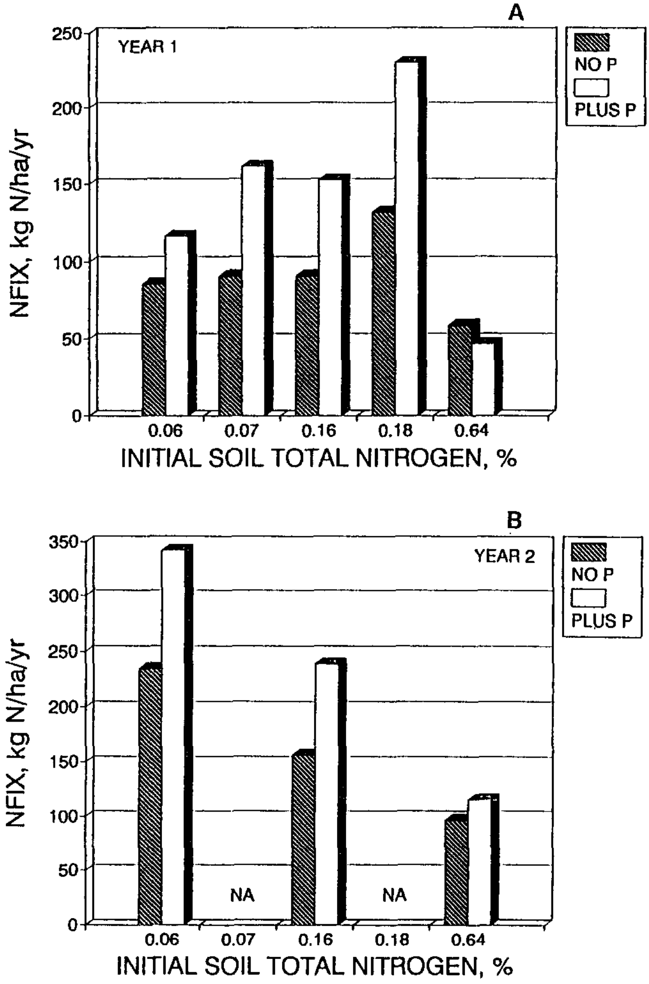

Fig. 3. Effects of initial soil nitrogen and rates of phosphorus fertilization on annual nitrogen fixation (NFIX, kg N ha^{-1} yr^{1}) in lucerne/ryegrass fields in Great Britain. (A) Year 1; (B) Year 2 (see text).

seedlings. Similarly, Guofan & Tingxiu (1991) explored the edaphic controls of nitrogen fixation by black locust (*Robinia pseudoacacia* L.) seedlings grown in pot cultures on 42 types of forest soils, and developed regression models relating N-fixation to soil chemistry. A plot of the predictions of their regression Eq. 1 (Table 2 in Guofan & Tingxiu 1991) illustrates the inverse relationship that they observed between rates of N-fixation and the total soil N: available P ratio (Fig. 4).

Effects of the nitrogen and phosphorus supply on non-symbiotic terrestrial associations

The effects of N:P supply ratios on terrestrial nitrogen fixation are not restricted to legume/grass mixtures. As was demonstrated above for legume productivity (Fig. 1), the abundance of non-symbiotic dinitrogen-fixing soil microorganisms such as *Azotobacter* is also positively correlated with soil phosphorus availability (Jensen 1965). A parallel response of non-symbiotic nitrogen fixation to N:P supply ratios thus would also be expected.

Until recently, the best evidence that the N:P supply ratio affects non-symbiotic nitrogen fixation was provided by a study of tallgrass prairie soils in eastern Kansas (USA) by Eisele et al. (1989). In a series of laboratory experiments using soil cores artificially inoculated with cyanobacteria,

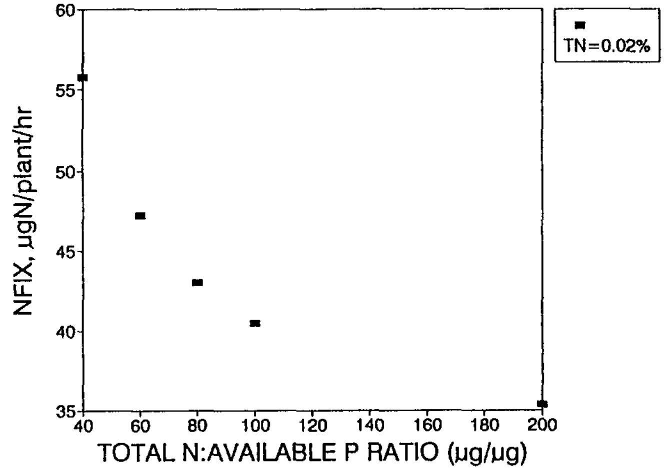

Fig. 4. Effects of soil total nitrogen:available phosphorus ratios on rates of nitrogen fixation (NFIX, ug N plant^{-1} h^{-1}) in pot cultures of black locust, as predicted by regression Eq. 1 in Guofan & Tingxiu (1991). Soil total N was assumed to be 0.02%, and the values for all other soil factors other than available P were set to their transformed mean values of 0.5.

these authors found that the rate of N-fixation (NFIX, μmol C_2H_4 m^{-2} hr^{-1}) was inversely related to the available N:P ratio (Fig. 5).

However, additional new evidence linking the soil N:P ratio to nitrogen fixation by non-symbiotic soil cyanobacteria can also be found in a recent study by Chapin et al. (1991). These authors examined the environmental regulation of nitrogen fixation in a high arctic lowland ecosystem, and found that acetylene reduction activity was present in virtually every community sampled. Although soil bacteria may also have been partly responsible for this activity, Chapin et al. (1991) concluded that the majority of nitrogen fixation was due to *Nostoc commune* and other heterocystous cyanobacteria. Soil nitrogen levels were found to be significantly and negatively correlated with NFIX (μmol C_2H_4 m^{-2} hr^{-1}) in a multiple regression analysis including water-extractable ammonia N, water-extractable P, and % soil water. In contrast to the coefficient for N, the coefficient for water-extractable P was positive in their model and a strong positive correlation between acid-extractable P and NFIX was also observed at three other sites.

As would be expected from the structure of their multiple regression model, a reduction in the effective N:P supply ratio of soil-plant cores from a *Carex* meadow by experimental phosphorus fertilization resulted in a significant stimulation of NFIX relative to unfertilized control cores (Table 1). These authors speculated that the phosphorus stimulation of N-fixation reflected an enhancement of cyanobacterial biomass and/or

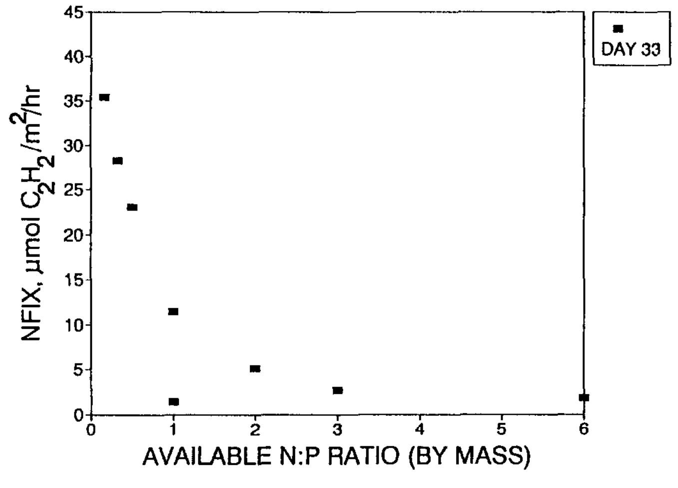

Fig. 5. Effects of the available N:P ratio on cyanobacterial nitrogen fixation (NFIX, μmol C_2H_2 m^{-2} h^{-1}) in soil cores from Kansas tallgrass prairies, as predicted by the regression model of Eisele et al. (1989).

Table 1. Effects of phosphorus additions on rates of nitrogen fixation (NFIX, μmol C_2H_4 m^{-2} hr^{-1}) in soil-plant cores from a *Carex* meadow in high arctic lowlands. Values having different letters differ significantly from each other (from Table 6 in Chapin et al. 1991).

Treatment	Mean	SE
Control	17.2a	4.06
+ 0 mg L^{-1} P + H_2O	46.5b	8.32
+ 100 mg L^{-1} P + H_2O	104.9c	3.87

heterocyst number, in addition to the direct effects of P on heterocyst activity.

Further independent support for the N:P ratio hypothesis derives from the data of long-term nitrogen balance measurements in British wheat fields (Wolf & van Kuelen 1989). These authors observed annual N-fixation rates of 28 kg N ha^{-1} yr^{-1} by non-symbiotic soil microflora in unfertilized control plots. My reanalysis of their data indicated that phosphorus fertilization alone increased non-symbiotic N-fixation to 35 kg N ha^{-1} yr^{-1}; this effect was apparently not noted by these authors. Moreover, when supplemental N was added to similarly P-fertilized plots, the rate of nitrogen fixation decreased exponentially to zero with increasing rates of N addition (Fig. 6). Although the microorganisms responsible were not identified, these field results are consistent both with the phosphorus dependence of dinitrogen fixing soil organotrophs observed by

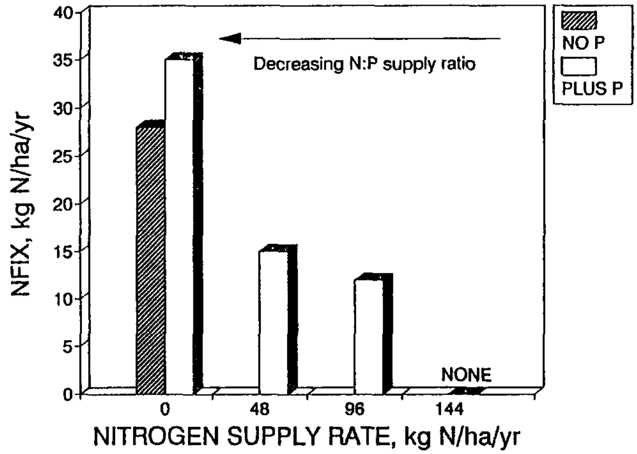

Fig. 6. Effects of nitrogen and phosphorus supply rates on non-symbiotic nitrogen fixation (NFIX, kg N ha^{-1} yr^{-1}) in the Broadbalk, U.K., experimental fields (see text).

Jensen (1965) and with the responses of soil cyanobacteria to phosphorus additions observed by Eisele et al. (1989) and Chapin et al. (1991).

Parallels with aquatic ecosystems

It is important to note that similar effects of N:P ratios have been observed in a variety of aquatic ecosystems. For example, phytoplanktonic nitrogen fixation in Green Bay, Wisconsin was found to be strongly dependent on the epilimnetic inorganic N:P ratio (Fig. 7; cf. Vanderhoef et al. 1974), and similar effects of the N:P loading ratio on N-fixation were found in experimentally fertilized lakes of the Experimental Lakes Area in Canada (Flett et al. 1980).

In a recent field test of resource-ratio theory, Smith et al. (in prep.) created a gradient of N:P ratios ranging from 5:1 to 50:1 by mass in a series of large (6 m^3) mesocosms filled with lakewater containing a constant concentration of total phosphorus (100 mg m^{-3}). The relative biomass of nitrogen fixing cyanobacteria was found to be inversely related to the experimental N:P ratio, and acetylene reduction measurements made by Brad Bebout (Institute of Marine Sciences, University of North Carolina) suggested that rates of nitrogen fixation exhibited a parallel trend. Similarly, Fairchild et al. (1985) used nutrient-diffusing clay pots to evaluate limiting factors for periphyton in oligotrophic Douglas Lake, Michigan, and their results revealed significant effects of N and P on both

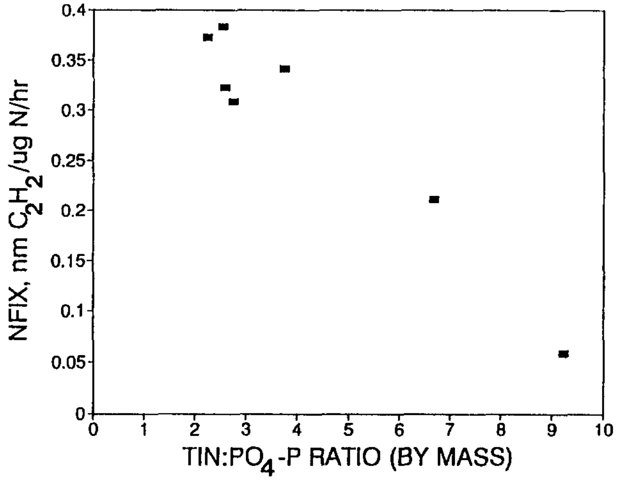

Fig. 7. Effects of the total inorganic nitrogen:orthophosphate (TIN:PO_4-P) ratio on rates of nitrogen fixation (NFIX, nmol C_2H_2 (μg N$)^{-1}$ h^{-1}) by epilimnetic phytoplankton in Green Bay, Wisconsin (data from Vanderhoef et al. 1974).

algal growth and community structure. The biomass of the nitrogen fixing cyanobacterium *Anabaena* responded positively to additions of P alone (i.e., low N:P supply ratios), and the authors suggested that the resource-ratio theory applied by Smith (1982) to phytoplankton communities applied equally well to their periphyton communities. Direct measurements of N-fixation in a phosphorus-fertilized lake in Sweden also suggested an inverse relationship between the N:P supply ratio and rates of nitrogen fixation by lake periphyton (Lundgren 1978).

Most relevant to the terrestrial analyses presented earlier, however, are effects of N:P supply ratios on N-fixation in aquatic plant-microbe associations. The *Azolla/Anabaena* symbiosis provides important inputs of new nitrogen to flooded rice fields, and such fields are often fertilized with phosphorus to enhance *Azolla* growth and rates of symbiotic N-fixation (Singh & Singh 1988, 1989). Experimental manipulations of the N and P supply to *Azolla* (Sah et al. 1989) revealed a strong dependence of nitrogen fixation by the symbiotic *Anabaena* on the relative supplies of N and P (Fig. 8). Similarly, *in situ* fertilization of the rhizosphere of the tropical seagrass *Syringodium filiforme* (Fig. 9) resulted in parallel effects on N-fixation by rhizosphere bacteria (Short et al. 1990; Smith 1992).

In both Fig. 8 and Fig. 9, the effects of the relative supplies of N and P on nitrogen fixation strongly resemble those observed in New Zealand grass/legume mixtures (Fig. 2), although the responses are much more clear in the aquatic systems. The greater variance observed in Fig. 2 may in part reflect the inadequacy of soil total N as an unambiguous indicator

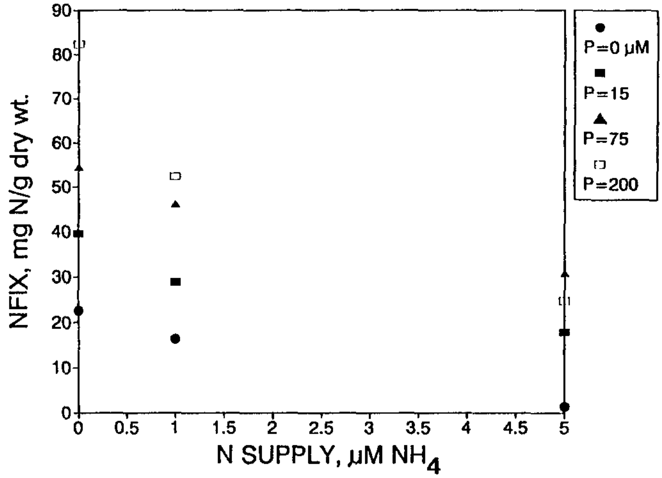

Fig. 8. Effects of phosphorus supply on nitrogen fixation (NFIX, mg N (g dry wt.)$^{-1}$) by the *Azolla/Anabaena* symbiosis at three supply levels of ammonia nitrogen (data from Sah et al. 1989).

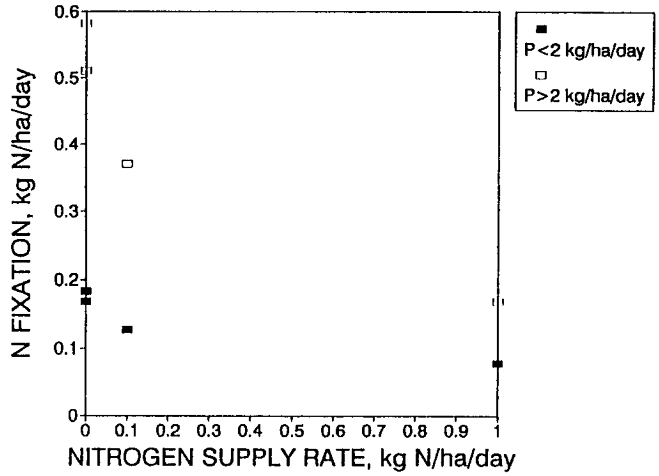

Fig. 9. Effects of phosphorus supply rates (P, kg P ha^{-1} d^{-1}) on nitrogen fixation by rhizosphere bacteria in the seagrass *Syringodium filiforme* at three levels of nitrogen supply (data from Short et al. 1990).

of N availability; it is the relative availabilities of ammonium, nitrate, and phosphate that underlie the physiological responses of N-fixing systems to N:P ratios.

Conclusions

The regulation of nitrogen fixation is clearly a complex phenomenon involving multiple controlling factors (cf. Howarth et al. 1988; Smith 1990; Vitousek & Howarth 1991). However, during the past 20 years an important emphasis has been placed on the potential role of phosphorus availability in the regulation of terrestrial nitrogen cycles (Walker & Syers 1976; Gorham et al. 1979; Cole & Heil 1981; Reiners 1981; Vitousek & Walker 1987; Eisele et al. 1989). In this paper, I have explored further the constraints imposed upon the biomass and activity of symbiotic and asymbiotic nitrogen fixers by their phosphorus supply, using data compiled from experimental studies of nitrogen fixation.

The data reviewed here support a developing consensus regarding the ecological importance of N:P ratios. These results confirm that changes in the relative availabilities of soil nitrogen and phosphorus can have significant effects on nitrogen cycles in a variety of terrestrial systems worldwide, including heavily managed pastures in New Zealand; lucerne/ryegrass fields in Great Britain; Kansas prairie soils; the soils of brackish and mesic to hydric arctic lowlands, and xeric arctic beach ridges; and British wheat fields. Moreover, these trends parallel closely the effects of N and P supplies on N-fixation observed in aquatic ecosystems.

We are still a long way away from a general predictive theory for nitrogen fixation in terrestrial ecosystems. Clearly, N:P ratios cannot explain everything. Other factors such as light availability, trace metal availability, pH, temperature, and soil moisture have important moderating effects in these and in other terrestrial ecosystems (cf. Chapin et al. 1991; Tamm 1991; Vitousek & Howarth 1991), and both nitrogen and phosphorus availability can have direct effects on nitrogenase activity via mechanisms that are independent of resource-ratio considerations (cf. Cole & Heil 1981; Hoglund & Brock 1987; Stock et al. 1990). Furthermore, the land surface is a complex mosaic of ecosystems, including deserts and forests where legumes are either absent or are present in only low abundance. Inhibitory effects of increasing N availability of N-fixation by associated bacteria have been observed for numerous non-legume plant species, but relatively little is currently known about the other edaphic factors affecting their nitrogenase activity (cf. Chalk 1991).

Nonetheless, Schlesinger (1991) has noted that the rate of accumulation of N generally tends to be greatest in soils with high phosphorus contents (Walker & Adams 1958), and he speculated that the high demand for P by dinitrogen-fixing organisms links the global cycles of N and P — with phosphorus availability being the ultimate limit on both N availability and net primary production. As has been stressed by Reiners (1986), there is a core stoichiometry of life, and I suggest that this basic elemental stoichiometry will result in predictable biological responses to N:P supply ratios in many different types of ecosystems worldwide. I urge other investigators to perform experimental and comparative analyses of the effects of N:P ratios on nitrogen fixation in a wide variety of terrestrial ecosystems to test this hypothesis further.

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