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**Climate constraint on nitrogen cycle determines evolution
of plant N₂ fixation strategies across biomes**

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Type of article: Letter

20 **Atmospheric dinitrogen (N₂) fixation by plants (in symbiosis with root bacteria) is a major source of**
21 **new nitrogen to land ecosystems¹. A long-standing puzzle² is that trees capable of N₂ fixation are**
22 **abundant in many tropical forests, but absent or restricted to early succession in extra-tropical**
23 **forests. This biome-scale pattern presents a paradox³ given the physiological growth penalty⁴**
24 **incurred by N₂ fixation: Why are fixers so successful in nitrogen-rich tropical soils, but out-competed**
25 **by non-fixers in nitrogen-poor temperate and Boreal soils? Here we present a theoretical analysis of**
26 **the emergence of N₂ fixation as an evolutionary stable strategy across climate- and biome-specific**
27 **differences in the nitrogen cycle. We demonstrate that a transient nitrogen deficit and rapid tree**
28 **growth in tropical forests favors a facultative fixation strategy (*i.e.*, the ability to down-regulate**
29 **fixation⁵⁻⁶ by sanctioning N₂-fixing bacteria⁷) and results in coexistence of fixers and non-fixers. In**
30 **contrast, a sustained nitrogen deficit in extra-tropical forests favors an obligate fixation strategy (*i.e.*,**
31 **little ability to down-regulate) and causes fixers to be excluded in late succession. We conclude that**
32 **biome-scale differences in N₂ fixation can be explained by the interaction between individual plant**
33 **strategies and climatic constraints on the nitrogen cycle over evolutionary time.**

34 Biological N₂ fixation is manifested in vastly different patterns across the biosphere⁸. In land
35 ecosystems, Hans Jenny first noted (ref. 2) that symbiotic N₂ fixers are common in tropical forests,
36 where soils are nitrogen-rich, but rare (and restricted to early-succession⁹⁻¹⁰) in extra-tropical forests,
37 where soils are nitrogen-limited. This biome-scale pattern offers an ecological and evolutionary
38 paradox³: Why is the energetically costly⁴ strategy of N₂ fixation widespread if non-fixing neighbors can
39 outcompete fixers in the nitrogen-rich conditions of tropical forests? Conversely, why is N₂ fixation not
40 widespread in extra-tropical forests, if fixers are competitively advantaged over non-fixers in nitrogen-
41 poor soils?

42 Jenny and others that followed him proposed that this biome-scale fixation pattern might be
43 due to differences in the physiological cost of fixation imposed by climate (*i.e.*, high energetic cost may

44 restrict fixation to warm climates^{8-9,11-12}), soil nutrients (fixation may be limited by phosphorus or
45 molybdenum^{8,12-15}), or the evolutionary history of fixers⁹ (diversification may be constrained in colder
46 regions). The first two explanations do not appear to resolve the paradox, however, as we now know
47 that: (i) fixation can be very high in early-successional forests in cold biomes¹³; and (ii) constraints on
48 fixation by phosphorus or molybdenum are not necessarily stronger in extra-tropical than tropical soils¹⁴⁻
49 ¹⁵. The third explanation remains unresolved, as recent findings^{16,17} show that potential N₂ fixers
50 (Fabaceae) radiated rapidly as modern-day forests emerged in the Paleocene, but it is unclear why fixers
51 have remained rare across vast areas of late-successional and nitrogen-poor extra-tropical forests.

52 A recent resource-optimization analysis¹² showed that the biome differences in fixation can be
53 generated by two combined mechanisms: (i) a proposed temperature constraint (discussed above) that
54 disproportionately penalizes fixation over other growth-related processes in extra-tropical forests, and
55 (ii) a proposed ability of fixers to use fixed nitrogen to compete for soil phosphorus (by exuding
56 phosphatase enzymes) in nitrogen-rich tropical forests. Recent findings suggest a different pattern,
57 however: fixers appear to rarely fix N₂ in tropical forests^{5,18-19}, but can transiently up-regulate fixation
58 when plant nitrogen demand is high following disturbance¹⁸.

59 More fundamentally, the evolutionary stability of N₂ fixation within biomes remains unresolved,
60 including why fixers have not radiated into late-successional extra-tropical forests when they are so
61 common across all stages of tropical succession? Any such analysis must account for evolved
62 differences in plant fixation strategies: Tropical fixers (primarily Fabaceae with rhizobia symbionts)
63 appear to have the facultative^{5,18} ability to down-regulate N₂ fixation in nitrogen-rich soils (thus avoiding
64 the energetic fixation penalty), while extra-tropical fixers (*e.g.*, *Alnus* or *Coriaria* with *Frankia* symbionts)
65 are less able to down-regulate fixation as soil nitrogen accumulates²⁰⁻²¹ (hereafter “obligate” strategy,
66 *sensu* refs. 3,6). A recent analysis²² predicted that facultative fixers should exclude obligate fixers over

67 ecological time when nitrogen is limiting, but did not resolve the corollary question: why have
68 facultative fixers diversified but remained limited to the tropical biome over evolutionary time?

69 We here use a game-theoretic approach²³ to evaluate the ecological and evolutionary conditions
70 under which individual fixation strategies emerge, and to resolve whether the evolutionary stable
71 strategy (ESS) depends on climatic constraints on the nitrogen cycle. Our analysis does not invoke any
72 mechanism (*e.g.*, temperature, phosphorus, or molybdenum effects on fixers) other than the costs and
73 tradeoffs of fixation strategies, and is based on climate- and biome-scale differences in the nitrogen
74 cycle.

75 Climate is a major determinant of soil carbon and nitrogen distributions across biomes.
76 Perennially warm and moist conditions in tropical forests promote decomposition (which progressively
77 removes carbon but recycles nitrogen) and cause soil C:N ratios near the threshold²⁴ at which nitrogen is
78 released to plants rather than immobilized (Fig. 1a). In contrast, seasonally cold forests accumulate
79 large quantities of un-decomposed carbon with C:N ratios that favor immobilization; in addition, cold
80 region coniferous forests produce high C:N ratio litter²⁵. The time it takes for nitrogen to be released as
81 litter decomposes is 1.6-16 times longer in deciduous, coniferous, and boreal forests compared to their
82 tropical counterparts²⁶. As a result, extra-tropical forests possess large soil nitrogen deficits, while
83 tropical forests are close to nitrogen sufficiency (Fig. 1a; *S11*).

84 To capture succession, our model (*Methods*) considers a stage-structured community in which
85 trees regenerate and spread in the landscape through seed, seedling, and sapling stages, before
86 maturing as canopy trees. Tree growth depends on nitrogen acquisition, mortality and density-
87 dependent competition for the globally constraining resource of access to the mature forest canopy
88 (*i.e.*, light). We can therefore examine different fixation strategies (no, obligate, facultative) in
89 landscapes that differ in disturbance regimes.

90 The relative biomass growth rate r of tree species j depends on nitrogen as:

$$91 \quad r_j = \omega(F_j) \left(\left[\frac{\nu(F_j)N}{c + N} \right] + F_j \right) - \theta F_j - \Psi_j F_j - \mu \quad (1)$$

92 where nitrogen enters from plant-available soil nitrogen (N ; c is the half-saturation constant) and/or N_2
93 fixation, with per unit biomass rates $\nu(F)$ and F respectively (F is constant for obligate but decreases
94 with N for facultative fixers; *S12*). Assimilated nitrogen is used to build plant biomass with a nitrogen-
95 use efficiency (NUE) $\omega(F)$. Both $\nu(F)$ and $\omega(F)$ decrease as F increases, representing tradeoffs
96 considered essential for the evolution of fixation²⁷: (i) between fixation and plant NUE ($\omega(F)$; N_2 -fixers
97 have lower C:N and thus lower NUE¹¹), and (ii) between fixation and soil nitrogen uptake ($\nu(F)$; N_2 -
98 fixers allocate carbon to N_2 fixation in nodules instead of fine roots for soil nitrogen uptake²⁸). We also
99 consider biomass mortality μ , the metabolic cost (θ) of fixation, and the opportunistic cost (Ψ) of
100 maintaining a root structure capable of both fixation and competition for soil nitrogen (*S13*).

101 Our ESS analysis shows that different fixation strategies emerge depending on soil nitrogen
102 conditions. We first analyzed the ESS in a system without the plant-soil nitrogen feedback, to evaluate
103 the ability of fixers to compete at any given stage of succession. (Inclusion of this feedback yields the
104 trivial result that there exists no non-zero ESS, since, at equilibrium, fixation always is excluded as soil
105 nitrogen accumulates over succession). A pairwise invasibility analysis shows (Figs. 1b,c) that there
106 exists a single fixation rate F^* that is continuously stable²⁹ (*i.e.*, cannot be invaded by any mutant F ; *S14*
107 & *S15*) and that sequential mutations with either higher or lower F converge to the F^* -ESS (arrows in
108 Figs. 1b,c). When evaluated across a continuum of soil nitrogen (Fig. 1d,e), F^* decreased with
109 increasing nitrogen as less fixation was needed to overcome nitrogen-limitation. We conclude that
110 there exists a non-zero fixation ESS whenever soil nitrogen limits fixer growth.

111 The F^* -ESS was sensitive to the magnitude of tradeoffs between fixation and other plant traits:
112 (i) F^* increased with NUE (parallel lines in Fig. 1d), and (ii) F^* decreased with increased soil nitrogen
113 uptake (Fig. 1e). The fixation vs. soil uptake tradeoff was non-linear, with F^* increasingly influenced by
114 the tradeoff as soil nitrogen increased. The biomass- and area- specific ESS fixation rates (0-0.06 gN gC⁻¹
115 yr⁻¹ and 0-240 kgN ha⁻¹ yr⁻¹) that emerged from this analysis were within the range observed in
116 nature^{5,11,17-18}. We conclude that fixers can be out-competed by non-fixers (*i.e.*, $F^*=0$) at progressively
117 lower soil nitrogen, either as fixer NUE decreases or fixer soil nitrogen uptake increases.

118 We next evaluated how the F^* -ESS influences the relative success of fixers vs. non-fixers across
119 a soil nitrogen gradient. For the obligate strategy, fixers dominated at low soil nitrogen but were
120 replaced by non-fixers at high soil nitrogen (Fig. 2a,b). The transition from fixers (gray/blue lines) to
121 non-fixers (red) occurred: (i) at intermediate fixer NUE (low or high NUE caused sole dominance of non-
122 fixers or fixers, respectively; Fig. 2a), and (ii) at lower soil nitrogen levels when fixer soil uptake increased
123 (Fig. 2b). For the facultative strategy, fixers dominated at low soil nitrogen, but, by down-regulating
124 fixation, they could coexist with non-fixers even at high soil nitrogen (Fig. 2c, *Extended Data Fig. 1*).

125 When we allowed obligate and facultative fixers to compete (Fig. 2d), obligate fixers dominated
126 at low soil nitrogen because their fixation rate was at or near F^* -ESS and they did not incur the
127 opportunistic cost of facultative fixation. At high soil nitrogen, however, obligate fixers could not adjust
128 fixation to match F^* -ESS whereas facultative fixers could. Obligate fixers were therefore replaced once
129 soil nitrogen was abundant enough not to penalize facultative fixers. The transition from obligate to
130 facultative fixation (intersection of solid vs. dashed lines, Fig. 2d) identifies the upper limit of the
131 obligate fixer niche. We conclude that the obligate fixer ESS can persist across a range of low soil
132 nitrogen conditions, but that the facultative strategy can be maintained independent of soil nitrogen
133 (*S16*).

134 To study successional dynamics, we allowed fixers (obligate or facultative) to compete with non-
135 fixers under changing soil nitrogen in the climate and biome conditions of Fig. 1a. We assumed rapid
136 (<20 yrs) correction of the soil nitrogen deficit in tropical soils^{18,30} and slow (>50 yrs) correction in extra-
137 tropical soils²⁰ (Fig. 1a, *Methods*). This is equivalent to viewing a landscape as consisting of forest
138 patches of different successional ages, and N₂-fixers as persisting in meta-populations across these
139 patches (videos in *S17*).

140 The success of obligate vs. facultative fixers depended on the timescale of two interacting
141 processes following disturbance: soil nitrogen recovery vs. closure of the mature forest canopy. In
142 tropical forests, soil nitrogen recovers rapidly and non-fixing trees out-compete obligate fixers before
143 forest biomass saturates (Fig. 3a). The facultative strategy, however, allows fixers to down-regulate
144 fixation to match the decline in F^* -ESS that occurs as soil nitrogen increases (Fig. 3b). Facultative fixers
145 thus successfully compete for canopy access and co-exist with non-fixers across all successional stages of
146 tropical forests (*S16, S17*).

147 In contrast, slow soil nitrogen recovery in extra-tropical forests allows obligate fixers to suppress
148 non-fixers as the canopy closes (*i.e.*, $F_{obligate} \approx F^*$)(Fig. 3c). We infer that the prolonged period of low
149 nitrogen and high sunlight gives obligate fixers enough time to complete their life cycle (~30-50 years for
150 *Alnus*¹⁰ and *Coriaria*²⁰) before becoming replaced by non-fixers (*i.e.*, $F_{obligate} \gg F^*$; *S16*). Facultative fixers
151 are less successful than obligate fixers, however, as they are penalized by the opportunistic cost of
152 down-regulating fixation (Fig. 3d, *S13*). This successional analysis supports our pairwise competition
153 results (Fig. 2d): obligate fixation is selected when soil nitrogen remains low, and facultative fixation
154 when soil nitrogen recovers rapidly.

155 Finally, we evaluated a landscape with 500 patches subject to stochastic disturbance and
156 succession (Fig. 3e; *S17*). Our results re-created the biome-scale pattern of disturbance, succession and

157 fixation observed in field studies: obligate fixers persisted alone in early successional stages of extra-
158 tropical landscapes, while facultative fixers co-existed with non-fixers across all stages of tropical
159 landscapes. We infer that facultative fixation has evolved as a solution for conditions where soil
160 nitrogen recovers faster than canopy closure, and obligate fixation where soil nitrogen is unlikely to
161 change appreciably over the life-span of the individual plant.

162 Our proposed explanation resolves two fundamental observations across biomes: (i) that
163 facultative fixers occur widely across successional age in tropical forests, while obligate fixers are
164 favored but limited to early succession in extra-tropical biomes, and (ii) that fixers and non-fixers co-
165 exist in tropical forests regardless of successional stage, while transient stands of obligate fixers occur in
166 early-succession in extra-tropical biomes but are replaced by non-fixers later in succession. Most
167 importantly, our analysis indicates that these patterns are evolutionarily stable to any mutant strategy
168 across a broad range of physiological tradeoffs.

169 Our findings identify a link between the evolution of nitrogen fixation, the evolutionary stability
170 of fixation strategies at the biome scale, and climate differences in nitrogen cycling across biomes. The
171 maintenance of N₂ fixers can be explained by interactions between individual fixation strategies, the
172 indirect influence of climate on the nitrogen cycle, and competition between fixers and non-fixers. Over
173 evolutionary time, these interactions appear to have led to biome-scale differences in plant fixation
174 strategies, and, in turn, the emergence of broad differences in the global nitrogen cycle.

175

176 **METHODS**

177 **Model description**

178 Our model evaluates the adaptive dynamics of a forest tree community in the context of species
179 with different nitrogen acquisition strategies (motivated by work by Menge et al.^{6,27}). Our ESS analysis
180 differs from optimization models by allowing incremental mutations in plant strategies/traits, and by

181 evaluating whether mutants can competitively exclude resident plants across strategy- and trait-space.
 182 The resulting ESS may or may not coincide with the results from an optimization analysis.

183 We examine the dynamics of four life-stages of a tree: biomass [kg Carbon ha⁻¹] of mature (seed
 184 producing) trees \mathbf{B} , and numbers [ha⁻¹] of seeds \mathbf{S} , “dormant” shaded seedlings in the understory \mathbf{D} , and
 185 juvenile saplings \mathbf{Y} (Extended Data Fig. 2); in a meta-community of L tree species j ($j = 1 \dots L$) distributed
 186 in M spatially implicit sites i ($i = 1 \dots M$), with equal distances among all sites. The dynamics of the forest
 187 community are modeled as:

$$188 \quad \frac{dB_j^i}{dt} = B_j^i r_j + \gamma Y_j^i \cdot z - B_j^i \cdot \frac{m_j}{K^i} \sum_{j=1}^L B_j^i \quad (2)$$

$$189 \quad \frac{dS_j^i}{dt} = \frac{1}{M} \left(\alpha \cdot \sum_{\Omega} B_j \right) - g \cdot S_j^i - d \cdot S_j^i - h \cdot S_j^i \quad (3)$$

$$190 \quad \frac{dD_j^i}{dt} = d \cdot S_j^i - \beta \cdot D_j^i - q \cdot D_j^i \quad (4)$$

$$191 \quad \frac{dY_j^i}{dt} = g \cdot S_j^i + \beta \cdot D_j^i - \gamma \cdot Y_j^i - \delta \cdot Y_j^i - Y_j^i \cdot \frac{p}{K^i} \cdot \sum_{j=1}^L B_j^i \quad (5)$$

192 Equation (2) describes the dynamics of the biomass of mature trees \mathbf{B} as a function of the growth
 193 rate r (see equation 1 in the main text), recruitment of saplings \mathbf{Y} with a biomass z that mature at a rate
 194 γ , and community-level density-dependent mortality at a rate m that depends on total forest biomass
 195 (of all species j) at the site and the per site i carrying capacity K^i . The rate of density-dependent
 196 mortality m_j may vary among different species, representing different sensitivities to competition for a
 197 global resource (*e.g.*, shade tolerance/intolerance).

198 Equation (3) describes per-species seed production as a proportion α [kg Carbon⁻¹ yr⁻¹] of adult
 199 biomass; and the successive germination of seeds into growing saplings (\mathbf{Y}) at a rate g and into

200 understory seedlings (D) at a rate d . The remaining seeds loose variability at a rate h adjusted at each
201 time step so no seed bank is formed.

202 Equations (4) and (5) describe the dynamics of the understory seedlings and saplings, respectively,
203 governed by germination, and mortality at rates q and δ , respectively. Seedlings develop into saplings at
204 a rate β , which represents the proportion of seedlings that are released from the seedling-bank after a
205 disturbance event. Sapling competition with adult trees for global resources (*e.g.*, light) is modeled with
206 a community level density-dependent mortality at a rate p that determines the sapling sensitivity to
207 competition with mature tree biomass.

208 To capture different strategies of N_2 fixation we used an exponential function:

$$209 \quad F_j = \bar{F}_j \cdot e^{-f_j \cdot N(t-T)} \quad (6)$$

210 For an obligate fixer $f_j = 0$ and the fixation rate F remains constant at \bar{F}_j , independent of soil N .

211 For a facultative fixer $f_j > 0$ which means that the rate of fixation f_j decreases exponentially from the initial
212 value \bar{F}_j at soil $N \approx 0$ (at time $t-T$) to approximate zero at high soil nitrogen (*S12*). T allows for a time-lag
213 between change in soil nitrogen and plant physiological response⁶, but here we use a default of $T=0$.

214 **Analysis of the evolutionary stable strategy**

215 We performed numerical analyses to examine the outcome of competition between all possible
216 combinations of a resident with N_2 fixation rate F_r and a mutant F_m under constant soil nitrogen
217 conditions over time (and all other parameters kept equal for all species; see Extended Data Table 1 for
218 the list of parameter values). The ESS rate of fixation F^* for each level of soil nitrogen availability is the
219 F_r that could not be invaded by any mutant type (Fig. 1b,c; see *S14* for sensitivity analysis and *S15* for ESS
220 analysis of a simple model). We searched for the ESS rate of fixation for different levels of tradeoffs to
221 fixation, modeled as negative linear functions $\nu(F)$ and $\omega(F)$ constrained between a minimum value

222 (v_{\min}) for species with the highest physiological rate of N₂ fixation \bar{F}_j and an added value (v_{\max}) as the
223 rate of fixation decrease to zero, such as:

$$224 \quad v(F_j) = v_{\min} + v_{\max} \left(1 - \frac{F_j}{\bar{F}_j}\right) \quad (7)$$

225 **Stable solutions along a range of soil nitrogen**

226 We ran numerical simulations to create bifurcation diagrams for the stable structure of the forest
227 community (20 sites, 500 years) with constant soil nitrogen availability. Initial conditions for the two
228 competing species were set equal. We tested the outcomes of pairwise competition between three
229 combinations of species: an obligate fixer with $\bar{F}_j=0.055$ kg N kg C⁻¹ year⁻¹ (F^* for low soil nitrogen) vs. a
230 non-fixer; a facultative fixer with $\bar{F}_j=0.055$ vs. a non-fixer; and an obligate vs. a facultative fixer.

231 **Successional analysis**

232 To examine the ecological dynamics of a given strategy, we also considered that soil nitrogen
233 increased over successional time. To simplify the model for each biome we imposed a rate of soil
234 nitrogen accumulation based on observed successional dynamics, which, in turn, reflect biome-scale
235 differences in soil nitrogen availability caused primarily by climate. We used a logistic model for soil
236 $N(t)$, with different parameters that determine the shape of the function (*e.g.* gray lines in Figs. 3a,c).
237 We analyzed the outcomes of two-way competition under different successional scenarios.

238 *Disturbance*

239 We introduced disturbance to a forest that consisted of 500 patches, where stochastic disturbance can
240 occur at each time step at each patch according to the probability of disturbance. A disturbance event:
241 (1) eliminates all mature tree biomass B and all saplings Y ; (2) transforms all seedlings D to saplings Y
242 (after disturbance $\beta=1$, without disturbance $\beta=0$); and (3) reduces soil nitrogen to $N \approx 0$. We simulated
243 the two-way competition of the different successional scenarios with increasing probability of
244 disturbance (15 replications per scenario).

245 **Soil nitrogen deficit**

246 We used a global data set of soil carbon and nitrogen contents (0-100 cm depth, see *S11*) to
247 compare carbon (C) and nitrogen (N) contents in the soil and calculate the soil nitrogen deficit in Boreal
248 ($n=264$), temperate ($n=1127$) and tropical ($n=457$) forest soils. The soil nitrogen deficit was calculated
249 relative to the theoretical C:N molar ratio of $\sim 6:1$ for the decomposing bacteria and a growth efficiency
250 of 0.5 (i.e. $C/12-N$)²³.

251

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317

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323 Author contributions: ES and LH designed research, ES, SB, LH and SL conceived the theoretical work, ES
324 performed modeling work and analyzed output data, SB provided field data, ES and LH wrote the
325 manuscript, and all authors contributed to revisions.

326

327 **Fig. 1. Climate- and biome-differences in soil nitrogen deficit and evolutionary stable strategies (ESS)**

328 **of N₂ fixation.** **a**, Mean (\pm SE) carbon, nitrogen, and nitrogen deficit in soils (0-100 cm) of tropical
329 (n=457), temperate (n= 1127) and Boreal forests (n=264) (30). Numbers above the soil carbon bars
330 represent mean soil C:N ratio; numbers above the nitrogen-deficit bars represent the timescale of
331 overcoming nitrogen limitation assuming a nitrogen input of 30 kgN ha⁻¹ year⁻¹. Letters indicate
332 significant differences. **b,c**, Numerical pairwise invasibility plots show the ESS rate of N₂ fixation F^* for
333 **b**, low, and **c**, high soil nitrogen (with medium nitrogen-use efficiency and soil nitrogen-uptake). Black
334 areas indicate combinations where the mutant strategy invades the resident. The ESS is the resident
335 rate F that cannot be invaded (indicated by arrows). **d,e**: ESS- F^* as a function of soil nitrogen with
336 varying: **d**, nitrogen-use efficiency ω and **e**, soil nitrogen uptake ν . Low, medium and high indicate the

337 N₂-fixer trait is 20, 50 and 80% of the non-fixer, respectively (with medium level for the other trait).

338 Large symbols in **d,e** correspond to the results in **b,c**.

339

340 **Figure 2. Forest composition as a function of soil nitrogen.** Mature tree biomass resulting from
341 pairwise competition between: **a,b**, non-fixers (red/orange lines) vs. obligate N₂-fixers (gray lines) with
342 varying levels of **a**, nitrogen use efficiency, and **b**, soil nitrogen uptake (blue lines); **c**, non-fixers vs.
343 facultative N₂-fixers (dashed lines) with varying levels of soil nitrogen uptake; **d**, obligate (*i.e.*, constant
344 rate) vs. facultative (*i.e.*, fixation decreases with soil nitrogen) fixers with different costs Ψ of being
345 facultative. Low, medium and high indicate the N₂-fixer trait is 20, 50 and 80% of the non-fixer,
346 respectively (with medium level for the other trait).

347

348 **Figure 3. Forest succession of N₂ fixers and non-fixers.** **a,b**, Tropical forests with rapid accumulation of
349 soil nitrogen (gray dashed line in **a**), with: **a**, obligate N₂-fixation strategy (blue solid line) or **b**, facultative
350 N₂-fixation strategy (blue dashed line). **c,d**, Extra-tropical forests with slow accumulation of soil nitrogen
351 (gray dashed line in **c**), with: **c**, obligate N₂-fixation strategy (violet solid line) or **d**, facultative N₂-fixation
352 strategy (violet dashed line). **e**, Landscape-scale abundance (proportion of total mature tree biomass) of
353 N₂-fixers as a function of forest biome, fixation strategy and probability of disturbance. Parameters used
354 as in Extended Data Table 1.

355

356 **Supplementary Information:**

357 Extended Data Figures 1 and 2

358 Extended Data Table 1

359 Supplementary note 1: Soil nitrogen deficits across forest biomes

360 Supplementary note 2: Influence of functional form of down-regulation of the rate of fixation for
361 facultative fixation

362 Supplementary note 3: Cost of fixation and the costs and limitations of the facultative fixation strategy

363 Supplementary note 4: Sensitivity analysis

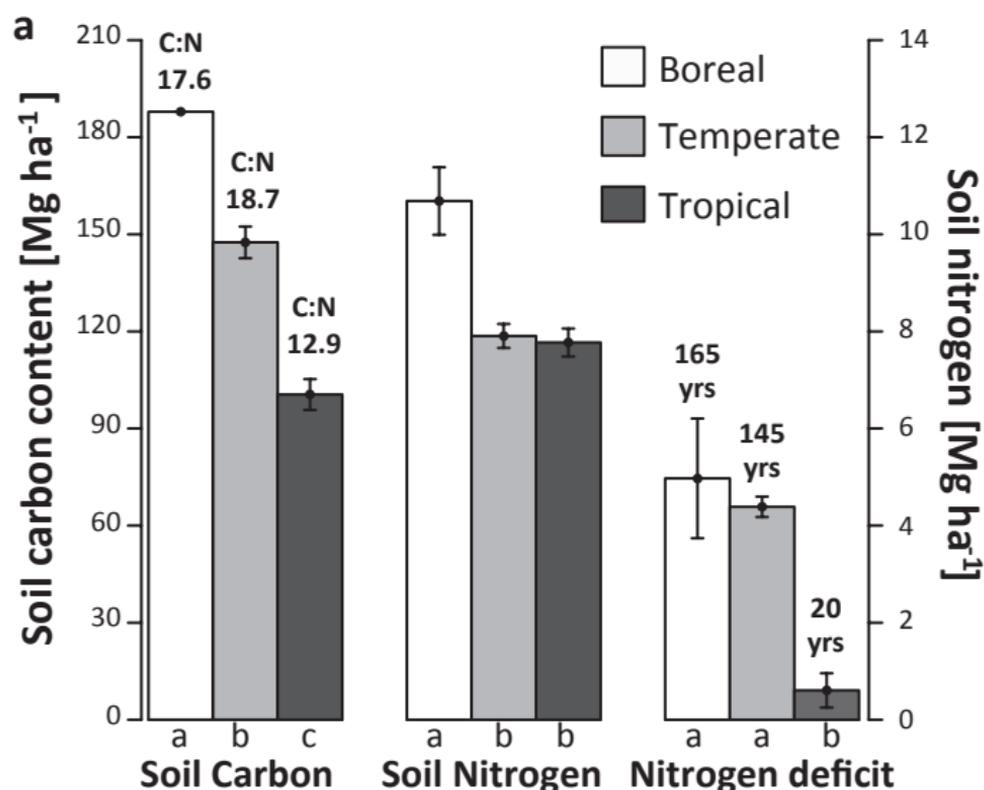
364 Supplementary note 5: ESS analysis using a simplified model

365 Supplementary note 6: Persistence of obligate vs. facultative strategies as a function of the rate of soil
366 nitrogen accumulation

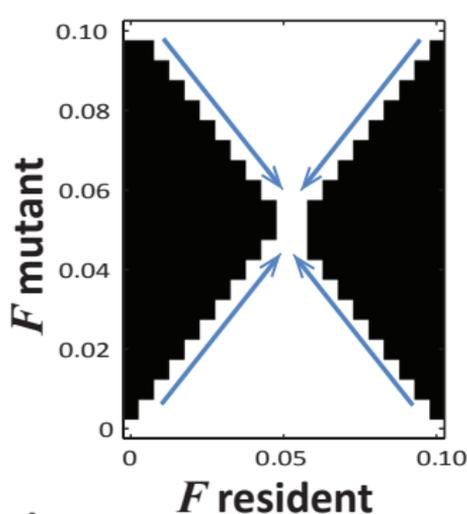
367 Supplementary note 7: Forest landscape dynamics with stochastic disturbance

368 References for supplementary information

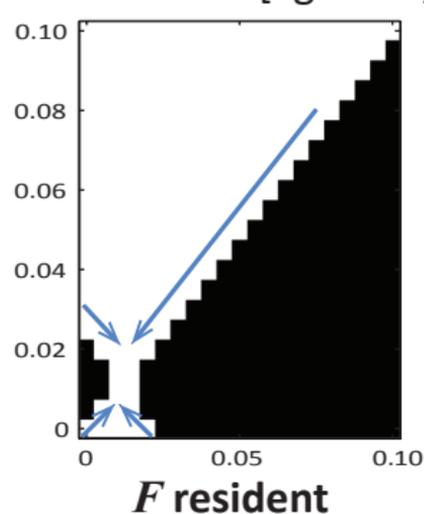
369 Movies S1-S8



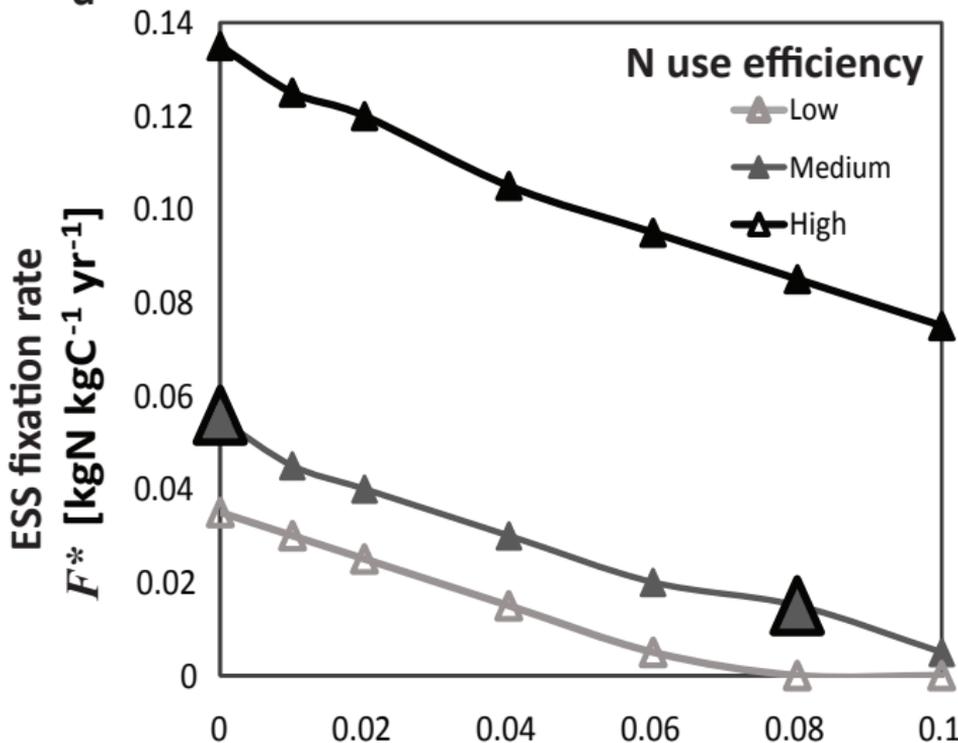
b Soil N = 0



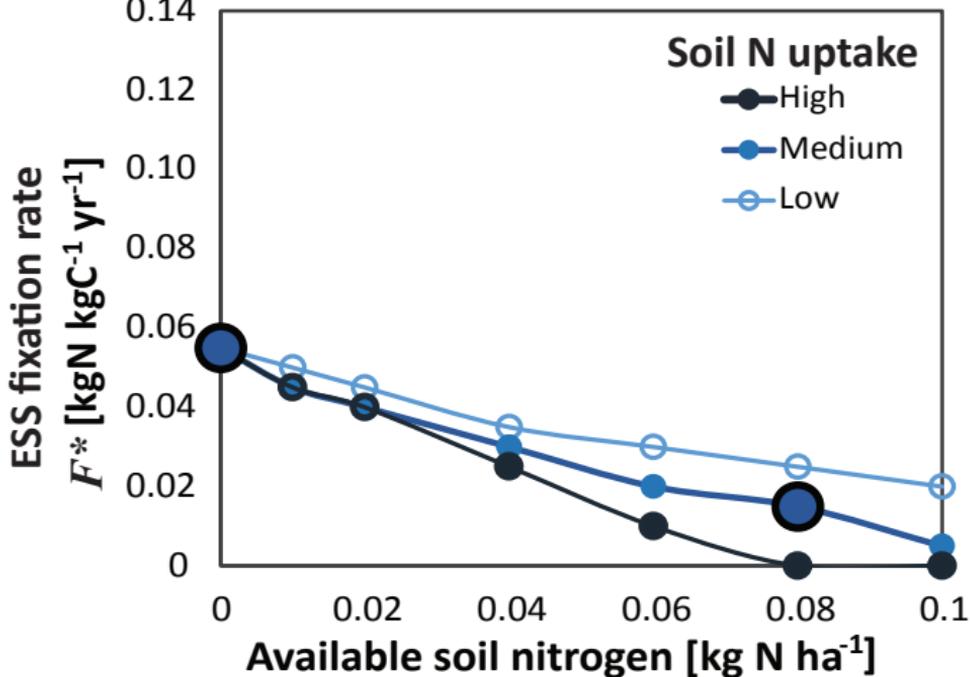
c Soil N = 0.08 [kgN ha^{-1}]

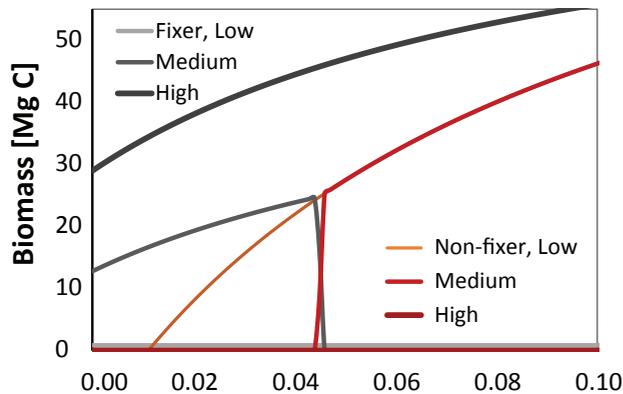
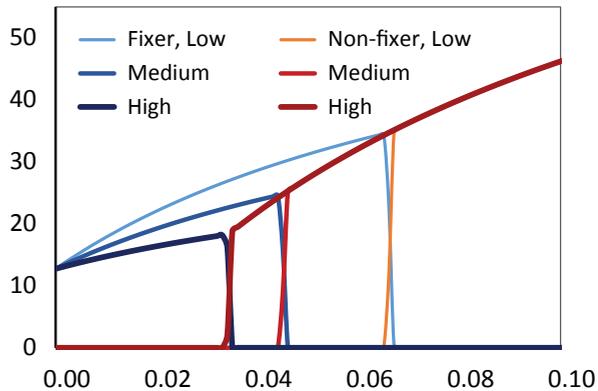
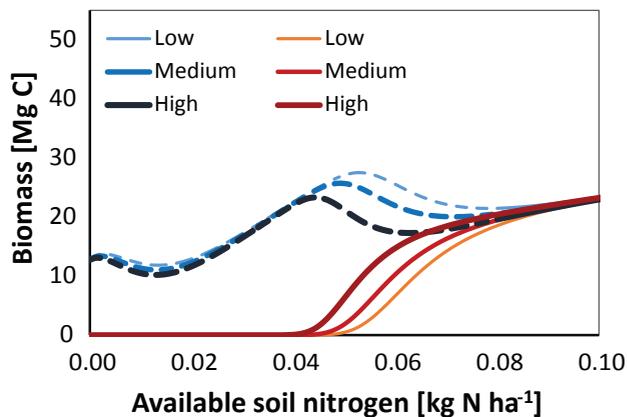
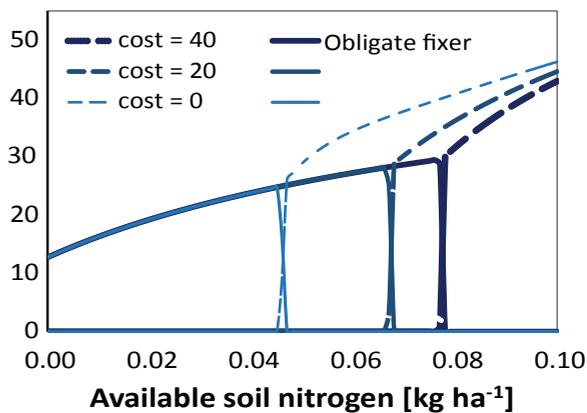


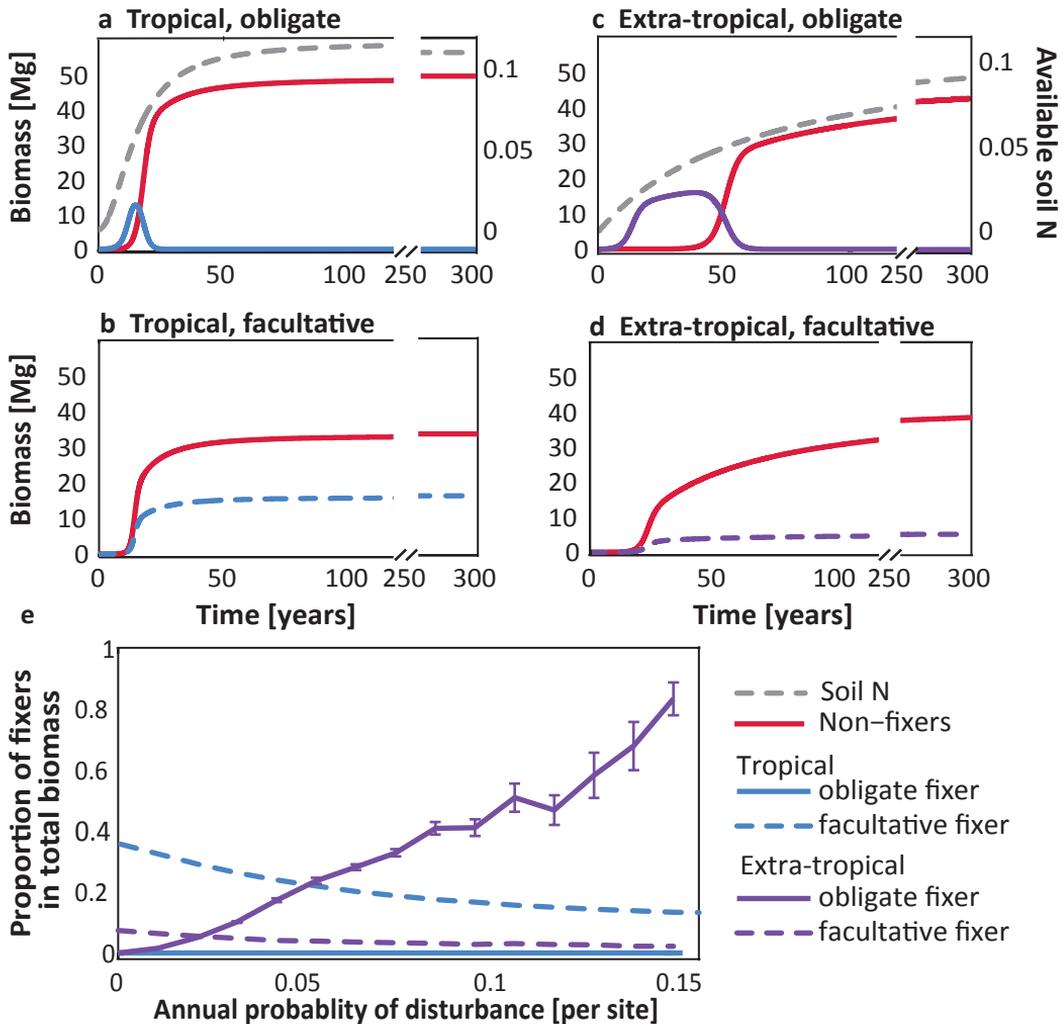
d



e



a N use efficiency, obligate fixer vs. non-fixer**b Soil N uptake, obligate fixer vs. non-fixer****c Soil N uptake, facultative fixer vs. non-fixer****d Cost of facultative, facultative vs. obligate fixer**



Supplementary Information

Climate constraint on nitrogen cycle determines evolution of plant N₂ fixation strategies across biomes

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Supplementary note 6: Persistence of obligate vs. facultative strategies as a function of the rate of soil nitrogen accumulation

Supplementary note 7: Forest landscape dynamics with stochastic disturbance

Supplementary Movies:

Movie S1. Extra-tropical forest with obligate N₂ fixers, and low disturbance frequency. Showing the coexistence of fixers and non-fixers, with increasing dominance of non-fixers over time, and occasional short-term dominance of fixers in disturbed sites.

Movie S2. Extra-tropical forest with facultative N₂ fixers, and low disturbance frequency. Showing the high dominance of non-fixers (no coexistence), and occasional short-term dominance of fixers in disturbed sites.

Movie S3. Extra-tropical forest with obligate N₂ fixers, and high disturbance frequency. Showing a shifting mosaic of patches either dominated by fixers or with coexistence of fixers and non-fixers, and low proportion of patches dominated by non-fixers.

Movie S4. Extra-tropical forest with facultative N₂ fixers, and high disturbance frequency. Showing a landscape dominated by non-fixers, with occasional post-disturbance dominance of fixers that are quickly outcompeted by non-fixers.

Movie S5. Tropical forest with obligate N₂ fixers, and low disturbance frequency. Like the case in Video S4, this scenario also results in a landscape dominated by non-fixers, with occasional post-disturbance dominance of fixers that are quickly outcompeted by non-fixers.

Movie S6. Tropical forest with facultative N₂ fixers, and low disturbance frequency. Showing the coexistence of fixers and non-fixers with a slow increase in the proportion of non-fixers.

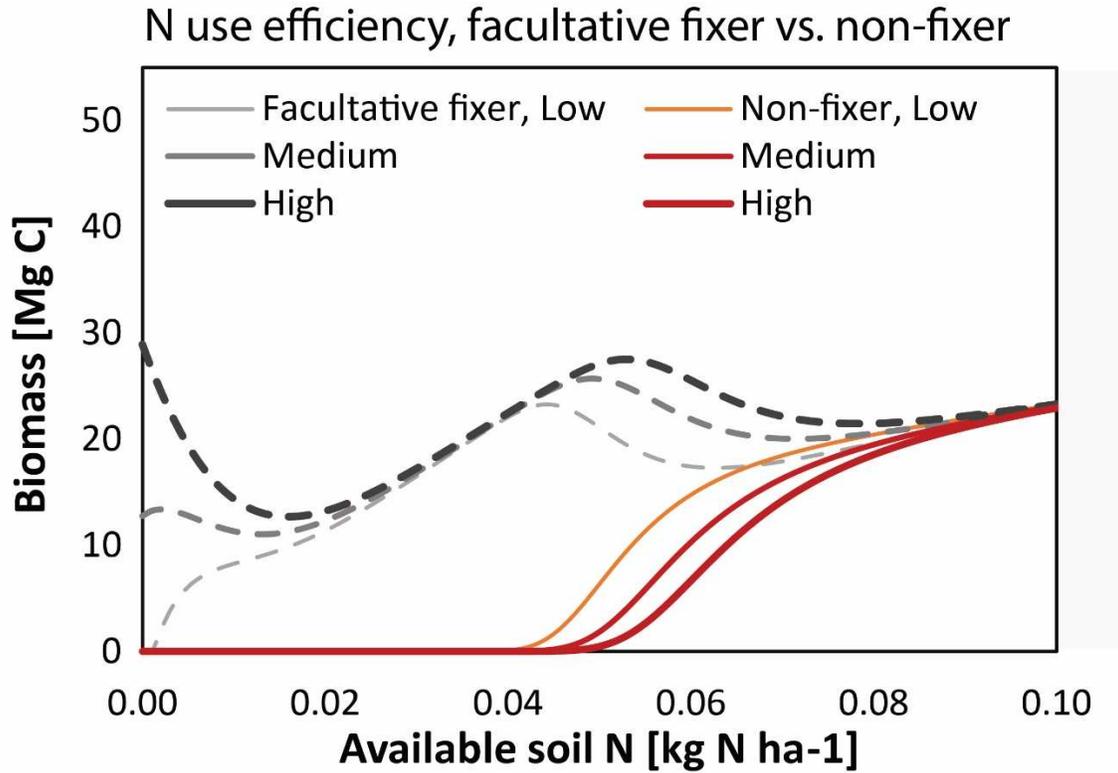
Movie S7. Tropical forest with obligate N₂ fixers, and high disturbance frequency. Like the case in Videos S4 & S5, this scenario also results in a landscape dominated by non-fixers, with post-disturbance dominance of fixers that are very quickly outcompeted by non-fixers.

Movie S8. Tropical forest with facultative N₂ fixers, and high disturbance frequency. Showing a landscape with a higher proportion of non-fixers compared to fixers in most patches and a slowly increasing dominance of non-fixers, with occasional post-disturbance fixer dominance.

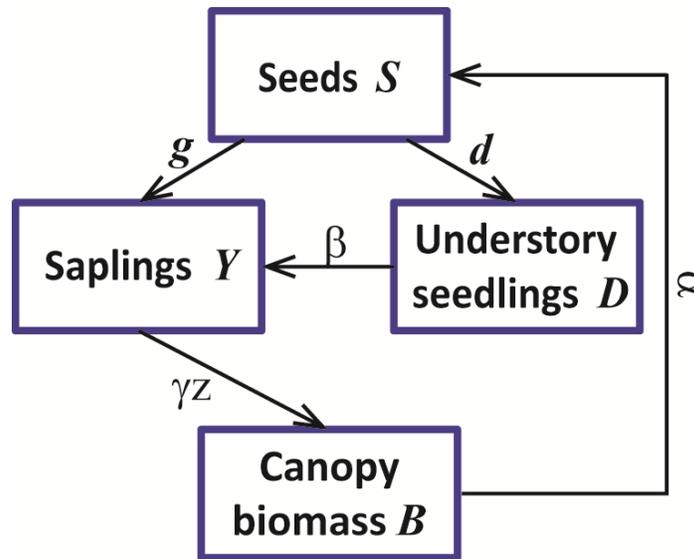
Supplementary Data Table 1. Parameters, and variables used for simulations

Parameter		Units	Value
$N(t)^i$	Soil available nitrogen at time t in site i (exogenous model)	kg N ha ⁻¹	0 – 0.2
c	Michaelis-Menten half-saturation constant for soil N uptake	kg N ha ⁻¹	0.1
v_{\max}	Soil N uptake rate of non-fixer	gN gC ⁻¹ yr ⁻¹	0.1
ω_{\max}	Nitrogen use efficiency of non-fixer	gC gN ⁻¹	50
\bar{F}_j	Maximal rate of N ₂ -fixation	gN gC ⁻¹ yr ⁻¹	0.055
f_j	Rate of exponential decrease in F as a function of $N(t)^i$	-	0-100
θ	Reduction of growth rate due to N ₂ -fixation (metabolic cost)	gC gN ⁻¹	10
Ψ_j	Reduction of growth rate due to facultative N ₂ -fixation (opportunistic cost)	gC gN ⁻¹	0-40
μ	Biomass mortality rate	yr ⁻¹	0.5
γ	Rate of sapling maturation	yr ⁻¹	0.01
y	Biomass of a maturing sapling	kgC	30
m	Density-dependent mortality rate	yr ⁻¹	0.5
K^i	Biomass carrying capacity at the i^{th} site	kgC	600
T	Time lag	yrs	0
α	Rate of seed production as a function of adult biomass	g ⁻¹ yr ⁻¹	0.1
σ	Biomass of a seed	gC	3
d	Rate of seed germination to seedlings	yr ⁻¹	0.015
g	Rate of seed germination to saplings	yr ⁻¹	0.015
h	Seed mortality rate $h = 1 - g - d$	yr ⁻¹	0.7
q	Seedlings mortality rate	yr ⁻¹	0.3
β	Rate of post-disturbance transition from seedlings to saplings	yr ⁻¹	1
δ	Sapling mortality rate	yr ⁻¹	0.3
p	Density-dependent sapling mortality rate	yr ⁻¹	0.1

Supplementary Figure 1. Forest composition as a function of soil nitrogen. Mature tree biomass resulting from pairwise competition between non-fixers (orange/red lines) vs. facultative N_2 -fixers (dashed gray lines) with varying levels of nitrogen use efficiency. Low, medium and high indicate the N_2 -fixer trait is 20, 50 and 80% of the non-fixer, respectively (with medium level for the other trait).



Supplementary Figure 2. Model structure. Boxes are the life-history stages of a tree represented in equations 2-5: adult biomass (B), seeds (S), seedlings in the “dormant” understory seedling bank (D), and saplings (Y). Arrows represent the rates of transition from one stage to another: seed production (α), germination to seedling or sapling (d and g , respectively), release from understory seedling bank (β , following a disturbance event), and sapling maturation (γ).



Supplementary note 1: Soil nitrogen deficits across forest biomes

We evaluated soil carbon and nitrogen contents and the deficit in soil nitrogen (calculated relative to the nitrogen needed to decompose the soil organic matter, as described below) from: (i) a global dataset³¹ of deep soil samples (0-100 cm) from 1,848 sites across tropical, temperate and Boreal forests (analyzed in Fig. 1a); and (ii) published data of shallow soil samples (0-10 cm) from tropical, temperate and boreal forests^{18,20,32,33-52} that either harbored or did not harbor significant populations of N₂-fixing trees^{20,32,35,46,47,50} (Fig. S1).

In both cases, we calculated the soil nitrogen deficit as the quantity of nitrogen present in the soil minus the quantity of nitrogen needed to fully decompose the pool of soil organic matter to the sampled depth. We used soil bulk densities (published in the same paper or elsewhere for the same soil type and approximate location) to convert %C and %N to per-area carbon and nitrogen contents [kg ha⁻¹]. We assumed a decomposer C:N molar stoichiometry of 6:1^{24,53} and a decomposer growth efficiency of 0.5^{24,54}. The calculation was:

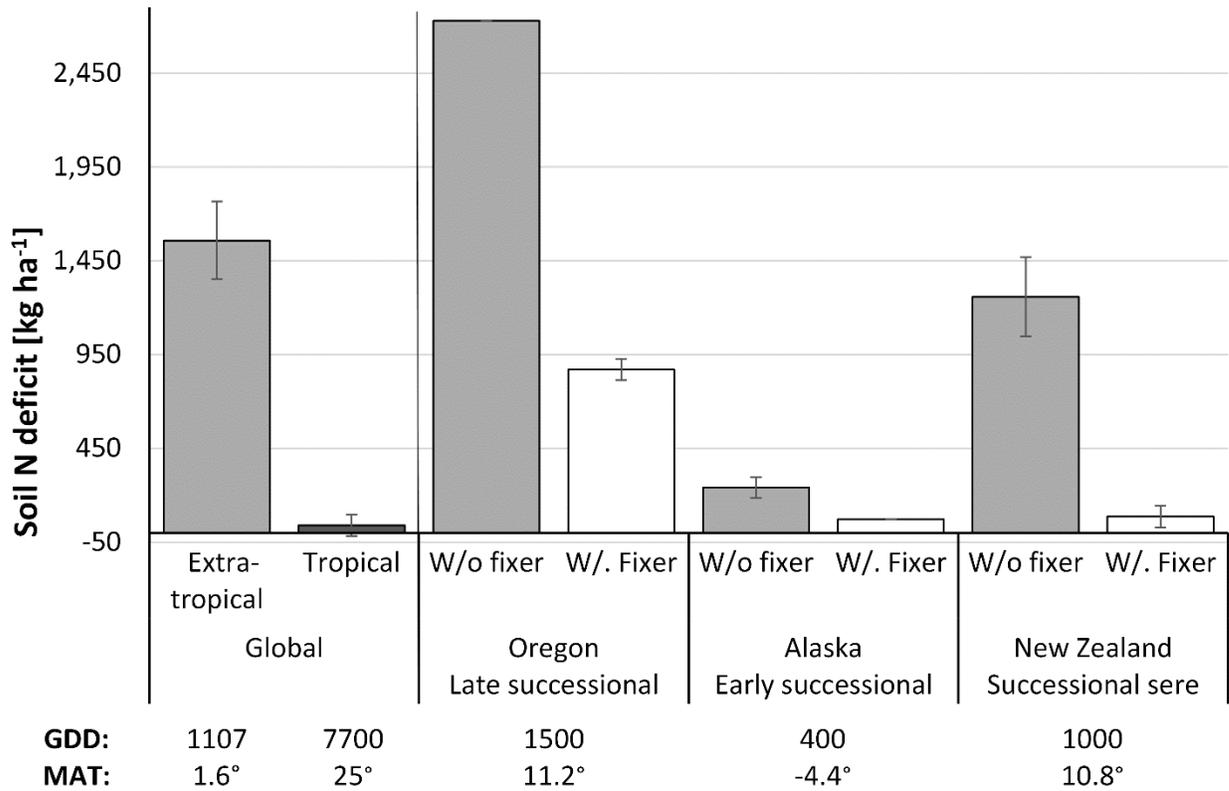
$$N \text{ deficit} = \frac{C_{soil}}{12} - N_{soil}$$

In the deep soil dataset, we found substantial deficits in soil nitrogen (relative to decomposer needs) in extra-tropical forests, but only minor or negligible deficits in tropical forests (Fig. 1a in the main text; ANOVA results for C: $F=19.378$, $p<0.0001$; for N: $F=12.956$, $p<0.0001$; and for N deficit: $F=25.967$, $p<0.0001$).

The shallow soil dataset showed the same broad trends. There were similarly large nitrogen deficits in extra-tropical forests where N₂ fixers were rare, but only minor deficits in tropical forests (Fig. S1.1). The nitrogen deficit was reduced, however, in extra-tropical forests where fixers were abundant (Fig. S1.1), showing that abundant fixers can enhance local soil nitrogen even though the soils of extra-tropical forests generally are nitrogen poor.

We infer from this analysis that: (i) extra-tropical forests with few to no N₂ fixers are characterized by high soil carbon (owing to low decomposition rates) and low soil nitrogen (owing to low nitrogen input and high C:N in plant litter), which creates high soil nitrogen deficits relative to decomposer demand; (ii) tropical forests are characterized by high soil nitrogen relative to soil carbon (owing to high nitrogen inputs, low C:N in plant litter, and rapid soil carbon decomposition), which causes low soil nitrogen deficits; and (iii) extra-tropical forests with high N₂ fixation input are characterized by high soil nitrogen relative to soil carbon, which may result in reduced soil nitrogen deficits.

Figure S1.1. Soil nitrogen deficit. Mean \pm SE soil nitrogen deficit in tropical ($n=35$) and in extra-tropical ($n=73$) forests, and in representative temperate and Boreal forests with and without N_2 -fixing trees. Nitrogen deficit was calculated as the quantity of added nitrogen necessary to reach a C:N ratio of 12:1 in the shallow soil (0-10 cm depth). Numbers in the bottom represent mean growing degree-days (GDD) and mean annual temperature (MAT) ($^{\circ}C$) of study sites.



Supplementary note 2: Influence of functional form of down-regulation of the rate of fixation for facultative fixation

We examined the sensitivity of our results to different rates and functional forms for the down-regulation of fixation with increasing soil nitrogen availability. We evaluated three classes of functional response (Fig. S2.1): (1) an exponential down-regulation of fixation with increasing soil available nitrogen (described in Methods) based on field observations^{5,22}, for which we compared sharper and milder declines in fixation rate as a function of soil nitrogen; (2) a linear down-regulation of fixation with increasing soil available nitrogen; and (3) an exponential function in which down-regulation is delayed and starts only at slightly elevated soil available nitrogen.

We found two classes of dynamics for these options: The first class of result is that facultative fixers dominated all forests globally when the decline function for fixation was sharp and/or the cost of down-regulation (*i.e.*, 'opportunistic' cost of being facultative) was low to negligible. This scenario is not realistic as (*i*) this pattern is not observed in the field, (*ii*) the ability to down-regulate fixation likely is associated with a cost (see *supplementary note 3*), and (*iii*) fixers are not perfectly able to predict soil nitrogen levels and thereby perfectly adjust their fixation rates (Fig. S2.2). The second class of result is that facultative fixers coexisted with non-fixers under certain conditions (*e.g.*, tropical forests with rapid soil nitrogen recovery) and were out-competed by non-fixers in other conditions (*e.g.*, extra-tropical forests with slow soil nitrogen recovery) when the decline function for fixation was less steep and/or when down-regulation was associated with a significant carbon cost (Fig. S2.3). Such a cost can be either direct or indirect, including the opportunity cost associated with re-building a root system optimized for fixation to one optimized for soil nitrogen uptake as further discussed in *supplementary note 3*.

Figure S2.1. Rate of N₂ fixation as a function of soil nitrogen availability in facultative fixers using different functional forms.

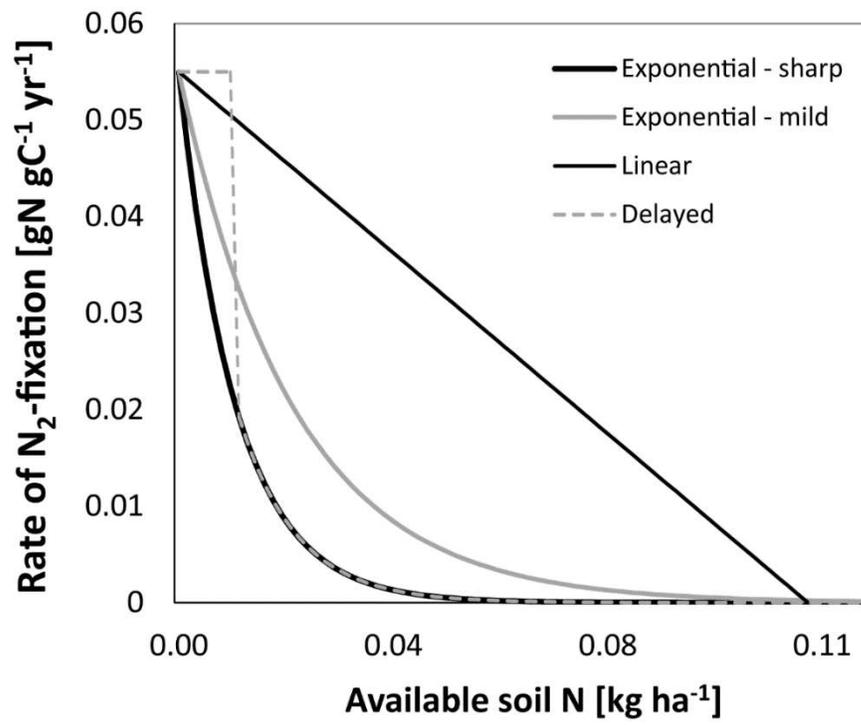


Figure S2.2. Successional biomass dynamics of non-fixers (red lines) and facultative fixers (dashed blue lines), assuming different functional forms (given in Fig. S2.1) for the down-regulation of fixation with increasing soil available nitrogen, and assuming no cost of being facultative ($\Psi=0$). Two successional scenarios are shown for each type of facultative fixation strategy: a tropical forest with rapid soil nitrogen recovery (left side panels) and an extra-tropical forest with slow soil nitrogen recovery (right side panels).

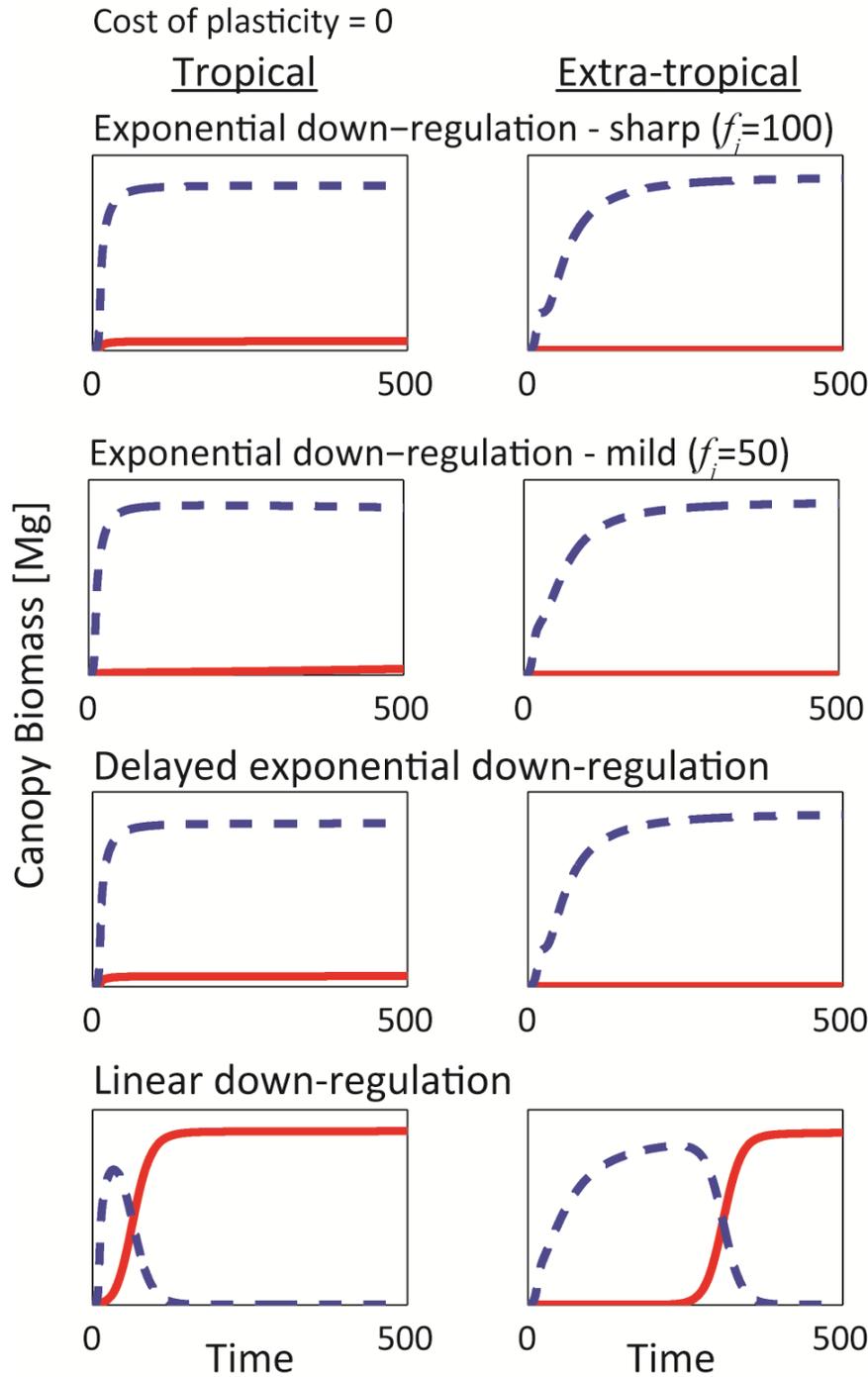
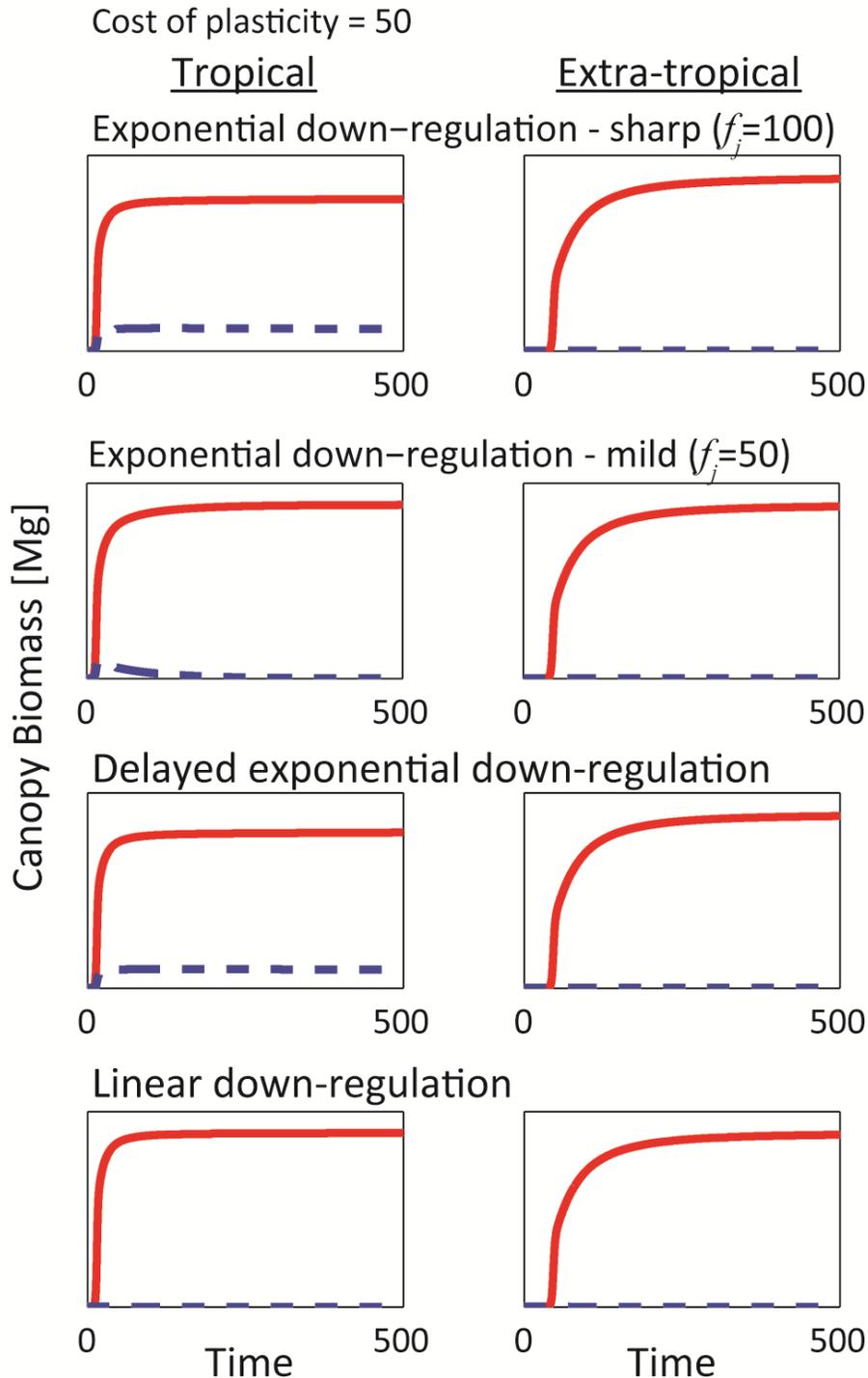


Figure S2.3. Successional biomass dynamics of non-fixers (red lines) and facultative fixers (dashed blue lines), assuming different functional forms (given in Fig. S2.1) for the down-regulation of fixation with increasing soil available nitrogen, and assuming a cost of being facultative ($\Psi=50$). Two successional scenarios are shown for each type of facultative fixation strategy: a tropical forest with rapid soil nitrogen recovery (left side panels) and an extra-tropical forest with slow soil nitrogen recovery (right side panels).



Supplementary note 3: Cost of fixation and the costs and limitations of the facultative fixation strategy

Calculations of the cost of N₂ fixation date back to Gutschick (1981)⁴, who focused on what is generally assumed to be the two most significant costs to individual plants: First, a direct metabolic cost based on the energetic demand of the biochemical process of fixing atmospheric N₂. Second, an indirect structural carbon cost associated with the construction and maintenance of root nodules, and any physiological structures that maintain the symbiotic microbes within these nodules. Gutschick and others following him^{6,12,28} estimated the total cost to the individual plant as ~10 g of plant carbon per gram of fixed nitrogen. Some¹² have used a slightly smaller value as the direct fixation cost (6 g carbon g N⁻¹ fixed), but we chose the larger value to include also indirect costs. We further assume that our value includes lesser (but poorly known) costs, associated with: (i) the emission of signaling compounds to attract potentially symbiotic soil bacteria; (ii) the potential mortality of N₂-fixing plants caused by herbivory; and (iii) the potential cost (over generations) of maintaining the capacity to fix N in a plant's genetic makeup. We separately consider the opportunistic costs described in the next paragraph.

Trees face both direct and indirect costs associated with the facultative fixation strategy. Direct physiological costs include: (i) sensing the availability of nitrogen in the soil in order to react to changes in soil nitrogen status, and (ii) the subsequent costs of building and maintaining fine roots when fixation is down-regulated and building root nodules when fixation is up-regulated. Indirect costs include: (i) the opportunity cost associated with the need to grow a root system with a structure that will be suitable for both hosting bacteria for fixation and competing for soil nitrogen when not fixing, and (ii) the inability of facultative fixers to precisely match their fixation rate to the instantaneous ESS- F^* rate called upon for each soil nitrogen condition (see Fig. 1 in the main text).

We use the parameter Ψ in our model to capture these costs that are uniquely associated with facultative fixation. Ψ scales with the rate of fixation F , so that the cost is zero when fixation is down-regulated ($F = 0$).

Supplementary note 4: Sensitivity analysis

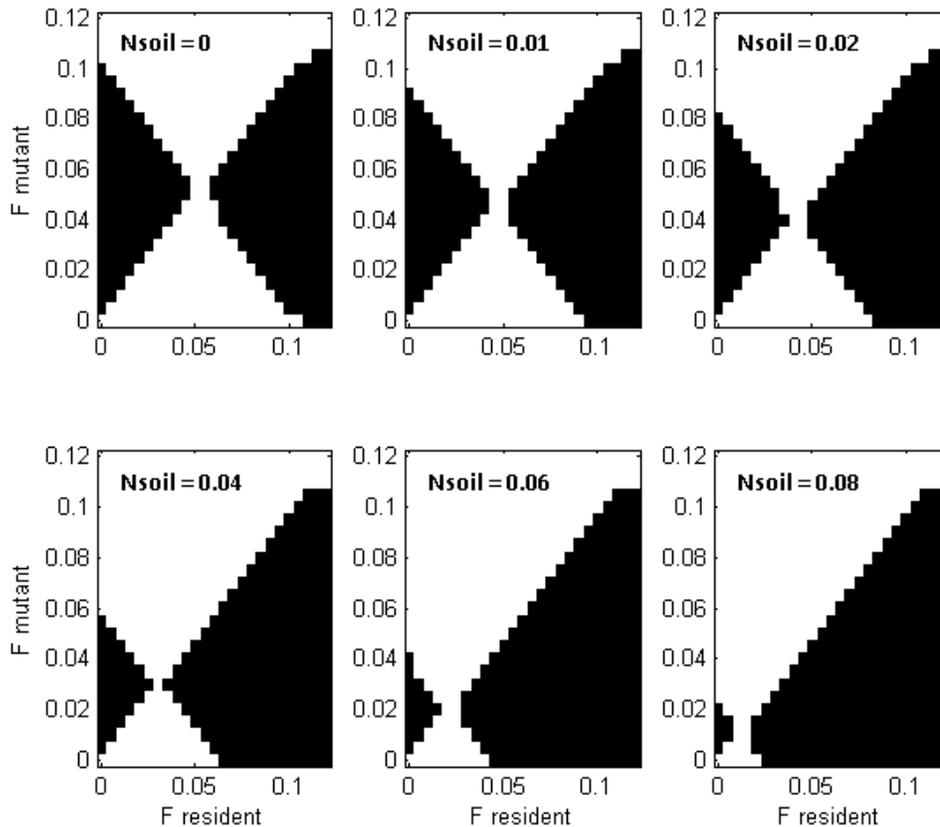
We examined the robustness of our results for the ESS rate of fixation F^* using larger and smaller values for the main parameters in equation (1) compared to the default parameters used (shown in Extended Data Table 1; baseline values are shown in the figures below as $parameter_0$).

$$r_j = \varpi(F_j) \left(\left[\frac{\nu(F_j)N}{c + N} \right] + F_j \right) - \theta F_j - \Psi_j F_j - \mu \quad (1)$$

In all cases, our sensitivity analyses show that our result of a convergent-stable ESS is insensitive to the parameter values. Black areas in the pairwise invasibility plots below correspond to successful invasion by the mutant genotype, and white areas correspond to species combinations in which the resident is not invaded by the mutant. Gray areas in the plots correspond to parameter ranges in which the resident genotype goes extinct. Our results show that for each parameter value there exists a convergent-stable ESS in which the resident cannot be invaded by any mutants (indicated by the existence of a white vertical line).

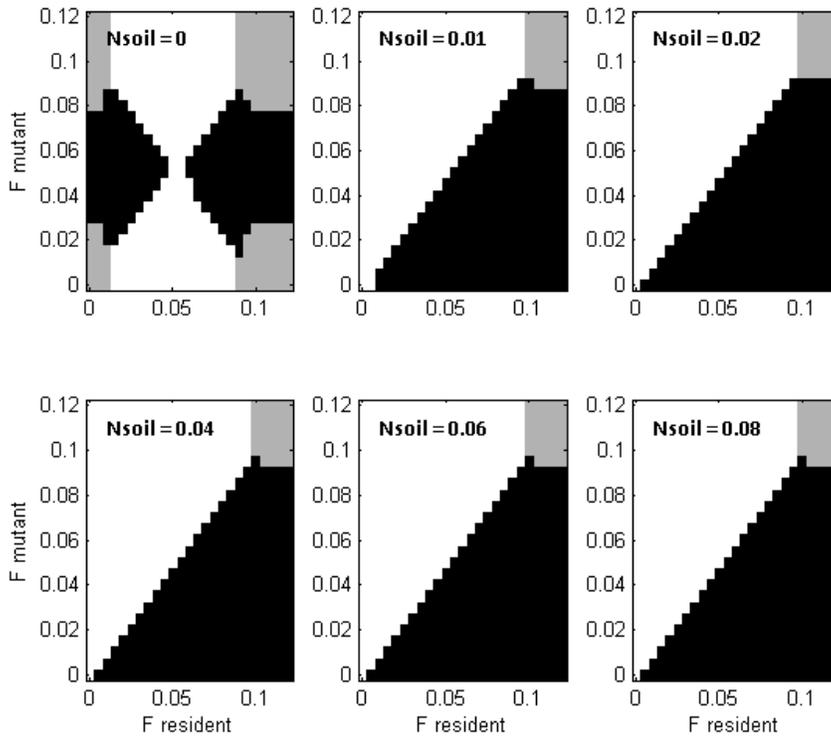
Parameter γ – rate of sapling maturation to adult biomass; $\gamma_0 = 0.01$

$\gamma = 0.1$

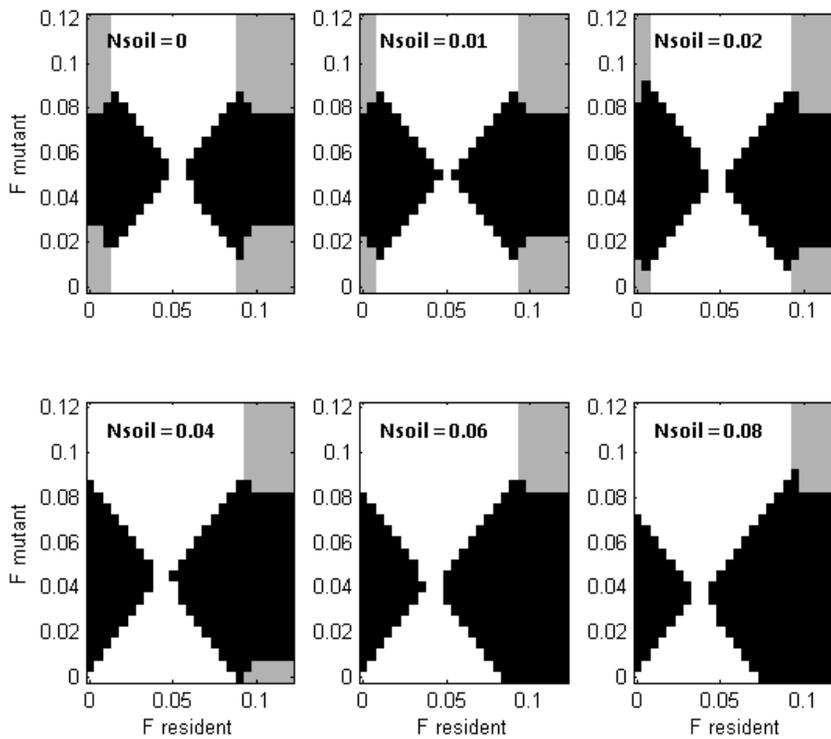


Parameter c - half-saturation value for nitrogen uptake from the soil; $c_0 = 0.1$

$c = 0.01$

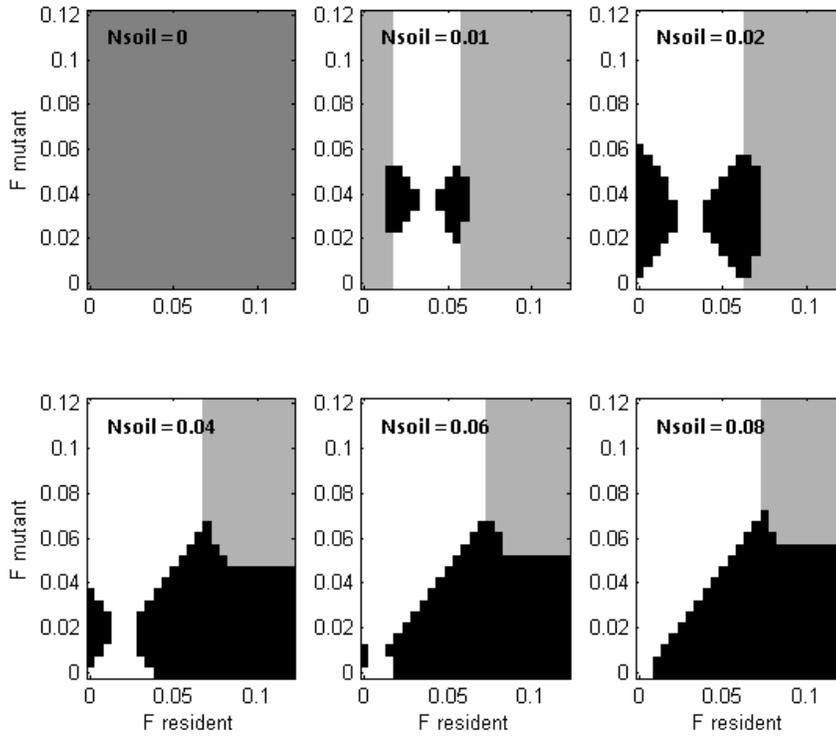


$c = 0.3$

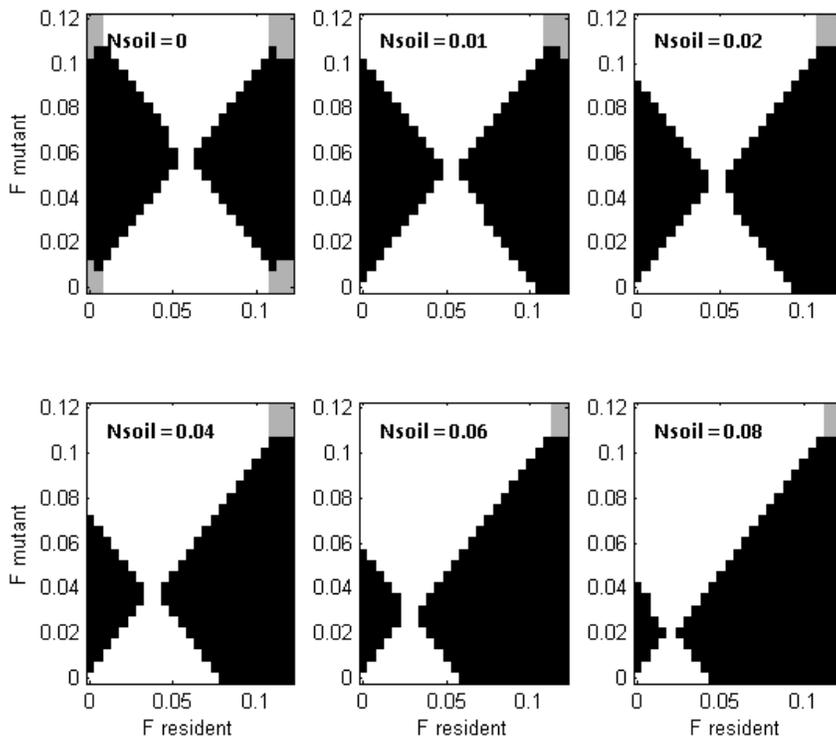


Parameter ω – nitrogen use efficiency; $\omega_0 = 50$ ($\omega_{\min}=25$ $\omega_{\max}=25$, the 50% tradeoff is maintained)

$\omega = 30$

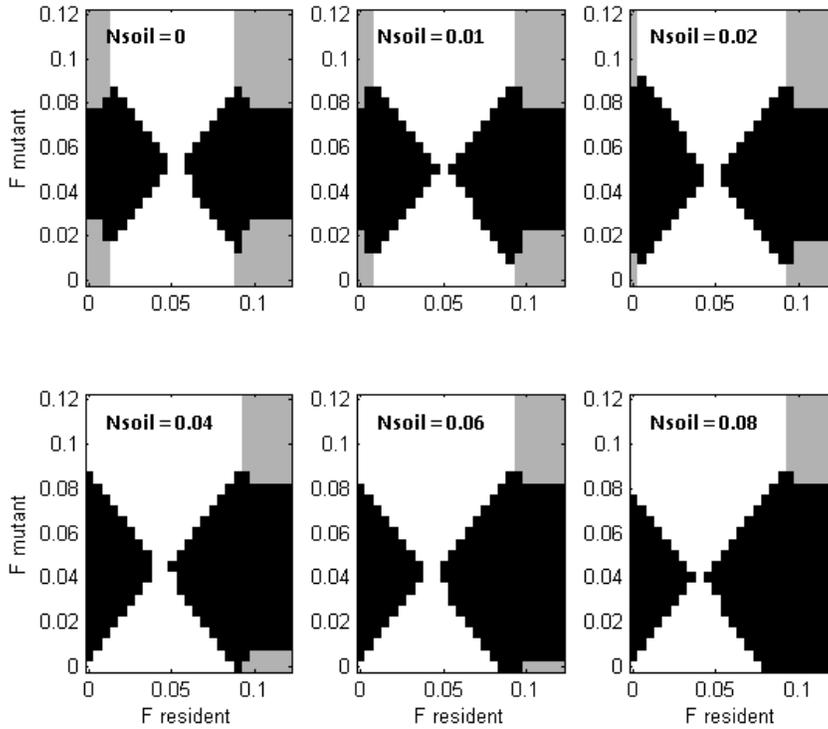


$\omega = 90$

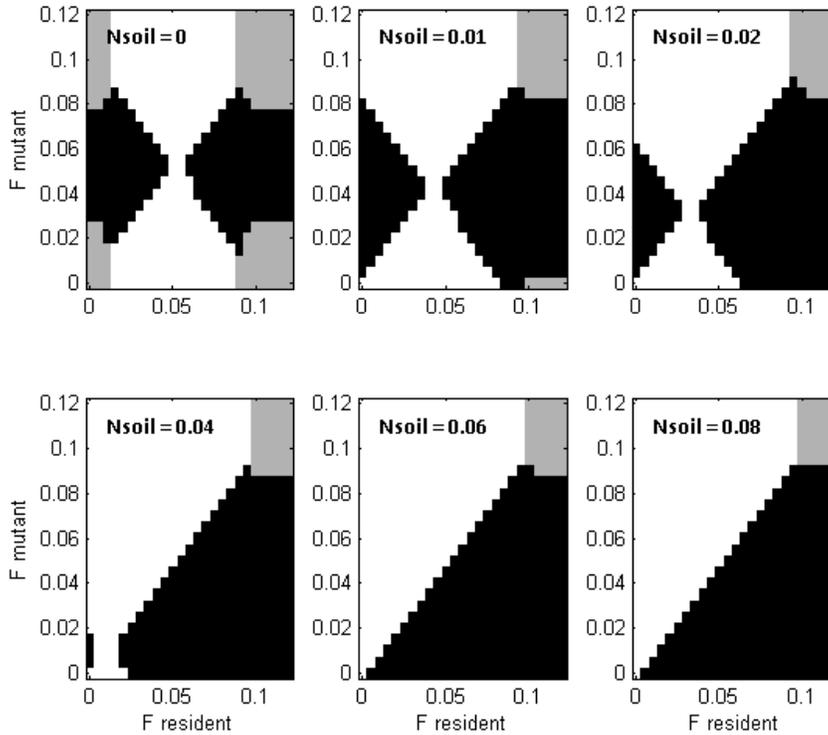


Parameter v – rate of soil nitrogen uptake; $v_0 = 0.1$ ($v_{\min} = 0.05$ $v_{\max} = 0.05$, the 50% tradeoff is maintained)

$v = 0.04$

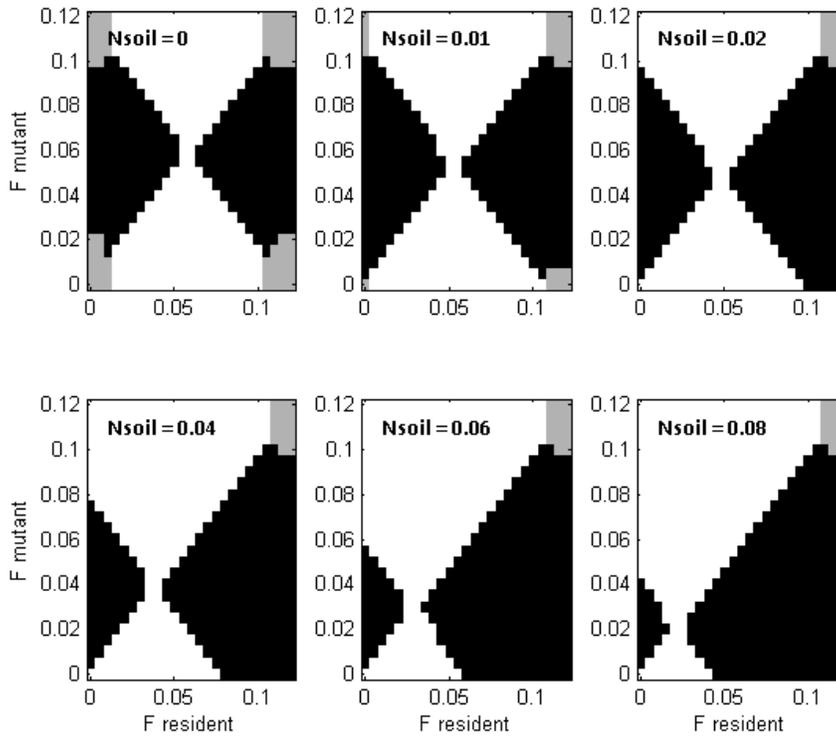


$v = 0.16$

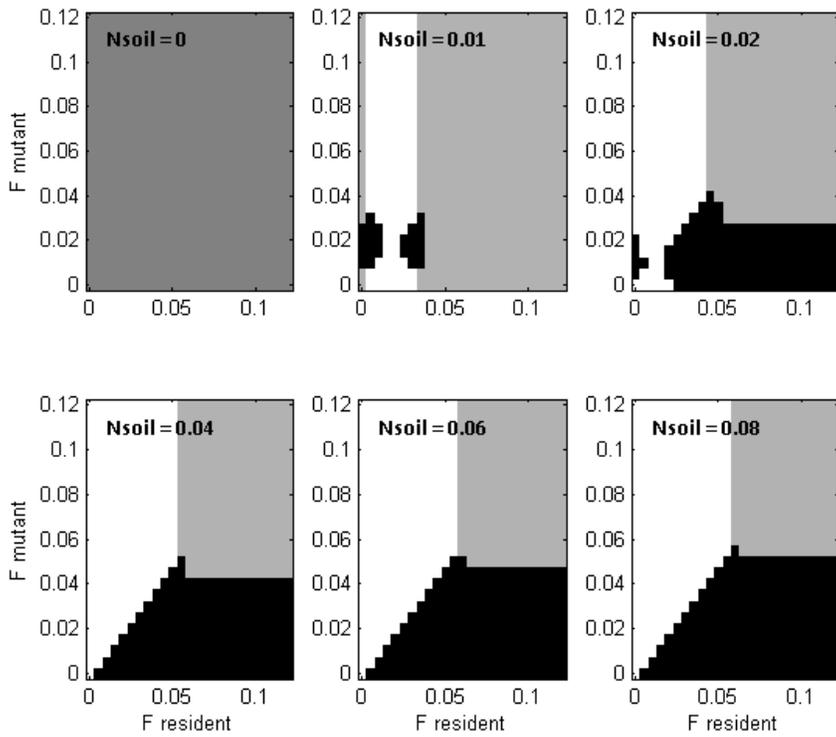


Parameter θ – metabolic cost of fixation; $\theta_0 = 10$

$\theta = 5$

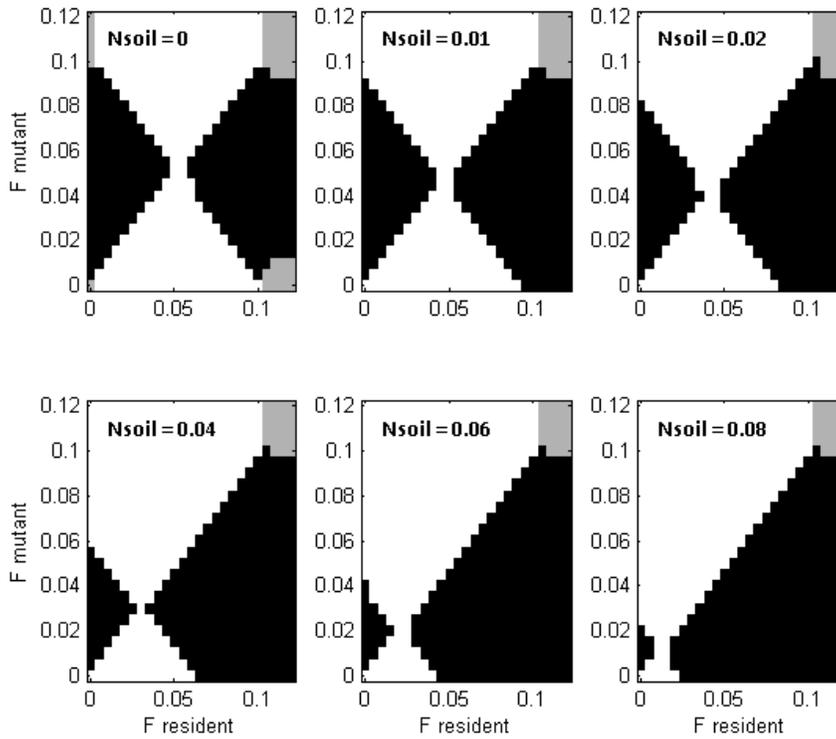


$\theta = 30$

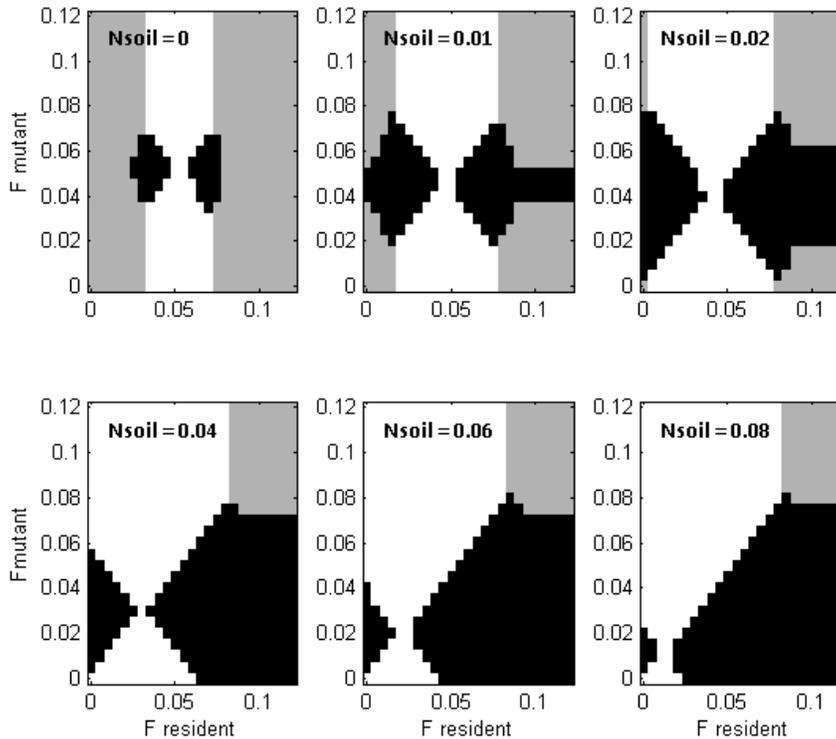


Parameter μ – rate of biomass mortality (turnover); $\mu_0 = 0.5$

$\mu = 0.1$



$\mu = 0.9$



Supplementary note 5: ESS analysis using a simplified model

We examined the robustness of our results for the ESS rate of fixation F^* using a simplified model with the biomass of adult trees only (*i.e.*, instead of eq. 2-5) as follows:

$$\frac{dB_j^i}{dt} = B_j^i r_j - B_j^i \cdot \frac{m_j}{K^i} \sum_{j=1}^L B_j^i \quad (8)$$

We found that the results of the ESS rate of fixation F^* are robust to the life-stage structure of the model (Fig. S5.1). The ESS rate of fixation F^* using the simplified model with a single life-stage decreased with increasing soil nitrogen availability (Fig. S5.2), using similar parameters.

Figure S5.1. Evolutionary stable strategies (ESS) of N_2 fixation. Numerical pairwise invasibility plots show the ESS rate of N_2 fixation F^* for increasing amounts of available soil nitrogen (N_{soil} , from upper left to lower right). Black areas indicate combinations where the mutant strategy invades the resident. The ESS is the resident rate F that cannot be invaded. The nitrogen-use efficiency ω and soil nitrogen uptake ν of fixers are medium, *i.e.*, the trait of fixer is 50% of the trait value of non-fixers.

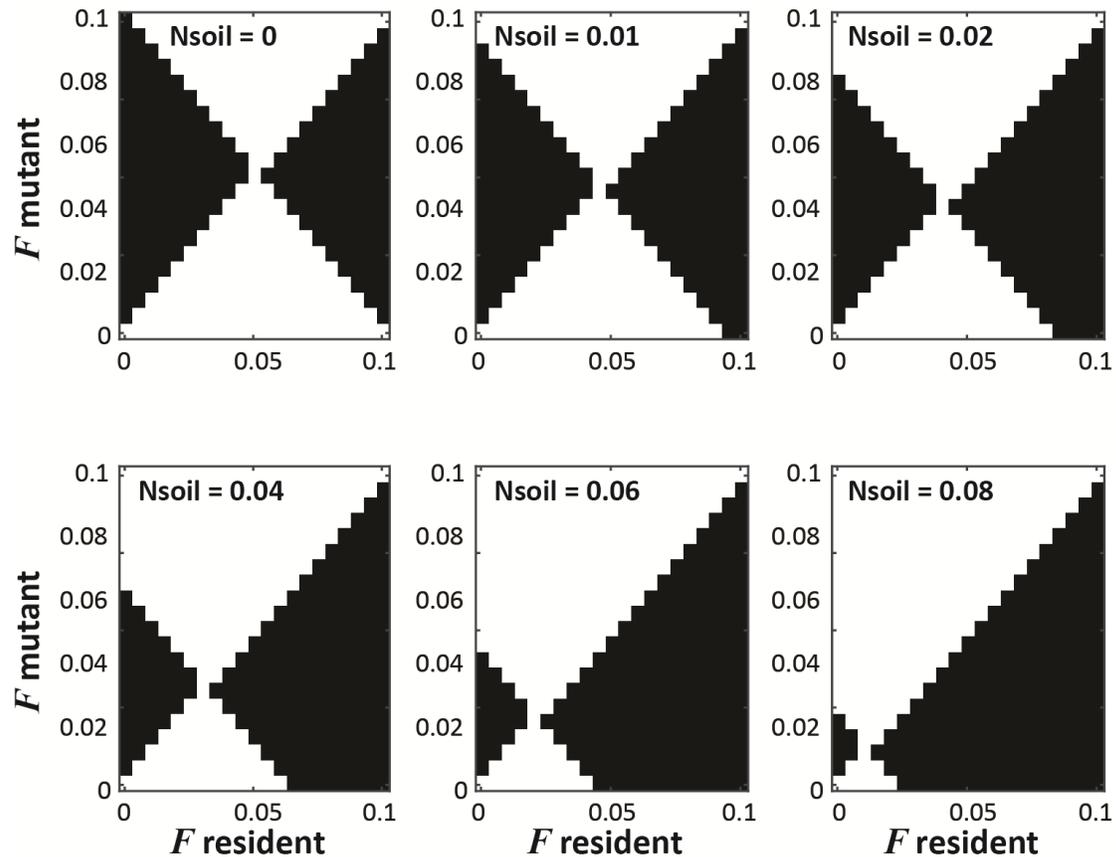
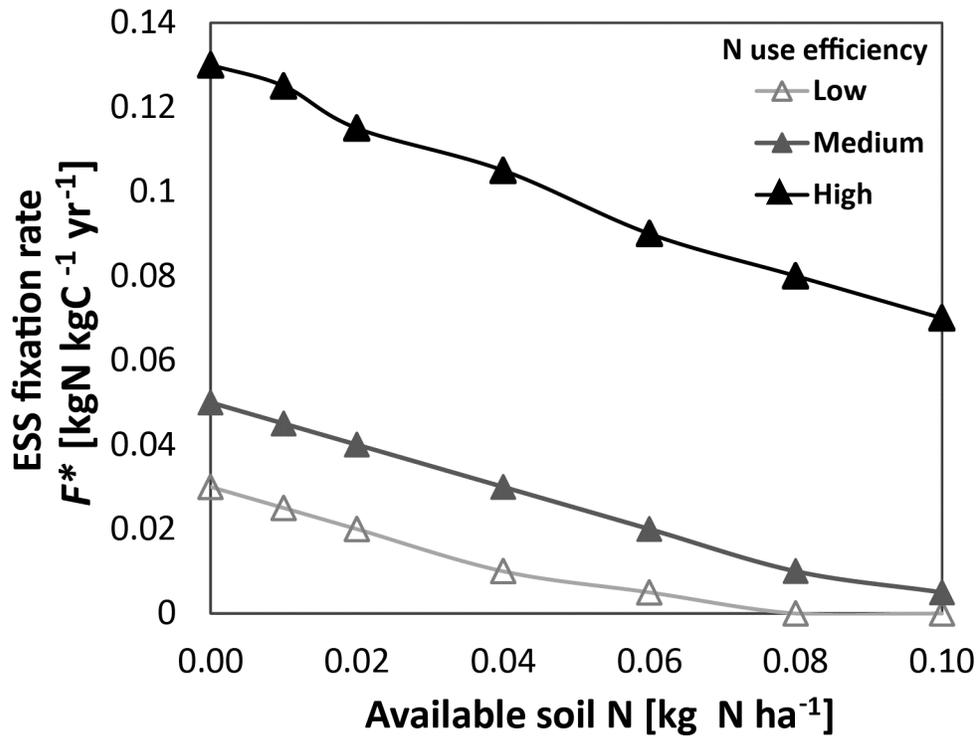


Figure S5.2. Evolutionary stable strategies (ESS) of N₂ fixation and nitrogen use efficiency. ESS rate of fixation F^* as a function of soil nitrogen with varying nitrogen-use efficiency ω . Low, medium and high indicate the N₂-fixer nitrogen-use efficiency is 20, 50 and 80% of the non-fixer, respectively (with medium level for the soil nitrogen-uptake rate). Results of Medium nitrogen use efficiency are based on the pairwise invasibility plots shown in Fig. S5.1.

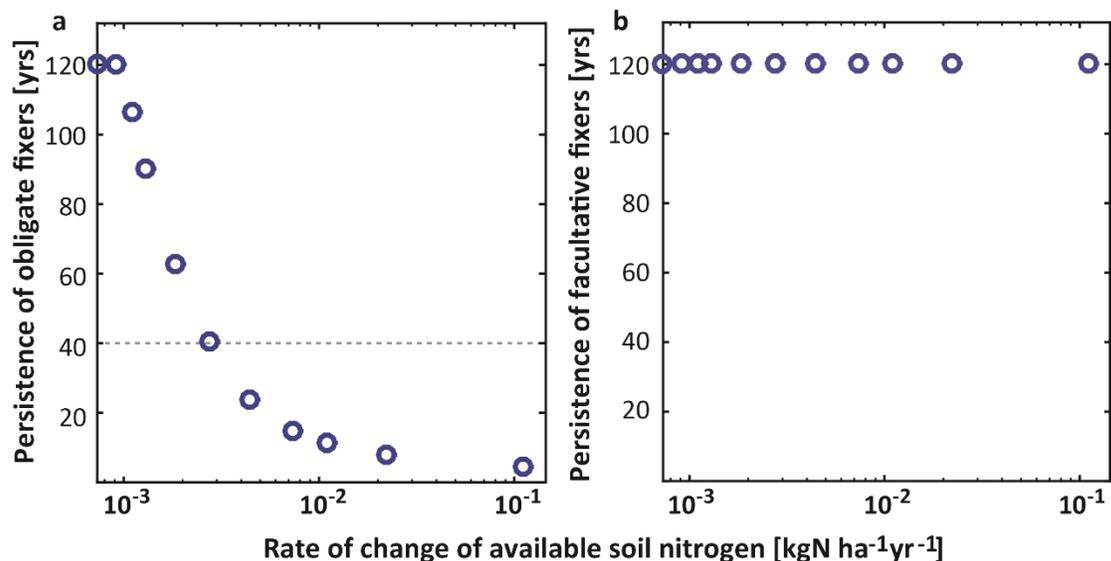


Supplementary note 6: Persistence of obligate vs. facultative strategies as a function of the rate of soil nitrogen accumulation

Since fixation itself can influence the rate of soil nitrogen accumulation, we evaluated whether the persistence of obligate and facultative fixation strategies depends on the rate of change of bioavailable soil nitrogen (equivalent to the net difference between nitrogen inputs minus microbial nitrogen immobilization). The nitrogen deficit in Fig. 1a indicates the extent to which soil from different biomes are at or near equilibrium with respect to microbial nitrogen immobilization.

Our results show that the obligate fixation strategy is sensitive to the rate at which nitrogen becomes available in the soil, while the facultative fixation is not. The persistence of obligate fixers decreases with increasing accumulation of soil available nitrogen (Fig. S6a). This means that the competitive dominance of obligate fixers (over non-fixers) is shortened if fixation itself increases the rate of soil nitrogen accumulation. Such a pattern is observed for the two temperate biome obligate fixers *Alder*¹⁰ and *Coriaria*²⁰ in nature, which both must mature and set seed early in succession, before being out-competed by non-fixers. In nature this typically occurs within no less than 40 years for *Alder* or *Coriaria*, as indicated by the dashed line in Fig. S6a. In contrast, facultative fixation is successful independent of the rate of change of soil available nitrogen (Fig. S6b), supporting the observation that facultative fixers persist throughout succession in nitrogen-rich tropical forests. In sum, we find no evidence that a possible effect of fixers on the rate of soil nitrogen accumulation will qualitatively change our overall results or conclusions.

Figure S6. Persistence of N₂-fixation strategies as a function of rate of change of available soil nitrogen. The length of time obligate **a**, and facultative **b**, N₂ fixers persist along forest succession, as a function of the rate of soil nitrogen accumulation (inputs to soil minus immobilization, logarithmic x axis). All analyses used the same parameters as in figure 3 in the main text.



Supplementary note 7: Forest landscape dynamics with stochastic disturbance

We simulated a landscape with 500 forest patches subject to stochastic disturbance. Local recovery from disturbance follows a successional trajectory with equal initial abundances of fixers (either facultative or obligate) and non-fixers, and soil nitrogen that accumulates over time. Disturbance is simulated as the mortality of the biomass of all mature canopy trees and sub-canopy saplings, the transition of “dormant” understory seedlings to growing saplings, and a reset of soil nitrogen to initial conditions from which succession restarts.

We evaluated forests with (1) different rates of succession (*i.e.*, timescales of soil nitrogen recovery) to simulate typical succession in tropical forests (fast recovery, <20 years) and in extra-tropical forests (slow recovery, >50 years), and (2) different disturbance frequencies (simulated as the probability of disturbance per year at each patch). The results are summarized in Fig. 3e (main text) and in the supplementary animation movies attached. The animations demonstrate changes in the composition of the forest with time (see time-bar at the bottom), where blue patches correspond to dominance of fixers and red patches correspond to non-fixer dominance, with intermediate colors (see color bar on the right side) indicating coexistence.

The following Supplementary videos are included:

Movie S1

Extra-tropical forest with obligate N₂ fixers, and low disturbance frequency. Showing the coexistence of fixers and non-fixers, with increasing dominance of non-fixers over time, and occasional short-term dominance of fixers in disturbed sites.

Movie S2

Extra-tropical forest with facultative N₂ fixers, and low disturbance frequency. Showing the high dominance of non-fixers (no coexistence), and occasional short-term dominance of fixers in disturbed sites.

Movie S3

Extra-tropical forest with obligate N₂ fixers, and high disturbance frequency. Showing a shifting mosaic of patches either dominated by fixers or with coexistence of fixers and non-fixers, and low proportion of patches dominated by non-fixers.

Movie S4

Extra-tropical forest with facultative N₂ fixers, and high disturbance frequency. Showing a landscape dominated by non-fixers, with occasional post-disturbance dominance of fixers that are quickly outcompeted by non-fixers.

Movie S5

Tropical forest with obligate N₂ fixers, and low disturbance frequency. Like the case in Video S4, this scenario also results in a landscape dominated by non-fixers, with occasional post-disturbance dominance of fixers that are quickly outcompeted by non-fixers.

Movie S6

Tropical forest with facultative N₂ fixers, and low disturbance frequency. Showing the coexistence of fixers and non-fixers with a slow increase in the proportion of non-fixers.

Movie S7

Tropical forest with obligate N₂ fixers, and high disturbance frequency. Like the case in Videos S4 & S5, this scenario also results in a landscape dominated by non-fixers, with post-disturbance dominance of fixers that are very quickly outcompeted by non-fixers.

Movie S8

Tropical forest with facultative N₂ fixers, and high disturbance frequency. Showing a landscape with a higher proportion of non-fixers compared to fixers in most patches and a slowly increasing dominance of non-fixers, with occasional post-disturbance dominance of fixers.

Additional reference for the supplementary material

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