N₂ Fixation in *Trichodesmium* Does Not Require Spatial Segregation from Photosynthesis

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**ABSTRACT** The dominant marine filamentous N₂ fixer, *Trichodesmium*, conducts photosynthesis and N₂ fixation during the daytime. Because N₂ fixation is sensitive to O₂, some previous studies suggested that spatial segregation of N₂ fixation and photosynthesis is essential in *Trichodesmium*. However, this hypothesis conflicts with some observations where all the cells contain both photosystems and the N₂-fixing enzyme nitrogenase. Here, we construct a systematic model simulating *Trichodesmium* metabolism, showing that the hypothetical spatial segregation is probably useless in increasing the *Trichodesmium* growth and N₂ fixation, unless substances can efficiently transfer among cells with low loss to the environment. The model suggests that *Trichodesmium* accumulates fixed carbon in the morning and uses that in respiratory protection to reduce intracellular O₂ during the mid-daytime, when photosynthesis is downregulated, allowing the occurrence of N₂ fixation. A cell membrane barrier against O₂ and alternative non-O₂ evolving electron transfer also contribute to maintaining low intracellular O₂. Our study provides a mechanism enabling N₂ fixation despite the presence of photosynthesis across *Trichodesmium*.

**IMPORTANCE** The filamentous *Trichodesmium* is a globally prominent marine nitrogen fixer. A long-standing paradox is that the nitrogen-fixing enzyme nitrogenase is sensitive to oxygen, but *Trichodesmium* conducts both nitrogen fixation and oxygen-evolving photosynthesis during the daytime. Previous studies using immunoassays reported that nitrogenase was limited in some specialized *Trichodesmium* cells (termed diazocytes), suggesting the necessity of spatial segregation of nitrogen fixation and photosynthesis. However, attempts using other methods failed to find diazocytes in *Trichodesmium*, causing controversy on the existence of the spatial segregation. Here, our physiological model shows that *Trichodesmium* can maintain low intracellular O₂ in mid-daytime and achieve feasible nitrogen fixation and growth rates even without the spatial segregation, while the hypothetical spatial segregation might not be useful if substantial loss of substances to the environment occurs when they transfer among the *Trichodesmium* cells. Our study then suggests a possible mechanism by which *Trichodesmium* can survive without the spatial segregation.

**KEYWORDS** *Trichodesmium*, nitrogen fixation, oxygen, temporal segregation

*Trichodesmium* sp. is a dominant contributor to marine microbial N₂ fixation (1–3), an essential process in marine ecology and biogeochemistry. N₂ fixation by *Trichodesmium* has been thought to be paradoxical, since it fixes N₂ and conducts O₂ evolving photosynthesis during the daytime (1), although nitrogenase, the enzyme catalyzing N₂ fixation, is highly sensitive to O₂ (4). One widely discussed hypothesis is that *Trichodesmium* may temporally segregate the two conflicting processes (5–10). Photosynthesis of *Trichodesmium* often peaks in the morning, while N₂ fixation mainly occurs at noon or in the afternoon, when the intracellular O₂ is low due to concurrent low photosynthesis and probably high respiration (5, 8, 9, 11, 12). This phenomenon of asynchrony in the peak timing of photosynthesis and N₂ fixation is commonly referred to as temporal segregation of the two processes,
although the two processes are not completely separated in time (5). The temporal segregation, however, is not always obvious in Trichodesmium (13–15), for unclear reasons.

In addition, it has been hypothesized that Trichodesmium segregates these two competing processes spatially (5, 7, 9, 10, 16). Trichodesmium exists as filamentous trichomes consisting of dozens to hundreds of cells (1), in which N₂ fixation may be allocated in specialized cell segments (termed diazocytes) (15) and thus be spatially segregated from photosynthesis (5, 9). The spatial segregation, if it exists, requires the transfer of substances among Trichodesmium cells, while it is unclear how it occurs (6). As supporting evidence, some studies have revealed that nitrogenase is only distributed in diazocytes (5, 17–19). However, contradictory results have also been reported in which nitrogenase is randomly distributed in some, or even all, Trichodesmium cells (20–22). ¹³C and ¹⁵N isotope measurements via nanometer-scale secondary ion mass spectrometry (NanoSIMS) also indicate that N₂ fixation of Trichodesmium might not be limited to specialized diazocytes (6). These results lead to controversy in the existence of spatial segregation between N₂ fixation and photosynthesis in Trichodesmium.

Multiple mechanisms have been found or proposed to be involved in the O₂ regulation of Trichodesmium. The reduced permeability of the Trichodesmium plasma membrane to O₂ can slow into-cell O₂ diffusion, which is possible considering that the membrane of Gram-negative Trichodesmium is surrounded by a cell envelope with multiple layers (23). A recent study also proposed that hopanoid lipid, a component of Trichodesmium’s membrane, may reduce the O₂ permeability (24).

Another mechanism for O₂ regulation is respiratory protection (RP). RP is active aerobic respiration of carbohydrates by diazotrophs to lower intracellular O₂ to protect nitrogenase, while the produced energy is lost as heat to the environment (5, 7, 25, 26). High RP might reduce the plastoquinone pool and send negative feedback to photosystem II (PSII), further lowering intracellular O₂ production in Trichodesmium (5).

Alternative electron transfer (AET), one of the photosynthetic electron transfer (PET) pathways, might also contribute to maintaining low intracellular O₂ (8, 26). Electrons produced from PSII via the decomposition of H₂O transfer to ferredoxin (Fd) and terminally return to H₂O by, e.g., the Mehler reaction, forming (pseudo)cyclic electron flows around photosystem I (PSI) and resulting in zero net O₂ production (27, 28). AET produces intracellular energy (ATP) but not the reducing agent NADPH (29). In marine diazotrophs, AET can be a complement to linear PET (LPET), in which ATP and NADPH are produced at a molar ratio (1.3:1) that is substantially lower than that (3:1) required by N₂ fixation (27, 28, 30–32). AET can therefore benefit N₂ fixation by providing ATP to energetically expensive N₂ fixation while not generating O₂ (8, 26, 33, 34).

Recently, the segregation of Trichodesmium N₂ fixation and photosynthesis has been studied using a physiological cell model (7). The model consists of coarse-grained metabolic fluxes resolving key metabolisms, such as N₂ fixation, respiration, and biomass synthesis, suggesting that combined mechanisms are essential in regulating intracellular O₂, including RP, low permeability of cell membranes, and temporal and spatial segregations of N₂ fixation and photosynthesis. However, the model predefined the temporal segregation of N₂ fixation and photosynthesis and thus did not test the possibility of Trichodesmium’s survival without spatial segregation. Exploring such a possibility is critical in reconciling conflicting observations in which nitrogenase is found in a small group of cells (5, 17–19) or all the cells (20–22).

In the present study, we constructed a systematic physiological model of a single trichome of Trichodesmium, tracking the fluxes of carbon, nitrogen, O₂, NADPH, and ATP through different intracellular pools and processes. An optimization method was applied to seek a model parameter set that maximizes the growth rate, which allows the model to self-organize its diurnal patterns of various physiological processes. Model experiments were conducted to quantitatively evaluate the importance and necessity of different strategies, including the temporal and spatial segregations of N₂ fixation and photosynthesis, for regulating the intracellular O₂ of Trichodesmium trichomes and accomplishing feasible N₂ fixation. The results show that the hypothetical spatial segregation can be useful but is not mandatory.
RESULTS

General model framework. The model (Fig. 1) estimates the growth of *Trichodesmium* trichome by simulating 12-h diurnal cycles of major intracellular processes involved in synthesizing organic carbon and fixing N\(_2\). The production and the consequent allocation of intracellular ATP and/or NADPH partly determine the rates of these processes. The rate of N\(_2\) fixation is also impacted by the temporal evolution of the intracellular O\(_2\) level. The modeled O\(_2\) inhibition on N\(_2\) fixation rate uses a Michaelis-Menten equation (35), in which the half-saturation coefficient (\(k^{\text{NF}}_{\text{O}_2} = 10^{-2}\) mol O\(_2\) m\(^{-3}\)) is determined by fitting a modeled gross fixed C-to-N ratio of 49:1 with the observed value of 47:1 from an experiment with *Trichodesmium* (6) (see further discussion and sensitivity tests of \(k^{\text{NF}}_{\text{O}_2}\) in the Discussion, below). The assimilated organic carbon and nitrogen accumulated at the end of the diurnal cycle are used to calculate a daily integrated growth rate. The spatial segregation of N\(_2\) fixation and photosynthesis into segments of model trichome is also tested. Here, we briefly introduce our model framework, while more details are described in Materials and Methods.

We first introduce our model case in which photosynthesis and N\(_2\) fixation are not spatially segregated (Fig. 1a). The model runs with diurnal variable light, which drives PET pathways, including LPET and AET. LPET produces O\(_2\), ATP, and NADPH, while AET only produces ATP (28). The ratio of LPET and AET is dynamically adjusted to fulfill the relative requirements of ATP and NADPH by all the processes. The sole N source of the model, N\(_2\) fixation, consumes ATP and NADPH (31, 32). CO\(_2\) and HCO\(_3^-\) are taken up (ATP is needed in HCO\(_3^-\) uptake) (36) and then fixed to carbohydrates by consuming ATP and NADPH. Some carbohydrates are further synthesized to form carbon skeletons (37). ATP is also needed for cell maintenance (38). Our model prioritizes using ATP and NADPH for N\(_2\) fixation over other processes, but N\(_2\) fixation can only proceed under low intracellular O\(_2\) levels (4).

The model implements RP by actively respiring carbohydrates to reduce the intracellular O\(_2\) while wasting the produced ATP (5, 7, 25, 26). RP, as discussed above, inhibits PET (5) and consequently slows O\(_2\) production. This intracellular production and consumption of O\(_2\), as well as the cross-cell exchange of O\(_2\), generate a dynamic level of intracellular O\(_2\) and largely regulate the sometimes-observed diurnal patterns of N\(_2\) fixation. Note that ATP and NADPH in the model are solely produced by LPET and AET during the daytime (39) and are instantaneously used (i.e., not stored). At the end of
the daytime, the model calculates the amount of accumulated fixed carbon that needs to be respired to produce ATP, which subsequently supports maximal biosynthesis from the remaining fixed carbon and nitrogen.

The model case with spatially segregated photosynthesis and N\textsubscript{2} fixation is constructed by modifying the model without the spatial segregation, to separate the trichome into N\textsubscript{2}-fixing (diazocytes) and photosynthetic cells (Fig. 1b). N\textsubscript{2} fixation is confined in diazocytes, which are set to make up 15% of total cells (2, 16), while LPET, AET, and carbon fixation only occur in the remaining photosynthetic cells. All the materials except O\textsubscript{2} are assumed to instantaneously and 100% efficiently transfer between diazocytes and photosynthetic cells and distribute evenly along the trichome (6), which is a best-case assumption for the growth of Trichodesmium with spatial segregation. Further evaluation and model experiments about this assumption are discussed later. Intracellular O\textsubscript{2} in diazocytes and in photosynthetic cells is simulated separately. A mixed layer of O\textsubscript{2} (see Fig. S1 in the supplemental material) is considered to form around the surface of the whole trichome, and the O\textsubscript{2} exchange among the mixed layer, the diazocytes and the photosynthetic cells, is calculated separately, following a scheme from Staal et al. (40).

**Growth rate and daily integrated carbon and N\textsubscript{2} fixation rates.** By optimizing model parameters, the model in which Trichodesmium trichome is not spatially segregated to diazocytes and photosynthetic cells achieves a maximal growth rate of 0.25 day\textsuperscript{-1} (Table 1). The modeled growth rate falls within the general observed levels of Trichodesmium (0.1 to 0.4 day\textsuperscript{-1}) (13, 41–45). The modeled daily integrated fixed N (0.05 mol N per mol C per day) is coupled with the growth rate, using the Redfield ratio (46).

After incorporating the spatial segregation into the model, it reaches a much higher rate of 0.51 day\textsuperscript{-1}, which is mainly attributed to the elevated daily integrated N\textsubscript{2} fixation rate (Table 1). However, the daily integrated carbon (carbohydrate) fixation rate is nearly the same in the two model cases (Table 1), indicating that much more fixed carbon is respired or wasted in the model without spatial segregation. The high growth and N\textsubscript{2} fixation rates in the model without spatial segregation will be discussed later.

Nevertheless, Trichodesmium that does not spatially segregate photosynthesis and N\textsubscript{2} fixation can still grow mainly, because it fixes a large amount of carbon in the early period and then uses that carbon in RP during the mid-day, resulting in more O\textsubscript{2} consumption than is produced by photosynthesis and creating a low-O\textsubscript{2} window for N\textsubscript{2} fixation (further details are provided in the Discussion section, below).

**Temporal segregation of carbon and N\textsubscript{2} fixations.** The simulated carbon and N\textsubscript{2} fixations segregate temporally in both models with and without spatial segregation (Fig. 2a and b). These optimized results represent the patterns via which the model can reach the maximal growth rate and tentatively support the necessity of the temporal segregation between photosynthesis and N\textsubscript{2} fixation in Trichodesmium, more of which will be discussed later. The carbon fixation rate increases to its daily peak in the first 2 h, gradually decreases to approximately half of its maximum until noon, remains nearly constant (Fig. 2a) or increases to a second peak (Fig. 2b) for another 4 h, and then reduces to 0 at the end of the light period. N\textsubscript{2} fixation mainly occurs in the middle light period, when the carbon fixation rate is down-regulated and a window of low intracellular O\textsubscript{2} emerges (Fig. 2c and d). Compared to the model without spatial segregation, the model spatially segregating photosynthesis and N\textsubscript{2} fixation has a wider low-O\textsubscript{2} window in diazocytes and a longer period of N\textsubscript{2} fixation (Fig. 2).

Our model generates a diurnal pattern of N\textsubscript{2} fixation with a single peak, which is consistent with most previous studies of single trichomes of Trichodesmium (5, 6, 8, 13, 39, 41, 44, 47). N\textsubscript{2} fixation in our model peaks during the later light period, which was observed in some of above studies (6, 13, 41, 47), although the exact peaking time of N\textsubscript{2} fixation varied substantially, probably due to different culture and physiological conditions.
Dynamic changes of O2 fluxes. We first compared the intracellular O2 budgets in the model without spatial segregation to those in the photosynthetic cells of the model with spatial segregation (Fig. 3a and b). In the early morning (0 to 4 h), a large amount of O2 was quickly produced from photosynthesis, which is consistent with an observation that the gross O2 evolution of *Trichodesmium* was high in the late morning or midday (48). The produced O2 then either diffuses to the ambient environment or is respired in both cases. After that period, when O2 production is moderate and N2 fixation increases rapidly, the RP dominates the removal of intracellular O2 in both cases. The low intracellular O2 in turn leads to a physical influx of O2 in the model without spatial segregation (Fig. 3a). Without N2 fixation, the photosynthetic cells of the model with spatial segregation, however, allow a lower RP than that without spatial segregation, and meanwhile an intracellular O2 concentration always higher than the extracellular level causes a continuous outflux of O2 (Figs. 2d and 3b). For the diazocytes of the model with spatial segregation, no O2 is produced inside, and there is only a relatively small influx of O2 due to the small area of the interface (7); consequently, a low RP is enough to create a low-O2 window in these cells (Figs. 2d and 3c).

In terms of the daily integrated O2 budget, both the O2 consumed by RP and its ratio to photosynthetic O2 production in the model without spatial segregation are higher than those with spatial segregation (Fig. 3d). This is mainly because of the lowered RP requirement in both diazocytes and photosynthetic cells of the model without spatial segregation. Furthermore, with a higher intracellular O2, the photosynthetic cells in the model with spatial segregation can diffuse a much larger amount of O2 than that without spatial segregation (Figs. 2c and d and Fig. 3d).

Carbon, ATP, and NADPH allocation. Mainly owing to the much higher fraction of gross fixed carbon consumed by RP, much less (13%) fixed carbon is synthesized to biomass in the model without spatial segregation than that with spatial segregation (30%) (Fig. 4a). To supply ATP for biosynthesis at night, more fixed carbon is respired in the model with spatial segregation than that without spatial segregation because of the higher growth in the former (Table 1 and Fig. 4a). Compared to the model without spatial segregation, ATP production is higher in the model with spatial segregation, mainly because it is inhibited less by lower RP, with slightly more ATP produced by LEPT than by AET in both cases (Fig. 4c). Hence, the model with spatial segregation is capable of supporting higher energy consumption than that without spatial segregation (Fig. 4d). In both cases, most ATP (81% and 71% in models without and with spatial segregation, respectively) is allocated...
to N\textsubscript{2} fixation (Fig. 4d). The fraction of NADPH allocated to carbon fixation is even higher (97% and 93% in models without and with spatial segregation, respectively), with the remaining 10% of NADPH used by N\textsubscript{2} fixation, reflecting that carbon fixation requires a higher ratio of NADPH:ATP than N\textsubscript{2} fixation (Fig. 4b).

DISCUSSION

Formation of the temporal segregation. Without representing the spatial segregation between photosynthesis and N\textsubscript{2} fixation in *Trichodesmium*, our model generates rhythms of carbon and N\textsubscript{2} fixation (Fig. 2) that are basically consistent with sometimes-observed rhythms (6). The modeled rhythms can be divided into four stages (Fig. 5).

In the first stage (hours 0 to 2), carbon is quickly fixed and accumulates, while N\textsubscript{2} is barely fixed due to high intracellular O\textsubscript{2} (see Fig. S2 in the supplemental material), resulting in an increasing ratio of particulate organic C to N, a phenomenon also found in culture experiments (49).

In the second stage (hours 2 to 4), the accumulation of carbon skeletons (see Fig. S2) increases the cellular demand for N\textsubscript{2} fixation, which in turn triggers RP (Fig. 3a). The elevated RP not only consumes more O\textsubscript{2} but also partly inhibits PET and O\textsubscript{2} production (Fig. 3a). These two effects, together with the diffusion of O\textsubscript{2} out of the cells, quickly reduce intracellular O\textsubscript{2} to a level lower than that in the environment (Fig. 2c).

**FIG 3** Modeled intracellular O\textsubscript{2} fluxes. (a to c) Photosynthesis and N\textsubscript{2} fixation are either not spatially segregated (a) or spatially segregated to photosynthetic cells (b) and diazocytes (c). (d) Daily integrated O\textsubscript{2} fluxes in models with and without spatial segregation, with the results for the photosynthetic cells and diazocytes in the model with spatial segregation also shown. Positive and negative values represent gain and loss of intracellular O\textsubscript{2} respectively. O\textsubscript{2} fluxes in the photosynthetic cells and diazocytes (b to d) are normalized to their own respective biomass.
In the third stage (hours 4 to 10.5), the majority of N₂ is fixed. To maintain a low intracellular O₂ for N₂ fixation (Fig. 2c), the RP is at its highest level (Fig. 3a) to consume not only all the O₂ that is photosynthetically produced at a moderate level (Fig. 2a) but also the O₂ in influx from the environment. This consequently results in a net consumption of organic carbon (see Fig. S2). The results are consistent with the net O₂ consumption observed around a period of active N₂ fixation in a cultured Trichodesmium experiment (48). Therefore, adequate carbon must be fixed and stored in the first two stages before substantial N₂ fixation occurs, which is a reason for the necessity of the temporal segregation between carbon and N₂ fixations.

In the last stage (hours 10.5 to 12), the accumulation of fixed N (see Fig. S2) and downregulated PET because of weakened light (Fig. 3a) causes a decrease in N₂ fixation and in turn slows down RP (Fig. 3a). There is still a small amount of carbon fixed in this last stage (Fig. 2a).

Additional model experiments without the spatial segregation (see Text S1 and Fig. S3) show that the degree of temporal segregation between photosynthesis and N₂ fixation largely determines daily integrated O₂ production and RP and the ratio of net carbon to N₂ fixations. The model reaches a maximal growth rate at an intermediate level of the temporal segregation.

**FIG 4** Modeled daily integrated carbon, NADPH, and ATP fluxes. Gross fixed carbon (a) and NADPH (b) allocation and ATP production (c) and allocation (d) were integrated over a diel cycle in both models, without and with spatial segregation. Note that the ordinary respiration of fixed carbon is calculated at the end of the daytime for the amount of ATP needed to synthesize biomass during the night (see text for details).
In summary, efficient carbon and N\textsubscript{2} fixations with dynamic regulation of intracellular O\textsubscript{2} and the requirement of sufficient accumulation of organic carbon before the period of high N\textsubscript{2} fixation are the two main reasons for the modeled temporal segregation between photosynthesis and N\textsubscript{2} fixation of \textit{Trichodesmium}. Our model provides a scenario in which, even without spatially segregating N\textsubscript{2} fixation and photosynthesis, \textit{Trichodesmium} can still grow at a moderate rate with the concurrence of the two processes.

Meanwhile, our model always produces the temporal segregation between N\textsubscript{2} fixation and photosynthesis, although some previous studies observed no temporal segregation in single trichomes of \textit{Trichodesmium} (13, 14). The mechanism for how \textit{Trichodesmium} grows without temporal segregation is certainly worthy of further investigations.

**Evaluation of the impacts from spatial segregation.** Meanwhile, the spatial segregation of photosynthesis and N\textsubscript{2} fixation in different cells can increase the modeled maximum growth rate by 104% (Table 1); this is mainly caused by the expanded low-O\textsubscript{2} window and the elevated N\textsubscript{2} fixation in diazocytes (Fig. 2) and the lowered consumption of fixed carbon in RP (Figs. 3 and 4a). This result, however, was obtained by assuming all the synthesized materials (except O\textsubscript{2}) can freely and efficiently transfer between diazocytes and photosynthetic cells in the model. Although direct transfer of substances among cells has been suggested for some terrestrial filamentous N\textsubscript{2}-fixing cyanobacteria, such as the channels found to connect cells in \textit{Anabaena} (50, 51), such channels or other similar mechanisms have not been discovered for \textit{Trichodesmium}. If the substances produced in certain cells of \textit{Trichodesmium} have to be otherwise released to extracellular environment before they can be retaken by other \textit{Trichodesmium} cells, the loss of the transferred substances to the environment would be unavoidable. By setting a lost fraction of the intercellularly transferred materials in the model with spatial segregation (see Materials and Methods), the growth rate decreases substantially, mainly caused by the loss of fixed N\textsubscript{2} and becomes even lower than that in the model without spatial segregation when the lost fraction is higher than 50% (see Fig. S4). A loss fraction lower than this level can be difficult to reach, considering the ocean environment is dynamic and other microorganisms inhabiting areas near \textit{Trichodesmium} can also take up these substances. Our model experiments then suggest that the benefit that \textit{Trichodesmium} can obtain from the spatial segregation is likely overwhelmed by the loss of substances during their transfer among cells.

**Intracellular O\textsubscript{2} management.** \textit{Trichodesmium} also adopts another suite of combined intracellular O\textsubscript{2} management mechanisms to protect nitrogenase. Considering the

![Intracellular O\textsubscript{2} management](image)
analyses above, we limit our discussion in this section only to the model results without spatial segregation.

Our model results suggest that proper low cell permeability to O$_2$ is important to maintain the low-O$_2$ window for N$_2$ fixation, which is consistent with the conclusions of other studies (7, 48). The multilayer cell envelope of *Trichodesmium* makes the O$_2$ diffusivity across the cell membrane thousands of times lower than that in water (7, 48, 52). Our model experiment estimates an O$_2$ diffusivity of $10^{-4}$ of that in water (Fig. 6), a value comparable to that in another study (7). When $\varepsilon$ is lower ($10^{-3}$), the O$_2$ produced in the early morning cannot quickly diffuse out of the cell, resulting in extremely high intracellular O$_2$ concentrations (about 60 times higher than the far-field ambient O$_2$ concentration) (Fig. 6c). Although not represented in our model, this high intracellular O$_2$ can cause strong oxidative stress (48). When $\varepsilon$ is higher ($10^{-3}$), the modeled cell needs to consume much more carbon in RP, so that the modeled gross fixed C-to-N ratio was substantially increased and the modeled growth rate was greatly decreased, unless the O$_2$ inhibition on N$_2$ fixation was weak (i.e., high $k_{NF}^{O_2}$) (Fig. 6a and b).

The half-saturation coefficient for the O$_2$ inhibition ($k_{NF}^{O_2}$) (equation 3), unknown but probably substantially lower than a typical ambient O$_2$ concentration (0.213 mol O$_2$ m$^{-3}$ at 34 practical salinity units (PSU) and 25°C), is estimated at $10^{-3}$ mol O$_2$ m$^{-3}$. This value of $k_{O_2}^{N_2}$, together with an $\varepsilon$ of $10^{-3}$, results in a ratio of modeled gross C-to-N fixations of 49:1 (Fig. 6), which fits well to an observed value of 47:1 from an experiment of *Trichodesmium* (6). By setting a stronger O$_2$ inhibition on N$_2$ fixation, i.e., a lower $k_{NF}^{O_2}$ of $10^{-3}$ mol O$_2$ m$^{-3}$ (see Fig. 55), more carbon is consumed in RP, resulting in a slightly higher ratio of modeled gross fixed C to N (55:1) and a slightly lower growth rate (Fig. 6a and b), while the pattern of the temporal segregation is basically unchanged (Fig. 6d). When the O$_2$ inhibition on N$_2$ fixation is weaker, by setting a higher $k_{NF}^{O_2}$ of $10^{-1}$ mol O$_2$ m$^{-3}$, the model reached a much higher growth rate (0.53 day$^{-1}$) with a lower gross fixed C-to-N ratio (23:1) (Fig. 6a and b). However, in this case, the...
modeled intracellular O$_2$ is higher than the ambient O$_2$, even when the N$_2$ fixation rates are high (Fig. 6e), contradictory to our intention of this model and the common understandings that *Trichodesmium* needs to substantially reduce intracellular O$_2$ to allow N$_2$ fixation. The results of this experiment show that the modeled degree of temporal separation depends on the parameter $k_{OS}^{NF}$, which sets the strength of the O$_2$ inhibition on N$_2$ fixation. The model can resolve a much less pronounced temporal separation (Fig. 6e) than that in the standard model (Fig. 2a) when the O$_2$ inhibition is set to be weaker, while the modeled growth rate of *Trichodesmium* is still comparable to other reported observations (41, 53).

Upon further consideration of other reported observations showing that the gross C/N fixation ratio mainly ranges between 30 and 50 (5, 6, 13, 47), our model sensitivity tests narrowed $\epsilon$ and $k_{OS}^{NF}$ estimates to considerably smaller ranges of $=10^{-4.5}$ to $10^{-4}$ and $10^{-2}$ to $10^{-1.5}$ mol O$_2$ m$^{-3}$, respectively, in which the modeled ratios of gross C and N fixations, growth rates, and intracellular levels are likely acceptable (Fig. 6).

Our model also reveals the importance of RP in regulating the intracellular O$_2$ of *Trichodesmium*, in which active RP not only directly consumes O$_2$ but also downregulates PET and thus photosynthetic O$_2$ production (5). Further model experiments without RP showed that the much higher intracellular O$_2$ levels inhibit N$_2$ fixation nearly entirely (see Fig. 56). The active role of RP can also be supported by the observed strong positive correlation between the expression of nitrogenase and cytochrome oxidase (the enzyme of respiration) in *Trichodesmium* (25). The RP of *Trichodesmium* is an extra-high indirect cost of N$_2$ fixation (36) and is a carbon biomass efficiency trade-off strategy commonly adopted by other marine diazotrophs (54–56).

AET can be another important mechanism in N$_2$-fixing *Trichodesmium*. As already discussed, AET partly satisfies the higher ATP demand by N$_2$ fixation. The fraction of PET electrons passing AET is substantially higher in *Trichodesmium* (48% ± 15%) than in nondiazotrophic cyanobacteria, such as *Synechococcus* (25%) (26, 57). Even the fraction of AET in *Trichodesmium* decreases when it grows with nitrate instead of N$_2$ (8). Our modeled fraction of AET is 39% on a daily basis and has a daily rhythm similar to that of N$_2$ fixation (see Fig. S7b), a phenomenon also found by Milligan et al. (8).

Another important role of AET in *Trichodesmium* is to scavenge O$_2$ produced in PSII (27, 28) and to thus protect nitrogenase (58, 59). In our model, AET scavenges 39% of O$_2$ produced in PSII, at rates comparable to those of a previous observation (26). Turning off AET in the model, the increased photosynthetic O$_2$ production (by 56%) elevates RP by 11% and reduces the growth rate by 62%.

There are other possible strategies that *Trichodesmium* may use to manage intracellular O$_2$, but they are not considered in our model. For instance, diazotrophs may dynamically adjust their membrane permeability to O$_2$ by redistributing hopanoid lipids in the membranes to cope with instantaneous requirements (24). The high abundance of *Trichodesmium* found on sinking particles implies that remineralization of particulate organic carbon may create a low-O$_2$ microenvironment for *Trichodesmium* (60). The constitution of *Trichodesmium* colonies may also protect N$_2$ fixation from into-cell O$_2$ diffusion by forming O$_2$-depleted microzones inside the colonies (61). High respiration rates of the heterotrophic bacteria attached to *Trichodesmium* colonies (62) might also help to create a hypoxic microenvironment. However, recent studies found that no anoxia formed inside the colonies during the light period (63, 64). Nevertheless, N$_2$ fixation of *Trichodesmium* colonies is often reported to be lower than that of free trichomes (48, 65, 66). How colony formation helps *Trichodesmium* manage O$_2$ and impacts its N$_2$ fixation, as well as its evolutionary reason, requires further research.

**Conclusions.** In this study, we constructed a physiological model of *Trichodesmium* to explore its conflict in O$_2$-evolving photosynthesis and O$_2$-inhibiting N$_2$ fixation. Our study shows that N$_2$ fixation of *Trichodesmium* is feasible without spatial separation from photosynthesis, consistent with observations in which it occurs in photosynthetic cells. Our model also suggests that the spatial segregation overall may not benefit *Trichodesmium* if substances lose during their transfer across cells. The model provides a mechanistic understanding behind the occurrence of N$_2$ fixation despite the
presence of photosynthesis across the trichome. Proper low cell permeability to O<sub>r</sub>, respiratory protection, and alternative electron transfer are key processes of *Trichodesmium* in its intracellular management to create the low-O<sub>r</sub> window for N<sub>2</sub> fixation. Given the diurnal changes of physiological activities simulated (e.g., photosynthetic electron transfer, carbon and nitrogen fixations), our model may be adapted in future studies to provide a further mechanistic insight regarding *Trichodesmium*, for example, into how limiting light intensity and other limiting nutrients such as iron can mediate the ATP and NADPH production and other processes and then regulate diurnal patterns of growth and N<sub>2</sub> fixation. Our model may also be used to advance our understanding of physiological processes in *Trichodesmium* colonies in their dynamic microenvironments by incorporating them into a proper physical framework.

**MATERIALS AND METHODS**

In the following, we briefly describe schemes of the model without spatial segregation. The full model description, parameter values, and variables of both models without and with spatial segregation can be found in Text S1 and Tables S1 and S2 in the supplemental material.

**Photosynthetic pathways.** A 12-h daylight cycle is set using a sine function (67) and drives a light-dependent PET rate (V<sub>PET</sub>, in moles electrons per mole C per second), which is further inhibited by RP as already discussed:

\[ V_{PET} = \frac{V_{PET}^d}{e^{\beta \cdot V_{PET}}} \]

where \( V_{PET}^d \) (in moles C per mole C per second) is the RP rate described later and \( \beta \) (in moles C<sup>−1</sup> per C<sup>−2</sup>seconds) is a parameter for the inhibition strength.

The modeled PET is divided into LPET and AET at variable fractions. For each electron through LPET, 0.65 ATP, 0.5 NADPH, and 0.25 O<sub>2</sub> are produced, while each electron through AET generates 0.65 ATP but no net NADPH or O<sub>2</sub> (27). Note that this ATP production rate by AET is based on a pathway in which electrons cycle through the Mehler reaction (27), which appears to be the dominant AET pathway in *Trichodesmium* (8), although other AET pathways can have different ATP production rates (27).

N<sub>f</sub> fixation and C fixation require different ratios of ATP to NADPH (3:1 and 1.9:1, respectively; see below). At each time step, after calculating the N<sub>f</sub> fixation rate, the model dynamically adjusts the fraction of AET in PET (\( f_{AET} \)), and consequently the ratio of produced ATP to NADPH, to fulfill the requirements of the N<sub>f</sub> fixation rate and maximize the C fixation (see Text S1 and Fig. S7a). Therefore, our model assumes a fully efficient adjustment of fractioning of PET into LPET and AET.

**N<sub>f</sub> fixation.** The N<sub>f</sub> fixation, including N<sub>f</sub> assimilation to NH<sub>4</sub><sup>+</sup> and NH<sub>4</sub><sup>+</sup> assimilation to glutamate, in the model consumes 9 ATP and 3 NADPH per fixed N atom (31, 32). A possible reason that N<sub>f</sub> fixation of *Trichodesmium* primarily occurs during the light period is that NADPH required by N<sub>f</sub> fixation may be completely provided by PET instead of respiring carbohydrates (39). Therefore, the maximal potential that N<sub>f</sub> fixation can reach \( V_{NADPH}^{N_max} \), in moles N per (moles C per second) in our model is when NADPH and ATP produced by PET are fully allocated to N<sub>f</sub> fixation:

\[ V_{NADPH}^{N_{max}} = V_{PET} \cdot \frac{(N_{max} - N)}{N_{NADPH}} \cdot \frac{NADPH}{PET} \]

where \( f_{AET}^{N} = 56.7\% \) is the required value of \( f_{AET} \) for PET to produce ATP and NADPH at the ratio (3:1) required by N<sub>f</sub> fixation, \( V_{NADPH}^{NADPH} = 0.5 \text{ mol NADPH (mol electrons)}^{-1} \) is the NADPH production quota of LPET, and \( V_{NADPH}^{NADPH} = 3 \text{ mol NADPH (mol N)}^{-1} \) is that required by N<sub>f</sub> fixation.

N<sub>f</sub> fixation in the model also depends on the carbon skeleton (CS; in moles C per mole C), fixed N (in moles N per mole C), and intracellular O<sub>r</sub> (in moles O<sub>r</sub> per cubic meter):

\[ V_{N_f} = V_{N_{max}} \cdot \frac{CS}{CS + k_{CS}} \cdot \left( \frac{N_{max} - N}{N_{max}} \right) \cdot \left( 1 - \frac{O_2}{O_2 + k_{O_2}} \right) \]

where \( k_{CS} \) (in moles C per mole C) is the half-saturating coefficient of the carbon skeleton for N<sub>f</sub> fixation. We assume that *Trichodesmium* tends to downregulate N<sub>f</sub> fixation when the fixed N is approaching maximal N storage (\( N_{max} \), in moles N per mole C) (7). The model's O<sub>r</sub> inhibition on N<sub>f</sub> fixation rate uses a Michaelis-Menten equation (35), in which the value of the half-saturation coefficient (\( k_{O_2} \)) for the inhibition has not been reported in the literature. Model experiments were then conducted to find and pair a \( k_{O_2} \) value with another parameter, \( \alpha \), as described below.

**Carbon fixation.** Each inorganic carbon (Ci, including CO<sub>2</sub> and HCO<sub>3</sub><sup>−</sup>) is fixed into carbohydrates using 2 NADPH and 3 ATP, based on the stoichiometry of the Calvin-Benson cycle (30). Additional energy of 0.8 ATP per fixed C is used by assuming 50% Ci leakage, 80% C, from HCO<sub>3</sub><sup>−</sup>, and a transport cost of 0.5 ATP per HCO<sub>3</sub><sup>−</sup> (68, 69). As mentioned above, the rate of carbon fixation is determined with \( f_{AET} \) after the N<sub>f</sub> fixation rate is calculated.

The carbon skeleton CS value in the model is produced from carbohydrates without energy consumption or carbon loss (37). The production rate of the carbon skeleton (\( V_{CS} \), in moles C per mole C per
second) is stimulated by the concentration of carbohydrates (CH₂O, in moles C per mole C), as shown using a Michaelis-Menten equation (35) and is inhibited by its own accumulation (7):

\[ V_{CS} = \frac{v_{CS}^{max} \cdot CH₂O \cdot CS_{max} - CS}{CH₂O + k_{CS}^{CH₂O} \cdot CS} \]  

where \( v_{CS}^{max} \) (in moles C per mole C per second) is the maximal production rate of the carbon skeleton, \( k_{CS}^{CH₂O} \) (in moles C per mole C) is the half-saturation constant of carbohydrates for carbon skeleton production, and \( CS_{max} \) (in moles C per mole C) is the maximal CS storage.

**Respiratory protection.** To create a low-O₂ environment for N₂ fixation, high intracellular O₂ stimulates RP. The rate of RP is also stimulated by the potential of N₂ fixation, which is in turn elevated by light and CS and is limited by fixed N (7, 56). We then parameterized the rate of RP (in moles C per mole C per second), as follows:

\[ V_{RP} = \frac{v_{RP}^{max} \cdot O₂ \cdot (1 - e^{-\frac{L}{R}})}{CS \cdot CS_{max} \cdot \left( \frac{N_{max} - N}{N_{max}} \right)} \]  

where \( v_{RP}^{max} \) (in moles C per mole C per second) is the maximal rate and \( v_{RP} \) (per micromole per square meter per second) is the initial slope of the photosynthesis versus light curve.

**O₂ diffusion.** The O₂ diffusion rate between the cell cytoplasm and ambient environment (\( T_{O₂} \), in moles O₂ per cubic meter per second) is simulated using a scheme from Staal et al. (40):

\[ T_{O₂} = \frac{-2 \cdot \pi \cdot d_{02} \cdot L}{V} \left( \frac{1}{\varepsilon} \ln \left( \frac{R}{R + L_g} \right) - \ln \left( \frac{R + L_g + L_d}{R + L_g} \right) \right)^{-1} \cdot (O₂^a - O₂) \]  

where \( O₂^a \) is the ambient far-field O₂ concentration set to a saturating concentration (0.213 mol O₂ m⁻³) under typical ocean conditions of 34 PSU salinity and 25°C (70), \( d_{02} \) (in square meters per second) is the O₂ diffusion coefficient in seawater, L (in meters) and V (in cubic meters) are the length and volume of the trichome (simplified to cylindrical geometry, respectively), \( \varepsilon \) is the ratio of the O₂ diffusion coefficient of the cell membrane to the \( d_{02} \), and is estimated to be 10⁻⁴ by model experiments (described below), R (in meters) is the radius of the cytoplasm, \( L_g \) (in meters) is the thickness of the cell membrane, and \( L_d = 1,024 \cdot (R + L_g) \) is the thickness of the boundary layer (64).

**Integration of state variables during the daytime.** The temporal change rates of state variables of carbohydrates, carbon skeleton, field N, and intracellular O₂ are represented in ordinary differential equations (ODEs), including all the fluxes described above. Note that NADPH and ATP are not stored but are fully consumed at each time step. Because all the rates described above have been normalized to carbon biomass, either volume, initial biomass, or the biomass concentration of *Trichodesmium* trichome does not need to be included in the model. An exception is for the ODE of intracellular O₂ (in moles per cubic meter), in which the cellular carbon biomass concentration (\( Q_{C} \), which is 18,333 mol C m⁻³) (71) is used to convert carbon-normalized biological fluxes of O₂:

\[ \frac{dO₂}{dt} = (V_{O₂} - V_{RP}^{max}) \cdot Q_{C} - T_{O₂} \]  

where \( (V_{O₂} - V_{RP}^{max}) \) is the biological production and consumption of O₂ (by RP) in moles of O₂ per mole C per second. These ODEs are integrated over the light period by using the MATLAB ode15s integrator (72).

**Biosynthesis and growth rate.** *Trichodesmium* might store fixed C and N during the dark period (6). Therefore, for simplification, the model calculates the amount of biomass (Bio, in moles C per moles C) that can be synthesized using the carbohydrates, carbon skeletons, and fixed N at the end of the light period. Bio is the smaller of N-based (BioN) and C-based biomass (BioC), with BioN being calculated by dividing fixed N by the molar ratio N:C (0.159) (46). BioC is calculated from the carbohydrates and carbon skeleton, considering the mass and energy balance. The energy needed for biosynthesis is derived from the respiration of carbohydrates (CH₂ORESP):

\[ BioC \cdot q_{ATP}^{RESP} \cdot (1 + \gamma_{ATP}) = CH₂O^{RESP} \cdot q_{ATP}^{RESP} \]  

where \( q_{ATP}^{RESP} \) (= 2 mol ATP per mol C) is the ATP requirement rate for biosynthesis (7), \( \gamma_{ATP} \) (= 10%) represents additional energy used in maintenance, referring to all cellular processes (e.g., nutrient uptake and DNA protection) that are incalculable but require energy (38), and \( q_{ATP}^{RESP} \) (= 5 mol ATP per mol C) is the ATP production rate from respiring carbohydrates (73). Then, the nonrespired carbohydrates and all carbon skeletons can be directly used to synthesize biomass:

\[ BioC = CH₂O - CH₂O^{RESP} + CS \]  

BioN, BioC, and CH₂O^{RESP} are the two unknown variables in equations 8 and 9 and thus can be solved. Noting that all the rates have been normalized to carbon biomass, Bio is therefore the relative increase in biomass over 1 day. The growth rate (G) is then the natural log of (1 + Bio) divided by 1 day.

**Optimization of model parameters.** Our optimality-based model assumes *Trichodesmium* can regulate its intracellular processes to maximize its growth (74). In the model without spatial segregation, several important parameters, whose values are largely unknown from the literature, were optimized in large bounded ranges by using the MATLAB global optimizer MultiStart (Table 2). These optimized parameters include those related to
**TABLE 2** Optimized model parameters

| Symbol | Units | Description | Initial range | Value after optimization |
|--------|-------|-------------|---------------|--------------------------|
| $K_{CS}^{NF}$ | mol C (mol C)$^{-1}$ | Half-saturating coefficient of carbon skeleton for $N_2$ fixation | [0, 1]$\ast$ | 0.06 |
| $v_{CS}^{max}$ | mol C (mol C)$^{-1}$ s$^{-1}$ | Maximal production rate of carbon skeleton | [0, $5.0 \times 10^{-4}$$\ast$] | $3.7 \times 10^{-6}$ |
| $K_{CH_2O}$ | mol C (mol C)$^{-1}$ | Half-saturating coefficient of carbohydrate for carbon skeleton production | [0, 1]$\ast$ | 0.58 |
| $v_{RP}^{max}$ | mol C (mol C)$^{-1}$ s$^{-1}$ | Maximal respiratory protection rate | [0, $5.0 \times 10^{-4}$$\ast$] | $4.5 \times 10^{-4}$ (without spatial segregation); $4.0 \times 10^{-4}$ (with spatial segregation) |

$\ast$The upper bounds are the maximal potential of organic carbon that can be fixed over the diurnal cycle.

Model availability. All data generated or analyzed in this study are included in this article and its supplemental material. The code of the model in this study is available on Zenodo (https://doi.org/10.5281/zenodo.6774659).

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

TEXT S1, PDF file, 0.2 MB.

FIG S1, PDF file, 0.1 MB.

FIG S2, PDF file, 0.1 MB.

FIG S3, PDF file, 1.8 MB.

FIG S4, PDF file, 0.1 MB.

FIG S5, PDF file, 0.1 MB.

FIG S6, PDF file, 0.1 MB.

FIG S7, PDF file, 0.6 MB.

TABLE S1, PDF file, 0.1 MB.

TABLE S2, PDF file, 0.1 MB.

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