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Spatial and Seasonal Patterns of Plastic Debris, Physicochemical Conditions, Hydrobiological Components, and Plastisphere Composition in the Sahline Salt Marsh, Tunisia

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Abstract

Plastic pollution has become a major environmental concern, particularly in coastal wetlands where debris accumulates and interacts with local biota. This study investigated the relationships among plastic debris, physicochemical conditions, hydrobiological components, and plastisphere-associated components in the Sahline Salt Marsh (Monastir, Tunisia). Seasonal sampling was conducted at five monitoring stations over one year. Water samples were analyzed for temperature, salinity, dissolved oxygen, pH, nutrients, and chlorophyll *a*. Plastic debris was assessed using an item-count approach, and biotic and abiotic components present in the surrounding water and associated with plastic surfaces were characterized.

Plastic bottles, bags, and fragments were the dominant debris types, with the highest abundances recorded at station S₅ (57.3 ± 3.01 to 93.0 ± 1.77 items station⁻¹). No plastic debris was detected at the salt-pan stations during winter and late spring. The water surrounding plastic debris was dominated by *Artemia*-like forms and insect fragments, whereas plastisphere-associated components included *Artemia*-like forms, insect fragments, salt crystals, plant fibres, and *Dunaliella*-like microalgae. Statistical analyses revealed significant associations between selected physicochemical parameters and specific plastic categories, particularly yogurt packaging, while plastic bottles showed an isolated association with dissolved oxygen in August 2022. Significant associations were also identified between selected physicochemical parameters and plastisphere-associated components.

These findings highlight marked spatial and seasonal variability in plastic debris distribution and plastisphere-associated components and indicate associations with the physicochemical gradients characterizing this hypersaline salt-marsh environment. These relationships should be interpreted as associations rather than evidence of direct causal effects.

Keywords: Marine litter; hypersalinity; environmental gradients; biological colonization; salt-pan ecosystem; Monastir.

Introduction

Plastic pollution has emerged as one of the most widespread environmental challenges affecting aquatic ecosystems worldwide. Due to their extensive production, persistence, and resistance to degradation, plastics represent a major fraction of marine litter and anthropogenic debris in coastal and marine environments (Derraik, 2002; Barnes et al., 2009; UNEP, 2016). Global plastic production has continuously increased over recent decades, resulting in substantial quantities of plastic waste entering aquatic systems through rivers, coastal activities, tourism, and inadequate waste management practices (Jambeck et al., 2015). Once released into the environment, plastic debris can accumulate in different aquatic compartments, including beaches, water columns, sediments, and transitional ecosystems.

Salt marshes and hypersaline wetlands are particularly vulnerable to plastic accumulation because they can act as retention zones for land-derived debris. Environmental factors such as solar radiation, temperature, and mechanical abrasion promote the fragmentation of plastic debris into smaller particles. In addition to their physical persistence, plastic materials provide artificial surfaces that can be colonized by microorganisms and other organisms, contributing to the formation of a specific ecological assemblage commonly referred to as the plastisphere (Zettler et al., 2013). The composition and development of plastisphere-associated components may be influenced by environmental conditions, including spatial variability, seasonality, salinity, and other physicochemical factors (Oberbeckmann et al., 2014; Amaral-Zettler et al., 2015; Basili et al., 2020). Although numerous studies have investigated plastic pollution in marine and coastal environments, relatively few have examined hypersaline salt-marsh ecosystems and the relationships among plastic debris, physicochemical conditions, and associated biological communities.

Plastic debris can interact with local environmental conditions and resident organisms, including microorganisms, microalgae, and invertebrates. Several studies have demonstrated that plastics can serve as substrates for diverse biological assemblages, although plastisphere-associated components may differ from those in surrounding environments (Bryant et al., 2016; Dussud et al., 2018b; Pinto et al., 2019; Delacuvellerie et al., 2019; Amaral-Zettler et al., 2020). In hypersaline environments, organisms such as *Artemia*-like forms and halotolerant microalgae may occur in association with plastic debris and its surrounding environment. However, the relationships between abiotic parameters, plastic debris distribution, and associated biological components remain insufficiently explored in hypersaline salt-marsh ecosystems.

In Tunisia, Mediterranean coastal ecosystems are increasingly exposed to plastic contamination due to coastal development, tourism activities, and other anthropogenic pressures. Despite the ecological importance of Tunisian salt marshes, information regarding plastic accumulation and its relationships with environmental and biological components remains limited. The Sahline Salt Marsh (Monastir Governorate) represents a particularly relevant study area because of its hypersaline conditions and ecological importance for halophilic and euryhaline organisms. Within the framework of the “Plasti-COMA” project developed by the Marine Biodiversity Laboratory, this study investigates the relationships between plastic debris, environmental conditions, and associated biological components in the Sahline Salt Marsh, complementing previous investigations conducted in Monastir Bay.

Therefore, this study aimed to assess the relationships among plastic pollution, physicochemical conditions, and hydrobiological components in the Sahline Salt Marsh. Specifically, we investigated (1) seasonal variations in physicochemical parameters and nutrient concentrations, (2) spatial and temporal patterns of plastic debris abundance, (3) biological components associated with the water surrounding plastic debris, and (4) the composition of

plastisphere-associated components associated with plastic surfaces. The relationships between environmental variables, plastic debris distribution, and associated biological components were also evaluated to better understand the distribution and ecological relevance of plastic pollution in hypersaline salt-marsh environments.

Materials and Methods

Study area and sampling design

The study was conducted in the Sahline Salt Marsh (35°45'N, 10°46'E), located along the central Tunisian coastline in the Monastir Governorate. A preliminary survey was conducted in May 2021 to identify areas of plastic debris accumulation within the salt marsh. From September 2021 to August 2022, five sampling campaigns were conducted at five stations (S₁–S₅) to assess environmental conditions, plastic debris distribution, and associated biological and abiotic components. Water samples were collected from the environment surrounding plastic debris for laboratory analyses of nutrient concentrations and chlorophyll *a*. Plastic debris was collected manually at each station, and its associated biological and abiotic components were subsequently examined and recorded.

Study area characteristics and sampling station selection

The Sahline Salt Marsh is a thalassohaline ecosystem consisting of a coastal sebkha extending into a series of salt pans. It is located near Monastir Airport and the industrial area of Sahline (Fig. 1). Five sampling stations (S₁–S₅) were selected to represent the main environmental gradients within the salt marsh. Stations S₁ and S₂ were located in the central part of the salt marsh, S₃ and S₄ within the salt-pan areas, and S₅ close to the zone of marine influence. Sediment characteristics differed among stations: S₁, S₂, and S₅ were characterized by muddy sediments with relatively high organic matter content, whereas S₃ and S₄ were mainly characterized by mineral deposits associated with salt-production areas.

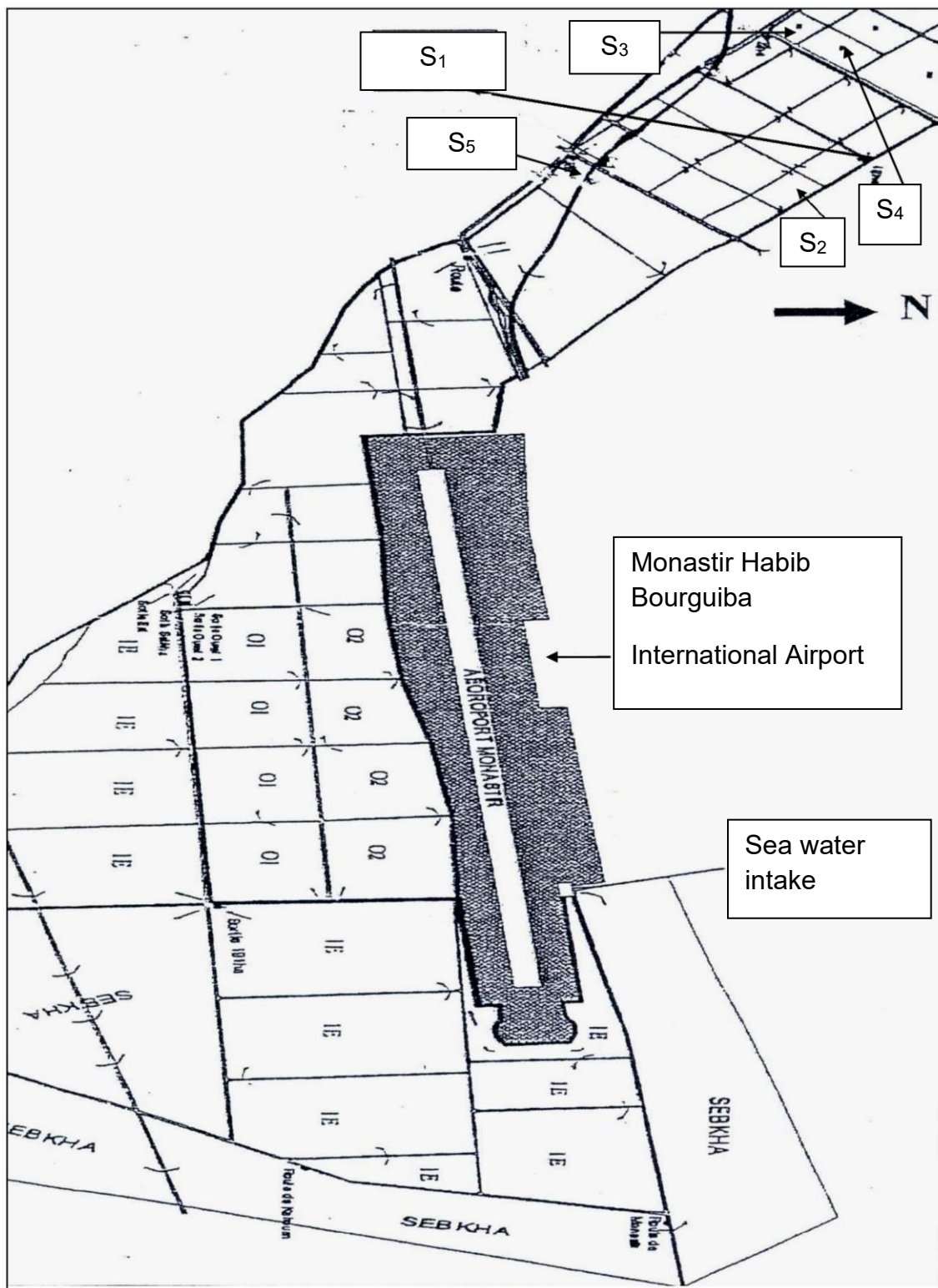


Fig. 1 Position of the study stations: Sahline salt marsh

Measurement of physicochemical and hydrobiological parameters

Physicochemical and hydrobiological parameters were measured during each sampling campaign, including temperature, salinity, dissolved oxygen, pH, nutrient concentrations (NH_4^+ , NO_2^- , NO_3^- , TN, PO_4^{3-} and TP), and chlorophyll *a*. Water temperature was measured using a thermometer with an accuracy of 0.5°C. Salinity was determined using a hand-held refractometer (ATAGO, Japan), while dissolved oxygen concentrations were measured using a portable oximeter (THERMO ORION model 810). pH measurements were performed using a calibrated pH meter (WTW) with standard buffer solutions (pH 7 and 9). Field measurements of temperature, salinity, dissolved oxygen, and pH were obtained during each sampling campaign, whereas nutrient and chlorophyll *a* analyses were conducted in the laboratory.

Nutrient concentrations were determined using filtered water samples (25 mL) passed through Whatman GF/A filters (47 mm diameter; 1.6 μm pore size) and analyzed by spectrophotometry using a Beckman Acta III spectrophotometer. Concentrations were determined using calibration curves established with standard solutions and expressed as $\mu\text{mol L}^{-1}$. Chlorophyll *a* concentrations were determined by filtering 100 mL of water through Whatman GF/A filters, followed by extraction with 90% acetone. Absorbance was measured spectrophotometrically at 665 and 750 nm before and after acidification, and chlorophyll *a* concentrations were expressed as mg m^{-3} .

Plastic debris sampling and plastisphere characterization

Sampling was conducted during morning hours (08:00–12:00). Plastic debris and water from the environment surrounding plastic debris were collected during each sampling campaign at the five sampling stations (S_1 – S_5). Plastic debris abundance was assessed using a semi-quantitative item-count approach. Plastic items were manually collected at each sampling station during each campaign and classified according to their type, including bottles, bags, fragments, caps, yogurt packaging, and PVC pipe fragments. Plastic abundance was expressed as the number of plastic items collected per station (items station⁻¹). This approach was used to compare the spatial and temporal distribution of plastic debris among the five sampling stations. Because the sampling areas were not standardized by surface area or plastic mass, the results represent item counts per station rather than area- or mass-based plastic density.

At each sampling campaign, environmental parameters, plastic debris, and water surrounding plastic debris were recorded at the five sampling stations. The sampling station was considered the observational unit for statistical analyses. Accordingly, the statistical dataset consisted of observations recorded at the five stations for each sampling campaign. No additional replicate observations were entered into the statistical analyses. The recorded observations were therefore compared among stations and sampling campaigns according to the statistical procedures described below.

In the laboratory, plastic surfaces were examined, and associated biological components and other materials were carefully collected using clean equipment. The collected material was transferred onto microscope slides and examined under an optical microscope at 40 $\times$ magnification for morphological characterization. Water samples were collected from the environment surrounding plastic debris using clean plastic containers. Plastic samples bearing associated biological material were preserved in 5% neutralized formalin until analysis. The biological components and other associated materials were identified and classified according to their morphological characteristics. These biological

and abiotic components observed in association with plastic surfaces were classified as plastisphere-associated components.

Laboratory characterization of plastic debris and plastisphere-associated components

In the laboratory, plastic debris surfaces and the material associated with them were examined to characterize plastisphere-associated components. Plastic items collected from the salt marsh environment, including bags, bottles, and other debris, were examined under a stereomicroscope. Material attached to the plastic surfaces was carefully removed and suspended in physiological saline solution (9‰ NaCl) to facilitate microscopic observation and morphological characterization. The composition of plastisphere-associated components was determined based on their morphological characteristics.

For plastic bottles, the recovered material was centrifuged at 5000–6000 rpm, and both the supernatant and pellet fractions were examined following the addition of physiological saline solution. The associated biological components were subsequently characterized. Microalgal forms morphologically resembling the genus *Dunaliella* and *Artemia*-like forms were recorded based on morphological characteristics observed under an optical microscope at 40× magnification. Species-level identification was not confirmed using molecular or culture-based methods; therefore, these components are referred to as *Dunaliella*-like microalgae and *Artemia*-like forms throughout the manuscript.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26. Descriptive statistics were used to summarize the physicochemical variables and plastisphere-associated components for each sampling station and sampling campaign. One-way analysis of variance (ANOVA) was used to evaluate differences in physicochemical variables among groups defined by the occurrence of plastic debris or plastisphere-associated components. ANOVAs were conducted separately for each sampling campaign and each component.

The statistical analyses were based on the observations recorded at the five sampling stations for each sampling campaign, with the station considered the observational unit. Statistical significance was set at $p \leq 0.05$. Results with $p < 0.01$ were considered highly significant. When the number of observations was insufficient or no within-group variance was available, the corresponding ANOVA could not be calculated and was not interpreted. All analyses were performed using IBM SPSS Statistics, and the complete statistical outputs are provided in Online Resource 2.

Results

Seasonal and spatial variation of physicochemical parameters

Seasonal and spatial variations in the main physicochemical parameters (temperature, salinity, dissolved oxygen, and pH) were monitored at the five sampling stations during the five sampling campaigns (Tab. 1). Water temperature exhibited a clear seasonal pattern, with minimum values recorded in winter and maximum values in summer. Salinity showed marked temporal and spatial variations among stations. Dissolved oxygen concentrations were highest in February 2022 and decreased during the summer period, whereas pH displayed moderate seasonal fluctuations.

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168 **Table 1** Seasonal variation of Temperature, Salinity, Dissolved oxygen and pH

Parameters Stations	Temperature (°C)					Salinity (psu)					Dissolved oxygen (mg L ⁻¹)					pH				
	Sep 21	Nov 21	Feb 22	May 22	Aug 22	Sep 21	Nov 21	Feb 22	May 22	Aug 22	Sep 21	Nov 21	Feb 22	May 22	Aug 22	Sep 21	Nov 21	Feb 22	May 22	Aug 22
S ₁	27.2± 1.88	18.8± 0.16	18.4± 0.29	22.56± 1.38	30.1± 3.81	242.8± 7.63	218.4± 24.09	246.5± 25.02	229.66± 15.10	231.6± 16.73	1.4± 0.08	1.13± 0.12	1.4± 0.016	1.46± 0.033	0.75± 0.03	8.45± 0.038	7.2± 0.01	7.8± 0.02	8.2± 0.009	7.6± 0.016
S ₂	25.8± 1.47	18.7± 0.30	17.6± 0.08	21.26± 0.71	30.46± 3.79	232.7± 8.31	215.5± 3.44	204.33± 18.73	255.66± 15.62	261.3± 8.05	3.95± 0.13	3.85± 0.05	3.98± 0.09	3.95± 0.04	0.85± 0.04	8.44± 0.04	7.4± 0.021	7.9± 0.008	7.7± 0.012	7.82± 0.024
S ₃	27.66± 2.62	19.2± 0.71	17.7± 0.20	23.23± 2.15	34.43± 0.54	336.6± 26.24	327.3± 20.80	305.83± 39.5	352.66± 21.31	368.7± 21.40	5.16± 0.06	5.35± 0.17	5.45± 0.20	5.41± 0.22	0.80± 0.05	7.96± 0.014	7.13± 0.016	7.4± 0.01	7.7± 0.02	7.87± 0.03
S ₄	28.1± 2.80	19.4± 0.64	17.7± 0.35	24.3± 2.82	34.83± 0.30	349.8± 21.43	349.6± 2.62	334.33± 45.05	357± 5.09	362± 10.70	1.18± 0.012	1.09± 0.008	5.41± 0.22	1.18± 0.021	0.97± 0.02	7.99± 0.032	7.14± 0.03	7.5± 0.026	7.8± 0.029	7.70± 0.012
S ₅	28.03± 2.43	18.7± 0.24	18.03± 0.04	23.33± 2.21	36.5± 2.19	176.2± 5.93	213.73± 18.20	178± 15.57	193.66± 9.67	191.6± 6.23	0.75± 0.03	0.85± 0.04	5.49± 0.008	0.85± 0.063	0.81± 0.028	8.59± 0.008	7.45± 0.024	7.8± 0.021	8.34± 0.03	7.79± 0.029

Seasonal and spatial variation of nitrogen nutrients

Seasonal and spatial variation of nitrogen nutrients

Mean ammonium (NH_4^+) concentrations showed marked spatial and seasonal variability throughout the study period (Fig. 2a). In September 2021, concentrations ranged from 0.517 ± 0.02 to $5.016 \pm 0.001 \mu\text{mol L}^{-1}$, with the highest value recorded at S_5 . Concentrations generally increased from November 2021 to May 2022, reaching their highest May values at S_1 and S_2 (4.760 ± 0.070 and $4.613 \pm 0.001 \mu\text{mol L}^{-1}$, respectively). Concentrations subsequently decreased in August 2022, with values ranging from 0.537 ± 0.008 to $1.403 \pm 0.004 \mu\text{mol L}^{-1}$.

Nitrite (NO_2^-) concentrations were relatively low in September 2021, ranging from 0.130 ± 0.003 to $0.617 \pm 0.001 \mu\text{mol L}^{-1}$ (Fig. 2b). Concentrations increased in November 2021, followed by a decline in February 2022 and relatively low values in May 2022. A marked increase was observed in August 2022, with the highest concentrations recorded at S_2 ($2.194 \pm 0.005 \mu\text{mol L}^{-1}$) and S_4 ($2.335 \pm 0.003 \mu\text{mol L}^{-1}$).

Nitrate (NO_3^-) concentrations ranged from 1.741 ± 0.002 to $6.512 \pm 0.003 \mu\text{mol L}^{-1}$ in September 2021 (Fig. 2c). Concentrations increased in November 2021, declined in February 2022, and showed marked spatial variability in May 2022. A pronounced increase was observed in August 2022, with the highest concentrations recorded at S_2 ($16.857 \pm 0.005 \mu\text{mol L}^{-1}$) and S_4 ($17.240 \pm 0.020 \mu\text{mol L}^{-1}$).

Mean total nitrogen (TN) concentrations ranged from 8.672 ± 0.001 to $14.980 \pm 0.005 \mu\text{mol L}^{-1}$ in September 2021 (Fig. 2d). Concentrations increased in November 2021, reaching the highest values at S_4 ($21.050 \pm 0.005 \mu\text{mol L}^{-1}$) and S_5 ($23.350 \pm 0.004 \mu\text{mol L}^{-1}$), followed by a decline in February 2022. Moderate spatial variation was observed in May 2022, whereas TN concentrations increased again in August 2022, reaching their highest values at S_2 ($23.413 \pm 0.004 \mu\text{mol L}^{-1}$) and S_4 ($24.955 \pm 0.002 \mu\text{mol L}^{-1}$).

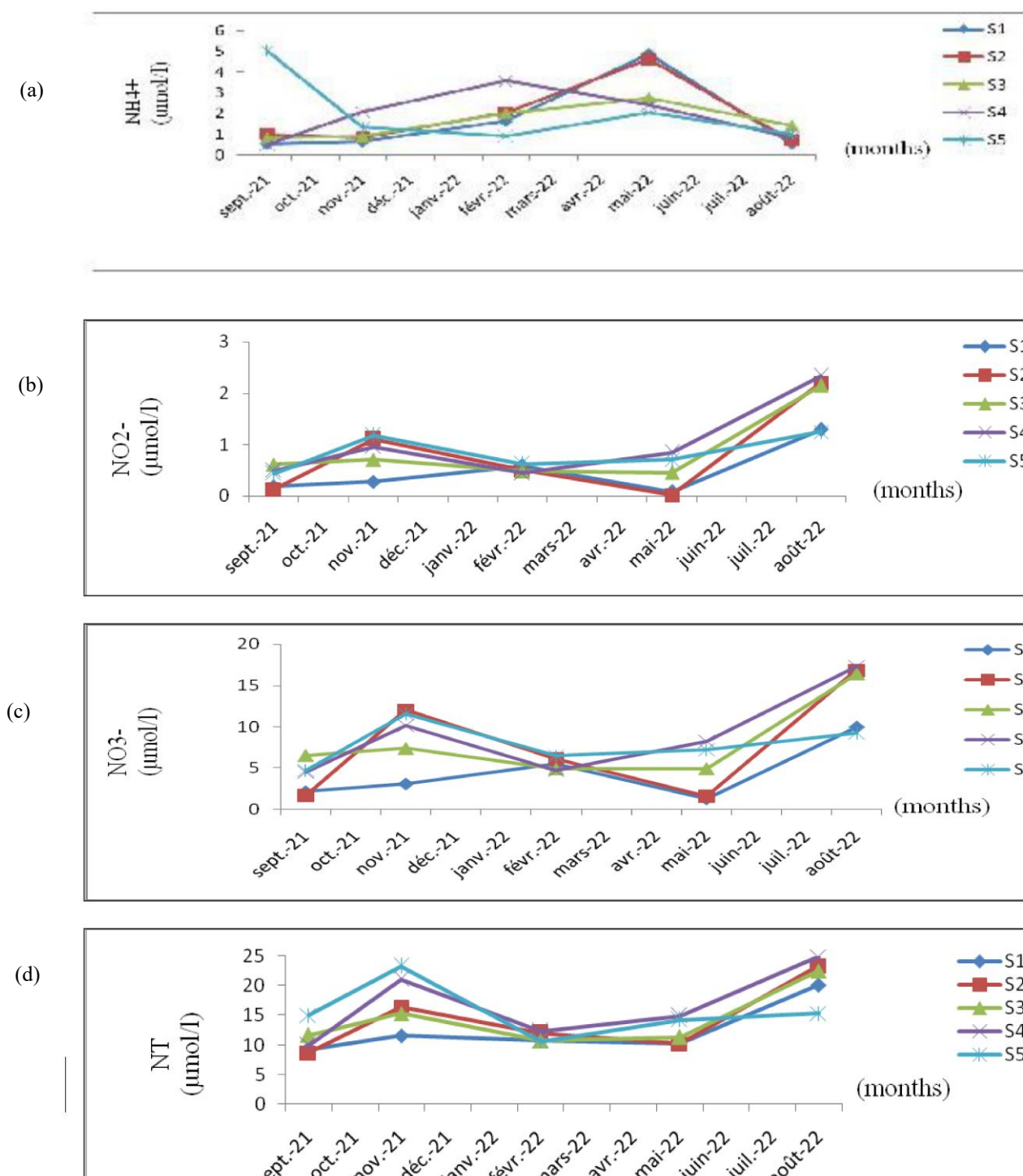


Fig. 2 Seasonal variation in nitrogenous nutrients (a) Ammonium (NH_4^+); (b) Nitrite (NO_2^-); (c), Nitrate (NO_3^-); (d) Total Nitrogen (TN)

Seasonal and spatial variation of phosphorus nutrients and chlorophyll *a*

Orthophosphate (PO_4^{3-}) concentrations were generally low in September 2021, ranging from 0.159 ± 0.003 to $0.260 \pm 0.005 \mu\text{mol L}^{-1}$, with the lowest and highest values recorded at S₃ and S₅, respectively (Fig. 3a). Concentrations increased

markedly in November 2021, reaching $2.452 \pm 0.001 \mu\text{mol L}^{-1}$ at S₂ and $2.583 \pm 0.004 \mu\text{mol L}^{-1}$ at S₅. Concentrations subsequently declined in February 2022 and remained relatively low in May 2022, with values ranging from 0.240 ± 0.002 to $0.735 \pm 0.003 \mu\text{mol L}^{-1}$. A pronounced increase was observed in August 2022, particularly at S₁ and S₂, where concentrations reached 4.130 ± 0.004 and $3.672 \pm 0.003 \mu\text{mol L}^{-1}$, respectively.

Total phosphate (TP) concentrations also showed marked seasonal and spatial variability throughout the study period (Fig. 3b). In September 2021, concentrations ranged from 1.992 ± 0.003 to $2.855 \pm 0.001 \mu\text{mol L}^{-1}$, with the lowest value at S₃ and the highest at S₁. Concentrations increased substantially in November 2021, reaching their highest values at S₅ ($18.983 \pm 0.006 \mu\text{mol L}^{-1}$) and S₄ ($11.321 \pm 0.003 \mu\text{mol L}^{-1}$). In February 2022, TP concentrations remained relatively high, particularly at S₅ ($11.571 \pm 0.007 \mu\text{mol L}^{-1}$) and S₃ ($9.992 \pm 0.001 \mu\text{mol L}^{-1}$), followed by a decline in May 2022. In August 2022, concentrations increased again, reaching their highest values at S₂ ($21.202 \pm 0.005 \mu\text{mol L}^{-1}$) and S₁ ($18.54 \pm 0.0012 \mu\text{mol L}^{-1}$).

Chlorophyll *a* concentrations showed comparatively moderate spatial and seasonal variability during the study period (Fig. 3c). In September 2021, concentrations ranged from 2.050 ± 0.002 to $3.574 \pm 0.003 \text{ mg m}^{-3}$, with the highest value recorded at S₃. Concentrations decreased in November 2021, reaching their lowest value at S₂ ($1.743 \pm 0.001 \text{ mg m}^{-3}$). In February 2022, chlorophyll *a* concentrations increased at most stations, with the highest value recorded at S₅ ($3.054 \pm 0.0033 \text{ mg m}^{-3}$). In May 2022, concentrations ranged from 2.014 ± 0.002 to $2.708 \pm 0.003 \text{ mg m}^{-3}$, with the highest value at S₁. A slight increase was observed at most stations in August 2022, with concentrations reaching 3.012 ± 0.005 and $2.992 \pm 0.006 \text{ mg m}^{-3}$ at S₁ and S₂, respectively.

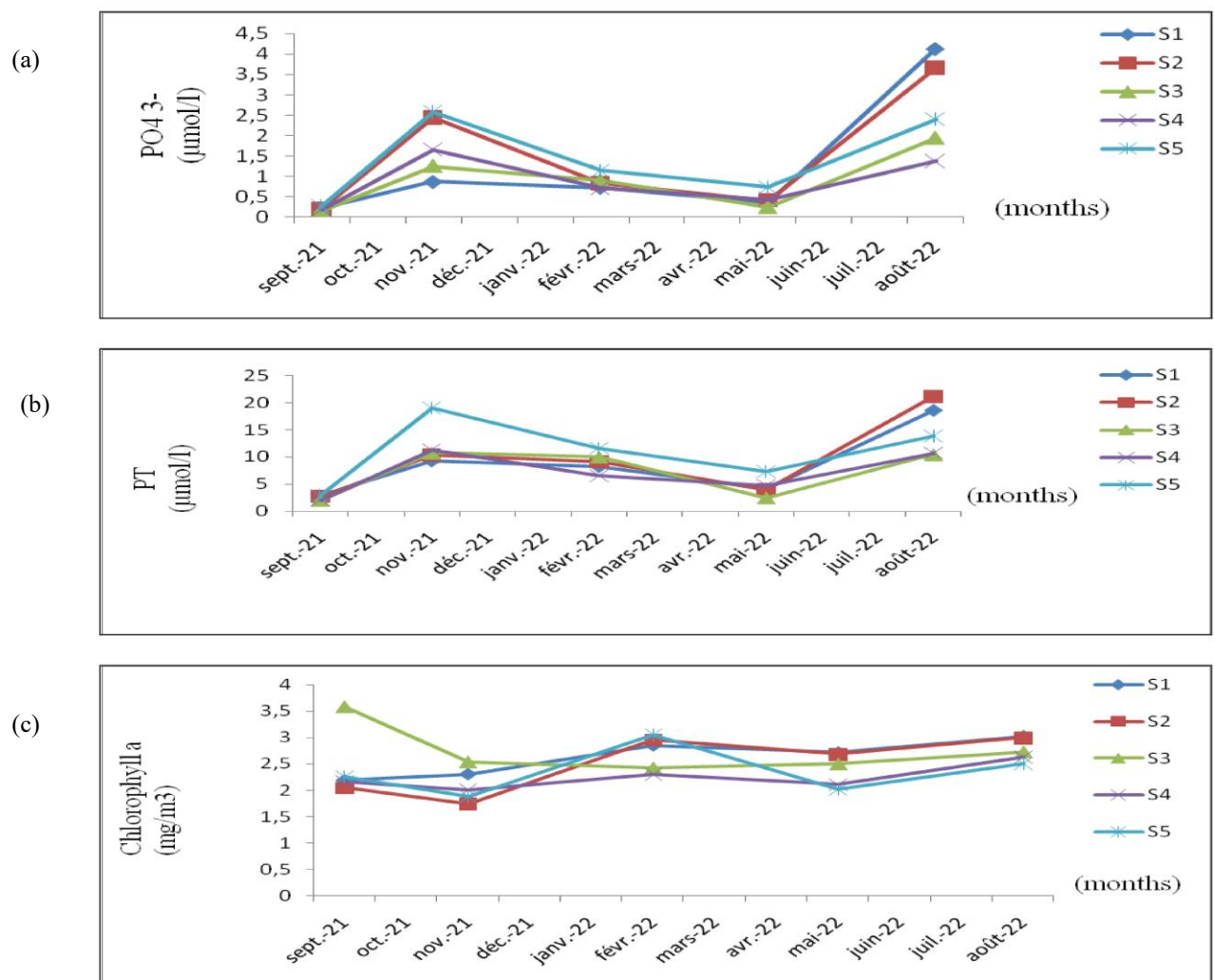


Fig. 3 Seasonal fluctuations in phosphorus nutrient and biomass (a) Orthophosphate (PO_4^{3-}); (b) Total Phosphorus (TP); (c) Chlorophyll *a*

Seasonal and spatial distribution of plastic debris

Abundance

Plastic debris abundance exhibited marked seasonal and spatial variations across the five sampling stations (Fig. 4). Plastic bottles, bags, and fragments were the dominant debris categories throughout the study period. Station S5 consistently exhibited the highest plastic debris abundance, ranging from 57.3 ± 3.01 items station⁻¹ in February 2022 to 93.0 ± 1.77 items station⁻¹ in August 2022. In contrast, the lowest abundances were recorded at S₃ and S₄, where no plastic debris was detected in February and May 2022. Intermediate levels were observed at S₁ and S₂, whereas other plastic items, including yogurt packaging and PVC pipe fragments, occurred sporadically and in low quantities.

One-way ANOVA showed no significant associations in the examined physicochemical parameters among groups defined by plastic bottle observations during most sampling periods (Table 7). An exception was observed for dissolved oxygen in August 2022 ($F = 357.000$, $p = 0.039$). Yogurt packaging was significantly associated with temperature in August 2022 ($F = 53.363$, $p = 0.018$), salinity and pH ($F = 44.367$, $p = 0.022$; $F = 182.416$, $p = 0.005$ respectively) in September 2021.

Plastic bags and fragments showed no significant associations with the examined physicochemical parameters. PVC observations likewise showed no significant associations with temperature ($p = 0.473\text{--}0.766$), pH ($p = 0.165\text{--}0.786$), salinity ($p = 0.304\text{--}0.963$), or dissolved oxygen ($p = 0.054\text{--}0.607$). The closest result to significance occurred for dissolved oxygen and PVC in February 2022 ($F = 9.545$, $p = 0.054$), but remained non-significant. Comparisons between temperature and plastic bottles could not be evaluated in November 2021 and February 2022 because no within-group variance was observed. Similarly, the comparison between temperature and yogurt packaging could not be evaluated in November 2021 for the same reason. Further statistical details are provided in Online Resource 2.

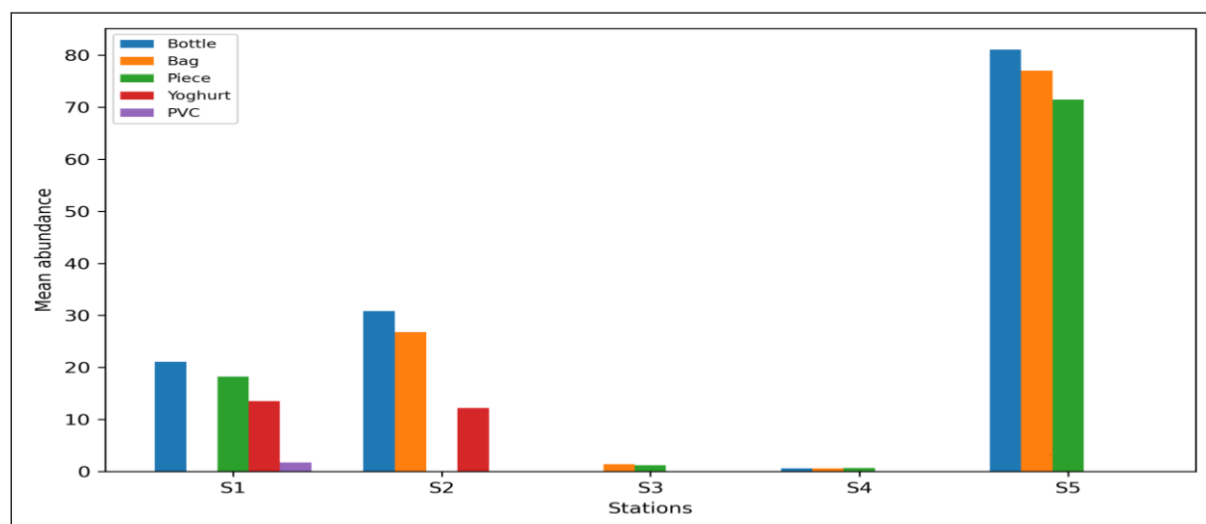


Fig. 4 Seasonal and spatial variation in plastic debris observations

Characterization of the components associated with the water surrounding plastic debris

The components associated with the environment surrounding plastic debris exhibited marked spatial and seasonal variations (Fig. 5). *Artemia*-like forms and insect fragments were the dominant components at S₁, S₂, and particularly S₅, where the highest numbers of *Artemia*-like forms ranged from 80.0 ± 4.10 to 98.56 ± 1.81 observations station⁻¹ during the study period. Salt crystals predominated at the salt-pan stations (S₃ and S₄), with the highest numbers recorded in May and August 2022, reaching 90 and 95 observations station⁻¹ at S₃ and 95 and 97 observations station⁻¹ at S₄, respectively. In the surrounding water, *Dunaliella*-like microalgae were detected only at S₁, whereas plant fibres occurred sporadically at S₂ and S₃. Insect fragments were detected at all stations throughout the study period.

One-way ANOVA showed significant associations in selected physicochemical parameters among groups defined by components observed in the water surrounding plastic debris (Table 8). For *Artemia*-like forms, significant associations were recorded with pH in September 2021 ($F = 42.225$, $p = 0.023$) and February 2022 ($F = 23.032$, $p = 0.042$) and with salinity in May 2022 ($F = 28.081$, $p = 0.034$). Insect fragments were significantly associated with pH in May 2022 ($F = 24.592$, $p = 0.039$) and with dissolved oxygen in November 2021 ($F = 21.408$, $p = 0.045$) and August 2022 ($F = 24.935$, $p = 0.039$). Salt crystals showed significant associations with temperature in August 2022 ($F = 53.363$, $p = 0.018$) and with salinity ($F = 44.367$, $p = 0.022$) and pH ($F = 182.416$, $p = 0.005$) in September 2021. No significant associations were found for *Dunaliella*-like microalgae or plant fibres ($p > 0.05$). Comparisons between temperature and *Artemia*-like forms

could not be evaluated in November 2021 and February 2022, while the comparison between temperature and salt crystals could not be evaluated in November 2021, because no within-group variance was observed. Further statistical details are provided in Online Resource 2.

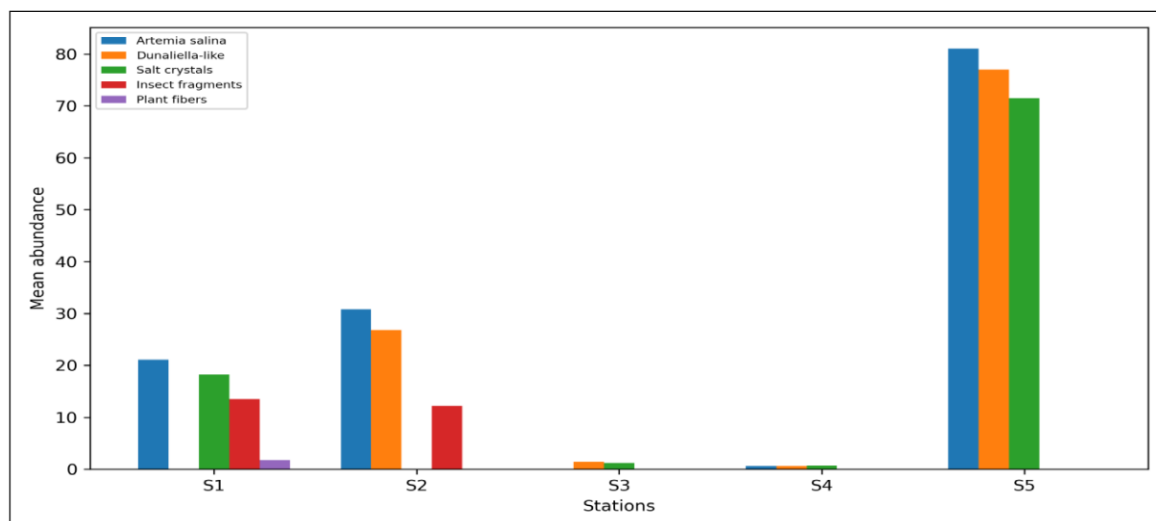


Fig. 5 Seasonal and spatial variation in biological and abiotic components associated with the water surrounding plastic debris

Composition of plastisphere-associated components

The composition of plastisphere-associated components exhibited marked spatial and seasonal variations across the five sampling stations (Fig. 6). The number of observations of *Artemia*-like forms, insect fragments, salt crystals, and plant fibres were the dominant plastisphere-associated components. The highest numbers of *Artemia*-like forms and other plastisphere-associated components were recorded at S₂ (24.2 ± 2.43 – 83.53 ± 1.79 observations per station) and S₅ (24.5 ± 0.62 – 98.0 ± 2.82 observations per station). On plastisphere-associated components, *Dunaliella*-like microalgae were detected at S₃ and S₄, with the highest abundance recorded at S₄ in August 2022 (88.3 ± 1.08 observations per station).

One-way ANOVA revealed significant associations in selected physicochemical parameters among groups defined by plastisphere-associated components (Table 9). Significant differences in pH and salinity ($F = 182.416$, $p = 0.005$; $F = 44.367$, $p = 0.022$ respectively) in September 2021 and temperature in August 2022 ($F = 53.363$, $p = 0.018$) were detected among groups defined by *Artemia*-like forms. For *Dunaliella*-like microalgae, a highly significant association with dissolved oxygen was detected in February 2022 ($F = 160.000$, $p = 0.006$), while plant fibres showed a significant difference in relation to salinity in August 2022 ($F = 41.390$, $p = 0.024$). In contrast, none of the examined physicochemical parameters differed significantly among groups characterized by salt crystals or insect fragments ($p > 0.05$). In November 2021, ANOVA could not be performed to assess the association between temperature and the occurrence of *Artemia*-like forms and insect fragments because no within-group variance was observed. Further statistical details are provided in Online Resource 2.

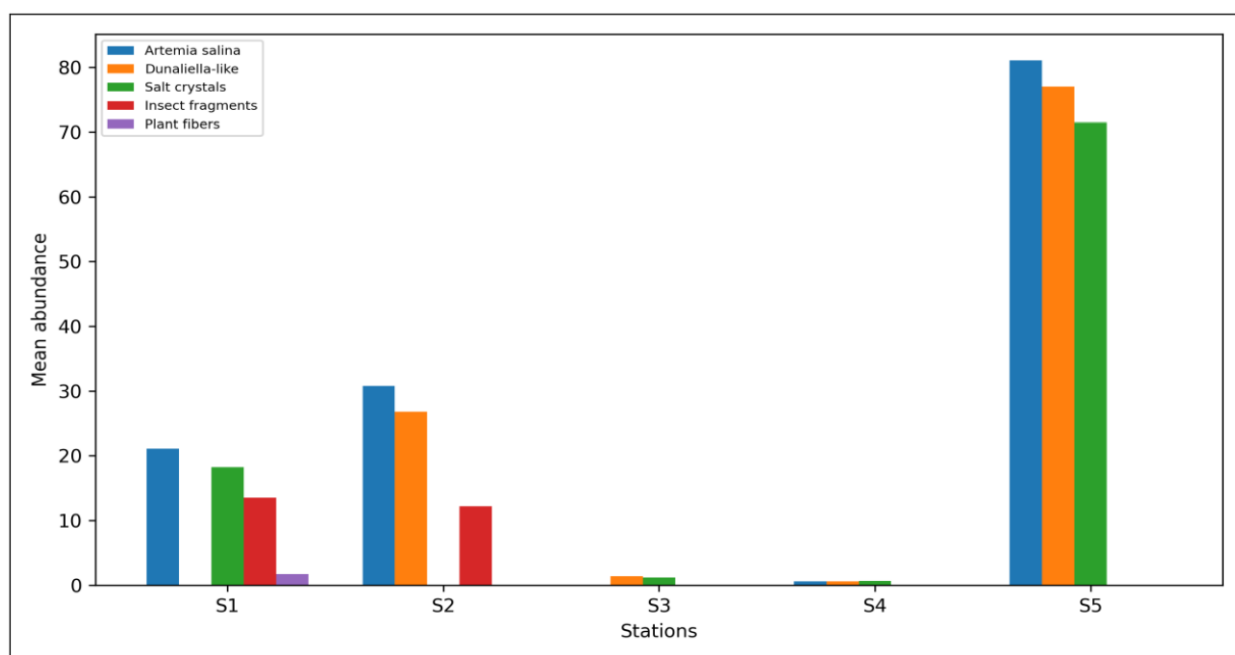
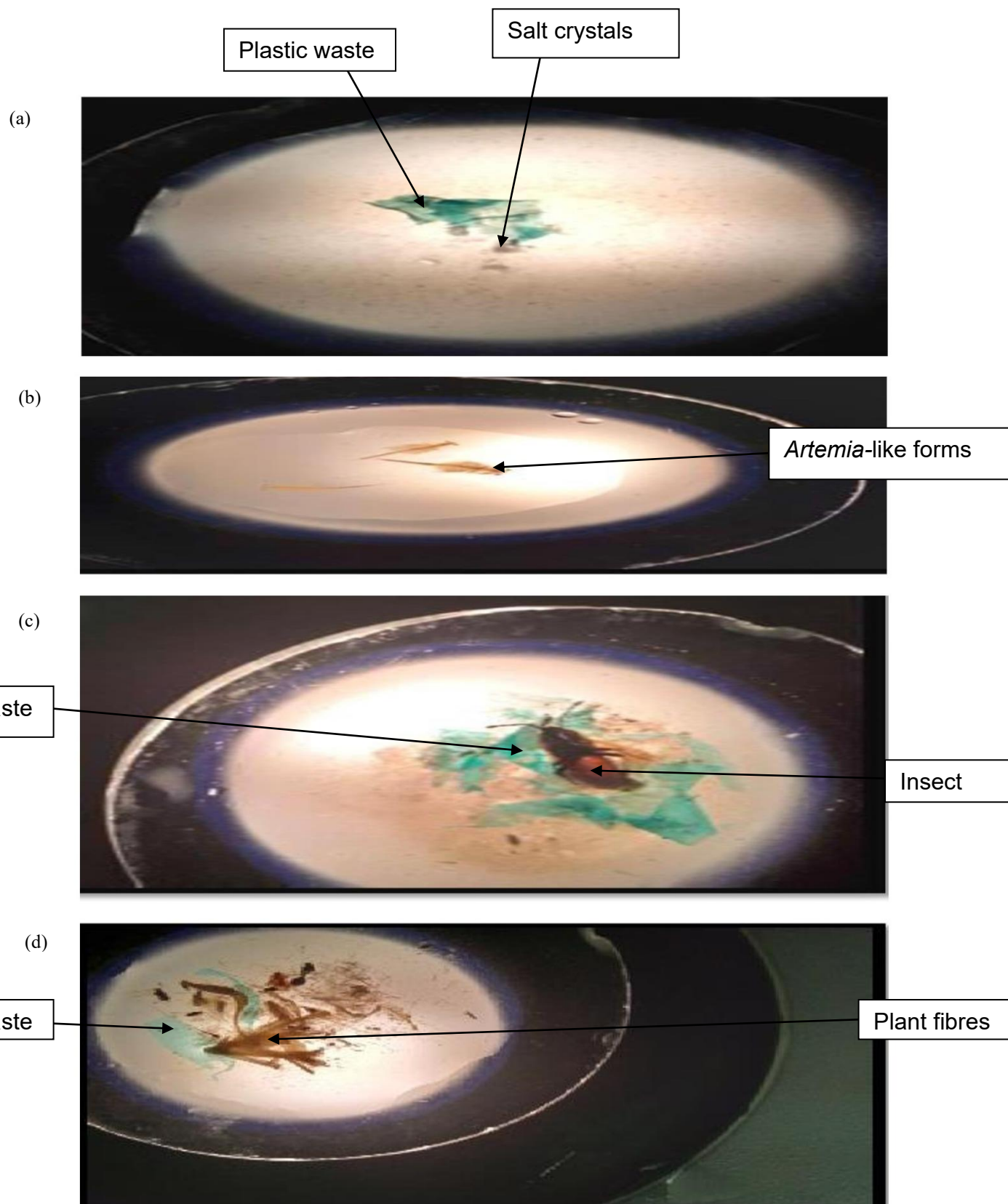


Fig. 6 Spatial and temporal distribution of plastsphere-associated components at the five sampling stations during the study period

Detailed quantitative data supporting the results presented in Figs. 2–6 are provided in Online Resource 1.

Representative microscopic observations of plastsphere-associated components and biological and mineral components in the water surrounding plastic debris are shown in (Fig. 7).



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285 **Fig. 7** Microscopic observations of plastsphere-associated components and biological and mineral components in the water
 286 surrounding plastic debris (a) plastic debris and salt crystals; (b) *Artemia*-like forms; (c) insect fragments; (d) plant fibres

Relationships between environmental variables, plastic debris, components associated with the water surrounding plastic debris and plastisphere-associated components

Table 7 Summary of one-way ANOVA results for the relationships between plastic debris types and physicochemical parameters

Variable	Temperature	Salinity	Dissolved oxygen	pH
Plastic bottles	Ns+ Nc (Nov+ Feb)	Ns	* (Aug)	Ns
Plastic bags	Ns	Ns	Ns	Ns
Plastic fragments	Ns	Ns	Ns	Ns
Yogurt packaging	* (Aug)+Nc (Nov)	* (Sep)	Ns	** (Sep)
PVC	Ns	Ns	Ns	Ns

Note: * = significant association detected in at least one sampling campaign $p < 0.05$

** = significant association detected in at least one sampling campaign $p < 0.01$

Ns = no significant association detected in any sampling campaign / Nc= not calculable

Table 8 Summary of one-way ANOVA results for the relationships between physicochemical parameters and components associated with the water surrounding plastic debris

Variable	<i>Artemia</i> -like forms	<i>Dunaliella</i> like-microalgae	Salt crystals	Insect fragments	Plant fibres
Temperature	Ns+ Nc (Nov+Feb)	Ns	* (Aug) + Nc (Nov)	Ns	Ns
Salinity	* (May)	Ns	* (Sep)	Ns	Ns
Dissolved oxygen	Ns	Ns	Ns	* (Nov+Aug)	Ns
pH	* (Sep+Feb)	Ns	** (Sep)	* (May)	Ns

Note: * = significant association detected in at least one sampling campaign $p < 0.05$

** = significant association detected in at least one sampling campaign $p < 0.01$

Ns = no significant association detected in any sampling campaign / Nc= not calculable

Table 9 Summary of one-way ANOVA results for the relationships between physicochemical parameters and plastisphere-associated components

Variable	Temperature	Salinity	Dissolved oxygen	pH
<i>Artemia</i> -like forms	* (Aug)+ Nc (Nov)	* (Sep)	Ns	** (Sep)
<i>Dunaliella</i> -like microalgae	Ns	Ns	** (Feb)	Ns
Salt crystals	Ns	Ns	Ns	Ns
Insect fragments	Ns+ Nc (Nov)	Ns	Ns	Ns
Plant fibres	Ns	* (Aug)	Ns	Ns

Note: * = significant association detected in at least one sampling campaign $p < 0.05$

** = significant association detected in at least one sampling campaign $p < 0.01$

Ns = no significant association detected in any sampling campaign / Nc = not calculable

Complete results of the statistical analyses examining the relationships between environmental factors, plastic debris, biological components associated with plastic debris in the surrounding waters, and plastisphere-associated components are provided in Online Resource 2.

Discussion

Physicochemical conditions

The Sahline Salt Marsh exhibited pronounced spatial and seasonal variability in its physicochemical and biological conditions, reflecting the dynamic characteristics of hypersaline ecosystems. Seasonal increases in temperature were accompanied by elevated salinity and reduced dissolved oxygen concentrations, whereas winter conditions were characterized by lower temperatures and greater oxygen availability. These patterns are consistent with the combined effects of intense evaporation, restricted water renewal, and the lower solubility of oxygen at higher temperatures, which are characteristic features of Mediterranean hypersaline wetlands. The physicochemical conditions recorded in the present study were generally consistent with those previously reported for Tunisian salt marshes. Temperature and pH values were comparable to those reported by Trigui et al. (2016), whereas the higher salinity values recorded in the present study support the highly hypersaline conditions prevailing in the Sahline Salt Marsh. In contrast, dissolved oxygen concentrations were lower than those reported for Sebkha El Adhibet in southern Tunisia (Ben Naceur et al., 2009), where lower salinity and higher phytoplankton biomass may have favoured greater oxygen availability.

These environmental conditions are likely to influence the composition and distribution of hydrobiological communities. The occurrence of halotolerant microalgae, including *Dunaliella*-like forms, is consistent with the ability of this group to tolerate highly saline conditions. Similarly, Masmoudi (2014) reported that the photosynthetic activity of *Dunaliella salina* and the cyanobacterium *Phormidium versicolor* was less affected by high salinity and intense light than that of the diatom *Cylindrotheca closterium*, highlighting the ecological advantage of halotolerant microorganisms under hypersaline conditions. Such physiological adaptations may help explain the occurrence of *Dunaliella*-like microalgae in hypersaline environments such as the Sahline Salt Marsh, although the *Dunaliella*-like forms observed in the present study should not be interpreted as a confirmed identification of *Dunaliella salina*.

Nutrient dynamics

The relatively high concentrations of some nitrogen and phosphorus compounds observed during the study indicate that the Sahline Salt Marsh remains a nutrient-rich environment despite its hypersaline conditions. Seasonal fluctuations in nutrient concentrations are likely associated with changes in hydrological conditions, biological activity, and nutrient recycling within the salt marsh. The marked increases in nitrate, total nitrogen, and total phosphorus observed during specific sampling periods may reflect enhanced mineralization of organic matter and reduced biological uptake under unfavourable environmental conditions, whereas subsequent decreases may have resulted from increased assimilation by primary producers.

Overall, the nutrient dynamics observed in the present study are broadly consistent with those previously reported for the Sahline Salt Marsh by Trigui et al. (2016). The persistence of relatively high nutrient concentrations may contribute to sustaining the productivity of halophilic communities, including microalgae, which constitute important primary producers in the salt-marsh food web. This nutrient availability may also indirectly support the development of biological communities associated with plastic debris by providing resources for primary producers and microorganisms colonizing plastic surfaces.

Phytoplankton biomass

The relatively high chlorophyll *a* concentrations recorded during the study indicate that the Sahline Salt Marsh supports relatively high phytoplankton biomass under hypersaline conditions. This relatively high biomass may be related to the availability of nitrogen and phosphorus compounds, which can provide favourable conditions for primary production. Seasonal fluctuations in chlorophyll *a*, characterized by increases during winter and late summer and lower concentrations during autumn, suggest that phytoplankton biomass may reflect seasonal variations in nutrient availability and physicochemical conditions.

Compared with the lower chlorophyll *a* concentrations reported for the hypersaline Lake Urmia, Iran (0.46 ± 0.20 – 2.40 ± 0.20 mg m⁻³, equivalent to $\mu\text{g L}^{-1}$) by Dahesht et al. (2013), the concentrations observed in the present study were within a relatively elevated range compared with those reported for some other hypersaline systems. The relatively high phytoplankton biomass may be supported by nutrient availability and the presence of halotolerant microalgae, particularly *Dunaliella*-like microalgae, which were identified morphologically in the present study and resemble members of the genus *Dunaliella*. Members of this genus have been reported to dominate the phytoplankton community of Lake Urmia under hypersaline conditions (Dahesht et al., 2010). Consequently, phytoplankton production may contribute to maintaining ecosystem productivity and may indirectly support the development of biological communities associated with plastic debris and plastisphere-associated components.

Plastic debris distribution and physicochemical associations

The spatial and seasonal distribution of plastic debris in the Sahline Salt Marsh suggests that plastic accumulation may result from the combined influence of local environmental conditions, hydrodynamic connectivity, and anthropogenic inputs. The predominance of plastic bottles, bags, fragments, caps, and yogurt packaging observed in the present study is broadly consistent with observations from Tunisian coastal environments, where plastic and packaging materials constitute major components of marine litter (Baccar-Chaabane et al., 2024). The highest accumulation recorded at station S₅ may reflect the greater connectivity of this station with the marine environment and its potential exposure to externally transported debris. However, because hydrodynamic processes and direct plastic inputs were not measured in the present study, these mechanisms should be considered plausible explanations rather than demonstrated causes. In contrast, the absence of plastic debris at the salt-pan stations during winter and late spring may be related to differences in hydrological connectivity, local retention conditions, and seasonal variations in plastic inputs. Overall, these spatial and seasonal patterns indicate that plastic accumulation within the salt marsh is heterogeneous and may reflect interactions among environmental variability, transport and retention processes, and local anthropogenic inputs.

One-way ANOVA identified significant associations in selected physicochemical parameters among groups defined by the most plastic debris categories. Plastic bottles showed no significant associations with temperature, salinity, or pH, whereas a significant association with dissolved oxygen was detected in August 2022. This isolated result should be interpreted cautiously because it occurred during a single sampling campaign and does not demonstrate a causal influence of dissolved oxygen on plastic bottle occurrence. Yogurt packaging showed significant associations with temperature (August 2022), salinity and pH (September 2021) during selected sampling periods, suggesting that its occurrence varied under particular physicochemical conditions. However, these associations may also reflect the spatial and seasonal distribution of this plastic category within the salt marsh rather than a direct environmental influence. The absence of significant associations for plastic bags, plastic fragments, and PVC further indicates that no consistent relationship was observed across plastic types. Overall, the ANOVA results suggest that the distribution of most plastic debris categories was not consistently related to the measured physicochemical conditions. Their distribution may instead be related to external inputs, transport, and retention processes, which were not directly quantified in this study. Nevertheless, this interpretation remains tentative because hydrodynamic transport, residence time, and source-specific inputs were not directly assessed.

The predominance of consumer-related plastic items observed in the present study is consistent with previous surveys conducted in Tunisia and neighbouring Mediterranean coastal environments. Chaabane and Chaabane-Baccar (2019) reported a substantial contribution of plastic debris, including consumer-related items, during surveys conducted on four Tunisian beaches. Similarly, field surveys conducted in the Kerkennah Islands in 2019 documented plastic as the dominant waste category, with bottles, caps, carrier bags, and fishing-related plastic items among the frequently observed debris (Chaabane, 2020). More recent monitoring of Tunisian coastal areas has likewise reported a high contribution of plastic and packaging materials to marine litter, with tourism and recreational activities identified as potential sources of contamination (Baccar-Chaabane et al., 2024). These observations are consistent with the present findings and suggest that consumer-related plastic waste represents an important component of plastic contamination along Tunisian coastal and wetland environments. Comparable observations have also been reported from the Gulf of Annaba, Algeria, where plastic packaging and bottles represented a major proportion of recorded macro-litter (Belabed et al., 2019).

Taken together, these findings support the importance of anthropogenic plastic inputs in coastal and hypersaline environments, while also highlighting the potential role of spatial heterogeneity, hydrodynamic connectivity, transport, and retention processes in determining plastic accumulation. However, the relative contribution of individual sources and transport mechanisms could not be determined in the present study and requires further investigation using direct measurements of hydrodynamic processes, plastic residence time, and source-specific inputs.

Components associated with the water surrounding plastic debris

The biological communities associated with the water surrounding plastic debris were dominated by halophilic organisms, particularly *Artemia*-like forms and insect fragments. The highest numbers of *Artemia*-like forms are consistent with the strong tolerance of this genus to hypersaline environments, where elevated salinity can limit the occurrence of many other aquatic organisms. The higher abundances observed during the warmer months further suggest that seasonal changes in temperature, together with salinity, may influence the development and distribution of these communities. Such seasonal patterns are also relevant to plastic-associated components in transitional environments. For example, a one-year study

conducted in a subtropical estuary showed that season had a stronger influence on plastisphere composition than polymer type or incubation time, with significant seasonal changes observed in bacterial, fungal, and eukaryotic communities (Sérvulo et al., 2023).

The significant differences detected among groups characterized by the occurrence of *Artemia*-like forms in relation to pH in September 2021 and February 2022 and salinity in May 2022 suggest that *Artemia*-like forms varied across distinct physicochemical conditions. Given the strong tolerance of *Artemia*-like forms to hypersaline environments, these relationships are ecologically plausible; however, the present ANOVA results indicate statistical differences between groups rather than causal effects. These findings suggest that one-way ANOVA identified significant associations in selected physicochemical parameters among groups defined by some components associated with plastic debris in the surrounding water, particularly temperature, salinity, and pH, but these statistical associations should not be interpreted as evidence of direct effects. Insect fragments also showed significant associations in relation to dissolved oxygen in November 2021 and August 2022 and to pH in May 2022, suggesting that their occurrence varied under specific local physicochemical conditions. Salt-crystal groups showed significant associations in relation to temperature in August 2022 and to salinity and pH in September 2021. These relationships are consistent with the strong influence of evaporation and physicochemical variability on salt precipitation in hypersaline environments, although causality cannot be established from the present analysis. In contrast, no significant associations were detected between the measured physicochemical variables and *Dunaliella*-like microalgae or plant fibres. Notably, *Dunaliella*-like microalgae were detected only at S₁ in the surrounding water, whereas they were recorded on plastisphere-associated material mainly at S₄, indicating that their distribution differed between the surrounding water and plastic surfaces. Overall, the results suggest component-specific associations between the physicochemical conditions of the surrounding water and the biological and mineral components observed around plastic debris. The relatively small number of observations available for some comparisons and the absence of within-group variance in selected analyses further limit the strength of ecological inference. Plastic surfaces may provide artificial substrates for the attachment and persistence of organisms originating from the surrounding environment. This process has also been documented in salt-marsh environments, where microorganisms from the surrounding sediment rapidly colonized different types of microplastics and the composition of the plastisphere changed dynamically over a one-year incubation period (Rosato et al., 2022).

In contrast, the relatively limited occurrence of *Dunaliella*-like microalgae in the surrounding water should not necessarily be interpreted as evidence of a minor ecological role. *Dunaliella*-like microalgae are characteristic components of hypersaline environments and can become the main, or even the sole, primary producer at very high salinities (Oren, 2014). Therefore, its relatively low abundance in the present samples may instead reflect spatial or temporal heterogeneity in its distribution, differences in local habitat conditions, or the availability of suitable substrates. Similarly, the absence of significant relationships between the measured environmental variables and *Dunaliella*-like microalgae and plant fibres suggests that their distribution may be influenced by additional ecological processes, including local habitat characteristics, hydrodynamic transport, substrate availability, or biological interactions. These factors were not investigated in the present study and should be considered in future investigations.

Plastisphere-associated components

The plastisphere-associated components developing on the surface of plastic debris comprised a diverse assemblage of halophilic or halotolerant organisms, biological residues, and abiotic components. The highest abundances of *Artemia*-like forms, insect fragments, salt crystals, and plant fibres, particularly at stations S₂ and S₅, indicate spatial variability in plastisphere-associated components under contrasting local environmental conditions. The occurrence of *Artemia*-like forms and other biological components at these stations may be related to the availability of organic matter and the physicochemical characteristics of the surrounding environment. In contrast, the more restricted presence of *Dunaliella*-like microalgae, mainly at station S₄, indicates that microalgal occurrence on plastic surfaces was spatially heterogeneous and may vary with local environmental conditions. Notably, *Dunaliella*-like microalgae showed contrasting distributions between the surrounding water and plastisphere-associated material, being detected mainly at S₁ in the former and at S₄ in the latter. This spatial difference suggests that their occurrence may vary between the surrounding-water compartment and plastic-associated surfaces.

One-way ANOVA identified significant associations in selected physicochemical parameters among groups defined by plastisphere-associated components. As shown in Table 9, *Artemia*-like forms were significantly associated with temperature (August 2022) and salinity (September 2021) and showed a highly significant association with pH (September 2021). *Dunaliella*-like microalgae showed a highly significant association with dissolved oxygen (February 2022), whereas plant fibres were significantly associated with salinity in August 2022. In contrast, no significant associations were detected between the measured physicochemical parameters and salt crystals or insect fragments. These results indicate that different plastisphere-associated components may respond differently to local physicochemical variability. The significant associations observed for *Artemia*-like forms are consistent with their ability to persist under broad fluctuations in hypersaline environments, although these statistical relationships do not demonstrate direct causal effects. Similarly, the association between dissolved oxygen and *Dunaliella*-like microalgae may indicate sensitivity to local environmental conditions, but cannot be interpreted as evidence of a direct effect. Because plastisphere-associated components were characterized primarily from morphological observations and the statistical analyses were based on a limited number of observations, these relationships should therefore be considered indicative. Further investigations using standardized plastic surface-area measurements and taxonomic or molecular approaches would help determine whether these patterns reflect environmental conditions or differences in colonization and retention on plastic surfaces.

These findings are consistent with previous studies describing plastic debris as a novel ecological substrate that can support the development of biological communities and biofilms (Zettler et al., 2013; Bryant et al., 2016; Dussud et al., 2018a; Pinto et al., 2019; De Tender et al., 2017; Jacquin et al., 2019). Previous investigations have shown that plastisphere composition and structure vary according to geographical location, seasonality, habitat characteristics, and environmental conditions (Oberbeckmann et al., 2014; Amaral-Zettler et al., 2015; Basili et al., 2020). More recent studies have reinforced these observations, reporting spatial and temporal variability in plastisphere-associated components along environmental gradients, including in salt-marsh and saline environments (Rosato et al., 2022; Meng et al., 2024; Yuan et al., 2024; Frias et al., 2025; Philip et al., 2025; Pinheiro et al., 2026). Studies conducted along freshwater–brackish–marine gradients have also identified salinity as an important factor associated with changes in plastisphere composition, while algae may contribute to the stability and functioning of plastic-associated communities under saline conditions (Chen et al., 2025). The spatial differences observed among the five sampling stations in the present study are consistent with these findings and suggest that local physicochemical conditions may contribute to variation in plastisphere-associated components in the hypersaline Sahline Salt Marsh. The occurrence of *Dunaliella*-like microalgae, particularly

at station S₄, is consistent with the capacity of halotolerant microalgae to persist under highly saline conditions. Experimental studies on *Dunaliella salina* have further shown that interactions with microplastics can influence algal growth and metabolic responses (Wang et al., 2023; Xiao et al., 2026). However, the occurrence of *Artemia*-like forms on plastic debris in the present study appears to be less documented in the plastisphere literature and may represent a noteworthy feature of hypersaline environments. Overall, the findings suggest that plastisphere-associated components are spatially variable and may be associated with local salinity, temperature, pH, dissolved oxygen, habitat characteristics, and biological colonization.

The observed assemblage suggests that plastic debris can provide a substrate for a diverse set of organisms and inorganic materials under hypersaline conditions. However, plastisphere characterization in the present study was based on optical microscopy; therefore, the observed organisms should be regarded as morphologically identified forms, with *Dunaliella*-like microalgae described at the morphotype level rather than as confirmed species-level identifications. In addition, the item-count approach provides a semi-quantitative assessment of plastisphere-associated components and does not allow direct comparison of abundance per unit plastic surface area or mass. Further molecular and quantitative approaches would therefore be useful to resolve the taxonomic and functional diversity of the plastisphere in this hypersaline environment.

Ecological relevance of plastic debris

Previous studies have demonstrated that microplastics can be ingested by planktonic organisms and may subsequently contribute to the transfer of plastic particles through aquatic food webs (Kedzierski, 2017; Chaabane and Chaabane-Baccar, 2019; Chaabane, 2020). Plastic ingestion may also cause physical effects, including digestive obstruction, reduced growth and impaired mobility, depending on the type, size and quantity of particles ingested (Van Franeker, Law, 2015; Chaabane, 2020).

Beyond these direct effects, plastic debris provides artificial surfaces that can be rapidly colonized by microorganisms and other organisms, resulting in the formation of a plastisphere-associated component. Biofilm formation may modify the chemical properties of plastic surfaces and influence their interactions with aquatic organisms. For example, plastic colonized by algae may produce chemical cues resembling natural food sources, potentially increasing plastic ingestion by fish (Chaabane and Chaabane-Baccar, 2019; Pösel et al., 2018). Thus, plastisphere-associated components may contribute to additional biological interactions beyond the physical persistence of plastic debris.

In the Sahline salt marsh, the occurrence of *Artemia*-like forms, insect fragments, plant fibres, salt crystals and *Dunaliella*-like microalgae associated with plastic debris indicates that plastic surfaces can serve as additional substrates for biological colonization under hypersaline conditions. The observed plastisphere-associated components therefore suggest potential interactions between plastic debris and the local biota, including possible effects on the distribution and transport of associated organisms. However, these observations do not demonstrate direct ecological impacts, and further taxonomic, molecular and functional investigations are required to determine the ecological significance of these plastic-associated components. Although these findings highlight the potential ecological relevance of plastic-associated communities in the salt marsh, the present study did not directly demonstrate adverse effects of plastic debris on the organisms or ecosystem processes.

More broadly, the presence of plastic debris in aquatic environments may also raise concerns regarding the transfer of plastic-associated contaminants and potential exposure through aquatic food webs (Baechler et al., 2020). However, these pathways were not directly investigated in the present study. The ecological relevance of plastic pollution in the Sahline salt marsh should therefore be considered in relation to the observed plastic abundance and colonization patterns, while further studies are needed to assess trophic transfer, contaminant accumulation and potential consequences for ecosystem functioning.

Study limitations and future perspectives

Several methodological limitations should be considered when interpreting the present findings. First, plastic debris abundance was assessed using an item-count approach, which provides a semi-quantitative estimate of plastic occurrence but does not include standardized measurements of mass, size distribution, or abundance per unit area. This limitation restricts direct quantitative comparisons with studies using standardized protocols for plastic contamination assessment. Future investigations should therefore incorporate standardized measurements of the number, mass, size distribution, and spatial density of plastic items, together with polymer identification where possible.

Second, the characterization of plastisphere-associated components was primarily based on morphological observations using optical microscopy. Although this approach allowed the main biological and abiotic components associated with plastic debris to be characterized, it does not provide sufficient taxonomic resolution to confirm the identity of all observed organisms or to assess their functional diversity. In particular, the designation of *Dunaliella*-like microalgae should be considered tentative in the absence of molecular or culture-based confirmation. Similarly, further taxonomic approaches would be required to confirm the identity and diversity of *Artemia*-like forms and other biological components associated with plastic surfaces. Despite these limitations, the methodology adopted in the present study was appropriate for the exploratory objectives of the investigation and enabled the identification of important spatial and seasonal patterns in plastic debris distribution and plastisphere-associated community composition within the Sahline Salt Marsh.

Future investigations should extend the present approach to the sediment compartment, where plastic particles may accumulate through sedimentation and interactions with suspended particulate and organic matter. Investigating the sediment compartment would provide a more comprehensive understanding of the links between plastic debris, sedimentary processes, and benthic communities in hypersaline environments. In addition, the application of standardized quantitative approaches and higher-resolution analytical techniques, including polymer characterization and molecular analyses of plastisphere-associated components, would allow a more comprehensive assessment of their taxonomic diversity, ecological functions, and environmental drivers. Such integrated approaches would contribute to a better understanding of the fate and ecological dynamics of plastic pollution in Mediterranean hypersaline salt-marsh ecosystems.

Conclusion

This study demonstrated marked spatial and seasonal variability in plastic debris distribution and in the biological and inorganic components associated with plastic surfaces in the Sahline Salt Marsh. Plastic accumulation was highest at S₅, while plastisphere-associated components were particularly abundant at S₂ and S₅. Statistical analyses revealed selective and campaign-dependent associations between physicochemical parameters and specific plastic types and plastisphere-

associated components, involving temperature, salinity, dissolved oxygen, and pH. These relationships varied among components and should therefore be interpreted as associations rather than evidence of direct causal effects. Overall, the findings highlight the role of the hypersaline salt-marsh environment in shaping plastic-associated assemblages and support the need for standardized plastic quantification, polymer characterization, and higher-resolution taxonomic approaches in future studies.

596 **Declarations**

597 **Author contributions**

598 KT and FA carried out all experiments, performed the data analysis, and prepared the manuscript. NA supervised the work
599 and reviewed and edited the manuscript. All authors read and approved the final manuscript.

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602 **Data availability**

603 The datasets generated during and/or analyzed during the current study are available from the corresponding author on
604 reasonable request.

605 **Ethics approval**

606 This research did not involve human or animal participants and was conducted in the Sahline Salt Marsh, with no work
607 conducted on private or protected areas. No specific permissions were required for the study locations.

608 **Consent to participate**

609 Not applicable.

610 **Consent for publication**

611 Not applicable.

612 **Competing interests**

613 The authors declare no competing interests.

614 **Ethical responsibilities of authors**

615 All authors have read, understood, and complied, as applicable, with the statement on “Ethical responsibilities of Authors”
616 found in the Instructions for Authors.

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