

## Research Article

**Cite this article:** Rekik A, Pagano M, Ayadi H, Guerrazi W, Elloumi J (2023). Spatial and seasonal changes in microphytoplankton and ciliate communities in a stressed area of the southeastern Mediterranean coast (Tunisia). *Journal of the Marine Biological Association of the United Kingdom* **103**, e71, 1–15. <https://doi.org/10.1017/S0025315423000462>

Received: 27 January 2023

Revised: 14 June 2023

Accepted: 16 June 2023

### Keywords:


Ciliates; microphytoplankton; physico-chemical parameters; seasonal distribution; southern coast of Sfax

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# Spatial and seasonal changes in microphytoplankton and ciliate communities in a stressed area of the southeastern Mediterranean coast (Tunisia)

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## Abstract

The spatial and seasonal variability of the microphytoplankton and ciliates communities in relation to the environmental factors were studied in the southern coastal area of Sfax. Results revealed a striking difference between seasons regarding pH, with strong acidification in autumn generated by industrial activity. Spatial distribution of pH in autumn impacted the microorganisms in different ways: acidic stations to the south showed significant correlations with Cyanobacteria, dinoflagellates and loricate ciliates whereas higher pH values in spring (pH > 8) were linked to diatoms richness. The high availability of inorganic phosphate is associated with the high release of phosphate due to residue from a phosphate treatment manufacture along the coast; consequently, N/P ratios were low (1.34–13.43) suggesting nitrogen limitation. Microphytoplankton abundance shifted from dinoflagellates dominance in autumn to dominance of diatoms during winter and of Euglenophyceae in summer. Loricate ciliates accounted for the largest proportion of the ciliates community while aloricate ciliates were relatively scarce during all seasons. Variability of ciliate community appeared not directly linked to environmental conditions, but significant positive relationships between abundance of loricate ciliates and microphytoplankton suggest that these ciliates may feed on microphytoplankton.

## Introduction

Due to their abundance and vital roles, microphytoplankton communities are fundamental to the functioning and evolution of marine ecosystems. They are the primary producers in the pelagic marine food web, representing the main pathway for transferring matter and energy to the higher trophic levels (Ben Salem *et al.*, 2015). Hence the diversity and fluctuations of microphytoplankton can affect the food web and the ecological functions and thus explicit knowledge of the structure of this component is major for identifying trophic regimes and investigating the general functioning of the marine ecosystems (Lagarria *et al.*, 2016). Moreover, the analysis of the composition, abundance and changes in the frequency of microphytoplankton are informative of the actual and future changes in water quality (Belén Sathicq *et al.*, 2016). Simultaneously of the most important components of plankton, ciliates are trophic link between the traditional food chain and microbial food web (Elloumi *et al.*, 2015). Marine planktonic ciliates are a major, ubiquitous and varied group of protozooplankton (Ying *et al.*, 2013). Their dynamics are closely related to variations in environmental parameters (Küppers and Claps, 2012), particularly in coastal ecosystems due to the combination of marine and land influences, making ciliates useful as indicators of ecosystem health status (Kchaou *et al.*, 2009; Elloumi *et al.*, 2015). With their fast growth, ciliates react more rapidly to environmental variations than most other microorganisms (Gong *et al.*, 2005).

Several studies have been undertaken in the southern coast of Sfax regarding the spatial distribution of plankton assemblages (Rekik *et al.*, 2013a, 2015a, 2016a, 2016b; Ben Salem *et al.*, 2015, 2016; Drira *et al.*, 2016) to compare the spatial and seasonal distribution of dinoflagellates and diatoms along Sfax northern and southern coasts (Rekik *et al.*, 2017a, 2017b). The present study is the first examining the distribution of microphytoplankton and ciliates assemblage through sampling these communities simultaneously at high spatial resolution sampling in the shallow coastal waters south of Sfax during four seasons. It is therefore of interest to assess to the high impact of human pressure, chiefly by phosphogypsum, on plankton assemblages in a stressed ecosystem (Rekik *et al.*, 2015a). Our objectives are (1) to study the spatial and seasonal distribution of ciliates in relation to microphytoplankton, that constitute one of their potential prey, in the shallow coastal waters of Sfax, (2) to determine their potential relationship with environmental factors by using statistical analyses and (3) to determine marine water quality based on biological parameters as a bioindicator.



## Material and methods

### Study site

The southern coast of Sfax, the second largest city in Tunisia (Figure 1) is marked by salt extraction ponds from solar salter located over an area of about 1500 ha (COTUSAL) (Kobbi-Rebai *et al.*, 2013). In addition, phosphogypsum, the residue of phosphate treatment, has been stored along the coastline at an uncontrolled dumpsite from the manufacture which produces phosphoric acid (SIAPE) (Rekik *et al.*, 2012). This coast is subject to degradation of water quality (Drira *et al.*, 2016), increasing eutrophication (Kobbi-Rebai *et al.*, 2013), green tides caused by coastal *Ulva rigida* replacing the *Posidonia oceanica* seagrass beds (Ben Brahim *et al.*, 2013) and thus degrading benthic habitats (Turki *et al.*, 2006). It also suffered over the last two decades from an important decrease in fish resources that might have resulted from industrial and urban activities, menacing Tunisia's socio-economic resources (Abdennadher *et al.*, 2012). Many studies have reported the high level of atmospheric pollution (Azri *et al.*, 2010), marine pollution such as hydrocarbon (Zaghden *et al.*, 2014), and heavy metal contamination (Serbaji *et al.*, 2012; Naifar *et al.*, 2018).

### Field sampling

Samples for nutrients, microphytoplankton and ciliates were taken during four one-day campaigns in winter (16 February), spring (22 May), autumn (11 October), and summer (15 July) 2011 along the southern coast of Sfax. During each campaign, water samples were collected in 20 stations, divided in to five transects from coast to open water (Figure 1). The stations were located at different depths due to different distances off the coast: S1, S5, S9, S13, and S17 with depth < 0.5 m; S2, S6, S10, S14, and S18 with depth varying between 0.5 and 3 m; S3, S7, S11, S15, and S19 with depth varying between 3 and 5 m; S4,

S8, S12, S16, and S20 with depth > 5 m. A total of 80 samples were collected with a Van Dorn-type closing bottle that was deployed horizontally and at a depth ranging from 0.5 to 7 m. Nutrient samples (120 ml) were kept immediately upon collection at  $-20^{\circ}\text{C}$  in the dark. Samples for microphytoplankton were preserved with acid Lugol solution (at 3%; Parsons *et al.*, 1984) and alkaline Lugol solution was used for fixation of ciliate samples (at 5%; Sherr and Sherr, 1993). Samples for microphytoplankton and ciliates were placed at  $4^{\circ}\text{C}$  in the dark for enumeration. Water samples for Chlorophyll-*a* (1 l) and suspended matter (0.5 l) analyses were filtered by vacuum filtration onto Whatman GF/F and Whatman GF/C glass fibre filters, respectively, which were then immediately stored at  $-20^{\circ}\text{C}$ .

### Physico-chemical variables

Physical parameters (temperature, salinity, and pH) were measured using a multi-parameter kit (Multi 340 i/SET) immediately after sampling. Subsamples for the nutrients (nitrite, nitrate, ammonium, orthophosphate, silicate, total nitrogen, and total phosphate) were collected in plastic containers of 4.5 ml previously washed with distilled water. They were analysed with a Bran and Luebbe type 3 autoanalyzer and concentrations were determined colourimetrically using a UV-visible (6400/6405) spectrophotometer (Grasshof, 1983). Analyses were independent. The automatic analysis system provides fast and accurate analysis of these nutrients. Although each nutrient is determined in a different way, but the method remains similar. It is used colourimetry to determine the dosage of each nutrient. Percentages of dissolved inorganic nitrogen were calculated from  $[(\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+)/\text{T-N}] \times 100$ . Percentages of dissolved inorganic phosphate were calculated from  $[\text{PO}_4^{3-}/\text{T-P}] \times 100$ . Suspended matter concentrations were measured using the dry weight of the residue after filtration of 0.5 l of seawater onto Whatman GF/C membrane filters and drying at  $60^{\circ}\text{C}$  during 24 h.

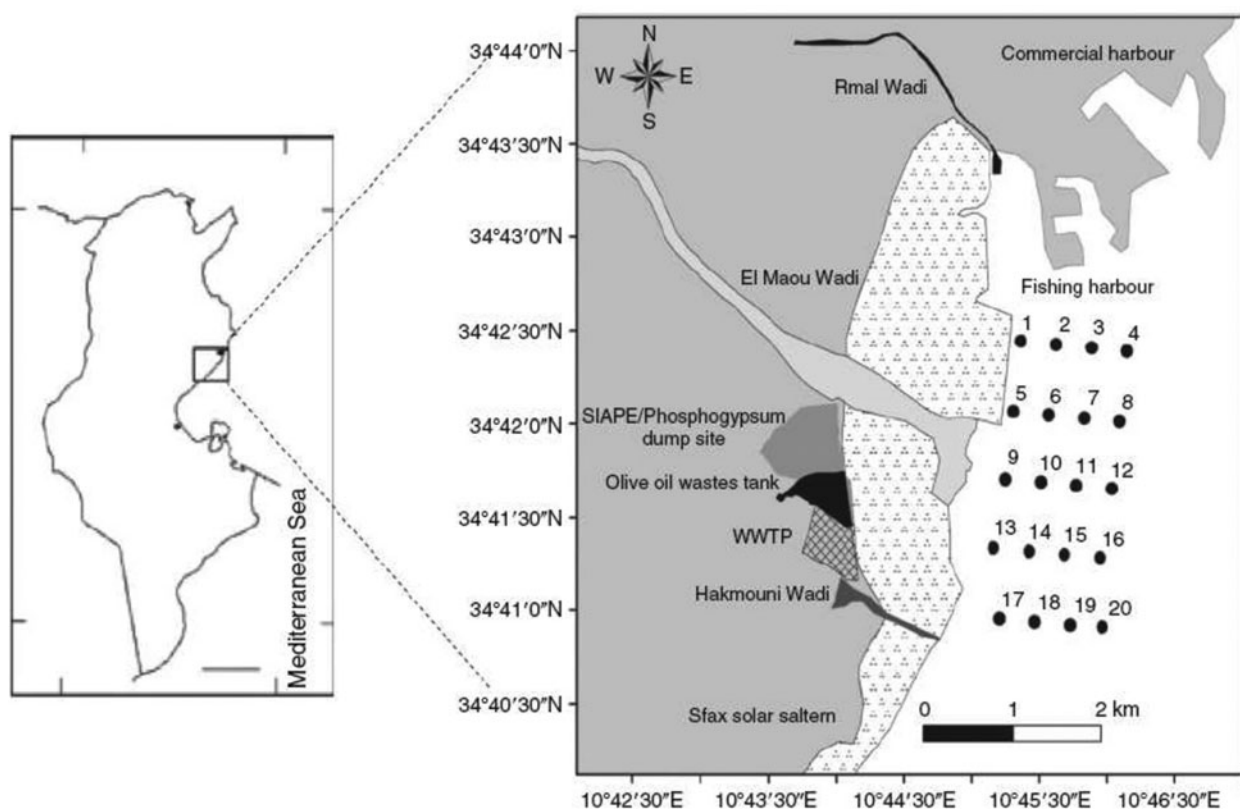


Figure 1. Location of sampling stations (1–20) in the south coast of Sfax.

### Ciliates and microphytoplankton enumeration

Sub-samples (50 ml) for microphytoplankton and ciliates counting to estimate the abundance were analysed under an inverted microscope (Leica) using the Utermöhl method (1958) after 24 h settling. Microphytoplankton and ciliates species counts were carried out on the entire sedimentation chamber with 40× magnified. Identification of microphytoplankton species was made according to various keys (Balech, 1959; Tomas *et al.*, 1996). Ciliates were identified to genus or species level after the works of Alder (1999), Petz (1999) and Strüder-Kypke and Montagnes (2002). The importance value for the different species was determined by their relative frequency.

### Chlorophyll-a

Chlorophyll-a was estimated by spectrometry, after extraction of the pigments in acetone (90%). The concentrations were then estimated using the equations of SCOR-UNESCO (SCOR-UNESCO, 1966). This method consists of filtering 1 l of sea water by vacuum filtration onto Whatman GF/F glass fibre filters, without exceeding 400 mmHg to prevent cell breakdown. A pinch of carbonate magnesium is added to avoid the degradation of pigments in pheopigments at the end of filtration. Filters are kept in aluminium paper and are dried under vacuum on silica gel during 24 h

and were then conserved at  $-20^{\circ}\text{C}$  until the time of extraction. The pigments extraction is carried out in 90% acetone in the dark and cold for 5 h. After 10 min of centrifugation at 3500g, the absorbance is measured using a Jenway spectrophotometer at 630, 645 and 663 nm.

### Data analyses

Means and standard deviations (SD) were reported when appropriate. The potential relationships between variables were tested with Pearson's coefficient correlation. One-way ANOVA followed by a post hoc comparison using Tukey's test was applied to identify significant differences between seasons.

The variations of phytoplankton and ciliate communities were investigated using multivariate analysis, specifically Nonmetric Multidimensional Scaling (NMDS). The mean percentage abundance of the taxa per transect and per seasonal period were square root transformed before estimation of resemblance using the Bray Curtis metric. The similarity matrix was then ordinated using NMDS. A SIMPER (percentage of similarity) analysis was performed to identify the species contributing most to similarity within and dissimilarity between clusters.

The physico-chemical and biological parameters assessed at 20 stations during four seasons were submitted to a normalized principal component analysis (PCA) (Dolédéc and Chessel, 1989).

**Table 1.** Seasonal variation of physical-chemical and biological parameters in the south coast of Sfax (Mean  $\pm$  SD;  $n = 20$ )

Variables	Autumn	Winter	Spring	Summer	F values	P values
Physical variables						
Temperature ( $^{\circ}\text{C}$ )	21.71 $\pm$ 0.49	15.55 $\pm$ 0.87	26.97 $\pm$ 3.25	31.75 $\pm$ 0.85	316.45	7.73 $\times 10^{-43}$ ***
Salinity (p.s.u.)	39.43 $\pm$ 0.47	36.50 $\pm$ 1.98	38.25 $\pm$ 1.40	37.05 $\pm$ 0.95	19.44	1.88 $\times 10^{-9}$ ***
pH	7.17 $\pm$ 0.08	7.80 $\pm$ 0.10	8.13 $\pm$ 0.29	7.91 $\pm$ 0.19	97.14	6.20 $\times 10^{-26}$ ***
Suspended matter ( $\text{mg l}^{-1}$ )	30.08 $\pm$ 3.38	38.60 $\pm$ 11.09	49.47 $\pm$ 11.86	34.58 $\pm$ 23.14	6.78	0.00***
Chemical variables						
$\text{NO}_3^-$ ( $\mu\text{M}$ )	10.39 $\pm$ 7.87	7.77 $\pm$ 2.77	7.37 $\pm$ 2.86	3.35 $\pm$ 2.34	8.12	9.28 $\times 10^{-5}$ ***
$\text{NO}_2^-$ ( $\mu\text{M}$ )	3.58 $\pm$ 2.40	1.54 $\pm$ 1.59	0.26 $\pm$ 0.09	0.31 $\pm$ 0.33	22.98	1.08 $\times 10^{-10}$ ***
$\text{NH}_4^+$ ( $\mu\text{M}$ )	6.43 $\pm$ 6.12	6.10 $\pm$ 1.55	4.53 $\pm$ 2.08	3.74 $\pm$ 5.10	1.85	0.14
T-N ( $\mu\text{M}$ )	34.14 $\pm$ 13.51	23.05 $\pm$ 5.28	21.12 $\pm$ 4.55	18.18 $\pm$ 9.62	12.01	1.61 $\times 10^{-6}$ ***
$\text{PO}_4^{3-}$ ( $\mu\text{M}$ )	3.42 $\pm$ 1.10	2.51 $\pm$ 1.09	11.35 $\pm$ 4.75	3.45 $\pm$ 3.13	39.31	1.92 $\times 10^{-15}$ ***
T-P ( $\mu\text{M}$ )	17.02 $\pm$ 4.62	10.61 $\pm$ 3.16	29.39 $\pm$ 13.37	13.53 $\pm$ 9.79	17.84	7.35 $\times 10^{-9}$ ***
N/P ratio	6.24 $\pm$ 3.23	13.43 $\pm$ 24.72	1.34 $\pm$ 0.80	3.00 $\pm$ 2.05	3.66	0.01*
Si (OH) <sub>4</sub> ( $\mu\text{M}$ )	24.69 $\pm$ 21.62	5.90 $\pm$ 3.14	30.92 $\pm$ 12.00	23.58 $\pm$ 21.98	8.37	7.10 $\times 10^{-5}$ ***
Biological variables						
Chlorophyll a ( $\text{mg l}^{-1}$ )	11.08 $\pm$ 11.67	0.42 $\pm$ 0.87	6.95 $\pm$ 4.27	0.58 $\pm$ 1.66	13.68	3.19 $\times 10^{-7}$ ***
Total microphytoplankton ( $\times 10^2$ cells $\text{l}^{-1}$ )	44.10 $\pm$ 41.92	32.75 $\pm$ 23.56	50.85 $\pm$ 48.45	84.10 $\pm$ 57.91	4.86	0.00**
Bacillariophyceae ( $\times 10^2$ cells $\text{l}^{-1}$ )	16.25 $\pm$ 22.23	25.35 $\pm$ 22.27	26.05 $\pm$ 26.86	21.45 $\pm$ 14.99	0.84	0.48
Dinophyceae ( $\times 10^2$ cells $\text{l}^{-1}$ )	21.60 $\pm$ 26.86	5.95 $\pm$ 8.00	16.45 $\pm$ 33.28	24.40 $\pm$ 32.17	1.81	0.15
Cyanobacteria ( $\times 10^2$ cells $\text{l}^{-1}$ )	1.27 $\pm$ 1.14	0.90 $\pm$ 1.74	3.03 $\pm$ 6.34	0.65 $\pm$ 1.22	1.99	0.12
Euglenophyceae ( $\times 10^2$ cells $\text{l}^{-1}$ )	2.05 $\pm$ 3.50	0.55 $\pm$ 1.31	2.30 $\pm$ 4.29	37.60 $\pm$ 27.31	33.29	7.61 $\times 10^{-14}$ ***
Dictyochophyceae ( $\times 10^2$ cells $\text{l}^{-1}$ )	0.05 $\pm$ 0.22	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.00	0.40
Chlorophyceae ( $\times 10^2$ cells $\text{l}^{-1}$ )	0.35 $\pm$ 1.34	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.35	0.27
Total ciliates ( $\times 10^2$ cells $\text{l}^{-1}$ )	7.15 $\pm$ 4.39	9.70 $\pm$ 4.99	4.15 $\pm$ 3.51	11.00 $\pm$ 8.89	5.39	0.00**
Loricated ciliates ( $\times 10^2$ cells $\text{l}^{-1}$ )	5.35 $\pm$ 4.31	7.30 $\pm$ 4.05	3.05 $\pm$ 3.54	9.00 $\pm$ 9.15	3.99	0.01*
Naked ciliates ( $\times 10^2$ cells $\text{l}^{-1}$ )	1.80 $\pm$ 2.06	2.40 $\pm$ 2.13	1.10 $\pm$ 1.55	2.00 $\pm$ 2.38	1.39	0.25

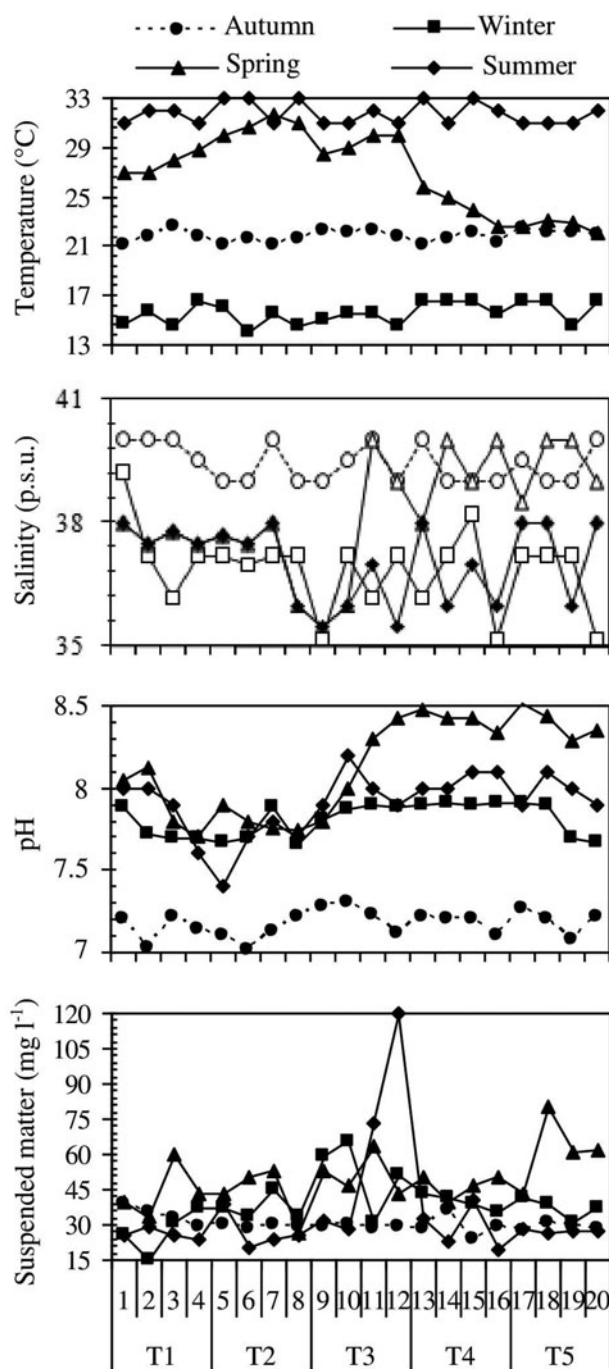
In the last column, results of one-way ANOVA analysis. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , show significant differences among sampled levels.

Simple log ( $x + 1$ ) transformation was applied to data in order to correctly stabilize variance (Frontier, 1973). These statistical analyses were performed using Primer 7 software.

## Results

### Hydrological features

The mean values of physical variables recorded at the 20 sampled stations are summarized in Table 1. Temperature varied among stations and seasons (Figure 2 and Table 1). The temperature was in the range 14–33°C, the lowest values being observed at stations 6 in winter and the highest at stations 5, 6, 8, and 13 in summer. At each station, temperature exhibited increasing values from winter to summer and a slight decline in spring compared



**Figure 2.** Spatial and seasonal variations of physical variables. Stations (1–20) and transects (1–5).

to summer. In winter, the observed temperatures were at their lowest (Figure 2 and Table 1). Thermal stratification did not develop because of the shallowness at the sampled stations (<7 m). Salinity varied from 35.2 in winter (stations 9, 16, and 20) to 40 in autumn (stations 1, 2, 3, 7, 11, 13, and 20) and spring (stations 11, 14, 16, 18, and 19). The pH values ranged from 7.01 (autumn, station 6) to 8.51 (spring, station 17). Concentrations of suspended matter varied between  $30.08 \pm 3.38 \text{ mg l}^{-1}$  during autumn and  $49.47 \pm 11.86 \text{ mg l}^{-1}$  during spring (Table 1).

### Nutrients

$\text{NO}_3^-$  concentration varied between 1.31 and  $39.66 \mu\text{M}$  in the study area, with the lowest concentration observed in summer at station 11 and the highest in autumn at station 17 (Figure 3). Mean values were also higher in autumn ( $10.39 \pm 7.87 \mu\text{M}$ ) than in summer ( $3.35 \pm 2.34 \mu\text{M}$ ), whereas winter and spring were intermediate ( $7.77 \pm 2.77$  and  $7.37 \pm 2.86 \mu\text{M}$  respectively; Table 1).  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and total nitrogen (T-N) concentrations were higher in autumn and winter than that in spring and summer. Nitrogen appeared mainly in its dissolved inorganic form (DIN,  $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$ ) representing 57.35% of the total nitrogen. Orthophosphate and total phosphate concentrations had almost the same distribution pattern (Figure 3), with low concentrations during winter and maximum values during spring (Table 1). The N/P ratio (dissolved inorganic nitrogen ( $\text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+$ ) to dissolved inorganic phosphate ( $\text{PO}_4^{3-}$ ) ratio), ranged from 1.34 in spring to 13.43 in winter (Figure 3). These values were less than the Redfield ratio (16), suggesting a potential N limitation. Silicate concentrations ranged from  $5.90 \pm 3.14 \mu\text{M}$  (winter) to  $30.92 \pm 12.00 \mu\text{M}$  (spring) (Table 1).

### Chlorophyll-a

Average Chl *a* concentrations remained  $<12 \text{ mg l}^{-1}$  (Table 1), but exhibited higher values like the maximum ( $39.40 \text{ mg l}^{-1}$ ) observed at station 9 in autumn. Meanwhile, Chl *a* was very low and sometimes undetected in some samples during winter and summer (Figure 4).

### Microphytoplankton

Mean microphytoplankton abundance was the highest in summer ( $84.10 \pm 57.91 \times 10^2 \text{ cells l}^{-1}$ ) and the lowest in winter ( $32.75 \pm 23.56 \times 10^2 \text{ cells l}^{-1}$ ) (Figure 5 and Table 1), and displayed significant differences from season to season ( $F = 4.86$ ;  $df = 80$ ;  $P < 0.01$ ). In the present study, 65 microphytoplankton taxa were observed, 25 among them were identified to the species level (Table 2). Diatoms were the most species-rich group with 30 taxa, followed by dinoflagellates with 29 taxa and Cyanobacteria with 3 taxa. Other groups such as Dictyochophyceae (*Dictyocha* sp.), Euglenophyceae (*Euglena acusformis*) and Chlorophyceae (*Merismopedia* sp.) were represented by only one species each. The genus *Protoperidinium* (9 taxa) was the most diverse among dinoflagellates and the genera *Lithodesmium*, *Skeletonema* and *Synedra* (2 taxa) among diatoms (Table 2). Diatoms were, on average, the most abundant group throughout the survey period (Table 1), but dinoflagellates and Euglenophyceae were punctually more abundant in autumn and summer respectively (Figure 6). Microphytoplankton diversity changed significantly throughout our study, shifting from the predominance of diatoms, particularly *Grammatophora* sp., *Navicula* sp., *Coscinodiscus* sp., *Pinnularia* sp., and *Bellarochea* sp. during the winter and spring, to that of dinoflagellates represented by *Gymnodinium* sp., *Prorocentrum gracile*, and *Protoperidinium steinii* in autumn (Table 2). The highest microphytoplankton abundance observed in summer ( $84.10 \pm 57.91 \times 10^2 \text{ cells l}^{-1}$ , Table 1), was associated with

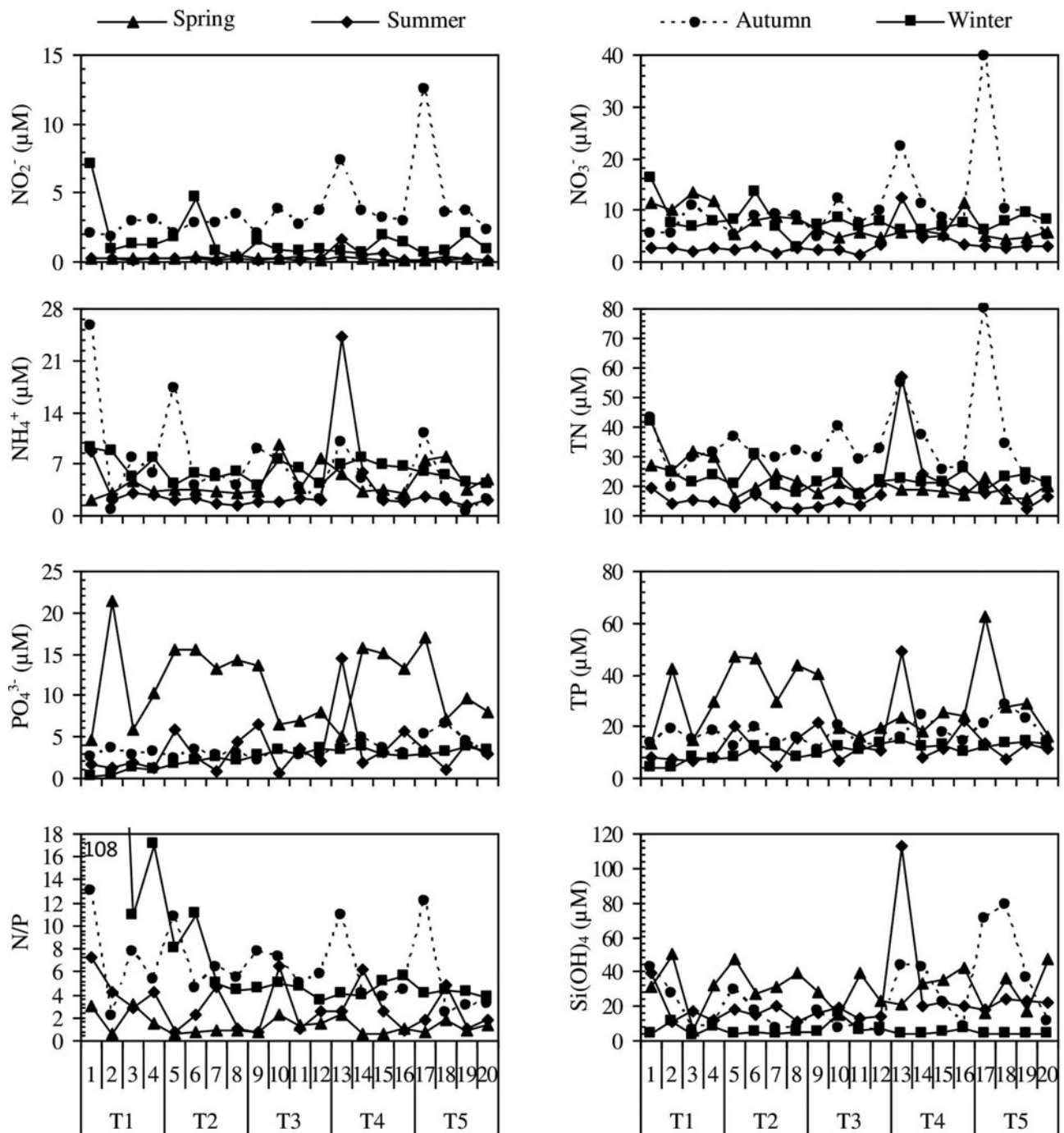


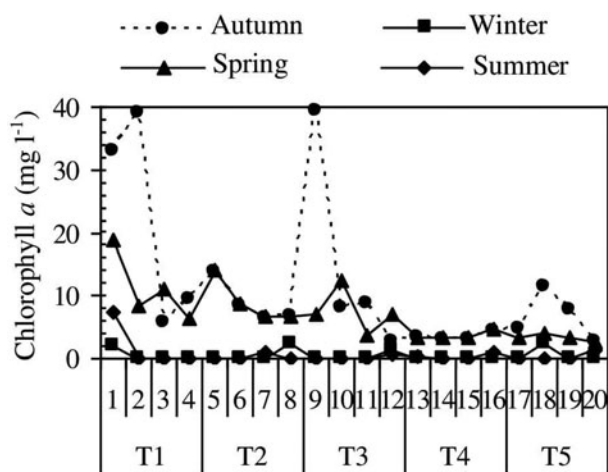
Figure 3. Spatial and seasonal variations of chemical parameters. Stations (1–20) and transects (1–5).

an important proliferation of Euglenophyceae ( $37.60 \pm 27.31 \times 10^2$  cells  $l^{-1}$ , Table 1), with *Euglena acusformis* accounting for 44.71% of total microphytoplankton abundance. The dominance of *E. acusformis* was coupled with a low number of microphytoplankton taxa (only 25 taxa, Figure 5), but no significant correlation was found between this species and physico-chemical variables.

### Ciliates

Ciliate abundance ranged from 0 (stations 1, 7, 12 (spring), and 20 (summer)) to  $32 \times 10^2$  cells  $l^{-1}$  (station 3, summer) (mean =  $8.00 \times 10^2 \pm 3.02 \times 10^2$  cells  $l^{-1}$ ). The highest ciliate abundance was recorded in summer and the highest number of ciliate taxa was observed in winter (34 taxa) (Figure 5). The ciliate community consisted of 64 taxa (33 taxa in autumn, 34 taxa in winter,

25 taxa in spring, and 20 taxa in summer) belonging to 32 genera and 2 groups: loricate ciliates and naked ciliates (Table 3). Loricate ciliates were the most diversified with 43 taxa and representing 73–82% of total ciliates abundance. The genus *Tintinnopsis* was dominant among loricate ciliates (13 taxa), followed by *Codonellopsis* and *Undella* (4 taxa) (Table 3). Loricate ciliates and total ciliate abundance showed the same temporal and spatial distribution patterns (Figure 7). Loricate ciliate abundance varied from 0 to  $32 \times 10^2$  cells  $l^{-1}$ , with the highest abundance at station 3 in summer, associated with an important reproduction of *Poroecus apiculatus* and *Tintinnopsis beroidea*. High abundances were also recorded at the same season at station 2 ( $26 \times 10^2$  cells  $l^{-1}$ , *Tintinnopsis aperta*) and station 4 ( $27 \times 10^2$  cells  $l^{-1}$ , *Tintinnopsis parvula* and *Tintinnopsis complex*) (Figure 7). Some loricate ciliates species (among which *Tintinnidium balechi*,



**Figure 4.** Spatial and seasonal variations of chlorophyll-*a* concentration. Stations (1–20) and transects (1–5).

*Tintinnopsis beroidea*, and *Tintinnopsis nana*) were omnipresent at all seasons (Table 3). Naked ciliates abundance varied from 0 to  $10 \cdot 10^2$  cells  $l^{-1}$  (maximum in summer at station 9), and showed its highest mean value ( $2.40 \pm 2.13 \times 10^2$  cells  $l^{-1}$ ) in winter and its lowest  $1.10 \pm 1.55 \times 10^2$  cells  $l^{-1}$  in spring (Figure 7; Table 1).

### Statistical analysis

#### Non-metric dimensional scaling (NMDS) and similarity (SIMPER) analyses on microphytoplankton and ciliate species

The NMDS ordination of relative abundances of the microphytoplankton species (stress value of 0.16 indicating a strong ordination) roughly identified four clusters corresponding to the four seasons (Figure 8A). However, in winter one transect (T5) clearly distinguished from the four other clusters, mainly due to some species (*Gonyaulax* sp., *Grammatophora* sp., *Licmophora* sp., *Bellarochea* sp., *Gymnodinium* sp., and *Prorocentrum triestinum*) that explained 62% of the dissimilarity with the four other transects (T1–T4). Also, in spring two transects differentiate from the three others: T3 due to *Anabeana* sp., *Coscinodiscus* sp., *Nitschia longissimi*, *Prorocentrum micans*, *Prorocentrum triestinum*,

*Navicula* sp., and *Polykrikos* sp. (50% cumulated dissimilarity) and T5 due to *Prorocentrum lima*, *Navicula* sp., *Anabeana* sp., *Coscinodiscus* sp., *P. micans*, and *Prorocentrum triestinum* (42% cumulated dissimilarity). The autumn group (60.45 average similarity) was mainly explained by *Gymnodinium* sp., *Grammatophora* sp., *Navicula* sp., *Achnanthes* sp., and *Anabeana* sp. that explained 72% cumulative similarity. The main winter group (70.73 similarity, without T5) was explained by *Navicula* sp., *Grammatophora* sp., *Bellarochea* sp., *Gymnodinium* sp., and *Pinnularia* sp. (72% cumulated), the main spring group (46.78 similarity, without T3 and T5) by *Navicula* sp., *Coscinodiscus* sp., *Euglena* sp., and *Prorocentrum triestinum* (62% cumulated), and the summer group (69.23 similarity) by *Euglena* sp., *Navicula* sp., *Grammatophora* sp., *Gymnodinium* sp., and *Prorocentrum triestinum* (75% cumulated).

The NMDS ordination of the relative abundances of ciliate species (stress value of 0.16 indicating a strong ordination) clearly identified three clusters corresponding to autumn, winter and summer transects, showing a relative spatial homogeneity of the ciliate communities at these three periods, whereas the five transects of spring were scattered (low similarity: 17.99), suggesting high spatial variability at this period (Figure 8B). The summer group had the highest similarity (60.87) mostly explained by *Tintinnopsis aperta*, *Tintinnopsis beroidea*, *Poroecus apiculatus*, and *Euplotes Charon* (72% cumulative). Winter and autumn groups were less homogeneous (38.52 and 35.07 similarity, respectively) and both highly explained by *Tintinnopsis beroidea* (>50%).

#### Relationships between biological and environmental variables

Simple correlation analyses between biological and environmental variables and between microphytoplankton and ciliate variables are detailed in Tables S1 and S2, respectively.

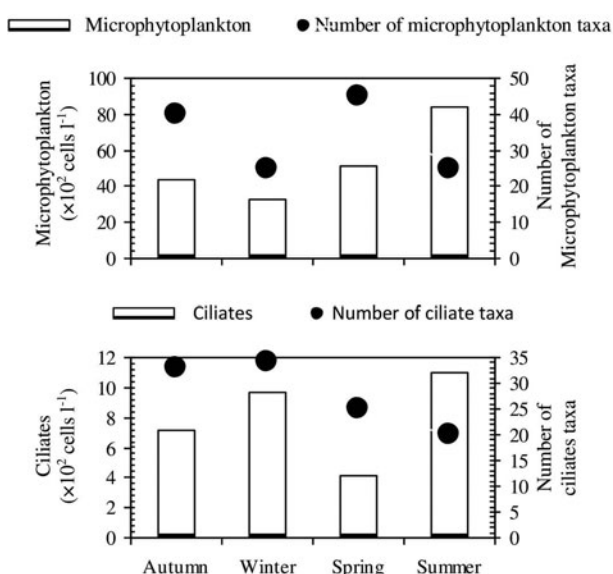
The PCA on the mean values per transect of the four seasonal sets of hydrological (temperature, salinity, pH, suspended matter, nutrients) and biological (Chlorophyll *a*, microphytoplankton groups' abundance and ciliates groups' abundance) variables (Figure 9) allowed clear discrimination of the four seasonal sampling groups around the F1 and F2 components. The F1 component axis (26% of the variance) opposed the autumn sampling points to the summer sampling points. The formers were characterized by high concentrations of N-nutrients and Chlorophyll *a* and by the presence of Dictyochophyceae and Chlorophyceae, and the latter by high temperature and pH and by high Euglenophyceae, diatoms and ciliate densities. The F2 component axis (23% of the variance) opposed spring points correlated with pH, temperature, P and Si nutrients to winter points correlated with loricate and naked ciliates.

### Discussion

The current study is the first report concerning the distribution of microphytoplankton and ciliates assemblage through high spatial resolution sampling in the shallow coastal waters south of Sfax during four seasons.

#### The south coast of Sfax, a typical stressed Mediterranean coastal zone

Our results allow characterizing the environmental context of a typical stressed area of the southeastern Mediterranean coast. The high values of temperature and salinity are in agreement with other studies performed in arid to semi-arid Mediterranean areas (Elloumi et al., 2015). A strong acidification of seawater was observed in autumn with pH values down to 7 (mean =  $7.17 \pm 0.08$ ), contrasting with the highest pH levels in spring ( $8.13 \pm 0.29$ ). Such low pH values could reasonably be attributed to the industrial activity still in operation along the



**Figure 5.** Seasonal variations of average values of microphytoplankton abundance, number of microphytoplankton taxa, ciliates abundance, and number of ciliates taxa.

**Table 2.** List and frequency of microphytoplankton species found in the southern coast of Sfax during the study conducted at four successive seasons

Microphytoplankton species (cells l <sup>-1</sup> )	Autumn	Winter	Spring	Summer
Cyanophyceae				
<i>Anabaena</i> sp.	C	–	C	–
<i>Oscillatoria</i> sp.	R	R	R	R
<i>Spirulina</i> sp.	R	–	–	–
Bacillariophyceae				
<i>Achnanthes</i> sp.	C	C	–	C
<i>Amphiprora</i> sp.	–	–	R	–
<i>Amphora</i> sp.	R	R	R	–
<i>Bellerochea</i> sp.	R	C		C
<i>Biddulphia</i> sp.	R	C	R	R
<i>Climacosphenia</i> sp.	R	R	R	–
<i>Cocconeis</i> sp.	–	R	R	R
<i>Coscinodiscus</i> sp.	–	R	C	R
<i>Diploneis</i> sp.	R	–	R	–
<i>Epithemia</i> sp.	–		R	–
<i>Grammatophora</i> sp.	C	C	R	C
<i>Gyrosigma</i> sp.	–	–	R	–
<i>Leptocylindrus danicus</i> (Cleve, 1889)	–	–	C	–
<i>Leptocylindrus</i> sp.	–	–	R	–
<i>Licmophora</i> sp.	R	C	R	–
<i>Lithodesmium</i> sp.	–	–	R	–
<i>Lithodesmium undulatum</i> (Ehrenberg, 1839)	R	–	R	–
<i>Navicula</i> sp.	C	C	A	C
<i>Nitschia longissima</i> (Ralf, 1861)	R	–	R	–
<i>Pinnularia</i> sp.		C	R	R
<i>Plagiotropis</i> sp.	–	–	R	–
<i>Pleurosigma</i> sp.	R	C	R	R
<i>Rhabdonema</i> sp.	C	R	–	–
<i>Rhizosolenia</i> sp.	R	R	–	–
<i>Rhizosolenia stolforthii</i> (Cupp, 1943)	R		–	–
<i>Skeletonema costatum</i> (Cleve, 1873)	–	–	R	–
<i>Skeletonema</i> sp.	–	–	R	–
<i>Striatella unipunctata</i> (Agardh, 1832)	–	R	–	R
<i>Synedra</i> sp.	R	–	–	–
<i>Synedra ulna</i> (Ehrenberg, 1832)	R	–	–	–
<i>Thalassiosira</i> sp.	R	–	R	–
Dinophyceae				
<i>Alexandrium</i> sp.	–	–	R	–
<i>Amphidinium</i> sp.	R	R	R	R
<i>Tripos lineatus</i> (Gomez, 2013)	R	–	–	–
<i>Dinophysis caudata</i> (Saville-Kent, 1881)	R	–	–	–
<i>Dinophysis</i> sp.	–	–	–	R
<i>Gonyaulax</i> sp.	–	C	R	R
<i>Gymnodinium marinum</i> (Saville-Kent, 1880)	R	–	–	–
<i>Gymnodinium</i> sp.	A	C	C	C
<i>Gyrodinium</i> sp.	R	R	–	R

(Continued)

Table 2. (Continued.)

Microphytoplankton species (cells l <sup>-1</sup> )	Autumn	Winter	Spring	Summer
<i>Noctiluca</i> sp.	–	–	R	–
<i>Peridinium</i> sp.	R	–	R	C
<i>Polykrikos</i> sp.	R	–	R	R
<i>Prorocentrum compressum</i> (Dodge, 1975)	–	–	R	–
<i>Prorocentrum gracile</i> (Schütt, 1895)	C	–	–	–
<i>Prorocentrum lima</i> (Stein, 1878)	R	–	C	R
<i>Prorocentrum micans</i> (Ehrenberg, 1834)	–	–	C	R
<i>Prorocentrum triestinum</i> (Schiller, 1918)	–	C	C	C
<i>Protoperidinium bipes</i> (Balech, 1974)	R	–	R	–
<i>Protoperidinium cerasus</i> (Balech, 1973)	–	–	R	–
<i>Protoperidinium conicoides</i> (Balech, 1973)	–	–	R	–
<i>Protoperidinium conicum</i> (Balech, 1974)	R	–	–	–
<i>Protoperidinium depressum</i> (Balech, 1974)	–	–	R	–
<i>Protoperidinium globulum</i> (Balech, 1974)	–	–	R	–
<i>Protoperidinium minutum</i> (Loeblich III, 1970)	–	R	R	R
<i>Protoperidinium</i> sp.	R	R	R	R
<i>Protoperidinium steinii</i> (Jorgensen, 1899)	C	–	–	–
<i>Pyrophacus</i> sp.	R	–	–	–
<i>Scrippsiella trochoidea</i> (Stein, 1883)	R	R	R	R
Euglenophyceae				
<i>Euglena acusformis</i> (Schiller, 1925)	C	R	C	A
Dictyochophyceae				
<i>Dictyocha</i> sp.	R	–	–	–
Chlorophyceae				
<i>Merismopedia</i> sp.	R	–	–	–

(–) en dash means not detected.

(R) Rare means 0–100 cells l<sup>-1</sup>.

(C) Common means 100–300 cells l<sup>-1</sup>.

(A) Abundant means >300 cells l<sup>-1</sup>.

south coast (Rekik *et al.*, 2013a). In particular, the phosphate processing industries (SIAPE-Sfax) generate very acidic residues (phosphogypsum) that can result in very low pH values of coastal marine water: in the Ghannouch-Gabes zone, values lower than 3.5 have been recorded close to the discharge (Ben Amor and Gueddari, 2016), and values close to or even lower than 7 can be observed at several kilometres off the coastline (El Kateb *et al.*, 2018). The high concentration of suspended matter may be the result of the shallow depth of the sampled area and the intensity of the dominant winds (southwest and north-east), which usually provoke not only sediment mixing but also remobilization from the surface deposits (Ben Salem *et al.*, 2015). The high concentrations of orthophosphate and total phosphate are associated with an important release of phosphate due to residue from the phosphate processing industries (SIAPE-Sfax) (Ben Brahim *et al.*, 2010). Additionally, the N/P–DIN to DIP ratio was highly variable and in average lower than the Redfield ratio (16) during the four periods. Strong variability in the N/P ratio characterizes coastal ecosystems, particularly under eutrophication conditions, where the high terrestrial inputs of nutrients, tide and turbulence-driven resuspension cause situations far from the relative equilibrium found in the open ocean (Ryther and Dunstan, 1971). Low N/P ratio during our study agrees with the results in the north

Sfax coast before the restoration process (Rekik *et al.*, 2012), also suggesting an overall nitrogen limitation in this stressed coastal zone.

#### Microphytoplankton community of the south coast of Sfax and its environmental drivers

With the oligotrophic Eastern Mediterranean Sea, the southern coast of Sfax stands out as a highly productive ecosystem (Rekik *et al.*, 2015a). The high productivity has been further confirmed by compiling satellite observations and biogeochemical data, which reinforce the contrast with the Eastern Mediterranean Sea (D'Ortenzio and Riberad'Alcalà, 2009; Ayata *et al.*, 2017). Microphytoplankton assemblages recorded in our study in the southern coast of Sfax showed some similarities compared to other coastal environments (Rekik *et al.*, 2013b). A high number of microphytoplankton taxa (65 species), with a prevalence of diatoms species was observed in agreement with previous studies conducted in the north Sfax coast, during the 2009–2010 period, showing a comparable number of taxa (70 taxa/90 taxa) at the surface and the water-sediment interface, respectively (Rekik *et al.*, 2013b, 2015b, 2016a). Microphytoplankton abundance shifted from dinoflagellates dominance in autumn to diatoms dominance in winter and spring and dominance of



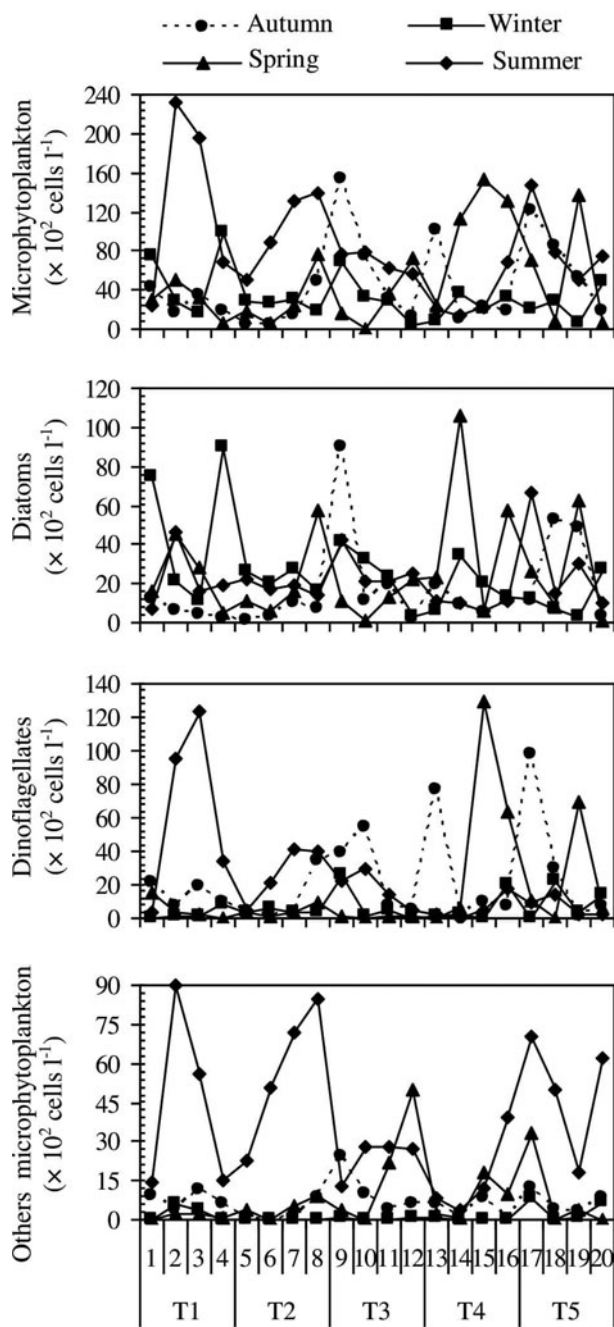


Figure 6. Spatial and seasonal variations of the abundance of microphytoplankton, diatoms (diat), dinoflagellates (dino), and other microphytoplankton (other micro).

Euglenophyceae in summer in the southern coast. On the north coast of Sfax, the microphytoplankton community consisted mainly of diatoms in autumn and winter, dinoflagellates in spring and Cyanobacteriae in summer (Rekik *et al.*, 2013b). The variations of microphytoplankton community were mainly related to nutrient and environmental parameters. Dinoflagellates were positively correlated to pH, TN and N/P ratio in autumn. The important abundance of dinoflagellates in autumn may be explained by their cell motility allowing them to explore different depths (Rekik *et al.*, 2017a). The abundance of dinoflagellates in such polluted situation (low pH, high nutrients) agrees with their cosmopolitan and less demanding character in terms of environmental conditions compared with other groups (Ben Salem *et al.*, 2015). In our study, dinoflagellates species composition showed similarity between the southern coast of Sfax and the Gulf of Gabes. Some dinoflagellates, such as *Gymnodinium*, *Gonyaulax*,

Table 3. List and frequency of ciliate species found in the southern coast of Sfax during the study conducted at four successive seasons

Ciliates species (cells l <sup>-1</sup> )	Autumn	Winter	Spring	Summer
Loricated ciliates				
<i>Acanthostomella norvegica</i> (Kofoid and Campbell, 1929)	-	R	-	-
<i>Amphorellopsis</i> sp.		R	-	R
<i>Ascampbelliella armilla</i> (Kafoid and Campbell, 1929)	R	-	-	-
<i>Ascampbelliella urceolata</i> (Ostenfeld, 1899)	-	R	R	-
<i>Codonellopsis cylindroconica</i> (Alder, 1999)	-	-	R	-
<i>Codonellopsis obesa</i> (Balech, 1948)	R	-	-	-
<i>Codonellopsis pusilla</i> (Jørgensen, 1924)	R	-	-	-
<i>Codonellopsis</i> sp.	-	R	-	-
<i>Cyttarocylis</i> sp.	-	-	R	R
<i>Favella errhenbergii</i> (Claparède and Lachmann, 1858)	R	-	R	-
<i>Favella serrata</i> (Möbius, 1887)	R	-	-	-
<i>Favella</i> sp.	-	R	-	-
<i>Helicostomella</i> sp.	R	-	-	-
<i>Helicostomella subulata</i> (Ehrenberg, 1833)	R	-	-	R
<i>Metacylis jorgenseni</i> (Cleve, 1902)	R	-	-	-
<i>Metacylis</i> sp.	R	R	R	-
<i>Ormosella acantharus</i> (Kofoid and Campbell, 1929)	R		-	-
<i>Ormosella cormicopia</i> (Campbell, 1929)	-	R	-	R
<i>Petalotricha ampulla</i> (Fol, 1881)	-	R	R	-
<i>Petalotricha</i> sp.	R	-	-	-
<i>Poroecus apiculatus</i> (Cleve, 1899)	R	-	R	C
<i>Proplectella ovata</i> (Jørgensen, 1924)		R	-	-
<i>Rhabdonella amor</i> (Cleve, 1900)	R	-	-	-
<i>Rhabdonella spiralis</i> (Fol, 1881)	-	R	-	-
<i>Steenstrupiella steenstrupii</i> (Claparède and Lachmann, 1858)		-	R	-
<i>Tintinnidium balechi</i> (Barra de Cao, 1981)	C	R	R	R
<i>Tintinniopsis campanula</i> (Ehrenberg, 1840)	-	R	-	R
<i>Tintinniopsis lobiancoi</i> (Daday, 1887)	R	R	R	R
<i>Tintinniopsis amphora</i> (Kofoid and Campbell, 1929)	-	-	R	-
<i>Tintinniopsis aperta</i> (Brandt, 1906)		R	R	C
<i>Tintinniopsis beroidea</i> (Stein, 1867)	C	C	R	C
<i>Tintinniopsis butschlii</i> (Daday, 1887)	R	-	-	-

(Continued)

Table 3. (Continued.)

Ciliates species (cells l <sup>-1</sup> )	Autumn	Winter	Spring	Summer
<i>Tintinnopsis complex</i> (Stein, 1867)	–	R	R	C
<i>Tintinnopsis fimbriata</i> (Meunier, 1919)	R	–	–	–
<i>Tintinnopsis nana</i> (Lohmann, 1908)	R	R	R	R
<i>Tintinnopsis parva</i> (Merkle, 1909)	R	–	–	–
<i>Tintinnopsis parvula</i> (Jørgensen, 1912)	R	R	–	R
<i>Tintinnopsis</i> sp.	–	R	–	–
<i>Undella claparedei</i> (Daday, 1887)	–	–	R	–
<i>Undella hemisphaerica</i> (Laackmann, 1910)	–	R	–	–
<i>Undella hyalina</i> (Daday, 1887)	–	–	R	–
<i>Undella</i> sp.	–	R	–	–
Naked ciliates				
<i>Aspidisca lynceus</i> (Ehrenberg, 1830)	R	–	–	R
<i>Aspidisca</i> sp.	–	R	–	–
<i>Balanion</i> sp.	–	R	–	–
<i>Enchelyodon laevis</i> (Quennerstedt, 1869)	R	–	–	–
<i>Euplotes charon</i> (Müller, 1786)	–	R	R	R
<i>Halteria</i> sp. (Claparede and Lachmann, 1853)	–	R	R	–
<i>Leegaardiella sol</i> (Lynn and Montagnes, 1988)	R	R	R	R
<i>Lohamaniella oviformis</i> (Leegaard, 1915)	R	R	R	–
<i>Mesodinium</i> sp.	R	–	–	–
<i>Monodinium balbianii</i> (Fabre-Domergue, 1888)	–	–	R	–
<i>Philasterine</i> sp.	–	R	–	–
<i>Pleuronema crassum</i> (Dujardin, 1841)	R	–	–	–
<i>Strobilidium</i> sp.	R	–	R	R
<i>Strombidium acutum</i> (Leegaard, 1915)	–	R	–	R
<i>Strombidium capitatum</i> (Leegaard, 1915)	–	R	–	–
<i>Strombidium chlorophilum</i> (Montagnes et al., 1988)	–	R	–	–
<i>Strombidium compressum</i> (Leegaard, 1915)	R	–	–	–
<i>Strombidium conicum</i> (Wulff, 1919)	R	R	–	–
<i>Strombidium dalum</i> (Lynn et al., 1988)	–	R	–	–
<i>Strombidium</i> sp.	R	–	R	R
<i>Tiarina fusus</i> (Claparede and Lachmann, 1857)	R	–	R	–
<i>Uronema marinum</i> (Dujardin, 1841)	R	R	–	R

(–) en dash means not detected.  
 (R) Rare means 0–100 cells l<sup>-1</sup>.  
 (C) Common means 100–300 cells l<sup>-1</sup>.

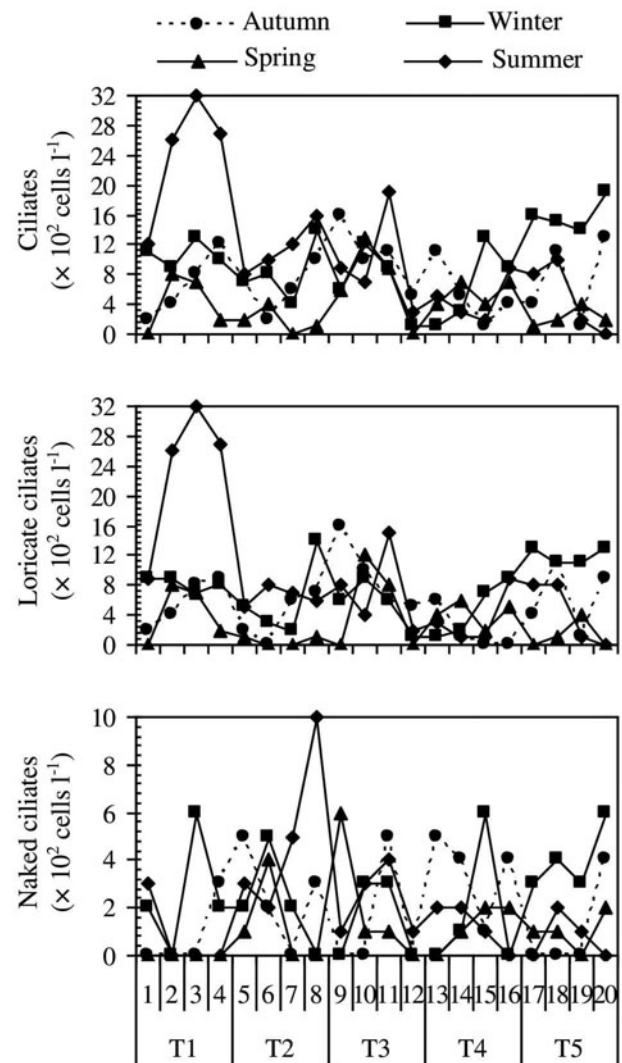
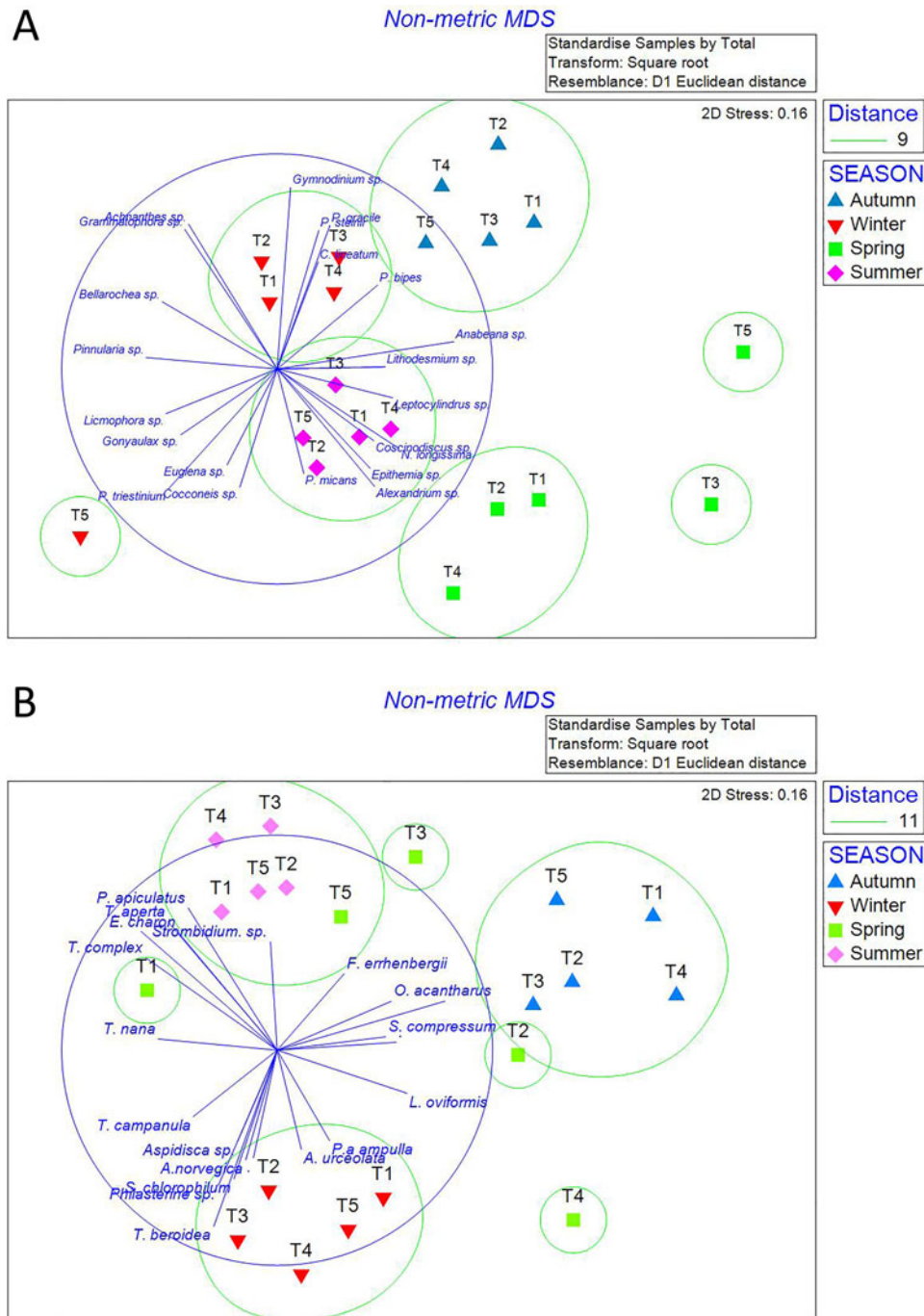


Figure 7. Spatial and seasonal variations of the abundance of ciliate (cil), loricated ciliate (loricate cil) and naked ciliates (naked cil).

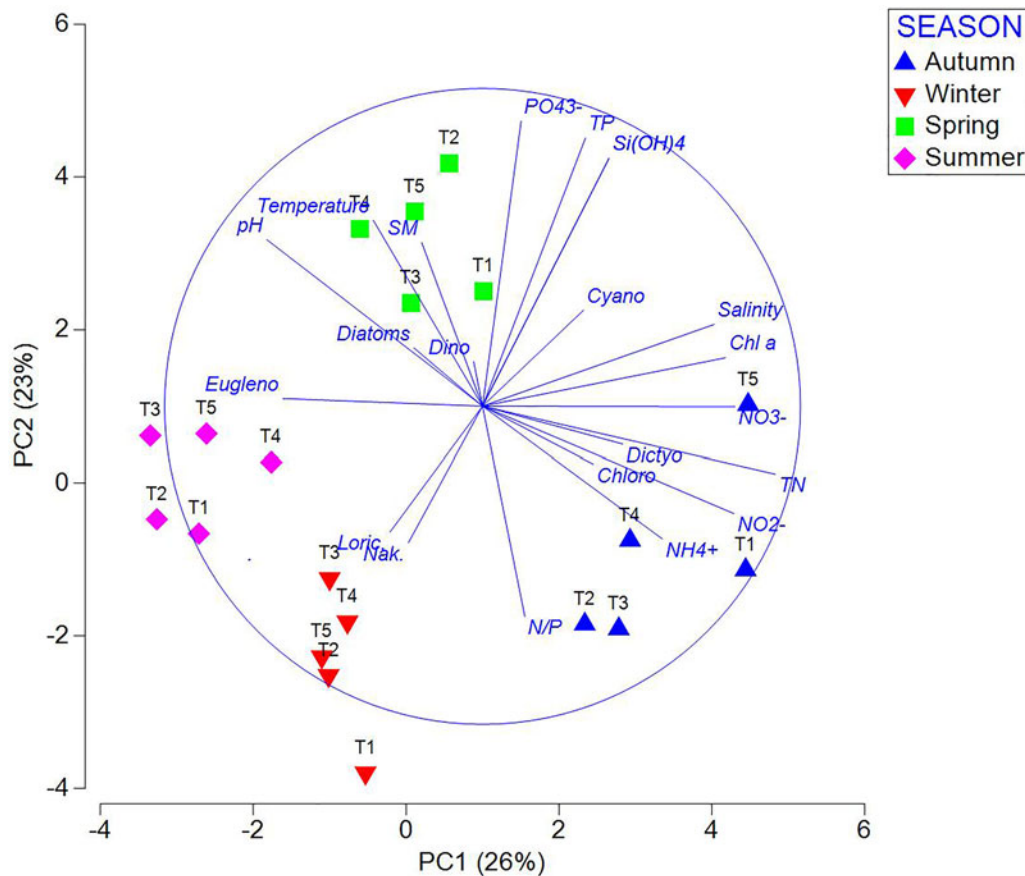
*Protoperidinium*, and *Prorocentrum* attained high abundance in stressed areas like in the northern coast of Sfax (Rekik et al., 2012), the southern coast of the Kerkennah islands (Rekik et al., 2018), the Kneiss island (Rekik et al., 2017b), and the Gulf of Gabès (Drira et al., 2008), indicating their tolerance to local environmental conditions. In fact, they can overcome the lack of nutrients by diversifying their trophic modes (autotrophic, mixotrophic, and heterotrophic; Jeong et al., 2010). About half of dinoflagellate species in marine plankton lack chloroplasts (Sherr and Sherr, 2007). Dinoflagellates comprise a large variety of toxic species, which can produce many different toxic compounds (Smayda, 1997) that can interfere with recruitment, growth and viability of an important range of marine organisms including their competitors (Plumley, 1997). In this study, potential toxic species such as *Protoperidinium depressum* (spring), *Protoperidinium steinii* (autumn), *Dinophysis caudata* (autumn), and *Prorocentrum lima* (autumn, spring, and summer) were recorded (Hallegraeff, 1993). The dinoflagellates assemblages also included high numbers of *Gymnodinium*, a genus that was reported to occur under high phosphate loading (Daly-Yahia Kéfi et al., 2005). However, in this study high *Gymnodinium* abundance occurred under low phosphate and high nitrogen concentrations, suggesting that the reproduction of dinoflagellates was mainly nitrogen-driven (Rekik et al., 2015a). High density of diatoms in winter and spring may be due to their quick growth



**Figure 8.** Non-metric dimensional scaling analyses (NBMDs) on mean values per sampling transects (T1–T5) and per season of abundance percentages of phytoplankton species (A) and ciliate species (B).

capacity under turbulent and high nutrient conditions (Maranon *et al.*, 2012). Diatoms are known to be opportunistic organisms (Fogg, 1991) having fast growth due to rapid nitrogen uptake (Lomas and Glibert, 2000). These large species (*Navicula* (95  $\mu\text{m}$ ), *Coccinodiscus* (160  $\mu\text{m}$ ) and *Leptocylindrus* (150  $\mu\text{m}$ )) are characterized by a high tolerance to various environmental parameters and physical stress characteristic of shallow coastal ecosystems, especially during spring blooms (Lomas and Glibert, 2000). During our survey the south coast of Sfax, Euglenophyceae, represented by one species, *Euglena acusformis*, displayed their highest abundance in summer (45% of the total microphytoplankton abundance) but in previous surveys, high abundance of this species was also recorded in winter in the same area (Ben Salem *et al.*, 2015). *Euglena acusformis* has been recognized as the most

opportunistic and saprobiontic species which assimilate lots of organic matter and might be an indicator of pollution (Barrera *et al.*, 2008). Because of their high surface to volume ratio, small cells like *Euglena acusformis* incorporate nutrients at low energy cost (Agawin *et al.*, 2000) and thus outperform large cells (Sin and Wetzel, 2000). In our study Cyanobacteria were not well represented in the microphytoplankton community but reached their maximal density in Spring mainly due to the nitrogen-fixing *Anabaena* sp., consistently with the lowest N/P ratio, indicating N limitation at this period (Table 1). On the north coast of Sfax, the main period for Cyanobacteriae growth was in summer with the dominant opportunistic and nitrogen-fixing species *Trichodesmium erythraeum* (Rekik *et al.*, 2013b) which can form important blooms in the gulf of Gabès during



**Figure 9.** Principal component analysis (PCA) (axis I and II) of mean values per sampling transects (T1–T5) and per season abundance and selected environmental variables.

warm periods (Hamza *et al.*, 2016) and which is known to dominate the microphytoplankton community in the oligotrophic Sea (Nausch *et al.*, 2008).

#### **Ciliate community of the south coast of Sfax and its environmental drivers**

A total of 64 ciliates taxa representing 46 different genera were identified during this study, few species of which could be characterized as rare, found at only one or two stations. The species number reported here is lower than the one reported by Rekik *et al.* (2015c) in the northern coast of Sfax (Tunisia), over four seasons (40 planktonic species at the surface and 53 at the bottom). The divergence in terms of species number between the north and the south coast of Sfax is probably partly due to difference in terms of sampling efforts applied to each study. Another reason may be the mixing of planktonic and benthic ciliates (thus increasing the number of sampled species) due to the shallow water depth of the south coast of Sfax. Ciliates community demonstrated a clear temporal pattern: high abundance values in winter and summer with an obvious peak at station 3 in summer, low abundance values in spring and autumn. Ciliates are dominant in southern coast of Sfax during summer as shown by the maximal abundance recorded for a wide range of ciliates (20 taxa) belonging to different size classes with different feeding strategies (autotrophic, heterotrophic, and mixotrophic ciliates). The ciliates community was dominated by loricate ciliates while aloricate ciliates were relatively rare, as also reported in the north coast of Sfax (Rekik *et al.*, 2015c), the Gulf of Gabès (Hannachi *et al.*, 2009; Kchaou *et al.*, 2009), the Adriatic Sea (Bojanić *et al.*, 2005), and the Yellow Sea (Jiang *et al.*, 2011).

The high abundance of loricate species is probably due to the eutrophic conditions in these marine areas, since these species might possess a higher adaptability to eutrophic environments than other ciliates (Bojanić *et al.*, 2005). Some loricate ciliates species, such as *Favella ehrenbergii*, *Helicostomella subulata*, *Tintinnopsis beroidea*, *Tintinnopsis campanula*, and *T. lobiancoi*, reach high abundances in highly anthropized marine coastal areas, showing their tolerance to environmental stress (Rekik *et al.*, 2015a). However, there are some exceptions to this composition pattern in some marine regions where aloricate species dominate the ciliate community; this is typical for many coastal and oceanic waters, such as the Irish Sea (Edward and Burkill, 1995), the Irminger Sea (Montagnes *et al.*, 2010), the Baltic Sea (Mironova *et al.*, 2009), and the North Sea (Stelfox-Widdicombe *et al.*, 2004).

In this study, we found no significant correlations between environmental factors and the abundance of the ciliates community. The same results were reported by Gong *et al.* (2005). The water temperature and various inorganic nutrients might not directly control the structure and dynamics of the ciliates community but indirectly influence it via food availability. For instance, the dominance of the agglutinated species *Tintinnopsis beroidea* was shown to be related to the availability of particles to construct the lorica in addition to the presence of its preferred food (Cyanobacteria) (Rakshit *et al.*, 2015).

#### **Relationships between ciliate and microphytoplankton communities in the south coast of Sfax**

Loricate ciliates are known to have significant relationships with microphytoplankton groups suggesting a close ecological link to

this type of food and revealing further insights into the ecological role of ciliates as grazers on microphytoplankton, especially in autumn and summer when microphytoplankton is very abundant (Yang *et al.*, 2015). The seasonal distribution of microphytoplankton and ciliates (dominated by the genus *Tintinnopsis*) suggests that ciliates community consume nanophytoplankton but also microphytoplankton, as shown in the north coast of Sfax (Rekik *et al.*, 2012). Significant relationships between loricate ciliates and microphytoplankton community abundance have been found in previous studies in Tunisian coastal areas such as the Gulf of Gabes (Hannachi *et al.*, 2009). In our study, there were significant correlations between loricate ciliates and Cyanobacteria ( $r = 0.85$ ,  $n = 20$ ,  $P < 0.05$ ) in autumn and between loricate ciliates and dinoflagellates ( $r = 0.56$ ,  $n = 20$ ,  $P < 0.05$ ) in summer. On the other hand, dinoflagellates may also be in direct feeding competition with ciliates for food. Indeed, *Protoperdinium* is a heterotrophic genus known to feed exclusively on diatoms (Sherr and Sherr, 2007) and several other dinoflagellates (including the genera *Gymnodinium*, *Gyrodinium*, *Gonyaulax*, *Triplos*, and *Alexandrium*) are considered as grazers, since most of them were previously shown to be mixotrophic (Stoecker, 1999). This competition for food between ciliates and dinoflagellates may constitute another hypothesis explaining their simultaneous presence and the correlations recorded between them. However, loricate ciliates rarely control the abundance or composition of their prey, as their aggregate feeding activity usually equates to clearing a maximum of 1–2% per day of the surface layer waters they occupy (Dolan *et al.*, 2013).

## Conclusion

The present study indicates that the environmental properties of the southern coast of Sfax have typical characteristics of a stressed area. The microphytoplankton community is highly tolerant and dependant on environmental variables in particular pH and nutrient availability. Diatoms are dominant in winter and spring taking advantage of their high growth capacity. Dinoflagellates dominate in autumn in low pH condition showing their high tolerance to environmental stress. Euglenophyceae are the most numerous in summer in the lowest nutrient condition, may be due to their high surface to volume ratio favouring nutrient assimilation at low energy cost. In contrast with current observations in the open Mediterranean Sea the ciliate community of the southern coast of Sfax is dominated by loricate ciliates (mostly the genera *Tintinnopsis*, *Codonellopsis*, and *Undella*) which are more abundant than naked ciliates. Ciliate abundance and community structure is highly variable between seasons but this variability seems not directly driven by environmental variables but indirectly through dependence on prey availability, resulting in a tight coupling with microphytoplankton community. Ciliates should exert a top-down control on microphytoplankton but the importance of mixotrophic and heterotrophic dinoflagellates (known to feed on diatoms) also suggests a feeding competition with this group.

At present, the phosphogypsum restoration had been acutely necessary allowing microphytoplankton and ciliate species to take optimal advantage of niche opportunities, which, in turn, improve water quality along the southern coast.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0025315423000462>.

**Acknowledgements.** This work was supported by the Taparura Project conducted in the Laboratory LR/18ES30 Marine biodiversity and environment at the University of Sfax. We have obtained permits for sampling and observation field studies from the Taparura Project.

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