Optimal control of root nodulation – Prediction of life history theory of a mutualistic system

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ABSTRACT

Legumes produce root nodules containing symbiotic rhizobial bacteria that convert atmospheric molecular nitrogen into ammonia or related nitrogenous compounds. The host plant supplies photosynthetic products to root nodules forming a mutualistic system. Legumes have physiological mechanisms for regulating nodule production with chemical signals produced in leaves, called the autoregulation of nodulation. In this paper, we discuss the optimal number of root nodules that maximizes the performance of the host plant. Here, we study two models. In the stationary plant model, the acquired photosynthetic products minus cost and loss are used for reproduction. In the growing plant model, the excess material is invested to produce leaves, roots, and root nodules, resulting in the exponential growth of the whole plant. The analysis shows that having root nodules is beneficial to the plant for a high leaf nitrogen content, faster plant growth rate, a short leaf longevity, a low root/shoot ratio, and low soil nutrient concentration. We discuss the long-distance control of nodulation-autoregulation and dependence on the environmental conditions of terrestrial plants considering these results.

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1. Introduction

Nitrogen is an indispensable component of proteins that form the structure of plant bodies, enzymes catalyzing various biochemical reactions, and the major components of chloroplasts. Nitrogen is often demanded strongly by terrestrial plants and is limited in soil, as shown by fertilization experiments (Vitousek and Howarth, 1991; Vitousek et al., 2002).

Some plant species receive nitrogen from symbiotic bacteria that fix atmospheric molecular nitrogen. Host plant and bacteria interact and form structures called "root nodules", which contain symbiotic nitrogen-fixing bacteria. The most famous example of nodule symbiosis is the one between legumes (Fabaceae family) and rhizobial bacteria.

Since host plants supply nutrition to their symbiont out of the photosynthetic products, having an excessive number of root nodules is maladaptive. This is demonstrated by *har1* mutants of *Lotus japonicus* (Nishimura et al., 2002) and the *klv* mutant, the latter being slow in growth, and smaller in size (dwarf phenotype), as well as having deformed leaf veins and markedly delayed flowering (Oka-Kira et al., 2005). In environments with abundant soil nitrate, the plants reduce the number of nodules to produce. There are mutants that cannot perform this adaptive response to enhanced soil nitrate concentrations (Carroll et al., 1985; Krusell et al., 2002; Okamoto et al., 2009; Reid et al., 2011).

Wild-type plants of legumes are equipped with a mechanism called the "autoregulation of nodulation" to maintain the number of root nodules at adaptive levels (Ferguson et al., 2010; Reid et al., 2011). This includes several phytohormones, such as auxin (van Noorden et al., 2006; Suzaki et al., 2012), cytokinin (Sasaki et al., 2014), and jasmonic acid (Suzuki et al., 2011). Recently, detailed molecular mechanisms have been studied (e.g., Okamoto et al., 2009, 2013). One notable aspect of the autoregulation of nodulation is that the regulation of the root nodule number is performed by phytohormones produced in the leaves, which is called "long-distance control" (Carroll et al., 1985; Krusell et al., 2002; Searle et al., 2003; Tsikou et al., 2018).

The ecology of nodule symbiosis has also been studied. One field study of *Acacia koa* showed that young trees (six years old) possessed more root nodules than did older trees (20 years old) (Pearson and Vitousek, 2001). In terrestrial ecosystems, nitrogen is an important factor limiting tree growth (Vitousek and Howarth, 1991; Vitousek et al., 2002). However, in the temperate and boreal forests, few trees adopt nodule symbiosis, except in the early stages of primary succession (Chapin et al., 1994).

Menge et al. (2008) explained the rarity of nodule symbiosis in old growth temperate/boreal forests by the large cost associated with nodule symbiosis by the host plant. In contrast, many tropical forests show a high activity of nitrogen-fixing bacteria (Vitousek et al., 2002; Hedin et al., 2009), which seems to be in conflict with the higher availability of soil nitrogen in tropical forests than in temperate or boreal forests (Martinelli et al. 1999). To understand the observation, studies of systems-ecological modeling have been performed, considering various aspects including the cost of nitrogen fixation, spatial structure, limitation of other nutrients, and intensive competition (Vitousek and Field, 1999; Rastetter et al., 2001; Fisher et al., 2010: Menge and Levin, 2017).

The importance of nitrogen to plants has been studied by physiological ecologists. In the leaves of terrestrial plants, a large fraction of nitrogen is contained in the chloroplasts. In particular, Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), a key enzyme for fixing CO_2, occupies a considerable fraction of the total nitrogen in leaves. Rubisco is the most abundant enzyme on earth (Ellis, 1979; Raven, 2013). The need for a large amount of nitrogen in the leaves is caused by the inefficiency of Rubisco. The amount of leaf nitrogen is almost proportional to the net photosynthesis rate when a sufficiently strong light is available, although various chlorophyll binding proteins also use a fraction of nitrogen in leaves (Mooney et al., 1981; Gulmon and Chu, 1981). However, under low light intensity, the photosynthetic rate is more weakly affected by the amount of nitrogen in leaves (Gulmon and Chu, 1981). Hence, nitrogen is very important for leaves receiving high light intensity, but not so much for leaves in low-light environments.

In this paper, we explore the idea that these diverse behaviors of the legumes controlling the root nodulation might be the optimal strategy of the host plant, considering the benefit of receiving nitrogen from the N-fixing bacteria and the cost of producing and maintaining the nodulation. Theoretical models based on similar idea have been successful in revealing various behaviors of the terrestrial plants, including the schedule of reproduction and growth (Gadgil and Bossert, 1970; Vincent and Pulliam, 1980: Iwasa and Cohen, 1989), the shoot/root balance (Iwasa and Roughgarden, 1984), and the allocation of alkaloid defense as a function of leaf age (Iwasa et al., 1996) among others.

To focus on the key logic, we deliberately choose the simplest possible model that can handle the questions we ask: what are the conditions in which the plant finds it profitable to possess root nodules, and, if so, what the optimal number of nodules produced by a plant would be. We adopt the models in which the benefit of nitrogen to the plant is explicitly considered. We develop two mathematical models for the root nodule regulation that achieves the best performance of the whole plant. In the "stationary plant model", we consider a plant in the reproductive phase with the numbers of leaves and nodules unchanged over time. Out of the photosynthesis products obtained, the plant pays the maintenance costs for leaves and nodules, replenishes the loss of leaves, and allocates the remaining amount of material to reproductive activities, forming flowers and fruits. The amount of excess material is the measure for the plant's performance. In contrast, the "growing plant model" describes a plant in the vegetative growth phase, which invests all the excess material obtained to increase its size. The growth rate of the whole plant is the measure of performance. The concept of "dilution effect" is a key to understand the difference in the leaf nitrogen content and the number root nodules between growing-plant model and the stationary-plant model in the same environmental conditions. We discuss the implications of the models to the autoregulation of nodulation pathways, and to the patterns of root nodulation dependence on the environment.

2. Stationary plant model

We consider a whole plant individual with the roots and shoot. Let y be the number of leaves. We assume that all the leaves are equal in size, the amount of nitrogen, and photosynthetic capacity. Please see Table 1 for the symbols.

The photosynthetic rate achieved per leaf per day increases with the light intensity at foliage, which is denoted by L. It also increases with the amount of nitrogen per leaf N (i.e. leaf nitrogen content, or leaf nitrogen concentration) (Gulmon and Chu, 1981). The enhancement of the photosynthetic rate by a higher nitrogen content is very strong for high light intensity but much weaker for low light intensity. This can be expressed by the following simple equation:

$$[\text{photosynthesis rate per leaf}] = \frac{aLN}{bL + N} \qquad (1)$$

Under low light intensity (small L), the photosynthetic rate Eq. (1) increases as aL, proportional to the light intensity, but under high light intensity (large L), the rate saturates to aN/b (the maximum photosynthetic rate). The light saturation level of photosynthesis increases linearly with the leaf nitrogen content N, as is shown in the physiological ecology (Mooney et al., 1981). In contrast, if we fix the light level L, the photosynthetic rate Eq. (1) increases with the leaf nitrogen content N. When N is small, the photosynthetic rate Eq. (1) increases in proportion to the nitrogen level as aN/b, but when N is large, the photosynthetic rate saturates to the level aL, which is proportional to the light. This is also qualitatively the same as the observed measurements (Gulmon and Chu, 1981).

To understand the meaning of Eq. (1) intuitively, we may rewrite it as $1/[1/aL + b/aN]$, which is interpreted as the rate regulated by two limiting processes connected in series: one for capturing energy from sunlight and the other for the assimilation of carbon dioxide, where the former is controlled by light availability L and the latter is controlled by the amount of leaf nitrogen. In fact, very roughly speaking, carbon dioxide assimilation is performed through two different processes: the first is to acquire energy by receiving light, in which chlorophyll receives light, and obtain ATP and NADPH using thylakoid components; and the second is to produce organic material using this energy. Rubisco plays a key role in the second process. When the light intensity is low, a small amount of Rubisco may be sufficient: however, when the light intensity is high, having a large amount of Rubisco is worthy for the plant, even if it is costly.

More exact formulas for the leaf photosythetic rate are available (e.g. Hirose and Werger, 1987; Hikosaka and Terashima, 1995): however, here, we adopt Eq. (1), which is the simplest possible formula considering the role of leaf nitrogen.

Table 1
A list of the variables and parameters included in the model.

Symbol	Definition
x	number of root nodules
y	number of leaves
ρ	root/shoot ratio (root biomass per leaf)
L	light intensity at leaves
N	nitrogen content per leaf
K	construction cost per leaf
m	maintenance cost per leaf
c	maintenance cost per nodule
h	nitrogen fixation rate per nodule
n_s	nitrate concentration in soil
g	efficiency of soil nitrate absorption per unit root biomass
δ	loss rate of leaf nitrogen
a, b	parameters included in the photosynthesis rate function

Let x be the number of nodules in the roots. We assume that the amount of photosynthetic products supplied by the host plant increases in proportion to the number of nodules: cx, where c is the maintenance cost per root nodule. Then, the amount of net gain of photosynthetic product for the plant is as follows:

$$F = \frac{aLN}{bL + N} y - my - cx \qquad (2)$$

where the first term on the right-hand side is for the photosynthetic products obtained per day by y leaves. The second term is for the maintenance cost of the leaves with m being the cost per leaf. The last term is for the maintenance cost of root nodules.

On the other hand, the amount of nitrogen is determined by the following balance equation:

$$\frac{d}{dt}(Ny) = hx + gn_s\rho y - \delta Ny \qquad (3)$$

where N is the nitrogen per leaf, and Ny is the total amount of leaf nitrogen. For simplicity, we consider that most of the plant nitrogen exists in leaves. The left-hand side represents the change in total nitrogen per day. The first term on the right-hand side represents the nitrogen fixation per day made by the root nodules, where h is the amount of nitrogen compounds per day per nodule. The second term is for the nitrate sequestration from the soil per day, where ρy is the root biomass. The root/shoot ratio (i.e. the root biomass per leaf) is denoted by ρ, and we here assume that ρ is a given constant. Let n_s be the nitrate concentration in the soil, and the amount of assimilated nitrogen compounds acquired from the soil per day per unit amount of root is gn_s, where g is the proportionality coefficient. The last term in the right-hand side of Eq. (3) represents the loss of nitrogen at a rate proportional to the amount of nitrogen contained in the leaves Ny.

In Eq. (3), δ is the rate of nitrogen loss per day per leaf. In plant physiological ecology, the inverse of δ is called the "mean residence time of nitrogen (abbreviated as MRT)". This loss of leaf nitrogen might be caused by herbivory, physical disturbances, or aging. The rate of loss decreases with the leaf longevity (Wright and Westby, 2003). In addition, plants relocate the nitrogen from aged leaves to young leaves, and only a fraction of the leaf nitrogen is lost when a leaf dies of old age. δ is $\delta = (1 - r_e)/$[leaf longevity], where r_e is the fraction of leaf nitrogen recovered until its death. The between-species variation of $\delta(= 1/$MRT$)$ is mostly caused by the variation in leaf longevity (Aerts and Chapin, 1999).

In the stationary plant model, the balance of nitrogen must be maintained. We hence set Eq. (3) equal to zero and obtained the leaf nitrogen content as follows:

$$N = \frac{1}{\delta}\left(\frac{hx}{y} + gn_s\rho\right) \qquad (4)$$

Eq. (4) indicates that the amount of nitrogen per leaf is the sum of two terms: the first is proportional to the number of root nodules per leaf (x/y) and the second is proportional to the soil nitrate concentration multiplied by the root/shoot ratio ($n_s\rho$). We also note that the number of nodules needs to be either positive or zero: $x \geq 0$. This leads to an inequality constraint of nitrogen content per leaf as $N \geq gn_s\rho/\delta$.

We then search for the optimal number of nodules x that maximizes the net rate of gain of the photosynthetic products, given by F in Eq. (2), under the constraint of Eq. (4) and the inequality constraint $x \geq 0$. We assume that the number of leaves y and soil nitrate concentration n_s and other parameters are given. (See SI Appendix A for the analysis).

2.1. Parameter dependence of the optimal nodulation

According to the analysis in SI Appendix A, we obtain the optimal number of root nodules per leaf as follows:

$$\frac{x}{y} = \frac{1}{h}\left[\delta L\left(\sqrt{\frac{abh}{c\delta}} - b\right) - gn_s\rho\right] \qquad (5)$$

if the right-hand side is positive. If it is negative, the optimal plant has no root nodules, $x = 0$.

Fig. 1A is a contour map of the optimal root nodule number per leaf (x/y), where the horizontal and vertical axes are the light intensity L and soil nitrate concentration n_s, respectively. We can see that more root nodules (per leaf) should be produced for strong light intensity (large L) and poor soil (small n_s).

Fig. 1B is a contour map of the leaf nitrogen content N when the optimal number of nodules is produced. The two axes are the same as those in Fig. 1A. According to the derivation in SI Appendix A, the leaf nitrogen content is given as follows:

$$N = max\left[L\left(\sqrt{\frac{abh}{c\delta}} - b\right), \frac{gn_s\rho}{\delta}\right] \qquad (6)$$

This indicates that the leaf nitrogen content should be controlled by (proportional to) the light intensity L when nodules are produced. In contrast, the leaf nitrogen content should be controlled by the soil nitrate concentration n_s when no nodules are produced.

Fig. 2A indicates that there exists a threshold light intensity $L_c = gn_s\rho/\delta\left(\sqrt{abh/c\delta} - b\right)$, and no nodule should be produced when $L \leq L_c$. When $L > L_c$, some nodules are produced and the number of nodules per leaf (x/y) increases linearly with the light intensity L. Fig. 2B indicates the leaf nitrogen content. When no nodules are produced, N is a level given by the soil nitrate concentration n_s. When nodules are produced, N increases linearly with the light intensity L.

Fig. 3A indicates the dependence of the optimal number of nodules per leaf x/y on the soil nitrate concentration. The horizontal axis is for n_s, the nitrate concentration in the soil. The optimal nodule number decreases linearly with n_s, when n_s is smaller than the threshold level, $n_{sc} = L\left(\sqrt{abh/c\delta} - b\right)/g\rho$. No nodule should be produced when $n_s \geq n_{sc}$. Fig. 3B indicates the leaf nitrogen content N. For $n_s < n_{sc}$, N is independent of the soil nitrate level, in which the plant produces root nodules and obtains nitrogen by the fixation of atmospheric molecular nitrogen. For $n_s \geq n_{sc}$, the N is directly proportional to the soil nitrate concentration, n_s.

Fig. 4 shows the dependence on the loss rate of leaf nitrogen δ, which is 1/MRT. Fig. 4A indicates that the number of root nodules per leaf x/y shows a nonmonotonic dependence on δ. No nodules should be produced either for very fast loss rates or very slow loss rates. Nodules may be produced for intermediate levels of loss rate: $\delta_1 < \delta < \delta_2$. As the loss rate δ increases starting from δ_1, the optimal nodule number per leaf x/y increases and has a peak at an intermediate value δ. As δ increases further, x/y decreases with δ, and finally becomes zero at $\delta = \delta_2$. Fig. 4B indicates that the leaf nitrogen content N declines with the loss rate δ.

If the loss rate δ is very small ($\delta < \delta_1$), no nodules are produced because the plant finds no need to obtain nitrogen beyond the level supplied by nitrate absorbed from the soil. As the loss rate δ increases, the plant finds it profitable to obtain more nitrogen, and having costly nodules becomes profitable. In contrast, it is not easy to intuitively explain the reason why no root nodules are produced for very high loss rates ($\delta > \delta_2$). Probably, the leaf nitrogen content quickly decreases as δ increases (as shown in
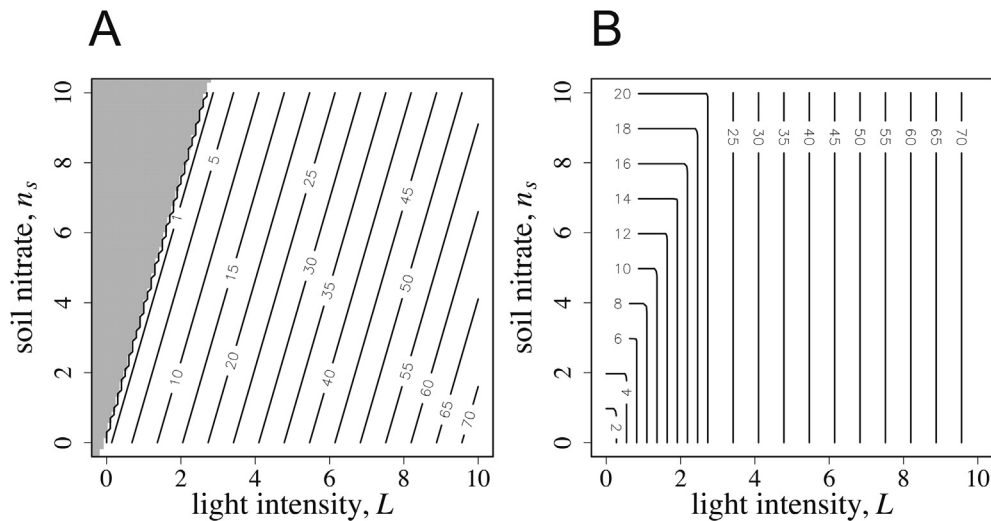
A

B

Fig. 1. Contour maps of the stationary plant model. The horizontal and vertical axes are the light intensity L and soil nitrate concentration n_s, respectively. (A) The optimal root nodule number per leaf (x/y). More root nodules per leaf should be produced for a stronger light intensity (larger L) and poor soil (small n_s). The shaded region indicates parameter combinations in which plant should produce no root nodule ($x = 0$). (B) The leaf nitrogen content N when the optimal number of nodules is produced. The parameters are: $\delta = 1$, $a = 15$, $b = 10$, $h = 1.0$, $c = 0.5$, $g = 1$, and $\rho = 2$, unless specified otherwise.
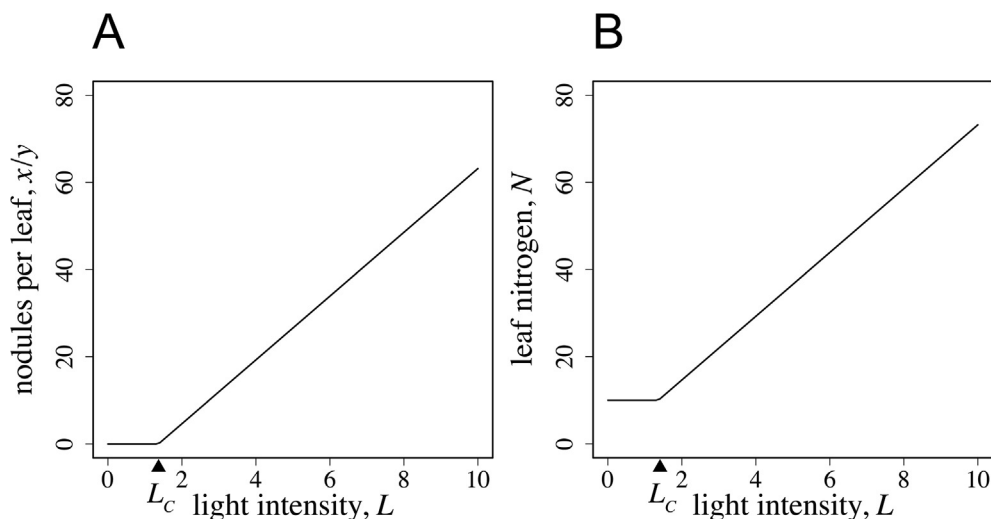
A

B

Fig. 2. The optimal solution of the stationary plant model. Horizontal axis is the light intensity at foliage L. (A) The number of root nodules per leaf, x/y. If L is lower than the threshold level L_c, no nodule should be produced. If L is higher than L_c, the number of root nodules should increase linearly with L. (B) The nitrogen content per leaf N. If $L < L_c$, N is a positive constant independent of L. However, if $L > L_c$, N increases linearly with L. We set $n_s = 5$. The other parameters are the same as in Fig. 1.

Fig. 4B), and the nitrogen absorbed from the soil nitrate would become sufficient for replenishing the leaves.

We note that neither the nodule number per leaf x/y nor leaf nitrogen content N is dependent on the maintenance cost m in the stationary plant model. If the maintenance cost m is enhanced, the amount of reproductive allocation from excess photosynthetic products declines, and the optimal root nodule number and leaf nitrogen content remain unchanged.

3. Growing plant model

The model studied in previous sections assumed that the size of the individual plant remains constant. All the income by photosynthesis minus maintenance cost will be allocated to the reproductive activities, e.g. producing flowers and fruits. This model is suitable for the optimal number of root nodules for a mature plant. However, a plant in vegetative phase performs no reproductive activity, and the income obtained by photosynthesis minus main-

tenance cost will be used to increase the number of leaves, the root size, and the number of root nodules. Reproduction will occur later when the plant reaches a sufficiently large size. In such a situation, the optimal allocation to the root nodule production, considering the cost and benefit, should be determined by considering the rate of growth of the whole individual. The use of the growth rate as a surrogate for the fitness is justified by the general life history strategy theory. For example, the optimal balance of multiple organs (e.g. shoot/root ratio) of plants in the vegetatively growing phase is realized by the one that achieves the fastest growth of the whole plant (e.g., Iwasa and Roughgarden 1984).

In Appendix B, we developed the analysis of this model. We set the following assumptions: The number of leaves, the size of the root , the number of root nodules, and the total amount of nitrogen in the body increase exponentially, in proportion to e^{rt}, where r is the exponential rate of growth of the whole plant size. We denote the number of leaves by $y(t)$ and the number of root nodules by $x(t)$. The plant can choose the number of root nodules per leaf
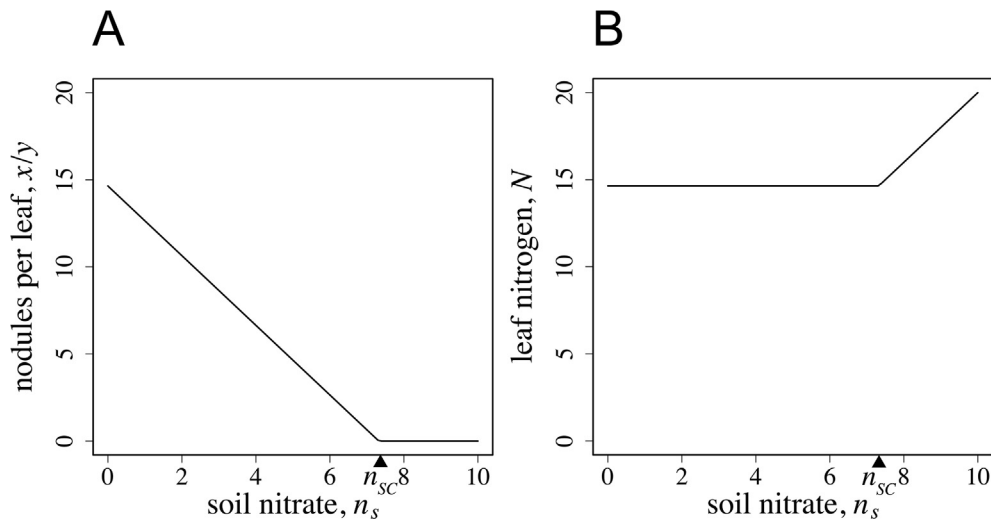
Fig. 3. The optimal solution of the stationary plant model. The horizontal axis is the soil nitration concentration n_s. (A) The number of root nodules per leaf, x/y. If the soil nitration concentration is lower than the threshold level n_{sc}, the number of nodules should decline linearly with n_s and becomes zero when $n_s = n_{sc}$. If $n_s > n_{sc}$, no root nodule should be produced. (B) The nitrogen content per leaf N. If $n_s < n_{sc}$, the leaf nitrogen level N is a positive constant independent of n_s. However, if $n_s \geq n_{sc}$, N increases linearly with n_s. We set $L = 2$. The other parameters are the same as in Fig. 1.
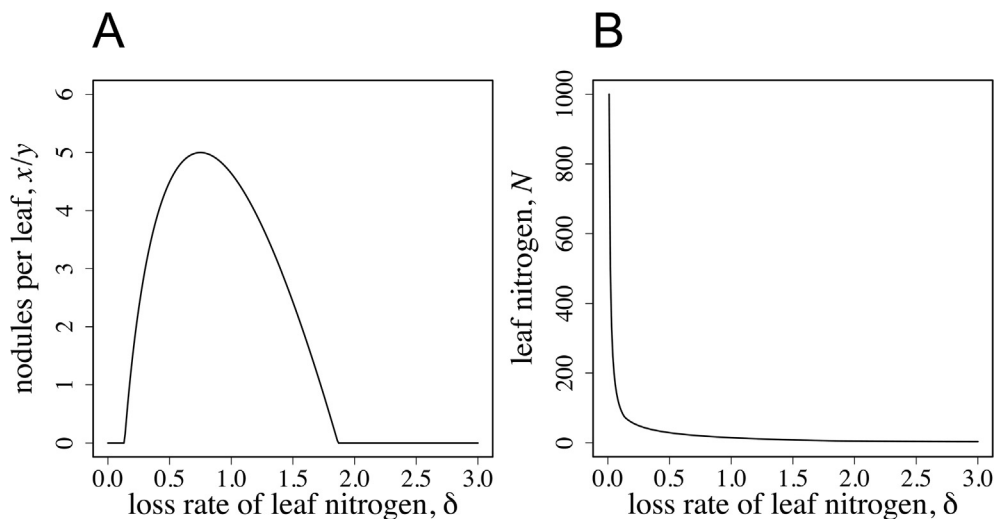

Fig. 4. The optimal solution of the stationary plant model. The horizontal axis is the loss rate of leaf nitrogen δ. (A) The number of root nodules per leaf, x/y. It is positive for an intermediate value of δ ($0.13 < \delta < 1.87$), but it is zero both for $\delta < 0.13$ and for $\delta > 1.87$. The largest number of root nodules per leaf should be produced for a plant with an intermediate rate of loss. (B) The nitrogen content per leaf N. It decreases rapidly with δ. We set $L = 2$, and $n_s = 5$ The other parameters are the same as in Fig. 1.

$x(t)/y(t)$ to maximize the exponential growth rate r. We have two equations indicating the balance of carbon and that of nitrogen, respectively. Using these formulas, we can see leaf nitrogen concentration N increases with the number of root nodules per leaf. Having more root nodules per leaf would increase nitrogen content N, which should enhance the photosynthetic rate (the income of carbon). However, due to the construction cost and the maintenance cost accompanied by root nodules, having an excess number of nodules may not improve the fitness. We can calculate the optimal leaf nitrogen content that achieves the fastest growth of the plant. However, there is another constant $x(t) \geq 0$, indicating that the number of root nodules cannot be negative. Hence, the optimal solution is either to produce some root nodules (i.e. $x(t)/y(t) > 0$), or to produce no root nodules ($x(t)/y(t) = 0$). All the analyses are explained in SI Appendix B. In the following we explain how the optimal strategy of the plant depends on parameters.

3.1. Behavior of the optimally growing plant

In contrast to the stationary plant model, the growing plant model includes a new parameter K, which is the cost of producing a new leaf. A larger K implies that more resources are needed to produce new leaves, leading to a slower growth rate of the whole plant. Another quantity that is new to the growing-plant model is r, the exponential rate of increase. Note that r is not a parameter but a quantity determined as a result of the dynamics (see Appendix B).

Fig. 5A illustrates a contour map for the optimal number of root nodules per leaf x/y. The horizontal and vertical axes are for L and n_s, respectively. The parameters are the same as those in the stationary plant model shown in Fig. 1A. In both figures, the optimal plant has no nodule when soil nitrogen n_s is high and light intensity L is low, but has some root nodules when n_s is low and L is
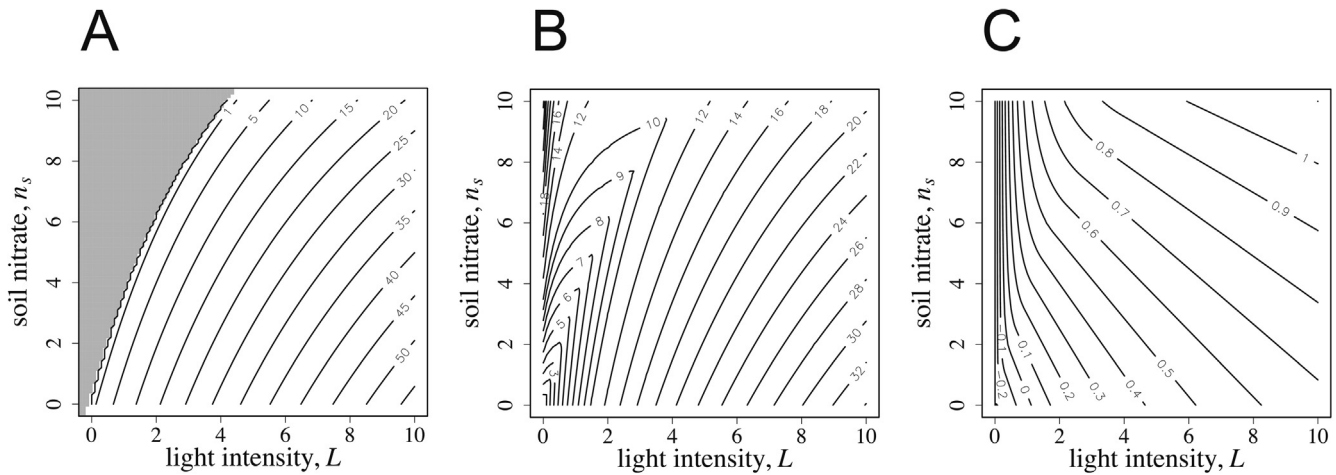
Fig. 5. Contour map of the growing plant model. The horizontal and vertical axes are the foliage light intensity L and soil nitrate concentration n_s, respectively. (A) The optimal number of root nodules per leaf x/y. The two regions are separated by a curve. Nodules are produced for large L and small n_s, but no nodules are produced for small L and large n_s, as indicated by the shaded region. (B) The leaf nitrogen content N. When nodules are produced, N is larger for large L and small n_s; when no nodules are produced, N is larger for small L and large n_s. (C) The plant growth rate r. The growth rate is larger for large L and large n_s. We set $K = 10$, and $m = 3$. The other parameters are the same as in Fig. 1.

high. The boundary between these two regions is a curve in Fig. 5A, in contrast to a straight line in Fig. 1A. The contours of the root nodule number per leaf are also curves with positive slopes in Fig. 5A. Note that nodule number per leaf x/y in Fig. 5A is smaller than that in Fig. 1A, at the same values of parameters L and n_s.

Fig. 5B illustrates a contour map for the leaf nitrogen content N. This looks much more complex than in the stationary plant model (Fig. 1B). In Fig. 5B, the contours between the region with no nodule produced ($x = 0$) and the one with some nodules produced ($x > 0$) are curves, instead of straight lines like in Fig. 5A. Interestingly, N is high for both large L and small n_s (the bottom-right corner) and small L and large n_s (the top-left corner). N has a valley near the boundary between the two regions.

Fig. 5C illustrates a contour map for the growth rate of the whole plant r. We can see that the growth rate of the plant is fast where both light L and soil nitrogen n_s are abundant. This is a plausible and intuitively understood result, although their exact values need to be calculated numerically.

To understand the complex-looking Fig. 5B intuitively, it is useful to note that the faster growth of a plant (larger r) tends to reduce the leaf nitrogen content (smaller N), owing to the "dilution effect" as follows: we may imagine two individual plants equal in the number of leaves, the number of root nodules, the nitrogen content, soil nitrogen level, and the foliage light intensity and all the other parameters, but they differ in the growth rate r. If the first plant has a smaller construction cost K and/or maintenance cost m per leaf, r is larger for the first plant than the second one. Thus the leaf nitrogen level must be smaller for the plant with the larger r. This is because the nitrogen currently held by a plant was either absorbed from soil or fixed by rhizobial bacteria in the past. The size of the plant in the past was smaller for the faster growing plant than for the slower growing plant. Hence, the amount of nitrogen for the faster growing plant must be lower than the slower growing one, which was pointed out by Hikosaka and Osone (2009). When there is no root nodule, all the leaf nitrogen originates from the soil absorption, and the dilution effect can be shown mathematically: from Eq. (B.8) (in SI Appendix B) with $x = 0$, we have a formula $N = g\rho n_s/(r + \delta)$, which indicates that the leaf nitrogen content N decreases with the growth rate r. From Fig. 5C, the plant growth rate r is faster when both L and n_s are larger, and then dilution effect would be stronger.

If we focus on the parameter region for no root nodule production, the stationary plant model would predict that the leaf nitro-

gen level is controlled by the soil nitrogen level n_s but is independent of foliage light intensity L, as indicated by the contours of N parallel to the L-axis in Fig. 1B. However, in the growing plant model, there exists the dilution effect in addition to the direct effect of n_s. This effect reduces the leaf nitrogen for a higher r, caused by higher L and n_s. This explains why N decreases with the light intensity L in the top-left portion of Fig. 5B.

As a consequence, we have a contour map for the leaf nitrogen content in Fig. 5B, which indicates that N is high with both high L and low n_s and with low L and high n_s, which are the bottom-right portion and the top-left portion of Fig. 5B. In between them, there is a valley where the leaf nitrogen content is low. The leaf nitrogen content becomes larger as the point moves away from the boundary between the two regions. The location of the valley is given by the boundary between the areas with nodules and without nodule, and it is indicated by the inflection points of the contours in Fig. 5B.

4. Discussion

Legumes are a very successful family of terrestrial plants (Yahara et al., 2013). They are distributed widely throughout tropical, temperate, and boreal forests, from dry regions to wet regions, and include trees, shrubs, and herbaceous forms (Sprent et al., 2017). Their success is owing to their ability to become symbiotic with nitrogen-fixing bacteria in nodules. Having more root nodules is not necessarily profitable for the plant, because the plant must supply some photosynthetic products to the root nodules. In this paper, we studied the optimal number of root nodules for the plant to maintain nitrogen-fixing rhizobial bacteria.

4.1. Having root nodules is advantageous to the plants in open habitats

As indicated in Fig. 1A for the stationary plant model, there are two separate regions and the behavior of the plant is quite different between them. The plant should produce some nodules when $\delta L\left(\sqrt{abh/c\delta} - b\right) > gn_s\rho$. The number of nodules per leaf increases with the light intensity L and decreases with the soil nitrate concentration n_s (Fig. 1A). The leaf nitrogen content N is proportional to the light intensity L (Fig. 1B). A higher light intensity at foliage indicates a higher demand of nitrogen to the plant, which increases the leaf nitrogen accordingly by producing more root nodules.

Interestingly, the leaf nitrogen content N is predicted to be independent of the soil nitrate level n_s.

In contrast, if the opposite inequality holds, the plant should produce no nodule $x = 0$, and the leaf nitrogen level is controlled by the soil nitrate level n_s, and is independent of the light level L in the stationary plant model.

Legumes have a competitive advantage to other plants that do not have nodule symbiosis when $\delta L\left(\sqrt{abh/c\delta} - b\right) > gn_s\rho$. Hence, legumes dominate in habitat with high light intensity (large L), frequent physical disturbances and short-lived leaves (large δ), poor soil (small n_s), and small root (small ρ). This predicts that legumes, nitrogen-fixing plants, should be more abundant in open habitats with high grazing pressure and/or physical disturbances (e.g. fire), rather than shaded environments (Arianoutsou and Thanos 1996, Hiers et al. 2000). Interestingly, once plants possess root nodules, the leaf nitrogen level of the stationary plant is predicted to be high and be independent of the soil nitrate level.

This prediction is consistent with recent experiments with *Medicago truncatula* and its symbiont *Ensifer medicae*, which demonstrated that legumes adjust the allocation to rhizobial nitrogen fixation in response to the light and nitrogen manipulation (Friel and Friesen, 2019).

4.2. Dilution effect

The optimal allocation of growing plants in the vegetative phase was formalized as the one achieving the fastest growth rate, an assumption common to the model of the root/shoot balance of a terrestrial plant (Iwasa and Roughgarden, 1984) and that for alkaloids in leaves of different ages (Iwasa et al., 1996). A similar assumption was also adopted for models of task allocation among workers in an ant colony (Iwasa and Yamaguchi, 2020).

The parameter dependence of the optimal leaf nitrogen content of the growing host plant (Fig. 5B) is qualitatively similar to but looks much more complex than that of the corresponding stationary plant (Fig. 1B). These differences can be explained by considering the "dilution effect" (Hikosaka and Osone, 2009), namely a plant growing faster tends to have lower nitrogen content in leaves than a plant in the stationary state, if other parameters are equal. Since the plant growth rate r is higher for larger L and larger n_s, this would distort the contours in Fig. 1B to those in Fig. 5B. This tendency is more exaggerated when the leaf production cost is smaller, resulting in faster plant growth, as illustrated in Fig. S2 in SI Appendix B. In a similar manner, the optimal number of root nodules is smaller for a fast-growing plant (Fig. 5A), than for a stationary plant (Fig. 1A), if L and n_s are the same between them.

We must note that the dilution effect is applicable to the comparison of a growing plant in the vegetative phase and another plant in the reproductive phase, the latter performing reproductive activities without size growth, given they live in the same environment (e.g. the same L and n_s). This is useful to understand the dependence of the growing plant shown in Fig. 5B. However, it is incorrect to interpret that the dilution effect might suggest that plants sampled from different locations should exhibit a negative correlation between the growth rate and the number of nodules or the leaf nitrogen content. If we just compared plants observed in field, we would certainly see many examples in which rapidly growing plants with many more nodules and have a higher nitrogen content per leaf than slowly growth plants, just because they live in the environments with different L and n_s. But the dilution effect indicates that the leaf nitrogen content of a rapidly growing immature plant is lower than a mature plant that does not grow if they live with the same L and n_s.

Dilution effect is a key concept in understanding the adaptation of nodulation in legumes, but testing it requires careful experiment and careful comparative study (e.g., Wright et al., 2005; Yahara et al., 2013).

4.3. Why should the root nodule number be controlled by signals from leaves?

One feature of the autoregulation of nodulation is long-distance control, i.e. chemical signals produced in the leaves regulate the root nodule production (Carroll et al., 1985; Krusell et al., 2002; Searle et al., 2003). This can be explained by the result of our analysis. Whether or not the plant benefits from having root nodules must be answered by considering the nitrogen needs of the plant. Nitrogen is important to the photosynthesis only for the environment with high light intensity. If the light intensity on the leaves is low, the plant does not need much nitrogen, and the absorption from the roots may be sufficient. Hence the autoregulation mechanism requires information concerning the need for nitrogen based on the light availability at the leaves.

All the physiological and molecular studies of the mechanism for the autoregulation of nodulation have been concerning the pathway realizing the response to soil nitrate concentration. According to our analysis, the nodulation also needs to respond to the light availability, and there can be pathways responsible for this dependence.

The light level L in the model implies the level expected in the near future, rather than the current one. In fact, the nodulation of *Lotus japonicus* is controlled by sensing the red/far-red (R/FR) ratio (Suzuki et al., 2011), indicating that the root nodule formation is suppressed under the presence of competing plants.

In Table 2, we listed up major predictions of the models and whether they have been tested by observation or by experiment.

4.4. Ecology of N-fixing plants and their distributions

Nitrogen-fixing terrestrial plants have a high species diversity in tropical forests (Yahara et al., 2013). Hedin et al. (2009) considered this to be a paradox because the soil nitrogen level is high in tropical forests.

One of the major conclusions of the model is the condition in which plants possess root nodules, which is given by the following inequality:

$$[\text{leaf nitrogen content}] \times \{[\text{plant growth rate}] + [\text{loss rate}]\}$$
$$> g \times [\text{root/shoot ratio}] \times [\text{soil nitrogen concentration}] \quad (7)$$

which is derived in SI Appendix B. g is a constant for the absorption rate of nitrogen per root mass per soil nitrogen concentration. The optimal number of nodules per leaf is proportional to the difference between both sides (see Eq. (B.8)).

Please note that, both [leaf nitrogen content] and [plant growth rate] in Eq. (7) are quantities determined by other parameters. The inequality (7) does not explicitly include the leaf maintenance and construction costs or the root nodule maintenance cost. This appears to be inconsistent with studies of systems ecological modeling which concluded that a lower cost of nitrogen fixation in tropical forests than in temperate and boreal forests should explain the greater nitrogen fixation in the tropics than in areas of higher latitudes (e.g. Menge et al., 2008; Fisher et al., 2010). In the formalism of this paper, a higher cost of nitrogen fixation reduces both the leaf nitrogen content N and plant growth rate r, and makes inequality (7) less likely to hold.

Based on comparative studies of 175 sites worldwide, Wright et al. (2005) discovered that the leaf nitrogen content is higher with both higher temperature and irradiance. They have also shown that the leaf longevity of evergreen trees decreases with the mean temperature, while that of deciduous trees increases

Table 2
The predictions of the models and empirical studies.

[Prediction 1]
The number of root nodules per leaf adaptive to the host plant varies with the nitrate availability in soil and with the light intensity at leaves. The number of root nodules should decrease with soil nitrate concentration and increase with the light intensity at leaves.
[Test] The predicted responses of plants have been demonstrated by manipulation experiments.
The molecular study on the pathway controlling nodulation is limited to the one suppressing the number of newly formed nodules by the amount of nitrogen in the soil or the presence of existing root nodules. No study has been conducted on the pathway by which the intensity of light received by the leaves increases the number of nodules.
[Prediction 2]
The number of nodules to produce should be determined by combining the information on the soil nitrate concentration and that on the light intensity at leaves.
[Test] A chemical signal is produced in the root tissue, reflecting the environment in the soil, which acts on the leaf tissue. In response to this, another chemical signal is produced in the leaf tissue and affect the root tissue in order to suppress nodule formation.
This is called long-distance control. We can infer that the plant needs long-distance control because the availability of two different resources at different locations (the nitrate availability in the soil and the light intensity in the leaves) must be combined to determine the optimal control of root nodulation.
[Prediction 3]
For a mature plant that does not grow fast, if it has no root nodule, the leaf nitrogen content increases with the nitrate concentration in the soil, and it is independent of the intensity of light at leaves.
In contrast, if the plant produces root nodules, the leaf nitrogen concentration in the soil increases with the light intensity at leaves and does not depend on the amount of nitrogen in the soil.
[Test] These interesting quantitative predictions have not been tested. It is probably because most experiments are carried out with small vegetatively growing plants. The model predicts different responses for stationary plants and for vegetative growing plants (see Growing Plant Models).
[Prediction 4]
Plants in the vegetative phase grow faster when the nitrogen availability in soil and the light intensity in leaves are higher.
[Test] The predictions are supported by observations.
[Prediction 5]
The leaf nitrogen content of a vegetatively growing plant is high both for low soil nitrate concentration and high leaf light availability and for high soil nitrate concentration and low leaf light availability. In between these two, the leaf nitrogen content tends to be lower.
[Test] The predictions have not been tested.
[Prediction 6]
The amount of nitrogen per leaf is higher for a plant in the reproductive phase than for a plant in the vegetative phase the latter growing in size rapidly, if both live in the environment with exactly the same light intensity and soil nitrogen concentration.
[Test] The prediction has not been tested.

with the mean temperature, which was the pattern predicted by the model on the economics of leaf longevity (Kikuzawa, 1996; Kikuzawa et al., 2013). The loss rate of leaf nitrogen (i.e. the inverse of the mean retention time of nitrogen) is about inversely proportional to the leaf longevity. Hence, among evergreen trees, the loss rate of leaf nitrogen might be faster in tropical forests than in temperate or boreal forests. We must be careful that the data analyzed by Wright et al. (2005) included herbs, shrubs, and trees, and also the data on plants other than legumes. However, we can see possibility of the general trend that the left-hand side of Eq. (7) can be greater in tropical forests than in temperate or boreal forests. A more careful quantitative study of functional traits other than just the soil nitrogen concentration is required. This might verify that our observation in different ecosystems is not a paradox, but adaptive responses of the plants.

CRediT authorship contribution statement

Ryota Kobayashi: Conceptualization, Formal analysis, Investigation, Visualization, Writing. **Sachi Yamaguchi**: Conceptualization, Formal analysis, Investigation, Visualization, Writing. **Yoh Iwasa**: Conceptualization, Formal analysis, Investigation, Visualization, Writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

R.K., S.Y. and Y.I. designed the research, performed the research, and wrote the paper.
Data availability
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtbi.2020.110544.

References

Aerts, R., Chapin III, F.S., 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30, 1–67.
Arianoutsou, M., Thanos, C.A., 1996. Legumes in the Fire-Prone Mediterranean Regions: an Example From Greece. Int. J. Wildland Fire 6 (2), 77–82.
Carroll, B.J., McNeil, D.L., Gresshoff, P.M., 1985. A supernodulation and nitrate-tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78, 34–40.
Chapin, F.S., Walkers, L.R., Fastie, C.L., Sharman, I.C., 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. Ecol. Monogr. 64, 149–175.
Ellis, R.J., 1979. Most abundant protein in the world. Trends Biochem. Sci. 4, 241–244.
Ferguson, B.J., Indrasumunar, A., Hayashi, S., Lin, M.H., Lin, Y.H., Reid, D.E., Gresshoff, P.M., 2010. Molecular analysis of legume nodule development and autoregulation. J. Integr. Plant Biol. 52, 61–76.
Fisher, J.B., Sitch, S., Malhi, Y., Fisher, R.A., Huntingloard, C., Tan, S.-Y., 2010. Carbon cost of plant nitrogen acquisition: a mechanistic, globally applicable model of plant nitrogen uptake, retranslocation, and fixation. Global Biogeochem. Cy. 24, GB1014.
Friel, C.A., Friesen, M.L., 2019. Legumes modulate allocation to rhizobial nitrogen fixation in response to factorial light and nitrogen manipulation. Frontier Plant Sci. 10, 1316.

Gulmon, S.L., Chu, C.C., 1981. The effects of light and nitrogen on photosynthesis, leaf characteristics, and dry matter allocation in the chaparral shrub, *Diplacus aurantiacus*. Oecologia 49 (2), 207–212.

Hedin, L.O., Brookshire, E.N.J., Menge, D.N.L., Barron, A.R., 2009. Nitrogen paradox in tropical ecosystems. Annu.l Rev. Ecol. Evol. S. 40, 613–635.

Hiers, J.K., Wyatt, R., Mitchell, R.J., 2000. The effects of fire regime on legume reproduction in longleaf pine savannas: is a season selective?. Oecologia 125, 520–530.

Hikosaka, K., Osone, Y., 2009. A paradox of leaf-trait convergence: why is leaf nitrogen concentration higher in species with higher photosynthetic capacity?. J Plant Res 122 (3), 245–251.

Hikosaka, K., Terashima, I., 1995. A model of the acclimation of photosynthesis in the leaves of C3 plants to sun and shade with respect to nitrogen use. Plant Cell Environ 18 (6), 605–618.

Hirose, T., Werger, M.J.A., 1987. Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. Physiol Plant 70 (2), 215–222.

Iwasa, Y., Roughgarden, J., 1984. Shoot/root balance of plants: Optimal growth of a system with many vegetative organs. Theor. Popul. Biol. 25 (1), 78–105.

Iwasa, Y., Yamaguchi, S., 2020. Task allocation in a cooperative society: specialized castes or age-dependent switching among ant workers. Sci. Rep. 10, 3339.

Iwasa, Y., Kubo, T., van Dam, N., de Jong, T.J., 1996. Optimal level of chemical defense decreasing with leaf age. Theor. Popul. Biol. 50, 124–148.

Kikuzawa, K., 1996. Geographical distribution of leaf life span and species diversity of trees simulated by a leaf-longevity model. Vegetatio 122 (1), 61–67.

Kikuzawa, K., Onoda, Y., Wright, I.J., Reich, P.B., 2013. Mechanisms underlying global temperature-related patterns in leaf longevity. Global Ecol. Biogeogr. 22, 982–993.

Krusell, L., Madsen, L.H., Sato, S., Aubert, G., Genua, A., Szczyglowski, K., Duc, G., Kaneko, T., Tabata, S., de Bruijn, F., Pajuelo, E., Sandal, N., Stougaard, J., 2002. Shoot control of root development and nodulation is mediated by a receptor-like kinase. Nature 420, 422–426.

Martinelli, L.A., Piccolo, M.C., Townsend, A.R., Vitousek, P.M., Cuevas, E., McDowell, W., Robertson, G.P., Santos, O.C., Treseder, K., 1999. Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. Biogeochemistry 46 (1-3), 45–65.

Menge, D.N.L., Levin, S.A., 2017. Spatial heterogeneity can resolve the nitrogen paradox of tropical forests. Ecology 98 (4), 1049–1061.

Menge, D.N.L., Levin, S.A., Hedin, L.O., 2008. Evolutionary tradeoffs can select against nitrogen fixation and thereby maintain nitrogen limitation. Proc. Natl. Acad. Sci. U.S.A. 105, 1573–1578.

Mooney, H.A., Field, C., Gulmon, S.L., Bazzaz, F.A., 1981. Photosynthetic capacity in relation to leaf position in desert versus old-field annuals. Oecologia 50 (1), 109–112.

Nishimura, R., Hayashi, M., Wu, G.-J., Kouchi, H., Imaizumi-Anraku, H., Murakami, Y., Kawasaki, S., Akao, S., Ohmori, M., Nagasawa, M., Harada, K., Kawaguchi, M., 2002. HAR1 mediates systemic regulation of symbiotic organ development. Nature 420, 426–429.

Oka-Kira, S., Tateno, K., Miura, K., Haga, T., Hayashi, M., Harada, K., Sato, S., Tabata, S., Shikazono, N., Tanaka, A., Watanabe, Y., Fukuhara, I., Nagata, T., Kawaguchi, M., 2005. *klavier* (*klv*), a novel hypernodulation mutant of *Lotus japonicus* affected in vascular tissue organization and floral indication. Plant J. 44, 505–515.

Okamoto, S., Ohnishi, E., Sato, S., Takahashi, H., Nakazono, M., Tabata, S., Kawaguchi, M., 2009. Nod factor/nitrate-induced CLE genes that drive HAR1-mediated systemic regulation of nodulation. Plant Cell Physiol. 50, 67–77.

Okamoto, S., Shinohara, H., Mori, T., Matsubayashi, Y., Kawaguchi, M., 2013. Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase. Nature Comm. 4, 2191.

Pearson, H.L., Vitousek, P.M., 2001. Stand dynamics, nitrogen accumulation, and symbiotic nitrogen fixation in regenerating stands of *Acacia koa*. Ecol. Appl. 11, 1381–1394.

Rastetter, E.B., Vitousek, P.M., Field, C., Shaver, G.R., Herbert, D., Agren, G.I., 2001. Resource optimization and symbiotic nitrogen fixers. Ecosystems 4, 369–388.

Raven, J.A., 2013. Rubisco: still the most abundant protein on earth?. New Phytol. 198, 1–3.

Reid, D.E., Ferguson, B.J., Gresshoff, P.M., 2011. Inoculation- and nitrate induced CLE peptides of soybean control NARK-dependent nodule formation. Mol. Plant Microbe In. 24, 606–618.

Sasaki, T., Suzaki, T., Soyano, T., Kojima, M., Sakakibara, H., Kawaguchi, M., 2014. Shoot-derived cytokinins systemically regulate root nodulation. Nature Comm. 5, 4983.

Searle, I.R., Men, A.E., Lanya, T.S., Buzas, D.M., Iturbe-Ormaetxe, I., Carroll, B.J., Gresshoff, P.M., 2003. Long-distance signaling in nodulation directed by CLAVATA1-like receptor kinase. Science 299, 109–112.

Sprent, J.I., Ardley, J., James, E.K., 2017. Biogeography of nodulated legumes and their nitrogen-fixing symbionts. New Phytol. 215, 40–56.

Suzuki, A., Suriyagoda, L., Shigeyama, T., Tominaga, A., Sasaki, M., Hiratsuka, Y., Yoshinaga, A., Arima, S., Agarie, S., Sakai, T., Inada, S., Jikumaru, Y., Kamiya, Y., Uchiumi, T., Abe, M., Hashiguchi, M., Akashi, R., Sato, S., Kaneko, T., Tabata, S., Hirsch, A.M., 2011. *Lotus japonicus* nodulation is photomorphogenetically conrolled by sensing the red/far red (R/FR) ratio through jasmonic acid (JA) signaling. Proc. Natl. Acad. Sci. U.S.A. 108, 16837–16842.

Suzaki, T., Yano, K., Ito, M., Umehara, Y., Suganuma, N., Kawaguchi, M., 2012. Positive and negative regulation of cortical cell division during root nodule development in *Lotus japonicus* is accompanied by auxin response. Development 139 (21), 3997–4006.

Tsikou, D., Yan, Z., Holt, D.B., Abel, N.B., Reid, D.E., Madsen, L.H., Bhasin, H., Sexauer, M., Stougaard, J., Markmann, K., 2018. Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA. Science 362, 233–236.

van Noorden, G.E., Ross, J.J., Reid, J.B., Rolfe, B.G., Mathesius, U., 2006. Defective long-distance auxin transport regulation in the *Medicago truncatula* super numeric nodules mutant. Plant Physiol. 140, 1494–1506.

Vitousek, P.M., Field, C.B., 1999. Ecosystem constrains to symbiotic nitrogen fixers: a simple model and its implications. Biogeochemistry 46, 179–202.

Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea. How can it occur?. Biogeochemistry 13, 87–115.

Vitousek, P.M., Cassman, K., Cleveland, C., Crews, T., Field, C.B., Grimm, N.B., Howarth, R.W., Marino, R., Martinelli, L., Rastetter, E.B., Sprent, J.I., 2002. Toward an ecological understanding of nitrogen fixation. Biogeochemistry 57/58, 1–45.

Wright, I.J., Westoby, M., 2003. Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. Funct. Ecol. 17, 10–19.

Wright, I.J., Reich, P.B., Cornelissen, J.H.C., Falster, D.S., Groom, P.K., Hikosaka, K., Lee, W., Lusk, C.H., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Warton, D.I., Westoby, M., 2005. Modulation of leaf economic traits and trait relationships by climate. Global Ecol. Biogeogr. 14, 411–421.

Yahara, T., Javadi, F., Onoda, Y., de Queiroz, L.P., Faith, D.P., Prado, D.E., Akasaka, M., Kadoya, T., Ishihama, F., Davies, S., Slik, J.W.F., Yi, T., Ma, K., Bin, C., Darnaedi, D., Pennington, R.T., Tuda, M., Shimada, M., Ito, M., Egan, A.N., Buerki, S., Raes, N., Kajita, T., Vatanparast, M., Mimura, M., Tachida, H., Iwasa, Y., Smith, G.F., Victor, J.E., Nkonki, T., 2013. Global legume diversity assessment: Concepts, key indicators, and strategies. Taxon 62 (2), 249–266.