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# **Trichodesmium and other Planktonic Cyanobacteria in New Caledonian waters (South West Tropical Pacific) during an El Niño Episode**

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**ABSTRACT.** Contributions of filamentous and picoplanktonic cyanobacteria to the phytoplankton community structure were examined in New Caledonian waters during the 2001-2003 El Niño period at three stations. Morphometric characteristics of diazotrophic filamentous cyanobacteria are given as well as the seasonal and inter-annual variations of their surface areas and integrated abundances. *Trichodesmium tenue* and *T. thiebautii* were the dominant species followed by *T. erythraeum*, altogether accounting for more than 51-80 % of the biomass of the free-living filamentous cyanobacteria. *Katagnymene* spp. accounted for a smaller percentage (<13.8 % at ocean stations, <3.6 % in the lagoon). *R. intracellularis* biomass was relatively small (<1 % of total surface area and volume of *Trichodesmium* trichomes) with the highest

concentration observed in summer (735 trichomes l<sup>-1</sup>). Colonies of unidentified cyanobacteria composed of spherical cells accounted on average for <1 % of the *Trichodesmium* biomass, with values exceeding 4000 cells l<sup>-1</sup>. Abundance of filamentous cyanobacteria varied according to environmental factors, with summer 2001-2002 characterized by low filamentous diazotroph abundance and summer 2003, occurring at the peak of the 2001-2003 El Niño, particularly rich in filamentous cyanobacteria (with a maximum *Trichodesmium* spp abundance of 4500 trichomes l<sup>-1</sup> in the Loyalty Channel). A similar variability pattern was observed for large diatoms and dinoflagellates, and for all picoeukaryotic populations. Different biomass estimators are provided as cell abundances, pigment concentrations including chlorophylls and phycoerythrin, and carbon content. The filamentous communities dominated in summer in oceanic waters while *Synechococcus* and picoeukaryote communities dominated in lagoon waters in winter at all stations.

## INTRODUCTION

In the Oceans, most of the subtropical and tropical pelagic areas are dominated by oligotrophic conditions and picophytoplankton, which are best adapted to nutrient-poor environments (Buitenhuis et al. 2012a, Luo et al. 2012). Unicellular picoplanktonic cyanobacteria are certainly the most abundant photosynthetic group and distributed throughout the tropical Pacific Ocean (Le Bouteiller et al. 1992, Ishizaka et al. 1994, Buitenhuis et al. 2012b). Other marine cyanobacteria, comparatively patchier and less abundant, also play a pivotal role in N-limited ecosystems, combining primary production and dissolved N<sub>2</sub> fixation (diazotrophy). This cyanobacterial diazotrophic community includes unicellular, filamentous, and symbiotic forms (Luo et al. 2012, Bergman et al. 2013). Pelagic filamentous cyanobacteria consist mainly of the *Trichodesmium* genus with two more frequently reported and quantified species: *T. erythraeum* Ehrenberg and *T. thiebautii* Gomont. Three other *Trichodesmium* species (*T. tenue* Wille, *T. contortum* Wille and *T. hildebrandtii* Gomont), and two *Katagnymene* species (*K. pelagica* and *K. spiralis* Lemmerman) are more scarcely reported. Furthermore, *Richelia intracellularis* has been regarded as one of the main tropical filamentous diazotrophs in marine pelagic waters forming large blooms in some regions as parts of Diatom-Diazotrophic Associations (DDAs). This heterocystous cyanobacterium is usually observed as an endosymbiont of the *Rhizosolenia* (sometimes referred as Het1 or RR) and *Hemiaulus* (Het-2 or HR) (Venrick 1974, Janson et al.

1999) diatoms and has also been reported as an epiphyte of *Chaetoceros* spp. (Gómez et al. 2005) or a free-living filament. The epiphytic form was identified as a separate species, *Calothrix rhizosoleniae*, based on genetic characterization (Janson et al. 1999, Foster et al. 2010). It has also been reported in cohabitation with *Trichodesmium* puff-form colonies (Jabir et al. 2013, Momper et al. 2015, Giraud et al. 2016). Besides *Trichodesmium* and other filamentous cyanobacteria, free-living unicellular cyanobacteria (UCYN-A, -B, and -C) are also recognized as possible major diazotrophs in tropical and subtropical areas (Zehr & Bombar 2015), occupying different ecological niches.

N<sub>2</sub> fixation by filamentous cyanobacteria in the oceans is estimated at around 80 Tg N per year (Capone et al. 1997) for a global oceanic N<sub>2</sub> fixation of 100-200 Tg N per year (Karl et al. 2002) and accounts for more than 70 % of global marine N<sub>2</sub> fixation (Luo et al. 2012, Bergman et al. 2013). The Southern Tropical Pacific is a region known for high N<sub>2</sub> fixation, as measured between New Caledonia and Vanuatu (Garcia et al. 2007, Masotti et al. 2007, Biegala et al. 2014), over a longitudinal transect at 17°S (Shiozaki et al. 2014) and in the lagoon (Biegala & Raimbault 2008, Bonnet et al. 2016). Surface blooms are frequently observed in the region, mainly composed of filamentous cyanobacteria (Dupouy et al. 2011).

Identification and counts of filamentous cyanobacteria at the species level are nevertheless scarce (Campbell et al. 2005, Luo et al. 2012) as recent work relies only on *Nifh* gene counts (Moisander et al. 2010, Bonnet et al. 2015, 2016). Moreover, few studies address their temporal variability as reported at HOT in the Northern Pacific (Letelier & Karl 1996), BATS in the Northern Atlantic (Orcutt et al. 2001), and in the Indian Ocean (Lugomela et al. 2002).

By quantifying the different species abundances and their specific relationship with nutrient and physical environmental conditions, it should be possible to more precisely identify the main factors stimulating filamentous cyanobacterial growth and how they affect the community structure. Such data are essential for estimating global N<sub>2</sub> fixation more accurately, improving our understanding of how N<sub>2</sub> fixing species respond to their environment, and anticipating how the phytoplankton community will develop in the context of global climate change (Dutkiewicz et al. 2015, Gruber 2016). *In situ* abundance of filamentous cyanobacteria and their content in pigment are also needed to calibrate recent bio-optical models aiming to determine their abundance from light reflectance of surface mats (Dupouy et al. 2011, Gower et al. 2014, McKinna 2015) or medium concentration blooms (Westberry & Siegel 2006, De Boissieu et al., 2014).

A careful study of the seasonal and inter-annual variations of different filamentous cyanobacteria species abundances was undertaken in 2001-2003 (Diapalis program) in New Caledonian waters and tentatively analyzed based on changes in environmental conditions and in the abundance of other components of the autotrophic community, particularly picoplankton. For this paper, data were obtained at three stations: an open-ocean station (Loyalty Channel), a large, deep and open bay (Santal Bay, Lifou Island) and a lagoon station (Ouinne, southeast coral reef lagoon). Taxonomic identification, measurements and counts of filamentous species were carried out by microscopy while data on picoplankton were analyzed by flow cytometry. The various estimators for determining filamentous cyanobacterial biomass were also compared. Nutrient data (Van Den Broeck et al. 2004, Moutin et al. 2005) and all N<sub>2</sub> fixation rates measured under the Diapalis program had been published earlier (Garcia et al. 2007).

## MATERIAL AND METHODS

**Sampling and environmental data.** Nine oceanographic cruises (Diapalis program: D1 to D9) lasting about a week each were conducted between October 2001 and October 2003 aboard IRD's R.V. *Alis* between 165°E and 168 °E and between 20°S and 23°S in the Eastern Coral Sea (Table 1, Fig 1). Samples were taken at three stations: the first at 167°30'E 20°30'S in the deep Santal Bay which is broadly exposed to the ocean, on Lifou Island's west coast (bottom depth: 1050 m, ST1), the second in open ocean waters in the middle of the Loyalty Channel at 21°30'S 167°E (bottom depth: 2000 m, ST2), and the third (166°45'E 21°57'S) in the south eastern coral-reef lagoon of New Caledonia (bottom depth: 40 m, ST3). The sampling covered different seasons (Table 1): summers 2002 (D2 to D4) - 2003 (D7), winters 2002 (D6) - 2003 (D8) and transition periods (D1, D5, D9). A sample was also collected from a surface accumulation of *Trichodesmium erythraeum* located in New Caledonia's southwestern lagoon.

During cruises, CTD casts were carried out at each station with water sampling down to 200 m for the deepest stations and down to 30 m in the lagoon. Temperature (T°C) and salinity were measured with Sea-Bird SBE 911 and water samples were collected with 8L-Niskin bottles attached to a CTD-rosette system. Nitrate + nitrite (NO<sub>x</sub>) and phosphate (PO<sub>4</sub><sup>3-</sup>) were preserved in Nalgene bottles with HgCl<sub>2</sub> prior colorimetric assays on a Technicon autoanalyzer II (Raimbault et al. 1990). Ammonium was measured immediately on board by fluorometry (Turner Design TD-700) according to the procedure described in Holmes et al. (1999). More details on

the methods and the full data set Diapalis are available on <http://www.obs-vlfr.fr/proof/vt/op/ec/diapazon/dia.htm>. The mixed layer depth (MLD) was estimated by taking the first depth where potential density ( $\sigma_t$ ) was  $0.03 \text{ Kg m}^{-3}$  greater than the density at 10 meters, based on Montegut et al. (2004).

**Filamentous cyanobacteria counting and morphometric characteristics.** Filamentous cyanobacteria were recovered from water samples taken at 4-6 depths down to 80 m, with a rosette of 8L Niskin bottles. Organisms were collected by filtering whole water bottles through a  $10 \mu\text{m}$  Nuclepore polycarbonate membrane. The filter was then placed in a 20 mL glass bottle containing a 4 % formalin solution for preserving photosynthetic organisms. In the laboratory, the filter was rinsed with a plastic wash bottle containing filtered seawater in order to remove all the organisms from the filter and collect them in a 100 mL glass cylinder. After processing, the filter was checked for any remaining photosynthetic organisms. A suitable quantity of an acidic formalin solution (1/1 mixture of 40 % formalin and acetic acid) was then added to obtain organisms in a final 0.4 % formalin solution. From this solution, filamentous cyanobacteria and other large phytoplanktonic species were measured and enumerated with an inverted microscope (OLYMPUS IM.,  $100\times$  magnification) using a standard sedimentation technique (Utermöhl 1931). According to Cronberg et al. (2004), the acetic acid breaks the gas vesicles facilitating the sedimentation of filaments in the sedimentation chambers. Sedimentation time was 24 hours in 5 and 10 mL chambers and at least 48 hours for the 25 and 50 mL chambers, depending on cell and filament abundances. A total of 88 samples were examined for the 9 cruises. For surface accumulations of *Trichodesmium erythraeum*, samples were collected directly by hand with a small bottle (500 mL), which was then processed using the same method as described above.

Identification of the filamentous *Trichodesmium*, *Katagnymene* (see review in Bergman et al. 2013) and *Richelia* (Foster et al. 2010) was carried out based on their morphological characteristics. Cell shape and dimensions (particularly cell diameter) were the main criteria used for classifying and identifying them. The sample processing method used to facilitate enumeration and morphometric examination at trichome level could not be used for observations at colony level, because most of them had disintegrated. Trichome surface and volume were then calculated on the assumption that they had an elongated cylindrical morphology. For *T. erythraeum* and *T. thiebautii*, identification was confirmed by molecular biology studies (unp. data). Colonies of unidentified cyanobacteria composed of spherical cells were also observed and

enumerated.

**Photosynthetic pigment analysis by spectrofluorometry.** Chlorophylls including divinyl-chl *a* and *b* for *Prochlorococcus* were measured by spectrofluorometry (F4500 HITACHI spectrofluorometer) based on Neveux & Lantoine (1993) and Tenório et al. (2005). From 0.25 to 0.5 L, water samples were filtered on 25 mm GF/F for the chlorophylls analysis in the total community. Chlorophylls were extracted in 90 % acetone after grinding the GF/F filter. Measurements in the >10 µm fraction of the community were also carried out by filtering a whole 8L Niskin bottle through 47 mm Nuclepore polycarbonate membranes. In the latter case, chlorophylls were extracted in 90 % Dimethylformamide (DMF) without grinding the membrane. For convenience's sake, the abbreviations Chla, DV-Chla and TChla were used for monovinyl-chlorophyll a, divinyl-chlorophyll a and the sum of these two pigments (total chlorophyll a) respectively. Concentrations of cyanobacteria phycoerythrin (PE) were assessed by spectrofluorometry both on the overall and >10 µm community, after extraction/cell resuspension in a 50/50 mixture of glycerol/phosphate buffer based on Wyman (1992). 1 to 3 L water samples were filtered through 47 mm 0.4 µm polycarbonate membranes for total PE and 8L onto 10 µm membranes for size-fractionated PE. Details of the PE spectrofluorometric analysis are given in Lantoine & Neveux (1997), Neveux et al. (1999) and Neveux et al. (2006).

**Picoplankton and nanoplankton analysis by flow cytometry.** Subsamples (1 mL) were stored in cryovials, and fixed with paraformaldehyde (0.2 % final concentration, Campbell & Vault 1993) and frozen in liquid nitrogen for a period of less than three months before flow cytometry analyses. Samples were counted on a Becton Dickinson FACSCan flow cytometer. For each cell, forward and side light scattering as well as red and orange fluorescence were quantified. The light signals were calibrated using 1.002 µm fluorescent beads (Polysciences Inc., Europe) as a reference. Four prevalent populations were distinguished and enumerated according to their scattering and fluorescence properties. Two of them belonged to picoplanktonic cyanobacteria, *Prochlorococcus* and *Synechococcus*, and two were eukaryotic groups, the smallest being deemed picoeukaryotes. Unicellular nanoplanktonic cyanobacteria such as *Crocospaera*, easily recognized by the fluorescence excitation spectra of their phycoerythrin and by their flow cytometric signature (Neveux et al. 1999), were not observed during the Diapalis cruises.

**Carbon biomass estimation.** Information on the relative size of each population within the total autotrophic biomass of the ecosystem was obtained by converting cell numbers into carbon units.

Conversion factors [ $\text{fg C cell}^{-1}$ ] for *Prochlorococcus* (36), *Synechococcus* (255) and picoeukaryotes (2590) were used according to Buitenhuis et al. (2012b) and for *Richelia* (10) from Luo et al. (2012). For all filamentous cyanobacteria, the carbon conversion factor determined in this study on *Trichodesmium erythraeum* was applied ( $17.22 \text{ ng C trichome}^{-1}$ ; see Table 8). The integrated carbon content of each group and their relative percentages were calculated for the upper layer (0-60 m ST1 and ST2, 0-30 m at ST3). Note that diatoms and dinoflagellates in the  $>10 \mu\text{m}$  fraction were not included in the analysis, since mean cell size or cell volume was not determined.

## RESULTS

### Environmental conditions during the 9 Diapalis cruises

The New Caledonia region (Fig. 1) is typical of the tropical regime in the South West Tropical Pacific with clear-air temperature and precipitation seasonality (Fig. 2). The Diapalis period (2001-2003) coincided with the 2002-2003 El Nino build-up, with a first period (2001) marked by a Neutral Multivariate El Nino Index (MEI) and the second (2003) by a stronger positive MEI, indicative of a strong Central Pacific El Nino during D7-D9 (Fig. 2). On New Caledonia's east coast, precipitations were more abundant during summer 2001-2002, with a peak of cumulative rain in January-March 2002, while summer 2003 was drier, except for the passage of Tropical Storm Beni, which crossed the area just before the February 2003 cruise. Beni reached peak intensity on January 29 with winds of 125 mph (205 km/h), before rapidly weakening, and dissipating on February 5. It brought heavy precipitation and floods lasting 6 days.

During the austral summer, seawater temperature increased to  $27^\circ\text{C}$  at ST1 and ST2 and up to  $28^\circ\text{C}$  in the lagoon at ST3 (Fig. 3, left panel), leading to a vertical stratification and the formation of a pronounced thermocline (Fig. 4). During the winter, cooling of surface waters induced vertical mixing in the water column at least down to the deepest sampling level, e.g. 90-100 m in Aug 02 at ST2. Minimum temperatures in the upper layer were  $23.4^\circ\text{C}$  at ST1 and ST2 and  $22.8^\circ\text{C}$  at ST3. The highest salinities (around 35.4) were measured in Oct 03 at all three study sites, and the lowest during the wet summer season, in Apr 02 (from 34.47 at ST3, close to the mouth of the Ouinne River to 34.86 at ST1). Mean salinities were similar at ST1 and ST2 stations,



but lower in summer 2002 than summer 2003, due to different precipitation regimes linked to the El Nino episode (Fig. 2). Mean phosphate ( $\text{PO}_4^-$ ) concentrations in the upper layer (Fig. 3, right panel) were weak throughout the sampling period, reaching maximum values of 0.06-0.12  $\mu\text{mol l}^{-1}$  in Oct 01 and Oct 03 during the transition periods and the minimum of 0.02  $\mu\text{mol l}^{-1}$  in summer. A small increase was observed in winter in Aug 02 due to a deepest MLD (Fig. 4). In wide Santal Bay (ST1),  $\text{NO}_x$  concentrations were also very low, except in Aug 02, when they reached 0.08  $\mu\text{mol l}^{-1}$ , linked to the deep winter mixing. The upper layer waters at ST2 oceanic station were  $\text{NO}_x$  poor with concentrations always  $<0.06 \mu\text{mol l}^{-1}$  (Fig. 3), even during deep vertical winter mixing as in Aug 02. Small maxima (0.05  $\mu\text{mol l}^{-1}$ ) were observed in Oct 03 as in Feb 03 despite warmer, saltier waters. At ST3 lagoon station, the  $\text{NO}_x$  concentrations varied from 0.01 to a maximum of 0.07  $\mu\text{mol l}^{-1}$  in Jan 02.  $\text{NH}_4^+$  concentrations (data not shown) at ST1 and ST2 were generally low  $<0.04 \mu\text{mol l}^{-1}$  and often close to the detection limit 0.01  $\mu\text{mol l}^{-1}$ . Higher concentrations were observed only in Feb 03 at both stations (0.11 and 0.19  $\mu\text{mol l}^{-1}$  at ST1 and ST2, respectively). In the lagoon, concentrations were on average slightly higher: up to 0.30  $\mu\text{mol l}^{-1}$ .

### **Filamentous cyanobacteria**

**Specific composition:** Five species of the *Trichodesmium* genus were observed (Fig. 5-6) and identified by their morphology and morphometric characteristics (Table 2). Three of them, *T. erythraeum*, *T. thiebautii* and *T. tenue* (Fig. 5A, B, H) were the most frequently observed at all three sampling sites. *T. contortum* was seldom observed (3 samples). *Katagnymene* (Fig. 5E, F, G) and *Richelia/Calothrix* (Fig. 6) genera were common (in 50 % and 75 % of samples, respectively), but generally in low abundance. *Richelia* was observed as an endosymbiont of *Rhizosolenia* and *Hemiaulus* and *Calothrix* as an epibiont on *Chaetoceros sp.* During the May 02 cruise, west of Lifou, we observed unidentified filaments very similar to *Trichodesmium*, but differing in their very small cellular diameter (2.7-3  $\mu\text{m}$ ). We also observed colonies of unidentified cyanobacteria (Fig. 5C, D), which formed clusters sometimes made up of several dozen cells. All filamentous species during Diapalis had dimensions (cell length and cell diameter) within the range published in the literature (Table 3 and 8) and the sizes of *T. thiebautii* and *T. erythraeum* species were in the low part of the size range.

***Trichodesmium* and *Katagnymene* abundance:** At ST1 and ST2, the highest abundances of filamentous cyanobacteria in the 0-60 m upper layer were noted in summer (Feb 03; Fig. 7), i.e.

3000-4500 trichomes l<sup>-1</sup> and 1000-3000 trichomes l<sup>-1</sup> between 5 and 10 m, respectively. However, the concentrations and vertical distribution could be highly variable from one day to another, as shown at ST1 in Feb 03 (Fig. 7). Some vertical profiles showed maximum concentrations at 40 m as at ST2 (mostly due to *T. erythraeum*). Vertical distributions were quite homogeneous at the period of low abundance, i.e. in the austral winter (Aug 02 and Jun 03). At ST3 (Fig. 7), concentrations did not exceed 1000 trichomes l<sup>-1</sup>. Tables 4 to 6 give general statistics on the abundances of the different filamentous cyanobacteria enumerated by inverted microscopy during Diapalis cruises 1-9 for each station. Filamentous cyanobacteria were extremely rare in Oct 03 regardless of depth.

Assuming that *Trichodesmium* can migrate vertically within one day (Villareal & Carpenter 2003), using integrated abundance seemed more appropriate for gauging their seasonal variations in the water column (Fig. 4). Thus, at ST2, 0-60 m integrated abundance showed a 500-fold variation between minimum in Oct 03 (<0.5 10<sup>6</sup> trichomes m<sup>-2</sup>) during the transition periods and maximum in summer (Feb 03: 235 10<sup>6</sup> trichomes m<sup>-2</sup>). At ST1, the seasonal variations appeared similar but the variation range (x 150) and the maximum summer concentrations (73 10<sup>6</sup> trichomes m<sup>-2</sup>) were lower than at ST2. At both stations, maximum abundance in summer 2003 was coincident with a shallow MLD, but low abundances were also observed in summer 2002 with a similar thermohaline structure. At ST3 station (Fig. 4), integrated concentrations (0-30 m only) were 15 times lower and varied from 0.9 10<sup>6</sup> trichomes m<sup>-2</sup> to 13.5 10<sup>6</sup> trichomes m<sup>-2</sup>, although ST3 was not sampled in Feb 03.

The three main *Trichodesmium* species together accounted for 85-100 % of the integrated biomass of filamentous cyanobacteria in terms of trichome numbers and always more than 51 % in terms of trichome volume (Fig. 8). *Trichodesmium tenue* and *T. thiebautii* were generally the dominant species followed by *T. erythraeum*. Their mean cell numbers per trichome were relatively similar (Table 7). As expected, the percentage contribution of slender *T. tenue* was higher in terms of total trichome number than total volume (40-49 % vs 33 %). Its percentage contribution (trichome numbers) was minimum at ST2 in Feb 03 (23 %) and maximum (>70 %) in Dec 01 at ST2 and ST3. *T. thiebautii* reached >40 % of the total trichome number in 65 % of the samples with minimal contributions in Dec 01 and May 02 at ST2 (<14 %) and a fairly stable one at ST1 and ST3 (37-47 %).

In terms of volumes, *T. thiebautii* contributions (reaches 50 % at ST2) were more variable, linked to a trichome length changes along the annual cycle. The third main species, *T.*

*erythraeum*, represented less than 18 % of total trichomes on average, with the minimum contribution at ST3 (<7 %) and maximum at ST1 and ST2 in Feb 03 during the peak abundance (Tables 4 to 6). Its relative importance increased as expressed in volume, especially in Feb 03 at the two open ocean stations (22-28 %). *T. contortum* and *T. hildebrandtii* (given as “others” in Fig. 8), contributed <3 % to total trichome number or volume. The unidentified trichomes observed in May 02 further north than ST2 represented less than 1 % of the integrated biomass.

The two *Katagnymene* species (*K. spiralis* and *K. pelagica*) were not numerous, but their contribution in terms of volume was significant. Each form accounted for less than 4 % of the total number of trichomes, except *K. spiralis* at ST1 in Oct 01 (10 %). *K. spiralis* was observed in 50 % of samples. Its highest contributions to the total volume of trichomes occurred at ST1 in Oct 01 (28 %) and Feb 03 at ST2 (12 %). The contribution of *K. pelagica* was around 20 % of the total volume in Feb 03 at ST1 and ST2, 15 % at ST2 in Oct 01, and was not observed at ST3. The relative abundance of species could change along depth. For example, during cruises D4 and D5 (Apr-May 02), *T. thiebautii* and *T. tenue* were numerous in the upper layer (60 %) and disappeared at 50 meters while *T. erythraeum* peaked at this depth. Obviously, the relative abundance depends on the biomass criteria considered. For example, at ST2 in Apr 02, *T. erythraeum* contributions at 30 to 50 m decreased in terms of trichome number (6 %) but increased (41 %) in terms of total volume, because trichome size increased. The opposite trend was observed for *T. thiebautii*.

***Richelia intracellularis/Calothrix rhizosolenia (RC) from DDAs and unidentified cyanobacteria abundance:*** RC (Tables 4 to 6) were observed in 75 % of samples, but their abundance was never very high. The highest RC abundances were recorded in Feb 03 at both ST1 and ST2 (64 trichomes l<sup>-1</sup> and up to 735 trichomes l<sup>-1</sup>, respectively), accounting for less than 1 % of total *Trichodesmium* trichome surface area and volume. Conversely, at ST3, peak abundance was observed for *R. intracellularis* in Oct 01 (up to 117 trichomes l<sup>-1</sup> at the surface) equivalent to 4.3 % of *Trichodesmium* trichome volume. In our samples, RC in DDAs experienced a considerable variation in filament length (10-88 µm) and cell diameter (vegetative cells 2-7 µm; heterocyst 3-10 µm) or trichomes per host cell (1-22). We also noted that in most cases, the host cell was almost empty. Apart from *Richelia*, other symbiotic coccoid-form cyanobacteria (not necessarily diazotrophs) were also observed in *Climacodium* sp. and *Ornithocercus* sp., though only in the New Caledonian lagoon.

Colonies of unidentified cyanobacteria composed of round-shaped cells observed in some samples reached less than 1 % of *Trichodesmium* biomass on average (Tables 4 to 6, Fig 5C, D). The highest concentrations were observed at ST2 during Jan 02 and May 02 with values exceeding 4000 cells l<sup>-1</sup>, which was equivalent to less than 1.6 % of *Trichodesmium* trichome volume. At ST1, these organisms were rarely observed and only at very low concentrations, i.e. <20 cells l<sup>-1</sup>. At ST3 they were observed only in Jan 02 with a maximum value at 10 m of 429 cells l<sup>-1</sup>. Tracking *Crocospaera* (2-10 µm cell size) by flow cytometric and phycoerythrin signatures was unsuccessful during Diapalis although it has been reported in the region (Saito et al. 2010 and references therein). However, Diapalis samples showed relatively high N<sub>2</sub> fixation in the <10 µm fraction, particularly in Oct 03 (Garcia et al. 2007), but it was not possible to link this fixation to specific organisms or biomass indicators.

### Abundance of other phytoplankton groups

**Picoplankton abundance:** *Prochlorococcus* concentrations at ST1 and ST2 (Tables 4-5 and Fig. 9A) was maximum (10-13 10<sup>12</sup> cells m<sup>-2</sup>) during the warm season (Dec 01-Apr 02 and in Feb 03). During transition periods, concentrations were lower by a factor of 3 with 3.5-3.9 10<sup>12</sup> cells m<sup>-2</sup> at ST2 (Oct 01 and Oct 03, respectively), while at ST1 the minimum concentration (4.5 10<sup>12</sup> cells m<sup>-2</sup>) was observed during winter. At these two oceanic stations, the vertical distribution of *Prochlorococcus* was quite similar, with a marked maximum below the thermocline (40-80 m) in summer and a more homogeneous distribution down to 60-80 m in winter. At ST3, *Prochlorococcus* abundance was much lower than at the oceanic stations, with values <2.3 10<sup>12</sup> cells m<sup>-2</sup> through 0-30 m (max 70 10<sup>3</sup> cells ml<sup>-1</sup>), except in Dec 02 when they reached 4.8 10<sup>12</sup> cells m<sup>-2</sup> (max 225 10<sup>6</sup> cells ml<sup>-1</sup> at depth).

Unlike *Prochlorococcus*, integrated abundance of *Synechococcus* (Tables 4 to 6, Fig. 9B), was on average 5 to 14 times higher at the lagoon station (ST3) than offshore (ST1 and ST2), except during the austral winter (Aug 02) when they were 24 % higher in oceanic waters at ST2 (1.91 10<sup>12</sup> cells m<sup>-2</sup>). This winter maximum was linked to thoroughly-mixed cool waters down to >60 m. The maximum *Synechococcus* abundance at the oceanic stations was generally located shallower than that of *Prochlorococcus*, i.e. in the upper mixed layer or at the top of the thermocline, except during the Oct 03 cruise when it peaked at the same depth (between 75 and 110 m).

Picoeukaryotes were the least abundant picoplankton organisms (Tables 4 to 6, Fig. 9C). Integrated abundances were more variable at ST2 (factor 6; 0.02 to 0.12  $10^{12}$  cells  $m^{-2}$ ) than at ST1 (0.07 to 0.14  $10^{12}$  cells  $m^{-2}$ ). At ST3, integrated abundances changed from 0.08 to 0.17  $10^{12}$  cells  $m^{-2}$ . Picoeukaryote maxima were consistently found below the thermocline and, therefore, deeper than the *Synechococcus* maxima, even in Aug 02.

**Large eukaryotes:** Large eukaryotes ( $>10\ \mu m$ ) described by microscopy and made up of dinoflagellates and diatoms (Fig. 10) were generally found together with *Trichodesmium* in open ocean waters at ST1 and ST2 and, to a lesser extent, at ST3 coastal site. Dinoflagellates showed a maximum of 60  $10^6$  cells  $m^{-2}$  during the maximum *Trichodesmium* abundance in Feb 03 at ST1 and ST2 and low abundance ( $<30\ 10^6$  cells  $m^{-2}$ ) at other periods. Diatoms also showed a maximum concentration (412  $10^6$  cells  $m^{-2}$ ) in Feb 03 at ST2 and, to a lesser extent, at ST1. At ST3 coastal site, no data were available in Feb 03, but there was a considerable maximum for both groups (17  $10^6$  cells  $m^{-2}$  for dinoflagellates; 240  $10^6$  cells  $m^{-2}$  for diatoms) in winter (Aug 02). The main diatoms belonged to 1) the *Navicula*, *Nitzschia* and *Pseudo-nitzschia* genera at ST1, 2) the *Chaetoceros*, *Navicula* and *Pseudo-Nitzschia* genera at ST2, 3) the *Chaetoceros*, *Navicula* and *Rhizosolenia* genera at ST3. *Rhizosolenia* was the main RC host. Among dinoflagellates, protoperidinians were represented at all stations, but few larger cells were found as *Ceratium* and *Amphisiolenia* species at ST3 in Oct 01.

### Chlorophyll and phycoerythrin distribution

Pigment content (Chla, PE) per cell or per trichome (Table 8) was calculated for Diapalis samples during the Feb 03 cruise at ST1 and ST2, and for a pure sample of *T. erythraeum*. Mean Chla concentration per trichome was 99 pg cell $^{-1}$  for pure *T. erythraeum* while the relationship between trichome numbers and Chla in the  $>10\ \mu m$  fraction suggested an upper limit for the mean Chla concentration per trichome of 43 pg for the mixed community of *Trichodesmium* (Table 8). Considering the diatoms and dinoflagellates in the  $>10\ \mu m$  fraction revealed by microscopy and by the presence of a significant quantity of Chlc, the Chla per trichome would be approximately 10 % lower (assuming a constant Chlc/Chla ratio of 0.2 for diatoms and dinoflagellates) than this upper limit. Similarly, the PE per trichome (Table 8) was 251pg trichome $^{-1}$  for pure *T. erythraeum* and 197 pg trichome $^{-1}$  for mixed *Trichodesmium* (Table 8).

Regarding phycoerythrin (PE), its fluorescence excitation spectra were typical of PE-

cyanobacteria according to Neveux et al. (2006) with either PE-*Synechococcus* or PE-*Trichodesmium* dominating or a clear mixture of both PEs. At ST1 and ST2 stations, PE-*Trichodesmium* dominated the community in Dec 02 and Feb 03. Integrated Total Chla change showed much less variation across cruises than trichome abundance (Fig. 11). The integrated proportion of Dv Chla to TChla was generally around 20 % in lagoon waters (ST3), 40 %-50 % at ST1 and over 50 % in the Loyalty Channel (ST2). The highest integrated values of PE at ST1 and ST2 in Feb 03 (69 mg.m<sup>-2</sup>, Fig. 11) was linked to the high abundance of *Trichodesmium* at the surface while the high value in Oct 03 at ST1 was related to a deep maximum of *Synechococcus*. In Feb 03, PE maxima measured in the upper layer were 4 to >10 times higher than during the other cruises, ranging from 0.61 µg l<sup>-1</sup> at ST1 to 1.27 µg l<sup>-1</sup> at ST2. In the >10 µm fraction, the PE in the upper layer (data not shown) was exclusively related to filamentous cyanobacteria, mainly *Trichodesmium*. At ST3 station (Fig. 11), PE was essentially related to *Synechococcus* and concentrations varied between 0.10 (Apr 02) and 0.62 µg l<sup>-1</sup> (Aug 02) at the surface with fairly even vertical distribution. The integrated concentrations varied from 4.30 (Apr 02) to 29.26 mg m<sup>-2</sup> (Aug 02). The integrated N<sub>2</sub> fixation rate for all species (Garcia et al. 2007) roughly followed the integrated PE pattern (Fig. 11) and are correlated with the integrated trichome concentrations (r<sup>2</sup>=0.73, N=11).

### **C biomass distribution in the microbial community**

The relative contribution of *Trichodesmium* + *Katagnymene* (TK) to C biomass of total picoplankton and filamentous cyanobacteria (Fig. 12) was generally high at ST2 with maxima in summer (80 % in Feb 03) and minima in winter (6 % in Aug 02), where TK was replaced by *Synechococcus* as the major contributor and in Oct 03 with only a few filaments observed. Comparatively, the contribution of TK at ST1 was lower, with a maximum of ~ 50 % in Feb 03. At ST3, *Synechococcus* was the main contributor to C biomass and the contribution of TK was generally low. At all stations, the contribution of RC to C biomass was negligible over the whole sampling period. Picoeukaryotes may account for a significant portion of the biomass in the lagoon near Ouinne and in winter at all stations.

### **Correlations between groups and environmental variables**

To determine the impact of environmental variables on the abundance variations of the different phytoplanktonic groups and species, Spearman correlations were calculated between integrated abundances of each filamentous species, integrated nutrients, and depth-averaged temperature and salinity, at each station/cruise (Table 9). Significant positive correlations were found between total trichome abundance, temperature and ammonium and a negative correlation with phosphate, but no significant correlation with MLD (see also Fig. 4). At the species level, *RC* showed a strong positive correlation with MLD and ammonium, but *K. pelagica* only with phosphate, while *K. spiralis* was strongly correlated with all the analyzed nutrients (NO<sub>x</sub>, phosphate and ammonium). Diatom and picoplanktonic species did not show any correlation with environmental parameters. Inter-species correlations revealed that *RC* was positively correlated with *T. thiebautii* and *K. spiralis*, but not with *T. tenue* (inversely correlated with *T. thiebautii*). Dinoflagellates were closely correlated with total trichome abundance and *T. erythraeum*, but inversely with *K. pelagica*. No correlations were evidenced between diatoms and the other groups. Finally, *Synechococcus* and picoeukaryotes showed a positive correlation.

## DISCUSSION

### Influence of methodology on enumeration and biomass estimates

As part of the effort of collecting biomass and metabolic rates specific to different phytoplankton functional groups, a database on diazotrophic organisms in the global pelagic upper ocean was built by Luo et al. (2012). Variability in counting and sampling procedures, however, hamper comparisons between studies around the world, especially for filamentous cyanobacteria. Significant error in the accuracy of low abundance measurements occurs depending on the sampling mode. In this case, the use of a plankton net increased the detection of *Trichodesmium* (0.1 against 1 trichome. l<sup>-1</sup>) by a factor of 10 compared to water samples collected with 2.5 L GO FLO bottles (Chang et al. 2000). When bottle sampling, it is essential to filter all the contents to prevent uneven distribution of *Trichodesmium*, which can occur when trichomes (and colonies) migrate due to cell-controlled or uncontrolled buoyancy (Bergman et al. 2013). When tightly-packed filaments of *T. erythraeum* are observed at the surface, manual sampling within the first few millimeters of depth with narrow-necked plastic bottles provides a fairly accurate picture of trichome abundance, despite the somewhat uneven distribution.

Abundance data are usually expressed as cell, trichome or colony numbers with or without specific information on the taxonomic composition of filamentous cyanobacteria. Conversion factors from one unit to another are, however, vague and based on the morphometric variability of filamentous diazotrophs (Luo et al. 2012), such as the number of trichomes per colony, cell numbers per trichome, and trichome dimensions, which are quite variable. For the Diapalis data set, trichome counts of mixed species were linearly correlated (Spearman's rank coefficient > 0.95,  $p < 0.001$ ) with the estimates of entire surface and total volume of trichomes, as previously pointed out by Neveux et al. (2006). So, replacing trichome numbers with trichome surface areas or volumes did not greatly modify the qualitative vertical distributions or seasonal trends in the filamentous biomass. This indicated a relatively constant composition of the filamentous community. Also, mean *T. erythraeum* carbon content per trichome (17.22 ng) and per cell (290 pg) were determined by our microscope counts. These values were in the upper range of the approximate cell carbon content previously published for *Trichodesmium* species assuming 100 cells trichome<sup>-1</sup> (110-250 pg C cell<sup>-1</sup>; Luo et al. 2012). Taking the measured value of 290 pg C cell<sup>-1</sup> of *T. erythraeum* (Table 8), TK accounted for up to 80 % of C cyanobacteria biomass in summer at the oceanic station of the Loyalty Channel, which confirms the essential role of filamentous cyanobacteria for the global food chain (Bergman et al. 2013) and in the south western tropical Pacific Ocean (SWTP) (Biegala et al. 2014, Bonnet et al. 2015).

### Comparison of *Trichodesmium* abundance with other studies

During the Diapalis program, *Trichodesmium* spp. abundance in the ocean varied from 1 to 4578 trichomes l<sup>-1</sup> found at 0 to 60 meters (average 586 trichomes l<sup>-1</sup>,  $n = 88$ ), which was much higher than previously published data in New Caledonian waters at the end of the summer (250-1000 trichomes l<sup>-1</sup> in April 1998; Campbell et al. 2005). The only other abundance data available for our region around New Caledonia are *nifH* gene copies (Moisander et al. 2010). In Ouinne lagoon, a maximum of 1200 trichomes l<sup>-1</sup> was found in summer 2007 and more than 10,000 trichomes l<sup>-1</sup> in accumulations (Rodier & Leborgne 2010). These concentrations are, however, lower than measured in a surface accumulation in the Southwest Lagoon, namely up to 30 10<sup>6</sup> trichomes l<sup>-1</sup> for a Chla value of 3 mg. l<sup>-1</sup> (Neveux et al. 2009), the highest values reported for the region. Such surface accumulations (>10,000 trichomes l<sup>-1</sup>) of mixed *Trichodesmium* species were collected 98 times in summer between New Caledonia and Vanuatu (1998 to 2010: Dupouy



et al. 2011, 2017). In the southernmost part of the SWTP at 28°S in the austral summer, Law et al. (2011) observed comparatively low surface *Trichodesmium* abundance, averaging 11 trichomes l<sup>-1</sup> in the upper 10 m to 6 trichomes l<sup>-1</sup> at 35-50 m. Some high values were found in the Solomons Islands, expressed as trichome number (7700 trichomes l<sup>-1</sup>; Giraud et al. 2016) or *NifH* copies (Bonnet et al. 2015). In the Western North Pacific Ocean, abundance was between 1 and 400 trichomes l<sup>-1</sup> (Marumo & Asaoka 1974, Marumo & Nagasawa 1976, Chen et al. 2003, Shiozaki et al. 2015) with some high values in accumulations (>20,000 filaments l<sup>-1</sup>) near the Miyako Islands (Shiozaki et al. 2015).

Comparing the Diapalis program with other time series conducted on *Trichodesmium* in subtropical waters raises a number of problems, as a different sampling strategy and sample processing method were used. During the Hawaiian (HOT) and Bermuda Atlantic Time-Series (BATS), *Trichodesmium* abundance was studied at approximately monthly intervals (HOT= October 1989-December 1992, Letelier & Karl 1996; BATS = 1995-1997, Orcutt et al. 2001) and free trichomes (water filtration sampling) and colonies (net sampling) were counted separately. So, a conversion factor of 200 trichomes per colony used in Orcutt et al. (2001) was applied to HOT and BATS counting data for comparisons with the Diapalis values, as in our study colonies were not counted (disintegrated during the counting) and only trichome abundance is available. This showed that trichome abundances in New Caledonian waters were generally higher than those observed at HOT and BATS stations. In fact, the integrated maxima observed in Feb 03 in the Loyalty Channel (240 10<sup>6</sup> trichome m<sup>-2</sup>) were 70 times and 400 times higher than at HOT (3.5 10<sup>6</sup> trichome m<sup>-2</sup>) and BATS (0.64 10<sup>6</sup> trichome m<sup>-2</sup>), respectively. Moreover, mean cell numbers per trichome were generally lower in Diapalis samples (50-60) than at HOT (100). At HOT, apical cells were counted and divided by two to obtain the trichome number (Letelier & Karl 1996). This precaution was not applied to Diapalis samples, leading to a possible overestimation of trichome numbers due to trichome breaking during sample processing. HOT and BATS studies have shown a maximum abundance in summer and relatively strong inter-annual variability, even if free trichome variations at BATS seemed identical from one year to another. At BATS, however, interannual variations deduced from only 7 integrated depth profiles for 3 years fitted by a third-degree polynomial function of Julian days (Fig 1A in Orcutt et al. 2001), are questionable.

#### **Filamentous diazotroph species and their relationship with environmental factors**

The SWTP around New Caledonia is a typical warm low-nutrient low-chlorophyll (LNLC) region (Ceccarelli et al. 2013), with phosphate recharged annually (presumably other nutrients as well) in the euphotic zone during the transition periods (Moutin et al. 2005). The phosphate can limit the growth of diazotrophs species and consequently controls nitrogen fixation rates in the ocean (Karl et al. 2002). In LNLC, diazotroph species such as *Trichodesmium* are better adapted as they are able to use atmospheric N<sub>2</sub> (Bergman et al. 2013). Diazotrophs are likely responsible for the lower phosphate concentrations in summer in the region due to their PO<sub>4</sub> uptake (Moutin et al. 2005, Shiozaki et al. 2014), which corroborates the negative correlation between *Trichodesmium* and phosphate found in this study. During Diapalis cruises the ammonium concentrations at ocean stations were only high in February 2003 coinciding with the highest concentrations of trichomes, PE and cyanobacteria carbon. The ammonium concentrations also were positively correlated to *Trichodesmium* densities. Therefore, the relatively high ammonium concentrations may be attributed partially to direct or indirect release of this nutrient by the diazotrophs (Mulholland 2007; Bergman et al. 2013). We did not find any correlation between *Trichodesmium* abundance and MLD. As stated by Hood et al. (2004), increased *Trichodesmium* concentrations are linked to a shallow MLD (resulting in high mean light levels) and low dissolved inorganic nitrogen (DIN) concentrations for extended periods of time. The relationship between nitrogen fixation rates in plankton net samples and MLD depth was significantly fitted by a hyperbolic function in the Atlantic Ocean ( $r^2 = 0.31$ ,  $p < 0.05$ ,  $n = 18$ , Agawin et al. 2013).

*T. thiebautii* and *T. tenue* were the predominant species in the oceanic waters around New Caledonia and in the open lagoon of the east coast, as previously stated in Rodier & Leborgne (2010). By contrast and according to these authors, *T. erythraeum* dominate in the SW lagoon of New Caledonia, which is a semi-closed and larger lagoon than the SE lagoon. Generally, *T. thiebautii* and *T. tenue* are poorly represented in coastal regions (Relevante & Gilmartin 1982). They dominate in the oligotrophic gyre of the Northern Pacific, Western Pacific (Marumo & Nagasawa 1976) and eastern part of the China Sea (Marumo & Asaoka 1974) and are likely better adapted to low-nutrient conditions than *T. erythraeum*. In our study, *T. tenue* was inversely correlated with *T. thiebautii*, which suggests competition between the two species for an apparently similar environment. According to Romans et al. (1994), *T. tenue* is a common species often confused with *T. thiebautii* in optical microscopy, which can explain the lack of information on the species' distribution and quantification. The predominance of *T. tenue* observed on

occasion during the Diapalis cruises is a relatively original finding. In this study around New Caledonia, *T. contortum* and *T. hildebrandtii* occur in very low densities as in most oceans (Bergman et al. 2013).

Information on *Katagnymene* species in the field is very scarce. They are mentioned in the Southwestern Pacific (Lundgren et al. 2001, Dupouy et al. 2011, Giraud et al. 2016). Both *Katagnymene* species, *K. pelagica* and *K. spiralis*, should now be considered as two morphological forms of the same species (Lundgren et al. 2005). In our study, both forms are frequently observed, but always in low abundance  $<284$  trichomes  $l^{-1}$ . The strongest concentrations were observed in the first 40 meters, although a deeper maximum (60 m) was found in the Loyalty Channel in Feb 03. At this maximum, the *K. spiralis* form accounts for 81 % of the *Katagnymene* genus. Along the Revelle cruise track between New Caledonia and the Fiji Islands (April 1998), Lundgren et al. (2001) found both *Katagnymene* forms at 34 of the 51 stations and counted up to 400 trichomes  $l^{-1}$  of *Katagnymene* in the 0-25 m layer, with 89 % *K. pelagica*. It should be noted that *Katagnymene* trichomes are generally long (1 cm) and extremely fragile and can be broken up by handling before counting; hence the recommended use here of surface and total volume for biomass estimates. The significant relationship of *K. spiralis* with all nutrients, while *K. pelagica* was correlated only with phosphate, may indicate a different ecological niche for these two species.

Like *Trichodesmium* and *Katagnymene*, the heterocystous *Richelia intracellularis* is widespread in warm tropical and subtropical waters (Luo et al. 2012, Bonnet et al. 2015), whereas *Calothrix rhizosolenia*, as epibionts of *Chaetoceros*, have been observed in the Indian and Western Pacific Oceans (Sarma 2012, Giraud et al. 2016). Around New Caledonia in this study, both *Richelia* and *Calothrix* species (*RC* groups) were sometimes present as free-living organisms, but more frequently in endosymbiotic association with diatoms (mainly in *Rhizosolenia* sp.). *RC* were present in all seasons with a maximum ( $>700$  trichomes  $l^{-1}$ ) in the Loyalty Channel in summer (Feb 03), as was *Trichodesmium*. Based only on samples where *RC* was detected, its biomass correlated with the number of diatoms in the  $>10$   $\mu m$  fraction (Spearman rank correlation between 0.52 and 0.57,  $p < 0.0001$ ; data not shown). In endosymbiosis, the host cell provides a protective micro-environment and positive buoyancy to the symbiont, which enables the cell to remain in the euphotic layer, as, unlike *Trichodesmium*, this species does not have gas vacuoles (Janson et al. 1999). *Richelia* has a very uneven space distribution in the oceans, like *Trichodesmium*, depending on whether the environment is conducive to their growth. Both

genera often inhabit the same types of ecosystems, as they have similar physiological properties, like all diazotrophs (Bergman et al. 2013 and references therein). In the case of cyanobacteria-diatom associations, environmental conditions must meet the needs of both organisms, and particularly sufficient silicate concentration for the diatom host growth. Iron and silica were not limiting factors in our area of study and are provided by leaching from terrestrial soils (Shiozaki et al. 2014). In our study, *RC* was more correlated with *T. thiebautii* and *K. spiralis*, and its positive dependence on the density structure of the water column (MLD) and ammonium needs to be further explored. In the Northern Pacific Gyre, the greatest abundance in *Richelia intracellularis* is also observed in summer, when the species can form blooms of about  $10^4$  filaments per liter (Venrick 1974), values which are thus greater than ours (max 734 filaments  $l^{-1}$ ). Around the Hawaiian Islands (Sohm et al. 2011), a high abundance of all three groups, i.e. *Trichodesmium*, *Katagnymene* and *Richelia*, was reported in late boreal summer 2002 (Sep-Oct) and summer 2003 (July-Aug), with *Richelia*-diatom associations more abundant to the south of the islands. The high variability in *Richelia* size in our samples is another reason to underline the inaccuracy of biomass measurements in terms of filament numbers rather than total filament surface area or volume. The contribution of *RC* in terms of volume (or carbon) was minor ( $<1\%$ ) but must not mask the great impact of this species on carbon sequestration by DDA's as shown in the North Tropical Pacific (Dore et al. 2008, Karl 2014; Karl & Church 2014).

In our study, large diatoms did not show significant correlations with any of the other groups enumerated nor with any of the environmental variables. Dinoflagellates correlated positively with total trichome abundance. Their positive correlation with the percentage of *T. erythraeum* and their negative correlation with the percentage of *K. pelagica*, suggested affiliation to more coastal than oceanic environments. The diversity of trophic status in dinoflagellates (photosynthetic, mixotroph, heterotroph) was, nevertheless, not considered in the analysis. In the Northern Tropical Pacific, the dinoflagellate group revealed no specific ecological pattern nor any characteristic assemblages (Giraud et al. 2016). During the Diapalis cruises, the picoplanktonic groups did not show any correlation with environmental parameters (Table 9), which was surprising, as *Synechococcus* and *Prochlorococcus* have distinct ecophysiology, *Synechococcus* being better adapted to mesotrophic conditions and *Prochlorococcus* to oligotrophic conditions (Partensky et al. 1996). This could be explained for *Prochlorococcus* by the fact that their abundance varied little over the annual cycles. *Prochlorococcus* never dominated the phytoplankton C biomass, but were often the second contributor to C biomass after filamentous

cyanobacteria in oceanic waters (Fig. 12). All phytoplankton share a requirement for light and nutrients. A more specific mechanism underlying the covariation between *Synechococcus* and the picoeukaryotes, as opposed to *Prochlorococcus*, is that they have a larger cell size than the latter, and are able to use nitrate. This could explain they tend to respond positively to enhanced nutrient supply as observed mostly in the lagoon station and at Santal Bay. The covariation between *Synechococcus* and the picoeukaryotes has been described before in the Atlantic subtropical gyres (Marañón et al. 2003) and in the Pacific Ocean (Worden et al. 2004). The significant positive correlation between *Synechococcus* and picoeukaryotes may also occur due to similarities in favorable light conditions (Gutiérrez-Rodríguez et al. 2016). *Synechococcus* and picoeukaryotes dominated the C biomass in the lagoon, but may also be large contributors in oceanic waters, as observed in winter (Fig. 12).

### 2001-2003 Inter-annual variability

The difference in large diazotroph abundance (and nitrogen fixation rates, Garcia et al. 2007; Fig. 12C) observed between the summers of 2001-2002 and 2002-2003 could be the result of a relatively low *in situ* sampling frequency. Satellite imagery nevertheless confirmed the very low chlorophyll values during summer 2001-2002 compared to summer 2003 (Dupouy et al. 2011). Moreover, satellite-classified *Trichodesmium* pixels in the region 5°S-25°S, 160°E-170°W determined using the TRICHOSAT model were clearly lower in summer 2001-2002 (La Niña years) than summer 2003 (an El Niño year). The spectral fluorescence signature of surface blooms as measured by the MERIS sensor (Gower et al. 2014) was also high during El Niño summers (2003, 2007, 2010) but low during La Niña summers. The difference in cumulative precipitation between 2001-2002 and 2003 summers is striking, with 2003 being drier despite the occurrence of two tropical storms, namely Beni in January 2003 and Erica in March 2003 (Neveux et al. 2009). Law et al. (2011) reported that nitrogen fixation increased owing to an iron supply from a wet-dust deposition 8 days after Tropical Cyclone Wati in the Tasman Sea. Australian dust peaking in 2001 and 2002 was cited as a potential factor in increasing occurrence in the seas to the east of Australia (estimated by satellite) in 2001-2005 (Mackie et al. 2008). The regional distribution of aerosols was probably also influenced by the continuous passive (non-explosive) volcanic degassing of the Melanesian Volcanic Arc, as indicated by sulfur dioxide (SO<sub>2</sub>), the composition of which has been analyzed using satellite data and modelling (Lefèvre et al. 2015).

Likewise, Sahara dust deposition at BATS corresponded to an increased abundance of *Trichodesmium* colonies as well as changes in their chemical composition, even though it did not enhance the N<sub>2</sub> fixation rate per colony (Orcutt et al. 2001). At BATS, inter-annual variability could also be related to the relative extent of vertical mixing from one year to another, including the effects of hurricane frequency, which would reduce *Trichodesmium* abundance (Orcutt et al. 2001). During the Diapalis program, this negative vertical-mixing effect was not confirmed, as the highest *Trichodesmium* abundance occurred just after Tropical Storm Beni struck New Caledonia in Late January 2003. Inter-annual variability would also have been related to changes in main-current transports, i.e. a westward Sverdrup transport and trade winds enhanced during El Niño periods as against falling Sverdrup transport and strengthening northwesterly winds during La Niña (Kessler & Cravatte 2013; Cravatte et al. 2015). Wind regime, which may impact mixed-layer formation is known to alternate around New Caledonia with a trade-wind regime during El Niño and more westerly winds during La Niña, which may increase surface currents in the Loyalty Channel (Lefèvre et al. 2010). How such large-scale current variability or atmospheric composition influence total biomass and *Trichodesmium* abundance in New Caledonia is not clear yet.

## CONCLUSION

In 2001-2003, the seasonal and inter-annual variations in cyanobacteria abundance were investigated in New Caledonian waters during the 9 Diapalis program cruises. Three sites representative of different biotopes were sampled, i.e. open ocean (Loyalty Channel), coastal (lagoon off the Ouinne River estuary) and deep bays (Santal Bay at Lifou). Abundance was determined at the species level for diazotrophic filamentous cyanobacteria. Our description of the organisms (*RC* and diatoms) in symbiotic associations was well detailed. Filamentous cyanobacteria were practically always present throughout the year, but with relatively high variations. The highest abundances occurred during the summer seasons. The dominant species were *Trichodesmium thiebautii* and *T. tenue*, followed by *T. erythraeum*. *Katagnymene* as well as *Richelia* species (mainly endosymbiotic in diatoms), which were consistently present in low abundance. Microscopic examination also revealed the presence of unknown filamentous species formed by round-shape cells not yet described by molecular tools. Picoplanktonic cyanobacteria (*Prochlorococcus* and *Synechococcus*) were consistently present in abundance with less

pronounced seasonal variations than filamentous cyanobacteria. *Trichodesmium* dominated in terms of carbon content in the Loyalty Channel and Santal Bay in summer, while *Synechococcus* dominated at the Ouinne station and in winter at all the three stations. The comparison of the 2002 and 2003 summers showed major inter-annual variability. Environmental factors that could explain this variability were not clearly identified. One hypothesis could be the effects of alternation between La Niña and El Niño with maximum diazotrophs and *Trichodesmium* abundance during a well-established El Niño. Some hypotheses relating to the El Niño/La Niña cycle need verifying, i.e. dust inputs and links with precipitations, winds, vertical movements and horizontal circulation within the upper 200-meter layer, gyre formation in relation to wind strength and direction and increased mesoscale circulation.

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# TABLES

**Table 1:** Dates of the 9 Diapalis cruises

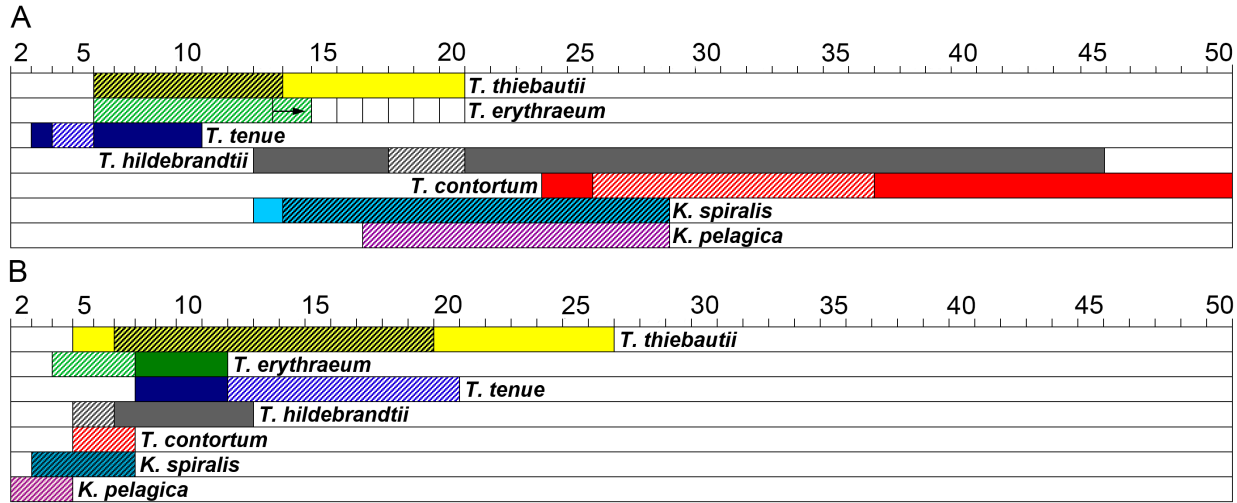
Diapalis cruises	Dates	Austral Season
D1	22-31 October 2001	transition period
D2	10-22 December 2001	Austral summer
D3	15-22 January 2002	Austral summer
D4	2-9 April 2002	Austral summer
D5	21-28 May 2002	transition period
D6	6-13 August 2002	Austral winter
D7	1-10 February 2003	Austral summer
D8	10-14 June 2003	Austral winter
D9	7-16 October 2003	transition period

**Table 2:** Morphometric characteristics of filamentous cyanobacteria, in the >10 µm size fraction, encountered during the Diapalis cruises. NDO: not distinctly observed.

Species	Cell diameter (µm)	Cell length (µm)	Cell shape
<i>T. erythraeum</i>	8 to 13.6	4 to 7	as wide as or up to twice as wide as long
<i>T. thiebautii</i>	6 to 14	7 to 19	as long as or up to twice as long as wide
<i>T. tenue</i>	4 to 5	12 to 20	3 to 4 x longer than wide
<i>T. contortum</i>	26 to 36	5 to 7	5 to 6 x wider than long
<i>T. hildebrandtii</i>	18-20	5 to 6	2.7 to 3 x wider than long
Unidentified filaments	2.7 to 3	NDO	NDO
<i>K. spiralis</i>	14 to 28	3 to 8	2.2 to 4.8 x wider than long
<i>K. pelagica</i>	17 to 28	2.5 to 3.5	4 to 14 x wider than long
<i>R. intracellularis</i> / <i>C. rhizosoleniae</i> group	cell 2-7 Ø heterocyst 3-10 Ø	-	-
Colonies of unidentified cyanobacteria (spherical cells)	type 1: 2.7-3 Ø type 2: 6 Ø	-	-

**Table 3:** A) Cell diameter (µm) and B) cell length (µm) of *Trichodesmium* and *Katagnymene* species. The colored areas represent size ranges published in the literature and shaded areas part

of the size range observed during the Diapalis cruises in the New Caledonian Lagoon. A black arrow indicates observation of a few *T. erythraeum* trichomes with a slightly wider diameter than previously published.





**Table 4.** Statistical data for the different groups enumerated by inverted microscopy in the >10 µm fraction and by flow cytometry in the picoplanktonic fraction (trichome number for identified filamentous cyanobacteria and cell number for all other groups and the colonies of unidentified cyanobacteria with spherical cells), at ST1, the Santal Bay station, during the 9 Diapalis cruises (2001-2003). Concentrations taken at 4-6 depths down to 60 m (l<sup>-1</sup>) and integrated values (m<sup>-2</sup>) in the 0-60 m layer. Trichomes: sum of *Trichodesmium* and *Katagnymene* trichomes.

ST1 Diapalis 01-09	min	max	average	stdev	n
Trichomes l <sup>-1</sup>	1	2929	651	648	50
10 <sup>6</sup> Trichomes m <sup>-2</sup>	0.06	89	38	30	12
10 <sup>6</sup> µm <sup>3</sup> Trichomes l <sup>-1</sup>	0.59	49.22	11.46	12.70	25
10 <sup>9</sup> µm <sup>3</sup> Trichome m <sup>-2</sup>	0.16	1.15	0.52	0.37	7
<i>R. intracellularis</i> / <i>C. rhizosoleniae</i> group l <sup>-1</sup>	0	64	8	16	25
10 <sup>3</sup> µm <sup>3</sup> <i>R. intracellularis</i> / <i>C. rhizosoleniae</i> group l <sup>-1</sup>	0.00	12.76	1.13	2.74	24
Unidentified cyanobacteria with spherical cells (cell l <sup>-1</sup> )	0	20	1	5	25
Dinoflagellates l <sup>-1</sup>	22	1927	322	405	31
Diatoms l <sup>-1</sup>	14	4516	640	1096	31
<i>T. tenue</i> l <sup>-1</sup>	20	947	285	255	28
<i>T. erythraeum</i> l <sup>-1</sup>	3	621	145	159	28
<i>T. thiebautii</i> l <sup>-1</sup>	18	1351	335	353	28
<i>K. spiralis</i> l <sup>-1</sup>	0	199	29	49	28
<i>K. pelagica</i> l <sup>-1</sup>	0	61	6	14	28
10 <sup>6</sup> µm <sup>3</sup> <i>T. tenue</i> l <sup>-1</sup>	0.09	7.82	2.20	2.25	27
10 <sup>6</sup> µm <sup>3</sup> <i>T. erythraeum</i> l <sup>-1</sup>	0.02	15.41	2.46	3.66	27
10 <sup>6</sup> µm <sup>3</sup> <i>T. thiebautii</i> l <sup>-1</sup>	0.18	13.90	3.67	3.64	27
10 <sup>6</sup> µm <sup>3</sup> <i>K. spiralis</i> l <sup>-1</sup>	0.00	6.90	1.33	1.98	27
10 <sup>6</sup> µm <sup>3</sup> <i>K. pelagica</i> l <sup>-1</sup>	0.00	31.40	1.54	6.04	27
10 <sup>4</sup> <i>Prochlorococcus</i> ml <sup>-1</sup>	3.73	46.21	13.37	8.13	76
10 <sup>4</sup> <i>Synechococcus</i> ml <sup>-1</sup>	0.22	3.58	1.50	0.86	76
10 <sup>4</sup> Picoeukaryotes ml <sup>-1</sup>	0.06	0.47	0.18	0.08	76
10 <sup>12</sup> <i>Prochlorococcus</i> m <sup>-2</sup>	3.41	14.80	8.76	3.96	11
10 <sup>12</sup> <i>Synechococcus</i> m <sup>-2</sup>	0.19	1.75	0.94	0.49	11
10 <sup>12</sup> Picoeukaryotes m <sup>-2</sup>	0.06	0.17	0.11	0.04	11

**Table 5.** Statistical data for the different groups enumerated by inverted microscopy in the >10 µm fraction and by flow cytometry in the picoplanktonic fraction (trichome number for identified filamentous cyanobacteria and cell number for all other groups and the colonies of unidentified cyanobacteria with spherical cells), at the ST2, Loyalty Channel station, during the 9 Diapalis cruises (2001-2003). Concentrations taken at 4-6 depths down to 60 m (l<sup>-1</sup>) and integrated values (m<sup>-2</sup>) in the 0-60 m layer. Trichomes: sum of *Trichodesmium* and *Katagnymene* trichomes

ST2 Diapalis 01-09	min	max	average	stdev	n
Trichomes l <sup>-1</sup>	4	4578	676	1089	45
10 <sup>6</sup> Trichomes m <sup>-2</sup>	0.46	235	44	66	11
10 <sup>6</sup> µm <sup>3</sup> Trichome l <sup>-1</sup>	0.03	62.22	9.53	15.29	36
10 <sup>9</sup> µm <sup>3</sup> Trichome m <sup>-2</sup>	0.16	3.05	0.63	0.92	9
<i>R. intracellularis</i> / <i>C. rhizosoleniae</i> group l <sup>-1</sup>	0	735	36	121	41
10 <sup>3</sup> µm <sup>3</sup> <i>R. intracellularis</i> / <i>C. rhizosoleniae</i> group l <sup>-1</sup>	0.00	117.33	7.22	21.64	30
Unidentified cyanobacteria with spherical cells (cell l <sup>-1</sup> )	0	4934	224	833	40
Dinoflagellates l <sup>-1</sup>	8	1205	289	306	40
Diatoms l <sup>-1</sup>	5	9969	834	2190	40
<i>T. tenue</i> l <sup>-1</sup>	13	1053	209	250	36
<i>T. erythraeum</i> l <sup>-1</sup>	2	417	50	78	36
<i>T. thiebautii</i> l <sup>-1</sup>	3	635	136	150	36
<i>K. spiralis</i> l <sup>-1</sup>	0	31	3	6	36
<i>K. pelagica</i> l <sup>-1</sup>	0	11	2	3	36
10 <sup>6</sup> µm <sup>3</sup> <i>T. tenue</i> l <sup>-1</sup>	0.04	17.28	2.88	3.49	32
10 <sup>6</sup> µm <sup>3</sup> <i>T. erythraeum</i> l <sup>-1</sup>	0.01	17.97	1.99	3.88	32
10 <sup>6</sup> µm <sup>3</sup> <i>T. thiebautii</i> l <sup>-1</sup>	0.13	21.03	2.92	4.66	32
10 <sup>6</sup> µm <sup>3</sup> <i>K. spiralis</i> l <sup>-1</sup>	0.00	15.89	0.82	2.90	32
10 <sup>6</sup> µm <sup>3</sup> <i>K. pelagica</i> l <sup>-1</sup>	0.00	2.07	0.18	0.46	32
10 <sup>4</sup> <i>Prochlorococcus</i> ml <sup>-1</sup>	2.59	29.02	13.05	5.95	111
10 <sup>4</sup> <i>Synechococcus</i> ml <sup>-1</sup>	0.05	4.58	0.99	1.24	111
10 <sup>4</sup> Picoeukaryotes ml <sup>-1</sup>	0.03	0.67	0.11	0.08	111
10 <sup>12</sup> <i>Prochlorococcus</i> m <sup>-2</sup>	3.53	14.87	8.76	3.59	17
10 <sup>12</sup> <i>Synechococcus</i> m <sup>-2</sup>	0.08	2.55	0.62	0.79	17
10 <sup>12</sup> Picoeukaryotes m <sup>-2</sup>	0.02	0.15	0.07	0.04	17

**Table 6.** Statistical data for the different groups enumerated by inverted microscopy in the >10 µm fraction and by flow cytometry in the picoplanktonic fraction (trichome number for identified filamentous cyanobacteria and cell number for all other groups and the colonies of unidentified cyanobacteria with spherical cells), at ST3, the Ouinne station, Eastern Lagoon, during the 9 Diapalis cruises (2001-2003). Concentrations taken at 4-6 depths down to 30 m (l<sup>-1</sup>) and integrated values (m<sup>-2</sup>) in the 0-30 m layer. Trichomes: sum of *Trichodesmium* and *Katagnymene* trichomes.

ST3 Diapalis 01-09	min	max	average	stdev	n
Trichomes l <sup>-1</sup>	10	854	172	219	32
10 <sup>3</sup> Trichomes m <sup>-2</sup>	0.38	15.00	4.74	5.30	9
10 <sup>6</sup> µm <sup>3</sup> Trichomes l <sup>-1</sup>	0.07	10.19	2.67	2.81	20
10 <sup>9</sup> µm <sup>3</sup> Trichome m <sup>-2</sup>	0.01	0.18	0.09	0.07	5
<i>R. intracellularis</i> / <i>C. rhizosoleniae</i> group l <sup>-1</sup>	0	117	15	25	25
10 <sup>3</sup> µm <sup>3</sup> <i>R. intracellularis</i> / <i>C. rhizosoleniae</i> group l <sup>-1</sup>	0	64.90	7.25	14.36	23
Unidentified cyanobacteria with spherical cells (cell l <sup>-1</sup> )	0	429	22	88	24
Dinoflagellates l <sup>-1</sup>	82	1000	344	251	22
Diatoms l <sup>-1</sup>	26	16893	2458	4345	22
<i>T. tenue</i> l <sup>-1</sup>	5	868	136	228	29
<i>T. erythraeum</i> l <sup>-1</sup>	0	132	16	28	29
<i>T. thiebautii</i> l <sup>-1</sup>	2	147	43	40	29
<i>K. spiralis</i> l <sup>-1</sup>	0	9	0	2	29
<i>K. pelagica</i> l <sup>-1</sup>	0	8	0	2	29
10 <sup>6</sup> µm <sup>3</sup> <i>T. tenue</i> l <sup>-1</sup>	0.08	6.09	1.61	2.10	18
10 <sup>6</sup> µm <sup>3</sup> <i>T. erythraeum</i> l <sup>-1</sup>	0.00	4.79	0.66	1.14	18
10 <sup>6</sup> µm <sup>3</sup> <i>T. thiebautii</i> l <sup>-1</sup>	0.02	2.41	0.89	0.68	18
10 <sup>6</sup> µm <sup>3</sup> <i>K. spiralis</i> l <sup>-1</sup>	0.00	0.50	0.03	0.12	18
10 <sup>6</sup> µm <sup>3</sup> <i>K. pelagica</i> l <sup>-1</sup>	0.00	1.42	0.10	0.35	18
10 <sup>4</sup> <i>Prochlorococcus</i> ml <sup>-1</sup>	1.01	24.17	7.28	4.40	66
10 <sup>4</sup> <i>Synechococcus</i> ml <sup>-1</sup>	1.14	13.82	6.60	2.91	66
10 <sup>4</sup> Picoeukaryotes ml <sup>-1</sup>	0.13	1.74	0.34	0.26	66
10 <sup>12</sup> <i>Prochlorococcus</i> m <sup>-2</sup>	0.48	4.75	2.13	1.05	11
10 <sup>12</sup> <i>Synechococcus</i> m <sup>-2</sup>	0.82	3.27	1.92	0.79	11
10 <sup>12</sup> Picoeukaryotes m <sup>-2</sup>	0.06	0.17	0.10	0.04	11

**Table 7:** Cell number per trichome of each *Trichodesmium* and *Katagnymene* species during the Diapalis cruises.

	<i>T. tenue</i>	<i>T. thiebautii</i>	<i>T. erythraeum</i>	<i>T. hildebrandtii</i>	<i>T. contortum</i>	<i>K. spiralis</i>	<i>K. pelagica</i>
min	3	3	4	16	19	2	10
max	206	209	235	132	62	140	334
Mean	54	55	64	62	40	42	57
STD	36	38	45	40	22	34	77
N	584	469	322	9	3	49	18

**Table 8:** Morphometric characteristics of *Trichodesmium* and *Katagnymene* species at the *Trichodesmium* peak abundance in February 03 (Diapalis 7). Their chlorophyll *a* (Chla), phycoerythrin (PE), carbon (C) content per trichome and volume of trichome. Comparison with cellular characteristics, pigments and carbon content obtained with *T. erythraeum* in New Caledonian waters (West Coast). TK: sum of *Trichodesmium* and *Katagnymene* trichomes.

	Mean	STD	Number
<b>D7 Cruise</b>			
pg Chla trichome <sup>-1</sup>	43.23	18.86	30
µg Chla mm <sup>-3</sup> trichome	3.47	1.71	23
pg PE trichome <sup>-1</sup>	196.86	58.34	30
µg PE mm <sup>-3</sup> trichome	16.71	7.94	24
TK trichome diameter (µm)	6.64	3.32	1884
TK trichome length (µm)	360	302	1884
TK trichome volume (µm <sup>3</sup> )	12808	28847	1884
<i>T. contortum</i> trichome diameter (µm)	33.00	-	2
<i>T. contortum</i> trichome length (µm)	128	-	2
<i>T. erythraeum</i> trichome diameter (µm)	8.01	1.11	402
<i>T. erythraeum</i> trichome length (µm)	320	245	402
<i>T. hidelbrandtii</i> trichome diameter (µm)	16.00	2.49	9
<i>T. hidelbrandtii</i> trichome length (µm)	269	308	9
<i>T. tenue</i> trichome diameter (µm)	4.49	0.57	530
<i>T. tenue</i> trichome length (µm)	472	357	530
<i>T. thiebautii</i> trichome diameter (µm)	6.06	0.28	735
<i>T. thiebautii</i> trichome length (µm)	327	273	735
<i>K. pelagica</i> trichome diameter (µm)	24.00	5.10	5
<i>K. pelagica</i> trichome length (µm)	403	558	5
<i>K. spiralis</i> trichome diameter (µm)	20.00	3.95	65
<i>K. spiralis</i> trichome length (µm)	170	189	65
<b><i>T. erythraeum</i></b>			
pg Chla cell <sup>-1</sup>	1.58	0.26	5
pg Chla trichome <sup>-1</sup>	99.00	14.62	5
ng Chla mm <sup>-3</sup> trichome	3.31	3.86	5
pg PE cell <sup>-1</sup>	4.01	0.76	5
pg PE trichome <sup>-1</sup>	251.57	41.26	5
µg PE mm <sup>-3</sup> trichome	8.37	1.21	5
pg carbon cell <sup>-1</sup>	289.89	-	2
ng carbon trichome <sup>-1</sup>	17.22	-	2
µg carbon mm <sup>-3</sup> trichome	588.06	-	2
PE/Chla	3.81	1.79	13
Carbon/Chla	173.21	55.96	9
Carbon/PE	71.15	33.54	6
Cell diameter (µm)	9.6	0.5	1849
Cell length (µm)	6.3	0.7	1849
Cell volume (µm <sup>3</sup> )	456	76	1849
Trichome length (µm)	402	157	1849

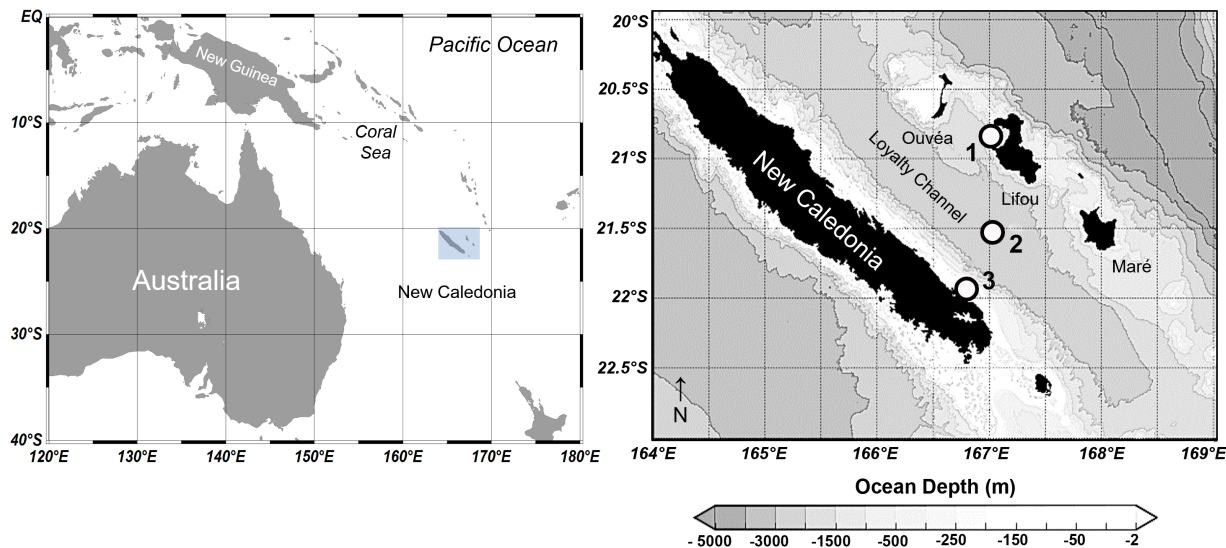
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**Table 9.** Spearman's correlation coefficient for the relationships between integrated abundance (cell or trichomes m<sup>-2</sup> over 60 m for ST1 and ST2 and 30 m for ST3) of autotrophic groups, the percentage of the different species of filamentous cyanobacteria and environmental parameters (n = 28). Data from the Diapalis cruises (2001-2003). \*p < 0.01, \*\*p < 0.05, ns: not significant; TK: sum of *Trichodesmium* and *Katagnymene* trichomes; Din: Dinoflagellates; Diat: Diatoms; *Rich*: *Richelia*; *Proc*: *Prochlorococcus*; *Syn*: *Synechococcus*; *Peuk*: Picoeukaryotes; *T.t*: *T. tenue*; *T. e*: *T. erythraeum*; *T. th*: *T. thiebautii*; *K. s*: *K. spiralis*; *K. p*: *K. pelagica*; MLD: mixed layer depth; T(°C): Temperature; Sal: Salinity.

Variables	Din	Diat	TK	<i>Rich</i>	<i>Proc</i>	<i>Syn</i>	<i>Peuk</i>	% <i>T. t</i>	% <i>T. e</i>	% <i>T. th</i>	% <i>K. s</i>	% <i>K. p</i>	MLD	T(°C)	Sal	NO <sub>x</sub>	NH <sub>4</sub>	PO <sub>4</sub>
Din	1	0.46**	0.63**	ns	ns	ns	ns	ns	0.50**	ns	ns	-0.57**	ns	ns	ns	ns	ns	ns
Diat		1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
TK			1	ns	0.65*	ns	ns	ns	ns	ns	ns	ns	ns	0.62*	ns	ns	0.51**	-0.48**
<i>Rich</i>				1	ns	ns	ns	ns	ns	0.61*	0.69*	ns	0.60**	ns	ns	ns	0.85*	ns
<i>Proc</i>					1	-0.42**	ns	ns	ns	-0.50**	ns	ns	ns	ns	ns	ns	ns	ns
<i>Syn</i>						1	0.52**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Peuk</i>							1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
% <i>T. t</i>								1	ns	-0.65*	-0.59**	ns	ns	0.49**	ns	ns	-0.57**	ns
% <i>T. e</i>									1	ns	ns	ns	ns	ns	ns	ns	ns	ns
% <i>T. th</i>										1	ns	ns	ns	ns	ns	ns	ns	ns
% <i>K. s</i>											1	ns	ns	ns	ns	0.64**	0.55**	0.59**
% <i>K. p</i>												1	ns	ns	ns	ns	ns	0.63**
MLD													1	ns	ns	-0.46**	ns	ns
T(°C)														1	-0.63*	ns	ns	-0.55*
Sal															1	ns	ns	0.52**
NO <sub>x</sub>																1	ns	0.48**
NH <sub>4</sub>																	1	ns
PO <sub>4</sub>																		1

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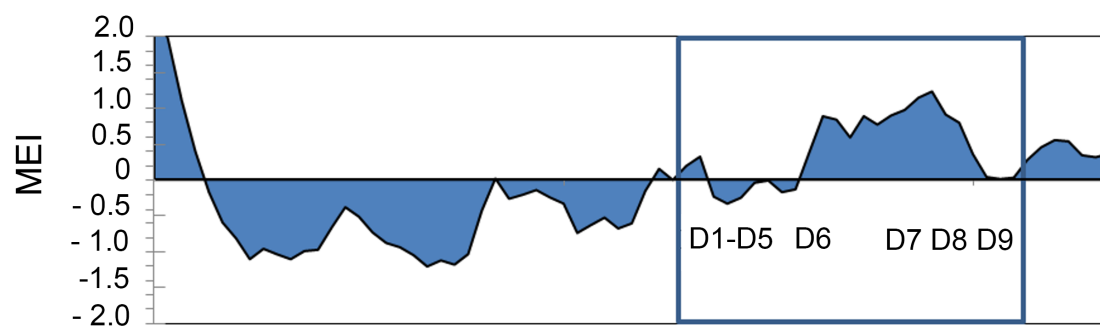
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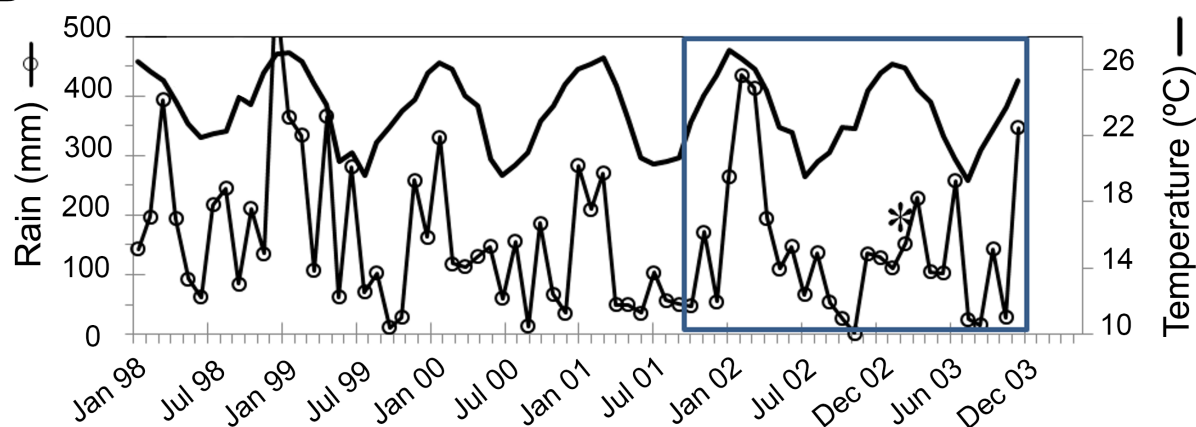
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**Figure 1.** Location of the 3 main stations sampled during the nine Diapalis cruises in the New Caledonian waters: ST1 (Santal Bay) on Lifou Island ( $167^{\circ}04'E$   $20^{\circ}51'S$ , 1050 m depth), ST2 (Loyalty Channel,  $167^{\circ}E$   $21^{\circ}30'S$ , 2100 m depth) and ST3 (Ouinne station) in the East Coast lagoon ( $166^{\circ}45'E$   $21^{\circ}57'S$ , 35 m depth). Figure Courtesy J. Lefèvre.

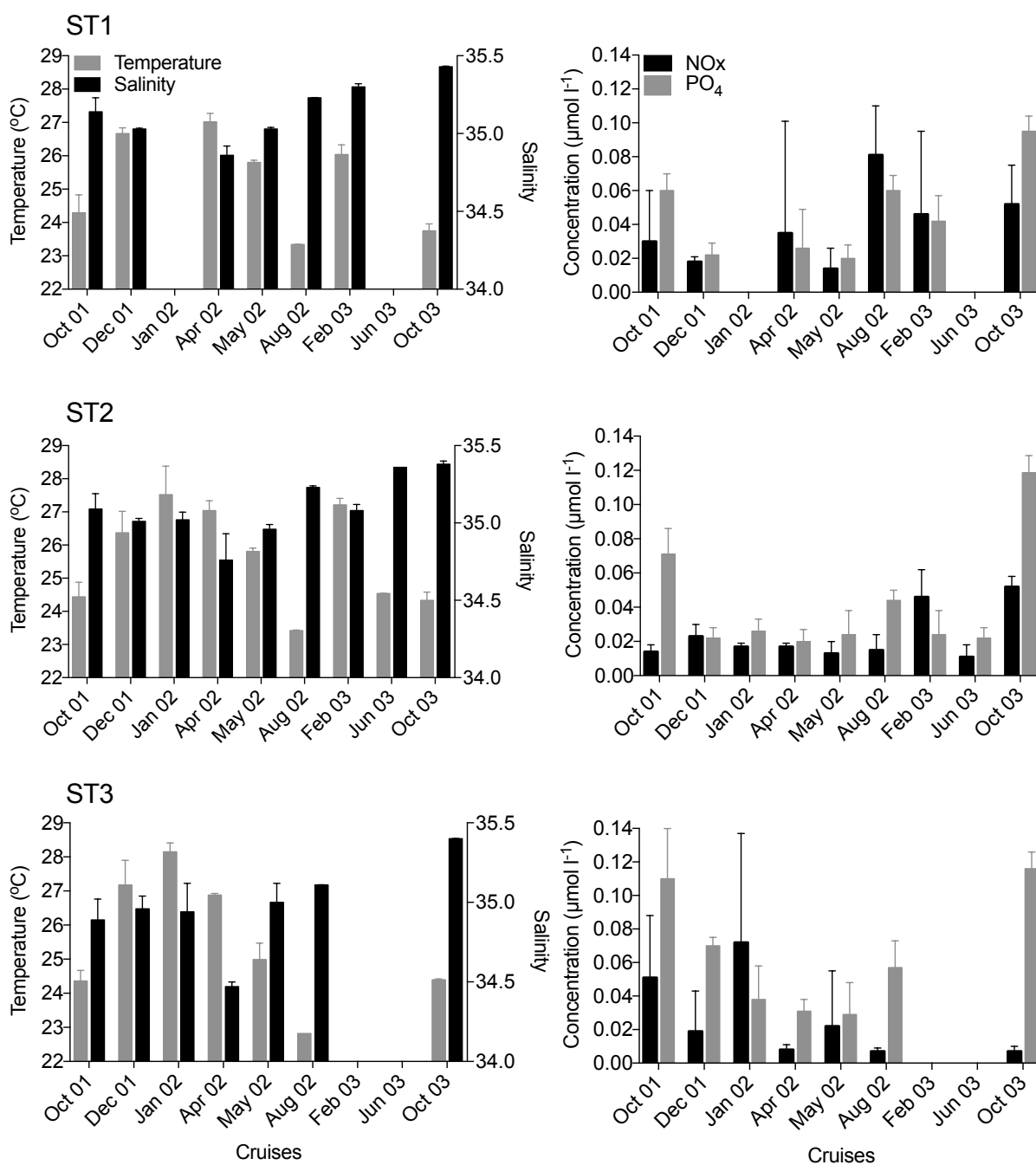
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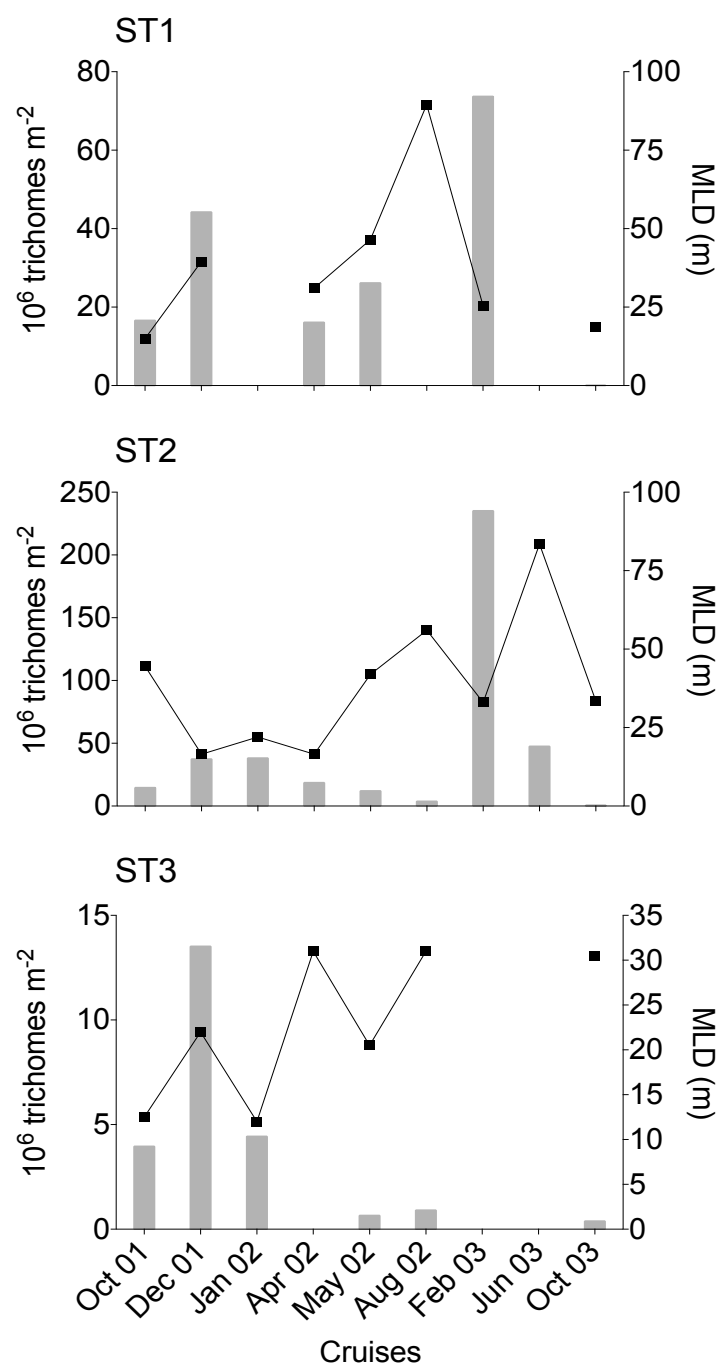


**Figure 2.** (A) Multivariate El Niño Index, (B) air temperature and (T °C) and monthly cumulative precipitation (mm) for 1998-2003 on New Caledonia's east coast (Thio station). The framed area represents the Diapalis sampling period. Tropical Storm Beni (29 Jan - 5 Feb 2003) is indicated as (\*). See Table 1 for the dates of the cruise.

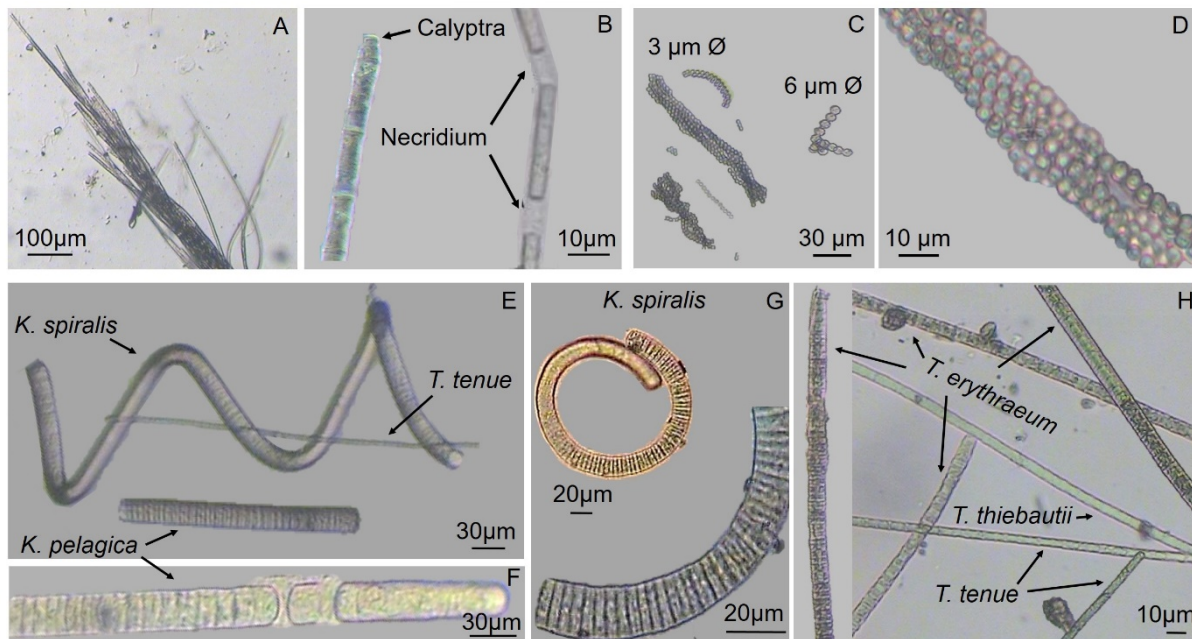


**Figure 3.** Mean temperature, salinity (left panel) and nutrients (NO<sub>x</sub>, PO<sub>4</sub>) (right panel) during the Diapalis cruises. Values are averaged from 0-60 m at ST1 and ST2 and 0-30 m at ST3. ST3 was not sampled in Feb 03 and Jun 03.

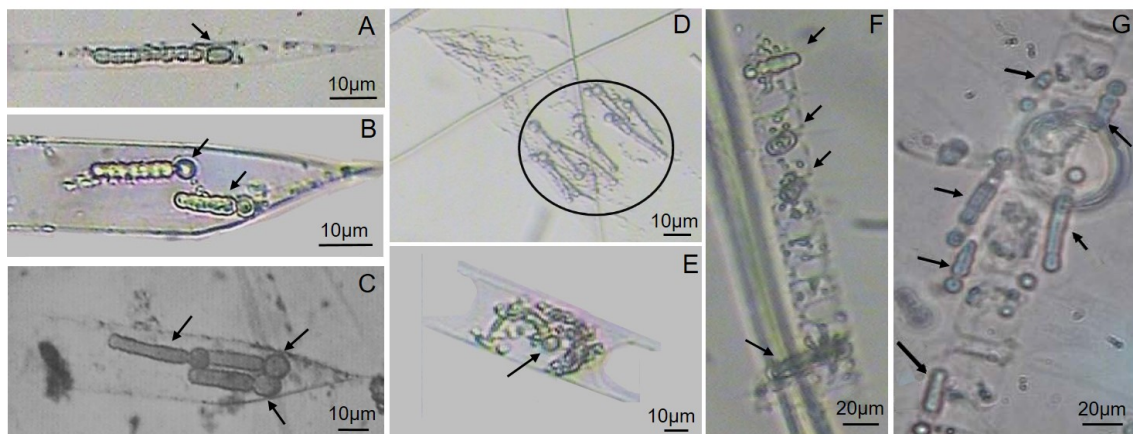




**Figure 4.** Integrated concentrations of the total filamentous cyanobacteria (trichomes m<sup>-2</sup>) in the >10 µm fraction (*R. intracellularis* and *C. rhizosoleniae* not included) (bars) and the mixed layer depth (MLD) (lines) during the Diapalis cruises. Integration depths: 0-60 m at ST1 and ST2 and 30 m at ST3.

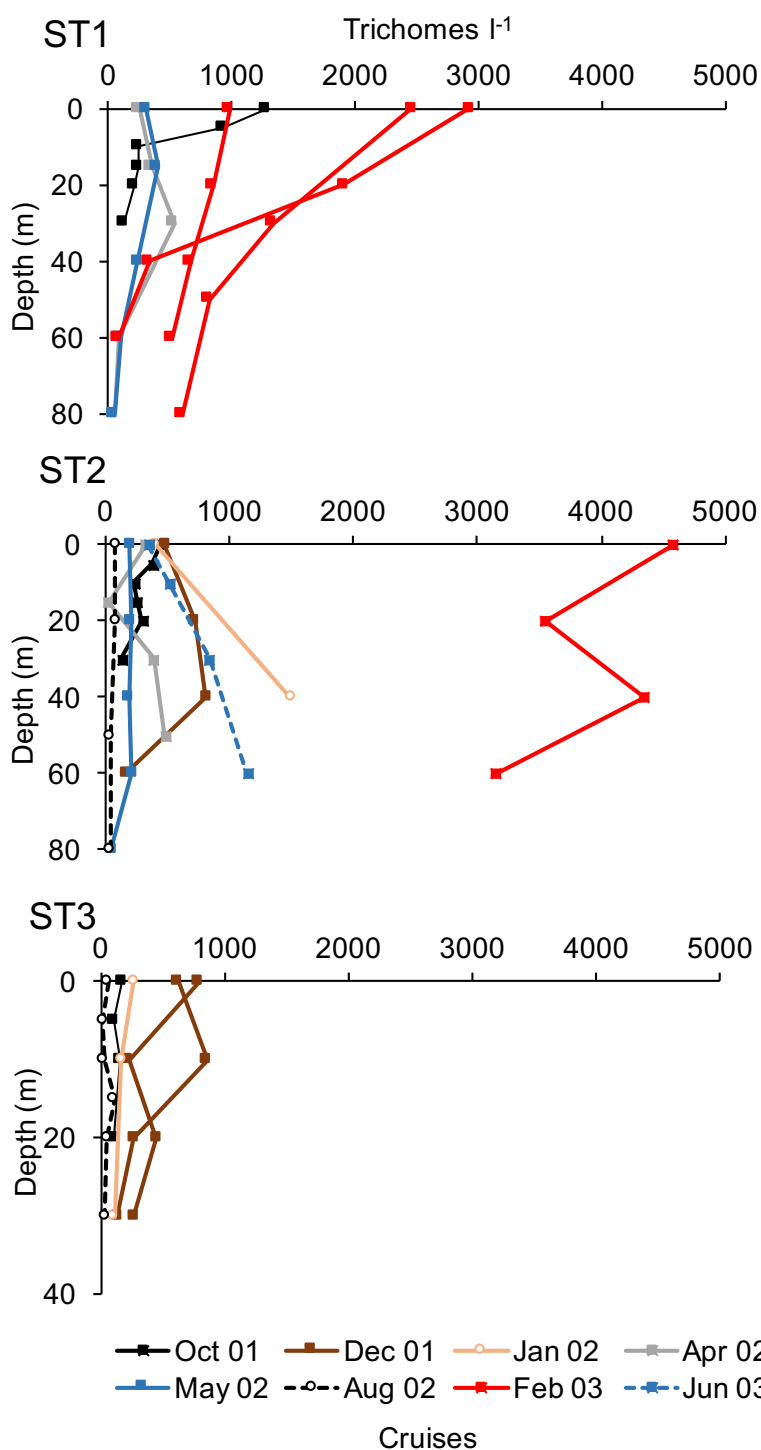


**Figure 5.** Photomicrographs of filamentous cyanobacteria obtained from the Diapalis cruises. A) *Trichodesmium tenue*, B) Details of *T. tenue* trichomes: note that cell length is about 3 times longer than wide; the calyptra (formation at the tip of the trichome) and the necridium (structure formed from a dead cell which constitutes a fracture point of the filament). C) Colonies of unidentified cyanobacteria composed of round-shaped cells. Type 1, 3µm Ø and Type 2, 6µm Ø and D) details of Type-1. E-G) *Katagnymene spiralis* and *Katagnymene pelagica*. Note the size difference between *Katagnymene* spp. and *T. tenue*. H) Mix of major species (*T. erythraeum*, *T. tenue*, *T. thiebautii*).



**Figure 6.** A-D) *Richelia intracellularis* symbiotic with *Rhizosolenia* spp. and E) *Hemiaulus* sp.; F-G) *Calothrix rhizosoleniae* epiphyte on *Chaetoceros* sp. Size and number of *Richelia* vary based on the size of the host. Photomicrographs obtained from the Diapalis cruises.

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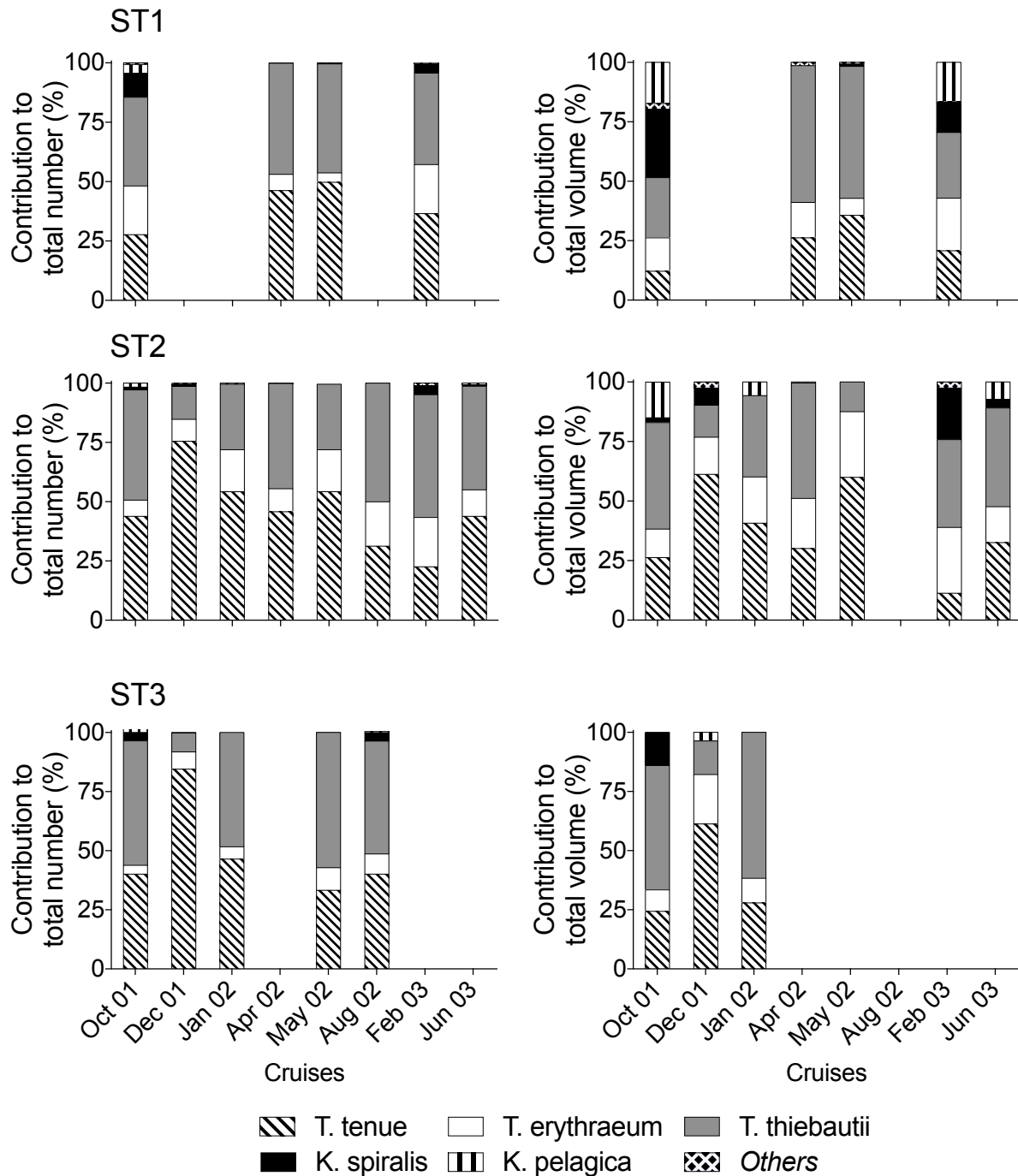


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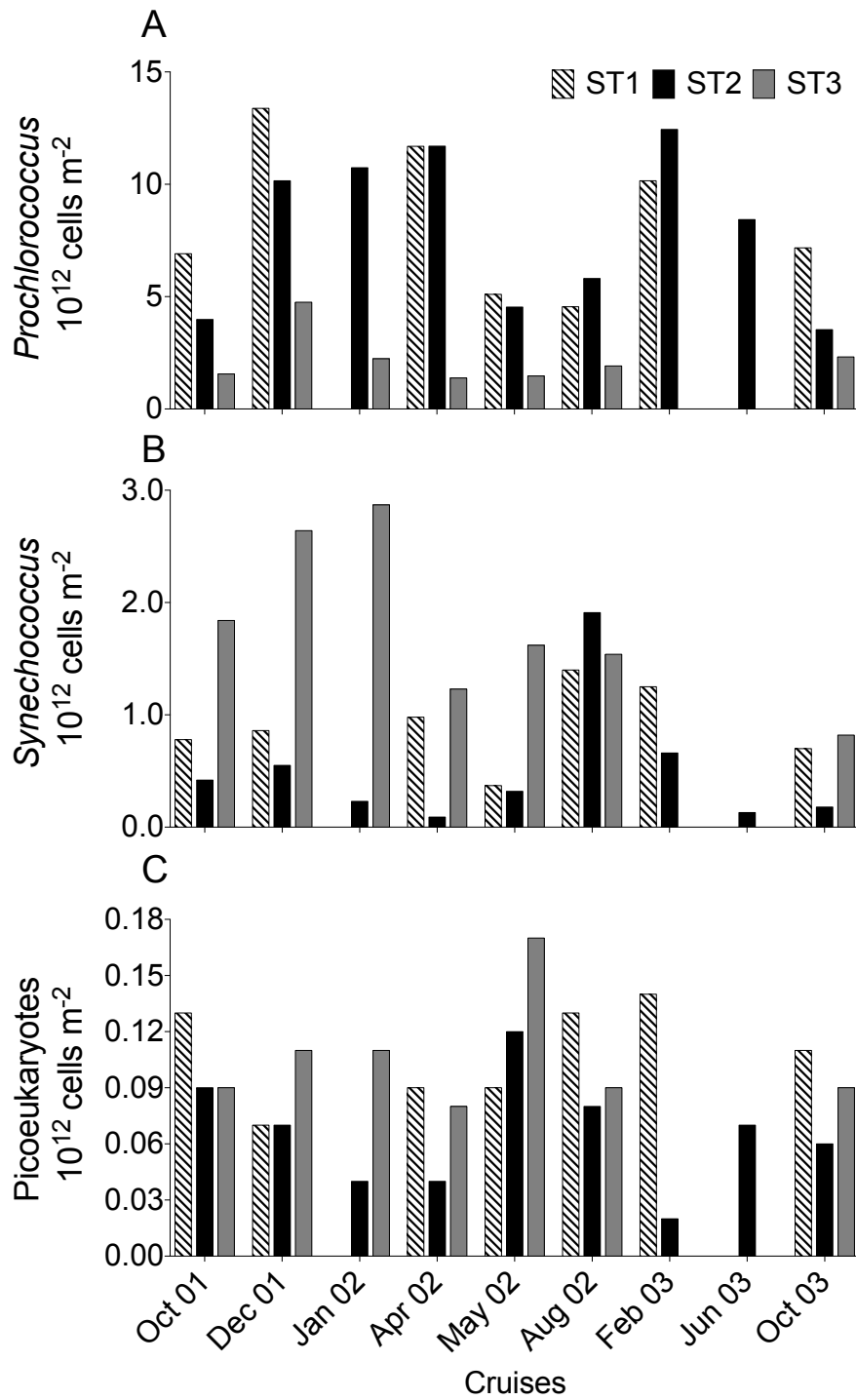
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1074 **Figure 7.** Vertical profiles for filamentous cyanobacteria abundance (Trichomes  $l^{-1}$ ) (except  
 1075 *R. intracellularis* and *C. rhizosoleniae*) during the Diapalis cruise stations. ST2 (open ocean,  
 1076 Loyalty Channel) is well representative of the seasonal variations (some cruises are missing  
 1077 at ST1 and ST3). Note that at ST1 in February 2003, 3 profiles were performed with intervals  
 1078 of approximately 24h.

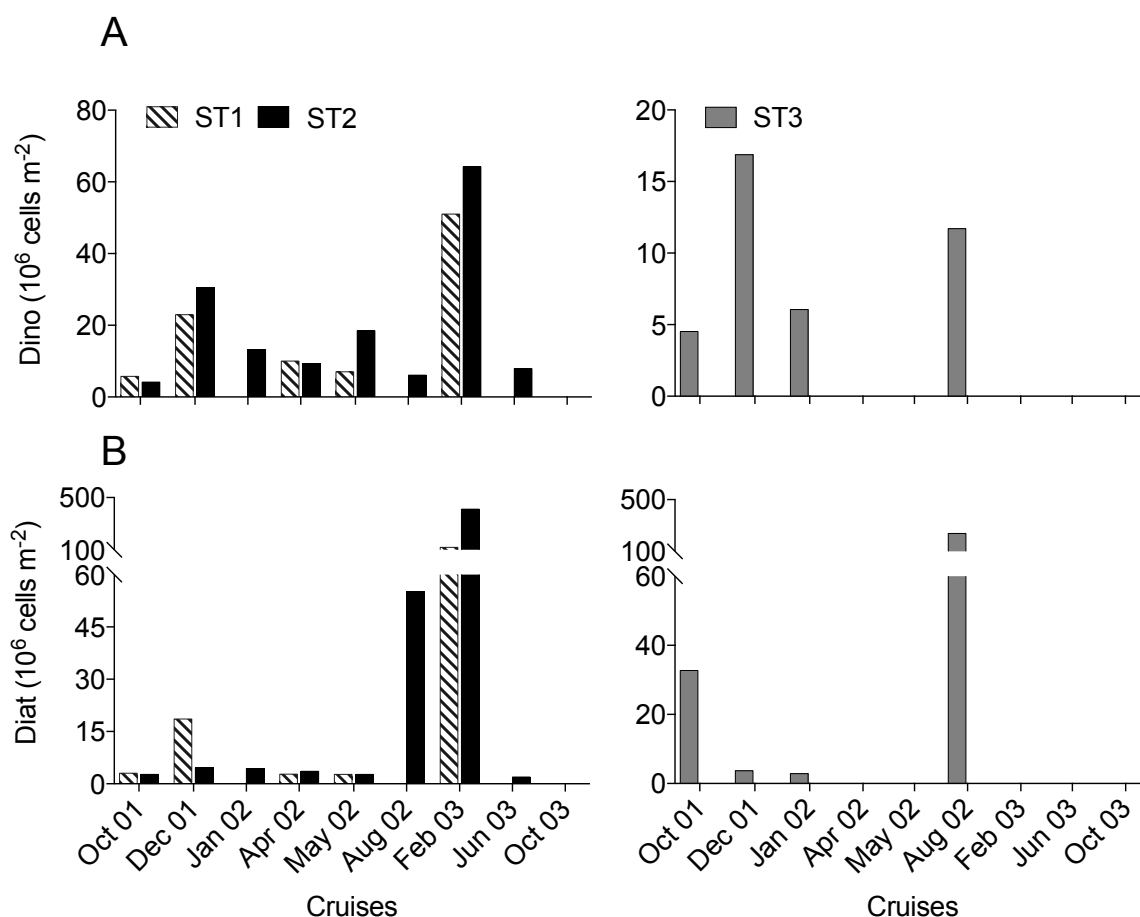
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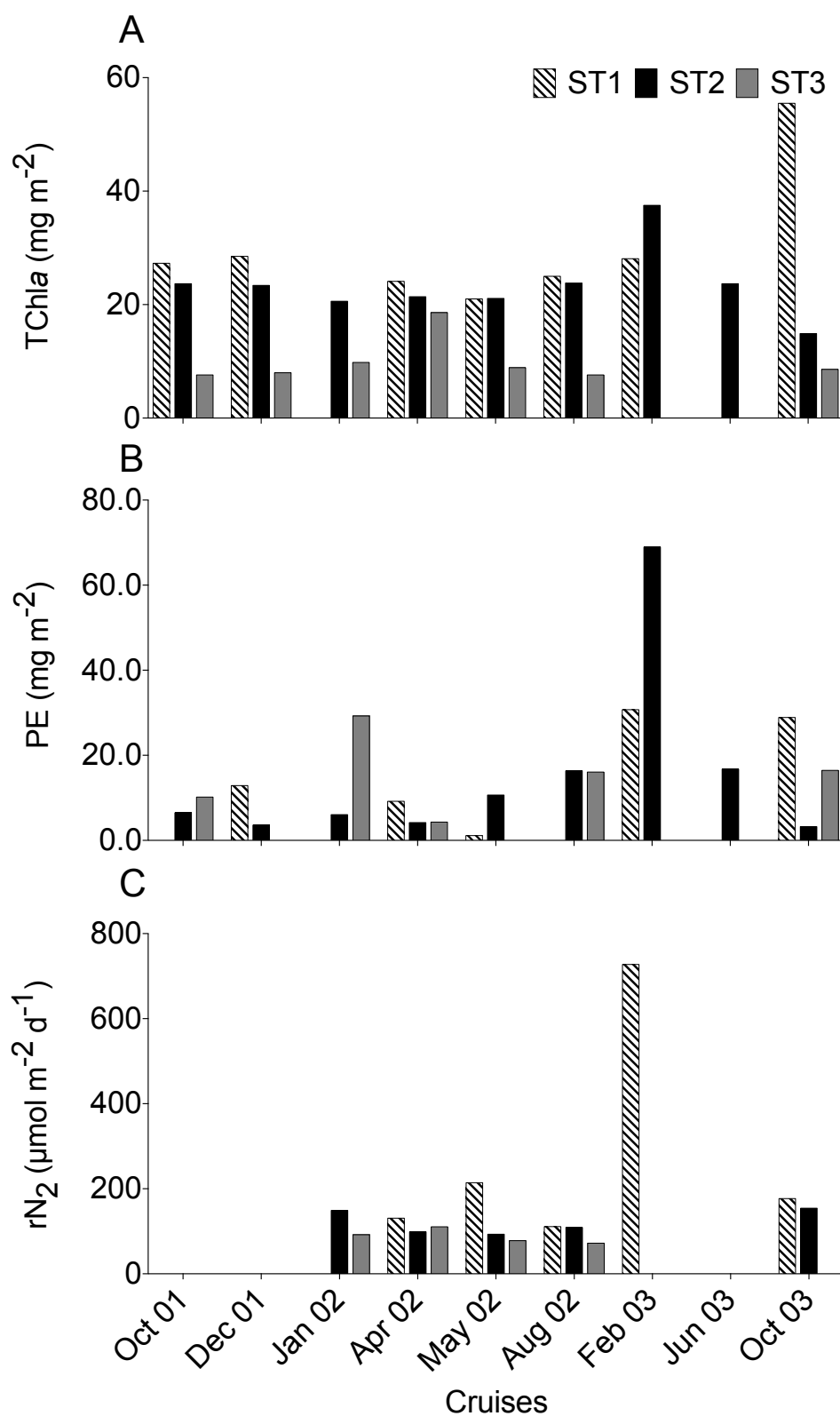
**Figure 8:** Contributions of the various species (%), in terms of trichome number (left panels) and trichome volume (right panel) to the total community of filamentous cyanobacteria (*R. intracellularis* and *C. rhizosoleniae* not included) at the three stations sampled during the Diapalis cruises. Integration depths: 0-60 m at ST1 and ST2; 0-30 m at ST3. At ST1 “Others” = *T. hildebrandtii* (October 2001, May 2002 and February 2003) or *T. contortum* (October 2001); At ST2 “Others” = *T. hildebrandtii*. At ST2, species contributions were determined at each cruise, except in August 2002 for the *Trichodesmium* volume contribution. Some cruises are missing at ST1 and ST3.



**Figure 9.** Integrated concentrations of (A) *Prochlorococcus*, (B) *Synechococcus*, and (C) picoeukaryotes at the three stations sampled during the Diapalis cruises. Integration depths: 0-60 m at ST1, ST2; 0-30 m at ST3.

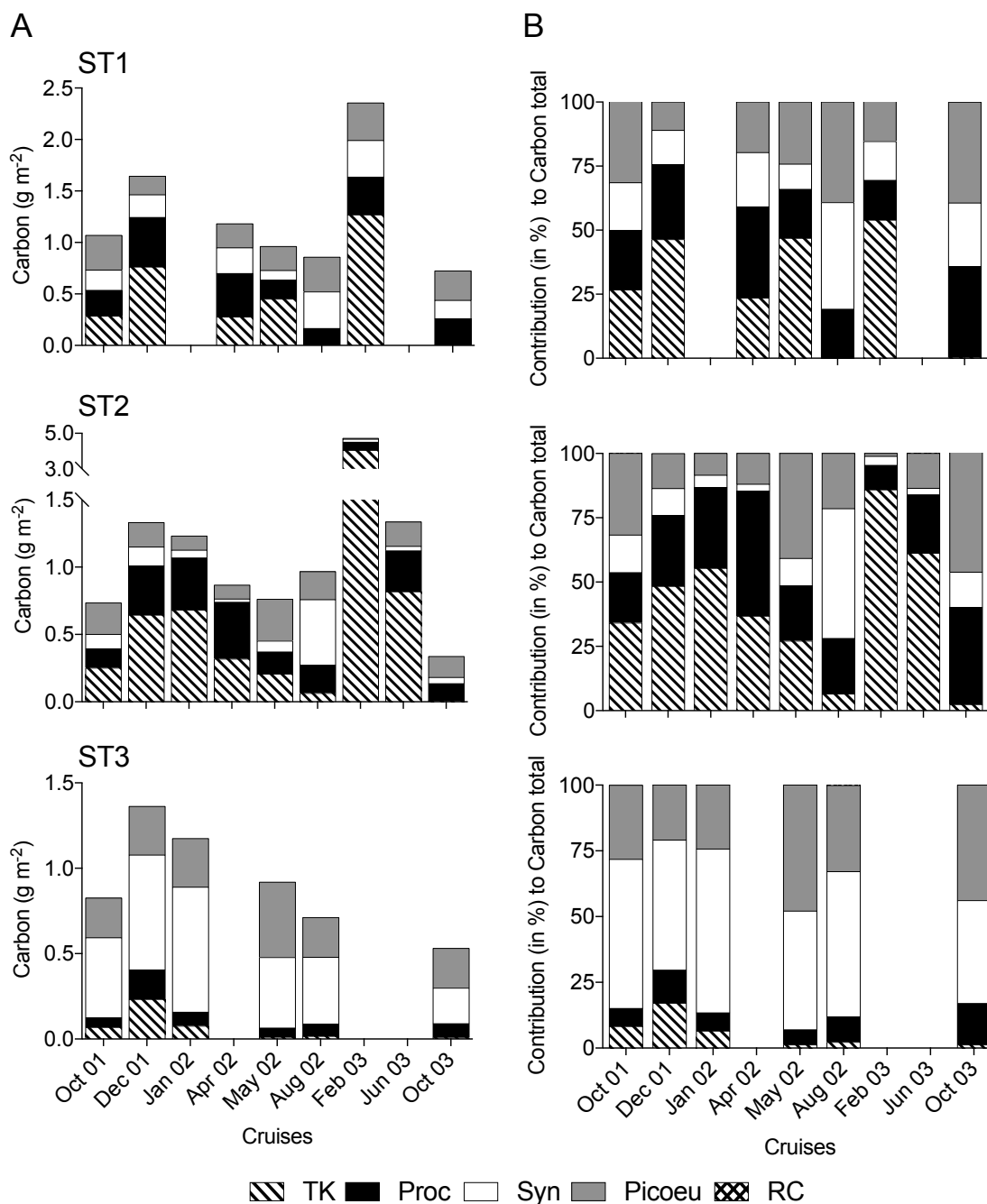


**Figure 10.** Integrated concentrations of (A) dinoflagellates (Dino) and (B) diatoms (Diat) in the  $>10\ \mu m$  fraction. Enumeration was carried out by inverted microscopy at the three stations sampled during Diapalis cruises. Integration depths: 0-60 m at ST1, ST2; 0-30 m at ST3. At ST2, integrated concentrations were determined at each cruise, except in August 2002. October 2003 abundance was too low for enumeration. Some cruises are missing at ST1 and ST3.



**Figure 11.** Integrated Tchl a (A) and phycoerythrin (B) concentrations and (C) N<sub>2</sub> fixation rates (Garcia et al. 2007) at the three stations sampled during the Diapalis cruises: Integration depths: 0-60 m at ST1 and ST2, and 0-30 m at ST3.

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1113 **Figure 12.** A) Carbon biomass of picoplankton and filamentous cyanobacteria at the three  
1114 stations sampled during the Diapalis cruises. The conversion factors were (fgC cell<sup>-1</sup>):  
1115 *Prochlorococcus* – Proc (36), *Synechococcus* - Syn (255), picoeukaryotes - Picoeu (2590),  
1116 *Richelia* - RC (10), sum of *Trichodesmium* and *Katagnymene* – TK (17.22 10<sup>6</sup> fgC trich<sup>-1</sup>). B)  
1117 Contribution of each group to the total carbon shown in A).