

# Benthic vegetation, chlorophyll $\alpha$ and physical-chemical variables in a protected zone of a Mediterranean coastal lagoon (Lesina, Italy)

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**Abstract** Phytoplankton and benthic vegetation biomass undergoes spatial-temporal changes in relation to their life cycle, but also to meteorological conditions, physical-chemical variables, organic input and internal dynamism. The main aim of this work was to observe the effect of all environmental variables on the vegetative dynamic process in a protected zone of a Mediterranean coastal lagoon (Lesina lagoon, SE Italy). Seven samplings were performed from 2010 to 2012 at 30 sites for nutrient and chlorophyll analyses, while TOC measurements and wet biomass evaluation were performed at 10 sites. Temperature, salinity and oxygen saturation were also measured by multiparametric probe and a visual census for vegetation was performed. Sites close to freshwater inflow were characterized by lower temperature and salinity, and high nitrate, with maxima of 191.05  $\mu\text{M}$  in May 2010 and more than 250  $\mu\text{M}$  in October 2010. Silicates drastically decreased from May 2010 (87.57  $\mu\text{M}$ ) to July 2010 (6.15  $\mu\text{M}$ ) and increased again in October (74.99  $\mu\text{M}$ ). Chl  $a$  concentrations were not on average higher than 6  $\text{mg m}^{-3}$ , but peaks of

20  $\text{mg m}^{-3}$  were observed during May 2011 and May 2012. Benthic vegetation wet biomass collected in 2010 was approximately twice that collected in 2012, with a maximum of 27,554  $\text{g m}^{-2}$  and a dominance of macroalgae (70 % in May 2010 and 40 % in August 2010). During period 2010, a simultaneous and drastic decreasing of both mean values of wet biomass and chl  $a$  was observed from May to October 2010. During period 2012 a shift of vegetation biomass was shown from May (phytoplankton prevalence) to August 2012, with angiosperm prevalence (more than 30 %).

**Keywords** Diversity · Biomass · Temperature · Salinity · Nutrients · Total organic carbon · Sediment

## Introduction

Coastal lagoons are characterized by high primary and secondary production and by important autotrophic and heterotrophic biomasses that explain their economic importance (Kjerfve 1994; Baran 2000). An intense primary production, from 200 to 400  $\text{g C m}^{-2}\text{y}^{-1}$  (Nixon 1982) and a high biodiversity, lead to its ecological considerable importance. Communities of species in these systems are connected through trophic relationships that form complex networks, whose resilience depends on environmental variables, food availability, resource abundance and consumer behaviour (Briand and Cohen 1984; Prado et al. 2013). The hydrological changes (freshwater inflow, water residence time, flushing rate) create environmental gradients that alter the chemical and biological properties of lagoons directly or indirectly (Ittkoot et al. 2000). This results in drastic changes in the ecology and ecosystem functioning (Setubal et al. 2013;

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Rakshesh et al. 2015). In addition, these environments are often severely influenced by human activities such species exploitation (fisheries, aquaculture and hunting) and the strong dependence from their watershed makes them vulnerable to human impact (agriculture, industry and urban development) which involve inputs of nutrients and organic matter of continental origin. Moreover, most of the particulate organic matter that reaches the bottom is mineralized in the surface layers of sediments, promoting a spatial variability in the exchange of nutrients at the water-sediment interface (Vidal and Morgu 1995). Nutrient additions stimulate the development of fast-growing macroalgal mats (Sfriso and Facca 2007) together with high phytoplankton crops due to low turnover rates (Monbet 1992), resulting in a subsequent eutrophication (Cloern 2001). The result is that these transitional environments exhibit a progressive and temporary decline of water quality with oxygen depletion and drastic and sudden changes of phytoplankton and benthic vegetation in both quality and quantity (Viaroli et al. 2008; Vadrucci et al. 2009; Basset et al. 2013; Orfanidis et al. 2014). Typically, the changing from seagrass meadows to bloom-forming macroalgae and vice versa causes relevant changes in the whole ecosystem metabolism and in the amounts of organic matter, which promote anaerobic processes and dystrophic crises (Viaroli et al. 2001; Specchiulli et al. 2009). This study was conducted from 2010 to 2012 in one of the largest transitional environments of the South Italy (Lesina lagoon), as part of a project aimed to study and estimate the potentiality and sustainability of the use of macroalgae in order to produce biodiesel. The study area is the extreme eastern part of the Lesina lagoon. Multiple factors as the massive presence of *Phragmites australis* and mats of macroalgae and angiosperms, strong local freshwater inputs that push seawaters westward and the hydraulic gradient between seaward channels during intense wind events (Ferrarin et al. 2014) are responsible for the confinement of the eastern area. This hydraulic condition reflects the lower water residence times and the presence of less saline waters, compared to those of the entire lagoon, affecting biogeochemical and hydrological processes in this area. The distribution of autotrophic biomass changes during the seasons, not only related to the life cycle of themselves, but also to winds, hydrodynamics, dissolved nutrients and organic matter in sediments (Lenzi et al. 2013). The purpose of the present study was to evaluate key abiotic and biotic environmental characteristics of the eastern zone of the Lesina lagoon (Sacca Orientale) through a multidisciplinary approach. In particular, the aim was to study: 1) the spatial-temporal variability of water and sediment physical-chemical variables with a focus on nutrients; 2) the spatial-temporal variability of chl *a* and benthic vegetation biomass; 3) the relationship between primary producer biomass and physical-chemical variables.

## Materials and methods

### Description of study area

The “Sacca Orientale” of the Lesina lagoon (Fig. 1) has been classified as a Special Protection Area (SPA) (IT9110031) since October 1988 (DM 03/04/2000 GU95 Environment of 04/22/2000). The reserve covers an area of 930 ha and it is an important habitat for migratory and sedentary species during autumn-winter seasons. Studies performed on hydrodynamic conditions of Lesina lagoon have low water residence time in the eastern area of the lagoon (50 days, Ferrarin et al. 2014). In addition, many drainage channels are present in the extreme zone and they could be the main source of pollutants during rainy periods (Fabbrocini et al. 2005). The balance between the hydrodynamics and the continue outflow from the Schiapparo channel characterize the low values of salinity in the eastern part of the lagoon, allowing dense meadows of angiosperms with a fresh water origin (e.g. *Zannichellia palustris*, *Ruppia cirrhosa*, *Myriophyllum spicatum*) (D’Adamo et al. 2009; Orfanidis et al. 2014).

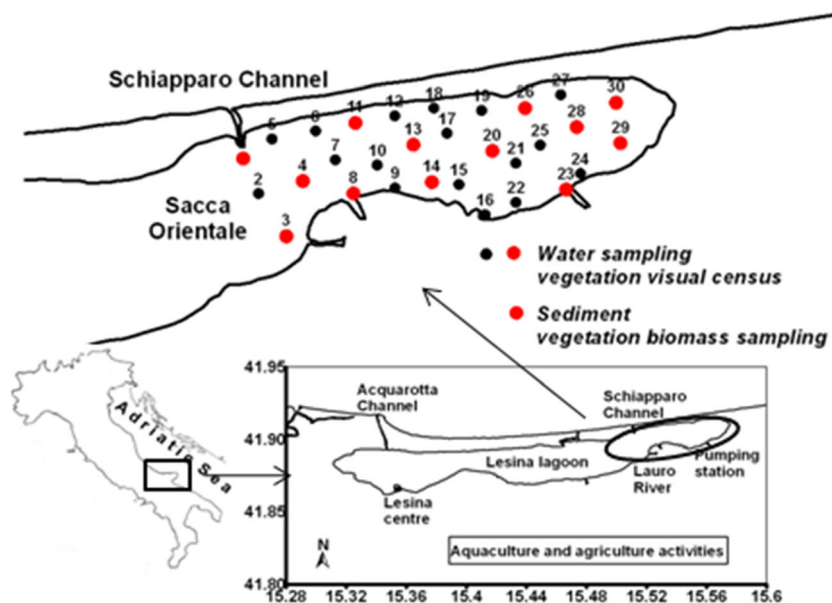
### Sampling procedure

Seven campaigns were performed in spring and summer seasons from 2010 to 2012 (May, July and October 2010; May and August 2011; May and August 2012). Due to the lack of details on environmental characteristics of the “Sacca Orientale”, a grid of 56 geo-referenced points according to west-east transects was initially chosen for the study purposes, but only 50 % of the total was investigated (30 sites, see Fig. 1), due to the difficulty of navigation for the massive presence of *Phragmites australis*. Temperature, salinity and oxygen saturation were measured at each site and in each sampling month by a calibrated multi-parameter probe (Corr-Teck Hydrometria) and duplicate surface water samples for nutrients and chlorophyll *a* analyses were collected from 30 sites during 2010 and 2011 and from 13 sites during 2012. Water samples for ammonium (NH<sub>4</sub>), nitrite (NO<sub>2</sub>), nitrate (NO<sub>3</sub>), soluble reactive phosphorous (SRP) and soluble reactive silicate (SRSi) were directly filtered through Whatman GF/F glass fiber syringes filter and frozen at -20 °C until analysis, while un-filtered water samples were taken for total phosphorous (TP) and total nitrogen (TN) analyses. For chlorophyll *a* (chl *a*) analysis, 500 ml of water samples were collected in plastic bottles and hold at 4 °C until laboratory filtration.

Duplicate surface sediment samples were collected during the samplings of 2010 and 2011 by a box-corer (15x15x15 cm) from 13 sites (see Fig. 1, red circles) and stored in plastic caps for total organic carbon (TOC) measurements.

For benthic vegetation, a visual census was performed over the entire observation period and at all the sampling sites.

**Fig. 1** Map of eastern Lesina lagoon area showing sampling sites



Based on the occurrence of all species in sites during the observation period, the frequency percentage (OP) was calculated as the presence of each species in relation to total number of sites. For wet biomass, macroalgae and angiosperms were collected during 2010 and 2012 from the surface of sediment core at the 13 sampling sites of sediments, kept within labelled plastic bags and transported to the laboratory for total wet biomass measurements.

### Laboratory analyses

Dissolved nutrient concentrations were measured on a three-channel Bran + Luebbe Autoanalyzer 3 Continuous Flow Analyzer (Bran + Luebbe, Norderstedt, Germany), using standard procedures reported in the manual (Bran and Luebbe 2004). TP and TN were analyzed at the same way, after persulphate digestion (Grasshoff et al. 1999). All units are reported as  $\mu\text{M}$ .

Water samples for chl  $a$  determinations were filtered through Whatman GF/F glass fiber filter and pigments were extracted with 90 % acetone (EPA Methods 445.0 1997) and measured with Trilogy Laboratory Fluorometer (Turner Design, V. 1.2) after its calibration with a commercially available chl  $a$  standard (Sigma Aldrich).

Chl  $a$  concentrations ( $\text{mg m}^{-3}$ ) were calculated according to the following equation:

$$\text{Chl } a = R \cdot (R-1)^{-1} \cdot C \cdot (F_0 - F_a) \cdot v \cdot V^{-1}$$

Where  $F_0$  = sample fluorescence;  $F_a$  = sample fluorescence after acidification;  $R = F_0/F_a$ ;  $C$  = Standard Chl  $a$  Concentration/ $F_0$ ;  $v$  = volume of extract (ml);  $V$  = volume of filtered sample (ml).

Sediment samples were pooled, homogenized and dried at  $60^\circ\text{C}$  in oven until constant weight and weighed ( $\pm 0.01$  g). Dried sediments were analyzed for TOC by direct total flash combustion, before and after acid digestion with HCl 19 %, using a CHNS Elemental Analyzer with a thermo-conductivity detector TCD (Perkin Elmer, mod. CHN/O 200) according with ICRAM methods (2001). Recoveries and reproducibility were checked by analysing procedural blanks and reference materials purchased from: National Institute of Standard and Technologies (NIST - NewYork waterway Sediment SRM1944). Measured average recoveries were a 98.8 % (s.d. 0.26 %).

Vegetation samples were weighted for wet biomass registration to the nearest 0.01 g and expressed in  $\text{g (ww)} \text{ m}^{-2}$ .

### Data handling and statistical analyses

Significant differences in variables among observation sites and periods were explored by means of the Kruskal-Wallis Test, a non-parametric ANOVA for ranks, by calculating H (statistics of the test) and p (level of significance). Box-Whisker plots were used on the water data matrix highlight the temporal trend and the spatial variability of all water variables. Spearman rank order was calculated using the data matrix (water and sediment) for each observation year to assess the degree of relationship among variables and to explore similar sources. STATISTICA 8.0 (StatSoftInc) was used for this preliminary approach. Principal component analysis (PCA) was performed on the data matrix of each sampling period to evaluate similarities/dissimilarities between periods and sites. Spatial and temporal differences were better highlighted, introducing the discriminating factors as fresh-intermediate-saline-waters, based on the distance of sites from Schiapparo Channel and river mouth, and sampling period

(May, July and October 2010; May and August 2011; May and August 2012). Multivariate analyses were developed using Primer-E Software package v6.0 (Plymouth Marine Laboratory, UK). As variables had different units, all data were normalized to their maximum value and log-transformed (Clarke and Green 1988). Distribution maps were produced as a contour plot based on Geographic Information System (GIS) technology. The software used was QGIS and the interpolation was carried out with Inverse Distance Weighted method (IDW) (Shepard 1968).

## Results

### Spatial changes of environmental variables and water sources

Wide spatial differences were observed for both temperature and salinity in almost sampling period (Fig. 2). Sites close to freshwater inflow (3, 23, 24, see Fig. 1) were characterized by both lower mean temperatures and salinities. An important variation in salinity occurred during 2012, reflecting a west-east gradient with the extreme eastern zone characterized by low salinities (values <5). The periods “a” (2010) and “b” (2011) were characterized by waters close to the saturation, with values lower than 60 % at sites located along the northern board (17, 18, 19) and confined in the eastern extreme zone (28, 29). In contrast, in the period “c” (2012) increased values from west (about 80 %) to east (120–140 %) were observed, contrary to salinity (Fig. 2).

Significant differences among sites were observed for nitrate and nitrite ( $p < 0.001$ ) and for TN ( $p < 0.05$ ) and TP ( $p < 0.01$ ) and the Kruskal-Wallis ranks analysis identified sites close to freshwater input (3, 8, 23, 24 and 25) and the sites confined in the extreme eastern area (27, 28, 29 and 30) as those with the highest variability in the nutrient concentrations. Ammonium showed maxima of 77.09  $\mu\text{M}$  in July 2010 near the pumping station (site 23) and 46.69  $\mu\text{M}$  in

August 2011 at site 4, far from direct input. High nitrite concentrations were measured near the pumping station (site 24) during the sampling period, with maxima of 5.61  $\mu\text{M}$  in October 2010 and 4.34  $\mu\text{M}$  in May 2011.

Sites 21, 23, 24 and 25 were also enriched with nitrate, with maxima of 191.05  $\mu\text{M}$  in May 2010, more than 250  $\mu\text{M}$  in October 2010 and 161.67  $\mu\text{M}$  in May 2011. The sites located near the Schiapparo Channel were characterized by high values of SRSi, with the highest value (285.67  $\mu\text{M}$ ) measured at site 1. In contrast, the sites in the more eastern zone were characterized by mean values lower than 100  $\mu\text{M}$ . SRP showed a not well-defined spatial distribution, with extreme values of 2.130  $\mu\text{M}$  (site 13) and 5.924  $\mu\text{M}$  (site 24) measured in May 2010 and May 2011, respectively. Spatially, TP accumulated in the extreme eastern zone (sites 26, 27, 28, 29 and 30), with peaks registered near the pumping station in May 2011 (6.34  $\mu\text{M}$ ) and along the northern edge (site 6) in August 2011 (3.94  $\mu\text{M}$ ). TN – enriched waters were observed in the area close to the pumping station (sites 21, 22, 23, 24 and 25), showing values 3–4 times higher than median in May 2010 (175  $\mu\text{M}$ , pumping station).

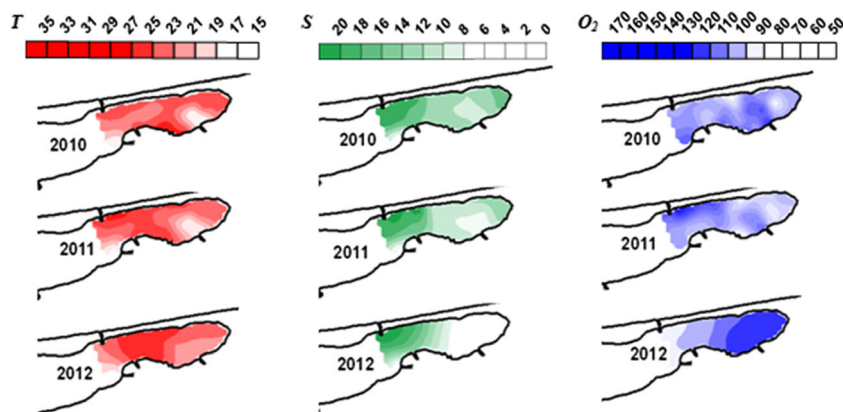
### Temporal variability of physical-chemical variables

The main descriptive statistics for each sampling year are reported in Table 1.

Temperature showed minimum (14.95 °C) and maximum (34.78 °C) values during 2010, in October and July, respectively, while salinity showed minimum of 1.56 in October 2010 and maximum of 25.95 in August 2012 (Table 1, Fig. 3a, b). Supersaturated waters were observed in all the 3 years (Table 1), and waters close to saturation were observed during July 2010, May 2011 and August 2012, while lower median were achieved in October 2010 (81 %) and August 2011 (71 %) (Fig. 3c).

Kruskal-Wallis analysis highlighted that all the nutrients changed significantly ( $p < 0.001$  for dissolved forms and  $p < 0.01$  for total forms) over the sampling periods and the

**Fig. 2** Spatial distribution of temperature, salinity and oxygen saturation during 2010, 2011 and 2012 observation period



**Table 1** Main descriptive statistics for temperature (T°C), salinity (S), oxygen saturation (O<sub>2</sub>%), chlorophyll *a* (chl *a* mg m<sup>-3</sup>), ammonium (NH<sub>4</sub> μM), nitrite (NO<sub>2</sub> μM), nitrate (NO<sub>3</sub> μM), phosphate (SRP μM), silicate (SRSi μM), total nitrogen (TN μM) and total phosphorous (TP μM), totalorganic carbon (TOC, %) and wet biomass (WB, g ww m<sup>-2</sup>), measured in the eastern area of the Lesina lagoon from 2010 to 2012. During the sampling of 2012 dissolved and total nutrients were not measured

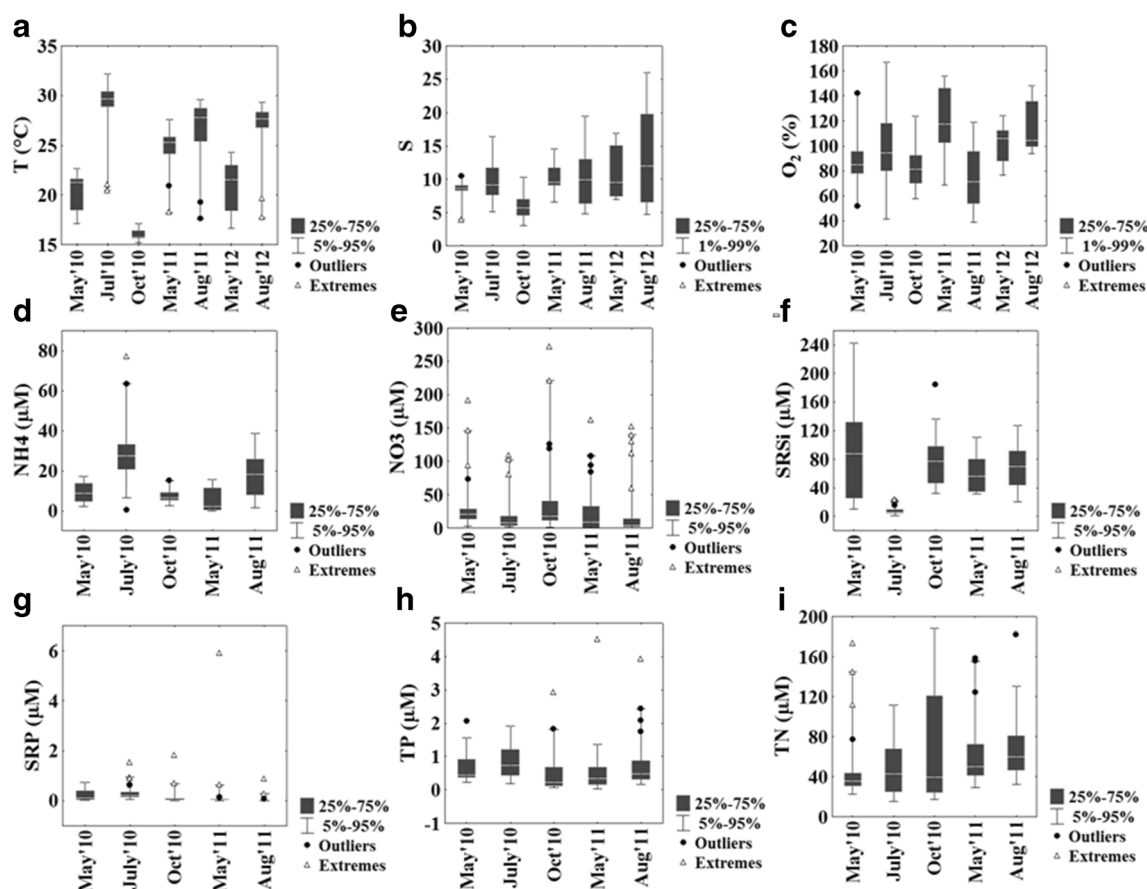
	T	S	O <sub>2</sub>	NH <sub>4</sub>	NO <sub>3</sub>	NO <sub>2</sub>	SRSi	SRP	TN	TP	Chla	TOC	WB
2010													
<i>N</i>	90	90	90	180	180	180	180	180	180	180	180	78	78
Mean	21.76	8.27	88	15.11	31.92	0.91	60.34	0.25	54.53	0.67	4.59	3.54	3525
Median	21.08	8.57	85	9.86	15.36	0.66	40.95	0.11	37.15	0.46	2.12	3.39	2639
Min	14.95	1.56	22	0.30	0.07	0.00	1.37	0.01	11.77	0.02	0.39	2.16	1393
Max	34.78	17.05	167	77.09	271.80	5.61	285.67	2.13	229.86	2.92	27.29	5.64	6543
SD	5.88	3.15	28	13.62	47.68	0.92	59.84	0.38	45.24	0.55	5.36	1.09	2687
2011													
<i>N</i>	60	60	60	120	120	120	120	120	120	120	120	52	-
Mean	25.67	10.53	97	12.18	24.64	0.54	66.35	0.17	65.23	0.76	5.63	5.06	-
Median	26.31	9.63	102	9.23	5.34	0.29	64.25	0.02	56.58	0.43	2.97	5.3	-
Min	17.58	4.80	39	0.02	0.02	0.01	14.96	0.00	25.44	0.00	0.09	3.08	-
Max	31.08	19.52	166	46.69	161.67	4.34	149.95	5.92	181.99	6.34	23.18	6.47	-
SD	3.46	3.50	34	12.08	43.07	0.78	31.05	0.80	34.10	1.04	6.21	1.09	-
2012													
<i>N</i>	60	60	60	-	-	-	-	-	-	-	120	-	52
Mean	23.64	12.14	108	-	-	-	-	-	-	-	3.83	-	1867
Median	23.60	10.42	106	-	-	-	-	-	-	-	1.42	-	1247
Min	16.68	4.77	77	-	-	-	-	-	-	-	0.64	-	495
Max	29.36	25.95	148	-	-	-	-	-	-	-	22.27	-	4029
SD	4.22	5.82	18	-	-	-	-	-	-	-	4.94	-	1231

analysis of ranks identified July 2010 and May 2011 as the months with the highest variability in nutrients. Ammonium showed significantly higher concentrations during summer months (Fig. 3d), while median values of nitrite never exceeded 1 μM (Table 1). Nitrate-enriched waters were observed in 2010 (Table 1), especially during the October sampling, when the highest values (extreme in Fig. 3e) were measured. Also, nitrate values higher than median were measured in May–July 2010 and August 2011. Median concentrations of SRSi were significantly different between the 2 years (Table 1), with a drastic decrease of values from May 2010 (87.57 μM) to July 2010 (6.15 μM) and a following increase in October (74.99 μM) (Fig. 3f). Phosphate (SRP) varied significantly from 1 month to the other, but its temporal variability (Fig. 3g) was not well defined. Anyway, median values <0.2 μM were measured in all the observation period, with an extreme value measured in May 2011 (Fig. 3g). Both total nutrients (TP and TN) showed values extremely higher than median during 2010 and 2012, respectively (Table 1). TP showed higher median values in July 2010 and extreme concentrations in October 2010 (2.92 μM), May 2011 (6.34 μM) and August 2011 (3.94 μM), while TN exhibited increased median values from May 2010 to August 2011 (Fig. 3h, i).

### Vegetation and chlorophyll *a*

For benthic vegetation, a total of 8 genera were recognized, whose 4 spermatophytæ and 4 macroalgæ and the results of the visual census are reported in Table 2. During 2010, the most frequent species identified in May were macroalgæ *Chara fragilis* var. *genuine* (33 %) and *Chaetomorpha linum* (37 %), while in July, a massive presence of spermatophytæ (*Zostera noltei* 23 % and *Zannichellia palustris* 23 %) was highlighted as well as *Chara fragilis*. In October, it can be noted an increase of percentage frequency of *Ruppia cirrhosa* (17 %) and a reduction of *Zostera noltei* (3 %). During 2011, there was a prevalence of spermatophytæ on macroalgæ in August, while during 2012 macroalgæ disappeared completely and occurrence of *Ruppia cirrhosa* and *Zostera noltei* ranged between 10 and 27 %.

Spatially, the biomass distributions showed clearly different accumulation zones over the observation period (Fig. 4). During 2010, high biomass zones were observed along the south-western edge (Fig. 4a, b), at site 3 near the Lauro river input, mainly represented by *Zostera noltei* (16,736 g m<sup>-2</sup>) and at lesser extent by *Chaetomorpha linum* (9818 g m<sup>-2</sup>). During 2012 it moved eastward, accumulating at site 16 in May (mainly *Zostera noltei*, 4344 g m<sup>-2</sup>) (Fig. 4c), while in August it



**Fig. 3** Temporal variation of temperature, salinity and oxygen saturation, nutrients dissolved ( $\text{NH}_4$ ,  $\text{NO}_3$ , SRSi and SRP) and total (Total Nitrogen-TN, Total Phosphorus-TP) during the studied period

accumulated near the pumping station (site 23, *Zannichellia palustris*  $6026 \text{ g m}^{-2}$ ) and in the extreme eastern part of the study area (site 30, *Ruppia cirrhosa*  $6044 \text{ g m}^{-2}$ ) (Fig. 4d). Wet biomass obtained from samples collected in 2010 was approximately twice that collected in 2012 (Table 1). Although the Kruskal-Wallis analysis showed no significant differences either between months nor between sites, a wet biomass of  $6543 \pm 8617 \text{ g m}^{-2}$  was calculated in May 2010, with a drastic decrease in July ( $2639 \pm 3389 \text{ g m}^{-2}$ ) and a settling around  $1100\text{--}1600 \text{ g m}^{-2}$  in the next months (Fig. 5).

Chl *a* concentrations were not on average higher than  $6 \text{ mg m}^{-3}$  (Table 1, Fig. 5); nevertheless, peaks higher than  $20 \text{ mg m}^{-3}$  were observed during periods of high phytoplankton bloom (May), as also highlighted by high standard deviation (Table 1). During 2010, the highest chl *a* concentrations were registered at site 22 ( $27.29 \text{ mg m}^{-3}$  May) and at site 21 ( $26.17 \text{ mg m}^{-3}$  July). In 2011, the maximum values were measured in the confined site 29 ( $21.07 \text{ mg m}^{-3}$ , May) and at site 4 far from direct freshwater input ( $23.18 \text{ mg m}^{-3}$ , August). During 2012, the highest chl *a* concentrations were again measured in the confined eastern area, the site 30, in May ( $22.27 \text{ mg m}^{-3}$ ) and August ( $11.73 \text{ mg m}^{-3}$ ).

### Sediment organic carbon

TOC showed median content lower in 2010 than that measured in 2011 (Table 1) and Kruskal-Wallis Anova by ranks highlighted significant temporal differences ( $p < 0.01$ ), with May 2010 and August 2011 being the months when lower and higher concentrations, respectively. TOC did not show significant differences among sites, although constantly lower values were observed at site 1 (Schiapparo Channel) than those measured at sites 26, 28 and 30 (the north-eastern zone), especially during spring periods (5–6 % in July 2010 and 6–7 % in August 2011).

### Relationship among variables

Spearman rank order ( $p < 0.05$ ) was applied to water-sediment data, taking into account data collected in each period “a”, “b” and “c”, separately. This analysis showed differences in variables correlations between sampling periods. During 2010, positive correlation was observed between temperature and salinity ( $r = 0.57$ ). No variable showed correlations with salinity, but a decrease of TN ( $r = 0.60$ ) and silicate ( $r = 0.57$ ) with increased temperature was observed, as well as a positive correlation

**Table 2** Occurrence of benthic vegetation species with the percentage frequency of each species (OF%) related to the total number of sampling sites, from 2010 to 2012

	Sites																														OP %
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
May 2010																															
<i>Zannichellia palustris</i> L.																						x							x		
<i>Myriophyllum spicatum</i> L.																							x		x						
<i>Ruppia cirrhosa</i>																													x		
<i>Zostera noltei</i>	x	x		x	x						x	x					x	x													
<i>Chara fragilis</i> var. <i>genuina</i>							x	x	x	x	x	x	x	x		x						x									
<i>Chetomorpha linum</i>			x		x	x	x	x	x	x			x					x											x		
<i>Ulva</i>																										x					
July 2010																															
<i>Zannichellia palustris</i> L.	x																						x	x			x	x	x	x	
<i>Myriophyllum spicatum</i> L.																							x								
<i>Ruppia cirrhosa</i>																		x							x						
<i>Zostera noltei</i>				x		x	x	x	x			x	x																		
<i>Chara fragilis</i> var. <i>genuina</i>		x					x		x		x	x	x	x																	
<i>Chetomorpha linum</i>	x		x		x												x								x						
<i>Ulva</i>																															
October 2010																															
<i>Zannichellia palustris</i> L.																					x					x		x	x		
<i>Myriophyllum spicatum</i> L.																															
<i>Ruppia cirrosa</i>																x	x	x				x				x					
<i>Zostera noltei</i>					x																										
<i>Chara fragilis</i> var. <i>genuina</i>						x	x	x		x	x	x					x														
<i>Chetomorpha linum</i>					x	x			x	x	x																				
<i>Ulva</i>																															
May 2011																															
<i>Zannichellia palustris</i> L.																												x		x	x
<i>Myriophyllum spicatum</i> L.																															
<i>Ruppia cirrosa</i>																															
<i>Zostera noltei</i>																															
<i>Chara fragilis</i> var. <i>genuina</i>												x	x																		
<i>Chetomorpha linum</i>										x		x	x				x	x													
<i>Chladophora</i> sp.																	x														
<i>Ulva</i>																							x								
August 2011																															
<i>Zannichellia palustris</i> L.																															
<i>Myriophyllum spicatum</i> L.																															
<i>Ruppia cirrosa</i>				x	x		x		x	x	x	x				x	x	x				x	x		x	x		x	x	x	
<i>Zostera noltei</i>						x								x																	
<i>Chara fragilis</i> var. <i>genuina</i>			x				x		x		x	x				x															
<i>Chetomorpha linum</i>			x																					x							
<i>Chladophora</i> sp.																															
<i>Ulva</i>																															
May 2012																															
<i>Zannichellia palustris</i> L.																									x						
																										x					

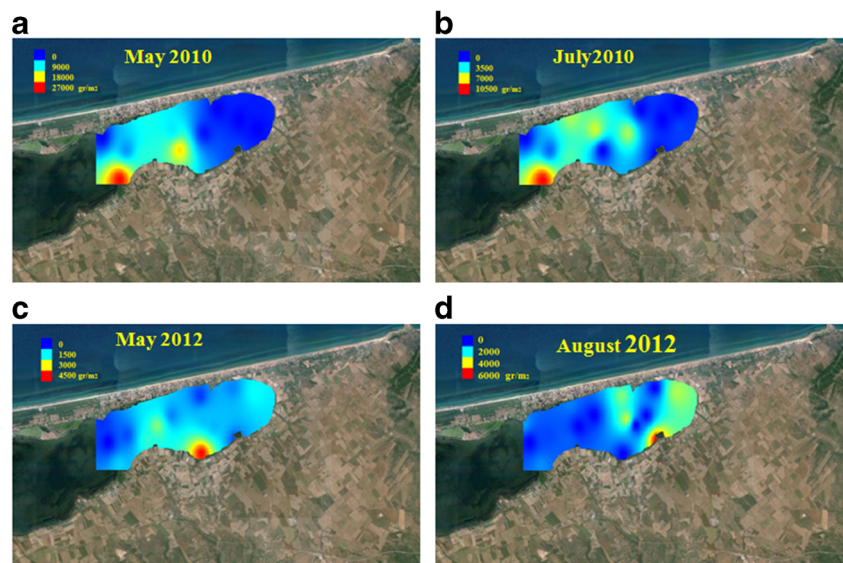
**Table 2** (continued)

	Sites																														OP %
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
<i>Myriophyllum spicatum</i> L.																															
<i>Ruppia cirrosa</i>									x			x							x	x									x	17	
<i>Zostera noltei</i>				x				x		x					x				x	x										20	
<i>Chara fragilis</i> var. <i>genuina</i>																														0	
<i>Chetomorpha linum</i>																														0	
<i>Ulva</i>																														0	
August 2012																															
<i>Zannichellia palustris</i> L.																								x						3	
<i>Myriophyllum spicatum</i> L.																															
<i>Ruppia cirrosa</i>																															
<i>Zostera noltei</i>	x	x	x				x			x		x	x							x	x							x	10		
<i>Zostera noltei</i>																															
<i>Chara fragilis</i> var. <i>genuina</i>																														0	
<i>Chetomorpha linum</i>																														0	
<i>Chladophora</i> sp.				x																										3	
<i>Ulva</i>																														0	

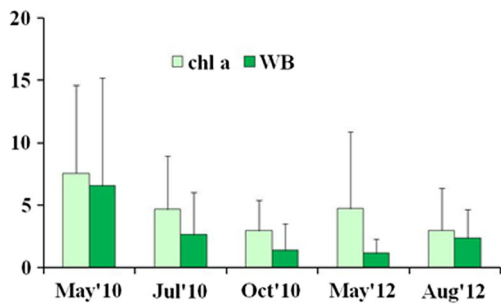
between chl *a* and temperature ( $r = 0.61$ ). Sedimentary organic carbon exhibited positive correlation with temperature ( $r = 0.30$ ) and TN ( $r = 0.29$ ) and negative correlation with SRP (0.26), while wet biomass was negatively correlated with TP ( $r = 0.55$ ).

During 2011, an increasing of TN with freshwater input ( $r = 0.58$ ) was seen and its inverse relationship with temperature was confirmed as in 2010 ( $r = 0.77$ ). Nitrate were well correlated with nitrite ( $r = 0.87$ ) and ammonia ( $r = 0.62$ ) and SRP presented negative correlations with temperature ( $r = 0.63$ ). TOC was not correlated with any variables. During 2012, a similar positive dependence on temperature was showed by chl *a* ( $r = 0.59$ ) and wet biomass ( $r = 0.67$ ),

while the analysis on monthly basis revealed a simultaneous increasing of chl *a* and oxygen saturation ( $r = 0.62$ ) in May 2012, while in August 2012 wet biomass showed a strong positive correlation with oxygen ( $r = 0.93$ ). PCA ordination of environmental variables performed in each year is presented in Fig. 6, taking into account “period” and “input” (fresh-intermediate-saline-waters) as segregation factors. We have avoided plotting high self-correlative variables (from Spearman rank analysis) to better represent the variability of the system. In 2010, the first three components accounted together for 58 % of the total variance, with PC1 (23.5 %) highly correlated with salinity, silicates and ammonia, PC2 (17.8 %) with oxygen

**Fig. 4** Spatial distribution of wet biomass during the spring-summer 2010 and 2012



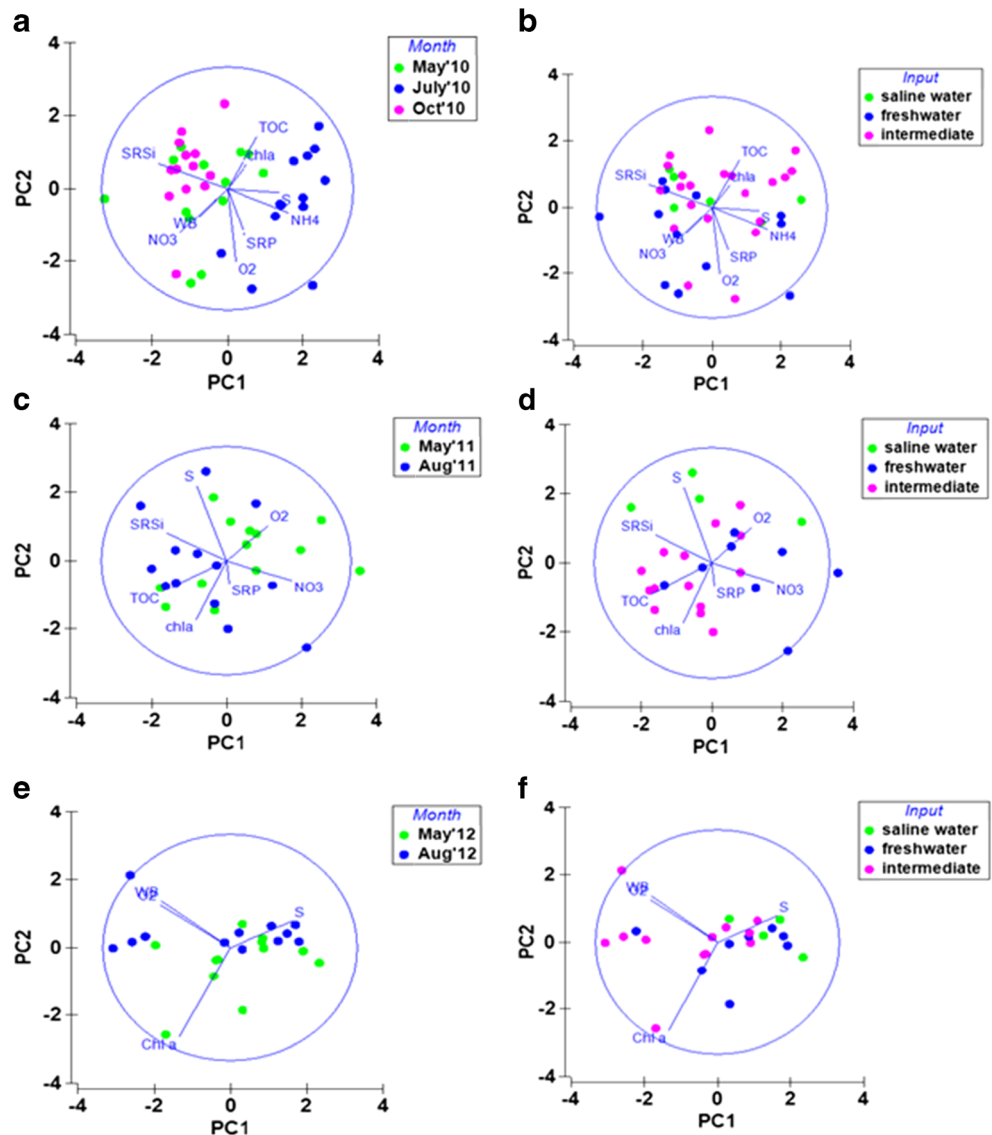


**Fig. 5** Comparison of temporal changes of chlorophyll  $a$  (chl  $a$ ) and benthic vegetation wet biomass (WB) from 2010 to 2012. WB is reported in  $\text{kg m}^{-2}$  in order to better highlight the differences along the ordinate axis ( $y$ )

saturation and TOC, PC3 (16.8 %) with biological components (chl  $a$  and wet biomass) and phosphate. July 2010 was well distinct (right side of the axis PC1) from May and October

2010, due to the lowest values of silicates and the highest values of ammonia (Fig. 6a), while sites segregation due to saline-freshwater input was well highlighted along the secondary axis PC2, with sediment TOC being the variable responsible of this difference (Fig. 6b). In 2011, 53.6 % of the total variability was explained by PC1 (31.3 %), correlated with nitrate, silicates and TOC, and PC2 (22.3 %), related to salinity and chl  $a$ . The difference between sampling effort was not well outlined as in 2010 (Fig. 6c), while sites affected by saline water input were separated by the others, due to the lowest values of phytoplankton biomass (Fig. 6d). In 2012, PC1 accounted for 62.9 % and PC2 for 19.4 %, for a total variance of 82.3 %. PC1 was highly correlated with wet biomass and salinity, while PC2 was inversely correlated to chl  $a$  the most variability of the system. Unlike other years, no significant differences between sampling effort and sites was observed (Fig. 6e, f).

**Fig. 6** Principal Component Analysis performed on environmental variables (water and sediment) for 2010 (a and b), 2011 (c and d), 2012 (e and f), taking into account “month” (a, c, e) and “input” (b, d, f) as segregation factors



## Discussion

Previous studies in the eastern area of the Lesina lagoon have been never performed, so it is not possible to compare our results to assess the inter-intra-annual trends. A previous study carried out in the entire Lesina lagoon (Roselli et al. 2009), except in the eastern area, showed mean values of oxidized nitrogen lower than our results and SRP concentrations comparable/higher than values of this study. In particular, these authors found  $N_{Ox}$  concentration range of 2.35–38.66  $\mu\text{M}$  (our study, 8.89–42.11  $\mu\text{M}$ ) and SRP concentration range of 0.08–0.90  $\mu\text{M}$  (our study, 0.08–0.39  $\mu\text{M}$ ). Moreover, Roselli et al. (2009, 2013) observed that the highest SRP concentrations were found at the stations near the inputs and specifically in the neighboring area to the eastern sector of the lagoon.

Temporal changing of hydrological parameters followed the seasonal variability, while spatial heterogeneity was affected by continental input and exchanges with the sea. In summer, when the input of freshwaters is low and the high temperatures enhance the evaporation, higher salinity values were recorded, due also to seawater inflows from Schiapparo channel (Ferrarin et al. 2014). A wide salinity range was observed during the observation period, ranging from 2 in October 2010 to 26 in August 2012.

The present study was carried out during a relatively dry period (spring and summer seasons), but the observed differences of nutrients between sampling period and sites suggest a significant role of local input (river, pumping station, sediment redox reactions) and/or vegetation/phytoplankton uptake in altering inorganic nutrients (Paudel and Montagna 2014). Higher concentrations of oxidized forms of inorganic nitrogen observed in almost all months near the pumping station and confined in the eastern area indicate that the “Sacca Orientale” of the Lesina lagoon was constantly supplied by nitrogen coming from agricultural activities. In contrast, ammonium showed its peaks during warmer months (July and August), suggesting a releasing through re-mineralization processes (Caffrey 1995), enhanced by high temperature, and/or excretion by benthic organisms, zooplankton and fish (Specchiulli et al. 2008).

The drastic decreasing of silicates concentrations from May to July 2010 indicates that this nutrient could be related to seasonal blooms of phytoplankton component, particularly diatoms and silicoflagellates (Valiela 1995), as also highlighted by strong and negative correlation with chl *a*. The relationship between benthic biogeochemical processes and nutrient dynamics in the water column has been well documented (Thouzeau et al. 2007; De Vittor et al. 2012). The sediments are the primary site for the organic matter mineralization and release of nutrients in the water column (Berelson et al. 1996). Shallow environments with limited hydrodynamic regimes, such as lagoons, are particularly vulnerable to the enrichment of organic matter and nutrients, and recent studies have shown that the sediments in these systems are the main source of

nutrients to the water column (Gikas et al. 2006). This has been also observed for Lesina lagoon (Roselli et al. 2009; Specchiulli et al. 2009). In our study, during 2010, the strong correlation between SRP and TOC suggest degradation processes of organic matter to be a probably source for phosphorous release along the water column, although low SRP concentrations observed for the entire observation period indicate phosphorous the limiting nutrient for the study area. A good correlation ( $p < 0.05$ ) observed during 2010 between TP and chl *a* indicates an involvement of the TP in the photosynthetic activity (Ferris and Tyler 1985; Molot and Dillon 1991; Roselli et al. 2009), similarly to the dissolved phosphorus (Larato et al. 2010).

The associations between nutrient loading, light availability and algal producers has been largely established (Menendez and Comin 2000; Hauxwell et al. 2003; Bartoli et al. 2008). Nitrogen loading from anthropogenic inputs has been demonstrated to contribute to extensive loss of seagrasses, as they appear to be sensitive indicators of nitrogen loading (Hauxwell et al. 2003; Olsen et al. 2015). The pattern of seasonal variations of occurrence and wet biomass observed in our study highlighted a dominance in spring-summer 2010 of macroalgae (*Chara fragilis* and *Chaetomorpha linum*), which were almost completely replaced by seagrasses (*Ruppia cirrhosa*, *Zostera noltei*) in 2011–2012. This suggest that the prevailing conditions in the study area (discrete nutrient contribution, good water transparency and light penetration) help the proliferation of seagrasses. With this data set it was not possible present in details the relationship between the observed vegetation changing and the abiotic variability.

Multivariate analysis pointed out significant intra-annual differences and highlighted spatial segregation related to marine–freshwater input. During period “a” (2010), nutrients (PC1) represented temporal variability, TOC (PC2) represented marine–freshwater inflows, while biological variables (wet biomass and Chl *a*, PC3) were responsible of both temporal and spatial variability. During period “b” (2011), no variable was representative of temporal variability, although May 2011 was characterized by slightly higher values of nitrates than those measured in August 2011, but Chl *a* represented the spatial variability and the segregation of sites close to freshwater inflow. During period “c” (2012), no significant spatial-temporal variability was observed, suggesting that neither wet biomass nor chl *a* were able to discriminate between spring and summer season and between marine and freshwater inflow. Nevertheless, this last result could be due to the absence of nutrient in the data set of 2012. However, a decreasing of Chl *a* from May (mean of 4.71  $\text{mg m}^{-3}$ ) to August (mean of 2.95  $\text{mg m}^{-3}$ ) 2012 was observed, contrary to wet biomass that doubled from 1158  $\text{g m}^{-2}$  to 2355  $\text{g m}^{-2}$ . Finally, differences among periods a, b and c can be identified when wet biomass and chlorophyll *a* data from the all sampling months and sites are combined.

During period “a”, a simultaneous and drastic decreasing of both mean values of wet biomass and Chl  $a$  was observed from May to October 2010 (Fig. 6). For wet biomass, the decreasing was mainly due to macroalgae, representing the dominant forms in May 2010 (70 %, especially *Chaetomorpha*) and July (40 %). This decrease was not due to a lack of nutrient availability, as this trend did not match neither a decreasing of nitrate nor a reduction of SRP (Fig. 4), and correlations with dissolved nutrients were not observed. In the period “c”, a shift of vegetation biomass was shown from May, with phytoplankton prevalence, to August 2012, with angiosperm prevalence (more than 30 %). In May 2012, light penetration is relatively high and high N loading could lead to a substantial decrease in macrophyte biomass due to an increase in phytoplankton, as also hypothesized by other authors (Pedersen and Borum 1996; Olsen et al. 2015).

## Conclusion

Despite the small quantity of freshwaters discharged during May and July–August samplings in the study area, the salinity range was large, due also to high evaporation during warmer sampling period, and a temporal and spatial variability of nutrients was observed. The high values of oxidized nitrogen confined near the pumping station identify the latter a possible input of nitrate. On the other hand, strong relationships between TOC-SRP and the increased values of ammonium in summer months indicate a possible influence of sedimentary degradation processes on ammonium and phosphorous concentrations in the water column. Our experimental approach took into account the spring-summer periods of greatest productivity (benthic vegetation and phytoplankton biomass). The study clearly showed that during spring-summer 2010 this productivity was strengthened by a higher nutrient availability (from both sediments and freshwaters input). Primary producers contend for nutrient availability which appeared to help a greater production of both phytoplankton and macroalgae during 2010. In May 2012, a greater phytoplankton production at the expense of benthic vegetation occurred, contrary to processes occurred in August 2012, probably due to a high N loading which lead to a substantial decrease in macrophytae biomass. The identification of processes conditioning the ecosystem, which should be maintained in ecological equilibrium, is very important, especially from the ecological point of view. We think that future works and research programs dedicated to the biogeochemical budget and biological processes study could give a further comprehension of the phenomenology studied here. A future approach should include a sampling design aimed at an intensification of measurements (more long-term) and at multi-disciplinary expertise.

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