LETTERS

A unifying framework for dinitrogen fixation in the terrestrial biosphere

Benjamin Z. Houlton[1,2]†, Ying-Ping Wang[3], Peter M. Vitousek[1] & Christopher B. Field[2]

Dinitrogen (N_2) fixation is widely recognized as an important process in controlling ecosystem responses to global environmental change, both today[1] and in the past[2]; however, significant discrepancies exist between theory and observations of patterns of N_2 fixation across major sectors of the land biosphere. A question remains as to why symbiotic N_2-fixing plants are more abundant in vast areas of the tropics than in many of the mature forests that seem to be nitrogen-limited in the temperate and boreal zones[3]. Here we present a unifying framework for terrestrial N_2 fixation that can explain the geographic occurrence of N_2 fixers across diverse biomes and at the global scale. By examining trade-offs inherent in plant carbon, nitrogen and phosphorus capture, we find a clear advantage to symbiotic N_2 fixers in phosphorus-limited tropical savannas and lowland tropical forests. The ability of N_2 fixers to invest nitrogen into phosphorus acquisition seems vital to sustained N_2 fixation in phosphorus-limited tropical ecosystems. In contrast, modern-day temperatures seem to constrain N_2 fixation rates and N_2-fixing species from mature forests in the high latitudes. We propose that an analysis that couples biogeochemical cycling and biophysical mechanisms is sufficient to explain the principal geographical patterns of symbiotic N_2 fixation on land, thus providing a basis for predicting the response of nutrient-limited ecosystems to climate change and increasing atmospheric CO_2.

The geographic occurrence of N_2-fixing organisms in the open ocean[4,5] and in lakes[6] makes sense. Where nitrogen (N) is in low supply, N_2 fixers have an advantage: they can fix N_2 into biomass and thus grow faster than their competitors. In contrast, where N is abundant, N_2 fixation is energetically costly and N_2 fixers are competitively excluded by non-fixing species. N_2-fixing organisms thereby bring the oceanic inventory of N into equilibrium with N losses over millennia[7], stabilizing nutrient demands of and supplies to marine primary producers[4]. However, this paradigm is inadequate to predict the global distribution of N_2 fixation in terrestrial environments[8]. Although models based on energetics, nutrients and other factors provide reasonable explanations for the distribution of symbiotic N_2-fixing plants in succession and as a function of soil fertility within regions, they do not explain the global distribution of N_2 fixation in forests. N_2-fixing trees (which are predominantly members of the Fabaceae family) are scarce in mature high-latitude forests despite the prevalence of N limitation there[8]; conversely, they account for a significant fraction of communities of many lowland tropical forests[9], despite the overall N-rich conditions of such ecosystems[10]. Here we build on earlier analyses to resolve the global pattern in terrestrial N_2 fixation, through a combination of empirical data synthesis and economic cost-benefit modelling.

We have developed two new hypotheses for understanding the distribution of N_2 fixation across global ecosystems. The first is that temperature constrains the distribution of N_2 fixation, contributing to the lack of symbiotic N_2-fixing trees in mature forests at high latitudes. N_2 fixation is enzymatic and incurs a substantial carbon (C) cost[11]; hence, the rate of N_2 fixation should increase with increasing temperature to some maximum rate. Whether N_2 fixation rates observed for different organisms and environments converge on a similar temperature maximum will be affected by the degree of acclimation to local climatic conditions. Any temperature constraint could reinforce the well-recognized influence of the energy cost of N_2 fixation[12]; the short growing season, relatively low net primary productivity (NPP), and low light availability in mature high-latitude forests could offset the advantages of N_2 fixation over plant N uptake from the soil[13].

The second hypothesis is that symbiotic N_2-fixing plants hold an advantage in phosphorus (P) acquisition, as recently described for N_2-fixing organisms in the P-deficient gyres of the North Atlantic Ocean[14]. N_2 fixers could rely on extracellular phosphatases, a constitutively N-rich (~15% N, ref. 15) class of enzymes involved in the breakdown of organic P (ref. 16), to enhance local P supplies. Once secreted into the soil by plants or microbes, extracellular phosphatases hydrolyze phosphodiester-bonded P (which accounts for 20% to 80% of soil organic P, ref. 17) to phosphate ions that are available for uptake by plant roots[16]. Although many factors control organisms' enzyme activities, phosphatase production increases substantially in response to added N (refs 15, 18), owing to the large N requirement associated with this P acquisition strategy. This hypothesis addresses the second part of the problem, offering an explanation for the persistence of N_2-fixing plants in mature lowland tropical forests and savannas, many of which seem substantially limited by P (ref. 19).

We found empirical support for both of these hypotheses. Our synthesis of the relationship between temperature and nitrogenase activity, spanning diverse species, strains, latitudes and environments, demonstrated a strong convergent effect of temperature on biochemical N_2 fixation (Fig. 1). Nitrogenase activity (that is, N_2 fixation) reaches a maximum at ~26 °C and decreases at higher temperatures, probably in response to depletion of C supplies. The data conform reasonably well to a single curve, suggesting little evidence for local acclimation across factors. The compiled data yield a slope (activation energy ~103 kJ mol^{-1}) that is substantially less than that for the nitrogenase enzyme itself (activation energy ~210 kJ mol^{-1}, ref. 20), although substantially greater than the temperature sensitivity of photosynthesis. This suggests that an interaction between energy supply (by means of photosynthesis) and the potential rate of N_2 fixation underlies the observed pattern. Overall, this shows a strong temperature constraint to maximal N_2 fixation rates under cooler climates, reinforcing the high energetic cost of fixing N_2, and reducing the marginal return of N per unit of C

[1]Biological Sciences, Stanford University, Stanford, California 94305, USA. [2]Department of Global Ecology, Carnegie Institution of Washington, Stanford, California 94305, USA. [3]CSIRO Marine and Atmospheric Research and Centre for Australian Weather and Climate Research, Aspendale VIC 3195, Victoria, Australia. †Present address: Department of Land, Air and Water Resources, University of California, Davis, California 95616, USA.

investment by plants in N_2 fixation compared to soil N uptake. In other words, as temperature declines, more enzyme is needed to achieve a given rate of N_2 fixation.

In support of the second hypothesis, phosphatase production rates were three times higher in soils sampled from beneath plants with known capacity to fix N_2 compared to that beneath non-fixing species (Fig. 2; $P < 0.001$; $n = 25$). This synthesis includes data from known rhizobial and actinorhizal N_2-fixing plants, differing biogeochemical conditions and plant functional growth forms (that is, herbaceous and woody vegetation), and across temperate/tropical latitudes. The higher phosphatase concentrations beneath these putative N_2 fixers probably function locally to mobilize organic P forms to organisms in low P environments. The origin of the phosphatase is uncertain; it could be produced directly by N_2-fixer roots[21], by microbes feeding on the N-rich litter of N_2 fixers, or (most probably) by both. In any case, the addition of N stimulates phosphatase production[15,18], thereby stimulating the availability of P and indicating an N-rich strategy of P acquisition that is particularly suited to N_2-fixing species.

We therefore propose that temperature amplifies the energetic constraint to N_2-fixing plants in mature high-latitude forests where N is often limiting, and also that N_2 fixers are adept at acquiring P by means of phosphatase enzymes, thus providing a means for their persistence in P-limited tropical ecosystems. Taken together and when combined with other costs of fixing N_2 identified previously[11–13] (for example, light, demand for other nutrients, and herbivory), we suggest that these mechanisms can explain patterns of N_2-fixing plants and N_2-fixation fluxes on land.

We addressed the sufficiency of this proposed framework for resolving the pattern of N_2 fixers across diverse terrestrial environments and conditions using a terrestrial biogeochemical model[22] (Supplementary Information) that simulates the economics (costs and benefits) of C, N and P acquisition and competition between N_2 fixers and non-fixers according to the resource optimization paradigm[23]. The model consists of 8 C pools, 9 N pools and 12 P pools, 6 of each of which are divided equally into the plants (N_2 fixer and non-fixer) and the remainder are in the soil. To calibrate the model, we varied maximal C, N and P uptake rates until all pool sizes at equilibrium agreed with empirical observations (average conditions) for four diverse terrestrial biomes: boreal forest, temperate forest, lowland tropical forest and tropical savanna (see Supplementary Table 1). The model is driven by the observed monthly soil mean temperature and maximal NPP of each biome, which implicitly depends on available light and water. Competitive outcomes are determined by NPP gains per unit of resource investment. We assume that NPP varies as a function of light interception aboveground and nutrient acquisition below-ground; the model predicts optimal strategies for maximizing NPP, adjusting N acquisition (N_2 fixation versus soil N uptake), P acquisition (phosphatase investment versus uptake of microbially mineralized P) and C allocation (shoots versus roots).

Building on our base model[22], we included the constraint of temperature on maximal N_2 fixation rates (Fig. 1; see also Methods equation (4)) and two different strategies of P acquisition by means of phosphatase investments: first, a global commons model in which P mineralized by phosphatase enzymes enters a common pool that is available to all plant and microbial competitors equally; and second, an individual-based strategy in which the plant producing phosphatase gets 'first crack' at the mineralized P (ref. 24). We explicitly assume a fixed cost of 15 g N per g P (ref. 15), the required investment of N in phosphatase production by roots or the microbial community. We implicitly assume that the N invested by the microbial community in phosphatase production is supplied by plant litter. We ran the model for three different situations to test our framework: (1) temperature-dependent N_2 fixation (on the basis of the empirical data in Fig. 1), individual based; (2) temperature-dependent N_2 fixation, global commons; and (3) constant N_2 fixation, individual based.

This model-based analysis agrees quantitatively with our proposed framework (simulation (1), described above) for controls of terrestrial N_2 fixation globally. The inclusion of temperature in our model results in the exclusion of N_2 fixers from mature forests at high latitudes (simulations (1) and (2), Fig. 3a, b). Despite profound N limitation (Fig. 3i, j), temperature limits N_2 fixation rates in temperate and boreal forests; here, the investment of C in soil N uptake yields the greatest NPP return. A temperature increase of 10 °C can alleviate this constraint on N_2 fixers, suggesting a potential interaction between climate warming and N_2 fixation at higher latitudes. Furthermore, according to our model, higher rates of N loss leading to more profound N limitation can overcome the energetic limitations on N_2 fixation in temperate forests (Supplementary Fig. A2). This latter result is consistent with the transient presence of N_2-fixing plants in early succession and disturbed temperate ecosystems[8] that have lost substantial quantities of N (refs 25, 26). Thus, our framework is able to reconcile both the presence and the absence of N_2-fixing plants in extra-tropical forests, and suggests that N_2 fixation

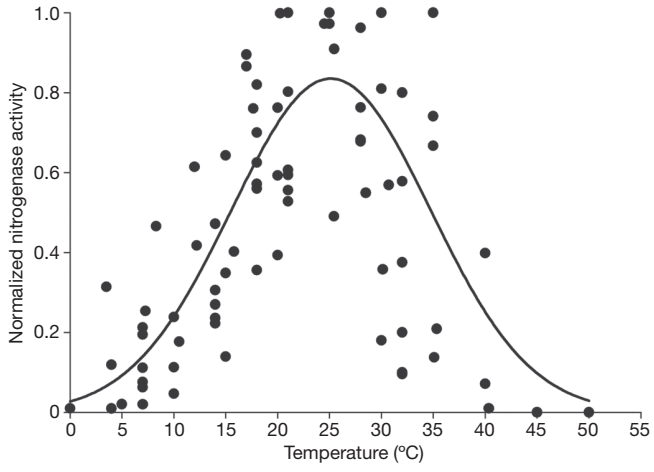

Figure 1 | Temperature dependence of terrestrial nitrogenase activity. We compiled these data from the primary literature and normalized them to the maximal activity observed for each individual study (see Supplementary Information). The data set spans a wide range of terrestrial conditions, latitudes, strains and species of N_2-fixing organisms, but can be fitted with an empirical equation with an optimal temperature of 25.2 °C ($n = 94$, $r^2 = 0.55$).

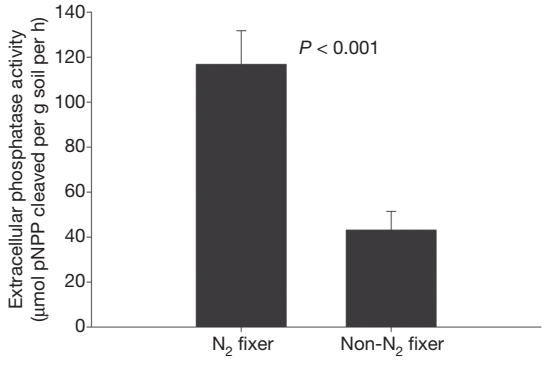

Figure 2 | Phosphatase enzyme rates in soils with and without N_2-fixing plants. We compiled these data from the primary literature, which included soils collected from actinorhizal and leguminous N_2-fixing plant species, herbaceous and woody plants, and across temperate to tropical latitudes (see Supplementary Information). Extracellular phosphatase fluxes were significantly higher in the presence of N_2-fixing plants (two-tailed *t*-test, $P < 0.001$; $n = 25$) compared to non-fixers only. pNPP, *para*-Nitrophenyl Phosphate. Error bars represent s.e.m.

can alleviate N limitation (for example, simulation 3; Fig. 3i, j) where it does occur.

Phosphatase enzymes and associated C, N and P interactions seem to be central to resolving the relatively high abundance of N_2-fixing plants in the tropics (Fig. 3c, d). Despite profound P limitation, our model simulates large contributions of N_2-fixing plants (from 20% to 50% of NPP) to tropical forest and savanna ecosystems when the P released by phosphatase is captured by the plant that produced the phosphatase (see cluster roots[24]; simulations (1) and (3); Fig. 3c, d). Our simulations indicate that newly fixed N is vital to stimulating phosphatase production and organic P mineralization rates; non-fixing plants and free-living microbes must rely on existing soil N pools, thus limiting their capacity for investment in N-rich phosphatase enzymes. According to our model, this proposed benefit to N_2-fixing plants sufficiently offsets the integrated costs (for example, C and light) associated with fixing N_2 in the tropics, where monthly mean temperatures are close to the optimal rate of N_2 fixation (Fig. 1) and P is often in low supply. If P is shared among competitors equally (that is, plants, microbes and geochemical sinks; global commons), this advantage is lost (simulation (2)), further illustrating the importance of below-ground competition for P in the tropics.

As a whole, our biogeochemical and biophysical mechanisms offer a unified explanation for global-scale patterns of terrestrial N_2 fixation; this working framework (which includes interactions between energy (C, light), nutrients (N, P) and temperature) seems sufficient to resolve the distribution of N_2-fixing plants across major forests worldwide. In tropical lowland forests, our model suggests that competition for P is central to the persistence of N_2 fixers. Future work could emphasize the sources and fates of P in different tropical soils, differences among the P-acquisition strategies of organisms, plant versus microbial phosphatase production rates, and the extent to which phosphatase producers receive a benefit over their competitors. Outside the tropics, temperature seems to impose a principal constraint to N_2 fixers in mature forests, whereas energy, other resources or plant traits may be important in controlling the abundance of N_2 fixers in other terrestrial high-latitude biomes, such as prairies and grasslands.

Finally, our proposed global framework (described by simulation (1)) and corresponding model indicate substantial symbiotic N_2 fixation rates by trees in lowland tropical forests and savannas ($\sim$20 to 60 kg N ha^{-1} yr^{-1}) and little to none in mature temperate and boreal forests (Fig. 3e–h). Both the magnitude and pattern of these modelled N inputs agree qualitatively with the relative abundance of potentially N_2-fixing legumes in those ecosystems[3,9]. They also agree with estimates of N_2 fixation rates extrapolated by way of environmental proxy data to continental scales[27], and the 'missing N' inputs observed for lowland tropical forest watersheds (see, among others, ref. 28 for example). We have not addressed free-living N_2 fixation here; future work might consider this pathway in the context of our framework and its potential magnitude globally. Furthermore, in addition to our framework, factors such as herbivory, trace metal availability, life history traits[9] and succession may help explain observations[29] for spatial and temporal patchiness in nodulation and in the contribution of N_2 fixation to the N economy of an individual tree[30] in tropical ecosystems.

METHODS SUMMARY

We performed boolean keyword searches using the ISI Web of Science to gain information on the temperature dependence of nitrogenase activity and phosphatase enzyme rates in soils sampled beneath N_2 fixers and non-fixers. For temperature effects on nitrogenase, we used the keywords 'temperature' and 'nitrogenase'; for phosphatase activities we used the keywords 'phosphatase', 'nitrogenase', 'nitrogen' and 'fix*'. Data for the effects of temperature on nitrogenase activity were normalized to the maximum rate observed for each individual experiment. Phosphatase data were lumped by functional group (that is, N_2 fixer versus non-N_2 fixer) and analysed for statistical significance using a Student's *t*-test. Details of the outcome or our literature search can be found in Methods.

Our model simulates the competition between N_2 fixers and non-fixers for light above-ground and nutrients below-ground. Details of the model equations can be found in the Methods and have been described previously[22]; details of the model calibration and simulation schemes are in the Supplementary Information. With the exception of fixing atmospheric N_2, model parameters are identical for N_2 fixers and non-fixers. Both N_2 fixers and non-fixers can take up soil N and can use any acquired N to produce phosphatases to increase soil labile-P availability. The successful invasion and establishment of N_2 fixers into a non-fixer dominant ecosystem depends on the return in NPP of its C investment into N_2 fixation

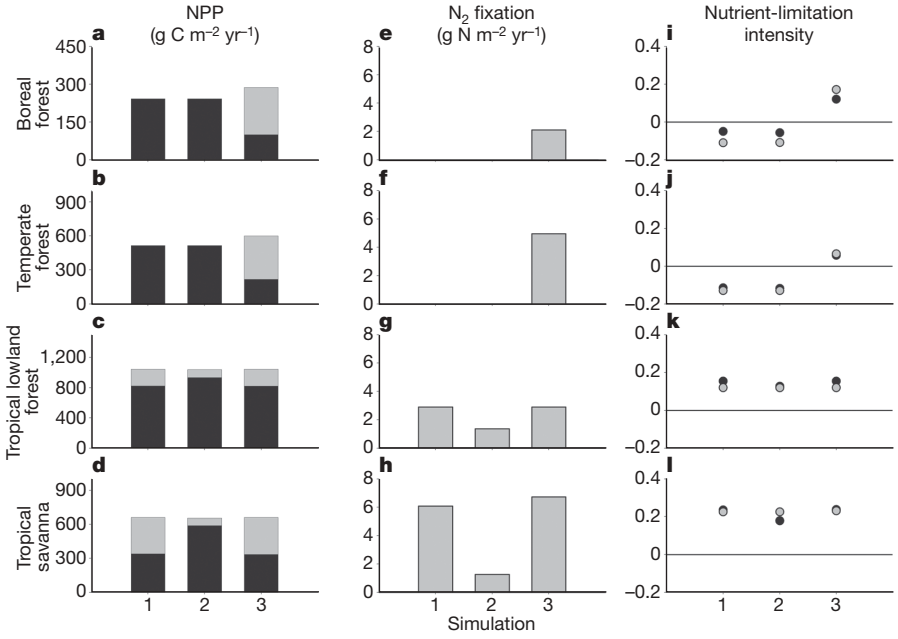

Figure 3 | Model results for different hypotheses across terrestrial biomes at steady state. a–l, The hypotheses tested are: (1), temperature-dependent N_2 fixation and individual-based P acquisition strategy (consistent with our proposed framework); (2), temperature-dependent N_2 fixation and global-commons P acquisition strategy; and (3), constant N_2 fixation (no temperature effect) and individual-based P acquisition strategy. **a–d**, NPP by N_2 fixers (grey) and non-fixers (black); **e–h**, the N_2 fixation rate; **i–l**, the nutrient-limitation intensity for N_2 fixers (grey) and non-fixers (black). Nutrient-limitation intensity was calculated as the difference in N availability minus P availability, such that positive values indicate P limitation and negative values indicate N limitation.

when NPP is N-limiting and also on the benefit of investing N into phosphatase production to reduce P limitation and increase NPP when P is limiting. All pool sizes of C, N or P at steady state are proportional to the uptake rate of C, N or P, respectively. The model has two unique and one non-unique equilibrium solutions for the parameters we used in this study.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Data compilation. Using our keyword search (see Methods Summary), we identified six studies[31–36] in the primary literature that examined nitrogenase activity across a range of temperatures in the laboratory, spanning multiple species, strains and growth forms of N_2 fixers, and terrestrial latitudes ranging from the extra-tropics to the tropics. We identified five studies[37–41] in the primary literature that evaluated soil phosphatase amounts in a paired design between known N_2 fixers and non-fixers, including data from temperate and tropical biomes, different biogeochemical conditions and across functional growth forms ranging from annual forbs to evergreen trees.

Curve-fitting statistics. We fitted a three-parameter equation of $y_{N,fix} = \exp[a + bT_s(1 - 0.5T_s/c)]$ to all data points in Fig. 1 using the nonlinear regression routine in Sigmaplot (version 10.0, 2006 Systat Software Inc.), where T_s is the rooting zone temperature. The best estimates of three parameters and one s.e.m. are $a = -3.62 \pm 0.52$, $b = 0.27 \pm 0.04$ and $c = 25.15 \pm 0.66$ ($n = 94$, $r^2 = 0.55$). The fitted equation was then normalized to give a value of $y_{N,fix} = 1.0$ at $T_s = c$ by multiplying by a scaling factor of 1.25.

The model. Our model simulates the competition between N_2 fixers and non-fixers for light above-ground and nutrients below-ground. Details of the model can be found in ref. 22. Changes in C, N or P in each pool are calculated by integrating a set of differential equations numerically. The two unique equilibriums are: exclusion of the N_2 fixer, with its NPP decreasing to zero (see Fig. 3a, simulations (1) and (2)); or invasion and establishment in the community (see Fig. 3a, simulation (3)). In the latter case, N_2 fixers will coexist with non-fixers. In case of a non-unique equilibrium solution, the initial fractions of N_2 fixers and its share of total NPP will be maintained (see Fig. 3c, d, simulation (2)).

Competition for light. We assumed that the NPP of N_2 fixers and non-fixers is proportional to the intercepted photosynthetically active radiation by its leaves, and that all leaves (either N_2 fixers or non-fixers) are randomly dispersed within the canopy. Therefore, the amount of intercepted light is proportional to the total leaf area of each species. NPP of non-N_2 fixers ($F_{C,nf}$) or N_2 fixers ($F_{C,fix}$) is modelled as:

$$F_{C,i} = U_{Cmax}\phi(x_{N,i}, x_{P,i})\frac{C_{i,1}}{\sum_i C_{i,1}}\left(1 - \exp(-k_l\sigma_1 \sum_i C_{i,1})\right) \quad (1)$$

where U_{Cmax} is a seasonally varying maximum NPP that reflects the seasonal variation of availability of light and water, k_l is the extinction coefficient (= 0.5), σ_1 is the specific leaf area ($m^2(g\,C)^{-1}$) and i = 'fix' for N_2 fixers or 'nf' for non-fixers. Function $\phi(x_{N,i}, x_{P,i})$ describes the dependence of NPP on N availability ($x_{N,i}$) or P availability ($x_{P,i}$). Nutrient availability is calculated as:

$$x_{N,i} = \frac{n_{i,1}}{n_{i,1} + K_n}$$

$$x_{P,i} = \frac{p_{i,1}}{p_{i,1} + K_p}$$

where K_n and K_p are two empirical parameters, $n_{i,1}$ and $p_{i,1}$ are the leaf N/C and P/C ratios of plant i, respectively. Nutrient limitation intensity (see Fig. 3), calculated as $x_{N,i} - x_{P,i}$, is positive when P is limiting and is negative when N is limiting.

Competition for soil mineral nitrogen. We assumed that all roots have equal access to soil mineral N and that the rate of root N uptake by N_2 fixers or non-fixers is proportional to their total root length in the soil. When the integrated C cost of root N uptake exceeds the C cost of symbiotic N_2 fixation, a fraction of the NPP allocated to root growth by N_2 fixers will be used to fix atmospheric N_2.

As described previously[13], we calculated the C cost of N uptake (λ_{up}) as the increase in NPP by plants for an extra amount of C invested to grow more leaves divided by the increase in NPP if that extra amount of C were allocated to roots. That is,

$$\lambda_{Nup} = \frac{\frac{\partial F_{C,fix}}{\partial C_{1,fix}}}{\frac{\partial F_{Nup,fix}}{\partial(F_{Nup}, C_{r,fix})}} = f_{Nup}\frac{\frac{\partial F_{C,fix}}{\partial C_{1,fix}}}{\frac{\partial F_{N,up,fix}}{\partial C_{r,fix}}} = f_{Nup}\lambda^*_{Nup} \quad (2)$$

where $F_{C,fix}$ and $F_{Nup,fix}$ are the net primary production ($g\,C\,m^{-2}\,yr^{-1}$) and N uptake ($g\,N\,m^{-2}\,yr^{-1}$) of N_2 fixers, f_{Nup} is the fraction of roots of the N_2 fixers that are not nodulated (= 1 for non-fixers), and λ^*_{Nup} is the C cost of N uptake if none of the roots are nodulated ($g\,C\,(g\,N)^{-1}$). $C_{l,fix}$ and $C_{r,fix}$ are the amount leaf and roots of N_2 fixers, respectively, in $g\,C\,m^{-2}$. The fraction of roots that are nodulated for fixing N_2 ($f_{N,fix}$) is modelled as:

$$f_{N,fix} = 1 - f_{Nup} = 1 - \min\left(1.0, \frac{\lambda_{N,fix}}{\lambda^*_{Nup}}\right) \quad (3)$$

where $\lambda_{N,fix}$ is the carbon cost of N_2 fixation (= 6.8 g C (g N)$^{-1}$).

The rate of symbiotic N_2 fixation ($F_{N,fix}$) is then modelled as:

$$F_{N,fix} = y_{N,fix} f_{N,fix} C_{r,fix} \quad (4)$$

Where $y_{N,fix}$ is the rate of N_2 fixed per unit of nodulated root C ($g\,N\,(g\,C)^{-1}\,yr^{-1}$) and is a function of soil temperature (see Fig. 1).

Competition for soil phosphorus. The rate of labile soil P uptake by roots of N_2 fixers or non-fixers is assumed to be proportional to its total root length. The rate of biochemical P mineralization increases with the difference between the N cost of labile soil P uptake and the N cost of phosphatase production (= 15 g N per g P). That is,

$$F_{ptase,i} = U_{ptase}(1 - 5\exp(-0.07P_o))\frac{\lambda_{pup,i} - \lambda_{ptase}}{\lambda_{pup,i} - \lambda_{ptase} + K_{ptase}} \quad (5)$$

where U_{ptase} is the maximum rate of biochemical P mineralization (= 0.01 g P m^{-2} yr^{-1}), P_o is the total amount of organic P in soil ($g\,P\,m^{-2}$), $\lambda_{pup,i}$ is the N cost of uptake of labile P in soil by roots of N_2 fixer (i = fix) or non-fixer (i = nf), and λ_{ptase} is the N cost of phosphatase production (= 15 g N per g P). The second term on the right-hand side of the equation accounts for the reduction of biochemical P mineralization rate as a result of decreasing amounts of organic P to be cleaved by phosphatases in the soil. We also assumed that up to 20% of soil organic P can be cleaved. The N cost of phosphorus uptake ($\lambda_{pup,i}$) was estimated as the increase in NPP for a further increase in P uptake divided by the increase in NPP per unit extra N uptake[22]. That is,

$$\lambda_{pup,i} = \frac{\partial F_{C,i}/\partial F_{P,i}}{\partial F_{C,i}/\partial F_{Nup,i}} \quad (6)$$

where $F_{P,i}$ is the rate of P uptake by N_2 fixers (i = fix) or non-fixers (i = nf) ($g\,P\,m^{-2}\,yr^{-1}$), and $F_{C,i}$ and $F_{Nup,i}$ are NPP ($g\,C\,m^{-2}\,yr^{-1}$) and N uptake rate by roots ($g\,N\,m^{-2}\,yr^{-1}$), respectively. Phosphatase production ($F_{ptase,i} > 0$) will start when $\lambda_{pup,i} > \lambda_{ptase}$.

Both N_2 fixer and non-fixers can produce phosphatases. When the biochemically mineralized P is not shared between N_2 fixers and non-fixers, the biochemically mineralized P by N_2 fixers or non-fixers is all taken up by the phosphatase producers; conversely, when the biochemically mineralized P is shared, the biochemically mineralized P enters the labile soil P pool that is assessable to both competitors equally.

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