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Space, the final frontier: The spatial component of phytoplankton–bacterial interactions

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Abstract

Microscale interactions between marine phytoplankton and bacteria shape the microenvironment of individual cells, impacting their physiology and ultimately influencing global-scale biogeochemical processes like carbon and nutrient cycling. In dilute environments such as the ocean water column, metabolic exchange between microorganisms likely requires close proximity between partners. However, the biological strategies to achieve this physical proximity remain an understudied aspect of phytoplankton–bacterial associations. Understanding the mechanisms by which these microorganisms establish and sustain spatial relationships and the extent to which spatial proximity is necessary for interactions to occur, is critical to learning how spatial associations influence the ecology of phytoplankton and bacterial communities. Here, we provide an overview of current knowledge on the role of space in shaping interactions among ocean microorganisms, encompassing behavioural and metabolic evidence. We propose that characterising phytoplankton–bacterial interactions from a spatial perspective can contribute to a mechanistic understanding of the establishment and maintenance of these associations and, consequently, an enhanced ability to predict the impact of microscale processes on ecosystem-wide phenomena.

KEYWORDS
marine microbiology, phytoplankton-bacteria interactions

1 | INTRODUCTION

Belying their minute size, marine microorganisms have an enormous effect on large-scale processes. For example, marine phytoplankton, through their collective photosynthesis, are responsible for almost 50% of global primary productivity and oxygen production (Field et al., 1998). Importantly, phytoplankton do not exist in isolation and it is known that these microalgae encounter and interact with other microorganisms, such as bacteria. These interactions can significantly impact the microscale cell environment, influencing carbon and nitrogen fixation rates, altering metabolic profiles and promoting cell growth or decline through chemical signalling (Amin et al., 2015; Kim et al., 2022; Samo et al., 2018; Segev et al., 2016; Seyedsayamdost et al., 2011). While the importance of microscale processes in regulating biogeochemical dynamics is widely acknowledged (Azam & Smith, 1991), studying these interactions at their scale is fundamental to understanding their vast contribution to global biogeochemical cycles.

Field and laboratory observations together suggest that the composition of bacterial communities associated with algae is different

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from those found in the surrounding seawater. For example, bacteria belonging to the genera *Marinobacter* and *Limnobacter* or to the family Roseobacteraceae are often reported as being particle-associated in environmental samples (e.g. Buchan et al., 2014) and are present in freshly isolated or cultured phytoplankton specimens (e.g. Ajani et al., 2018; Mönnich et al., 2020) despite not being the dominant taxa found in bulk seawater. This suggests that some heterotrophic bacterial taxa are adapted to exploit the immediate environment around phytoplankton cells (Eigemann et al., 2023; Kieft et al., 2021; Sarmento & Gasol, 2012), which has given rise to the emerging concept of a phytoplankton-specific microbiome (Helliwell et al., 2022). Following historical definitions (Berg et al., 2020; Whipps et al., 1988), the microbiome can be defined as a 'distinct microbial community surrounding an algal cell in a zone exhibiting physico-chemical properties different from the surrounding bulk water column'. Microbiome interactions with the algal host can include mutualistic (e.g. Amin et al., 2009, 2015; Cooper et al., 2019; Croft et al., 2005; Haines & Guillard, 1974), pathogenic (e.g., Cai et al., 2023; Doucette et al., 1999; Segev et al., 2016; Seyedsayamdost et al., 2011), or neutral outcomes in terms of growth (e.g., Gärdes et al., 2011). Interactions can be variable, with effects oscillating between different states depending on the host's metabolic status (e.g., Barak-Gavish et al., 2018). These effects impact both algal and bacterial physiology, such as algal productivity and the recycling efficiency of algal-derived organic matter (Ramanan et al., 2016 and references therein).

Bacterial growth can sometimes be supported by bulk organic matter concentrations in the oceans. This is the case for oligotrophic bacteria, adapted to thrive in the ocean's often nutrient-poor conditions (Carini et al., 2013) and also for copiotrophic bacteria in specific conditions such as phytoplankton blooms, where dissolved organic matter (DOM) concentrations in the bulk seawater are high (Thornton, 2014). In both cases, nutrient uptake occurs at average bulk concentrations, obviating the need for bacteria to seek the sources of chemical gradients. However, in many other situations, the rapid dissipation of compounds from their source by diffusion or fluid flow requires close spatial proximity of bacteria to the source in order to benefit, especially in a dilute environment such as the ocean. Thus, it stands to reason that these interactions between bacteria and phytoplankton in aquatic environments require mechanisms by which proximity can be established and maintained. The sustained physical contact between interacting partners is a critical component of the *senso-lato* definition of symbiosis, which was originally stated as 'all associations where two unlike organisms lived in or on each other for a substantial part of their life cycles' (de Bary, 1879; Frank, 1877).

The spatial associations observed in marine symbioses are a reflection of the strategies employed by both partners to maintain a sustained interaction and these have been best characterised in photosymbioses (those between a photosynthetic symbiont and a eukaryotic host, Figure 1a–c). Among unicellular organisms, intracellular symbioses represent the most intimate form of association, in which the symbiont becomes an integral part of the host's structure. This integration can take different forms, with exciting cases

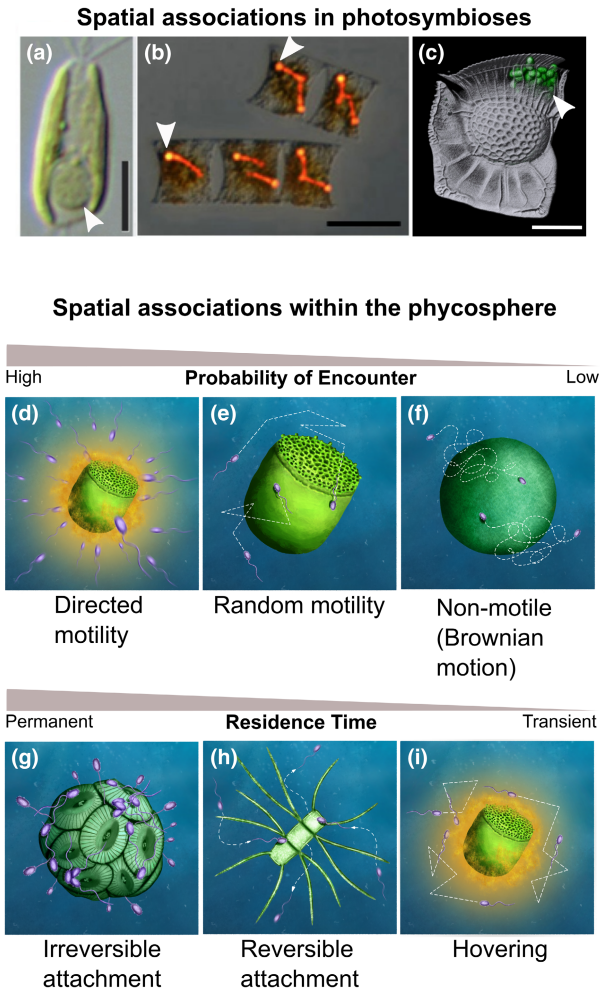

FIGURE 1 Strategies among marine unicellular organisms to achieve spatial proximity between interacting partners. (a–c) Microscopy images of mutualistic photosymbioses in the ocean. Arrowheads indicate the cyanobacterial symbionts. (a) N_2-fixing organelle, or 'nitroplast' (previously considered an endosymbiosis of the unicellular cyanobacterium Ca. *Atelocyanobacterium thalassa*) in the haptophyte *Braarudosphaera bigelowii* (Suzuki et al., 2021). Differential interference contrast (DIC) microscopy, scale bar: 5 μm. (b) Filamentous cyanobacterial symbionts inside the silica wall housing the diatom *Hemiaulus membranaceus* (Hilton et al., 2013). Fluorescence microscopy, scale bar: 50 μm. (c) Ectosymbionts of the dinoflagellate *Ornithocercus* sp. found on the balcony-like enlarged girdle (Gavelis & Gile, 2018). Confocal laser scanning microscopy, scale bar: 20 μm. (d–i) Sketches representing mechanisms that enable spatial association in the phycosphere between heterotrophic bacteria and diverse unicellular algae. In the absence of specialised host structures, these associations depend on the behaviour of the partners or on chance encounters. (d–f) Strategies leading to partner encounters, with decreasing probability: (d) directed motility or chemotaxis, (e) motility without chemotactic cues and (f) random encounters (e.g. via Brownian motion) in the absence of motile partners. (g–i) Strategies leading to prolonged contact between partners, beginning with those expected to result in longer-lasting interactions: (g) irreversible attachment via specialised bacterial structures (e.g. stalks), (h) reversible attachment and (i) 'hovering', hypothesised strategy that keeps cells in close proximity without attachment (potentially via directed motility). The latter has not yet been directly observed, so its role in extending interactions with the host remains unclear.

discovered where intracellular systems evolve beyond endosymbiosis to transition into a host's organelle (Coale et al., 2024; Moulin et al., 2024; Yoon et al., 2006) (Figure 1a). An intermediate state between intra- and extracellular symbiont location occurs in some cyanobacteria–diatom symbioses (e.g. Villareal, 1990), where symbionts are positioned between the diatom's cytoplasmic membrane and its silicified cell wall (Figure 1b).

Extracellular symbionts are frequently observed residing on the host cell surface (e.g., Hilton et al., 2013) and sometimes inhabit specialised host anatomical structures. This is the case in symbioses between dinoflagellate hosts and cyanobacteria and bacteria symbionts, which reside externally in the host girdle (Decelle et al., 2015), a groove-like structure that traverses the cell's equator. In some specific associations with cyanobacterial symbionts, the girdle has been reduced to a small chamber with an opening that corresponds to the diameter of the symbiont cell (Lucas, 1991). Other dinoflagellate hosts have broad girdles (Figure 1c) with less symbiont specificity and are often found with a community of cyanobacterial and bacterial populations of different cell sizes. Given the large percentage of the host cell volume taken up by its symbiotic partners and the morphological adaptations to house them, it seems likely that the relationships are important or necessary (Decelle et al., 2015). However, the function of the symbionts for their hosts is still unknown.

Interactions between non-photosynthetic bacteria and phytoplankton represent a taxonomically and geographically widespread mode of symbiotic association in the ocean. While endosymbioses have also been described between heterotrophic bacteria and algae (e.g. Tschitschko et al., 2024), interactions with free-living bacteria occur in the absence of dedicated host structures for physical confinement. Here, interactions are constrained to a space close to the algal cell, where diffusing chemical compounds can be available at concentrations that support growth. The concept of the 'phycosphere' defined this zone surrounding algal cells, 'in which bacterial growth is stimulated by extracellular products of the alga' (Bell & Mitchell, 1972). By highlighting the significance of spatial proximity to the alga, this founding definition incorporated a spatial component into our understanding of these interactions. Even though the role of spatial proximity in algal–bacterial interactions is generally acknowledged (e.g. Seymour et al., 2017), a fundamental challenge to the investigation of interactions at the microscale is the lack of a clear boundary to define the phycosphere. The zone for interaction is defined by metabolite concentrations that are able to influence bacterial metabolism, but these concentrations are often unknown. Furthermore, their residence time, impacted by compound-specific diffusivity, determines how long these compounds remain at sufficiently high concentrations while they are dispersing into the surrounding seawater, consequently determining the phycosphere's size and stability. Therefore, defining relevant spatial distances for cell–cell interactions is challenging and this complicates efforts to understand how these distances between partners are maintained.

Early insights into microscale interactions were obtained from studies that, rather than examining mutualistic partnerships, focused on bacteria as grazers of phytoplankton-derived biomass (e.g. Azam et al., 1994) or as prey for phytoplankton (e.g. Kiørboe & Titelman, 1998) and pioneered the consideration of motility and chemotaxis in facilitating spatial associations (Azam et al., 1994; Bell & Mitchell, 1972; Pomeroy, 1974). One of the first empirical descriptions of spatial proximity as a vehicle for mutualistic phytoplankton–bacterial interactions came from observations of the colonisation of growing diatoms by bacteria within a mesocosm bloom (Smith et al., 1995). The unexpected abundance of bacteria on actively growing diatoms contradicted the conventional notion that healthy algae are typically free from bacteria and actively discourage bacterial attachment (e.g. Sieburth, 1960). This finding expanded the possible roles of bacteria in their interactions with algae to include a range from pure opportunists scavenging on senescent algal populations to potential mutualists associated with growing algal populations.

Historically, however, work to gain a mechanistic understanding of marine algal–bacterial interactions has emphasised a reductionist approach studying only two interacting partners, rather than the entire microbial community associated with an algal host. Furthermore, potential interactions were primarily characterised by observing whether they led to enhanced, impeded, or unchanged growth in each partner (Gyurjan et al., 1984; Humenik & Hanna, 1971; Karakashian, 1975; Kuentzel, 1969; Lange, 1967; Lee & Zucker, 1969). While this approach has provided valuable insights, its focus on the growth phenotype seldom considered how spatial proximity was achieved and what role it plays in mediating these interactions. This disconnect makes it difficult to draw comparisons between biological systems when addressing fundamental questions, such as: How do partners establish a spatial relationship? How is the spatial association maintained? Is spatial proximity necessary for the interaction to occur? Answering such questions is critical to understanding how spatial proximity impacts phytoplankton and bacterial ecology.

The challenges associated with microscale investigations require a multifaceted approach (Figure 2), taking into account the diversity in bacterial behavioural strategies to establish and maintain physical proximity to phytoplankton (Figure 1), as well as the factors that influence them. Technological and theoretical advances now allow us to meet this challenge for many aspects of phytoplankton–bacteria interactions. Over recent decades, the field of microbiology has undergone a significant transition, shifting focus from population level analysis to that of individual cells. The development of single-cell approaches such as microfluidics and advances in mass spectrometry imaging (e.g. high spatial resolution secondary ion mass spectrometry, NanoSIMS) and microscopy techniques (e.g. atomic force microscopy) have enabled the study of single-cell processes, including physicochemical interactions between microorganisms and their environment (Brehm-Stecher & Johnson, 2004; Grujcic et al., 2022). These technologies have also allowed the investigation of the spatial realm governing algal–bacterial interactions.

While these methods have provided important insights, current knowledge is scattered across systems that have been studied with distinct approaches and goals. In this review, we highlight key examples encompassing the behavioural and metabolic evidence for spatial interactions in phytoplankton–bacterial relationships. Our aim is

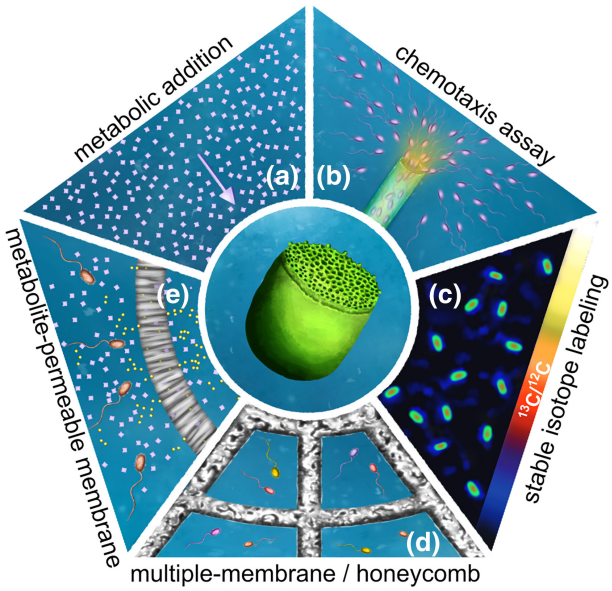

FIGURE 2 Schematic overview of the ways in which the spatial component of interactions between unicellular algae (centre) and bacteria can be studied experimentally. These methods can (a) mimic local metabolite concentrations when partners are spatially close and (b) assess the mode of encounter via bacterial directed motility. They also allow for (e) the study of algal and/or bacterial interactions with restrained spatial proximity or (c, d) as a function of distance between partners. (a) Metabolite addition, whereby the algae are exposed to extracted or synthetic bacterial metabolites (e.g. Amin et al., 2015). (b) Microfluidics or capillary assays to study chemotaxis of bacteria to phycosphere compounds (e.g., Lambert et al., 2017). (c) NanoSIMS allowing for visualisation of isotope ratio maps of algal-associated bacteria after incubation with isotope-labelled phytoplankton exudates e.g. using the $^{13}C/^{12}C$ ratio (Samo et al., 2018). (d) 'Honeycomb' microfluidic device used to control the distance between algal and bacterial cells (Kim et al., 2022). (e) A metabolite-permeable membrane, which can be used in bulk culture or microfluidic applications, to study algal–bacterial metabolite exchange (e.g. Luo et al., 2016).

to emphasise that the methods to characterise how spatial proximity contributes to phytoplankton–bacterial interactions are often available and we hope to encourage a consistent assessment of the spatial component of these interactions across different systems.

2 | BEHAVIOURAL AND METABOLIC EVIDENCE FOR THE ROLE OF SPATIAL PROXIMITY IN PHYTOPLANKTON–BACTERIAL INTERACTIONS

The most intuitive approach to characterising the role of distance between interacting bacteria and algae has been to directly observe how the interactions change when the host and symbiont are located at different distances from each other. However, control over the spatial separation of the two partners and direct visual observation can be challenging and sometimes evidence for the necessity of spatial proximity can instead be found through proxies. These include demonstrating phenotypic responses to high concentrations of metabolites as would be found in close proximity to the algal host (Figure 2a), or chemotaxis (directed motility) towards secreted metabolites (Figure 2b).

2.1 | High concentration single-metabolite approaches: Auxotrophies and signalling molecules

Metabolite approaches rely on isolating the chemicals released by algae or bacteria that mediate their interactions. Among those produced by bacteria are compounds essential for algal growth, such as vitamins and signalling molecules, such as plant growth hormones. Heterotrophic bacteria rely for growth, in turn, on photosynthetically derived organic carbon produced by the alga (Mayali et al., 2008). Identifying these key chemicals represents an often challenging but highly insightful step in understanding algal–bacterial interactions. It allows a detailed characterisation of how one member of the association (either the phytoplankton or the bacteria) responds to the metabolites in question. Although this approach, at first glance, seems to inherently dissociate the interactions from its spatial component, the relatively high concentrations of metabolites that are required to elicit a response suggest that these interactions would only function with a close spatial association between the partners.

2.1.1 | Auxotrophies: The case of phytoplankton responses to vitamin B12

Metabolite exchange between interacting microorganisms can fulfil nutrient demands that are not met otherwise. The frequent occurrence of microorganisms incapable of producing a specific organic compound that is essential for their growth (known as 'auxotrophs') suggests a high degree of metabolite exchange within the community (Yu et al., 2022). The existence of widespread vitamin auxotrophies among marine phytoplankton has perplexed researchers since their discovery (Droop, 1970; Menzel & Spaeth, 1962), given the extremely low free concentrations of vitamins in the marine environment (Sañudo-Wilhelmy et al., 2012) and has led to the suggestion that these algae form symbiotic associations with bacteria to obtain vitamins (Croft et al., 2005). Among these interactions, the provision of B vitamins to algae by bacteria, which in turn use algal-derived DOM, has been extensively studied (Croft et al., 2005; Cruz-López & Maske, 2016; Durham et al., 2015; Haines & Guillard, 1974).

The case of cobalamin (vitamin B12) has been of particular interest, since in marine ecosystems only certain heterotrophic bacteria and some members of the archaeal phylum Thaumarchaeota (known as B12 'prototrophs') can produce this compound (Heal et al., 2017; Shelton et al., 2019). While some mechanisms for survival without vitamin B12 have been reported for certain algae (Helliwell et al., 2011), the absence of such mechanisms in many phytoplankton species has led to the theory that the genes encoding for B12-independent metabolisms may have been lost due

to a sustained external supply of the vitamin (Croft et al., 2005). Experimental evolution under conditions of continual B12 addition has provided empirical evidence of a similar process, demonstrating the evolution of B12 auxotrophy via gene loss in *Chlamydomonas reinhardtii*, an algal species that is naturally B12 independent (Helliwell et al., 2015). This supports how auxotrophies in algae could arise as a result of sustained spatial associations with prototrophs. However, the causality in the evolution of natural auxotrophies remains an open question, whether this metabolic specialisation leads to or arises as a consequence of persistent associations (Kazamia et al., 2016).

Tackling the role of space in alleviating auxotrophies and thus understanding the impact of bacteria in this aspect of algal ecology is still challenging. Algal auxotrophy has often been investigated in batch cultures devoid of vitamin B12, where the addition of prototrophic bacteria results in a recovery in algal growth (Croft et al., 2005; Durham et al., 2017; Kazamia et al., 2012; Sultana et al., 2023; Wagner-Döbler et al., 2010), supporting the hypothesis that bacteria are capable of alleviating algal vitamin dependence. The mechanisms for sustained vitamin provision are not universal across model algal–bacterial systems. In some cases, there is evidence of bacterial attachment to algae, either directly to their surface, e.g., *Dinoroseobacter shibae* DFL12^{T} to the dinoflagellate *Prorocentrum lima* (Wagner-Döbler et al., 2010), or to their extracellular muciferous layer, as observed for *Halomonas* sp. and the red alga *Porphyridium purpureum* (Croft et al., 2005), with attachment suggested as the mechanism for metabolite exchange. Other studies suggest that physical contact may not always be essential, as bacterial production of vitamin B12 can facilitate algal growth via diffusion through a membrane, without physical attachment (Kazamia et al., 2012).

In the case of diatom hosts, an important group of algae known for widespread vitamin B12 auxotrophy (Croft et al., 2005; Helliwell et al., 2011), the findings are less conclusive. Although attachment is observed, it appears to be a minor aspect of the interaction, with low numbers of bacterial cells (typically 2–3) attached to exponentially growing diatoms (Durham et al., 2017). This has led to the suggestion that chemical diffusion may play a more significant role, although it is unclear how many attached bacterial cells would be required for significant vitamin provision. Furthermore, coculture experiments are typically performed using significantly higher densities of diatoms and bacteria than those found in natural environments; for example, 100-fold and 1000-fold greater for diatoms and bacteria, respectively (Durham et al., 2017), than in natural settings (Fu et al., 2020; Karentz & Smayda, 1984) and use batch culture approaches in which metabolites accumulate over time (Biddanda & Benner, 1997; Chorazyczewski et al., 2021). This likely results in concentrations of bacterial-produced vitamin B12 and algal-derived DOM in the culture medium that exceed averages in the open ocean, which could eliminate the need for a spatial association between the partners.

The mode of provision of B12 by bacteria to algae also remains largely an open question, with debate over whether it involves active secretion by bacteria or bacterial cell lysis (Droop, 2007; Wienhausen et al., 2022). While the potential for vitamin release has traditionally been inferred from the presence and expression of vitamin biosynthetic pathways (Bertrand et al., 2015; Doxey et al., 2015; Gómez-Consarnau et al., 2018; Haiwei & Moran, 2014; Shelton et al., 2019), not all marine prototrophic bacteria predicted to produce vitamin B12 have been found to in fact secrete it (Sultana et al., 2023). This has led to a recent distinction between B12-provider and B12-retainer strains where, intriguingly, most B12-provider strains were isolated as algal-associated microorganisms, whereas most B12-retainer strains were isolated as free-living in the ocean (Sultana et al., 2023). This correlation supports the view that, for prototrophic bacteria, living in close proximity to algae favours active vitamin release. An exciting open question then is to directly test whether B12-provider strains require close association in space with algal cells for vitamin transfer.

2.1.2 | Signalling molecules: The case of bacterial secretion of auxins

Phytohormones (signalling molecules within plants that regulate their physiology) are an important class of infochemicals, well studied in the plant rhizosphere and with the potential to also be important mediators in marine interactions. Similar to their role in short-scale interactions between microorganisms and plant roots, it is likely that these molecules are effective at short distances between marine algae and bacteria. Among phytohormones, indole-3-acetic acid (IAA) is the most abundant of the auxin class (Teale et al., 2006). IAA can be produced by both terrestrial plants and rhizosphere-associated bacteria (Khalid et al., 2004; Zhao, 2010) and is considered an effector molecule for bacteria–plant interactions, since it can affect gene expression in both partners (Spaepen & Vanderleyden, 2011). While evidence for the production of IAA by marine unicellular algae is currently limited to a few cases (Labeeuw et al., 2016), multiple metabolic pathways for IAA synthesis have been described for diverse marine bacterial groups, including many members of the *Roseobacter* group (Moran et al., 2007), commonly observed in association with unicellular algae (Buchan et al., 2014; Haiwei & Moran, 2014). Considering the known role of IAA in interactions between terrestrial plants and rhizosphere bacteria (Spaepen & Vanderleyden, 2011), it has been suggested that this metabolite is commonly exchanged between bacteria and unicellular algae in the ocean (Amin et al., 2015) and likely requires close proximity to the host to be effective.

The effects of IAA on algae are diverse and potentially species-specific (Amin et al., 2015). Early investigations found that the secretion of IAA allows the bacterium *Sulfitobacter pseudonitzschiae* to promote cell division of the diatom *Pseudo-nitzschia multiseries* (Amin et al., 2015). Similar to the effects of IAA in terrestrial plants in which the hormone enhances growth at low concentrations while causing physiological damage at high concentrations (Persello-Cartieaux et al., 2003), high IAA concentrations

have been observed to negatively affect phytoplankton growth (Amin et al., 2015). A striking example is the provision of IAA by *Phaoebacter inhibens*, a bacterium belonging to the *Roseobacter* clade, to the coccolithophore *Gephyrocapsa huxleyi* (formerly known as *Emiliania huxleyi*, Filatov et al., 2021). In coculture, *P. inhibens* initially stimulate algal growth through the release of IAA. The production of IAA is further enhanced by the algal supply of the biosynthetic precursor tryptophan. However, later in the interaction, a shift in bacterial strategy is observed as the bacteria trigger algal cell death by activating oxidative stress pathways (Segev et al., 2016). Such a shift from mutualistic to pathogenic relationship was originally described as a 'Jekyll and Hyde' behaviour (Seyedsayamdost et al., 2011). This observation has led to the suggestion that the bacteria are essentially 'farming' the algae, which is supported by the significant increase in bacterial growth (100,000-fold) over a 20-day period in *P. inhibens*–*E. huxleyi* co-cultures (Segev et al., 2016). The shift in algal phenotypic response could be reproduced and quantified with artificial IAA additions. When *E. huxleyi* is supplemented with 1 μM IAA, its growth is enhanced by almost 20%. However, when provided with 1000 μM IAA, the auxin acts instead as an algicide, leading to a crash of *E. huxleyi* cultures (Segev et al., 2016).

In both the *S. pseudonitzschiae*–*P. multiseries* and *P. inhibens*–*E. huxleyi* systems, the phenotype of the algae observed in co-culture could be replicated by the exogenous addition of synthetic IAA. Notably, however, this response only occurred in the presence of IAA concentrations orders of magnitude higher (in the nanomolar and micromolar range, respectively; Amin et al., 2015; Segev et al., 2016) than the concentration of bacterial IAA detected in the co-culture media or in surface ocean waters (picomolar range, Amin et al., 2015). This suggests that the response naturally induced by the bacteria is dependent upon close spatial proximity, so that the algae are exposed to a much higher local concentration of IAA produced by the bacteria than that present in the bulk. This evidence is, however, indirect and whether and how spatial proximity is established and maintained remain important open questions. While this has not been addressed for *S.pseudonitzschiae*, attachment has been observed for *P. inhibens* to different *E. huxleyi* strains (Bramucci et al., 2018; Segev et al., 2016). However, the Jekyll and Hyde behaviour was not reproducible in all *E. huxleyi* strains: for in some, only the algal crash and not the initial algal growth enhancement was observed (Bramucci et al., 2018; Bramucci & Case, 2019). Interestingly, the outcome of the interaction (Jekyll and Hyde, algicidal or neutral) appears to be dependent on the strain of *E. huxleyi* (Bramucci et al., 2018; Segev et al., 2016) and not on whether attachment occurs. Thus, whether attachment behaviour influences the IAA concentrations experienced by the host is yet unknown. Across these studies of auxotrophies and the exchange of signalling molecules, while the metabolite-addition approach only provides indirect evidence, nonetheless, carefully calibrated measures of the concentrations necessary to elicit a response in one partner and spatially resolved measures of concentrations produced by the other partner can provide a strong case for the importance of spatial proximity.

2.2 | Chemotaxis

Directed motility along chemical gradients, known as chemotaxis, in response to algal compounds provides another source of indirect evidence for the importance of space in the interactions of bacteria and phytoplankton. Bacterial investment in motility and chemotaxis, despite the high costs of these behaviours (Keegstra et al., 2022), can result in enhanced encounters between partners and may represent pervasive mechanisms for the establishment and maintenance of symbioses in the ocean (Raina et al., 2019).

There is evidence from theoretical and laboratory-based studies that diverse marine bacteria and some phytoplankton are capable of chemotaxis (Adler, 1966; Choi et al., 2016; Sjoblad et al., 1978; Stocker & Seymour, 2012). The ability of phytoplankton exudates to act as a chemotactic cue has also been demonstrated for diverse bacteria (Hallstrøm et al., 2022; Miller et al., 2004; Seymour et al., 2010). Laboratory observations of natural microbial communities have shown that bacteria exhibit chemotaxis to exploit the DOM released by lysing algae (Smriga et al., 2016) (Figure 3e). While this

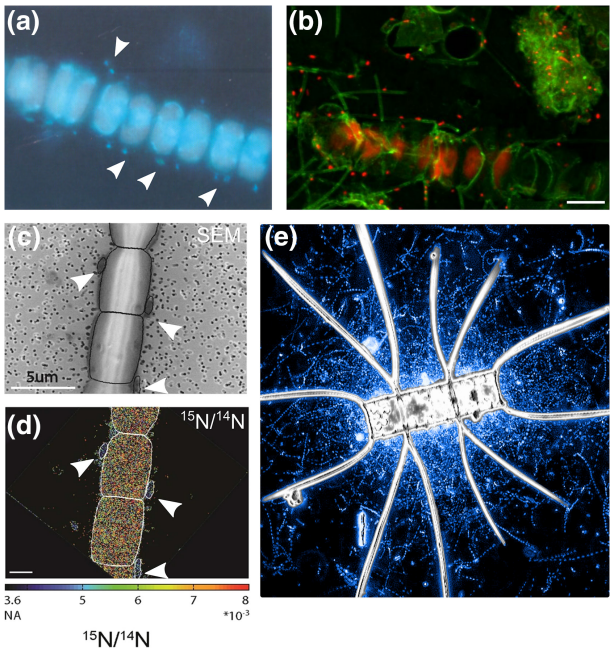

FIGURE 3 Examples of the direct visualisation of algal–bacterial interactions. (a) Epifluorescence microscopy image of DAPI-stained bacteria (arrows) attached to diatom cells during a mesocosm bloom (Smith et al., 1995). (b) False-colour confocal laser scanning micrograph of Alexa594-stained *Polaribacter* bacteria (POL740 probe, *red*) attached to the fucose-specific lectin AAL-Alexa488 (*green*), present in EPS aggregates and the setae of the diatom *Chaetoceros* sp. (*dark red*, chlorophyll autofluorescence). Scale bar: 10 μm (Bennke et al., 2013). (c, d) The diazotrophic cyanbacterium *Aphanizomenon* with associated bacteria (arrows), imaged with (c) scanning electron microscopy (SEM) and with (d) NanoSIMS, where nitrogen uptake and transfer is shown as $^{15}N/^{14}N$ ratio. NanoSIMS scale bar: 2 μm (Schoffelen et al., 2019). (e) Chemotactic clusters of bacteria around chains of the diatom *Chaetoceros affinis* undergoing lysis. Blue tracks are bacterial trajectories over 1–2 s (Smriga et al., 2016).

study provided direct evidence that bacteria can perform chemotaxis towards algal metabolites, it left open the question of whether this chemotactic attraction also occurs towards healthy algal cells and can therefore contribute to mutualistic interactions.

Expanding on the role of bacterial chemotaxis, it is increasingly evident that marine bacterial species exhibit a high chemotactic precision (Brumley et al., 2019; Stocker et al., 2008; Xie et al., 2011), enabling the sensing of smaller targets than traditionally assumed (Seymour et al., 2024). Subsequent research demonstrated the advantages of this precision in substantially enhancing metabolic exchange with small algal hosts, as shown in studies of bacteria and picophytoplankton (Raina et al., 2023). Stable-isotope tracking and measures of single-cell uptake via NanoSIMS revealed reciprocal uptake of exudates from the picocyanobacteria *Synechococcus* and the heterotrophic bacterium *Marinobacter adhaerens*. Comparison with measurements from bacterial mutants deficient in motility and chemotaxis revealed that chemotaxis increased nitrogen and carbon uptake of both partners by up to 4.4-fold, demonstrating that associations between heterotrophic bacteria and these minute picophytoplankton are mediated by bacterial behaviour. Numerical simulations further suggested that short-lived yet repeated encounters and intermittent pulses of exudates are the typical manner by which the two partners exchange compounds. This frequent repositioning of the bacteria to maintain close proximity to the host suggests a behaviour involving bacterial 'hovering' in the very small phycospheres predicted for picophytoplankton (Seymour et al., 2017). While the exact mode of interaction is yet to be experimentally identified, these observations suggest that interactions can be much more dynamic than traditionally assumed.

Bacterial chemotaxis may be a significant factor contributing to the observed specificity in algal–bacterial interactions, with growing evidence of distinct algal-associated bacterial communities, different from the bulk community and varying among phytoplankton hosts (Ajani et al., 2018; Behringer et al., 2018; Mönnich et al., 2020; Stock et al., 2022). The chemical composition of phytoplankton-derived DOM is complex and highly dependent on phytoplankton taxonomy (Aluwihare & Repeta, 1999; Becker et al., 2014; Castillo et al., 2010; Heal et al., 2021; Landa et al., 2017) and has been suggested to favour the presence of specific bacterial taxa (Fu et al., 2020; Helliwell et al., 2022). As a result, chemotactic attraction of bacteria to specific algal compounds could represent a mechanism that enables specialisation in colonisation among algal hosts and the formation of taxon-specific microbiomes.

Recent studies have offered experimental evidence for this hypothesis in the environment. The chemotactic ability of microbial populations has recently been measured directly in the ocean using a new approach, the *in situ* chemotaxis assay (ISCA), a field-based microfluidic platform that generates diffusive point sources of chemicals and allows for selective capture of live bacteria from the environment as a result of their chemotactic behaviour (Clerc et al., 2020; Lambert et al., 2017). Studies using this device to examine behavioural responses to various algal exudates have shown that the ability to perform chemotaxis towards algal-derived DOM is widespread among marine bacterial and archaeal taxa (Clerc et al., 2023; Raina et al., 2022). The distinct DOM composition of each phytoplankton species attracted phylogenetically distinct populations of microorganisms, providing empirical support for the hypothesis that specialised chemotactic responses promote the establishment of specific associations between phytoplankton and prokaryotes (Raina et al., 2022). While the individual compounds triggering specificity have not yet been identified, exploring chemotactic behaviour in this way provides exciting hints into the potential links between the chemical composition of the phycosphere and the microbiomes that are assembled. On the other hand, within the same algal species, nutrient limitation has been shown to increase the quantity (Guerrini et al., 2000; Myklestad & Haug, 1972; Staats et al., 2000) and induce changes in composition (Ai et al., 2015; Urbani et al., 2005) of secreted DOM (in particular, carbohydrates). This raises new questions about the microbiome's sensitivity to changes in the composition and concentration of algal-derived DOM. In addition to understanding the establishment and maintenance of associations, this connection is important to be able to predict community dynamics in changing environmental conditions.

3 | DIRECT VISUAL EVIDENCE FOR SPATIAL INTERACTIONS

Observations of attachment and quantification of the growth benefits resulting from these associations offer direct evidence of the significance and impact of spatial proximity in these interactions. We currently lack an understanding of the duration for which physical closeness must be sustained to trigger a phenotypic response in both bacteria and the host, or whether physical proximity alone is sufficient for an interaction to occur. Developing and applying appropriate visualisation techniques to observe the maintenance of spatial proximity and the resulting phenotypic responses (such as growth or metabolic exchange) has the potential to provide important new insights into these mechanisms of association (Figure 3).

3.1 | Growth behaviour

One of the most commonly used approaches to studying the interaction between two microorganisms is by characterising the impact of the interaction on their growth behaviour. The traditional and methodologically simplest way of doing this is by monitoring growth of the algae when they are kept physically separate but metabolically connected to the bacteria (and vice versa) (Figure 2d,e). This has been achieved through a variety of methods, most exploiting semi-permeable membranes that allow for metabolite exchange between partners by contact-independent mechanisms (e.g., Moutinho et al., 2017) (Figure 2e). In bulk cultures separated by membranes, these approaches effectively address the complication of distinguishing between organisms within bulk co-cultures; however, in most cases, this approach fails to capture direct cell–cell

interactions, as the two organisms are physically separated. In addition, this approach relies on large numbers of cells to produce sufficient concentrations of metabolites in the bulk to induce a response. Nevertheless, the inherent ease of using growth as a proxy to deduce whether an interaction is taking place is clear and thus methodologies that can measure growth effects as a function of distance from a nutrient source or an algal cell are powerful tools to characterise an interaction.

Though the idea of using semi-permeable membranes is most intuitively applied in keeping organisms physically separated and thus preventing spatial interactions from taking place, it can also be harnessed to explicitly resolve the influence of space on algal–bacterial interactions. By creating a multi-layer microfluidic system, it is possible to keep bacteria and phytoplankton physically separated but metabolically connected using semi-permeable membranes as walls (Kim et al., 2022) (Figure 2d). Using this design, it is possible to arrange symbionts at different distances from each other by inoculating them into closer or more distant wells for example, in steps of 10 mm separation between wells (Kim et al., 2022). This method has already allowed resolution of the spatially dependent behaviour of an algal-associated *Marinobacter*, which, when inoculated into this device, showed enhanced cell growth in chambers further away from the algal host during early stages of bacterial growth, but the reverse pattern at later experimental timepoints. This observation was explained with the creation of a decreasing inorganic nutrients concentration gradient towards the algal host which impeded bacterial growth closer to the algae at early stages of the experiment, but once this gradient had settled, the production of algal exudates promoting bacterial growth overpowered this effect. Furthermore, when an entire bacterial community was inoculated into the wells, the community diverged significantly in composition between wells close and more distant from the algae (Kim et al., 2022). These observations represent a powerful way of using the phenotypic response of growth to resolve the effects of distance on algal–bacterial interactions. Additionally, they show that the influence of spatial proximity on bacterial and phytoplankton growth may change over the course of a longer interaction.

3.2 | Attachment behaviour

Attachment between partners is arguably the best strategy to maintain a close and lasting association between unicellular organisms. Cell-to-cell adhesion can increase nutrient availability (Arandia-Gorostidi et al., 2022) and decrease the risk of separation, for example in turbulence, as opposed to interactions in which organisms are not attached (Berne et al., 2018; Costerton et al., 1995). A large number of marine pelagic bacteria are found in pairwise attached associations with other cells, including picocyanobacteria, revealing a prevalence of spatially intimate interactions in the upper ocean (Malfatti & Azam, 2009). However, the nature of the interactions cannot be deduced by this observation alone: bacterial attachment has been observed in mutualistic (Durham et al., 2017), algicidal (Furusawa et al., 2003; Segev et al., 2016) and neutral (Gärdes et al., 2011) associations.

Attachment mechanisms of bacteria have been extensively reviewed (e.g., Berne et al., 2018). For example, members of the marine *Roseobacter* clade attach via polysaccharides in their cell pole (Segev et al., 2015). There are several other bacterial structures that can mediate attachment. Perhaps the most striking are the stalks of members of the order Caulobacterales, where a polysaccharide-based adhesin at one cell pole allows strong attachment to surfaces, with forces in the microNewton range (Hershey et al., 2019; Tsang et al., 2006). The importance of spatial proximity is suggested in these two bacterial orders by the fact that attachment often leads to permanent inhibition of motility. This results in a biphasic 'swim and stick' lifestyle in *Roseobacters* (Geng & Belas, 2010) and forms part of a highly complex life cycle in Caulobacterales.

The most intuitive benefit of bacterial attachment to algal cells is the continuous access to the highest concentrations of algal metabolites in proximity to the host cell, yet it is not always apparent that attachment brings a direct benefit to the algal host. Both attachment and degradation of algal-derived polysaccharides have been described for members of the class Flavobacteriia (Bacteroidota). This bacterial group, particularly abundant in diatom blooms (Teeling et al., 2012, 2016), can dominate the bacterial fraction attached to phytoplankton in field studies (Bennke et al., 2013) and microcosm experiments (Sapp et al., 2007). Many marine Flavobacteriia are specialised in polysaccharide degradation (Kappelmann et al., 2019; Reintjes et al., 2017) and microscopy observations of colonies on algal polysaccharides (Bennke et al., 2013) suggest active growth through substrate consumption at the attachment site. Bacterial consumption of the very substrates that mediate attachment seems counterproductive to establishing long-lasting interactions with phytoplankton and indeed bacteria that are highly adapted to metabolising sugars and algae-derived polysaccharides are often categorised as commensal or opportunistic rather than mutualistic (Bennke et al., 2013; Teeling et al., 2012). This interpretation is further supported by the observation that bacterial attachment to visually healthy algal cells is relatively uncommon (Droop & Elson, 1966). Instead, bacterial attachment is often reported on senescent and damaged algal cells, (e.g., Jones, 1976; Oppenheimer & Vance, 1960), algal aggregates (e.g., Grossart et al., 2006), as well as free-floating extracellular polymeric substances (EPS) secreted by the algae (e.g., Alldredge et al., 1993; Long & Azam, 1996). Moreover, some algicidal bacteria require attachment to their prey for algal lysis to occur (Mayali & Azam, 2004). While historical observations led to the assumption that bacterial attachment to eukaryotic phytoplankton is detrimental, it is now recognised that this is not always the case (Smith et al., 1995) (Figure 3a). On healthy cells, a single attached bacterium per phytoplankton cell is the most common scenario, with few phytoplankton cells bearing multiple attached bacteria (Kogure et al., 1981). Thus, it is also possible that healthy cells actively deter attachment of bacteria, whereas unhealthy cells may lose this deterrence ability. An open question then is

whether bacterial attachment is actively prevented by algae and, if so, through what mechanism.

Early studies suggested that phytoplankton defence mechanisms against bacterial attachment are mediated by the production of antibiotics (Droop & Elson, 1966; Sieburth, 1960; Trick et al., 1984). However, it is likely that algal mechanisms to regulate bacterial colonisation are more nuanced and complex. Research on the cosmopolitan diatom *Asterionellopsis glacialis* found that, upon re-introduction of the diatom's natural bacterial community to an axenic culture, the diatom's metabolic profile was significantly altered, including the production of a secondary metabolite, rosmarinic acid (Shibl et al., 2020). Rosmarinic acid was found to reduce the motility of several strains of beneficial bacteria, whilst increasing the motility of parasitic strains. This impact on bacterial motility was suggested to encourage attachment by beneficial bacteria while discouraging attachment by parasitic species, an example of how phytoplankton may regulate their associated bacterial communities through secreted metabolites.

Algae could also mediate attachment directly by modulating the cell surface. However, the extent to which algae actively regulate their surface properties to selectively enhance the colonisation of certain bacteria over others remains unknown. The chemical composition of the algal surface and secreted polysaccharides, particularly in diatoms, has been proposed to be an important mediator of bacterial host recognition (Bennke et al., 2013) and potentially influences the establishment of bacterial–algal interactions. The diatom cell surface, a complex structure encompassing the cell wall and surface-coating chemical moieties (Tesson et al., 2009), has a composition that differs among species (Haug & Myklestad, 1976). Among these chemical moieties is a group of polysaccharides known as glycoconjugates, which coat diatom surfaces and, when found in the water column, are known as EPS. It has been proposed that distinct binding sites on glycoconjugate molecules mediate species-specific attachment. Lectin staining of glycoconjugates during a diatom bloom (Bennke et al., 2013) revealed distinct preferences for attachment among different flavobacterial clades towards specific polysaccharides characteristic of different phytoplankton species (Figure 3b). For example, members of the genera *Formosa* and *Polaribacter* were respectively attached to galactose- and fucose-rich surfaces on the diatom genus *Chaetoceros*, while *Ulvibacter* spp. showed a clear preference for mannose-containing binding sites on another blooming diatom species, *Asterionella* sp. While the preferential colonisation of fucose-rich glycans by specific bacterial species (*P. frisia* and *G. forsetii*) in diatom cells has also been observed in culture experiments (Den et al., 2023), the mechanisms and consequences of this specificity remain unknown. Other glycans are less favourable for bacterial attachment. This is the case for sulfated fucans (e.g. fucoidans), which coat many species of diatoms and are also present in EPS aggregates (Vidal-Melgosa et al., 2021). Given their recalcitrance to bacterial degradation in comparison to other algal-derived polysaccharides (Arnosti, 2011; Sichert et al., 2020), fucoidans have been suggested to serve as a protective barrier from bacterial predation (Bligh et al., 2022; Vidal-Melgosa et al., 2021). However, it is possible that they are also employed by diatoms as a deterrent against bacterial attachment, a hypothesis that has not yet been addressed.

Both field (Bennke et al., 2013) and co-culture (Den et al., 2023) studies have also revealed selective bacterial attachment to specific regions on algal cells and chains of cells, such as on chitin fibres and setae (long silica extensions from diatom valves), each possessing a distinct structure and chemical composition from the cell body (Durkin et al., 2009; Herth, 1979; Mayzel et al., 2021; Owari et al., 2022). This observation further implies that the location of attachment on the cell can be influenced by the surface properties. Still, the ecological consequences of this positioning are not yet known. Beyond diatoms, surface structure and/or chemical composition have been observed to mediate bacterial attachment to other unicellular algae, such as coccolithophores. For instance, some studies in the co-culture model system of *E. huxleyi* and *P. inhibens* have observed attachment exclusively on algal cells that lack their calcium carbonate shell (Eliason & Segev, 2022; Segev et al., 2016). However, it has been shown that *P. inhibens* itself has no influence on algal calcification (Eliason & Segev, 2022) and other studies have reported attachment to both calcified and decalcified cells (Bramucci et al., 2018), suggesting that the influence of calcification state on bacterial attachment might be controlled by multiple factors or specific conditions (Eliason & Segev, 2022; Segev et al., 2016). Loss of the algal shell, a condition commonly seen in older, nutrient deficient, or unhealthy cultures, likely represents an unhealthy state (Gerecht et al., 2018; Jakob et al., 2018; Walker et al., 2018). Observations of attachment of pathogenic *P. inhibens* to exclusively decalcified cells under certain conditions thus supports the hypothesis that attachment is more likely to occur on unhealthy cells. Taken together, these observations suggest that the shell normally functions as protection and its loss for reasons other than pathogenic attachment leaves the cells vulnerable to bacterial adherence. None of these hypotheses have been directly addressed, however, and it is not yet known if the coccolithophore calcium carbonate shell itself prevents attachment or if the observations are confounded by additional, unaccounted-for cellular fitness factors.

Beyond growth and mortality, observed in bulk in cultures, the further effects of attachment are more subtle and require single-cell methodology to be quantified, such as stable isotope probing combined with NanoSIMS (Figure 2c). These approaches have revealed how the physiology of attached bacteria can be modulated by the algal host. This has been best characterised in mutualist interactions of diazotrophic symbiotic cyanobacteria and phototrophic eukaryotes (reviewed in Foster et al., 2022), where the symbionts have been shown to fix more nitrogen than they need for their own cellular requirements in order to supplement their host's needs (Foster et al., 2011; Martínez-Pérez et al., 2016). Free-living diazotrophic cyanobacteria are also found as hosts of bacteria and it has been observed that attached bacteria similarly receive fixed nitrogen (Schoffelen et al., 2019) (Figure 3d). Attachment to the host increased when rates of nitrogen excretion increased, suggesting an active and dynamic response of symbionts to their host metabolism (Schoffelen et al., 2019).

Attachment-mediated mutualism is likely species-specific, as indicated by varying responses to attachment among different algae species (Samo et al., 2018). Experiments using a combination of stable-isotope enrichments and NanoSIMS in a mesocosm revealed that mutualist bacteria not only attach to and take up more carbon from the diatom *Phaeodactylum tricornutum* relative to other bacteria, but they are also associated with increased carbon fixation by the alga itself (Samo et al., 2018). Different effects were observed for the diatom *Nocardiopsis salina*, which fixed more carbon in axenic conditions and harboured fewer attached bacteria per algal cell compared to *P. tricornutum* in non axenic conditions. Since bacterial attachment was not directly beneficial for *N. salina* (with the interaction instead characterised as parasitic, with bacteria that 'syphon' carbon without a positive effect on the algae), it was concluded that this algal species actively prevented bacterial colonisation.

Current evidence suggests that the identity and location of bacteria attaching to phytoplankton can profoundly impact both bacterial and host physiology, leading to outcomes ranging from enhanced growth to the death of the algal host. Further exploration of the mechanisms enabling diatoms and other phytoplankton species to actively regulate their interactions with bacteria is crucial for understanding how they can avoid opportunistic or pathogenic bacteria while promoting beneficial associations.

4 | CONCLUSIONS

Traditional approaches to the study of phytoplankton–bacterial interactions have often looked exclusively at the end result of an interaction, qualifying the effect of an association via its impact on growth rate. Though this is certainly an essential part of characterising algal–bacterial interactions and can help categorise them as mutualistic, commensal, pathogenic, or parasitic, looking exclusively at growth responses neglects other components of an interaction such as metabolite exchange, surface attachment and impacts on the wider symbiont community. Approaches centred on growth as a phenotypic outcome often simplify the system by examining two interacting partners in isolation or one partner alone in response to introduced exogenous metabolites from the other. Extrapolation of the findings from these approaches to the natural environment is challenging, especially when considering that algal microbiomes may involve tens to hundreds of different species, as estimated from cultured consortia (Ajani et al., 2018; Behringer et al., 2018; González et al., 2000; Green et al., 2015; Mönnich et al., 2020). For instance, competition for vitamins with phytoplankton and among different members of the bacterial community (e.g., Bertrand et al., 2015) or the inhibition of a phenotype in the presence of other bacteria as reported for the *P. inhibens–E. huxleyi* system (Beiralas et al., 2023) could mitigate the effects observed in one-strain interactions. However, these challenges underscore the importance of understanding the effects of the microbiome as a whole on algal physiology.

Interactions between phytoplankton and bacteria are largely mediated by phytoplankton-derived DOM that serves marine heterotrophic bacteria as an energy and carbon source (Azam et al., 2022). Bacteria, in turn, are significant contributors of specific metabolites, such as vitamins and signalling molecules, which are expected to be more available in close proximity to the cells producing them. The necessity of close proximity for metabolite exchange is supported by measures of vitamin and auxin concentrations in seawater, which are lower than those required for microbial growth (Amin et al., 2015; Bertrand et al., 2007; Sañudo-Wilhelmy et al., 2012). In addition, there is compelling evidence that providing significantly higher metabolite concentrations (in comparison with concentrations measured in the bulk ocean) leads to enhanced phytoplankton growth rates (Gobler et al., 2007; Koch et al., 2011), yielding values consistent with growth rates measured in co-culture with bacteria (Amin et al., 2015; Croft et al., 2005; Segev et al., 2016). However, explicit attempts to assess the importance of spatial proximity in cell signalling and the evolution of metabolic dependencies among microorganisms are lacking. In addition to direct observations of interactions, manipulation of metabolite concentrations could be used to draw inferences about the required distance between unicellular organisms. Additionally, exploring these questions in dilute systems (as opposed to the current studies in batch cultures with elevated nutrient concentrations) holds promise as an approach to better reflect algal–bacterial associations in natural settings.

Another indication of the importance of spatial proximity in mediating phytoplankton–bacteria interactions comes from the proxy of bacterial chemotaxis to algal-derived compounds. The widespread existence of marine bacteria capable of chemotaxis towards algal exudates or known algal metabolites could be interpreted as evidence for their association. However, whether chemotaxis is a trait prevalent in algae-associated bacteria is an open question. Understanding its prevalence could shed light on whether chemotaxis is a dominant strategy of colonisation and the establishment of associations (in comparison to random encounters, for example). Moreover, there is growing evidence that phytoplankton can influence bacterial chemotaxis, as indicated by distinct chemotactic responses of different bacterial populations to different phytoplankton exudates. While this is likely a result of particular chemoattractants that trigger responses from specific bacterial communities, the identification of these compounds and whether they are secreted by algae in different metabolic states remain exciting unknowns. Additionally, whether chemoattractants simply function as cues or are themselves the metabolic substrates capable of supporting bacterial growth has received little attention (Clerc et al., 2023). In the context of interactions, addressing this aspect could provide further insights into the regulatory mechanisms employed by algae to prevent or enhance colonisation by specific bacteria.

Ample visual evidence of attachment of bacteria to different algal hosts and the quantification of metabolite exchange using NanoSIMS represent direct insights into the mechanisms and effects of physical proximity. Attachment is the most obvious evidence

for spatial associations. Increasingly, studies suggest that algae can regulate the number and taxa of attached bacteria. While the exact mechanisms remain unclear, parallels have been drawn with our understanding of animal defence mechanisms against bacterial and viral infections (Bligh et al., 2022). For instance, similar to the increased mucus synthesis observed during infections in nasal and lung airways, algae secrete extracellular matrix glycans in the presence of bacteria and viruses (Bligh et al., 2022). It would be of interest to explore whether specific glycans function as cellular protection from the degrading enzymes of bacteria or prevent active attachment by bacteria. Insights and methodological approaches can be gleaned from studies on multicellular macroalgae, in which isolation of algal surface compounds has allowed identification of chemical cues that regulate the abundance of bacterial colonisers (Lachnit et al., 2013).

In addition, work on bacterial polysaccharide degradation can contribute to completing the picture of the mechanisms, regulation and consequences of bacterial attachment. Questions remain open regarding whether polysaccharide-degrading bacteria consume secreted or surface-coating polysaccharides and whether they influence algal physiology when attached and the answers are important to the understanding of algal bloom dynamics. Integration of knowledge from both culture and field studies will be critical for moving beyond mere descriptions of attachment and advancing our understanding of its effects on both bacterial and algal physiology. Ultimately, this broader perspective will allow for a more nuanced understanding of opportunistic and mutualistic behaviours, contributing to an improved assessment of the wider ecological impacts arising from these single-cell processes.

Beyond the regulation of attachment, research hints that phytoplankton may also regulate bacterial motility. Indeed, fascinating evidence has been presented in this regard, including upregulation of the motility genes of parasitic bacteria and downregulation of the motility genes of beneficial bacteria in the presence of certain algal species (Shibl et al., 2020). This phenomenon has yet to be observed directly in terms of actual changes in swimming speed. If phytoplankton can indeed regulate the swimming speed of bacteria, this also has consequences on chemotaxis, since swimming speed can directly impact chemotactic precision (Son et al., 2016). The presence and nature of algal regulation of bacterial motility is not yet fully characterised, but represents another behavioural strategy that may vary across different algae and bacteria or change over the course of an interaction. This remains open for exploration.

Initially limited to the realm of the impact on bacterial growth, the concept of the phycosphere has now expanded to encompass the zone where reciprocal interactions between phytoplankton and bacteria unfold. Direct assessment of the spatial component of algal–bacterial interactions remains a technical challenge; however, many of the tools needed to more fully understand the spatial dimension, such as microfluidics allowing for fine spatial control, NanoSIMS for the characterisation of metabolite exchange as a function of distance, and chemotaxis assays to assess directed motility, are already available. We argue that comparing mechanisms that facilitate spatial proximity across symbiotic systems is currently challenging due to the scattered and disparate nature of existing studies. The wide range of approaches used, from methodologies that provide no insight into spatial dimensionality to studies that directly observe the spatial component, makes it difficult to evaluate the generality of current observations. A systematic approach across diverse systems will pave the way to identifying common traits and determining the importance of specific bacterial and phytoplankton behaviours for the establishment and regulation of associations.

While we are now assembling a more complete picture of the variety and mechanisms of bacterial–phytoplankton interactions, we anticipate that similar approaches, which have allowed the first insights into the spatial component in unicellular associations, will also open avenues to address many other questions. Unresolved issues include the frequency with which bacteria enter and leave the phycosphere, the length of time they must remain there for an exchange to occur, and whether metabolic exchange takes the form of a constant trickle of nutrients or pulses associated with different phases of growth or varying conditions. Addressing these questions, in turn, can provide the first insights into the vulnerability of interactions in algal-associated bacterial communities to environmental fluctuations. The resulting physiological changes carry ecological consequences, emphasising the importance of studying these microscale interactions for a predictive assessment of their resilience amid environmental changes and their impact on global elemental cycles.

AUTHOR CONTRIBUTIONS

Clara Martínez-Pérez: Conceptualization; writing – original draft; writing – review and editing; funding acquisition. **Sophie T. Zweifel:** Conceptualization; writing – original draft; writing – review and editing. **Roberto Pioli:** Visualization; writing – review and editing. **Roman Stocker:** Conceptualization; writing – review and editing; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no competing interests.

DATA AVAILABILITY STATEMENT

In this literature review, all data and information presented are derived from publicly available and previously published sources. The references and citations for each study or article included in this review are provided to facilitate access to the original works. No new data were generated or collected for the purpose of this review.

ETHICS STATEMENT

This review does not involve experimentation with neither human participants nor with animals. We therefore declare that no ethical, legal, or societal issues arise from this manuscript.

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