

Effects of the colonial cyanobacterium *Trichodesmium* spp. on bacterial activity

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ABSTRACT: We measured rates of particulate and dissolved primary production (DOCp) as well as bacterial production (BP) in order to determine the effect of freshly produced photosynthates on heterotrophic bacteria. During a 1 mo study carried out on samples from the southwestern lagoon of New Caledonia, we observed 2 different phytoplanktonic communities. The first was a mixed community, dominated by diatoms and with a low abundance of *Trichodesmium* spp. (10% total phytoplankton cells) and the second community was dominated by *Trichodesmium* spp. (up to 98% total phytoplankton cells). Chlorophyll *a* normalised total (particulate + dissolved) primary production rates were twice as high in the mixed community compared to the *Trichodesmium* spp. dominated community. The percentage of extracellular release (PER), calculated as the percentage of DOCp divided by total primary production (TOCp), reached 8% in the mixed community; in contrast, rates were much lower in the *Trichodesmium* spp. community and remained close to 1%. In terms of BP, activities were higher in the mixed community and were generally higher in light incubations than in the dark. In contrast, in the *Trichodesmium* spp. dominated community, bacterial activity was much lower and decreased with increasing *Trichodesmium* spp. abundance. This decrease in bacterial activity could not be explained by a decrease in bacterial abundance. This suggests that the proportion of active bacteria was much lower during the *Trichodesmium* spp. bloom.

KEY WORDS: Dissolved primary production · Bacterial–phytoplankton coupling · Bacterial production

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INTRODUCTION

In aquatic systems, the importance of the microbial loop in the flow of carbon through the system predicts that there should be at least some degree of coupling between primary producers and bacterial production (BP). During photosynthesis, phytoplankton produce and release a range of dissolved organic carbon (DOC) compounds into the water column and thus represent one of the major sources of potentially labile DOC (Biddanda & Benner 1997, Marañón et al. 2004). This freshly released DOC can represent an important source of energy and nutrients to heterotrophic bacteria and the microbial loop (Baines & Pace 1991, Legendre & Rassoulzadegan 1996), and so it seems logical that there should be a link between a major

source (phytoplankton photosynthesis) and a major sink (heterotrophic bacterioplankton). In their cross system overview of the apparent coupling between BP and particulate primary production (POCp), Cole et al. (1988) showed that these 2 processes are indeed linked, with BP increasing with POCp. Moreover, Moràn et al. (2002a) recently examined relationships between dissolved primary production (DOCp) and bacterial carbon demand (BCD) in a cross system comparison, and showed that while BCD was correlated with DOCp in oligotrophic regions, this relationship was either weak or non-existent in eutrophic systems.

Although total concentrations of DOC vary widely among ecosystems, it is estimated that only a small fraction of DOC is highly labile (utilizable on the scale of minutes to hours) and that the rest comprises an

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array of compounds with varying degrees of lability and recycling time (Carlson & Ducklow 1996). Two recent reviews have provided comprehensive summaries that highlight the importance of DOC released during photosynthesis (Carlson 2002, Bertilsson & Jones 2003). Despite recent increases in the number of DOCp measurements (e.g. Karl et al. 1998, Mara \acute{n} on et al. 2004), interest has tended to focus on rates of DOCp and the underestimation of primary production rates when the dissolved fraction is not taken into account, rather than on the fate of newly produced DOC and its impact on the microbial loop. Indeed, Bertilsson & Jones (2003) recently commented on the need for more measurements focused on the 'functional links between heterotrophic microorganisms and primary producers'.

It is an accepted idea that newly produced DOC (DOCp) should be labile to bacteria; however, this hypothesis remains to be tested on a variety of phytoplankton groups and species assemblages. It has been known for some time that different phytoplankton species have different rates of carbon fixation per unit chlorophyll, and different nutrient requirements for growth. Moreover, the rates and types of organic carbon moieties released during photosynthesis are also known to differ between different phytoplankton groups (Biddanda & Benner 1997, Biersmith & Benner 1998, Carlson 2002): even on a simple, visual level, the differences between mucous-producing species such as *Phaeocystis* spp. and *Trichodesmium* spp. and non-mucous-producing species are easily recognizable. However, despite an increasing amount of evidence to indicate that there are large differences in the rates and types of compounds released by different phytoplankton species, little is known about the effect of these exudates on associated heterotrophic bacterial populations.

In tropical and sub-tropical waters, the cyanobacterium *Trichodesmium* spp. can often dominate phytoplankton assemblages, particularly in oligotrophic regions (Carpenter 1983). Despite the apparent ubiquity of this cyanobacterium in warmer waters, and perhaps as a consequence of the random nature of blooms, *Trichodesmium* spp. was for many decades considered as a minor player in the biogeochemical cycling of carbon and nitrogen in the oceans (Dugdale & Goering 1967). This idea has recently been challenged, and it has been revealed that *Trichodesmium* spp. activity can be responsible for a major proportion of organic matter production in oligotrophic regions (Capone 2001, Michaels et al. 2001). Nevertheless, although many investigations have focused on the nitrogen fixing properties of this cyanobacterium, few have concentrated on the fate of the organic matter produced during photosynthesis (Hawser et al. 1992, O'Neil 1998), or on the production of DOC during photosyn-

thesis (Shimura et al. 1978). Furthermore, few studies have examined the subsequent bioavailability of this freshly produced, and potentially labile, DOC to heterotrophic bacteria (e.g. Paerl et al. 1989).

Here we present the results of an investigation into the effects of photosynthetic exudates of natural samples of the colonial cyanobacterium *Trichodesmium* spp. on surrounding heterotrophic bacterial populations. We were able to sample the phytoplanktonic community present before, during, and after an accumulation of the colonial cyanobacterium *Trichodesmium* spp., which enabled us to determine: (1) the rates of both DOCp and POCp, and the relative percentage of DOCp of total primary production (TOCp) (percentage extracellular release: PER); and, (2) the effects of this freshly produced DOC on surrounding heterotrophic bacterial populations.

MATERIALS AND METHODS

Sampling site. Our sampling site was located in the outer parts of the bay of St. Marie in the southwestern lagoon of New Caledonia (Fig. 1a). Samples were collected 2 to 3 times wk⁻¹ throughout March 2004 using a 35 μ m plankton net. Plankton tows were conducted early in the morning before the onset of the trade winds to take advantage of the calmer waters during which *Trichodesmium* spp. accumulate at the surface. Dependent upon the abundances observed, between 6 and 10 samples were collected and were returned to the laboratory in acid cleaned (10% HCl) and Milli-Q rinsed 4 l polycarbonate bottles. Samples were kept in a cooler during storage and transit.

During the second half of the month, a large accumulation of *Trichodesmium* spp. was found within the sampling area. The extremely high biomass observed meant that due to clogging of the net by the mucous matrix, samples were collected directly into several 4 l polycarbonate bottles. Upon return to the laboratory, never more than 90 min after the first sampling, samples were transferred into a decanting ampoule of 4 l volume after pre-filtration through a 100 μ m screen. The positive buoyancy of *Trichodesmium* spp. resulted in accumulation of this phytoplankton at the surface, and a light source directed towards the lower half of the ampoule aided the removal of most copepods present in the sample through a tap at the bottom. The decanting method, although less precise than collecting the individual colonies with an inoculating loop, permitted a more rapid and less physiologically stressed concentration of the sample.

High abundances of *Trichodesmium* spp. during the second half of the month meant that concentration of the samples was not necessary; therefore, samples

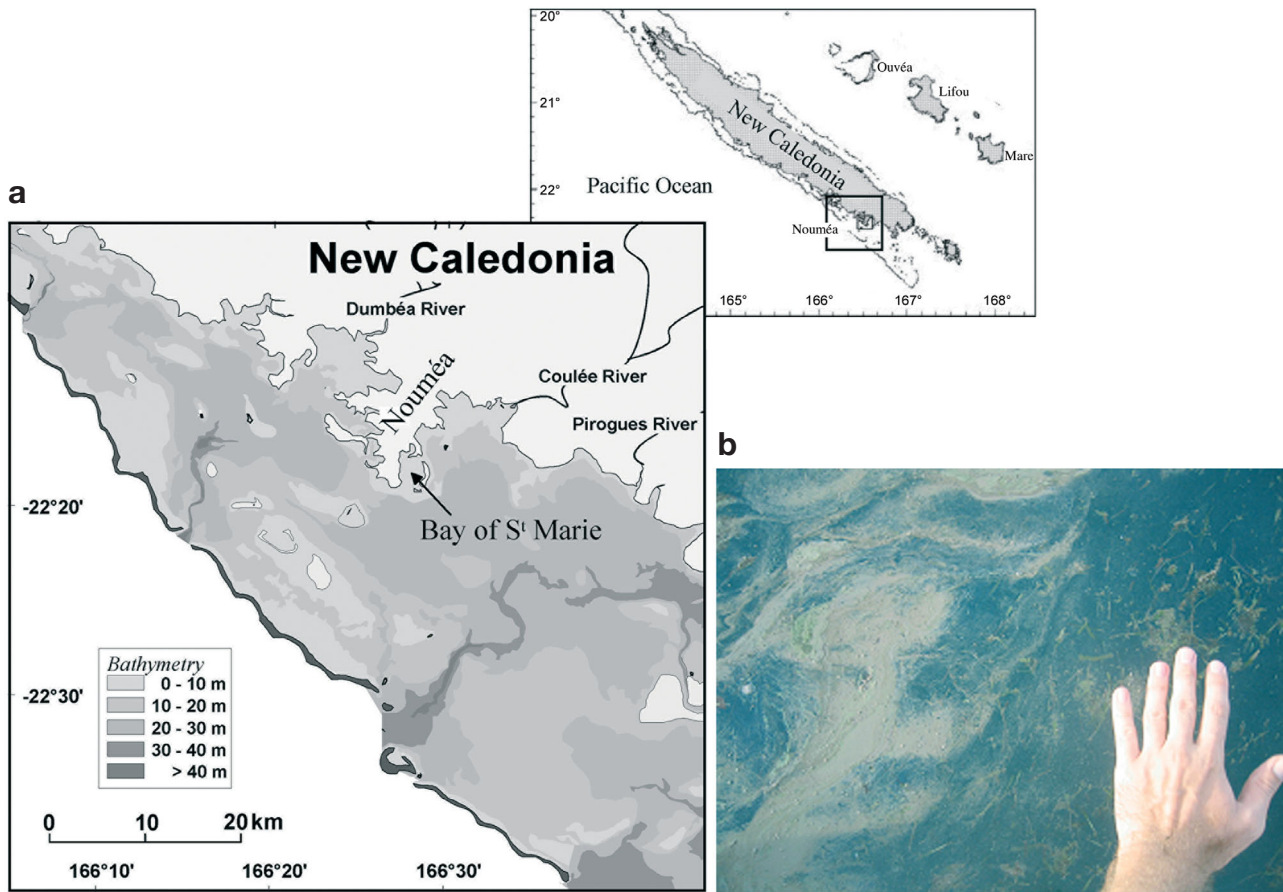


Fig. 1. (a) Study site. (b) Accumulation of *Trichodesmium* spp. at the water surface. Photograph taken on 24 March, which corresponded to the maximum *Trichodesmium* spp. abundance observed during the 1 mo survey

were pre-filtered through a 100 μm screen to remove the larger particles before transfer into the decanting ampoule to facilitate distribution of the samples into experimental flasks. The content of the ampoule was then carefully divided between 3 identical incubation flasks (Fig. 2). The flasks were double-walled to allow control of the incubation temperature via a temperature-controlled re-circulating water bath. Each flask held 202 ml and was closed by a silicone rubber stopper to ensure air-tightness. All incubations were initiated in the morning, upon return to the laboratory.

Primary production. Primary production was measured in Flask 1 (Fig. 2) using the ^{14}C method. For each incubation, ^{14}C -bicarbonate was added to the flask to achieve a final concentration of 0.75 MBq ml^{-1} . The flask was

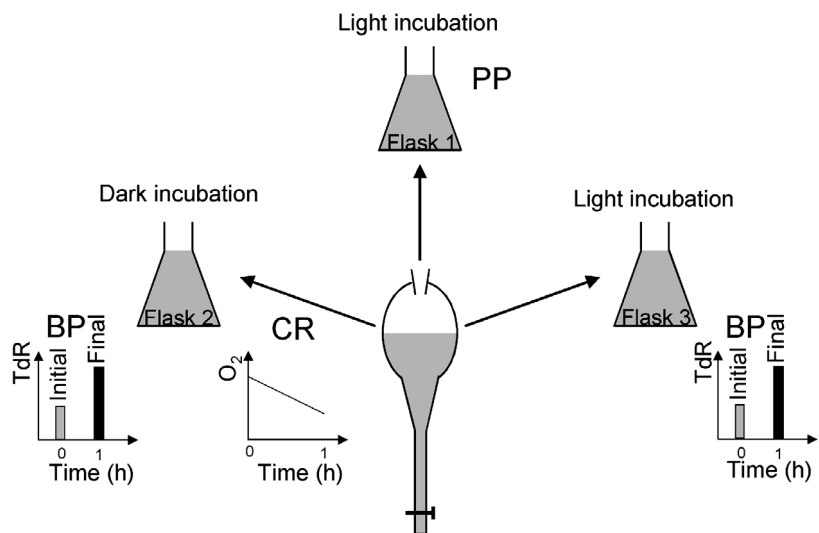


Fig. 2. Diagram of experimental protocol. After decantation, water was distributed into 3 flasks in order to measure bacterial production (BP), primary production (PP), and community respiration (CR). Inset graphs represent units of measurement over time (CR: O_2 consumption; BP: [*methyl*- ^3H] thymidine [TdR] incorporation) for respective parameters

then incubated in the light at *in situ* temperatures ($\pm 1^\circ\text{C}$) for 1 h. Light was provided by an incandescent lamp and incubations were performed at $550 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. After 1 h, 25 ml of sample was filtered (Whatman filter GF/F) for estimation of incorporation of the isotope into the particulate fraction (i.e. POCp). After acidification and drying, 5 ml of scintillation cocktail (Ultima Gold MV, Packard) was added to the filters. For measurement of DOCp, 5 ml of the filtrate was acidified with $100 \mu\text{l}$ 50% HCl, and left in open vials for 12 h on an orbital shaker to remove any unused ^{14}C -bicarbonate. After removal of the inorganic carbon, 15 ml of scintillation cocktail (Ultima Gold XR, Packard) was added. Samples were then counted on a Tri-Carb Scintillation Counter (Packard). All measures were conducted in duplicate. The TOCp was calculated from addition of the rates of POCp and DOCp. The PER was calculated from:

$$\text{PER} = \frac{\text{DOCp}}{\text{DOCp} + \text{POCp}} \times 100 \quad (1)$$

Bacterial production. BP was estimated at the beginning and end of each incubation in Flasks 2 and 3 (Fig. 2) by [*methyl*- ^3H] thymidine (TdR) incorporation ($1.44 \text{ TBq mol}^{-1}$) (Amersham) according to Fuhrman & Azam (1982) as modified by Torréton & Dufour (1996). Rates of ^3H -TdR incorporation were converted to production in terms of carbon using a conversion factor of $2.91 \times 10^{18} \text{ cells mol}^{-1} \text{ TdR}$ (S. Jacquet unpubl. data) and carbon conversion factor of $12.4 \text{ fg C cell}^{-1}$ (Fukuda et al. 1998).

BP was measured in both dark and light flasks, in order to test the hypothesis that primary production stimulates bacterial activity as suggested by Church et al. (2004). Prior to incubation, initial BP was measured; samples were then incubated in the dark (Flask 2) and in the light (Flask 3) (Fig. 2) for 1 h. At the end of the incubation, BP was again measured in both samples and compared to the initial value. The initial BP measurement was performed to assess the impact of the bottle effect on BP.

Community respiration. Community respiration (CR) was measured in the dark (Flask 2) using an oxygen microprobe, according to the procedure described by Briand et al. (2004). Oxygen concentration was continuously monitored during the incubation of 60 min. Respiration was then computed from the slope of O_2 vs. time and converted to carbon consumption using a respiratory quotient of 1.

Chlorophyll concentration and phytoplankton abundances. Chlorophyll *a* (chl *a*) concentration was estimated in duplicate in Flasks 2 and 3 at the beginning of the incubation. After filtration of 20 ml onto a 25 mm GF/F filter, pigment concentrations (chl *a* and phaeopigments) were determined fluorometrically (Turner

TD700) according to the formula of Lorenzen (1966) as modified by Jeffrey & Humphrey (1975).

Planktonic cell abundance (*Trichodesmium* spp., other phytoplankton, zooplankton, and bacterioplankton) was also estimated. Immediately after filling the incubation flasks, a 20 ml aliquot of the sample was preserved with $0.2 \mu\text{m}$ pore-size filtered borax-buffered formalin (2% final concentration). Phytoplankton and zooplankton abundances were estimated using the Utermöhl method (Utermöhl 1958) with a 10 ml sedimentation column (Hydro-Bios), after a sedimentation period of 24 h. The entire slide was counted using an inverted microscope (Leitz Fluovert, magnification $\times 125$). The number of *Trichodesmium* spp. cells (counted individually and not as trichomes) was differentiated from other phytoplanktonic groups and zooplankton (mainly copepods and ciliates).

Bacterial abundance was measured by a direct count method using epifluorescent microscopy and DAPI-stained samples (Porter & Feig 1980); 10 ml samples were stained with $0.2 \mu\text{m}$ filtered DAPI solution for 10 min before filtering onto a black $0.2 \mu\text{m}$ pore size Nuclepore filter. Filters were mounted on slides with non-fluorescent immersion oil and stored frozen until enumeration. For enumeration, at least 20 to 30 randomly selected microscope fields on each filter were counted with a Leitz Laborlux D at a magnification of $\times 1250$.

Statistics. Linear regression, correlation analysis and *t*-tests were used to determine relationships between variables. Relationships were considered significant when $p < 0.05$.

RESULTS

Phytoplankton and bacterioplankton abundance

During March (late austral summer), 2 main phytoplankton populations were observed. Microscopic observation of samples from the first half of the month revealed the presence of a mixed phytoplankton assemblage that was dominated by diatoms of the *Chaetoceros* genus. The abundance of *Trichodesmium* spp. in the samples was low during this period, and did not exceed 10% of the total phytoplankton cells (Fig. 3a). During the second half of the month, *Trichodesmium* spp. became the dominant phytoplankton and represented up to 98% of the phytoplankton community in the samples. Due to a period of calm weather, dense accumulations of *Trichodesmium* spp. were observed at the water surface (Fig. 1b). These accumulations covered approximately several hundred square meters and persisted up to 1 April. After 1 April, the sampling area was exposed to strong trade

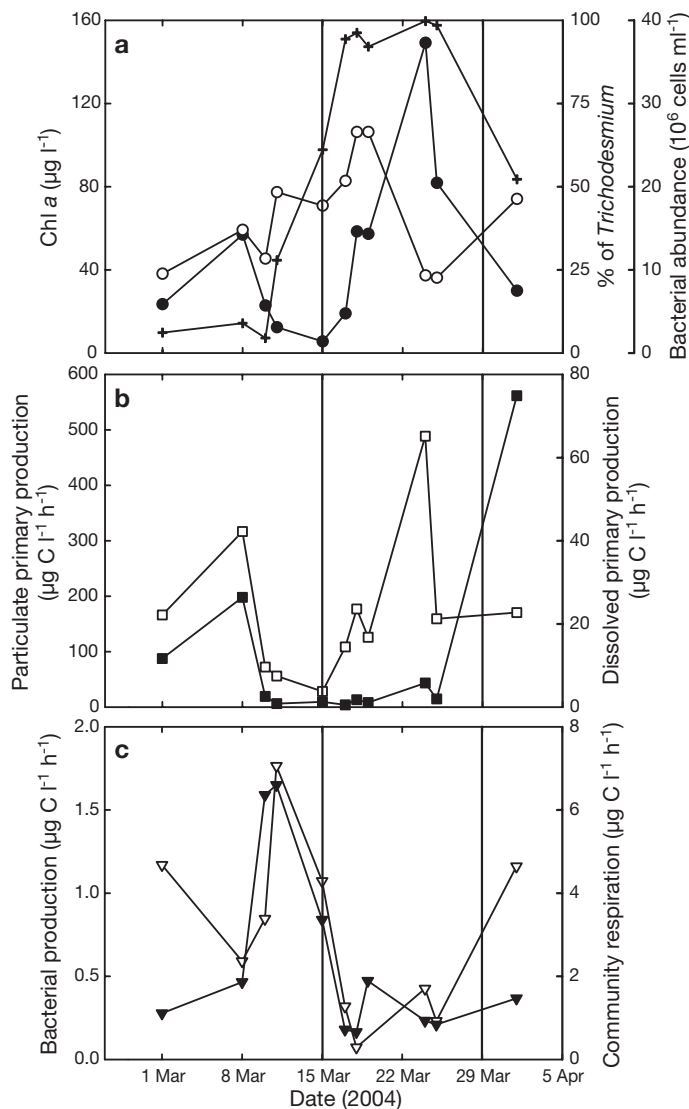


Fig. 3. Abundance and activity over time during 1 mo survey. (a) Chl *a* concentration (●), bacterial abundance (○), and proportion of *Trichodesmium* spp. (% total phytoplankton cells) (+). (b) Particulate (POCp: □) and dissolved (DOCp: ■) primary production. (c) Bacterial production (BP: ▽) and community respiration (CR: ▼). Note difference in scales between y-axes. Vertical lines separate different phases of the bloom during the 1 mo survey: pre-bloom, bloom and bloom collapse, respectively

winds (above 20 knots) leading to a total disappearance of the bloom due to strong mixing of the water column. We therefore arbitrarily divided the sample period as a function of the 2 different phytoplankton communities observed. Thus, hereafter, we refer to those samples collected before 15 March as belonging to the mixed community, and those from the second half of the month as belonging to the *Trichodesmium* spp. dominated community.

Bacterial abundances varied with time. Lowest values (around 9.3×10^6 cells ml^{-1}) were observed con-

comitant with the maximum of relative *Trichodesmium* spp. abundance and chl *a* concentration (Fig. 3a). Prior to the *Trichodesmium* spp. bloom, bacterial abundances were around 17 to 19×10^6 cells ml^{-1} .

Primary production and bacterial production

Particulate primary production (POCp) was always higher than DOCp (Fig. 3b); POCp varied by a factor of 17 and ranged between 28 and $488 \mu\text{g C l}^{-1} \text{h}^{-1}$, reflecting the wide range of chl *a* concentrations observed (5 to $149 \mu\text{g l}^{-1}$) in the concentrated samples used for incubations. DOCp rates were up 200 times lower than POCp rates; however, the range of variation was much larger than that of POCp. At the beginning of the month, DOCp rates were relatively high (11.7 and $26.4 \mu\text{g C l}^{-1} \text{h}^{-1}$, for 1 and 8 March, respectively); rates then declined to around $1 \mu\text{g C l}^{-1} \text{h}^{-1}$ after 11 March and remained low until 1 April, when a rate of $74 \mu\text{g C l}^{-1} \text{h}^{-1}$ was observed. This high DOCp rate was concomitant with the decline of the bloom (Fig. 3b). BP and CR (Fig. 3c) exhibited maximum rates around 10 March with values of 1.76 and $6.6 \mu\text{g C l}^{-1} \text{h}^{-1}$, respectively. During the bloom of *Trichodesmium* spp., both metabolic processes strongly decreased to reach the lowest values observed during the 1 mo survey (0.07 and $0.66 \mu\text{g C l}^{-1} \text{h}^{-1}$ for BP and CR, respectively). After the decline of the bloom, BP and CR increased again, with BP exhibiting a larger increase.

TOCp, calculated from the sum of POCp and DOCp, was correlated with chl *a* for both communities (Fig. 4a). Interestingly, TOCp rates were significantly higher (*t*-test, $p < 0.01$) per unit chl *a* in the mixed community (slope of regression: $6.2 \mu\text{g C } \mu\text{g chl } a^{-1} \text{h}^{-1}$, $r = 0.96$, $p < 0.01$) than in the *Trichodesmium* spp. community ($2.9 \mu\text{g C } \mu\text{g chl } a^{-1} \text{h}^{-1}$, $r = 0.94$, $p < 0.01$). In other words, chl *a* normalized primary production (assimilation number) was higher in the mixed community and declined with increasing *Trichodesmium* spp. dominance ($r = 0.79$, $p < 0.01$). Thus, for the same chl *a* concentration, the *Trichodesmium* spp. community fixed half as much carbon as the mixed community.

In terms of POCp and DOCp and their relative proportions to TOCp, there were also differences between the 2 communities (Fig. 4b). Rates of DOCp were relatively higher in the mixed community than in the *Trichodesmium* spp. dominated community. For example, at the beginning of March we observed a POCp of $166 \mu\text{g C l}^{-1} \text{h}^{-1}$ and a DOCp of $12 \mu\text{g C l}^{-1} \text{h}^{-1}$, whereas on 18 March, during the period of *Trichodesmium* spp. dominance, for a very similar POCp of $177 \mu\text{g C l}^{-1} \text{h}^{-1}$ we observed DOCp rates of only $1.8 \mu\text{g C l}^{-1} \text{h}^{-1}$. DOCp was positively correlated with POCp ($r = 0.99$, $p < 0.001$ for the mixed community and $r = 0.94$, $p < 0.01$ for the

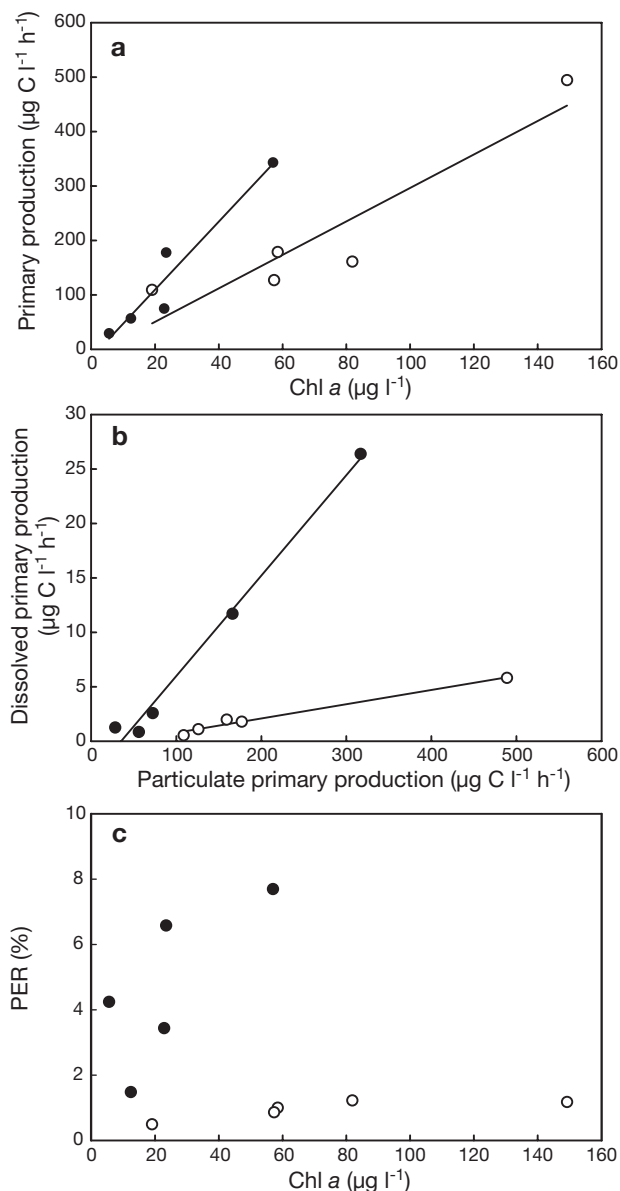


Fig. 4. Primary production and chl *a*, both communities. (a) Total primary production (TOCp = POCp + DOCp) as a function of chl *a* concentration. Slopes of lines represent average assimilation index of each community ($\mu\text{g C } \mu\text{g chl } \text{a}^{-1} \text{h}^{-1}$). (b) Relationship between POCp and DOCp. (c) Relationship between chl *a* and percentage of extracellular release (PER). ●: Mixed community; ○: *Trichodesmium* spp. dominated community

Trichodesmium spp. community) in both communities with a slope of 0.09 and 0.01 for the mixed community and *Trichodesmium* spp. community, respectively. In other words, for the same carbon production, the *Trichodesmium* spp. community released almost 10-fold less dissolved organic matter (DOM) than the mixed community. This reduction in the proportion of DOCp relative to POCp translated into a decrease in

the relative PER. Thus, it was clear that the PER was not constant between the 2 communities; furthermore, this difference was visible over a range of chl *a* concentrations (Fig. 4c).

In the mixed community, BP was strongly correlated ($r = 0.93$, $p < 0.05$) with phytoplankton abundance. In contrast, during the *Trichodesmium* spp. bloom, no significant correlation ($r = 0.36$, $p = 0.55$) was observed between BP and *Trichodesmium* spp. abundance (Fig. 5a). The degree of stimulation of BP over the 1 h incubation (in terms of percentage stimulation relative to BP at the start of the incubation) is shown in Fig. 5b. At the beginning of March, BP was stimulated by up to 380% during the incubation, whereas during the second half of the month (the period corresponding to the

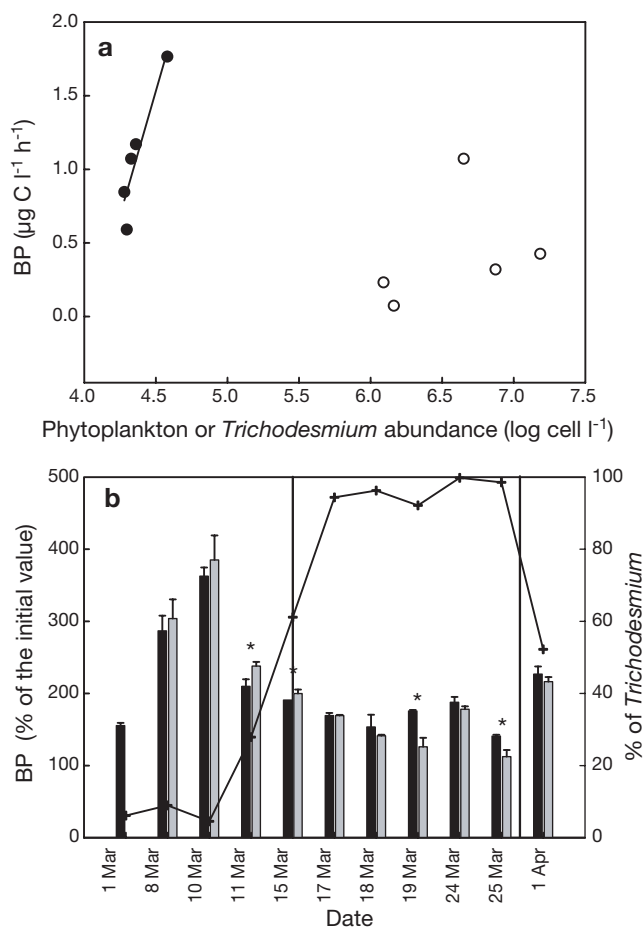


Fig. 5. (a) Bacterial production (BP) as a function of phytoplankton and *Trichodesmium* spp. abundance; ●: mixed community; ○: *Trichodesmium* spp. dominated community. (b) Percentage stimulation of BP over 1 h incubation as a function of light conditions; black bars: dark incubations (Flask 2); light grey bars: light incubations (Flask 3). Relative abundance of *Trichodesmium* spp. also shown (+). Vertical lines separate different phases of the bloom during the 1 mo survey: pre-bloom, bloom and bloom collapse, respectively; *: significant differences ($p < 0.05$) between light and dark incubations

highest *Trichodesmium* spp. abundance) the percentage stimulation was considerably lower and was on average $155 \pm 24\%$ (Fig. 5a). Furthermore, in both the dark and the light incubations, the percentage stimulation of BP decreased with increasing *Trichodesmium* spp. abundance (Fig. 5b). During the first half of the incubation, BP was generally higher in the light than in the dark and was significantly higher (*t*-test, $p < 0.05$) on 11 and 15 March (Fig. 4b). In contrast, during the period of *Trichodesmium* spp. dominance, BP rates tended to be lower during the light incubation (and hence during active photosynthesis) than in the dark incubation. Indeed, rates were significantly lower (*t*-test, $p < 0.05$) on 19 and 25 March (Fig. 4b).

DISCUSSION

The PER, assimilation number, and bacterial activity and degree of stimulation showed that structural changes in phytoplankton community composition led to functional changes that were measurable in terms of carbon cycling during the 1 mo period of sample collection. We observed 2 main phytoplankton communities in our net samples. In the first community—the mixed, mostly diatom dominated assemblage—primary production and PER rates and assimilation numbers were higher than in the second, *Trichodesmium* spp. dominated community.

The collection of *Trichodesmium* spp. samples by plankton net is a frequently used method; indeed, it is perhaps the only effective way of collecting sufficient colonies during periods of low abundance. We therefore used this method during the first half of the sampling in order to attain sufficient biomass. However, during the second half of the sampling period, the appearance of a large accumulation of *Trichodesmium* spp. in the sample area meant that net sampling was neither necessary nor possible; consequently, samples were collected directly into 4 l polycarbonate bottles. Thus, while it can be considered that phytoplankton abundances and, hence, chl *a* concentrations were higher during the first half of the sampling period than is normally observed in this area, the abundances observed during the second half of the month can be considered as representative of *in situ* concentrations within a bloom.

We observed trichome and chl *a* concentrations of up to 150 ml^{-1} and $150 \mu\text{g l}^{-1}$, respectively, at the peak of the bloom. These values are comparable to those reported by Carpenter & Capone (1992) for trichome abundance within blooms (average of 100 trichomes ml^{-1}), and are well within the range reported by Karl et al. (1992), who observed chl *a* concentrations of up to $1168 \mu\text{g l}^{-1}$ in the North Pacific. Furthermore, the

assimilation index (chl *a* normalized primary production) calculated during the *Trichodesmium* spp. bloom ($2.9 \mu\text{g C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$) is comparable to the assimilation index estimated by Paerl (1994) and by Letelier & Karl (1996), who observed values around 2.5 to $3.5 \mu\text{g C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$ for both natural and cultured populations.

Although our rates of PER were low for both communities, they fell within the ranges reported by Nagata (2000) and Morán et al. (2002b), and our average PER value of 8% in the community dominated by diatoms was close to that of Morán et al. (2002b), who observed PER production values of around 7% in the northeast Atlantic Ocean. Previously reported measurements of DOM released by *Trichodesmium* spp. are higher (ca. 8%) (Shimura et al. 1978) than the value that we calculated for the community dominated by *Trichodesmium* spp. (ca. 1%); however, the reason for this difference could be due to several factors, some of which are methodological. As outlined by several authors (Karl et al. 1998, Morán & Estrada 2001, Marañón et al. 2004), a number of precautions need to be observed when measuring DOCp. Sufficiently high concentrations of radioactivity are required to ensure a good sample to blank ratio (in this study the average ratio was 2.6), short incubation times are necessary to avoid too much recycling of DOCp (Gilbert & Bronk 1994), and incubations should be terminated by simple filtration to avoid the use of fixatives that are known to induce large amounts of DOC release (up to 78% of the PER) (Shimura et al. 1978). Finally, although our use of GF/F filters in this study may have resulted in an underestimation of the DOCp (Karl et al. 1998, Morán et al. 1999), this underestimation was probably the same for both communities, as the same method was used. Therefore, in a comparative sense, it is still evident that release rates were lower in the *Trichodesmium* spp. community. Furthermore, even if GF/F filtration did significantly decrease the PER values, the apparent suppression of bacterial activity in the *Trichodesmium* spp. incubations remained unaffected by this methodological problem.

We observed a decrease in bacterial activity and CR with increasing *Trichodesmium* spp. dominance. Furthermore, both parameters remained low during the persistence of the bloom (Fig. 2c). Indeed, at the end of the bloom we observed very high levels of DOCp ($72 \mu\text{g C l}^{-1} \text{ h}^{-1}$) in incubations that were coincident with the lowest values of BP and CR (Fig. 3c). Sellner (1997) previously reported decreases in bacterial abundance and activity during a bloom of the cyanobacteria *Aphanizomenon* sp. We also observed parallel decreases in bacterial activity and abundance; however, these 2 parameters were not significantly correlated in our data set ($R^2 = 0.0032$). Thus, the decrease in

BP was not wholly due to a decrease in bacterial abundance and was probably due, at least in part, to a decrease in the proportion of active bacteria. Additionally, dark CR was lowest during the period of highest *Trichodesmium* spp. dominance, similar to that of BP (Fig. 3c), which suggested a potential inhibition of the heterotrophic community. This apparent inhibition of bacterial activity was also noted by Nausch (1996), who observed higher bacterial activities in the surrounding water than within *Trichodesmium* spp. colonies. In addition, for those light and dark incubations that were statistically significantly different, we observed lower BP in the light relative to dark incubations in the *Trichodesmium* spp. dominated samples (Fig. 5b).

The inverse was true in the mixed community. The latter observation was in agreement with the study of Church et al. (2004), who observed in the North Tropical gyre that BP, measured by ^3H -leucine, was strongly stimulated by light indicating a tight coupling between primary production and BP. Conversely, the apparent inhibition of BP in light, as observed for the *Trichodesmium* spp. community, was supported by the observation of Moràn et al. (2001), who reported that BP in the Mediterranean waters was strongly inhibited by light. Our results confirmed that the effects of light on BP is dependent on the environment as well as on both phytoplankton and bacterial structure, as suggested by Church et al. (2004). In addition, the apparent inhibition of bacterial activity in the *Trichodesmium* spp. dominated community could be due to low DOCp from the cyanobacteria. This was probably not the sole reason for lower bacterial activities, as BP in the dark control with lower DOCp concentrations was higher than that in the light. An alternative explanation is perhaps that the quality or 'bioavailability' of exudates produced by *Trichodesmium* spp. was lower. Many cyanobacteria are known to release toxic compounds that are inhibitory or toxic to both vertebrates and invertebrates (Codd 1999): e.g. the cyanobacterium *Nodularia spumigena* produces the toxin Nodularin, which has been shown to be resistant to bacterial degradation (Mazur & Plinski 2001). Care needs to be taken when interpreting our results due to the small data set available.

Despite being potential sources of inhibitory compounds, cyanobacterial colonies are known to have a complex mix of associated bacterial symbionts (Paerl & Pinckney 1996). Colonies of *Nodularia spumigena* have been shown to possess at least 13 bacteria strains (Salomon et al. 2003). Similarly, *Trichodesmium* spp. are associated with a wide range of microbes that includes bacteria, dinoflagellates, amoebae, ciliates, and diatoms (Sheridan et al. 2002). Moreover, it is also probable that free bacteria and filament-associated bacteria differ in terms of their diversity and physio-

logical activity (Paerl & Pinckney 1996, Salomon et al. 2003). Thus, if we can assume that associated bacteria obtain some benefits from association with cyanobacteria, for example the transfer of organic matter and nutrients (Paerl et al. 1989), it would appear, at least in this data set, that any benefit obtained comes at the price of lower activities. Lower rates of TdR uptake within colonies have also been observed by Nausch (1996) and are in accordance with the results of Alldredge et al. (1986), who observed reduced bacterial activities on marine snow particles. We measured TdR uptake on whole water samples and so therefore took into account the uptake rates of the entire bacterial pool and not just of colony-associated bacteria; thus, when considering the results of Nausch (1996), it is possible that during a *Trichodesmium* spp. bloom bacterial activity is reduced both in the colony-associated bacteria and within free bacteria. However, as we have no abundance estimates of associated bacteria, or any measure of their diversity or specific activity, these hypotheses remain difficult to elucidate. Nevertheless, our study, which included the entire bacterial pool, may better reflect what happens in the field during a bloom. Lastly, although the presence of a consortium of associated bacteria within the *Trichodesmium* spp. colony may partly explain the small observed values of DOCp and PER (through rapid transfer and recycling), this cannot be the sole explanation. Indeed, if there was a rapid recycling of DOCp by the bacteria, we would have observed elevated bacterial activity levels in the entire bacterial pool instead of the converse.

The apparent inhibition of bacterial activity by *Trichodesmium* spp. may have important implications for the cycling of carbon in open ocean areas. The low bacterial activity and CR rates observed during the bloom suggests that organic matter fixed by these cyanobacteria is removed from the water column by a mechanism other than bacterial uptake and remineralisation, such as through coagulation and sedimentation (X. Mari et al. unpubl. data). However, if the composition of organic matter produced by *Trichodesmium* spp. changes throughout the development and decline of the bloom, as has been observed for other phytoplankton species (Biersmith & Benner 1998, Mari et al. 2005), then the bioavailability of these exudates may not always be low. If this is the case, then the accumulation of non-utilizable DOM in the surface waters may only occur during active growth. As we only have 1 data point collected after the collapse of the bloom, it is difficult to draw any conclusions on this. Nevertheless, BP and CR were higher towards the end of the bloom, potentially indicating either an adaptation by the bacteria to the substrates and/or a reduction in the inhibitory compounds present. Undoubtedly, a more focused investigation on the fate of DOM produced

during a *Trichodesmium* spp. bloom is required to address these questions.

Our study of primary production (POCp and DOCp) and of BP showed that the 2 communities were different in terms of photosynthetic efficiency, DOCp rates, and bioavailability of that DOCp to the surrounding heterotrophic bacterial population. The apparent inhibition of bacterial activity that we observed is contrary to what has been previously observed for other phytoplankton, and further highlights the need for more studies that examine the functional and structural nature of the relationships between phytoplankton and bacteria in the natural environment.

Acknowledgements. This research was funded by the French Institut de Recherche pour le Développement. Robert Leborgne is thanked for his help with the *Trichodesmium* counting.

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Editorial responsibility: Edna Granéli,
Kalmar, Sweden

Submitted: May 19, 2005; Accepted: September 7, 2005
Proofs received from author(s): December 20, 2005