

Review



Cite this article: Epihov DZ, Batterman SA, Hedin LO, Leake JR, Smith LM, Beerling DJ. 2017 N₂-fixing tropical legume evolution: a contributor to enhanced weathering through the Cenozoic? *Proc. R. Soc. B* **284**: 20170370. <http://dx.doi.org/10.1098/rsob.2017.0370>

Received: 22 February 2017

Accepted: 12 July 2017

Subject Category:

Palaeobiology

Subject Areas:

ecology, plant science, palaeontology

Keywords:

tropical forests, N₂-fixation, legume trees, rock weathering, CO₂ sequestration, Cenozoic

Author for correspondence:

Dimitar Z. Epihov

e-mail: dzepihov1@sheffield.ac.uk

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.3832519>.

N₂-fixing tropical legume evolution: a contributor to enhanced weathering through the Cenozoic?

Dimitar Z. Epihov¹, Sarah A. Batterman^{2,3}, Lars O. Hedin⁴, Jonathan R. Leake¹, Lisa M. Smith¹ and David J. Beerling¹

¹Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

²School of Geography and Priestley International Centre for Climate, University of Leeds, Leeds LS2 9JT, UK

³Smithsonian Tropical Research Institute, Balboa, Ancon, Panama

⁴Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA

ID DZE, 0000-0001-5711-5480; SAB, 0000-0002-7703-9873; LMS, 0000-0003-2364-8187; DJB, 0000-0003-1869-4314

Fossil and phylogenetic evidence indicates legume-rich modern tropical forests replaced Late Cretaceous palm-dominated tropical forests across four continents during the early Cenozoic (58–42 Ma). Tropical legume trees can transform ecosystems via their ability to fix dinitrogen (N₂) and higher leaf N compared with non-legumes (35–65%), but it is unclear how their evolutionary rise contributed to silicate weathering, the long-term sink for atmospheric carbon dioxide (CO₂). Here we hypothesize that the increasing abundance of N₂-fixing legumes in tropical forests amplified silicate weathering rates by increased input of fixed nitrogen (N) to terrestrial ecosystems via interrelated mechanisms including increasing microbial respiration and soil acidification, and stimulating forest net primary productivity. We suggest the high CO₂ early Cenozoic atmosphere further amplified legume weathering. Evolution of legumes with high weathering rates was probably driven by their high demand for phosphorus and micronutrients required for N₂-fixation and nodule formation.

1. Introduction

Biogeochemical weathering of silicate rocks (e.g. basalt, andesite, dunite) is a key process in the carbon cycle that acts as a long-term sink of atmospheric carbon dioxide (CO₂) [1]. Consumption of CO₂ by weathering is small (0.10–0.12 Gt C yr⁻¹) on an annual basis [2] compared with carbon transfers in photosynthesis or respiration. However, net CO₂ consumption by weathering is the dominant sink in the global carbon balance thus controlling atmospheric CO₂ and climate patterns at scales of millennia or longer [2].

Numerous field studies have shown that plants accelerate rock weathering through a suite of increasingly well understood processes [3] (electronic supplementary material, figure S1). By increasing the soil pools of H⁺ ions, carbonic (H₂CO₃, from plant or soil respiration) and chelating organic (RCOO⁻) acids, plants and their symbiotic partners cause the weathering release of base cations (electronic supplementary material, figure S1) that ultimately lead to the formation of marine carbonates on the seafloor [2]. The rise of the first forests during the Devonian (419–359 Ma) [4] probably accelerated silicate weathering, contributing to the drawdown of atmospheric CO₂ and establishing the basic features of the modern land carbon cycle. Today, forests are thought to enhance rock weathering by a factor of 2–10 compared with unvegetated catchments [5].

During the Cenozoic (past 65 Ma), the global biome transformation from palm-dominated Late Cretaceous forests to the highly productive and carbon-rich tropical forests that exist today, discussed in more detail in the next section, included the rise of trees in the ecologically important legume family

(Leguminosae, or ‘legumes’). Legumes dominate large areas of modern tropical forests in both total number of tree species and in abundance within local forests [6].

Four lines of evidence suggest that the evolution of the dinitrogen (N_2)-fixing rhizobial symbiosis (in which dinitrogen-fixing rhizobial bacteria are housed within specialized root nodules [7]) occurred as legumes radiated and spread in the early Cenozoic [9]. First, a whole-genome duplication event in the Papilionoideae clade, molecularly dated to 58 Ma, probably created the gene copies necessary for nodulation and N_2 -fixation to evolve [10]. Second, many modern rainforest -fixing legume trees are nodulated by β -rhizobia in the *Burkholderia* group [11]. Horizontal transfer of symbiotic *nod* genes between α -rhizobia and South American *Burkholderia* is dated to 60–50 Ma [12], indicating that compatible N_2 -fixing host trees may have appeared at that time. Third, the presence of fossil legume genera recovered from early Cenozoic deposits with present-day relatives capable of N_2 -fixation also supports the view that this capacity was developed in early members of the family, with our synthesis indicating that the majority of fossil taxa identified at the genus level of Palaeocene and Eocene age belong to N_2 -fixing genera (25 taxa) relative to non-fixing (16 taxa; electronic supplementary material, figure S2). Fourth, an increased proportion of legume fossil leaves recovered from 56 Ma old strata correlate with intensification of insect damage. This is a pattern consistent with the influx of fresh, fixed nitrogen (N) into the ecosystem [13].

Fossil genera, the symbiotic status of their nearest living relatives (electronic supplementary material, figure S2), evidence of increased insect damage in the fossil record in likely response to high foliar N and molecular clock dating therefore appear to indicate that N_2 -fixation and diverse mycorrhizal symbioses had evolved in legumes by the early Cenozoic.

Here, we review the rise of N_2 -fixing legume-rich tropical forests early in the Cenozoic and propose a new testable hypothesis for how the evolution of this biome may have strengthened the long-term carbon cycle feedbacks that helped shape Earth’s CO_2 and climate history in the Cenozoic.

2. Global rise of nitrogen-fixing legume-rich tropical forests

Late Cretaceous tropical floras were dominated by widely distributed palm communities from Africa to South America, a floristic region known as the Palmae Province [14–16]. Communities in both the Palaeo- and Neotropics contained abundant palms, including those resembling extant *Nyssa* palms and suggestive of coastal intertidal habitats similar to mangrove forests, while other areas harboured palm-dominated dry forest communities. Unlike modern tropical forests, both of these communities were deprived of abundant dicot arboreal flora [14,15]. In Africa, leaf fossil and pollen evidence indicate that the dominant palm lineages began to decline around the Cretaceous–Palaeogene boundary [16] and completely disappeared in the fossil record during the Miocene [15]. Similarly, palm abundance in Neotropical areas decreased in the early Cenozoic, although palms remain an important element of these forests today [17]. The Palmae Province was replaced in Africa and assimilated in South America by the rise of modern tropical forests during the early Cenozoic. The earliest record of modern Neotropical forests—found in Colombia and dated to the

Late Palaeocene (58 Ma)—indicates that the flora resembled the current day composition of plant families with abundant fossilized dicot and palm leaves, including numerous legumes [18]. Pollen records from Africa similarly show the rise of modern families of dicot trees following the Palaeocene [15,16].

Pollen and leaf macrofossils indicate that legume taxa have comprised a key component of tropical forests since the early Cenozoic (figure 1a; electronic supplementary material, figure S2). While it is difficult to translate a taxon’s abundance in the fossil record to abundance in a forest, the persistent recovery of legume pollen, leaves, flowers, fruits and wood indicate that legume trees were present and widespread in the flora of the Americas and Africa. The following observations can be drawn from early Cenozoic records: (i) legume leaves made up 21–73% of all fossilized leaves in South and North American forest assemblages [18,24]; (ii) legumes comprised 14–33% of all recorded taxa across tropical forests (figure 1) [25–27]; (iii) single legume tree species represented up to 7% of all fossil leaves (greater than 200 leaves) in species-diverse South American dry forests [28,29]; (iv) one legume tree species (the non-fixing *Cynometra*) formed a monodominant forest in Africa 46 Ma [26], with further monodominance indicated by the presence of Eocene fossils that belong to modern monodominant genera such as the non-fixing *Brachystegia* and *Julbernardia* (Eurasian deposits) and the non-fixing *Peltogyne* (South American formations) (electronic supplementary material, figure S2); (v) rainforests with abundant presence of caesalpinoid and mimosoid (many modern representatives of which are N_2 -fixing [7]) legumes were recorded in central Africa [15]; and (vi) tropical and temperate N_2 -fixing legume trees may have coexisted during warm Eocene climates in higher latitude boreotropical forests (England, Hungary, North America) [30].

Fossil evidence, therefore, indicates that early Cenozoic tropical forests (wet, dry and boreotropical) had evolved abundant legumes across continents (figure 1a). The timing of the early Cenozoic assembly of legume-rich tropical forests (58–42 Ma) as documented by the fossil record is similar to the molecular clock-dated diversification events in the legume clade (figure 1b; for recent changes in legume taxonomy, see [31]). Beneath these emerging tropical forests were substantial areas of unweathered rocks in tropical India [32], in South America, including the southeast part of the Amazon basin, and in the Amazon deltaic area [22] coinciding with peaks in terrestrial weathering (figure 1b) as evident from the recovery of highly weathered palaeosols [20].

3. Mechanisms of N_2 -fixing legume-driven enhanced weathering

Here, we propose that the rise of N_2 -fixing legume trees enhanced weathering through a series of processes associated with three abilities especially well developed in this group of trees: (i) to fix atmospheric N_2 , (ii) to build disproportionately N-rich leaf tissue, and (iii) to stimulate the primary production in ecosystems by redistributing fixed N to the soil and to neighbouring trees.

First, N_2 -fixing legumes have the ability to fix N at high rates in natural ecosystems [33]. Over time, fixers bring in substantial quantities of N and can provide the largest natural source of new N to ecosystems [34]. Soil N is high and nitrate and denitrification losses large (exceeding or rivaling many

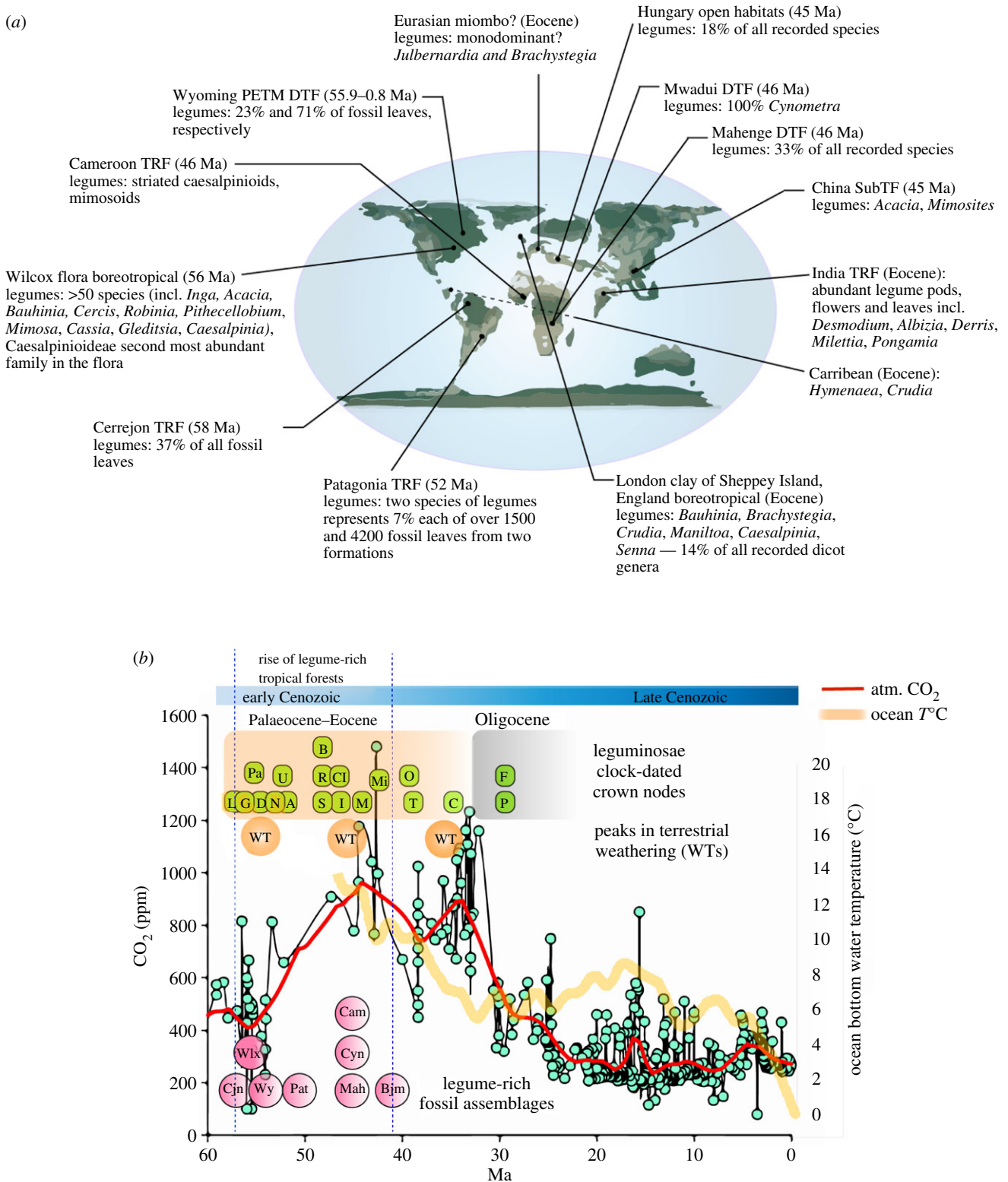


Figure 1. Global rise of legume-rich tropical forests during the early Cenozoic (58–42 Ma). (a) Global map of the major legume fossil records plotted on the Eocene continental configuration. Lines and their ball ends point to approximate locations. Caesalpinioids in the Wilcox flora are according the old pre-molecular taxonomy with a family status. DTF, dry tropical forest; SubTF, sub-tropical forest; TRF, tropical rainforest, boreotropical or BTF, a forest with mixed tropical and temperate species which is sometimes referred to as boreotropical. (b) Summary of the notable legume-rich fossil assemblages and all major molecular clock-dated crown nodes in the Leguminosae marking the rise of the legume-rich forests in the Palaeocene–Eocene plotted against atmospheric CO₂ records (light blue dots and red Loess curve) using data from [19] and ocean bottom water temperature (orange semi-transparent curve) using data from [21]. Peaks in terrestrial weathering (WTs = 55, 48, 35 Ma) are estimated as levels of lateritization and bauxitization in [20]. Cjn, Carrejon rainforest formation; Wlx, Wilcox boreotropical flora; Wy, Wyoming flora; Pat, Patagonia dry forests; Mah, Mahenge dry tropical forest; Cyn, *Cynometra*-monodominant stands in Mwadui; Cam, Cameroon tropical rainforest; Bjm, putative *Brachystegia*-*Julbernardia* miombo (macrofossils but not assemblage). Crown nodes include the divergence of L, Leguminosae; Pa, Papilionoideae; G, Genistoids; D, Dalbergioids; N, *Senna* clade; U, *Umtiza* clade; A, Amherstieae tribe (contains the majority EM taxa) after [23]; S, *Swartzia* clade; R, Robinioids; B, Mirbelioids; I, Indigoferoids; Cl, *Cladrastis* clade; M, Millettoids; Mi, Mimosoideae; O, *Peltophorum* clade; T, *Trifolium* (IRLC) clade; C, *Cercis* clade; P, *Poëppigia* clade; F, Fossil not supported *Brachystegia* clade (because fossils of *Brachystegia* and *Julbernardia* found much earlier and new estimates show that this divergence occurred 52.1 Ma—here marked as clade Amherstieae). Clock data references: all clade ages unless otherwise stated are after [9].

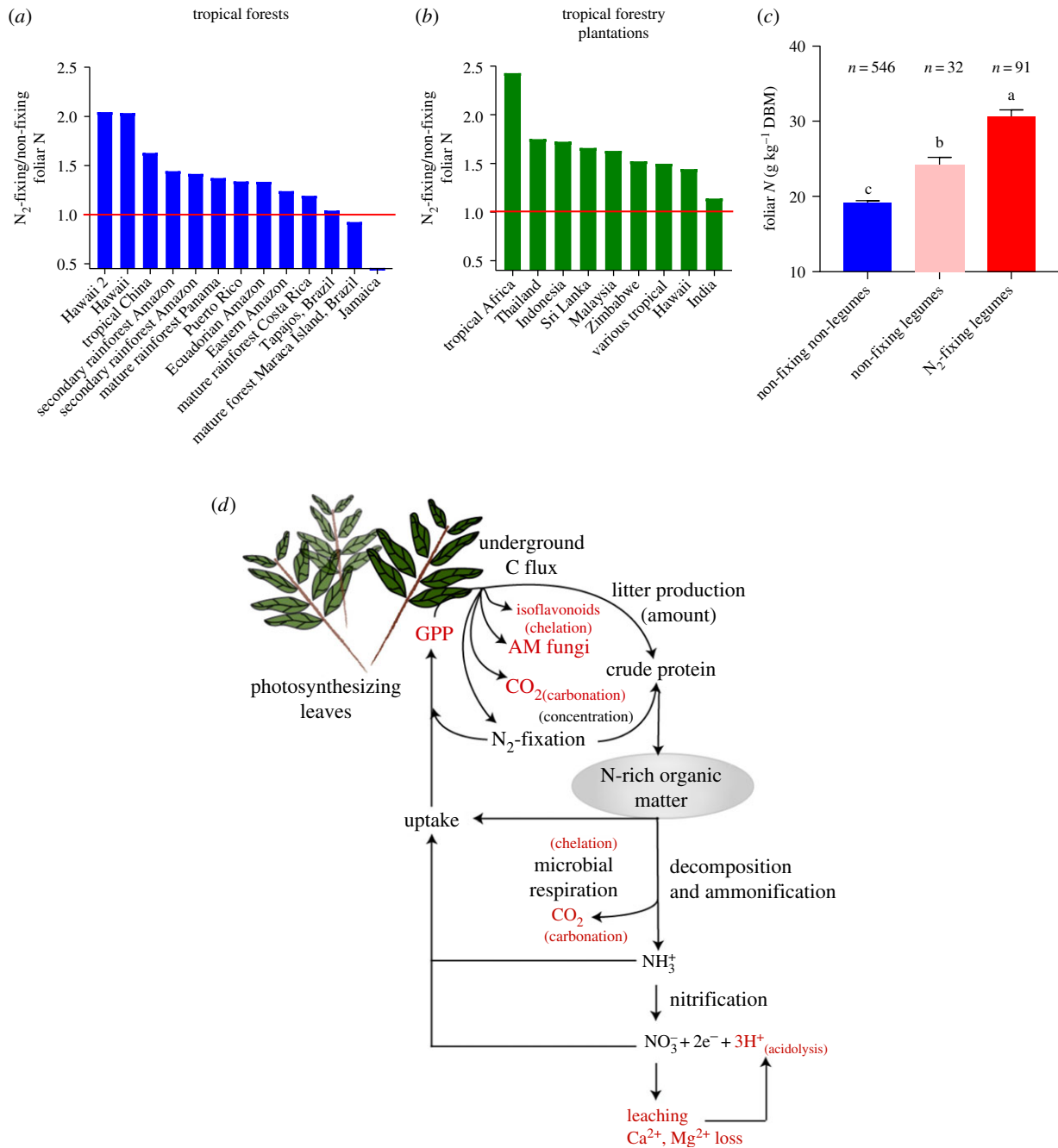


Figure 2. Foliar N ratios between N₂-fixing and non-fixing non-legumes in (a) tropical forests, (b) tropical forestry plantations and (c) between the three functional groups and (d) pathways of the nitrogen-weathering feedback hypothesis. Red typeface depicts factors stimulating weathering with specific weathering reactions associated with those factors in brackets. In tropical forests, N₂-fixing legumes exhibit an average of 34.58% (s.e.m. = 11.73%) higher leaf crude protein content than non-fixing tree species. In forestry plantations, N₂-fixing legume species reveal on average 64.50% (s.e.m. = 11.57%) higher leaf crude protein content than non-fixing trees. Raw data and references are available in the electronic supplementary material. In (c), 'n' stands for number of species and DBM stands for dry biomass.

temperate forests exposed to N deposition) in tropical forests that harbour N fixers [33]. In a survey across 55 tropical forests, these systems naturally sustained loss rates of 4–6 kg N ha⁻¹ nitrate, 6–10 kg N ha⁻¹ of total dissolved N and 4–5 kg N denitrified; when corrected for low levels of atmospheric N deposition, these rates could only be explained by fixation [35].

Second, N₂-fixing legumes contain substantially higher leaf N than non-fixing tree species [36]. We performed a meta-analysis of 31 studies encompassing 561 tropical tree species ($n = 680$ measurements) to evaluate the N content of N₂-fixing and non-fixing trees in natural forests and plantations across 22 different tropical regions (figure 2*a,b*). Our analysis shows that, despite considerable variation across sites, N₂-fixers exhibit higher mean leaf N content than non-

fixers (by 35% in natural tropical forests and by 65% in tropical forestry plantations) and non-fixing legumes (by 21%). These findings are consistent with a study of leaf N across Amazonian tropical forests that also reported N₂-fixing legumes had higher leaf N content than both non-fixers as a whole and non-fixing legumes [37].

Third, this N-rich leaf tissue would cause increased input of N-rich compounds including proteins and amino acids to soils via litterfall. Such increased N input, in turn, would enrich soils in N and probably cause higher rates of productivity for non-fixing as well as N₂-fixing trees. Evidence for such a major ecosystem impact comes from recent field studies: N₂-fixing legumes provided approximately 50% of the N required for early growth of Panamanian secondary rainforests, supported

rapid carbon accumulation in biomass of both fixers and non-fixers [38] and enhanced soil N [39] during periods of N limitation. Levels of N₂-fixation in early Cenozoic fixers are hard to establish empirically but indirect evidence of greater insect damage from fossil leaves together with greater palatability and protein content of N₂-fixing trees [13] support the assumption that ancient N₂-fixers were capable of generating high N foliage.

We suggest these three characteristics of N₂-fixing legumes probably entrain a suite of direct and indirect mechanisms that can enhance rates of rock weathering, as discussed below.

(a) N₂-fixing legume litter decomposition and microbial respiration

Litterfall and the decomposition of protein-enriched biomass would ultimately increase the flux of new fixed N into several linked soil processes (soil respiration, ammonification, nitrification) and pools (soil organic matter, dissolved organic N). The input of new N would trigger several weathering-related mechanisms (figure 2d).

First, the low C/N ratio of N₂-fixing legume litter implies fast decomposition, greater microbial respiration and greater CO₂ production than non-legume litter [40,41]. During decomposition, the majority of N-rich leaf tissue and its amino acids, amino sugars and other N-rich monomers will undergo ammonification and nitrification. Decomposition also generates organic acids and faster decomposition rates may facilitate passing the organic acid concentration threshold necessary to drive mineral weathering [42].

Second, N-rich organic matter can itself stimulate soil microbial activity and respiration. Although C inputs would have similar effects regardless of whether derived from decomposition of leguminous N-rich or non-leguminous N-poor litter, the lack of sufficient N can ultimately down-regulate microbial respiration specifically under high CO₂ regimes [43], such as those seen during the early Cenozoic (figure 1b). Addition of N₂-fixing legume-derived N-rich litter may therefore have a dual function. First, it will fuel microbial respiration with the energy stored in the carbon–hydrogen (C–H) and carbon–carbon (C–C) bonds of its carbohydrate component. Second, because of its abundance in N and protein, it will promote microbial respiration by alleviating any existing N-limitation on microbial metabolism. *In situ* studies in tropical soils confirm augmented rates of microbial respiration in the combined glucose and N treatment compared with the glucose treatment alone [44].

Third, the dissolved CO₂ generated by microbial respiration forms carbonic acid (H₂CO₃) which, in turn, acts as a major weathering agent [45] (electronic supplementary material, figure S1). Increased microbial respiration also positively correlates with the production of chelating organic acids, e.g. gluconic acid, a secreted by-product of microbial catabolism [46].

(b) N₂-fixing legume-driven soil acidification

Ammonia generated by ammonification during litter decomposition can undergo nitrification. In the process, each molecule of ammonia converted to nitrate generates three by-product H⁺ ions. Although these H⁺ ions are typically counterbalanced by plant secretion of anions (bicarbonate or organic acids) for each acquired NO₃⁻, nitrate leaching can uncouple this relationship and promote the build-up of H⁺

in the soil. High levels of N₂-fixation can exceed the rates at which N is immobilized within the system, resulting in enhanced NO₃⁻ leaching (as discussed above) and enhanced transport of H⁺ to deeper soil horizons (where contents of unweathered minerals may be high). Tree ring data from tropical fossil woods indicate that climate seasonality was largely similar between early Cenozoic and modern tropical forests [47], supporting the view that nitrification patterns as affected by soil moisture/dryness [48] probably were comparable.

During the leaching of NO₃⁻ large amounts of counterbalancing cations (Ca²⁺, Mg²⁺, K⁺) released by cation exchange reactions with nitrification-generated H⁺ are leached too, resulting in the decline of soil cation exchange capacity and soil pH buffering capacities. This phenomenon has been recorded for N₂-fixing forests of *Alnus rubra* in which large inputs of fixed N caused leaching, decreased cation concentration and increased soil acidification [49].

Despite the tight N budget of most tropical forest systems, substantial levels of nitrate leaching still occurs [33], suggesting that similar mechanisms probably operate in tropical forests rich in N₂-fixing legumes. In addition, because of their N₂-fixation, fixers tend to acquire lower relative amounts of negatively charged ions and produce larger organic acid loads per unit N resulting in the balancing H⁺ extrusion into the rhizosphere [50].

Consequently, pronounced soil acidification has been recorded in various N₂-fixing species from herbs [50,51] to trees and shrubs of temperate forest [52,53] and tropical rainforest [39] areas. Recent analysis of tropical rainforests at four Neotropical locations revealed that forests rich in N₂-fixers exhibited increased soil acidity (pH 4.1) and lower Ca²⁺ and Mg²⁺ concentrations than forests poor in N₂-fixing legumes (pH 5.2) [54]. N₂-fixing legume-driven acidification can promote weathering not only by acid attack (acidolysis) of the mineral lattice (electronic supplementary material, figure S1) but also by depleting soil cations through cation exchange, thus shifting the equilibrium towards further mineral dissolution.

(c) N₂-fixing legume-driven stimulation of net primary productivity

Ultimately, inorganic forms of fixed N are acquired from the soil solution by roots stimulating the N input into biomass, including that of neighbouring non-fixing trees. For instance, the non-fixing tropical trees *Peschiera*, *Psidium* [55], *Eucalyptus* [56] and *Terminalia* [57] all exhibited increased foliar N levels in N₂-fixing legume-rich neighbourhoods compared with legume-poor settings. As foliar N correlates with increased levels of crude leaf protein, including the photosynthetic enzyme RUBISCO [58], the photosynthetic rates of individual trees and the net primary production (NPP) of such mixed fixer/non-fixer forests may be upregulated. Indeed, N₂-fixing legumes exhibit up to twofold greater photosynthetic rates than the less N-rich leaves of non-fixing trees in Zimbabwe [59]. Similarly, non-fertilized mixed non-fixer/N₂-fixer forestry plantations reveal augmented NPP rates compared with non-fixing forests in Brazil and Puerto Rico [60,61].

Fossil evidence supports N₂-fixing legume-driven N-fertilization on productivity of tropical ecosystems. Presumed N₂-fixing legume-dominated assemblages exhibited insect damage (linked to higher leaf N content) spread across fossil taxa relative to systems with fewer legumes in which foliar damage was more concentrated on legume leaves [13]. This observation indicates that as legume domination was

established, N redistribution triggered by the input of N-rich litter increased N levels of neighbouring non-legumes (as observed in modern systems). The source of this N buffering effect is better explained by legumes capable of N₂-fixation than non-fixing legumes because the patterns are consistent with the influx of new fixed N to the system.

Some canopy photosynthate from highly productive N₂-fixing legume-rich forests will be allocated to symbiotic mycorrhizal fungi. The mycelial networks of these fungi grow in intimate contact with mineral grains, thus driving enhanced rock weathering and inorganic nutrient release via chelation, carbonation and acidolysis (electronic supplementary material, figure S1) [45]. Greater gross primary production (GPP) and its related NPP rates also correlate with greater root respiration (with associated production of carbonic acid) and organic acid leaching, which promotes further weathering [45] (electronic supplementary material, figure S1). N₂-fixing legume-enhanced forest NPP can also increase the demand for nutrients and thus further necessitate more extensive soil exploration via roots and mycorrhizal fungi, and eventually enhanced rock weathering. Therefore, increased N inputs could indirectly increase rock weathering via stimulation of rainforest NPP in legume-rich communities compared with *Nyssa* and other Late Cretaceous palm forests as well as to legume-poor early Cenozoic analogues.

(d) Accessory mechanisms of N₂-fixing legume-driven weathering

The unique ability of legumes (including many rainforest N₂-fixing legume trees [62,63]) to synthesize and exude isoflavonoids [64] may also impact weathering rates. Isoflavonoids enhance phosphorus (P) and iron (Fe) solubilization from the mineral vivianite by acting as soil chelators (electronic supplementary material, figure S1) as well as by decreasing organic acid decomposition [65]. Comparison between the estimated low-molecular organic acid exudation by lowland tropical rainforest trees (approx. 25 µg C g⁻¹ dry biomass (DBM) root h⁻¹) [66] and isoflavonoid exudation of the N₂-fixer *Lupinus albus* (approx. 31 µg C g⁻¹ DBM root h⁻¹) [67] (see the electronic supplementary material for detailed calculations) suggests that isoflavonoids could contribute to the pool of plant-derived chelating agents in legume-rich forest soils.

Isoflavonoids are crucial in establishing the N₂-fixing legume-rhizobial symbiosis by enabling both attraction and priming of rhizobial partners [68]. They attract larger soil rhizobial populations [69] of nodulation-competent strains of *Burkholderia*, *Rhizobium* and *Mesorhizobium*—members of all of these genera have been shown to exert strong chelating activities [70]. Soil pH, C, N and C/N ratio are also important determinants of microbial community structure [71]. Finally, legume-mediated changes in soil chemistry may change microbial community of the mineralosphere selecting for nitrophilic and acidophilic bacterial taxa.

4. N₂-fixing legume-rich forest responses to a CO₂-rich early Cenozoic atmosphere

The rise of N₂-fixing legume-rich tropical forests during the early Cenozoic coincides with elevated atmospheric CO₂ concentrations, with potential feedbacks on primary

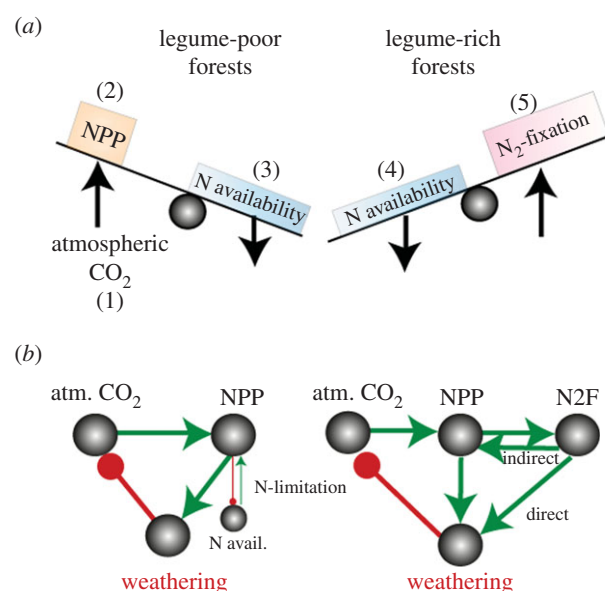


Figure 3. Atmospheric CO₂, NPP, weathering and N feedbacks. (a) Ecosystem effects of elevated CO₂ levels in legume-poor and rich forests; (b) differences in feedback relationships between rich and poor forests. In both forest types, high atmospheric CO₂ levels (1) promote a proportional NPP increase (2) which transitions the system to low N-availability (3). Ultimately, in poor forests that would result in a negative feedback on NPP. In rich forests, however, low N-availability (3) can upregulate N₂-fixation rates and recruitment of N₂-fixers (4) thus alleviating N limitations and allowing for an unchanged CO₂-NPP relationship. Green arrows indicate positive relationships, whereas red ball-ending lines—negative relationships; N₂F, N₂-fixation.

production and weathering (figure 1b, figure 3). Evidence for the mechanisms that may govern this potential feedback comes from free air CO₂-enrichment (FACE) experiments. In the Oak Ridge, TN, USA, FACE experiment, the non-fixing AM *Liquidambar styraciflua* trees showed a 24% increase in NPP during the first 6 years of exposure to elevated CO₂ [72]. However, over the next 5 years the positive CO₂-enrichment effect decreased to +9% in 11-year old stands as ecosystem N stocks declined [72], suggesting progressive soil N-limitation on tree NPP in the long-term under high CO₂ [72,73]. N₂-fixing legumes may mitigate this N-limitation mechanism under a high CO₂ atmosphere because N-limitation would favour recruitment of N₂-fixing legumes and/or upregulate their fixation rates [74,75]. Fossil evidence suggests that N₂-fixing legumes may increase in abundance under such conditions. During the transient climate warming event across the Palaeocene–Eocene thermal maximum (PETM; 55.8 Ma) that is linked to a rise in atmospheric CO₂ and continental weathering regimes [76], the abundance of fossilized leguminous leaf specimens increased to 73% and then declined to 21% post-PETM in the Bighorn Basin, USA [23]. Further evidence from PETM sites dominated by legumes corroborates extensive N₂-fixation capacity increasing N availability to the system (as discussed above) [13].

Physiologically, elevated CO₂ can promote nodulation and N₂-fixation [77–79], mycorrhization [80] and photosynthetic rates, and therefore may allow N₂-fixing legume productivity to increase proportionally more in response to CO₂ than non-legumes [77,79]. Furthermore, nodules represent additional sinks exchanging the increased flux of assimilates for fixed N thus curtailing the photosynthetic acclimation to elevated CO₂ when unconstrained by other factors [81], allowing higher

photosynthetic rates to persist. Those effects could promote N₂-fixer recruitment, upregulated N₂-fixation rates and greater dominance at high CO₂ concentrations [82]. A FACE experiment at Oak Ridge analysed the CO₂ response of over 2000 seedlings from 14 different temperate tree species. After 5 years, the N₂-fixing legume *Robinia pseudoacacia* exhibited an order of magnitude higher biomass response than all of the non-fixing angiosperm trees [83]. Controlled environment pot-based CO₂-enrichment experiments indicate that the photosynthesis and growth responses of nodulated N₂-fixing Leguminosae rainforest trees were significantly greater than that of non-leguminous species investigated [84]. Although there are clear limitations in extrapolating from these studies to legumes of early Cenozoic tropical forests, the mechanistic basis of the CO₂ response—linked to alleviation of N-limitation—would still hold.

Based on these findings, we conceptualize that different feedback loops operated between non-legume and N₂-fixing legume forests, atmospheric CO₂ and climate in the Cenozoic (figure 3). In *non-fixing forests* like those that existed prior to legume evolution or in legume-poor tropical forests of the early Cenozoic, increased atmospheric CO₂ would stimulate NPP until available soil resources—probably N and P in many locations—are exhausted (figure 3a: feedbacks 1-2-3). Progressive N-limitation could therefore uncouple the ‘standard’ relationship between NPP, CO₂ and weathering [85] in legume poor forests (figure 3b). By contrast, however, in *legume-rich forests*, progressive N-limitation would probably further promote recruitment of N₂-fixers and the up-regulation of N₂-fixation rates (figure 3a: feedbacks 1-2-3-4-5), as observed in modern N-limited rainforests [38]. This could allow NPP to respond to increasing CO₂ and help promote continued weathering (figure 3b). Additionally, biological weathering processes are strengthened by inputs of N-rich legume litter and associated downstream processes. Combined, this evidence indicates that in CO₂-rich conditions, the significant role of legumes in maintaining enhanced weathering regimes in early tropical forests may be amplified.

5. Evolutionary drivers of enhanced weathering by N₂-fixing legumes

Central to our feedback analyses (figure 3) is the idea that N₂-fixing legumes are associated with higher weathering rates than non-legume trees. This effect, in turn, may have evolved in response to a disproportionately high demand for P, molybdenum (Mo) and Fe across legume taxa. P and Mo have been identified as potentially limiting factors of N₂-fixation within tropical forests [86–88]. These limitations may occur because the most common type of nitrogenases involved in symbiotic N₂-fixation requires an Fe/Mo complex acting as a cofactor [86] while high P intake accommodates for enhanced production of energy-rich metabolites (e.g. ATP) and membranes during nodule organogenesis [89]. Linked to the probable greater P demand driven by higher rates of growth, some but not all N₂-fixing legumes may have higher foliar P levels than non-fixing trees (electronic supplementary material, table S1). Fe is also required for production of leghaemoglobin in nodules for oxygen binding [90]. Fe is very abundant in tropical soils but it is highly insoluble. Most P in soils is also insoluble in complexes with aluminium (Al)- and Fe-bearing secondary minerals, and

fresh Mo and P inputs originate from weathering of otherwise plant-unavailable mineral sources. Both the dissolution of insoluble P and Fe and the release of mineral-bound Mo rely upon the same weathering mechanisms that include chelation and acidolysis [91] (electronic supplementary material, figure S1). Al and iron phosphate minerals such as variscite and vivianite, respectively, dissolve faster at pH < 6, a process exacerbated by organic acids [91,92].

Overall, the processes of N₂-fixation and nodule formation require an array of sparingly soluble (P, Fe) or scarce soil minerals (Mo). This observation suggests that the mechanisms of enhanced weathering overlap with those driving acquisition of elements essential for N₂-fixing legumes. It provides a mechanism that would promote the evolution of adaptive strategies in tropical legumes leading to enhanced weathering and thereby unlocking sparingly soluble limiting nutrients. Our hypothesized mechanisms that relate N₂-fixing legume functioning to weathering rates are suitable for direct investigation in the field and laboratory, and future studies will hopefully further elucidate the relative importance of each of the mechanisms of the hereby proposed hypothesis.

6. Conclusion

Fossils and molecular dating suggest that a worldwide shift from palm-dominated communities to ‘modern’ tropical forests occurred early in the Cenozoic and involved the development of N₂-fixing legume-rich and symbiotically diverse communities. Based on our analyses of potential effects on forest ecosystem biogeochemical C and N cycling, we propose that the increasing abundance of N₂-fixing legumes in tropical forests amplified weathering rates through several interconnected pathways. Firstly, N₂-fixing legumes increased soil inputs of N-rich organic matter (by an estimated 35–65% based on modern analogues) which can promote microbial respiration and carbonation as well as progressive soil acidification resulting from leaching and compensatory H⁺ extrusion. Subsequently, increased N inputs may have fuelled greater N-availability stimulating forest NPP, thus driving further carbonation, organic acid chelation and rhizospheric weathering activities. Lastly, exudation of N-costly isoflavonoids unique to legumes could have provided an additional source of chelating activities that cause rock weathering. Together with soil acidification and decreasing C/N ratios these effects could have indirectly driven shifts in the weathering potential of the soil microbial community.

We suggest the global evolution of tropical forests rich in N₂-fixing legumes in the early Cenozoic in concert with abiotic drivers, including reduced subduction of oceanic crust and the rise of the Himalayas/Tibetan plateau [32,93], could have contributed to regimes of enhanced weathering over pantropical areas with consequent feedbacks on global climate. Furthermore, N₂-fixing legumes help maintain the NPP response to atmospheric CO₂ concentration. In an evolutionary context, tropical N₂-fixing legumes appear to enhance rock weathering as a possible adaptation to unlock previously unavailable P, Mo and Fe mineral sources, thus alleviating limitations on N₂-fixation processes.

Data accessibility. This article has no additional data.

Authors' contributions. D.Z.E., S.A.B., L.O.H. and D.J.B conceived the review; D.Z.E. compiled the first draft; all authors contributed to revisions of the paper.

Competing interests. We declare we have no competing interests.

Funding. D.Z.E. is supported by an ERC advanced grant awarded to D.J.B. (CDREG, 322998). S.A.B. was supported by a UK Natural

Environment Research Council (grant NE/M019497/1) and a Princeton University Climate Mitigation Initiative Young Investigator Fellowship (with funding from BP).

References

- Pagani M, Caldeira K, Berner R, Beerling DJ. 2009 The role of terrestrial plants in limiting atmospheric CO₂ decline over the past 24 million years. *Nature* **460**, 85–88. (doi:10.1038/nature08133)
- Goudie AS, Viles HA. 2012 Weathering and the global carbon cycle: geomorphological perspectives. *Earth Sci. Rev.* **113**, 59–71. (doi:10.1016/j.earscirev.2012.03.005)
- Taylor LL, Leake JR, Quirk J, Hardy K, Banwart SA, Beerling DJ. 2009 Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm. *Geobiology* **7**, 171–191. (doi:10.1111/j.1472-4669.2009.00194.x)
- Algeo TJ, Scheckler SE, Scott AC. 1998 Terrestrial-marine teleconnections in the Devonian: links between the evolution of land plants, weathering processes, and marine anoxic events. *Phil. Trans. R. Soc. Lond. B* **353**, 113–130. (doi:10.1098/rstb.1998.0195)
- Moulton KL, West J, Berner RA. 2000 Solute flux and mineral mass balance approaches to the quantification of plant effects on silicate weathering. *Am. J. Sci.* **300**, 539–570. (doi:10.2475/ajs.300.7.539)
- ter Steege H *et al.* 2006 Continental-scale patterns of canopy tree composition and function across Amazonia. *Nature* **443**, 444–447. (doi:10.1038/nature05134)
- Sprent JI. 2009 *Legume nodulation: a global perspective*. New York, NY: John Wiley & Sons.
- Dilworth MJ, James EK, Sprent JI, Newton WE. 2008 *Nitrogen-fixing leguminous symbioses*.
- Lavin M, Herendeen PS, Wojciechowski MF. 2005 Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. *Syst. Biol.* **54**, 575–594. (doi:10.1080/10635150590947131)
- Werner GDA, Cornwell WK, Sprent JI, Kattge J, Kiers ET. 2014 A single evolutionary innovation drives the deep evolution of symbiotic N₂-fixation in angiosperms. *Nat. Commun.* **5**, 4087. (doi:10.1038/ncomms5087)
- Barrett CF, Parker MA. 2005 Prevalence of *Burkholderia* sp. nodule symbionts on four mimosoid legumes from Barro Colorado Island, Panama. *Syst. Appl. Microbiol.* **28**, 57–65. (doi:10.1016/j.syapm.2004.09.002)
- Walker R, Agapakis C, Watkin E, Hirsch A. 2015 Symbiotic nitrogen fixation in legumes?: perspectives on the diversity and evolution of nodulation by *Rhizobium* and *Burkholderia* species. In *Biological nitrogen fixation*, vol. 2 (ed. F de Bruijn), pp. 913–923. New York, NY: John Wiley & Sons.
- Currano ED, Laker R, Flynn AG, Fogt KK, Stradtman H, Wing SL. 2016 Consequences of elevated temperature and pCO₂ on insect folivory at the ecosystem level: perspectives from the fossil record. *Ecol. Evol.* **6**, 4318–4331. (doi:10.1002/ece3.2203)
- Vajda V, Bercovici A. 2012 Pollen and spore stratigraphy of the Cretaceous–Paleogene mass-extinction interval in the Southern Hemisphere. *J. Stratigr.* **36**, 153–164.
- Maley J. 1996 The African rain forest: main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proc. R. Soc. Edinb. B* **104**, 31–73. (doi:10.1017/S0269727000006114)
- Jacobs BF. 2004 Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Phil. Trans. R. Soc. Lond. B* **359**, 1573–1583. (doi:10.1098/rstb.2004.1533)
- Burnham RJ, Johnson KR. 2004 South American palaeobotany and the origins of neotropical rainforests. *Phil. Trans. R. Soc. Lond. B* **359**, 1595–1610. (doi:10.1098/rstb.2004.1531)
- Wing SL, Herrera F, Jaramillo CA, Go C, Labandeira CC. 2009 Late Paleocene fossils from the Cerrejon Formation, Colombia, are the earliest record of Neotropical rainforest. *Proc. Natl Acad. Sci. USA* **106**, 18 627–18 632. (doi:10.1073/pnas.0905130106)
- Beerling DJ, Royer DL. 2011 Convergent Cenozoic CO₂ history. *Nat. Geosci.* **4**, 418–420. (doi:10.1038/ngeo1186)
- Retallack GJ. 2010 Lateritization and bauxitization events. *Econ. Geol.* **105**, 655–667. (doi:10.2113/gsecongeo.105.3.655)
- Cramer BS, Miller KG, Barrett PJ, Wright JD. 2011 Late Cretaceous–Neogene trends in deep ocean temperature and continental ice volume: reconciling records of benthic foraminiferal geochemistry ($\delta^{18}\text{O}$ and Mg/Ca) with sea level history. *J. Geophys. Res. Ocean* **116**, 1–23. (doi:10.1029/2011JC007255)
- Putzer H. 1984 The geological evolution of the Amazon basin and its mineral resources. In *The Amazon: limnology and landscape ecology of a mighty tropical river and its basin* (ed. H Sioli). Dordrecht, The Netherlands: Dr. W. Junk Publishers.
- Bruneau A, Mercure M, Lewis GP, Herendeen PS. 2008 Phylogenetic patterns and diversification in the caesalpinoid legumes. *Botany* **86**, 697–718. (doi:10.1139/B08-058)
- Jaramillo C *et al.* 2010 Effects of rapid global warming at the Paleocene–Eocene boundary on neotropical vegetation. *Science* **330**, 957–961. (doi:10.1126/science.1193833)
- Currano ED, Labandeira CC, Wilf P. 2010 Fossil insect folivory tracks paleotemperature for six million years. *Ecol. Monogr.* **80**, 547–567. (doi:10.1890/09-2138.1)
- Cantrill DJ, Bamford MK, Wagstaff BE, Sauquet H. 2013 Early Eocene fossil plants from the Mwadui kimberlite pipe, Tanzania. *Rev. Palaeobot. Palynol.* **196**, 19–35. (doi:10.1016/j.revpalbo.2013.04.002)
- Ettingshausen CB. 1879 Report on phytopalaeontological investigations of the fossil flora of Sheppey. *Proc. R. Soc. Lond.* **29**, 388–396. (doi:10.1098/rspl.1879.0065)
- Wilf P, Johnson KR, Cúneo NR, Smith ME, Singer BS, Gandolfo MA. 2005 Eocene plant diversity at Laguna del Hunco and Río Pichileufú, Patagonia, Argentina. *Am. Nat.* **165**, 634–650. (doi:10.1086/430055)
- Barreda V, Palazzesi L. 2010 Vegetation during the Eocene–Miocene interval in central Patagonia: a context of mammal evolution. In *The paleontology of Gran Barranca: evolution and environmental change through the Middle Cenozoic of Patagonia* (eds RH Madden, AA Carlini, MG Vucetich, RF Kay). Cambridge, UK: Cambridge University Press.
- Doyle JJ, Luckow MA. 2003 The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. *Plant Physiol.* **131**, 900–910. (doi:10.1104/pp.102.018150)
- LPWG. 2017 A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny—the Legume Phylogeny Working Group (LPWG). *Taxon* **66**, 44–77. (doi:10.12705/661.3)
- Jagoutz O, Macdonald FA, Royden L. 2016 Low-latitude arc–continent collision as a driver for global cooling. *Proc. Natl Acad. Sci. USA* **113**, 4935–4940. (doi:10.1073/pnas.1523667113)
- Hedin LO, Brookshire ENJ, Menge DNL, Barron AR. 2009 The nitrogen paradox in tropical forest ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **40**, 613–635. (doi:10.1146/annurev.ecolsys.37.091305.110246)
- Vitousek PM, Menge DN, Reed SC, Cleveland CC. 2013 Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. *Phil. Trans. R. Soc. B* **368**, 20130119. (doi:10.1098/rstb.2013.0119)
- Brookshire ENJ, Hedin LO, Newbold JD, Sigman DM, Jackson JK. 2012 Sustained losses of bioavailable nitrogen from montane tropical forests. *Nat. Geosci.* **5**, 123–126. (doi:10.1038/ngeo1372)
- Rascher KG, Hellmann C, Máguas C, Werner C. 2012 Community scale ¹⁵N isoscapes: tracing the spatial impact of an exotic N₂-fixing invader. *Ecol. Lett.* **15**, 484–491. (doi:10.1111/j.1461-0248.2012.01761.x)

37. Fyllas NM *et al.* 2009 Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate. *Biogeosci. Discuss.* **6**, 3707–3769. (doi:10.5194/bg-d-6-3707-2009)
38. Batterman SA, Hedin LO, van Breugel M, Ransijn J, Craven DJ, Hall JS. 2013 Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* **502**, 224–227. (doi:10.1038/nature12525)
39. Shebitz DJ, Eaton W. 2013 Growth after deforestation of Costa Rican lowland forests. *Int. Sch. Res. Not.* **2013**, 27–31.
40. Milcu A, Partsch S, Scherber C, Weisser WW, Scheu S. 2008 Earthworms and legumes control litter decomposition in a plant diversity gradient. *Ecology* **89**, 1872–1882. (doi:10.1890/07-1377.1)
41. Schwendener CM, Lehmann J, Rondon M, Wandelli E, Fernandes E. 2007 Soil mineral N dynamics beneath mixtures of leaves from legume and fruit trees in central Amazonian multi-strata agroforests. *Acta Amazonica* **37**, 313–320. (doi:10.1590/S0044-59672007000300001)
42. Jones D, Dennis P, Owen A, van Hees P. 2003 Organic acid behavior in soils—misconceptions and knowledge gaps. *Plant Soil* **248**, 31–41. (doi:10.1023/A)
43. Hu S, Chapin FS, Firestone MK, Field CB, Chiariello NR. 2001 Nitrogen limitation of microbial decomposition in a grassland under elevated CO₂. *Nature* **409**, 188–191. (doi:10.1038/35051576)
44. Ilstedt U, Singh S. 2005 Nitrogen and phosphorus limitations of microbial respiration in a tropical phosphorus-fixing Acrisol (ultisol) compared with organic compost. *Soil Biol. Biochem.* **37**, 1407–1410. (doi:10.1016/j.soilbio.2005.01.002)
45. Taylor LL, Banwart SA, Valdes PJ, Leake JR, Beerling DJ. 2012 Evaluating the effects of terrestrial ecosystems, climate and carbon dioxide on weathering over geological time: a global-scale process-based approach. *Phil. Trans. R. Soc. B* **367**, 565–582. (doi:10.1098/rstb.2011.0251)
46. Velizarov S, Beschkov V. 1998 Biotransformation of glucose to free gluconic acid by *Gluconobacter oxydans*: substrate and product inhibition situations. *Process Biochem.* **33**, 527–534. (doi:10.1016/S0032-9592(98)00000-4)
47. Wheeler EA, Baas P. 1991 A survey of the fossil record for dictyodermous wood and its significance for evolutionary and ecological wood anatomy. *Iawa Bull.* **12**, 275–332. (doi:10.1163/22941932-90001256)
48. Birch HF. 1960 Nitrification in soils after different periods of dryness. *Plant Soil* **7**, 81–96. (doi:10.1007/BF01377763)
49. Van Miegroet H, Cole DW. 1984 The impact of nitrification on soil acidification and cation leaching in a red alder ecosystem. *J. Environ. Qual.* **13**, 586. (doi:10.2134/jeq1984.00472425001300040015x)
50. Raven JA, Franco AA, De Jesus EL, Jacob Neto J. 1990 Proton extrusion and organic acid synthesis in nitrogen fixing symbioses involving vascular plants. *New Phytol.* **114**, 369–390. (doi:10.1111/j.1469-8137.1990.tb00405.x)
51. Bolan NS, Hedley MJ, White RE. 1991 Processes of soil acidification during nitrogen cycling with emphasis on legume based pastures. *Plant Soil* **134**, 53–63. (doi:10.1007/BF00010717)
52. Leary JK, Hue N V., Singleton PW, Borthakur D. 2006 The major features of an infestation by the invasive weed legume gorse (*Ulex europaeus*) on volcanic soils in Hawaii. *Biol. Fertil. Soils* **42**, 215–223. (doi:10.1007/s00374-005-0018-9)
53. Homann PS, van Miegroet H, Cole DW, Wolfe G V. 1992 Cation distribution, cycling, and removal from mineral soil in Douglas-fir and red alder forests. *Biogeochemistry* **16**, 121–150. (doi:10.1007/BF0002828)
54. Powers JS, Treseder KK, Lerdau MT. 2005 Fine roots, arbuscular mycorrhizal hyphae and soil nutrients in four neotropical rain forests: patterns across large geographic distances. *New Phytol.* **165**, 913–921. (doi:10.1111/j.1469-8137.2004.01279.x)
55. Laclau JP *et al.* 2008 Mixed-species plantations of *Acacia mangium* and *Eucalyptus grandis* in Brazil. 1. Growth dynamics and aboveground net primary production. *For. Ecol. Manage.* **255**, 3905–3917. (doi:10.1016/j.foreco.2007.10.049)
56. Bini D, Figueiredo AF, da Silva MCP, Vasconcelos RLDF, Cardoso EJB. 2013 Microbial biomass and activity in litter during the initial development of pure and mixed plantations of *Eucalyptus grandis* and *Acacia mangium*. *Rev. Bras. Ciênc. Solo* **37**, 76–85. (doi:10.1590/S0100-06832013000100008)
57. Nichols JD, Carpenter FL. 2006 Interplanting *Inga edulis* yields nitrogen benefits to *Terminalia amazonia*. *For. Ecol. Manage.* **233**, 344–351. (doi:10.1016/j.foreco.2006.05.031)
58. Evans JR. 1989 Photosynthesis and nitrogen relationship in leaves of C3 plants. *Oecologia* **78**, 9–19. (doi:10.1007/BF00377192)
59. Tuohy JM, Prior JAB, Stewart GR. 1991 Photosynthesis in relation to leaf nitrogen and phosphorus content in Zimbabwean trees. *Oecologia* **88**, 378–382. (doi:10.1007/BF00317582)
60. Parrotta JA. 1999 Productivity, nutrient cycling, and succession in single- and mixed-species plantations of *Casuarina equisetifolia*, *Eucalyptus robusta*, and *Leucaena leucocephala* in Puerto Rico. *For. Ecol. Manage.* **124**, 45–77. (doi:10.1016/S0378-1127(99)00049-3)
61. Santos FM, Balieiro FDC, Ataíde DHDS, Diniz AR, Chaer GM. 2016 Dynamics of aboveground biomass accumulation in monospecific and mixed-species plantations of *Eucalyptus* and *Acacia* on a Brazilian sandy soil. *For. Ecol. Manage.* **363**, 86–97. (doi:10.1016/j.foreco.2015.12.028)
62. Kraft C, Jenett-Siems K, Köhler I, Siems K, Abbwi D, Bienziele U, Eich E. 2002 Andriol A and B, two unique 6-hydroxymethylpterocarpenes from *Andira inermis*. *Z. Naturforsch C* **57**, 785–790. (doi:10.1515/znc-2002-9-1005)
63. Leuner O, Havlik J, Hummelova J, Prokudina E, Novy P, Kokoska L. 2013 Distribution of isoflavones and coumestrol in neglected tropical and subtropical legumes. *J. Sci. Food Agric.* **93**, 575–579. (doi:10.1002/jsfa.5835)
64. Yu O, Jung W, Shi J, Croes RA, Fader GM, McGonigle B, Odell JT. 2000 Production of the isoflavones genistein and daidzein in non-legume dicot and monocot tissues. *Plant Physiol.* **124**, 781–794. (doi:10.1104/pp.124.2.781)
65. Tomasi N *et al.* 2008 Flavonoids of white lupin roots participate in phosphorus mobilization from soil. *Soil Biol. Biochem.* **40**, 1971–1974. (doi:10.1016/j.soilbio.2008.02.017)
66. Aoki M, Fujii K, Kitayama K. 2012 Environmental control of root exudation of low-molecular weight organic acids in tropical rainforests. *Ecosystems* **15**, 1194–1203. (doi:10.1007/s10021-012-9575-6)
67. Weisskopf L, Tomasi N, Santelia D, Martinoia E, Langdale N, Tabacchi R, Abou-Mansour E. 2006 Isoflavonoid exudation from white lupin roots is influenced by phosphate supply, root type and cluster-root stage. *New Phytol.* **171**, 657–668. (doi:10.1111/j.1469-8137.2006.01776.x)
68. Oldroyd GED. 2013 Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nat. Rev. Microbiol.* **11**, 252–263. (doi:10.1038/nrmicro2990)
69. Odee DW, Sutherland JM, Kimiti JM, Sprent JI. 1995 Natural rhizobial populations and nodulation status of woody legumes growing in diverse Kenyan conditions. *Plant Soil* **173**, 211–224. (doi:10.1007/BF00011458)
70. Uroz S, Kelly LC, Turpault M-P, Lepleux C, Frey-Klett P. 2015 The mineralosphere concept: mineralogical control of the distribution and function of mineral-associated bacterial communities. *Trends Microbiol.* **23**, 751–762. (doi:10.1016/j.tim.2015.10.004)
71. Lauber CL, Hamady M, Knight R, Fierer N. 2009 Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* **75**, 5111–5120. (doi:10.1128/AEM.00335-09)
72. Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE. 2010 CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proc. Natl Acad. Sci. USA* **107**, 19 368–19 373. (doi:10.1073/pnas.1006463107)
73. LeBauer DS, LeBauer DS, Treseder KK, Treseder KK. 2008 Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**, 371–379. (doi:10.1890/06-2057.1)
74. Vitousek PM *et al.* 2002 Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* **57/58**, 1–45. (doi:10.1023/A:1015798428743)
75. van der Heijden MG, De Bruin S, Luckerhoff L, van Logtestijn RS, Schlaeppi K. 2015 A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME J.* **10**, 389–399. (doi:10.1038/ismej.2015.120)
76. Penman DE. 2016 Silicate weathering and North Atlantic silica burial during the Paleocene-Eocene thermal maximum. *Geology* **44**, 731–734. (doi:10.1130/G37704.1)

77. Polley HW, Johnson HB, Mayeux HS. 1997 Leaf physiology, production, water use, and nitrogen dynamics of the grassland invader *Acacia smallii* at elevated CO₂ concentrations. *Tree Physiol.* **17**, 89–96. (doi:10.1093/treephys/17.2.89)
78. Arnone III JA, Gordon JC. 1990 Effect of nodulation, nitrogen fixation and CO₂ enrichment on the physiology, growth and dry mass allocation of seedlings of *Alnus rubra* Bong. *New Phytol.* **116**, 55–66. (doi:10.1111/j.1469-8137.1990.tb00510.x)
79. Thomas RB, Bashkin MA, Richter DD. 2000 Nitrogen inhibition of nodulation and N₂ fixation of a tropical N₂-fixing tree (*Gliricidia sepium*) grown in elevated atmospheric CO₂. *New Phytol.* **145**, 233–243. (doi:10.1046/j.1469-8137.2000.00577.x)
80. Quirk J, Andrews MY, Leake JR, Banwart SA, Beerling DJ. 2014 Ectomycorrhizal fungi and past high CO₂ atmospheres enhance mineral weathering through increased below-ground carbon-energy fluxes. *Biol. Lett.* **10**, 20140375. (doi:10.1098/rsbl.2014.0375)
81. Irigoyen JJ, Goicoechea N, Antolín MC, Pascual I, Sánchez-Díaz M, Aguirreolea J, Morales F. 2014 Growth, photosynthetic acclimation and yield quality in legumes under climate change simulations: an updated survey. *Plant Sci.* **226**, 22–29. (doi:10.1016/j.plantsci.2014.05.008)
82. Rogers A, Ainsworth EA, Leakey ADB. 2009 Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiol.* **151**, 1009–1016. (doi:10.1104/pp.109.144113)
83. Mohan JE, Clark JS, Schlesinger WH. 2007 Enrichment of a forest ecosystem: implications for forest regeneration and succession. *Ecol. Appl.* **17**, 1198–1212. (doi:10.1890/05-1690)
84. Dobritsa AA, Winter K, Martínez C, Correa E, Aranda J, Garcia M, Jaramillo C, Turner BL. 2015 Responses of legume versus nonlegume tropical tree seedlings to elevated CO₂ concentration. *Plant Physiol.* **157**, 372–385. (doi:10.1104/pp.111.1)
85. Brantley SL *et al.* 2011 Twelve testable hypotheses on the geobiology of weathering. *Geobiology* **9**, 140–165. (doi:10.1111/j.1472-4669.2010.00264.x)
86. Vitousek PM, Howarth RW. 1991 Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* **13**, 87–115. (doi:10.1007/BF00002772)
87. Barron AR. 2007 Patterns and controls of nitrogen fixation in a lowland tropical forest, Panama. PhD thesis, Department of Ecology and Evolution, Princeton University, Princeton, NJ, USA.
88. Wurzbarger N, Bellenger JP, Kraepiel AML, Hedin LO. 2012 Molybdenum and phosphorus interact to constrain symbiotic nitrogen fixation in tropical forests. *PLoS ONE* **7**, e33710. (doi:10.1371/journal.pone.0033710)
89. López-Lara IM, Sohlenkamp C, Geiger O. 2003 Membrane lipids in plant-associated bacteria: their biosyntheses and possible functions. *Mol. Plant Microbe Interact.* **16**, 567–579. (doi:10.1094/MPMI.2003.16.7.567)
90. O'Hara GW, Dilworth MJ, Boonkerd N, Parkian P. 1988 Iron deficiency specifically limits nodule development in peanut inoculated with *Bradyrhizobium* sp. *New Phytol.* **108**, 51–57. (doi:10.1111/j.1469-8137.1988.tb00203.x)
91. Gardner WK, Parbery DG, Barber DA. 1983 The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant Soil* **70**, 107–124. (doi:10.1007/BF02374724)
92. Roncal-Herrero T, Oelkers EH. 2011 Does variscite control phosphate availability in acidic natural waters? An experimental study of variscite dissolution rates. *Geochim. Cosmochim. Acta* **75**, 416–426. (doi:10.1016/j.gca.2010.10.012)
93. Wu L, Huh Y, Qin J, Du G, van Der Lee S. 2005 Chemical weathering in the Upper Huang He (Yellow River) draining the eastern Qinghai-Tibet Plateau. *Geochim. Cosmochim. Acta* **69**, 5279–5294. (doi:10.1016/j.gca.2005.07.001)