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Better together? Lessons on sociality from Trichodesmium

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Review

Better together? Lessons on sociality from *Trichodesmium*

Meri Eichner,[1,*] Keisuke Inomura,[2] Juan José Pierella Karlusich,[3] and Yeala Shaked[4,5]

The N_2-fixing cyanobacterium *Trichodesmium* is an important player in the oceanic nitrogen and carbon cycles. *Trichodesmium* occurs both as single trichomes and as colonies containing hundreds of trichomes. In this review, we explore the benefits and disadvantages of colony formation, considering physical, chemical, and biological effects from nanometer to kilometer scale. Showing that all major life challenges are affected by colony formation, we claim that *Trichodesmium's* ecological success is tightly linked to its colonial lifestyle. Microbial interactions in the microbiome, chemical gradients within the colony, interactions with particles, and elevated mobility in the water column shape a highly dynamic microenvironment. We postulate that these dynamics are key to the resilience of *Trichodesmium* and other colony formers in our changing environment.

Highlights

Trichodesmium is globally abundant, both as single filaments (trichomes) and as colonies, but the mechanisms that govern the distribution of these morphotypes are poorly understood.

Colony formation affects all major life challenges, including nutrient acquisition, photosynthesis, mobility, defense against biotic and abiotic stressors, and resilience and adaptability in a changing environment.

The multitude of microbial interactions, variable chemical microenvironments, and mobility in the water column make *Trichodesmium* colonies highly dynamic systems.

The ability to shift between filament and colony morphology likely allows *Trichodesmium* to exploit the benefits of each form according to the conditions.

Despite ongoing methodological advances, key questions remain regarding the environmental controls, dynamics, and future prevalence of colony formation.

From single trichomes to colonies: teamwork is a crucial element of *Trichodesmium's* ecological success

Trichodesmium is a globally abundant, marine N_2-fixing **cyanobacterium** (see Glossary). Recordings of the vast surface blooms formed by this organism date back to the late 1700s, including the famous expeditions led by James Cook [1] and Charles Darwin [2]. In the last decade, high-throughput genetic surveys targeting the abundance and/or transcription of *nifH* genes (encoding a subunit of the N_2-fixing protein **nitrogenase**) and data-driven modeling confirmed the wide spatiotemporal distribution of *Trichodesmium* [3–7], (Figure 1A). Notably, *Trichodesmium* occurs in **trichomes** of tens to hundreds of cells that can aggregate to form millimeter-sized colonies, both forms being widely distributed across the tropical and subtropical oceans (Figure 1B). As it contributes up to one-half of the total **N_2 fixation** in these areas, it is a significant source of new nitrogen in marine ecosystems and fuels productivity in eutrophic ocean regions. Several climate-change studies have predicted that its global distribution and N_2 fixation rates will increase as temperatures and atmospheric CO_2 levels rise [8–11].

Although its ecology and physiology have been studied intensely in the past decades, *Trichodesmium* continues to surprise us with its special adaptations to life in the oligotrophic environment – be it the vast array of nutrient sources it can access, ranging from multiple organic and inorganic P sources to mineral iron (Fe) [12–16], the coordinated movement of dust particles along its filaments [17–19], the concerted coordination of N_2 fixation and photosynthetic activity within filaments [20–22], the multitude of interactions with its **microbiome** [23–25], or the recent finding of active N_2 fixation by *Trichodesmium* down to 1000 m depth [26].

Interestingly, all of these features have something in common: they are facilitated by interactions – either between individual cells within a *Trichodesmium* filament (trichome), or between trichomes within a **colony** or even with the diverse microorganisms that coinhabit *Trichodesmium* colonies. Speculating that *Trichodesmium's* ecological success is linked to the ability of trichomes to come together and form colonies, here we systematically analyze the positive and negative implications

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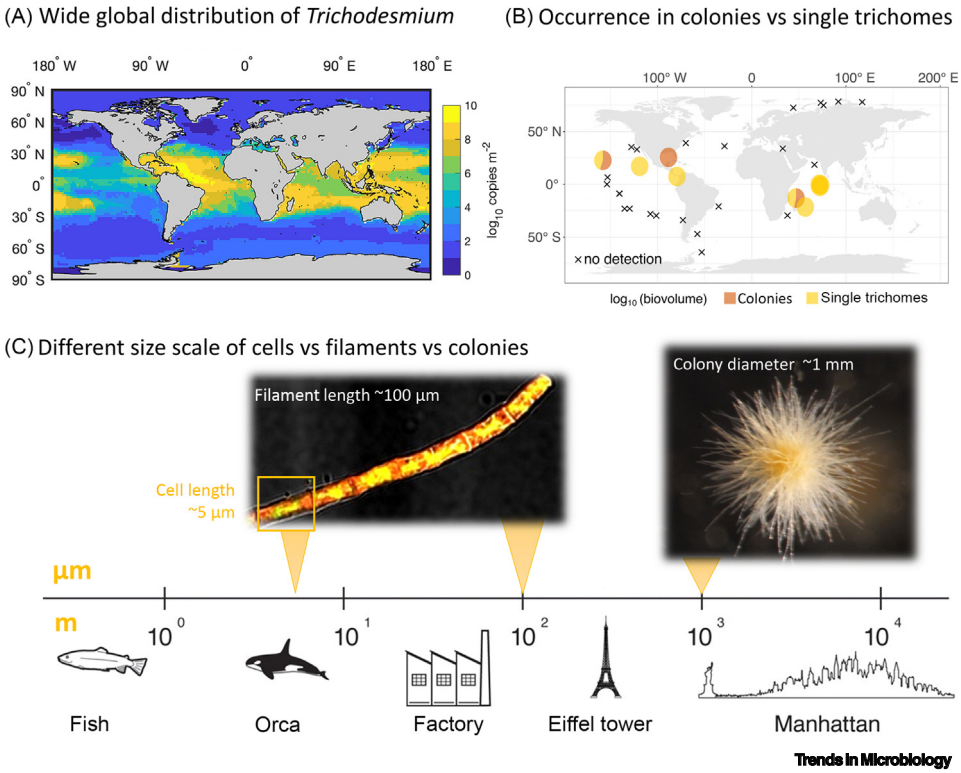


Figure 1. Global distribution of *Trichodesmium* in different morphologies. (A) Predicted global distribution of *Trichodesmium* (plot from [6], with permission). (B) Relative occurrence of *Trichodesmium* as colonies versus single trichomes (based on data in [5]). (C) Vast size difference between single cells, trichomes, and colonies (size scale and comparative images from [87], with permission). Position on the x-axis indicates equivalent size differences: if a single cell were equal to an orca, a colony would be about the size of Manhattan.

of the colonial lifestyle. Doing so, we consider chemical, physical, and biological mechanisms (Box 1). We structure the discussion according to five universal 'life challenges' that have to be met for an organism to be successful. In our analysis, we explore the implications of colony formation across a wide range of spatial scales, from nanometer scale all the way to the global distribution at kilometer scale.

Life as a colony: colony formation affects all major life challenges

Acquisition of nutrients

Induction of colony formation from single filaments due to P and Fe limitation in laboratory cultures points to a link between colony morphology and nutrient acquisition [27]. In fact, colony formation has various contrasting effects on nutrient availability (Figure 2). On the one hand, nutrient uptake by many cells concentrated in the small volume of the colony can induce limitation in dissolved inorganic nutrients [28]. On the other hand, high cell densities may facilitate the transfer of signaling molecules as well as the efficiency of release-based uptake via **siderophores** or excreted enzymes such as alkaline phosphatase [29]. Crucially, colony morphology allows for interactions with particles as a source of mineral nutrients [12,17–19,30]. Also, microbes associated with colonies can release additional organic and inorganic nutrients. Indeed, **metagenomes** of *Trichodesmium* colonies revealed nearly ten times more unique functions in the **epibiont** community than in *Trichodesmium* alone [31], including unique transporters for P and Fe that *Trichodesmium* does not encode [32] and various **hydrolytic enzymes** [33].

Glossary

Colony: an aggregate of filaments.
Cyanobacterium: an oxygenic photosynthetic bacterium.
Denitrification: the process of reducing nitrate and nitrite to dinitrogen and nitrous oxide.
Diazocyte: a group of cells that is specialized for fixing nitrogen.
Epibiont: an organism living on the surface of another organism.
Holobiont: an entity of a host organism and its symbionts.
Horizontal gene transfer: the transfer of genetic material between organisms, excluding that from parent to offspring.
Hydrolytic enzyme: an enzyme that facilitates the reaction of splitting one molecule into two involving water (H_2O).
Metagenome: the entire genome sequences in a bulk sample with mixed organisms.
(Meta)Proteome: an entire set of proteins of an organism (or mixed community).
Metatranscriptomics: a study based on the analysis of gene expression in a bulk sample with mixed organisms.
Microbiome: a community of microorganisms in a certain microenvironment.
N_2 fixation: the process of converting dinitrogen to ammonia.
NanoSIMS: an experimental technique that measures elemental and isotopic compositions of samples at nanometer resolution.
Nitrogenase: a nitrogen-fixing enzyme complex.
Phosphonate: an organophosphorus compound containing a C–$PO(OR)_2$ group.
Phytoplankton: plankton that conducts photosynthesis.
Quorum sensing: the ability to respond to changes in population density by excretion and sensing of specific molecules.
Siderophore: a molecule with high binding affinity for iron.
Stable isotope incubation: an experiment for quantification of elemental fluxes by labeling of elemental pools with rare isotopes.
Superoxide dismutase: an enzyme that converts the superoxide radical into oxygen and hydrogen peroxide.
Transcriptome: the sum of all initial products of genome expression.
Trichome: a filament of *Trichodesmium* cells.

Box 1. Universal effects of colony formation – physical, chemical, and biological mechanisms

When single phytoplankton cells aggregate to form a colony, the immediate changes in physical properties propagate to changes in chemical conditions and further affect biological processes ([88,89]; Figure I). These interconnected physical, chemical, and biological effects are related to one of the following processes: (i) In a first instance, the change in shape affects hydrodynamics. Specifically, due to the change in drag force, colonies move more rapidly than single cells up or down in the water column. (ii) As distances between cells are smaller, diffusive losses are reduced and the transfer of substances between cells is more efficient. For example, acquisition of dissolved nutrients from minerals, or transfer of DNA or signaling molecules (quorum sensing) is more efficient in a colony. Also, the close physical interaction among genetically identical cells allows for work division, or cell specialization. For example, specialization of cells for N_2 fixation or photosynthesis with direct cell-to-cell transfer of carbon and nitrogen, becomes possible. (iii) When many cells of the same species (same metabolic functions) are concentrated in a small space, effects of their own metabolic activity on the microenvironment are more pronounced. For example, chemical gradients in the diffusive boundary layer, caused by nutrient depletion, are larger in a colony than around a free-floating cell. (iv) When many different taxa (different metabolic functions) are concentrated in a small space, the holobiont has a greater number of ecological functions, and work division among different taxa opens possibilities for new pathways. For example, interactions with siderophore-producing bacteria allow for exploiting mineral Fe sources.

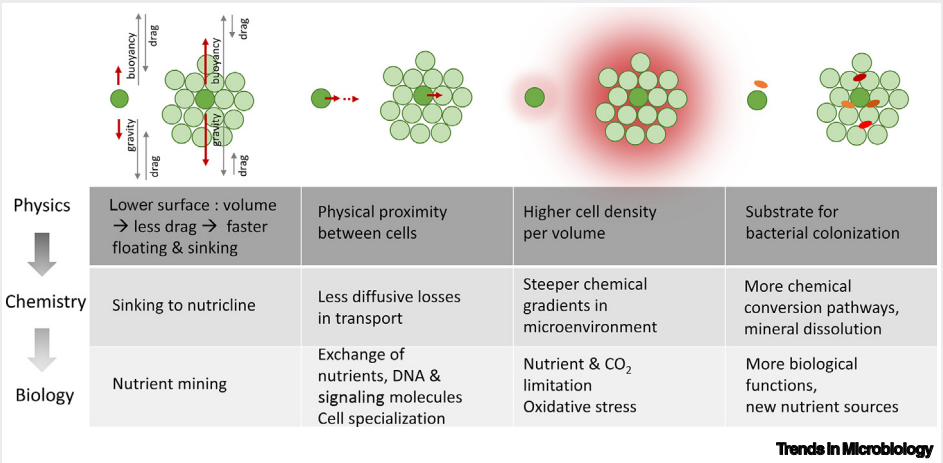
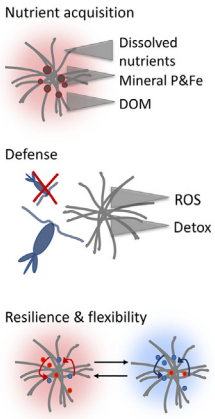


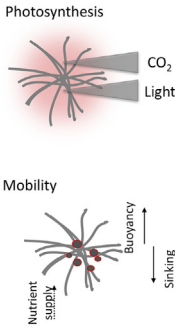
Figure I. Overview of the universal physical, chemical, and biological implications when single phytoplankton cells aggregate to form a colony.

These complex interactions of *Trichodesmium* with its microbiome in the acquisition and transfer of nutrients require a concerted coordination of the metabolic activities of the individual players. **Metatranscriptomics** revealed a close synchronization of bacterial and *Trichodesmium* **transcriptomes** over day–night-cycles [23], potentially facilitated by signaling molecules such as nitric oxide synthase as well as auxin efflux and sensing genes [31]. Moreover, several studies indicated that **quorum sensing** is an important mechanism for cell-to-cell signaling in the *Trichodesmium* microbiome, yet as *Trichodesmium* itself does not produce quorum sensing molecules, the exact mechanisms of communication between *Trichodesmium* and its microbiome remain to be resolved [14,31,33,34].

Given the O_2-sensitivity of the N_2-fixing enzyme complex nitrogenase [35], early studies suggested that anoxic microzones forming within colonies act to protect nitrogenase from O_2 [36]. However, elevated O_2 concentration measured within colonies in the light [37,38] – as well as higher N_2 fixation rates in colonies than in free trichomes [22] – question that hypothesis. While the accumulated experimental evidence regarding single-cell specialization (**diazocytes**) is conflictive (reviewed in [39]) and one recent model study questioned its benefit [40], other modeling approaches suggest that cell specialization combined with low membrane permeability provides



Life challenge	Variable	Single trichome	Colony	Example reference
Nutrient acquisition	Dissolved inorganic nutrients	+	–	[28]
	Dissolved organic nutrients	–	+	[57]
	Particulate nutrients	–	+	[12,18]
	N_2 fixation	+/-	+/-	[22,36]
Photosynthesis	Light utilization	+	–	[55]
	CO_2 availability	+	–	[56]
Defense	Grazing pressure	-?	+?	[72,73]
	ROS levels	+?	-?	[51,75]
Mobility	Vertical migration to nutricline	–?	+?	[26,60]
	Surface blooms	+/-	+/-	[61,62]
	Nutrient resupply by flow	-?	+?	[67]
	Sinking by mineral ballasting	–	+	[64]
Resilience & flexibility	Diverse microbial community	-	+	[31,33]
	Adaptation to variable conditions	-	+	[56]
	Crossing critical boundaries	+	-	[56]



Trends in Microbiology

Figure 2. Overview of pros and cons of single trichome versus colony morphology sorted according to different life challenges, as detailed in the text. The plus sign indicates an advantage for the respective morphology, the minus sign a disadvantage. Question marks indicate that a lack of studies prevents conclusions on the benefits for either morphology. Abbreviations: DOM, dissolved organic matter; Fe, iron; P, phosphorus; ROS, reactive oxygen species.

a more feasible mechanism for nitrogenase protection, even in a high-O_2 microenvironment [22,41]. Apart from O_2, nitrogen cycling mediated by the multitude of associated bacteria provides a potential control on N_2 fixation rates in the colony. Interestingly, N_2 fixation could be modulated by experimental addition of quorum sensing molecules to natural colonies, suggesting that the microbiome controls *Trichodesmium* N_2 fixation by quorum sensing [34]. Several metagenome and transcriptome studies have implied **denitrification** in *Trichodesmium* colonies [33,42,43]. By contrast, **stable isotope incubations** indicated that nitrogen gain (N_2 fixation) and recycling processes dominate in the colony, whereas nitrogen loss via denitrification was negligible, preserving new nitrogen in the system [28]. Model calculations of nitrogen gradients based on these incubations suggest complete nitrate depletion in the center of colonies but up to sixfold higher ammonium concentrations in colonies as compared to the boundary layer around single trichomes [28]. The 'trichosphere' (defined as the region enriched or depleted with nutrients or gases by more than 2% of ambient concentrations) was predicted to be 4- to 13-fold larger than the colony itself, which likely attracts associated bacteria [28]. Notably, this study predicted steep nutrient gradients in the colony even though assuming diffusivity close to seawater conditions (measured O_2 gradients were reproduced well by assuming a porosity of >0.996, i.e., the volume in the colony occupied by *Trichodesmium* cells was negligible [28]). Other studies have suggested that nutrient uptake can be limited by cell surface area available for transporters ('membrane crowding' [44]), which would be reduced when filaments are in direct contact in a colony, and that diffusion of inorganic nutrients in *Trichodesmium* colonies might be further reduced by the presence of viscous polymers or mucus [44], yet this has not been experimentally proven.

The acquisition of Fe, a major limiting nutrient for *Trichodesmium* [45], is critically linked to colony formation. Natural *Trichodesmium* colonies can actively collect and aggregate dust particles within their colony cores through coordinated movement of/along filaments [17–19]. While this facilitates Fe mining from particles in colonies, *Trichodesmium* cultures grown as single trichomes were not able to access mineral Fe from dust [19]. Various mechanisms by which *Trichodesmium* can access mineral Fe are under investigation, including reductive and ligand-promoted dissolution pathways [46]. Marker genes for siderophore utilization were enriched in *Trichodesmium*

colonies compared with free-living microbial communities in the same ocean regions [31] and siderophore production by bacteria associated with natural *Trichodesmium* colonies from the Gulf of Eilat was confirmed bioinformatically [47] as well as experimentally [48,49]. Interestingly, the addition of siderophores was found to increase Fe uptake from minerals by both *Trichodesmium* and its associated bacteria, suggesting a mutualistic interaction for Fe acquisition [12,48]. Several associated bacteria can synthesize photolabile siderophores (e.g., vibrioferrin, rhizoferrin, and petrobactin) [47]. In the sunlit surface waters, photolabile siderophores can enhance the bioavailability of particulate Fe to the entire consortium regardless of whether or not *Trichodesmium* colonies contain siderophore utilization genes [47]. H_2 produced during N_2 fixation and accumulating within colonies may act as an electron source for reductive dissolution, yet the exact mechanism requires further investigation [50]. Single-colony metaproteomics revealed a multitude of proteins associated with the presence of dust, including multiple Fe-acquisition pathways, the Fe storage protein ferritin, chemotaxis regulators, but also metalloproteins containing other trace metals such as Fe and nickel [51]. pH and O_2 gradients within colonies that are induced by photosynthesis and respiration may in principle alter Fe availability, yet in colonies in the Gulf of Eilat the effects were significant only in dense surface blooms [37].

Interactions of *Trichodesmium* with the colony microbiome in P acquisition have been extensively studied since it was discovered that *Trichodesmium* can not only utilize organic P but also produce **phosphonates** [13,52]. Omics-based field studies strongly suggest that P cycling within colonies, including phosphonate metabolism and alkaline phosphatase activity, is crucial in enabling *Trichodesmium* to thrive in P-limited ocean regions [31,33,53,54]. Regulation of alkaline phosphatase activity by quorum sensing suggests that microbial P cycling in *Trichodesmium* colonies is tightly controlled [14]. Recently, it was found that *Trichodesmium* colonies collect and retain not only Fe-rich but also P-rich mineral particles in their center [18]. Co-release of P and Fe from these particles by dissolution provides yet another P source that is directly linked to colony morphology [30].

Photosynthesis and carbon acquisition

Photosynthesis is directly affected by the gradients in light and in chemical conditions that are induced by colony formation (Figure 2). Self-shading within cultured *Trichodesmium* colonies has been observed to result in ca 40% of ambient light remaining at the point with lowest light intensity [55]. While in deeper water layers, this may lead to light limitation, self-shading may present an advantage under the high light intensities experienced in surface blooms. Uptake of inorganic carbon leads to formation of pH, CO_2, and bicarbonate gradients within colonies, which enhance energy requirements for carbon-concentrating mechanisms, yet diffusion-reaction-modeling based on O_2 and pH profiles suggested that the buffer capacity of open ocean seawater ensures that CO_2 does not become fully depleted in *Trichodesmium* colonies [56]. These gradients are the combined result of photosynthesis by *Trichodesmium* as well as respiration of dissolved organic matter (DOM) followed by CO_2 release by associated bacteria within the colonies, and the relative contribution of bacteria versus *Trichodesmium* to carbon turnover in colonies has not been quantified [56]. Stable isotope labeling experiments with *Trichodesmium* colonies in the Southwest Pacific showed dissolved organic carbon (DOC) uptake at rates similar to previously published inorganic carbon uptake, yet those experiments could not distinguish between direct DOC uptake by *Trichodesmium* and that mediated by associated bacteria [57].

Mobility

Trichodesmium was described early on to regulate its buoyancy in the water column by means of exceptionally robust gas vesicles [58]. Generally, the surface-to-volume ratio of a colony is smaller

than that of a free-floating filament due to increased cell-to-cell contact areas (Box 1). This decreased surface-to-volume ratio facilitates larger vertical mobility when in a colony morphology. However, due to a lack of experimental validation, sinking and floating velocities of **phytoplankton** aggregates, and specifically *Trichodesmium* colonies, are subject to considerable uncertainties [26,59–61]. A part of the uncertainties lies in how the micro-scale hydrodynamics are influenced by the morphology, which requires further experiments. Also, the surface-to-volume ratio may vary among colonies depending on their morphology, providing an additional dimension to sinking speed. While active migration of colonies in the water column has not been experimentally proven, vertical mobility theoretically enables colonies to exploit the natural gradients in the water column to balance light and nutrient demands and may furthermore increase nutrient supply due to reduction of diffusive boundary layer thickness (Figure 2).

Trichodesmium blooms often accumulate in the P-poor upper water column. Vertical migration to the P-rich nutricline (125–150 m depth), facilitated by buoyancy regulation through carbohydrate ballasting and gas vesicles, was suggested as a strategy to replenish the colony P pool [62,63]. Model calculations of carbohydrate ballasting suggested that reaching the nutricline requires a minimum colony size of 1 mm [64], which fits sizes observed in nature. Surprisingly, recent studies observed not only intact *Trichodesmium* colonies [61] but also active N_2 fixation by *Trichodesmium* [26] down to 1000 m depths. In model calculations, the authors show that this deep N_2 fixation can be supported by cellular carbon reserves, depending on the balance between the initial carbon storage and sinking speed [26]. However, sinking speed can be increased not only through carbohydrate accumulation but likely also through mineral ballast such as dust particles accumulated by colonies [65]. While a recent modeling approach has provided theoretical estimates of the impacts of mineral ballast on sinking speed [66], experimental quantification of the dependence of *Trichodesmium's* sinking speed on particle load will provide data to validate these assumptions (Wang *et al*. in preparation).

Concentrations of gases or nutrients within and around sinking aggregates directly depend on sinking velocity. O_2 concentration fields within sinking porous aggregates have been accurately reproduced with a newly described model of mass transfer (by advection and diffusion) and reaction [67]. At common Reynold's numbers for sinking marine aggregates, a plume of elevated nutrient concentrations develops at the rear end of the sinking aggregate that likely attracts motile chemotactic microbes [67,68]. Furthermore, flow-induced removal of breakdown products in sinking organic particles was suggested to drastically increase bacterial degradation rates [69]. For *Trichodesmium*, it was predicted that shear forces at the colony surface, due to turbulence, decrease the thickness of the boundary layer, from 500–1000 µm under still conditions to 200–500 µm under common shear rates in surface waters and thus increase nutrient concentrations at the cell surface [28]. Finally, mobility of colonies by rapid sinking and floating may also increase encounter rates with bacteria as well as organic and inorganic particles.

Defense against biotic and abiotic stressors

Protection from grazing is a classic example of a benefit of colony morphology. Indeed, formation of colonies can be induced by the presence of grazers in other phytoplankton such as *Phaeocystis* and *Microcystis* [70,71]. With a maximum ingestion size of 100 µm observed for crustacean zooplankton species [72], formation of 200–2000 µm (diameter) colonies enables *Trichodesmium* to pass this critical size. Zooplankton grazing on *Trichodesmium* is typically not considered to play a large role, yet ingestion of *Trichodesmium* by zooplankton has been occasionally reported (e.g., [73]). In addition to size, production of toxins such as saxitoxins associated with aggregation of *Trichodesmium* may hinder grazing [74]. In the relatively thick diffusive

boundary layer of a colony, toxic compounds may accumulate more easily above a critical level (as compared to single cells or filaments with the same rate of toxin excretion).

Regarding abiotic stressors, in parallel with elevated O_2 levels induced by photosynthesis [38], reactive oxygen species (ROS) can accumulate within colonies, making *Trichodesmium* a significant source of superoxide in the water column [75]. The presence of bacteria within colonies may increase their superoxide production compared with single trichomes [75], but bacteria may also contribute antioxidant activity, hence it is hard to predict the colony's overall ROS flux. For colonies collected at the Great Barrier Reef, trichome-normalized net superoxide production rates were ca 20-fold lower than gross production rates of laboratory cultures [76]. However, it should be noted that such comparisons are complicated by differences among *Trichodesmium* species and growth conditions in culture and in nature, all of which may mask the actual effect of colony lifestyle. Single-colony proteomics recently revealed elevated amounts of **superoxide dismutase**, suggesting an elevated need for ROS detoxification, in the presence of mineral particles [51].

Resilience and adaptability in a changing environment

Chemical conditions in the microenvironment within and around colonies are more variable over time and space than along single filaments, implying that colony formers are adapted to dynamic conditions (Figure 2). Light-dependence of O_2, pH, and H_2 gradients in colonies implies fluctuations over the day–night cycle [37,38,50], and recent observations of faster oscillations in the *Trichodesmium* **proteome** (three or four times per diel cycle) further highlight that colonies are a highly variable system [66]. While the mechanisms and dynamics of colony formation remain major open questions (see Outstanding questions), it is evident that aggregation and disaggregation of filaments can change the chemical and biological microenvironment of cells at relatively short time scales. In the laboratory, by applying nutrient stress, colony formation has been induced within 10 h to several days, while addition of nutrients to the media caused dissociation of aggregates within a day [27]. Chemical microenvironments and physiological activity may also differ among colony morphotypes. While tuft and puff-shaped colonies were found to represent different genotypes, a recent metagenome study revealed that a single *Trichodesmium thiebautii* genotype occurred in two different puff morphologies ('thin' and 'dense' puffs) which differed, for example, in their tendency to interact with dust, suggesting that *Trichodesmium* may be able to adjust its colony morphology via gene regulation [77].

Considering evolutionary timescales, the close spatial interaction within colonies may facilitate **horizontal gene transfer**, which is considered an important driving force for evolution in bacteria [78] and may thus foster adaptation. Indeed, several studies have found indications for horizontal gene transfer in *Trichodesmium* colonies [13,31,79]. Co-occurrence of several species of *Trichodesmium* in one colony has been reported based on visual inspection [38], which can be expected if colonies form by active aggregation of free filaments (as shown in the laboratory [27]) as opposed to monoclonal colonies expected if colonies grow merely by cell division. Yet, to the best of our knowledge, the diversity of *Trichodesmium* within single colonies has not been stringently assessed. A high level of functional redundancy in the microbiome may enhance its resilience to environmental changes [47]. Broadly speaking, the benefits of biodiversity under fluctuating conditions (described as the 'portfolio effect' in analogy to the benefits of a diverse portfolio in a fluctuating stock market [80]) may act favorably for the *Trichodesmium* colony **holobiont** in a changing environment.

Regarding climate change, adaptation to variable carbonate chemistry within colonies (specifically, to low night-time pH levels) may be an advantage under ocean acidification, while on the

downside, there is also a greater danger that critical boundaries in pH levels will be crossed earlier in colonies [56]. Conversely, it was suggested that dissolution of carbonates in the mineral particles collected by natural colonies may act as a buffer, as implied by pH measurements on *Trichodesmium* colonies amended with dust [37]. Interactive effects of ocean acidification with other chemical factors that vary within the colony microenvironment [81,82] can modulate climate change responses. In contrast to laboratory studies on cultures of *Trichodesmium* IMS101 grown as single filaments [10,83], several studies on natural communities of *Trichodesmium* did not show significant responses of ocean acidification treatments (e.g., [38,84]), yet, in lack of a direct comparison, it is not clear whether this is due to colony formation in the field or other factors such as the species or nutrient conditions. Overall, the complex interactions with the microbiome and the dynamic chemical microenvironment within colonies most likely play key roles in the resilience of *Trichodesmium* in our changing environment and thus require consideration in climate-change studies.

Taken together, colony formation has vital effects on all the key areas of life, and is thus inextricably linked to the physiology and ecology of *Trichodesmium*. As most of the life challenges are affected by both positive and negative factors, the balance between these pros and cons is key in understanding the ecological function of colony formation (Figure 2). Importantly, this balance may vary depending on environmental conditions (such as nutrient limitation or light) as well as metabolic state (such as respiration rates or the balance between carbon and nitrogen fixation), as recently demonstrated using a metabolic model of carbon fluxes [85].

From cellular interactions to global distribution: implications of colony formation across spatial scales

In summary, colony formation has effects across a remarkable range of spatial scales (Figure 3A, Key figure). At the smallest, nanometer scale, the close interactions among single cells and organic as well as inorganic material within the colony foster chemical conversions and exchange of nutrients, signaling molecules, and genetic material between cells of *Trichodesmium* and associated bacteria (Figure 3B). At the next scale, aggregation leads to formation of micrometer-scale physicochemical gradients, for example, in light, pH, O_2, nutrient, and toxin concentrations (Figure 3C). At even larger scale, colony formation enables meter- or kilometer-scale vertical migration along light and nutrient gradients in the water column, which has further impacts on encounter rates with bacteria and particles (Figure 3D). Jointly, these processes enable the formation of kilometer-scale blooms and finally determine global distribution, where the versatile lifestyle associated with colony morphology likely plays a key role in making *Trichodesmium* one of the globally dominant N_2 fixers.

Concluding remarks and future perspectives

Examining different life challenges and effects across spatial scales, we clearly show that life in a colony is different from that as a single cell or trichome. Differences are evident in all key areas of life, from acquisition of nutrients and photosynthesis, which are both closely linked to mobility in the water column, to defense against biotic and abiotic stressors and resilience to environmental changes. While, as far as we know, *Trichodesmium* does not exist as single cells, it is poorly quantified to which percentage it occurs in free filaments as opposed to colonies. A new data compilation based on Tara Oceans shows that both forms are globally abundant (Figure 1B, [5]). At most sampling stations, exclusively free filaments or colonies were observed, yet there are also areas where both morphologies were observed, with a higher prevalence in colonies (note logarithmic scale in Figure 1B). Given the long list of pros and cons of colony formation (Figure 2), the fact that they coexist in both forms means that there must be a

Outstanding questions

What governs the occurrence of colonies versus single filaments in natural systems?

Colony dynamics over time: how transient are they, do they form and open up, how does the abundance, composition, and activity of the associated bacteria change with time?

What determines the colony morphology (tufts versus puffs)?

Which biochemical mechanisms lead to colony formation? What is the 'glue' that keeps them together?

Does colony formation facilitate nitrogen fixation or work against it?

Can colonies be considered as 'reactors' catalyzing biochemical transformations of minerals and organic molecules?

What is the contribution of associated bacteria to carbon turnover in colonies?

What are the molecular mechanisms by which bacteria and *Trichodesmium* communicate?

How does climate change impact the niche of *Trichodesmium* and its colony formation?

Are colonies more resilient than single filaments to environmental changes?

Key figure

Potential implications of colony formation across spatial scales

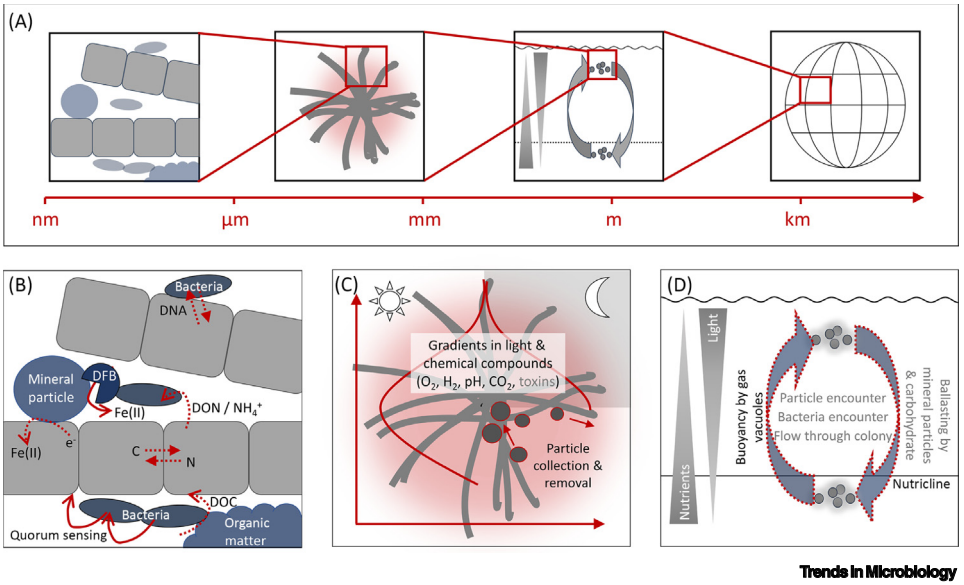


Figure 3. (A) Effects of colony formation reach from nanometer to kilometer scale. (B) At nanometer scale, colony formation may facilitate transfer between *Trichodesmium* cells [carbon (C) and nitrogen (N)], between *Trichodesmium* and associated bacteria [DNA, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and ammonia (NH_4)], between bacteria (quorum sensing) and release of dissolved iron [Fe(II)] from mineral particles fueled by siderophores such as desferrioxamine B (DFB) and electrons (e^-). (C) At micrometer scale, gradients in light and chemical compounds form, which vary over day–night cycles, while colony morphology enables active collection and removal of dust particles. (D) At kilometer scale, increased buoyancy and ballasting may promote movement along light and nutrient gradients and enhance encounters with particles and bacteria as well as flow-induced nutrient supply. Processes in which experimental evidence is scarce or conflicting are indicated by broken arrows and gray font.

fine balance between the negative and positive effects of forming a colony. How this balance is affected by environmental conditions is something we are far from understanding; while a few recent studies reported colony formation induced in the laboratory [22,27], to what extent the same mechanisms apply in the field is still a major open question (see Outstanding questions). Effects of climate change on the tendency to form colonies have not been directly examined, yet elevated production of extracellular polymeric substances under ocean acidification [86] might favor the formation of colonies. We suggest that the ability to switch between single filaments and colonies, as observed in the laboratory [27], allows *Trichodesmium* to exploit the benefits of both morphologies and is thus an important component of its ecological success.

Advances in method development in terms of both spatial resolution and sample size are allowing us to get more and more detailed insights into the chemical and physiological processes within colonies (Box 2). Yet, key questions remain in the basic understanding of the frequency, mechanisms, dynamics, and ecological benefits of colony formation by *Trichodesmium* (see Outstanding questions). From the available data, it is evident that the colonial lifestyle opens the door for an array of special functions that distinguish *Trichodesmium* from other phytoplankton and thus shape its specific niche. With a broader

Box 2. Tools for studying colony function and distribution

Resolving the composition and small-scale processes in colonies requires methods that have (i) high enough spatial resolution to characterize spatial gradients within colonies and/or (ii) high enough sensitivity to analyze compounds extracted from single-colony samples. In the past decades, various such techniques have been developed and successfully applied (Figure I), allowing intriguing insights into the chemical and physiological characteristics of colonies. Microscopy in combination with staining or observations of particle interactions over time provides information on the structure, composition, and behavior of colonies [17]. A classical tool for high-resolution chemical analysis is microsensors. Microelectrodes for various compounds, including O_2, pH, H_2, H_2O_2, and redox state are used either to measure absolute concentrations or to calculate fluxes from measured small-scale gradients [37,50,90], while optical fibers are used for light measurements or optode systems [55,91]. Chemical composition and uptake of nutrients and carbon by *Trichodesmium* have been visualized using nanoscale secondary ion mass spectrometry (**nanoSIMS** [17,38,92]), radio-imaging [48], and X-ray fluorescence analysis (MicroXRF [51]). Catalyzed reporter deposition (CARD)-FISH is widely applied to identify and localize specific microorganisms, but can also localize gene expression [mRNA CARD-FISH (Hania *et al*. in preparation)]. Enzyme activity assays such as the alkaline phosphatase activity assay have been applied to single colonies ([93]; Wang *et al*. in preparation). Advances in 'omics techniques have pushed the boundaries of required sample size, already allowing for successful single-colony metaproteomics [51] and metagenomics (Bizic *et al*. in preparation). Our understanding of processes within colonies can further be improved by combination of experimental techniques with mathematical models of different resolution [94], from simple models as a module in ecological modeling [95,96] to detailed models for reconstructing reactions from 'omics data [97]. Coarse-grained models compute intracellular molecular mass and fluxes of elements [98], and simple diffusion models are used to predict intracellular concentrations of O_2 [22,41]. To describe the spatiotemporal distribution of *Trichodesmium*, recent developments in high-throughput imaging (Underwater Vision Profiler, FlowCam, ZooScan) have enabled rapid enumeration of *Trichodesmium* filaments and colonies [5,99,100].

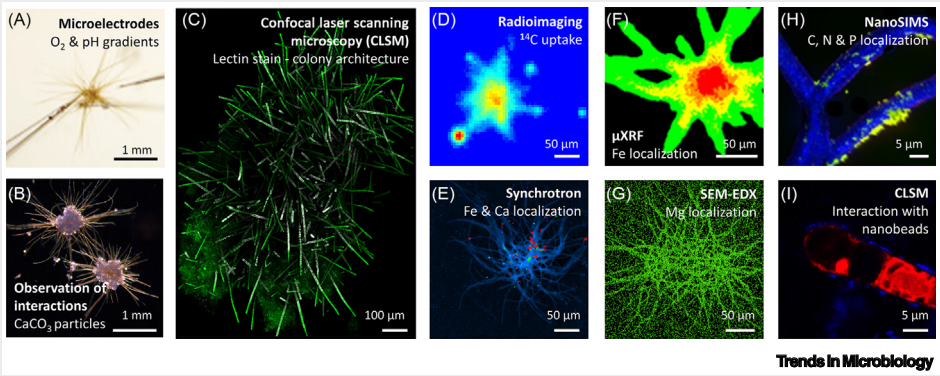


Figure I. Examples of experimental techniques used to analyze structure, composition, behavior, and physiology of *Trichodesmium* colonies. We acknowledge contributions from our collaborators: N. Kessler and M. Fine (panel B), T. Neu and U. Kuhlicke (panel C), S. Basu, S. Wang, and D. de Beer (panel D), S. Myneni, N. Kessler, R. Sanders, and D. Schlesinger (panel E), A-N. Visser, F. Zhang, O. Qafoku, and R. Boiteau (panel G), A. Mijovilovich, H. Kuepper, A. Colussi, G. Konert, and O. Prášil (panel F), M. Kienhuis, R. Lopez Adams, and L. Polerecky (panel H), G. Konert (panel I). Abbreviations: µXRF, MicroXRF (microX-ray fluorescence analysis); nanoSIMS, nanoscale secondary ion mass spectrometry.

perspective, we therefore call for considering the vital importance of both intra- and interspecific interactions in the definition of an ecological niche.

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Declaration of interests

No interests are declared.

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