Direct exchange of vitamin B$_{12}$ is demonstrated by modelling the growth dynamics of algal–bacterial cocultures

Matthew AA Grant$^{1,3}$, Elena Kazamia$^{2,3}$, Pietro Cicuta$^{1}$ and Alison G Smith$^{2}$

$^{1}$Cavendish Laboratory, University of Cambridge, Cambridge, UK and $^{2}$Department of Plant Sciences, University of Cambridge, Cambridge, UK

The growth dynamics of populations of interacting species in the aquatic environment is of great importance, both for understanding natural ecosystems and in efforts to cultivate these organisms for industrial purposes. Here we consider a simple two-species system wherein the bacterium Mesorhizobium loti supplies vitamin B$_{12}$ (cobalamin) to the freshwater green alga Lobomonas rostrata, which requires this organic micronutrient for growth. In return, the bacterium receives photosynthate from the alga. Mathematical models are developed that describe minimally the interdependence between the two organisms, and that fit the experimental observations of the consortium. These models enable us to distinguish between different mechanisms of nutrient exchange between the organisms, and provide strong evidence that, rather than undergoing simple lysis and release of nutrients into the medium, M. loti regulates the levels of cobalamin it produces, resulting in a true mutualism with L. rostrata. Over half of all microalgae are dependent on an exogenous source of cobalamin for growth, and this vitamin is synthesised only by bacteria; it is very likely that similar symbiotic interactions underpin algal productivity more generally.

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Introduction

The diverse photosynthetic eukaryotes collectively known as algae are found in all marine and freshwater environments, and may account for up to 50% of the world’s carbon fixation (Field, 1998). Primary production in aquatic systems is generally at equilibrium, and the community composition of the algae, as well as the abundance of individual species, is dependent on the available nutrients. The role of phosphorus and nitrogen has been studied extensively, largely because eutrophication by these elements (for example, by run-off from agriculture, or sediment discharge from rivers) alters the rate of production and community composition in most systems, inducing an algal bloom (Heisler et al., 2008). Iron has also been shown to have a key role, particularly in high-nutrient, low-chlorophyll regions (HNLC) as in the subarctic Northeast Pacific and Southern Oceans (for example, Moore et al., 2001). The importance of iron in limiting productivity was demonstrated experimentally by enriching surface waters of the Southern Ocean with acidic iron sulphate dissolved in sea water, eliciting a strong response in phytoplankton productivity monitored by satellite imaging of chlorophyll fluorescence (Coale et al., 2004).

In addition to these inorganic nutrients, it is becoming increasingly recognised that organic micronutrients in the form of vitamins may also have a role in controlling algal growth. Over half of all microalgal species surveyed (from fresh, marine and brackish habitats) are auxotrophic for cobalamin (vitamin B$_{12}$) that is, they are unable to grow in its absence. Similarly, 20% require thiamine (vitamin B$_{1}$) and 5% require biotin (vitamin B$_{7}$) (Croft et al., 2006). Several studies have demonstrated that fertilisation by B vitamins of natural systems has consequences equivalent to Fe (for example, Sanñudo-Wilhelmy et al., 2006; Gobler et al., 2007). Vitamin auxotrophy is randomly distributed across the algal lineages, with no phylogenetic relationship between those organisms that require the nutrients. For example, even within a single genus there are both requirers and nonrequirers: the heterokont Navicula pelliculosa requires thiamine but other members of the genus do not; Chlamydomonas reinhardtii is vitamin B$_{12}$ independent, whereas C. nivalis is an auxotroph (Provasoli and Carlucci,
The widespread prevalence of auxotrophy would suggest that there is a readily available source of these nutrients in natural ecosystems; otherwise, B12 requirers would suffer from a fitness disadvantage. In fact, the opposite appears to be the case: in a survey of 29 different bloom-forming species the majority were found to be auxotrophs for both thiamine and cobalamin (Tang et al., 2010), suggesting that these species are ubiquitous.

The majority of algae surveyed can synthesise thiamine, and those that require it appear to be missing one or more of the genes for thiamine-biosynthesis enzymes (Croft et al., 2006; Worden et al., 2009; Bertrand et al., 2012). In contrast, vitamin B12, one of the most complex primary metabolites in nature (Figure 1a), is synthesised only by bacteria, requiring more than 20 enzyme-catalysed reactions to do so (Warren et al., 2002). Algal B12 auxotrophs appear to have lost a key enzyme, cobalamin-independent methionine synthase (METE), and instead rely on a form of methionine synthase, METH, which needs B12 as a cofactor (Helliwell et al., 2011). Laboratory studies indicate that a minimum of 20–50 ng l^{-1} exogenous cobalamin is necessary to sustain algal growth axenically (Bertrand et al., 2012; Kazamia et al., 2012). An important question that arises therefore is how, in the natural environment, do algal auxotrophs obtain cobalamin in sufficient quantities for growth from the bacteria that synthesise it? In this context, it should be mentioned that only about one-third of prokaryotes encode genes for the complete cobalamin biosynthesis pathway (Warren et al., 2002).

Measurement of cobalamin levels free in solution in the natural environment indicate that it is in the range of 10–70 ng l^{-1} (San˜udo-Wilhelmy et al., 2006, 2012; Bertrand et al., 2007; Panzeca et al., 2009), although in certain lakes or in some coastal waters, where there are high bacterial loads and/or contamination by sewage, up to 600 ng l^{-1} have been measured (Okbamichael and San ˜udo-Wilhelmy, 2004). Nonetheless, in most habitats there is insufficient vitamin B12 free in solution to support the growth of algal auxotrophs in the photic zone. It has been proposed that vitamin B12 is delivered passively to algae as part of the microbial loop (Karl, 2002). In this model, vitamin B12-synthesising bacteria grow at depth on organic material that sinks from surface waters after organisms in the photic zone die. The bacteria that grow at depth lyse and release macronutrients and micronutrients (including vitamin B12), forming nutrient-rich deep water that is periodically transported back to the surface by upwelling, thus fertilising algal production and closing the loop. An alternative possibility is that algae obtain the vitamin directly from synthesising bacteria associated with them in the same environment, and that the heterotrophic bacteria get photosynthate in return. Symbiosis between algae and bacteria has been widely described, and ranges from obligate interactions, such as that described between the cyanobacterium UCYN-A and a unicellular prymnesiophyte (Thompson et al., 2012), to

Figure 1  Vitamin B_{12}-requiring algae are only able to grow in the presence of cobalamin, which can only be synthesised by some bacteria. (a) The structure of cobalamin (vitamin B_{12}). (b) The model laboratory system developed by Kazamia et al. (2012) to study this interaction. L. rostrata, a vitamin B_{12} auxotroph, grows in the presence of M. loti in autotrophic medium that does not contain cobalamin. Controls indicate that this is because of the exchange of a carbon source for vitamin B_{12}.
facultative mutualisms centred on nutrient exchange. Amin et al. (2009) showed that bacteria within the boundary layer of dinoflagellate algae promoted algal assimilation of Fe by facilitating photochemical redox cycling of this critical nutrient. Similarly, growth of several species of B12-dependent marine algae has been shown to be supported by the presence of the bacterium Halomonas spp. (Croft et al., 2005), and the symbiotic Dinoroseobacter shibae delivered both vitamin B12 and vitamin B to its dinoflagellate host (Wagner-Döbler et al., 2010).

The biological implications of the two methods of vitamin B12 delivery—passive exchange versus direct symbiosis—are widely different. Within the microbial loop, there is no interaction between algae and bacteria, and no need for them to coordinate metabolism. In contrast, if the symbiosis model prevails, it implies that organisms must have a way of recognising each other in order to undergo targeted nutrient exchange. It may also mean that bacteria need to synthesise more vitamin B12 to support the growth of algae in addition to satisfying their own requirement. The provision of fixed carbon would thus compensate for this, whereas in the microbial loop there is no guarantee that B12 producers would have preferential access to algal photosynthate.

Studying interactions between algae and bacteria in natural environments is challenging because of the open and highly complex nature of such systems. With reference to vitamin auxotrophy, which shows no phylogenetic relationship, species-specific interactions are the most important and yet the hardest to infer. For example, it was only after a large-scale metagenomic analysis that it was possible to identify in natural samples the nitrogen-fixing cyanobacterium UCYN-A, whose highly reduced genome indicated that it must have an obligate symbiotic lifestyle. Identifying facultative mutualists would be impossible in this way, as the genomic markers would be more subtle. In this regard, model laboratory systems offer the opportunity to study species-specific interactions that are likely to parallel those in natural systems, but under defined experimental conditions. We have previously developed such a model system between a vitamin B12-dependent freshwater green alga Lobomonas rostrata and a vitamin B12-producing bacterium Mesorhizobium loti (Kazamia et al., 2012), which has the following characteristics: (1) The two organisms can grow in coculture in medium that lacks both B12 and fixed carbon, whereas neither can grow separately in this medium (Figure 1b). (2) In cocultures, algal and bacterial cells reached a steady-state ratio of ~1:30, regardless of the starting population densities of the two organisms. This is true both for batch cultures and semi-continuous cultures where the medium was replenished regularly. (3) The carrying capacity reached in coculture was lower for both algae and bacteria than in axenic controls supplemented with vitamin B12 and a source of carbon, respectively. (4) It was possible to perturb the established equilibrium in cocultures by supplementation with either vitamin B12 or a source of carbon. When vitamin B12 was added to the cocultures, this favoured algal growth without concomitant increase in bacterial densities. Conversely, when a source of carbon was added, bacterial growth was exponential and did not lead to an increase in algal density.

Here, we formulate a mathematical description of the cocultures of L. rostrata with M. loti based on the physiological observations outlined above. The quantitative model arrived at allowed us to test specific hypotheses, particularly whether exchange is due to regulated transfer of nutrients, or is instead mediated by the death and lysis of organisms, as implied by the microbial loop. Although there have been some previous quantitative studies of algal uptake of vitamin B12, these examined certain aspects only, such as the impact of vitamin B12 concentration on algal growth rate (Droop, 1968), and did not consider the environmental source of vitamin B12. The mathematical models presented here take a radical step away from this approach, by considering directly and with minimal complexity the impact of M. loti on the growth of L. rostrata, and vice versa. By considering the biological relevance of the necessary parameters in the model, it is possible to distinguish between mutualism and lysis as a means of delivery of vitamin B12. Shedding light on algal–bacterial dynamics is important both for our understanding of natural aquatic systems and for industrial-scale cultivation of algae. Currently, algae are grown commercially for high-value products and there is considerable interest in cultivating algae for biofuels (Scott et al., 2010). However, the scale at which the latter will be necessary will obviate the possibility to maintain sterile conditions, and thus contamination by bacteria is inevitable. Our model of algal–bacterial interactions could be used to predict quantitatively growth dynamics in such systems.

Materials and methods

Numerical integration
The way in which the mathematical model was developed is detailed in Supplementary Materials Section 1. The model equations were integrated using a custom C++ implementation of the fourth-order Runge-Kutta method.

Measurement of vitamin B12 levels
To determine the amount of vitamin B12 produced by M. loti, axenic cultures of the bacterium and cocultures with L. rostrata were grown in batch culture in TP medium, as described in Kazamia et al (2012). Cells and medium were separated by centrifugation for 5 min at 13 000 g. The supernatant was filtered through a 0.22-μm filter to remove any
remaining cells, whereas the pellet was resuspended in the equivalent volume of TP+ medium and boiled at 100 °C for 15 min to lyse cells and release contents. For measurement of total vitamin B12, the cells were not separated from the medium, and the cultures were boiled to lyse all cells directly. For all boiled samples, there was a filtration step to remove the cell debris. Vitamin B12 levels were determined using the Salmonella typhimurium bioassay (Raux et al., 1996), which provides a semiquantitative estimation of the amount of B12 in a sample by reference to a standard curve.

Results

A mathematical model provides an objective way in which to understand better the interactions between the algal and bacterial cells. To establish such a model, it is necessary to construct equations that are able to describe the behaviour of the model, it is necessary to construct equations that are

1. *L. rostrata*, *a*, and *M. loti*, *b*, cannot grow axenically (by themselves, as a monoculture) in a medium that lacks vitamin B12, V, and a carbon source, C, respectively: if b, V = 0 and b = 0 if a, C = 0.

2. The algae and bacteria reach a steady ratio, r: a/b → r.

3. The carrying capacity (subscript ‘max’) reached in coculture (superscript ‘c’) is lower for both algae and bacteria than in axenic controls (supplemented with vitamin B12 and carbon, respectively—superscript ‘m’ (monoculture)): a\[\text{max}^c < a\[\text{max}^m, b\[\text{max}^c < b\[\text{max}^m.

4. The coculture is perturbed by nutrient add-back. When the system is perturbed by supplementing the medium with vitamin B12, the algal population reaches a higher carrying capacity. If a carbon source is added, then the equilibrium ratio is broken in favour of bacteria, which reach a greater carrying capacity: a/b > r if V > 0, C = 0. a/b < r if C > 0, V = 0.

Starting from a logistic equation for each of *L. rostrata* and *M. loti*, as described in Supplementary Materials Section 1, it was immediately apparent that, to satisfy the conditions, the model cannot have independent equations, as neither the algae nor the bacteria can grow alone without a source of vitamin B12 or fixed carbon, respectively. We then added further terms until, by construction, a minimal form was achieved that can describe the experimental results. In the following, a and b denote the number (population) of algae and bacteria, α and β their respective growth rates and Kc and Kb their carrying capacities. Two models were able to satisfy the experimental observations:

Model 1

This model features a carrying capacity term for the culture that is dependent on the population density of each organism, and which saturates at high numbers. There is also an independent term (Kc or Kb) for when there is add-back of nutrients (vitamin B12 or fixed carbon, respectively). In this model, provision of the nutrient is maximal at equilibrium; an increase in numbers of bacteria will have no effect on algal growth, and vice versa. This ‘unregulated’ model can be interpreted in different ways, and fits the suggestion that algae can grow using vitamin B12 released by bacteria following death and cell lysis (Droop, 2007). A form of Model 1 that reflects this explicitly, and that satisfies conditions 1–4, is

\[
\begin{align*}
\dot{a} &= \alpha - a \left( K_c b b + K_b \right)^{-1}, \\
\dot{b} &= \beta - b \left( K_a - K_c a + K_b \right)^{-1} - \delta b.
\end{align*}
\]

The lysis term, δ, describes the fraction of the bacterial cells that die per unit time, and it is these cells that enable the growth of *L. rostrata* in cocultures with *M. loti*. The composite term \(K_c b b/(b_c + \delta b)\) defines the carrying capacity of the algae that is due to bacterial presence (following lysis). This approach of modelling the carrying capacity of an organism as a function of its symbiont was first proposed by Yukalov et al. (2012). \(K_b\) is the maximum number of bacteria that the algae are able to support and \(\delta b\) is the total number of bacteria in the culture that die per unit time. When vitamin B12 is provided in the medium, growth is dependent on \(K_c\), which is the maximum number of algae that any externally added vitamin B12 provided can support.

Model 2

This model is similar to Model 1, but it includes an additional term to describe the dynamics during nutrient add-back. Effectively, it allows for a change in behaviour between algae and bacteria when nutrients are externally supplied. This is achieved through the use of the Heaviside function. For both algal and bacterial growth, the Heaviside step function, defined as

\[
H(x) = \begin{cases} 
0, & x \leq 0 \\
1, & x > 0, 
\end{cases}
\]

is incorporated to reflect the hypothesis that vitamin B12 is only made available to algae when there is no additional carbon in the medium. In other words, when fixed carbon is provided in
the medium, the Heaviside function takes on the value of 0 (zero) and the size and influence of nutrient exchange in the carrying capacity shrinks.

The motivation for inclusion of this term is that the experimental data suggest a reduction of the algal population when bacterial growth is stimulated by the addition of fixed carbon. Model 2 takes the following form:

\[
\dot{a} = \alpha a \left[ 1 - a \left( \frac{K_a b (1 - H(K_v) \delta_b)}{b_c + b} + K_v \right) \right]^{-1},
\]

(4)

\[
\dot{b} = \beta b \left[ 1 - b \left( \frac{K_d a (1 - H(K_v) \delta_a)}{a_c + a} + K_v \right) \right]^{-1}.
\]

(5)

Here, the carrying capacity of the algae is modelled with two terms. A composite term \(K_c b (1 - H(K_v) \delta_b)/(b_c + b)\) defines the carrying capacity of the algae that is due to the presence of bacteria. When there is no vitamin B12 added to the medium, Model 2 assumes that algal growth is dependent entirely on this composite term; however, when exogenous B12 is added, growth is also dependent on \(K_v\), which is the maximum number of algal cells that can be supported by the concentration of the added vitamin.

Bacterial growth is modelled similarly, with \(K_c\) representing the carrying capacity when carbon is added externally into the medium, and the composite term \(K_d a (1 - H(K_v) \delta_a)/(a_c + a)\) representing the carrying capacity due to algal presence. It should be noted that Model 2 reduces to Model 1, upon rescaling some of the parameters, and setting \(\delta_a = \delta_b = 0\).

Choosing parameter values. The parameters in Table 1 were fitted to the data, using values from the literature where possible and varying the rest until a satisfactory qualitative fit was achieved. The results are shown in Figure 2a, where the values of numbers of algal and bacterial cells that would arise from the two models with four different starting values of \(a\) and \(b\) are shown as lines. These trend lines fit the experimental data (shown as individual symbols) very closely, indicating that the models are able to describe the regulation of growth within the coculture as it tends to the equilibrium ratio of \(\approx 1:30\) algal to bacterial cells. Both models fit equally well. Figure 2b shows the trend lines of the models for the algal and bacterial cells after the addition of nutrients. Again the models are able to recreate the qualitative effects seen in the experimental data (shown as symbols), namely that addition of the carbon source results in an increase in bacterial numbers but no increase in algal cells, whereas addition of vitamin B12 causes an increase in algal cells with no corresponding increase in bacterial numbers. However, the experimental data suggest that the algal and bacterial numbers might actually decrease when carbon and vitamin B12, respectively, are added to the medium. Model 2 has the additional parameters \(\delta_a\) and \(\delta_b\) that enable it to capture such a decrease (Figure 2b Model 2, top panel), whereas Model 1 does not (Figure 2b Model 1, top panel).

Distinguishing between the two models. Because of the biological variability associated with the experimental measurements, we carried out further laboratory experiments to enable us to distinguish with more certainty which model best describes the

![Table 1](image)

<table>
<thead>
<tr>
<th>Parameter Symbol</th>
<th>Description</th>
<th>Valuea</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(x)</td>
<td>Growth rate of (L.\ rostrata)</td>
<td>1.5 log2 days(^{-1})</td>
<td>Harris (2001)</td>
</tr>
<tr>
<td>(K_a)</td>
<td>Maximum number of algae that the bacteria can support</td>
<td>(4 \times 10^6) ml(^{-1})</td>
<td>Fitted</td>
</tr>
<tr>
<td>(b_c)</td>
<td>Number of bacteria required to reach half the maximum carrying capacity, (K_c)</td>
<td>(10^6) ml(^{-1}) (M1)</td>
<td>Fitted</td>
</tr>
<tr>
<td>(\delta_a)</td>
<td>Fractional decrease in carbon production by algae when vitamin B12 is provided externally in the medium</td>
<td>0.4</td>
<td>Fitted</td>
</tr>
<tr>
<td>(K_v)</td>
<td>Maximum number of algae when vitamin B12 is provided externally in the medium</td>
<td>(4 \times 10^6) ml(^{-1})</td>
<td>Kazamia et al. (2012)</td>
</tr>
<tr>
<td>(\beta)</td>
<td>Growth rate of (M.\ loti)</td>
<td>4 log2 days(^{-1})</td>
<td>Vincze and Bowra (2006)</td>
</tr>
<tr>
<td>(K_b)</td>
<td>Maximum number of bacteria that the algae can support</td>
<td>(10^7) ml(^{-1})</td>
<td>Fitted</td>
</tr>
<tr>
<td>(a_c)</td>
<td>Number of algae required to reach half of the maximum carrying capacity, (K_v)</td>
<td>(5 \times 10^7) ml(^{-1})</td>
<td>Fitted</td>
</tr>
<tr>
<td>(\delta_b)</td>
<td>Fractional decrease in the production of vitamin B12 by bacteria if carbon is provided externally in the medium</td>
<td>Experimentally determined</td>
<td></td>
</tr>
<tr>
<td>(K_c)</td>
<td>Maximum number of bacteria if carbon is provided externally in the medium</td>
<td>(2 \times 10^8) ml(^{-1})</td>
<td>Kazamia et al. (2012)</td>
</tr>
<tr>
<td>(\epsilon)</td>
<td>Proportion of lysed cells</td>
<td>Varied</td>
<td></td>
</tr>
</tbody>
</table>

\(b\) takes different values in Model 1 (M1) and Model 2 (M2). Values were taken from the literature where possible, and then the rest varied until a satisfactory qualitative fit was achieved. The numerical values shown are those used in the solutions plotted in Figure 2.

Parameter values used in the solutions plotted in Figure 2.
observed algal–bacterial interactions. By design, and with an appropriate choice of parameter values, both models can fit the data in a satisfactory manner, but it is possible to ask which of the two is the most biologically plausible. To answer this, two approaches were taken: first, Model 2 predicts the presence of the parameter $d_b$, representing a change in production of vitamin B$_{12}$ by $M. loti$ in the presence of the algae. If $d_b$ is non-zero, this means the bacteria regulate the production of vitamin B$_{12}$, increasing it in the presence of the algae. This can be tested by measuring the levels of vitamin B$_{12}$ in monocultures of $M. loti$ and in cocultures of $L. rostrata$. Second, it is possible to estimate the number of bacteria that would need to lyse in order to support the algal growth, and consider if this figure is realistic. As little is known about the form of carbon provided by the algae, it is not possible to test experimentally the value of the parameter $d_a$, which represents a change in the supply of carbon by the algae to the bacteria.

To determine the amount of B$_{12}$ produced by $M. loti$ in the different cultures, we measured the total B$_{12}$ content in the culture, and also the B$_{12}$ in the cells and the medium (Figure 3). There was good agreement between the measurements (i.e. medium + cells = total) indicating that the bioassay was reliable. It is clear that when grown with $L. rostrata$ the amount of B$_{12}$ produced per bacterial cell is $\sim$10-fold higher than in monoculture.

Using the method described in full in Supplementary Materials Section 2, the rate of vitamin uptake per algal cell, $u$, can be estimated to be

$$u = 2 \times 10^{-7} \text{ng alga}^{-1} \text{day}^{-1} \quad (6)$$

which is in good agreement with that obtained by Droop (1968).

Disagreement with the ‘unregulated’ Model 1. It is possible to estimate the total rate of uptake of vitamin B$_{12}$ by all the algal cells in a coculture, $u_{\text{tot}}$. Using the value of $u$ mentioned above (equation (6)), together with the average carrying capacity of $3 \times 10^8$ cells per L (shown in Figure 2a, top panel), this is calculated to be:

$$u_{\text{tot}} = 60 \text{ ng l}^{-1} \text{ day}^{-1} \quad (7)$$

From the experimental data in Figure 3, and noting that there are approximately $10^{10}$ bacterial
cells per litre in coculture (Figure 2a, bottom panel), it is apparent that there is $<10 \text{ng l}^{-1}$ stored in all the cells in coculture. Thus, the amount of vitamin B$_{12}$ required by the algae per day is greater than the amount of vitamin B$_{12}$ stored in all the bacterial cells. As a result, the lysis interpretation of the unregulated model, Model 1, is unrealistic, as even in the implausible scenario in which all the bacteria in the culture lysed in a single day not enough vitamin B$_{12}$ would be released to support the number of algae present in a coculture for that day. This suggests that the vitamin B$_{12}$ is produced by the bacteria and taken up by the algae in a continuous manner, rather than being produced by the *M. loti* and retained by them, only to be transferred when they lyse.

**Agreement with ‘regulated’ Model 2.** Model 2 predicts the presence of a parameter $\delta_b$, which reflects the hypothesis that the bacteria decrease the production of vitamin B$_{12}$ when there is carbon present in the medium. To probe the value of this parameter, and test if it is non-zero, we took the values shown in Figure 3 (details in Supplementary Materials Section 3), obtaining the value:

$$\delta_b \approx 0.9.$$  

(8)

This value describes a situation in which, in a monoculture, the production rate of vitamin B$_{12}$ by *M. loti* is a factor of ten lower than in coculture. The precise value is less important than the fact that it appears to be non-zero, which lends weight to the scenario in which the production of vitamin B$_{12}$ by *M. loti* is reduced upon introduction of carbon into the medium.

**Discussion**

The dynamics of *M. loti* and *L. rostrata* growth observed in cocultures is not simple, as seen in the detailed experimental observations over a range of experimental conditions (Kazamia *et al*., 2012). An equilibrium in the number of cells of the two organisms is reached in media in which neither can grow alone. The equilibrium is broken when the exchanged nutrients are added externally, but enhanced growth of one organism does not lead to a corresponding increase in the other, even though, before nutrient add-back, there is a strong indicator of co-dependence. The ‘regulated’ Model 2 presented here can capture these dynamic interactions in a relatively simple set of equations by expressing the carrying capacity of each organism as a function of the other’s abundance, while incorporating a ‘switch’ to a state where that is not the case when either fixed carbon or vitamin B$_{12}$ is added to the medium. The model fits the data very well (Figure 2); there are only slight discrepancies in the perturbed region, where the data become noisy beyond the description of any deterministic model. However, the key features of the perturbed system, such as the observation that the algal numbers do not increase when a carbon source is added (and vice versa for the bacteria with B$_{12}$), are captured by the model.

Previous attempts have been made to describe quantitatively the aspects of algal B$_{12}$ auxotrophy. It was observed by Droop (1966) that the growth rate of algae did not depend on the concentration of vitamin B$_{12}$ in the classic manner of Monod dynamics. To circumvent this failure of standard growth models, additional terms were included alongside the usual Monod description to give a better empirical fit to the data. The form of these terms led Droop to hypothesise that a ‘vitamin-binding protein’ was secreted by the algae, which sequestered, and thus prevented access to, the vitamin (Droop, 1968). However, in evolutionary terms, the benefit of rendering an essential nutrient inaccessible seems questionable, and while B$_{12}$-binding proteins have been characterised from different algal species these are much more likely to be involved in the uptake of the vitamin. Work with *Euglena gracilis* (Watanabe *et al*., 1988) and *Thalassiosira pseudonana* (Sahni *et al*., 2001) found high-affinity vitamin B$_{12}$-binding factors in the growth medium of these two organisms. Similarly, a candidate B$_{12}$-binding protein named CBA1 (for cobalamin acquisition) has recently been identified based on a transcriptomic and proteomic analysis of *T. pseudonana* and a second diatom *Phaeodactylum tricornutum* (Bertrand *et al*., 2012). The protein was up to 160 times more abundant in vitamin B$_{12}$-deficient medium, suggesting a role in acquiring this compound.

The benefit of the approach we have used here, describing the interaction of the species through their population dynamics alone, means that, for example, knowledge of the precise growth rate dependence of the algae on vitamin B$_{12}$ concentration is not required; only the dependence on the number of bacteria is needed. This enabled us to test the models against the wide set of data from different experimental protocols keeping additional assumptions to a minimum; in particular, we were able to test whether the provision of nutrients could be explained by lysis. First, the levels of B$_{12}$ produced by *M. loti* in both cocultures and when grown in monoculture were measured (Figure 3). Even though these are much higher in the former, more than the total number of bacteria present would have to lyse in order to satisfy the observed growth requirements for vitamin B$_{12}$ of *L. rostrata* (equations (6) and (7)), leading us to reject conclusively the hypothesis that release of the vitamin by bacterial cell lysis could account for its provision in the coculture. Moreover, the data show that *M. loti* actively increased the rate of vitamin B$_{12}$ synthesis to satisfy algal requirements. Together, these observations support the conclusion that true mutualism through direct and
regulated exchange of vitamin B₁₂ is occurring between *L. rostrata* and *M. loti*.

While our model describes the exchange of nutrients in a laboratory context, we believe that similar associations of algae with bacteria are likely to be found in the natural environment, particularly as vitamin B₁₂ auxotrophy is widespread and not confined to particular habitats or algal phyla. Indeed, there have been previous reports of algal–bacterial assemblages for the provision of this micronutrient (Gillespie and Morita, 1972; Haines and Guillard, 1974). More recent oceanographic studies have reported the significance of vitamin B₁₂ in shaping algal growth and dynamics (San˜udo-Wilhelmy et al., 2006; Gobler et al., 2007; Koch et al., 2011), but the likely role of bacteria as symbionts to the algae has been overlooked. A recent study examined concentrations of vitamins in the ocean, arguing that the distribution of B-vitamins can be explained by seasonal patterns and ocean currents (San˜udo-Wilhelmy et al., 2012). Profiles for average vitamin B₁₂ abundance showed that the vitamin was present at depth, with little accumulating in the surface. The role of bacteria as B₁₂ producers was acknowledged, but presumed to happen at depth, whereas in surface waters bacteria are described as ‘scavengers’ that compete with algae for the available vitamin B₁₂. Similarly, in a study of nutrient supplementation on primary production in the Ross Sea (one of the most productive areas in the Southern Ocean), the addition of vitamin B₁₂ was investigated alongside iron (Bertrand et al., 2007). Nutrient add-back experiments conducted on collected sea water samples showed that significantly higher chlorophyll a concentrations were measured upon the addition of iron and B₁₂, relative to iron additions alone, in two out of three experiments. In the third experiment that did not show this stimulation, initial bacterial abundances were significantly higher. Despite these observations, no study to date has analysed whether there is a species-specific correlation of vitamin B₁₂ requirers and vitamin B₁₂ producers in the photic zone.

The biological implications of vitamin B₁₂ delivery via symbiosis or lysis are also widely different at the molecular and physiological levels. The symbiosis model implies that organisms must have a way of recognising each other in order to undergo targeted nutrient exchange. It may also mean that bacteria actively synthesise more vitamin B₁₂ to support the growth of algae in addition to satisfying their own needs. Conversely, if vitamin B₁₂ were provided via lysis, there would be no interaction between algae and bacteria, and thus the latter would not alter their level of vitamin B₁₂ production in response to the presence of algae; clearly, in our system this is not the case.

Moreover, direct vitamin B₁₂ exchange via symbiosis would also explain why vitamin B₁₂ auxotrophy has evolved so many times throughout the algal kingdom, and in all habitats (Croft et al., 2005). The molecular basis of this auxotrophy was uncovered by analysis of sequenced algal genomes, revealing that it is the result of loss of a single gene, *METE*, which encodes vitamin B₁₂-independent methionine synthase (Helliwell et al., 2011). Expression of *METE* is repressed by the addition of vitamin B₁₂ to the external medium of diverse algae (Croft et al., 2005; Helliwell et al., 2011), which then rely on the activity of cobalamin-dependent methionine synthase, encoded by METH. In the absence of selective pressure, METH can accumulate deleterious mutations over time, and indeed *METE* pseudogenes have been found in algal auxotrophs (Helliwell et al., 2011). Thus, to account for the widespread occurrence of vitamin B₁₂ auxotrophy by this process, there must be sustained supplies of cobalamin in the natural environment. These conditions would be obtained if there were direct algal–bacterial interactions to allow exchange of nutrients. Passive exchange via lysis would be unlikely to provide cobalamin free in solution for extended periods of time at sufficient levels to repress METH expression. In contrast, direct algal–bacterial interactions would allow a sustained exchange of nutrients. Interestingly, there is evidence that auxotrophy for other vitamins may also have arisen as a result of repression of gene expression, including for thiamine (B₁) and biotin (B₃) (Helliwell et al., 2013). Vitamins might be considered the ideal signalling currency between microbes: because they provide essential cofactors, they are ubiquitously required and recognised.

Our findings add to the tide of evidence of close bacterial associations with algae that are often highly evolved (e.g. Grossart et al., 2005; Wagner-Döbler et al., 2010; Gärdes et al., 2011; Hollants et al., 2011). Recently, Goecke et al. (2013) argued that algae may be viewed as an important environment for bacterial growth. They conducted a phylogenetic study based on 16S rRNA for 101 described bacterial species isolated from eukaryotic macro- and micro-algae from marine and freshwater environments, and found that bacterial species and strains that carried out similar metabolic functions were likely to colonise similar algal taxa or algal groups. This echoes earlier studies, such as that by Cole et al. (1988), which looked at bacterial production in fresh and saltwater ecosystems and found that those with high rates of algal production such as coral reefs have bacterial biomass greater than that predicted by the amount of bioavailable organic carbon alone. The data were interpreted as suggesting either competition between algae and bacteria and hence their growth in response to common factors or that phytoplankton were an important substrate for bacterial growth.

In summary, the work presented here illustrates how regulated symbiosis can be modelled and will make it possible to explore, and better understand, more complex interactions present in biology and biotechnology.
Conflict of Interest
The authors declare no conflict of interest.

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