



Microplankton Community Composition Associated With Toxic *Trichodesmium* Aggregations in the Southwest Atlantic Ocean

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The spatial distribution and species identification of *Trichodesmium* was assessed during two fall cruises along the Southwest Atlantic Ocean shelf break. Organisms from the microplankton >50 μm were collected using a vertical plankton net for quantification and identification of the microplanktonic community associated with the genus. Additional sub-samples were filtered and prepared for quantification and discrimination of phycotoxins from the particulate matter using High Performance Liquid Chromatography. Physical parameters such as temperature, salinity, wind speed, and mixed layer depth were used in order to evaluate the environmental conditions at the time of sampling and correlate with *Trichodesmium* occurrence. Overall, *Trichodesmium* abundances were higher in the northernmost stations under wind speeds of <8 knots and shallow mixed layer depths <40 m. Besides frequent reports on the occurrence of *T. erythraeum* and *T. thiebautii* for this region, we identified three species as *T. clevej*, *T. hildebrandtii*, and *T. radians*. In the majority of stations where *Trichodesmium* was not the dominant organism, other microplanktonic groups were present such as centric diatoms and dinoflagellates. The toxin analysis was positive for saxitoxins predominantly when *Trichodesmium* was at high numbers of trichomes per liter in the stations; however, there was an inverse relationship between abundance of trichomes and toxin concentrations. Using information from the environmental variables and *Trichodesmium* abundance, we suggest that the toxin production might take place during the aggregation phase of trichomes at surface, and that saxitoxins could be inhibiting the growth of other microplanktonic organisms.

Keywords: marine diazotroph, microbial community composition, phycotoxin, saxitoxins, STX

INTRODUCTION

The marine cyanobacteria *Trichodesmium* (Ehrenberg, 1830) form colonies that aggregate in the water surface as visible patches in tropical and subtropical oceans. The high abundance of trichomes in the upper water layer is often associated with environmental factors such as hot and calm weather and the presence of a shallow mixed layer (Karl et al., 2002; Agawin et al., 2013). The global

occurrence of *Trichodesmium* is of undoubted importance to biogeochemical cycles, especially nitrogen and carbon (Capone et al., 1997; Westberry and Siegel, 2006); however, the majority of studies involving *Trichodesmium* are restricted to the North Atlantic and North Pacific Subtropical Gyres (Villareal and Carpenter, 2003; LaRoche and Breithbarth, 2005), and the genus is often under-studied in other oceans. More recent reports of *Trichodesmium* in the Andaman Sea (Arun Kumar et al., 2012), coast of India (Srinivas and Sarin, 2013) and Southwest Atlantic Ocean (Silva, 2005; Silva et al., 2008; Detoni et al., 2016a,b; Bif and Yunes, 2017) suggest they are important members of the microbial community composition in those regions.

In terms of ecological role, *Trichodesmium* is often reported in co-occurrence with other members of phytoplankton and zooplankton. The symbionts *Rhizosolenia hebetata* and *Richelia intracellularis*, for example, were present with dense aggregations of *Trichodesmium* (Madhu et al., 2013). The association between the harpacticoid *Macrosetella gracilis* and *Trichodesmium* colonies are thought to benefit the zooplankton, since trichomes were reported containing eggs and larvae attached, and served as food and substrate (Björnberg, 1965; O'Neil et al., 1996). Understanding the microbial community structure surrounding *Trichodesmium* aggregations is therefore an important key to understand its ecology and species interactions, especially in understudied regions such as the South Atlantic Ocean where the genus is often reported (Gianesella-Galvão, 1995; Rörig et al., 1998; Carvalho et al., 2008; Monteiro et al., 2010, 2012). Recent works from our group have identified colonies in the Southwest Atlantic Ocean associated with iron-rich dust particles (Bif and Yunes, 2017) and producing toxins (Detoni et al., 2016a); however, the association of these potentially toxic aggregations with other microbial communities was never assessed. Previous works associated the occurrence of large aggregations of *Trichodesmium* (*T. erythraeum* and *T. thiebautii*) with anoxia-related mortality of fish and crabs in India (D'Silva et al., 2012 and references therein) and bryozoans in the South Brazilian coast (Silva, 2005); however, no toxin analysis was performed at that time (Silva et al., 2008). Given the reports of *Trichodesmium* aggregations along the Brazilian coast (Silva et al., 2008 and references therein), our work aims to investigate their distribution, morphological taxonomy, association with microplanktonic groups and the potential toxicity to these organisms.

MATERIALS AND METHODS

Study Area and Analysis of Physical Parameters

This study was carried out in the area encompassing the Brazilian shelf slope on board the R/V Atlântico Sul with TALUDE project (Table 1). Samples were collected between 24° and 35°S (Figure 1) during two consecutive fall cruises, the first between June 4th and 11th of 2013 and the second between May 10th and 20th of 2014. Vertical profiles of temperature and salinity were obtained using a CTD sensor (911 SeaBird®). The surface water masses were classified based on the thermohaline intervals

described by Möller et al. (2008). The mixed layer depth (MLD) was determined from vertical density profiles ($\partial\rho/\partial z$), excluding the first 5 meters of depth due to noise data. Wind speed and direction were obtained from an anemometer mounted on the ship's foremast ~18 m above the sea surface. Both sea surface temperature (SST) and salinity (SSS) were then averaged for the upper 30 m of water depth in order to correlate with other environmental parameters, especially community composition.

Analysis of Biological Samples

Samples were collected during two different conditions: areas of high density aggregations of *Trichodesmium*; and in the absence of visible surface aggregations. The sampling efforts were carried out during calm seas and clear skies. During the 2013 cruise we sampled the northernmost section (stations 1 to 4; Figure 1) and focused solely in high density of trichomes. The 2014 cruise was carried out in the southernmost stations and sampling was performed during both presence and absence of trichomes.

The organisms were collected by vertical hauls using conical plankton net with 30 cm mouth diameter and 50 μm mesh size, down to 30 m depth of the water column. These samples were preserved in 4% buffered formalin. *Trichodesmium* species were identified following Komárek and Anagnostidis (2005) and quantified as number of trichomes per liter. For the description of each species of *Trichodesmium*, it was necessary to distinguish among cell, trichome (cells filaments) and colony (clusters of trichomes organized as puffs or tufts) (Anagnostidis and Komárek, 1988). As far as possible, co-occurring microplankton (>50 μm) were also identified and counted (minimum 100 cells per group) using an inverted microscope (ZeissAxiovert A1) coupled with a camera (Axio Cam MRc) using the sedimentation chamber technique (Utermöhl, 1958). Samples were counted at $\times 200$, $\times 400$, and $\times 1000$ of magnification and the species were identified according to specific literature for each group i.e., diatoms (Hasle and Syvertsen, 1996) and dinoflagellates (Steindinger, 1996).

Since *Trichodesmium* spp. trichomes were very concentrated in many samples, it was not possible to estimate their abundance per species; instead, the total abundance was estimated by the sum of free trichomes plus number of colonies $\times 200$, as the average of trichomes on each colony (Carpenter, 1983). Total abundance of *Trichodesmium* and other taxa were, then, divided by the 30-m of water column \times 0.03-m of mouth diameter of the plankton net, so the final abundance is shown as trichomes (*Trichodesmium* spp.) or cells (other microplankton, excluding *Trichodesmium* spp.) per liter.

Analysis of Toxins

Samples from 6 different stations were collected from visible aggregations of *Trichodesmium* during December-2014, at the same region where biological samples took place. Using a GF/F glass fiber filter of 0.7 μm pore size, the water was filtered until the filter was clogged (60–470 mL of sample), washed with distilled water and maintained at -80°C until chemical analysis. We analyzed two kinds of toxins using High Performance Liquid Chromatography (HPLC UV-DAD): Domoic Acid (DA) (Miguez et al., 1996; Costa et al., 2003)

TABLE 1 | Major parameters of water and weather conditions at the stations.

Stations	Lat (S)	Long (W)	Sampling dates	Local depth (m)	SST**	T (°C)	SSS***	Salinity	MLD* (m)	Surface watermass	Wind speed (knots)	Wind direction
#1	24°16.6'	43°22.2'	06/11/13	692	24.0	23.7	36.8	36.9	45	TW	6	SE
#2	25°07.6'	44°51.4'	06/08/13	190	23.6	24.6	36.2	37.3	17	TW	4.5	NO
#3	25°48.8'	45°04.2'	06/07/13	1287	23.2	22.9	35.8	35.8	40	TW	8	SE
#4	27°6.4'	46°24.7'	06/04/13	830	23.2	23.1	36.8	36.8	80	TW	4.6	SE
#5	28°6.6'	48°00.0'	05/20/14	138	21.8	22.9	36.0	36.7	14	TW	5	NW
#6	29°22.9'	47°08.8'	05/19/14	1876	22.8	22.8	36.8	36.8	35	TW	11.6	SW
#7	29°26.0'	48°24.8'	05/18/14	140	22.7	22.8	36.7	36.8	35	TW	10.4	SW
#8	30°45.6'	48°24'	05/17/14	1505	20.6	20.7	34.3	34.5	80	STSW	12.2	W
#9	31°39.3'	49°29.8'	05/15/14	1816	21.8	22.9	36.0	36.7	43	TW	15.7	NW
#10	31°56.5'	49°57.2'	05/14/14	680	20.6	20.7	34.3	34.5	18	STSW	4	SE
#11	32°12.9'	49°41.1'	05/14/14	1870	21.8	22.9	36.0	36.7	40	TW	5.8	SW
#12	32°18.2'	50°06.6'	05/14/14	806	21.8	22.9	36.0	36.7	40	TW	11	SW
#13	32°33.6'	50°16.9'	05/13/14	575	20.6	20.9	31.1	31.4	47	PPW	18	W
#14	32°55.3'	50°17.6'	05/11/14	676	20.8	22.3	35.0	36.1	15	TW	6	NW
#15	33°04.0'	50°05.0'	05/12/14	1166	23.5	23.5	36.9	36.9	20	TW	6.7	W
#16	33°40.4'	51°23.5'	05/10/14	127	20.6	20.8	31.1	31.3	18	PPW	24	SE

Temperature (°C) and salinity values are averaged for 30 m of water column. *Mixed Layer Depth, **Sea Surface Temperature, ***Sea Surface Salinity.

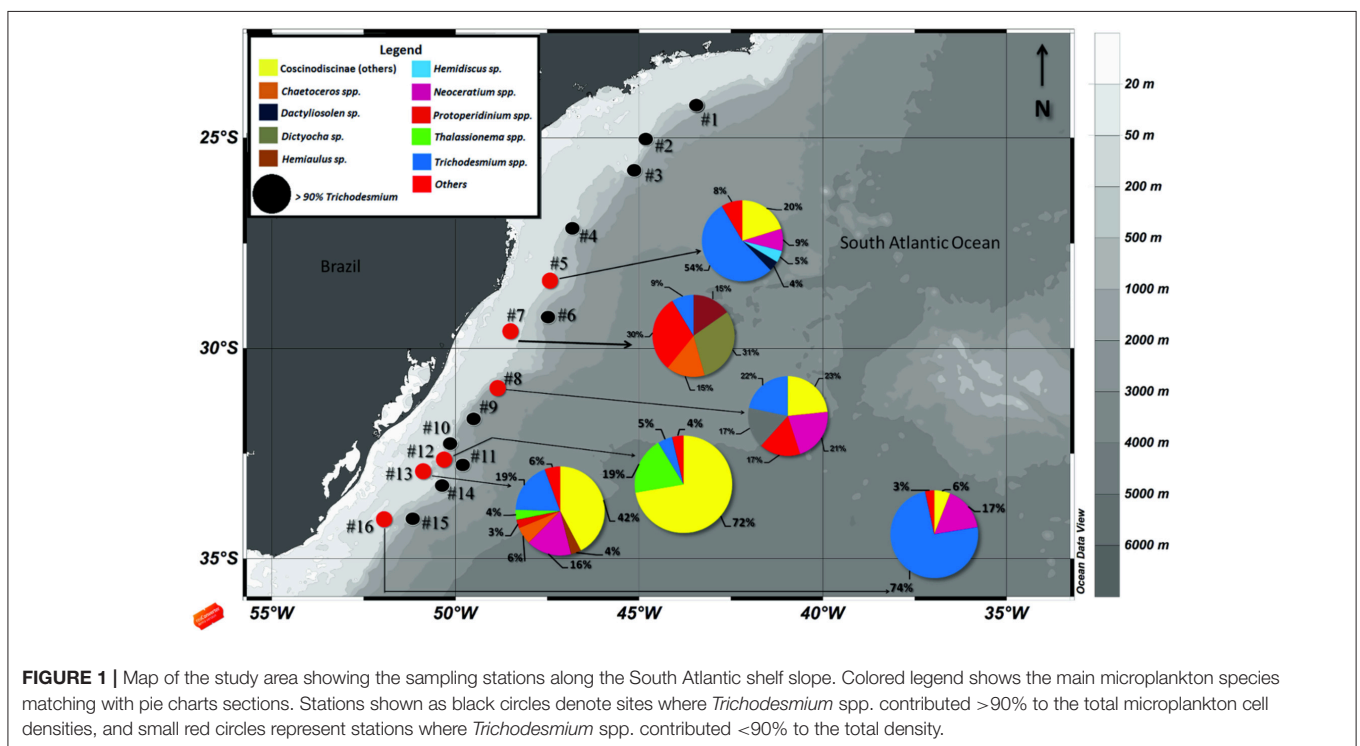


FIGURE 1 | Map of the study area showing the sampling stations along the South Atlantic shelf slope. Colored legend shows the main microplankton species matching with pie charts sections. Stations shown as black circles denote sites where *Trichodesmium* spp. contributed >90% to the total microplankton cell densities, and small red circles represent stations where *Trichodesmium* spp. contributed <90% to the total density.

and saxitoxins (STX), (Rourke et al., 2008). The collection of samples and analysis of STX were reported in detail (Detoni et al., 2016b), and the same samples were used for the analysis of domoic acid. The abundance of *Trichodesmium* was correlated with STX concentrations at the stations. We then calculated the STX:trichome ratio, and reported STX as equivalent saxitoxin concentrations (STX-eq) $\mu\text{g L}^{-1}$ that

is, the sum of all the variants present in the analyzed sample.

Statistics

A series of multivariate analyses were conducted in order to verify any spatial pattern in the species distribution, and to verify the relationship between environmental factors (i.e., temperature,

water masses, MLD) and the presence of the different taxonomic groups of microphytoplankton.

A non-metric multidimensional scaling (nMDS) was applied together with a dissimilarity matrix based on the non-metric Bray-Curtis index (Bray and Curtis, 1957) in order to group the taxa using an ordination diagram (Wickelmaier, 2003). An analysis of similarities (ANOSIM) was then applied in order to verify possible differences among the stations (Clarke, 1993). The analyses were carried out using the free software Past (v.1.81) (Hammer et al., 2008).

The Canonical Correspondence Analysis (CCA) was performed in order to identify patterns and variabilities of microplankton species with respect to environmental variables (TerBraak and Prentice, 1988). Biotic variables were represented by total abundances of *Trichodesmium* and other microplankton taxa. Environmental variables included: sea surface temperature; salinity; MLD; wind speed; latitude; and water mass. That latter variable was ascribed as follows: Tropical Water (TW) = 1, Subtropical Shelf Water (STSW) = 2, and Plata Plume Water (PPW) = 3, using latitude as a covariate. All variables were log-transformed before analysis in order to normalize the sets of variables. To test for the CCA significance, we run Monte-Carlo tests based on 499 permutations under a reduced model ($p < 0.05$). Three stations (#1, #15, and #16) were excluded from the final CCA analysis because they represented very extreme conditions in comparison to others.

RESULTS

Physical Conditions

Considering the physical conditions during the two fall cruises (Table 1), the Tropical Water (TW, Sal. ≥ 36 and Temp. $>22.3^\circ\text{C}$) mainly influenced the region. The Sub-Tropical Shelf Water (STSW, $33.5 < \text{Sal.} < 35.5$ and Temp. $\sim 20.7^\circ\text{C}$) was the dominant surface water mass at stations #8 and #10, while the Plata Plume Water (PPW, Sal < 33.5 and Temp $\leq 20.9^\circ\text{C}$) was noticeable at stations #13 and #16 (Möller et al., 2008). The wind direction varied among stations, but was dominated by Southerly or Westerly directions. Stations under the influence of relatively high wind speed >10 knots were always related to a non-dominant *Trichodesmium* spp. in the total abundance of trichomes (Figure 1; Table 1). MLD was also variable among stations, from 14 m down to 80 m of depth, and was not correlated to the local wind speed (Table 2).

Microplankton Identification and Spatial Distribution

Figure 1 compiles the map of the study area with the relative contribution of specific microplanktonic groups to the total abundance of organisms at each station. The results show that *Trichodesmium* spp. were the greatest contributors for both fall cruises, comprising more than $>90\%$ of total abundance in many stations. In a few southernmost stations, the diatoms from Coscinodiscineae group and *Thalassionema* spp., as well as the dinoflagellates *Neoceratium* spp. and *Protoperidinium* spp. co-occurred with the cyanobacterial trichomes at elevated cell numbers (Figure 1). Stations #2 and #3 had the most

elevated concentrations of trichomes at surface: 2.1×10^6 and 4.4×10^6 trichomes L^{-1} , respectively. These two stations and other four (#1, #4, #10, and #11) had trichome abundances $>8 \times 10^3$ trichomes L^{-1} . At these stations, the accumulation of *Trichodesmium* at surface was visible to the naked eye. In contrast, aggregations were not visible in the remaining stations, where abundances ranged from only ~ 4 trichomes L^{-1} to 2.5×10^3 trichomes L^{-1} (Table 2).

Our statistical analysis separated the stations where *Trichodesmium* was more abundant from those where their abundance was fairly comparable to the abundance of other organisms. The ordination diagram derived from the nMDS analysis defined the group A as composed by stations with $> 8,000$ trichomes L^{-1} and a group B with those with densities $<2,500$ trichomes L^{-1} (Figure 2). These two groups were significantly different (ANOSIM, $r^2 = 0.89$, $p < 0.0001$) and, interestingly, separate visible aggregations from those undetected to the naked eye.

In the stations where *Trichodesmium* spp. contributed $<90\%$ for the total abundance of organisms, the species composition varied between different groups of microplankton (Figure 1; Table 2). Stations #14 (18 taxa), #10 (17 taxa) #1, and #5 (13 taxa each) had a relatively high diversity and densities, while #6, #8, #12, and #15 had concentrations of <500 cells L^{-1} (Table 2). In considering the main microplanktonic organisms present in the area, the centric diatoms from the sub-order Coscinodiscineae, dinoflagellates *Neoceratium* spp. and silicoflagellates (Class Dictyochophyceae) were fairly common across the region and in relatively high abundance (Table 2). Interestingly, the harpacticoid copepod *Macrosetella gracilis* was present along most of the stations with eggs and larvae attached to the *Trichodesmium* trichomes (Figure 3a). In the Southernmost stations, an unidentified pteropod mollusk appeared in high abundances (data not shown). Symbioses between microplanktonic organisms and N_2 - fixers cyanobacteria were pretty common along the stations such as with Foraminifera members and the diatoms *Rhizosolenia* sp. and *Chaetoceros* sp. (Figures 3b–d).

The CCA analysis coupled with a Monte-Carlo test of the F-ratio associated the presence of microplankton groups correlated with environmental variables (Figure 4). Results indicated that five environmental variables (temperature, salinity, MLD, wind speed and water mass types) and the covariate latitude influenced significantly the spatial distribution of the different groups of microplankton ($p < 0.01$). In fact, environmental variability explained 44% of the community composition, and the first two significant canonical roots cumulatively explained 71.5% of the observed variance in taxa.

Taxonomy of *Trichodesmium* Based on Morphological Features

We identified 5 different species of *Trichodesmium* (Anagnostidis and Komárek, 1988; Komárek and Anagnostidis, 2005), which were mainly based on biometric parameters (length and width of cells within trichomes), as well as phenotypic features (presence/absence of calyptra and shape of apical cell). The

TABLE 2 | Abundance of microplankton groups (trichomes L⁻¹ for *Trichodesmium* spp. and cells L⁻¹ for others).

Taxa	Stations															
	#01	#02	#03	#04	#05	#06	#07	#08	#09	#10	#11	#12	#13	#14	#15	#16
CYANOBACTERIA																
<i>Trichodesmium</i> spp.	9 × 10 ⁵	4 × 10 ⁶	2 × 10 ⁶	1 × 10 ⁵	1180	263	4	13	936	8185	2 × 10 ⁴	26	1138	2500	461	1504
Other oscillatoriales	268	108	90	30						17						9
DIATOMS																
<i>Chaetoceros</i> spp.	670			36			7			22				834		
<i>Climacodium frauenfeldianum</i>					35			10		65		5		194		
Coscinodiscineae	804				444	29	14	14	21	117	10	381	26	5592	9	120
<i>Dactyliosolen</i> sp.					91					4				139	5	
<i>Hemiaulus</i> sp.	1116		45		18		7		11	104			13	500	5	
<i>Hemidiscus</i> sp.					104									46		
<i>Planktoniella sol</i>	45				5					4						
<i>Pleurosigma</i> sp.	42		23	6						9				19		
<i>Rhizosolenia</i> spp.	357		67	30	36					26		10		56		9
<i>Thalassionema</i> spp.										9		100	13	472		86
DINOFLLAGELLATES																
<i>Amphisoleniabidentata</i>	90			24												
<i>Ceratocoryshorrida</i>														9		
<i>Dinophysis</i> spp.														9		
<i>Neoceratium</i> spp.*	2411	54	402	269	194			13	11	294		30	10	2167	13	334
<i>Ornithocercus</i> spp.	268		67	6	9					16	65	5		65		9
<i>Protoperdinium</i> spp.	581	54	45	12										408		9
<i>Pyrophacus</i> sp.					22			5	11	43						
<i>Schuetiella mitra</i>																9
OTHERS																
Dictyochophyceae	45		23		9		14	5		22	5			83		
Ciliate										4				9		
Radiolarian					5					30	5			65		26
Foraminiferan	328		23	96	9					39				56		
Total abundance	9 × 10 ⁵	4 × 10 ⁶	2 × 10 ⁶	1 × 10 ⁵	2161	292	46	60	1006	9059	2 × 10 ⁴	557	1200	1 × 10 ⁴	493	2115

**N. concilians*, *N. contortum* var. *karstenii*, *N. extensum*, *N. fusus*, *N. horridum*, *N. pentagonum*, *N. praelongum*, *N. symmetricum*, *N. teres*, *N. trichoceros*, *N. vultur* var. *sumatranum*.

morphologically different forms generally co-occurred at the different stations; however, we did not differentiate the species composition for each station (Figures 5, 6).

T. clevei (J. Schmidt) (Anagnostidis and Komárek, 1988) was found as single trichomes or in colonies containing 20–30 trichomes, with cylindrical and slightly coiled cells. Most of the trichomes were blue-green, but were also found as dark reddish. The cell diameter ranged from 5.9 to 7 μm and were longer than wide, with apical cell rounded and without calyptra. Their occurrence was already registered in Gulf of Siam and Australia, but this is the first time this species is described in South Atlantic waters. *T. erythraeum* (Ehrenberg ex Gomont) was found as both free-floating or in colonies as purple-red trichomes, constricted at the cross-walls that were gradually attenuated at the ends. Cells were 6–11 μm long, with the apical cell containing a distinctive calyptra. The species is documented as forming dense aggregations in tropical and subtropical oceans, including the Atlantic, and was already described in the present region

(Rörig et al., 1998; Siqueira et al., 2006; Carvalho et al., 2008). *T. hildebrandtii* (Gomont) was easily distinguished among the other species because of the diameter ranging from 13 to 22 μm with significantly shorter cells. It was found as single trichomes or in colonies with a dark-reddish color, had attenuated cells at the ends and apical cells containing a calyptra. The species can be found in warm seas all over the world, but has not been previously described in South Atlantic Ocean. *T. radians* (Wille) (Golubié) had the most distinctive morphology, characterized by wavy trichomes that were either free or aggregated as star-shaped colonies (Figure 6). The cells were always blue-green with large gas vesicles and a cell diameter range of 6–9 μm, longer than wide. Apical cells were rounded and without calyptra. This species is largely distributed among the oceans, and this is the first report for the South Atlantic waters. *T. thiebautii* (Gomont ex Gomont) was found as single trichomes or colonies that were partly rope-like contorted or radially-arranged. Cells were nearly isodiametric, not constricted at the cell walls, with rounded

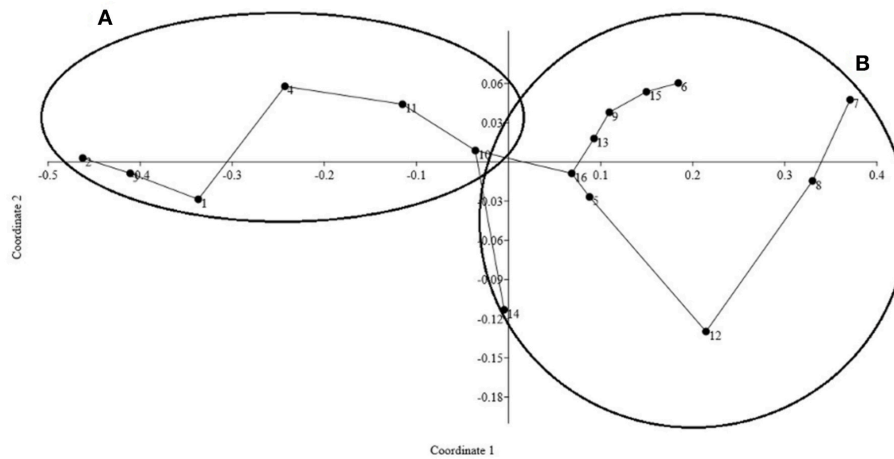


FIGURE 2 | nMDS ordination diagram dividing the stations into two distinctive groups according to *Trichodesmium* abundance of trichomes per liter. Group **(A)** where the stations presented $> 8,000$ trichomes L^{-1} and Group **(B)** contains stations with $< 2,500$ trichomes L^{-1} . The ANOSIM test revealed statistical differences between the two groups: $p < 0.01$, $R^2 = 0.58$ and Stress = 0.04 (< 0.5).

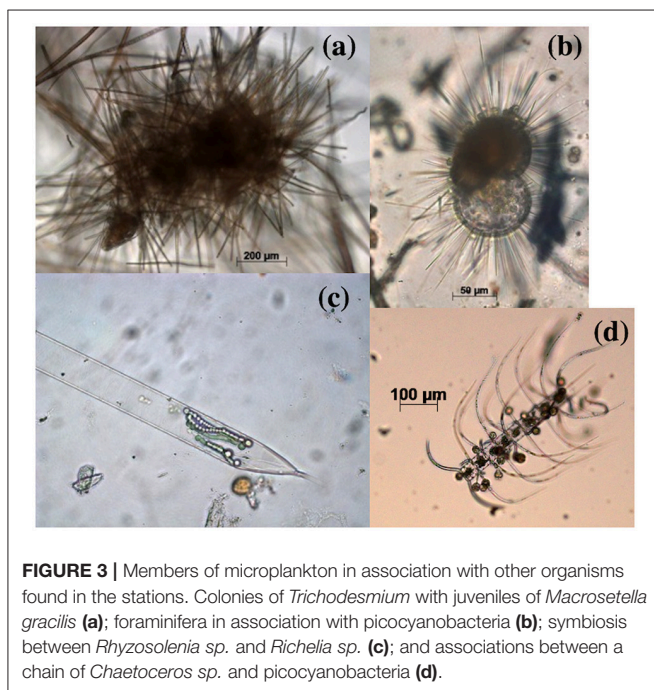


FIGURE 3 | Members of microplankton in association with other organisms found in the stations. Colonies of *Trichodesmium* with juveniles of *Macrosetella gracilis* **(a)**; foraminifera in association with picocyanobacteria **(b)**; symbiosis between *Rhyzosolenia* sp. and *Richelia* sp. **(c)**; and associations between a chain of *Chaetoceros* sp. and picocyanobacteria **(d)**.

apical cells. This species has a wide distribution range and was previously found offshore the Brazilian coast (Björnberg, 1965).

Toxicity of *Trichodesmium*

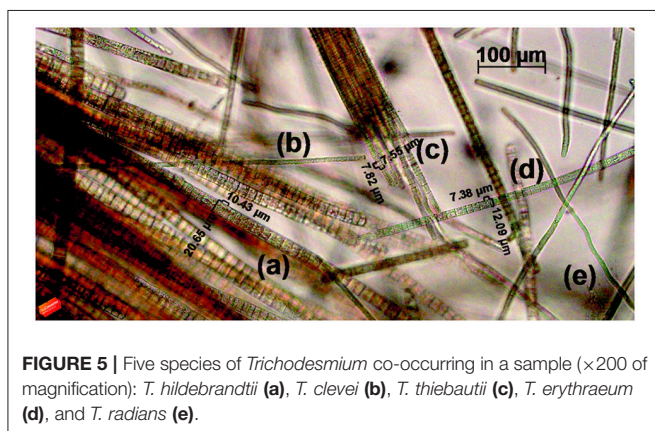
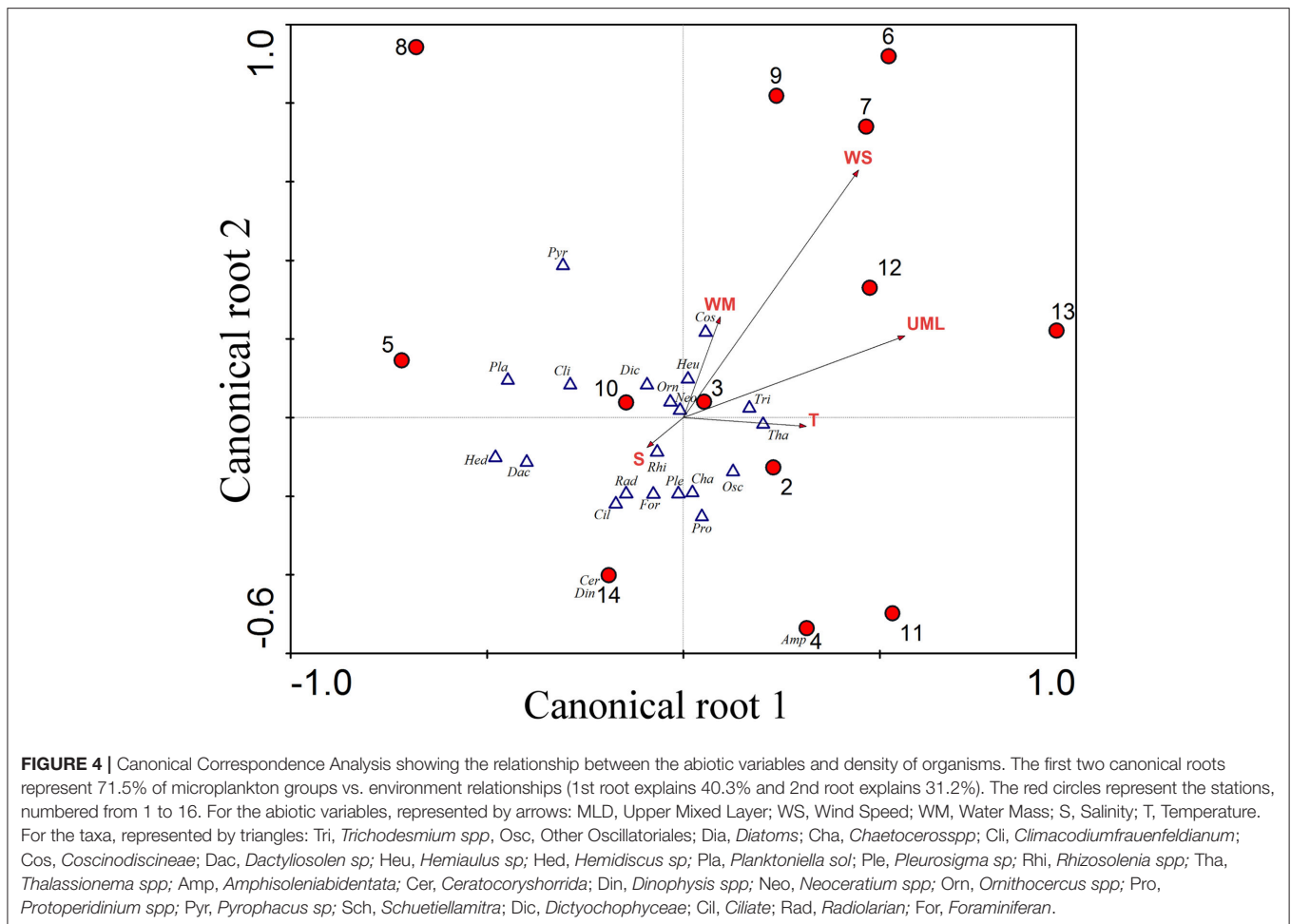
Our toxin analysis were negative for domoic acid and positive for STX concentrations in all samples analyzed (Figure 7). There was an inverse association between density of trichomes and STX concentrations. At the same time, *Trichodesmium* was dominant in terms of abundance for these stations (Figure 1). In relation to STX concentration, concentrations ranged from 0.45 to ~ 4

(STX-eq) $\mu g L^{-1}$, with the qualified and quantified variants as gonyautoxins (GTX), specifically GTX-4 and GTX-2. The GTX-4 was the main variant contributing for total STX (STX-eq) toxicity.

DISCUSSION

The aggregations of *Trichodesmium* were observed during clear skies, calm seas and when wind speed was relatively low < 10 knots; such conditions were reported elsewhere as suitable for trichome aggregations (Villareal and Carpenter, 2003; LaRoche and Breithbarth, 2005; Agawin et al., 2013), and this relationship is confirmed by statistical analysis (Figure 4). A noticeable feature observed at station 2 is that the aggregation was accompanied by floating objects such as feathers, wood and plastic. This suggests the objects and organisms were concentrating at the convergence region of a Langmuir cells, in agreement with previous observations elsewhere (Evans and Taylor, 1980). A shallow MLD of 17 m depth kept the trichome aggregations at surface, facilitated by gas vesicles inside the cells that give a positive buoyance to the trichomes (Capone et al., 1997; Villareal and Carpenter, 2003). Moreover, Since the genus is well-known to have very low growth rates $\sim 0.14 d^{-1}$ (LaRoche and Breithbarth, 2005), it was unlikely that trichomes formed blooms, such as harmful algal blooms (HABs).

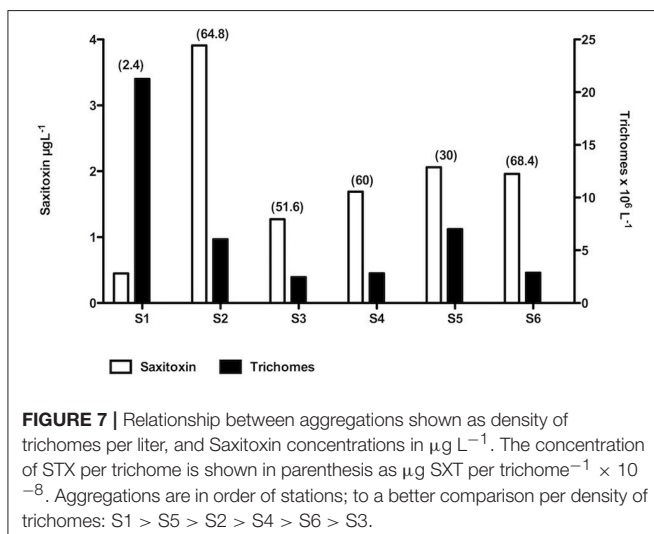
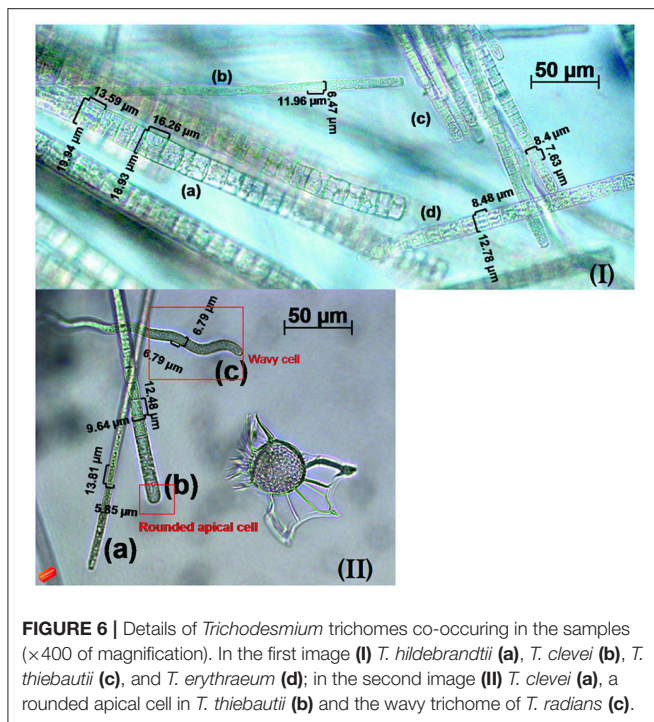
Intermediate densities of *Trichodesmium* $\sim 8 \times 10^3$ trichomes L^{-1} (some stations from group B, Figure 2) could represent a transient condition before the mixed layer depth (MLD) stabilization and associated with wind speeds < 8 knots, suitable for *Trichodesmium* aggregation and colony formation. On the other hand, the deepening of MLD might dilute *Trichodesmium* aggregations; in this case, our vertical net hauls of 30 m deep would not account for these organisms. The importance of MLD on the community structure was confirmed in the CCA's ordination diagram (Figure 4).



In stations where *Trichodesmium* was present in high densities, we found low densities of microphytoplankton co-occurring (Table 2; Figure 1). Inorganic nutrient concentrations through our study area, however, were present at high concentrations (Bif and Yunes, 2017) and do not support such low densities of microphytoplankton. From these organisms, typical representatives of open oceans were found at the stations,

such as the dinoflagellates *Neoceratium* spp., *Dinophysis* spp. and *Ornithocercus* spp., and the heterotroph *Protoperidinium* spp. These genera have been classified as the major consumers of phytoplankton biomass, where *Protoperidinium* spp. is especially important in open oceans (Sherr and Sherr, 2007). Two genera were found in symbiosis with picocyanobacteria, as the case of *Ornithocercus* spp. and *Rhizosolenia* spp.; such associations were previously seen in other oceans (Madhu et al., 2013). The co-occurrence of *T. hildebrandtii* with other microplankton organisms was already registered in the southern Brazilian coast (Guimarães and Rörig, 1997), but were never detailed.

The presence of STX, thus, is another relevant factor that should possibly control the distribution of microplankton groups across stations. Based on the relationship between trichome densities and toxin production, we found STX (STX-eq) associated with the presence of *Trichodesmium* (Figure 7). Higher STX (STX-eq) concentrations were correlated with lower trichomes per liter; thus, we suggest that *Trichodesmium* produces the toxin during the early stage of trichome aggregation. STX are allelochemical compounds produced as secondary metabolites which are not required for metabolism (i.e., growth, development and reproduction), but are considered an important defense against herbivory (Fraenkel, 1959; Stamp, 2003). Our work suggests that STX could be play a central



role during initial aggregations of *Trichodesmium* in order to minimize bottom-up (i.e., competition for nutrients) and top-down (i.e., herbivory) controls (Smayda, 1997). This is corroborated by the fact that other organisms were less abundant in the presence of *Trichodesmium*, but were dominant in regions where trichomes were not accumulating or forming colonies (Figure 1). Although we identified other potential toxin producers (i.e., STX and domoic acid) in the stations such as *Gonyaulax spinifera*, *Protoceratium reticulatum*, *Chaetoceros* spp., and *Pseudo-nitzschia seriata* (Table 3), they were not abundant enough to be responsible for the high toxin concentrations.

Despite the solely morphological/traditional identification of *Trichodesmium* performed in our work, previous studies already linked the traditional taxonomy with molecular approaches and found high agreement between techniques. Some approaches include 16S rRNA gene sequencing (Komárek, 2010), and genetic and biochemical informations that add to the development of a functional classification scheme (Hynes et al., 2012). A detailed description of *Trichodesmium* spp. species was never assessed for the Southwest Atlantic Ocean (Silva et al., 2008 and references therein), so the identification of three new species for the region (*T. clevei*, *T. hildebrandtii*, and *T. radians*) adds to the previous reports of *T. erythraeum* (e.g., Barbosa, 1944; Gíanesella-Galvão, 1995; Rörig et al., 1998) and *T. thiebautii* (Björnberg, 1965). Nevertheless, we suggest that further local studies based on the polyphasic approach (i.e., using morphological, ultra-structural, biochemical and genetic features) are still necessary (Komárek et al., 2014).

From the five species of *Trichodesmium* found in the region, *T. erythraeum* and *T. thiebautii* are already known to be toxic to the environment. In ecological tests performed with both species collected and isolated from Caribbean waters, *T. thiebautii* was highly toxic for *Artemia salina* and copepods, and was neurotoxic to mice and some non-grazing copepods (Hawser and Codd, 1992; Hawser et al., 1992). *T. erythraeum* toxicity was assessed in populations from the Northeast of Brazil through HPLC, Elisa and ecotoxicological tests, and was positive for STX and microcystins (Proença et al., 2009). In this same region, the species was found in co-occurrence with toxic dinoflagellates; but analogous to our study, the organisms were present in small abundances. The toxigenic potential of *T. erythraeum* was recently validated through brine shrimp toxicity assay, showing 100% of mortality after 48 h of incubations using aggregations from the Gulf of Mannar (Shunmugam et al., 2017). The study also demonstrated that *T. erythraeum* is able to synthesize other potent multi-class neurotoxins such as anatoxin-a and gonyautoxin, and microcystins. The toxicity of other *Trichodesmium* species, however, requires further investigation, and the Southwest Atlantic seems to be a good candidate to perform similar studies, given the diversity of *Trichodesmium* species and high abundance along the shelf break.

SUMMARY

The Southwest Atlantic Ocean shows suitable conditions for growth and colony formation of *Trichodesmium* spp. Five species of *Trichodesmium* (*T. clevei*, *T. erythraeum*, *T. hildebrandtii*, *T. radians*, and *T. thiebautii*) were identified, and three out five reported for the first time at the region. During non-*Trichodesmium*-dominated assemblages in the water surface, dinoflagellates and relatively large diatoms were the dominant groups. Our results on microplankton densities in the stations combined with toxin analysis revealed that STX was likely produced by *Trichodesmium* aggregations. Higher concentrations of toxins correlated with moderate numbers of trichomes per liter; lower concentrations were

TABLE 3 | Checklist of organisms found with *Trichodesmium* aggregations.

Taxa	Patch 2, 0 m #45	Patch 2, 15 m #46	Patch2, 0 m #24	Patch (net sample 6)
CYANOBACTERIA				
<i>Trichodesmium</i> spp. (abundance of trichomes and tufts)				
High	X		X	
Low		X		X
COCCOLITHOPHORES				
<i>Umbilicosphaera sibogae</i>				X
DIATOMS				
<i>Bacteriastrum delicatulum</i>				X
<i>Bacteriastrum furcatum</i>				X
<i>Chaetoceros aequatorialis</i>				X
<i>Chaetoceros coarctatus</i>				X
<i>Chaetoceros compressus</i>				X
<i>Chaetoceros dadayi</i>				X
<i>Chaetoceros messanensis</i>				X
<i>Chaetoceros peruvianus</i>				X
<i>Chaetoceros tetrastichon</i>				X
<i>Climacodinium frauenfeldianum</i>				X
<i>Corethron pennatum</i>				X
<i>Coscinodiscus</i> sp.	X			
<i>Cylindrotheca closterium</i>	X			
<i>Dactyliosolen</i> spp.				X
<i>Hemiaulus membranaceus</i>	X			X
<i>Hemiaulus sinensis</i>				X
<i>Leptocylindrus mediterraneus</i>				X
<i>Lioloma</i> sp.				X
Naviculaceae <20 μm		X		
<i>Nitzschia longissima</i>			X	
<i>Pleurosigma/ Gyrosigma</i>				X
<i>Proboscia alata</i>	X	X	X	X
<i>Pseudoguinaridia recta</i>				X
<i>Pseudo-nitzschia complex seriata</i> (t.a. >5 μm)				X
<i>Rhizosolenia styliformis</i>		X		
<i>Rhizosolenia</i> sp.	X	X	X	
<i>Thalassionema bacillare</i>				X
<i>Thalassionema frauenfeldii</i>		X		X
<i>Thalassiosira</i> sp.				X

(Continued)

TABLE 3 | Continued

Taxa	Patch 2, 0 m #45	Patch 2, 15 m #46	Patch2, 0 m #24	Patch (net sample 6)
DINOFLAGELLATES				
<i>Gonyaulax spinifera</i>				X
Gymnodiniales <20 μm		X		
<i>Oxytoxum</i> sp.	X			
<i>Prorocentrum compressum</i>		X		
<i>Protoperidinium conicum</i> *				X
<i>Protoperidinium curtipes</i> *				X
<i>Protoperidinium divergens</i> *				X
<i>Protoperidinium leonis</i> *				X
<i>Protoperidinium oblongum</i> *				X
<i>Protoperidinium movum</i> *		X	X	X
<i>Protoperidinium pellucidum</i> *				X
<i>Protoperidinium</i> sp.*	X			
<i>Protoceratium reticulatum</i>	X			X
<i>Ptychodiscus noctiluca</i>				X
<i>Pyrophacus</i> sp.	X		X	X
<i>Tripos concilians</i>				X
<i>Tripos furca</i>				X
<i>Tripos fusus</i>				X
<i>Tripos horridus</i>				X
<i>Tripos muelleri</i>				X
<i>Tripos teres</i>				X
<i>Tripos trichoceros</i>				X
Cyst	X		X	
Association/symbioses				
<i>Rhizomonas setigera</i> * + <i>Leptocylindrus</i> sp.			X	
Foraminifera*				X
CILIOPHORA*				
<i>Amphorellasp.</i>				X
<i>Climacocylis</i> cf. <i>scalaria</i>				X
<i>Dadaiella ganymedes</i>				X
<i>Eutintinnus</i> sp.				X
<i>Favella</i> sp.				X
<i>Salpingella</i> spp.				X
<i>Tintinnopsis</i> sp.				X
<i>Undella</i> sp.				X
<i>Xystonella treforti</i>				X
<i>Xystonellopsis</i> spp.				X
<i>Zoothamnium</i> sp.				X
Harpacticoida*				X
Nauplius and copepodites of <i>Macrosetella gracilis</i> *	X		X	X

*Heterotrophic species.

present during high densities of the genus. The lack of microplankton diversity during *Trichodesmium* dominance might be associated with the toxin production, which seems to be especially important during the early aggregation phase at the surface.

AUTHOR CONTRIBUTIONS

MB developed the research, collected, and analyzed the biological and wrote the article as a result of her Master's Thesis. MdS helped with plankton identification and calculations. LC analyzed the toxins. JY helped with data interpretation. All authors revised and made contributions to methodology, results and discussion in this manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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