



## Spatial variation in key functional traits of Mediterranean furoid algae: Insights from *Cystoseira sensu lato* intertidal canopies

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### ARTICLE INFO

#### Keywords:

*Cystoseira sensu lato*  
Rocky shores  
Algal forests  
Biomass  
Total organic carbon  
Restoration ecology

### ABSTRACT

*Cystoseira sensu lato* are furoid algae that form dense stands on intertidal and subtidal rocky reefs sustaining species-rich associated assemblages. The increasing human pressure is causing the decline of these marine forests, raising wide concerns on the ecological consequences of their loss. Yet, little is known about functional trait variables of *Cystoseira s.l.* species, which are essential to a deeper understanding of their contribution to the functioning of coastal ecosystems. We analysed the intraspecific spatial variation of the total organic matter, C<sub>org</sub>, N and P contents in *Ericaria amentacea* from intertidal rocky shores in the W Mediterranean Sea. Specifically, we quantified spatial patterns of variation of traits at large (100s km), small (1000s m), and local (100s cm) scale. We also explored potential interspecific variation by comparing the combined functional profile of trait variables between *E. amentacea* and *Cystoseira compressa*, an often co-occurring or vicariant species. Tissue contents of organic matter, C<sub>org</sub>, N and P, were consistent among *E. amentacea* stands, supporting the hypothesis of a large-scale (100s km) spatial homogeneity of such key traits in this species. Overlapping functional profiles between *E. amentacea* and *C. compressa* also suggested putative interspecific trait congruencies. However, a small-scale (1000s m) significant variability was found for all trait variables in *E. amentacea*. Variance components at small (1000s m) and local (100s cm) scale accounted for the largest contribution to the total spatial variation for all traits, recommending caution in the use of small and local-scale trait values to generalize functional performances of *Cystoseira s.l.* forests over large areas. This study represents one of the first attempts to shed light on the variability of key functional attributes of *Cystoseira s.l.* species at a range of spatial scales, which could assist more reliable assessments of their functional role and improve their management and conservation.

### 1. Introduction

Intertidal and subtidal forests of brown macroalgae account for about 15% of the global net primary production in coastal ecosystems (Duarte et al., 2022), sustain composite food webs (Graham, 2004; Koenigs et al., 2015), confer high structural complexity to coastal habitats and provide nursery and spawning grounds for a number of marine species (Graham, 2008; Cheminée et al., 2013; Bertocci et al., 2015). In the Mediterranean Sea, the main representatives of these macroalgae

belong to *Cystoseira sensu lato* species (including the genera *Cystoseira*, *Ericaria*, and *Gongolaria*, Molinari-Novoa and Guiry (2020)), which colonize rocky bottoms from the intertidal to >50 m depth (Hereu et al., 2008) and whose known distribution in the basin extends over 30% of rocky coasts (Fabrizzi et al., 2020). *Cystoseira s.l.* are furoid algae forming dense stands on rocky reefs that, analogously to kelp in cold-temperate marine regions, act as habitat-formers allowing species-rich sessile assemblages to thrive in the understory or as epiphytes, and providing feeding grounds and shelter to many vagile

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<https://doi.org/10.1016/j.ecss.2023.108524>

Received 20 July 2023; Received in revised form 27 September 2023; Accepted 4 October 2023

Available online 10 October 2023

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invertebrates and fishes (Ballesteros et al., 1998; Cheminée et al., 2013; Mineur et al., 2015; Blanfuné et al., 2016; Mancuso et al., 2021).

Due to their ecological relevance, *Cystoseira s.l.* species have been included in several international protection agreements and have become conservation targets in Marine Protected Areas, although this does not seem to have prevented the decline of these important habitat-formers in the Mediterranean Sea (Fabbrizzi et al., 2020; Tamburello et al., 2022). Land-based pollution, increased sediment load, direct physical disturbance and coastal artificialization seriously threaten *Cystoseira s.l.* species, causing local extinctions and large-scale reduction of their populations (Benedetti-Cecchi et al., 2001; Strain et al., 2014; Mancuso et al., 2018; Bevilacqua et al., 2021). In subtidal reefs, *Cystoseira s.l.* populations may also be strongly affected by the indirect effects of overfishing that may lead to severe reduction of the abundance of predator fish of commercial interest (e.g., sparids) and release from predation the main herbivores (e.g., sea urchins) which, in turn, may exert excessive grazing pressure on macroalgal canopies (Sala et al., 1998; Guidetti, 2006). In addition, the increasing frequency and intensity of thermal anomalies and extreme weather events probably exacerbate the effects of other human pressures by disrupting the reproductive cycles of *Cystoseira s.l.* species and, ultimately, erode the resilience potential of these marine forests (Bevilacqua et al., 2019; Peleg et al., 2020; Falace et al., 2021).

The ongoing reduction of *Cystoseira s.l.* stands across the basin, replaced by less complex algal turfs or barren grounds, has raised wide concerns about potential consequences for marine biodiversity and ecosystem functioning (Sales et al., 2011; Bianchelli and Danovaro, 2020; Peleg et al., 2020), fuelling intense efforts to restore lost populations (e.g., Verdura et al., 2018; De La Fuente et al., 2019; Clausen et al., 2023; Orlando-Bonaca et al., 2022). Despite the current emphasis on the potential depletion of ecosystem functions (e.g., carbon sequestration, nutrient cycling) associated with the decline of coastal marine forests (Krause-Jensen et al., 2018; Filbee-Dexter et al., 2022), information about traits of macroalgae (including all biological and ecological features of species affecting growth, reproduction and survival of individuals or that, at higher levels of biological organization, may reflect on community and ecosystem functioning; see Violle et al., 2007) is often scattered and inconsistent requiring renewed efforts for data collection and re-organization (Vranksen et al., 2023).

For *Cystoseira s.l.* species, little is known on basic functional attributes such as their organic carbon and key nutrients (e.g., nitrogen and phosphorous) contents, and very few attempts have been carried out to fill this gap (e.g., Delgado et al., 1994; Ballesteros and Sant, 2022). Actually, the available data are too sparse and fragmented to allow conservative estimates of trait values for organic matter and contents of key chemical elements for most *Cystoseira s.l.* species. For example, one of the largest databases on biomass and biochemistry of aquatic organisms, provided by Brey et al. (2010), reports in total only 22 single data entries for carbon, nitrogen and phosphorous contents for the 15 *Cystoseira s.l.* species listed. A major issue in this respect is that possible spatial and temporal intra- and interspecific variation of such trait values remain largely unexplored, which increases the uncertainty about their potential use for functional assessments into wide ecological and geographic contexts.

*Cystoseira s.l.* canopies, especially in rocky intertidal habitats, experience a wide range environmental disturbances (e.g., air exposure, UV radiation, temperature fluctuations, nutrient enrichment, trampling) that may affect their metabolism and, thus, their organic components (AbouAisha et al., 1997; Milazzo et al., 2004; Nygård and Dring, ; Mancuso et al., 2019). As these perennial and semi-perennial brown macroalgae generally alternate between vegetative and dormant phases, nutrient uptake and energy contents may vary according to changes in phenology and/or seasonal fluctuations (Delgado et al., 1994; Celis-Plá et al., 2016). Understanding whether environmental changes turn into significant spatial and temporal variability in biomass, contents of carbon and nutrients (e.g., nitrogen and phosphorous) in *Cystoseira s.l.*, and

identifying the relevant scales of variation, is essential for accurate quantifications of the contribution of *Cystoseira s.l.* stands to the overall functioning of coastal ecosystems in terms of matter and energy fluxes at different scales.

In this study, we analysed the intraspecific spatial variability, from local (100s cm) to large-scale (100s km), of key functional trait variables of *Ericaria amentacea* (C. Agardh) Molinari & Guiry canopies from intertidal rocky reefs in the W Mediterranean Sea, and specifically the contents of total organic matter, organic carbon ( $C_{org}$ ), nitrogen (N), and phosphorous (P) per unit of dry mass. We also explored possible inter-specific variation in these trait variables by comparing the combined functional trait profile of total  $C_{org}$ , N and P between *E. amentacea* and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin.

## 2. Materials and methods

### 2.1. Study area and sampling design

Patterns of spatial variation in contents of total organic matter,  $C_{org}$ , N and P per unit of dry mass were assessed by sampling *E. amentacea* populations at two locations, the Portofino Marine Protected Area (PFN, Ligurian Sea) and the Isole Ciclopi Marine Protected Area (CIC, South Ionian Sea) (Fig. 1). Sampling locations were selected within Marine Protected Areas (MPAs) to reduce as much as possible the potential influence of human activities on the investigated variables. At each location, samples were collected within continuous *E. amentacea* stands (belts of *E. amentacea*  $\geq 10$  m in length, average total substrate cover in  $50 \times 50$  cm quadrats  $>80\%$ ,  $n = 10$ ) on sub-horizontal rocky reefs in the intertidal fringe (0–1 m depth) at three sites (1–2 km apart) which were characterized by comparable environmental conditions representative of each location in terms of type and slope of rocky substrate, wave exposure, currents and prevailing winds. At each site, five random replicate samples (a few meters apart) were collected.

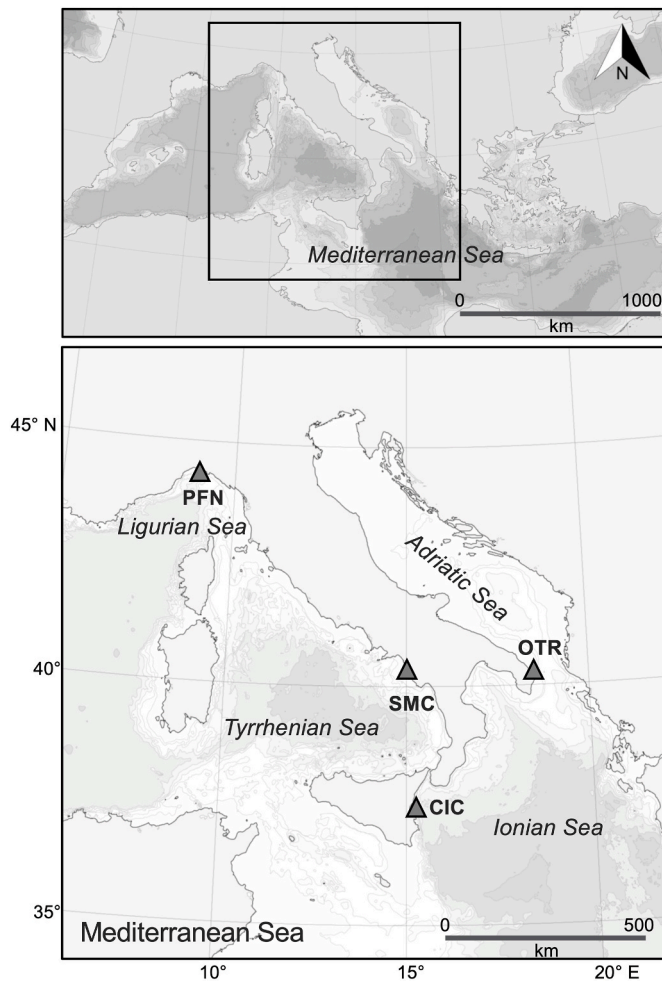
### 2.2. Total organic matter content

For total organic matter contents, samples were collected following the sampling design described in section 2.1. Sampling consisted of the complete removal of the *E. amentacea* thalli from the rocky substrate on a surface of approximately  $160 \text{ cm}^2$  for each replicate. Samples were collected using a PVC cylinder placed upon the canopy and an inox blade which was slid under the cylinder in order to cut off all *E. amentacea* thalli in the sampling unit. Samples were then preserved in formaldehyde (4%) and sea water solution and stored under cold and dark conditions, until biomass assessment.

A first sampling was carried out in October 2020 (T1), when algae were in the dormant phase. The hypothesis was that the total organic matter content of *E. amentacea* may not vary even across distant ( $\sim 700$  km) locations from different biogeographic regions (i.e., the NW Mediterranean, for PFN, and the Ionian regions for CIC; *sensu* Spalding et al., 2007) when the photosynthetic and metabolic activity of the *Cystoseira s.l.* species is strongly reduced, as occurs during its dormant phase (Sales and Ballesteros, 2012; Celis-Plá et al., 2014a; Kaleb et al., 2023). Sampling was then repeated in July 2021 (T2) to explore spatial patterns of variation in total organic matter content after *E. amentacea* experienced the intense regrowth phase typical of the vegetative period.

### 2.3. Total $C_{org}$ , N, and P contents

A separate set of samples of *E. amentacea* thalli was collected at each site in each location for the analysis of total  $C_{org}$ , N, and P contents. However, to limit the damage on macroalgal stands, these samples were collected only in T2 (with the exception of PFN, where samples were taken also in T1 to obtain a more representative range of  $C_{org}$ , N, and P contents to use in the construction of the functional profile of *E. amentacea*, see section 2.6), with three replicates per each site. Each



**Fig. 1.** Study area and sampling locations. PFN: Portofino Marine Protected Area; CIC: Isole Ciclopi Marine Protected Area; SMC: Santa Maria di Castellabate Marine Protected Area; OTR: European Special Area of Conservation of Otranto. PFN and CIC were the two main study locations, where data on the biomass, total  $C_{org}$ , N and P contents of *E. amentacea* were collected on two times of sampling and in multiple sites within locations. Additional data on total  $C_{org}$ , N and P contents were also collected in a single site and a single time of sampling at PFN and OTR for *C. compressa*, and at SMC for *E. amentacea*. See text and Table 1 for further details on sampling.

sample consisted of one 100 ml centrifuge tube filled with randomly-selected *E. amentacea* thalli (approximately 3–4 thalli per tube) cut off from the basal part to the apex. Samples were stored under cold and dark conditions and immediately brought back to the laboratory for further processing.

Finally, in order to investigate  $C_{org}$ , N, and P contents of *E. amentacea* from other geographic areas and/or in *C. compressa*, additional samples were collected in T2 following the sampling protocol described above and, specifically in the Santa Maria di Castellabate MPA (SMC, Tyrrhenian Sea, three samples of *E. amentacea* from one site), in the Portofino MPA (PFN, three samples of *C. compressa* from one site), and in the European Special Area of Conservation of Otranto (OTR, Southern Adriatic Sea, three samples of *C. compressa* from one site). The study areas are shown in Fig. 1, whereas sampling details are summarized in Table 1.

#### 2.4. Sample processing

Samples of *E. amentacea* for the analysis of biomass were soaked in sea water for 24 h to remove formaldehyde residues and were carefully

**Table 1**

Summary of sampling designs for all study areas. T1 = October 2020, T2 = July 2021. PFN = Portofino MPA (Ligurian Sea), CIC = Ciclopi MPA (Ionian Sea), SMC = Santa Maria di Castellabate MPA (Tyrrhenian Sea), OTR = European Special Area of Conservation of Otranto (Southern Adriatic Sea). See also Fig. 1.

Species	Variable(s)	Time	Sampling design	Sampling unit	Total no. of samples
<i>E. amentacea</i>	Total organic matter	T1	3 sites × 2 locations (PFN and CIC)	160 cm <sup>2</sup>	30
<i>E. amentacea</i>	Total organic matter	T2	3 sites × 2 locations (PFN and CIC)	160 cm <sup>2</sup>	30
<i>E. amentacea</i>	$C_{org}$ , N, P	T2	3 sites × 2 locations (PFN and CIC)	100 ml	18
<i>E. amentacea</i>	Combined functional profile for $C_{org}$ , N, P	T1 (PFN); T2 (PFN and CIC)	PFN: 3 sites × 2 times, $n = 3$ ; CIC: 3 sites, $n = 3$	100 ml	27
<i>E. amentacea</i>	$C_{org}$ , N, P	T2	1 site (SMC), $n = 3$	100 ml	3
<i>C. compressa</i>	$C_{org}$ , N, P	T2	1 site (OTR), $n = 3$	100 ml	3
<i>C. compressa</i>	$C_{org}$ , N, P	T2	1 site (PFN), $n = 3$	100 ml	3

cleaned from epiphytes. *E. amentacea* thalli were wiped up to remove the excess water and weighed altogether to determine the total wet mass (WM) per sample. All thalli in each sample were weighed again after drying at 80 °C for 48 h to obtain the total dry mass (DM). Samples were finally burnt in a muffle at 550 °C for 4 h, the ashes were then weighed, and the total organic matter was determined by subtraction as ash-free dry mass (AFDM). The total organic matter contents were expressed as AFDM per unit of DM ( $g \cdot kg^{-1}$ ).

As soon as possible after field sampling, thalli of *E. amentacea* and *C. compressa* sampled for the analysis of total  $C_{org}$ , N and P contents were placed in seawater, carefully cleaned from epiphytes, wiped up and frozen at −20 °C to avoid loss of organic compounds. Prior to analysis, samples were first dried in oven at 40 °C to constant weight and then lyophilized to completely remove water under low temperature conditions in a freeze-dryer (FD-1A-50, Boyikang Laboratory Instruments Ltd.).

Dried samples were finally homogenized by accurately cutting lyophilized algal tissues. Total  $C_{org}$ , N, and P were determined by acid digestion and chemical titration (UNI EN 13657:2004). Contents were expressed in grams per unit of DM ( $g \cdot kg^{-1}$ ). Limit of quantifications were 0.01  $mg \cdot g^{-1}$  for total N and P, and 0.1  $mg \cdot g^{-1}$  for total  $C_{org}$ .

#### 2.5. Univariate statistical analysis

Analysis of Variance (ANOVA) was performed to test for differences in total organic matter contents of *E. amentacea* samples between locations and among sites within locations, separately for T1 and T2. The design for the analysis consisted of two factors: Location (Lo, random, with two levels, PFN and CIC), and Site (Si(Lo), random and nested in Lo, with three levels), with  $n = 5$ . Variance components associated to each of the three investigated spatial scales, namely locations (100s km), sites (1000s m) and replicates (100s cm) were also estimated using the maximum likelihood estimators for two-way nested random models with balanced data (Searle et al., 1992).

The whole set of data on *E. amentacea* biomass (i.e., including samples from PFN and CIC collected in T1 and T2) was used to quantify weight-to-weight conversion factors of *E. amentacea*. Specifically, data were used to convert WM to DM and DM to AFDM. Conversion factors were estimated as the coefficients of a linear regression ( $y = ax$ ) fitted to DM against WM and AFDM against DM, respectively. Pairwise Pearson's product-moment correlations ( $r$ ) were also calculated.

ANOVA and the estimation of variance components were also performed for total  $C_{org}$ , N and P contents of *E. amentacea*. The design for the ANOVA included factors Location (Lo, random, with two levels, PFN and CIC) and Site (Si(Lo), random and nested in Lo, with three levels), with  $n = 3$ . For this analysis, only sample collected in T2 (which fell in the typical period of maximum growth of the algae) from PFN and CIC were used, as spatial replication in T1 and in other locations prevented any formal comparison.  $C_{org}$ , N, and P contents of *Cystoseira s.l.* from these additional sites were used for the functional analysis described in section 2.6.

For all response variables, the assumption of normality was tested with the Shapiro–Wilk test and the Cochran's C-test was used to test the assumption of homogeneity of variances prior to analysis. In all cases the assumptions of normality and variance homogeneity were respected, except for data on P contents, which were forth root transformed to stabilize variances (Underwood, 1997).

## 2.6. Comparison of functional profiles

To compare the functional trait profile of total  $C_{org}$ , N and P (i.e., the integrated functional range encompassing the three investigated traits) between *E. amentacea* and *C. compressa* from single additional sites and *E. amentacea* from PFN and CIC, a functional hypervolume of total  $C_{org}$ , N, and P trait variables was constructed for *E. amentacea* following the approach proposed by Blonder et al. (2014). This procedure allows inferring the shape and volume of an  $n$ -dimensional real-valued continuous space that encloses a set of  $m$  observations through kernel density estimations (Blonder et al., 2014; Blonder, 2017), and can be applied to model species distribution, traits and niche hyperspace (for an application to plants and phytoplankton see Lamanna et al., 2014; Blonder et al., 2017; de Vries et al., 2021). Here, we employed this approach to infer the combined functional range of total  $C_{org}$ , N, and P contents, that is the three-dimensional functional space identified by these traits, for *E. amentacea*.

All samples from the two locations were used for the analysis ( $m = 27$ ), and the obtained hypervolume was assumed to be representative of *E. amentacea* in the whole study region. The hypervolume was inferred based on three dimensions (i.e., total  $C_{org}$ , N, and P) using a Gaussian kernel density estimate with a bandwidth defined through the Silverman estimator, a quantile threshold of 0.95, and 1999 random replicate samples per point (Blonder et al., 2022). No normalization of data was applied prior to analysis as the three dimensions represented homogeneous variables (i.e., contents of chemical elements per unit of DM) expressed in the same units (i.e.,  $g \cdot kg^{-1}$ ).

We used the hypervolume to (1) check if the functional range of total  $C_{org}$ , N, and P contents of *E. amentacea* can actually be representative of the whole study region by testing whether an independent set of samples of *E. amentacea* collected at SMC (Tyrrhenian Sea) fit the hypervolume. We also (2) checked for homogeneity of  $C_{org}$ , N, and P contents between *C. compressa* and *E. amentacea* by testing whether the set of samples of *C. compressa* from PFN and OTR fit the hypervolume of *E. amentacea*. This was done using the hypervolume inclusion test (Blonder et al., 2022), which determines if a set of points (representative of a set of samples) fall within a hypervolume with a priori fixed probability threshold. The probability threshold below which the points can be considered to be outside the hypervolume (and thus not congruent with the  $n$ -dimensional functional range of *E. amentacea*) was fixed to 0.5.

All analyses were done in R version 4.2.2 (R Development Core Team, 2022) using the packages “GAD” (Sandrini-Neto and Camargo,

2022), “vegan” (Oksanen et al., 2022) and “hypervolume” (Blonder et al., 2022).

## 3. Results

ANOVA detected a significant variation in the total organic matter contents of *E. amentacea* in T1 at the scale of sites (1000s m) and locations (100s km) (Table 2, Fig. 2). The amount of total variation associated to these two spatial scales was comparable, whereas a smaller portion was ascribable to among-replicate variability (Table 2). In T2, ANOVA detected again a significant variability at the scale of sites, whereas no differences in the total organic matter contents were detected between the two locations (Table 3, Fig. 2). In this time of sampling, the largest variance component was due to the smaller scale (i.e., among replicates), followed by the variation among sites, with a null contribution estimated for locations (Table 3).

Dry mass (DM) of *E. amentacea* was strongly correlated to its wet mass (WM) ( $r = 0.94$ ,  $P < 0.001$ ) following a linear relationship (Fig. 3), with an estimated weight-to-weight conversion factor of  $0.16 \pm 0.01$  (Table 4). Similarly, ash-free dry mass (AFDM) was in turn strongly correlated to DM ( $r = 0.99$ ,  $P < 0.001$ ) following a linear relationship (Fig. 3), with an estimated weight-to-weight conversion factor of  $0.71 \pm 0.01$  (Table 4).

ANOVA detected a significant variation among sites within locations in total  $C_{org}$ , N and P contents of *E. amentacea*. However, differences among locations were not significant for all the considered variables (Table 5, Fig. 4). In all cases, the largest portion of variance was associated to the scale of sites within locations, followed by replicates within sites, with a lower or null contribution of locations to the total variance (Table 6).

Average values of  $C_{org}$ , N and P contents of *E. amentacea* from PFN and CIC were quite similar to those of the *E. amentacea* samples from SMC and the *C. compressa* samples from OTR, while *C. compressa* samples from PFN had lower  $C_{org}$ , N and P contents than the other locations (Table 7).

The three independent samples of *E. amentacea* from SMC fell within the hypervolume with an average probability of inclusion of 73%, indicating that the tested samples were highly compatible with the functional space of  $C_{org}$ , N and P contents inferred based on pooled data of *E. amentacea* from PFN and CIC. The same occurred for samples of *C. compressa* from PFN and OTR, which showed, respectively, an average probability of 75% and 70% to belong to the inferred hypervolume. Fig. 5 depicts the graphical comparison of the hypervolume of *E. amentacea* and test samples.

## 4. Discussion

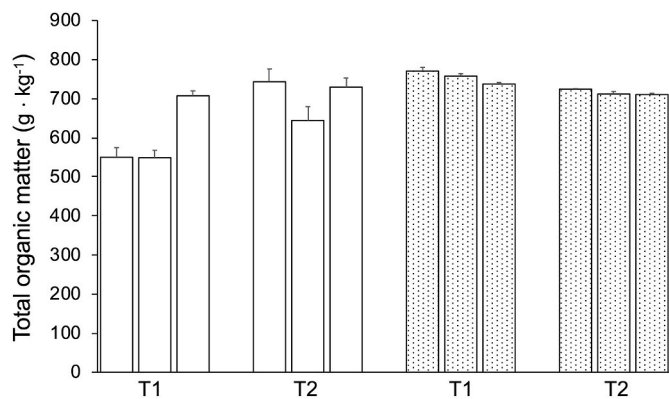
We quantified patterns of variation in organic matter,  $C_{org}$ , N and P tissue contents of *E. amentacea* at a range of spatial scales, including replicates (100s cm), sites (1000s m) and locations (100s km). Overall, we found a larger intraspecific variability of traits at the scales of sites and among replicates with respect to what observed at the scale of locations, supporting the hypothesis that factors and processes at a small and/or very local scale may have a greater influence on functional trait variables than those acting at a larger spatial scale.

The two investigated locations were at the extremes of a broad latitudinal gradient and were characterized by different levels of coastal urbanization and associated human pressures (Cannarozzi et al., 2023). However, differences in average values of  $C_{org}$ , N and P contents were not statistically significant among *E. amentacea* stands from the two study locations, with very low or null contribution of differences between locations to the total variance, indicating a large-scale (100s km) spatial homogeneity of such key functional trait values in this species despite potential effects of changes in environmental conditions. In Mediterranean intertidal and subtidal rocky reefs, low large-scale (10s–100s km) variability can be recurrent (Fraschetti et al., 2005),

**Table 2**

Results of ANOVA testing for variation in the total organic matter content per unit of dry mass of *E. amentacea* between PFN (Portofino MPA) and CIC (Cicliopi Islands MPA) in T1 (October 2020). Variance components associated to the three investigate spatial scales were also estimated; % contribution of each component to the total spatial variation were given in brackets. Results of tests for normality and variance homogeneity are also reported. NS = not significant.

Source	d.f.	SS	MS	F	P	Variance component
Lo	1	176050.0	176050.0	8.209	0.0457	4438.5 (46%)
Si(Lo)	4	85787.0	21447.0	20.469	0.0000	4079.9 (43%)
Res	24	25146.0	1047.7			1047.7 (11%)
Data transformation	None					
Shapiro–Wilk test	$W = 0.952^{NS}$					
Cochran's C-test	$C = 0.314^{NS}$					



**Fig. 2.** Mean ( $\pm$ SE,  $n = 5$ ) content of total organic matter (expressed as grams of ash-free dry mass per kilogram of dry mass) of *E. amentacea* in each site and time of sampling (T1, October 2020; T2, July 2021) at PFN (Portofino MPA, blank bars) and CIC (Cicliopi Islands MPA, dotted bars).

although patterns of variation at different spatial scales can be habitat and/or taxon-specific, or may vary depending on the investigated response variables (e.g., biomass, cover, diversity) (e.g., Frascchetti et al., 2005; Terlizzi et al., 2007; Dal Bello et al., 2017). Differences in nutrient availability, for example, could have little effects on macroalgae tissue contents. Previous studies on *Cystoseira s.l.* species found no significant correlations between tissue nutrients and dissolved nutrients in the ambient water (e.g., *Ericaria mediterranea* (Sauvageau) Molinari & Guiry; Delgado et al., 1994) whereas, species experimentally exposed to nutrient enrichment, showed either a transient effect of increasing

**Table 4**

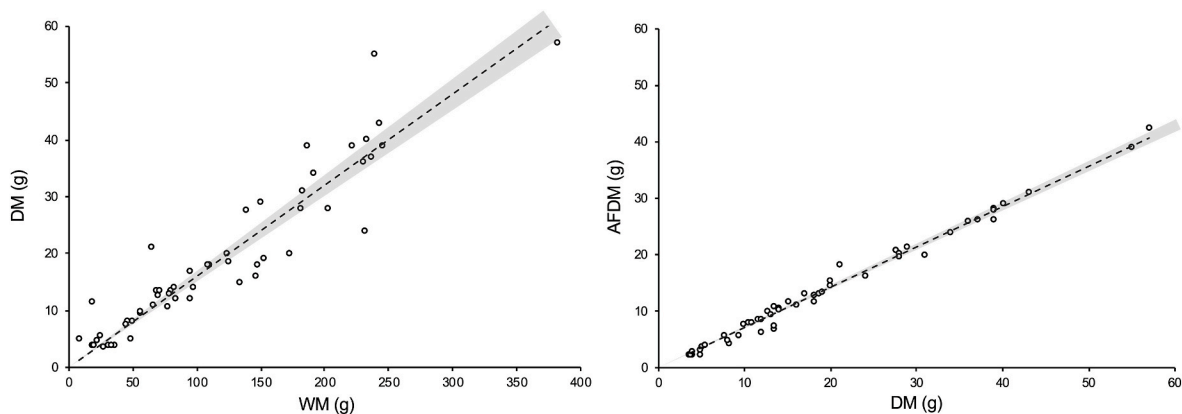
Weight-to-weight conversion factors of wet mass to dry mass (WM/DM) and dry mass to ash-free dry mass (DM/AFDM) estimated as coefficients of linear regression models (a summary of results of regression analysis is reported) fitted to the whole dataset ( $n = 60$ ).

Conversion	Coefficient	SE	$\pm 95\%CI$	Adj. $R^2$	P
WM/DM	0.16	0.005	0.169–0.151	0.941	>0.001
DM/AFDM	0.71	0.006	0.726–0.701	0.977	>0.001

**Table 3**

Results of ANOVA testing for spatial variation in the total organic matter content per unit of dry mass of *E. amentacea* between PFN (Portofino MPA) and CIC (Cicliopi Islands MPA) in T2 (July 2021). Variance components associated to the three investigate spatial scales were also estimated; % contribution of each component to the total spatial variation were given in brackets. Results of tests for normality and variance homogeneity are also reported. NS = not significant.

Source	d.f.	SS	MS	F	P	Variance component
Lo	1	737.6	737.6	0.100	0.7676	0.0 (0%)
Si(Lo)	4	29480.0	7369.9	3.029	0.0373	722.0 (23%)
Res	24	58396.0	2433.2			2433.2 (77%)
Data transformation	None					
Shapiro–Wilk test	$W = 0.959^{NS}$					
Cochran's C-test	$C = 0.452^{NS}$					

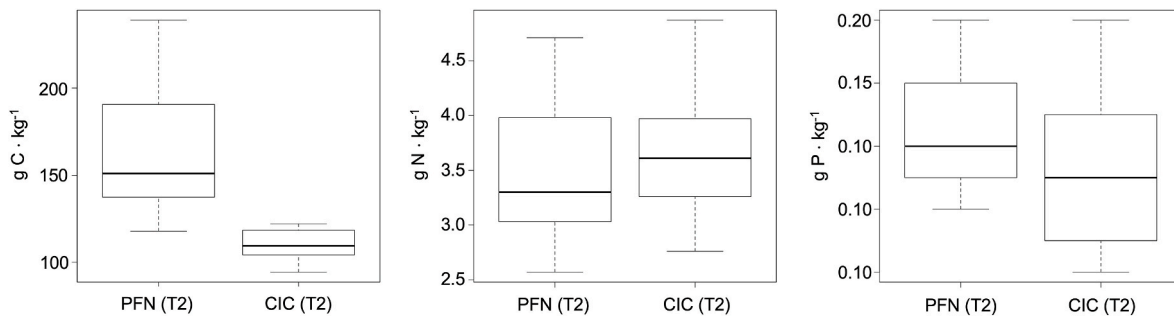


**Fig. 3.** Linear models of dry mass (DM) against wet mass (WM) (left panel) and ash-free dry mass (AFDM) against DM (right panel) of *E. amentacea*. Shaded grey bands represent the  $\pm 95\% CI$  of the linear model fitted to the data ( $n = 60$ ). Results of linear regression analysis were reported in Table 3.

**Table 5**

Summary of ANOVAs testing for spatial variation in the total  $C_{org}$ , N and P contents of *E. amentacea* between PFN (Portofino MPA) and CIC (Ciclopi Islands MPA) (T2 only). Results of tests for normality and variance homogeneity are also reported. \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ ; NS = not significant.

Source	d.f.	$C_{org}$			N			P	
		MS	F		MS	F	MS	F	
Lo	1	15129.0	5.413 <sup>NS</sup>		0.2402	0.168 <sup>NS</sup>	0.0025	0.547 <sup>NS</sup>	
Si(Lo)	4	2795.0	10.062 <sup>***</sup>		1.4255	7.842 <sup>**</sup>	0.0032	7.548 <sup>**</sup>	
Res	12	277.8			0.1818		0.0004		
Data transformation		None			None			$\sqrt{\text{root}}$	
Shapiro-Wilk test		$W = 0.963^{\text{NS}}$			$W = 0.913^{\text{NS}}$			$W = 0.902^{\text{NS}}$	
Cochran's C-test		$C = 0.352^{\text{NS}}$			$C = 0.275^{\text{NS}}$			$C = 0.297^{\text{NS}}$	



**Fig. 4.** Box plots of total  $C_{org}$ , N and P values in *E. amentacea* at PFN (Portofino MPA) and CIC (Ciclopi Islands MPA) (T2 only), expressed as grams per kilograms of dry mass. The black central line indicates the median, boxes indicate the 2nd and the 3rd quartile, and whiskers correspond to the 1st and 4th quartile.

**Table 6**

Variance components associated to the three investigated spatial scales for the total  $C_{org}$ , N and P contents of *E. amentacea* (T2 only). The contribution (%) of each component to the total spatial variation were also reported.

Source	$C_{org}$		N		P	
	Var. comp.	% contrib.	Var. comp.	% contrib.	Var. comp.	% contrib.
Lo	529.9	32%	0.0	0%	0.0	0%
Si(Lo)	839.1	51%	$2.0 \cdot 10^{-1}$	53%	$5.0 \cdot 10^{-4}$	57%
Res	277.8	17%	$1.8 \cdot 10^{-1}$	47%	$4.0 \cdot 10^{-4}$	43%

**Table 7**

Mean ( $\pm$ SE) total  $C_{org}$ , N and P contents ( $g \cdot kg^{-1}$  of dry mass) of *E. amentacea* and *C. compressa* in the study locations. T1 = October 2020, T2 = July 2021. PFN = Portofino MPA, CIC = Ciclopi Islands MPA, SMC = Santa Maria di Castellabate MPA, OTR = European Special Area of Conservation of Otranto.

Location (Time)	Species	$C_{org}$	N	P	Total no. of samples
PFN-CIC (T1-T2)	<i>E. amentacea</i>	134.42 ( $\pm 14.71$ )	4.02 ( $\pm 0.40$ )	0.11 ( $\pm 0.01$ )	27
PFN (T2)	<i>C. compressa</i>	90.33 ( $\pm 9.69$ )	2.88 ( $\pm 0.27$ )	0.04 ( $\pm 0.02$ )	3
SMC (T2)	<i>E. amentacea</i>	135.13 ( $\pm 15.91$ )	3.22 ( $\pm 0.54$ )	0.13 ( $\pm 0.02$ )	3
OTR (T2)	<i>C. compressa</i>	148.37 ( $\pm 19.50$ )	3.63 ( $\pm 0.37$ )	0.07 ( $\pm 0.02$ )	3

nutrient contents (e.g., *Ericaria brachycarpa* (J.Agardh) Molinari & Guiry; Gennaro et al., 2019) or a lower nutrient uptake with respect to turf-forming algae (e.g., *Ericaria selaginoides* (Linnaeus) Molinari & Guiry; Stengel et al., 2014). Comparable temporal patterns of nutrient concentrations during the period of study (Teruzzi et al., 2021, see Fig. S1 in supplementary material) may have further contributed to reduce the variability between the two locations.

The largest variance components were associated to the scale of sites within locations (1000s m) and, secondarily, to variability among replicates (100s cm) for all trait variables, suggesting that smaller-scale variation in environmental conditions (e.g., habitat patchiness, local nutrient load) can exceed average large-scale effects on biomass production and composition, so that the amount of nutrients in macroalgal tissues might be strongly dependent on local environmental features and the historical nutrient regimes of sites (Gennaro et al., 2019). *Cystoseira s.l.* species generally undergo significant seasonal changes in nutrients and organic carbon in response to phenology during their annual cycle (Delgado et al., 1994; Celis-Plá et al., 2014b, 2016). Interestingly, contents of total organic matter differed between PFN and CIC in T1 (the unique case in which variance components at the scales of sites and locations were comparable), but not in T2. This finding could seem counterintuitive, as differences in organic matter, if any, are expected to emerge in the vegetative-growing phase rather than during the resting period. Indeed, this result can be explained by the different onset and duration of the vegetative phase of *E. amentacea* between the two locations, as a consequence of the different biogeographic position and, therefore, in seasonality of irradiance and seawater temperature, which are among the main factors affecting algal productivity along with nutrient availability. In autumn, *E. amentacea* in Sicily (CIC) could experience a vegetative resumption accompanied by fructification, while in Liguria (PFN) it sheds its fronds, and the reproductive period concludes by the end of August. Most probably, environmental conditions in T1, although falling in autumn, were still suitable to maintain a relatively consistent productivity of *E. amentacea* in the South Ionian Sea, where CIC is located, but not at PFN, ~700 km north in the NW Mediterranean. A note of caution on this explanation is necessary, however, as factors other than the biogeographic position, such as for instance context-dependent environmental peculiarities of locations, could underlie the differences of total organic matter observed in T2.

Variations in environmental conditions, especially nutrient concentrations, irradiance and seawater temperature, may strongly affect the physiology, nutrient uptake, and photosynthetic performance of macroalgae determining, ultimately, their biomass production and composition (e.g., Delgado et al., 1994; Nygård and Dring, ; Celis-Plá et al.,

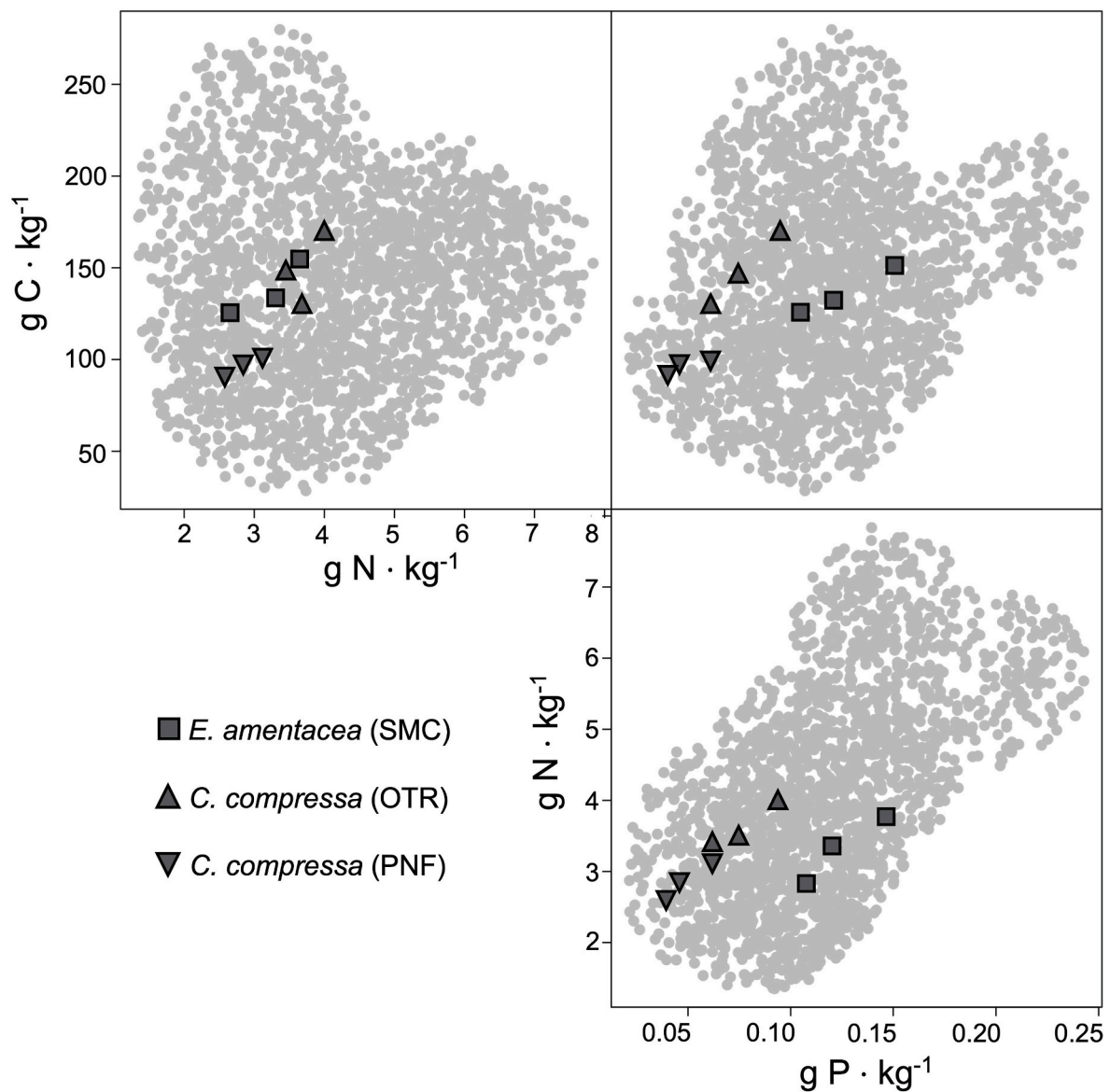


Fig. 5. Hypervolume of *E. amentacea* in the three dimensions corresponding to the total  $C_{org}$ , N and P contents. The hypervolume is shown as 2-d projections for all combinations of the three functional trait axes. In each panel, the projection is represented by a cloud of (light grey) points which were randomly sampled (i.e., they represent a stochastic description of the hypervolume) from the hypervolume inferred based on real data on total  $C_{org}$ , N and P contents of *E. amentacea* at PFN (Portofino MPA) and CIC (Ciclopi Islands MPA) ( $m = 27$ ). Dark symbols in each panel indicate real values of samples of *E. amentacea* from SMC (Santa Maria di Castellabate MPA), and *C. compressa* from PFN and OTR (European Special Area of Conservation of Otranto).

2015, 2016; Mancuso et al., 2019; Gennaro et al., 2019; Falace et al., 2021; Sánchez de Pedro et al., 2023). Responses to such variations and, more generally, the functional trait variables like organic matter,  $C_{org}$ , N and P contents, can nevertheless be species-specific or group-specific, depending on differences in life strategies, morphology and phenology among macroalgae (e.g., Stengel et al., 2014; Celis-Plá et al., 2015; Mauffrey et al., 2020). In contrast, the comparison of  $C_{org}$ , N and P cumulative profiles between *E. amentacea* and *C. compressa* indicated the potential interspecific overlapping for tissue contents of these key trait variables, although further assessments are certainly required to confirm this preliminary result. The fact that these two species have a similar morphotype (i.e., caespitose thallus), size and phenology (Falace et al., 2005), could be reflected in similar responses in terms of nutrient uptake and organic matter content (e.g., Wallentinus, 1984; Phillips and Hurd, 2004). Moreover, different *Cystoseira s.l.* species may exhibit comparable tissue contents of nutrients and organic carbon when sharing the same habitat and/or geographic location (Ballesteros and Sant, 2022).

*E. amentacea* and *C. compressa* seemed to have lower tissue contents of  $C_{org}$ , N and P compared to other *Cystoseira s.l.* species (Brey et al., 2010; Ballesteros and Sant, 2022). It is worth noting here that we focused on populations inhabiting the midlittoral rocky fringe, while most species in other studies typically occurred (or were sampled) in subtidal rocky habitats. Lower tissue contents of nutrients and organic carbon can therefore be due to differences in phenology, reservoir allocation, structural compounds (e.g., cellulose, alginates) among species inhabiting different habitats, or to gradients of decreasing nutrient availability with decreasing depth (Ballesteros, 1989; Sant and Ballesteros, 2020, 2021).

Traits analysed in this study are only a subset of ‘effect’ traits (i.e., traits potentially impacting the functioning of community and ecosystems; Violle et al., 2007) from a much wider spectrum of traits which also encompass morphological, physiological and phenological features of macroalgae (e.g., Violle et al., 2007; Cappelatti et al., 2019; Mauffrey et al., 2020). However, as brown canopy-forming macroalgae are crucial

for biomass production and energy flows along marine trophic networks (Umanzor et al., 2019; Duarte et al., 2022; Pessarodona et al., 2022), baseline information on such key functional traits of *E. amentacea* and other *Cystoseira s.l.* species is essential for a deeper understanding of their contribution to the functioning of Mediterranean coastal ecosystems. Although our assessment is clearly not exhaustive in terms of spatial and temporal extent, it provides some cues on the potential role of *E. amentacea* in coastal carbon cycling. A rough estimate of biomass turnover (dry weight) of *E. amentacea* from previous works in the investigated locations accounts for  $1059.3 \pm 566.7 \text{ g m}^{-2} \text{ y}^{-1}$  (Cannarozzi et al., 2023), and our estimates of  $C_{\text{org}}$  content averaged  $134.3 \pm 15.0 \text{ g C kg}^{-1}$  dry weight, resulting in a mean total organic carbon fixation of  $1.423 \text{ t C ha}^{-1} \text{ y}^{-1}$ .

The magnitude of carbon fixation rates per unit area in *E. amentacea* could therefore be comparable to those of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile, whose most recent estimates equal to  $1.302 \text{ t C ha}^{-1} \text{ y}^{-1}$ , including leaves, sheaths and rhizomes fixation (Pergent-Martini et al., 2021). The overall contribution of *Cystoseira s.l.* forests to the total carbon budget of Mediterranean coastal areas is probably paltry by comparison with *P. oceanica* seagrass beds, which extend over a larger bottom surface than *Cystoseira s.l.* in the basin. It should also be noted that *Cystoseira s.l.* forests could not be considered long-term carbon sinks, as they primarily grow on rocky bottoms and unlike seagrasses, which allow carbon deposition in large structures made by the intricate complex of roots and dead rhizomes buried in soft sediments (i.e., mattes), lack substantial organic accretions that can underlay significant carbon sequestration (Duarte et al., 2013). However, macroalgae export about 43% of their production as both particulate and dissolved organic carbon (Krause-Jensen et al., 2018), and the net transport of carbon towards deep systems can double that of seagrasses ( $8.5 \text{ mol C m}^{-2}$  for macroalgae in contrast to  $4.4 \text{ mol C m}^{-2}$  for seagrass; Barrón and Duarte, 2015). In this view, *Cystoseira s.l.* stands can represent important carbon donors to neighbouring systems, where this carbon can be sequestered in sediments and deep waters over a long time-scale (Hill et al., 2015).

From a more practical perspective, our findings allow some pragmatic considerations to account for in applied ecological research on *E. amentacea* and other *Cystoseira s.l.* species. Since organic carbon, nitrogen and phosphorous contents can largely vary at a very local scale, extrapolating functional properties or goods and services of these macroalgal stands (e.g., their natural capital) over large geographic areas could significantly under- or overestimate the ensuing quantifications, if based on data restricted to single, small spatial extents.

This high small-scale variability could also have important implications for conservation and active restoration strategies involving *Cystoseira s.l.* species. For example, restoration interventions on *Cystoseira s.l.* stands are often restricted to relatively small areas (Verdura et al., 2018; De La Fuente et al., 2019), which could match the scale at which key functional traits are more variable. Given that restoration costs for *Cystoseira s.l.* ranges between 57,000 and 7,000,000 €/ha, depending on the restoration techniques, logistics, and environmental context (Cebrian et al., 2021 and references therein), preliminary assessments on key functional traits of neighbouring and/or resident populations could help identify sites that could maximize the return of economic investments in terms of functional performance of restored *Cystoseira s.l.* stands.

The baseline knowledge of productivity, biomass, key functional traits, and especially their spatial and temporal variations from local to large scale, is still very limited for *E. amentacea* and, more generally, for many *Cystoseira s.l.* species, hindering reliable quantifications of uptake, fixation, export and storage of carbon and key elements of intertidal and subtidal stands of Mediterranean furoid algae. This study is one of the first attempts to fill these gaps, which could contribute to refine assessments of the functional role of *Cystoseira s.l.* forests and to improve their management and conservation.

## Funding

This research was part of the PhD program in 'Environment and Life' of L.C. funded by the University of Trieste.

## CRediT authorship contribution statement

**Laura Cannarozzi:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Stanislao Bevilacqua:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Monia Renzi:** Writing – review & editing, Methodology, Investigation. **Serena Anselmi:** Writing – review & editing, Methodology, Investigation. **Giuseppina Alongi:** Writing – review & editing, Investigation. **Valentina Asnaghi:** Writing – review & editing, Investigation. **Mariachiara Chiantore:** Writing – review & editing, Investigation. **Annachiara Pagnotta:** Writing – review & editing, Investigation. **Chiara Paoli:** Writing – review & editing, Investigation. **Ilaria Rigo:** Writing – review & editing, Investigation. **Annalisa Falace:** Writing – review & editing, Supervision, Methodology, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgments

This research was sustained by the MPAs of Portofino and Isole Ciclopi, which also gave special permission for undertaking destructive sampling in the no-take, no-access zone. The authors also greatly acknowledge the support of the entire staff of the MPAs for logistic assistance and invaluable help during sampling campaign.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2023.108524>.

## References

- AbouAisha, K.M., Shabana, E.F., ElAbyad, M.S., Kobbia, I.A., Schanz, F., 1997. Seasonal changes in *Cystoseira myrica* and phosphorus input at two sites of the Red Sea Egyptian coast. *Water Air Soil Pollut.* 93, 199–211.
- Ballesteros, E., 1989. Production of seaweeds in Northwestern Mediterranean marine communities: its relation with environmental factors. *Sci. Mar.* 53, 357–364.
- Ballesteros, E., Sant, N., 2022. Homogeneity of photosynthetic features in canopy-forming macroalgae of the order Fucales from shallow and sheltered environments. *Cryptogam. Algol.* 43, 107–115.
- Ballesteros, E., Sala, E., Garrabou, J., Zabala, M., 1998. Community structure and front size distribution of a deep Water stand of *Cystoseira spinose* (Phaeophyta) in the Northwestern Mediterranean. *Eur. J. Phycol.* 33, 121–128.
- Barrón, C., Duarte, C.M., 2015. Dissolved organic carbon pools and export from the coastal ocean. *Global Biogeochem. Cycles* 29, 1725–1738.
- Benedetti-Cecchi, L., Pannacciulli, F., Bulleri, F., Moschella, P.S., Airoldi, L., Relini, G., Cinelli, F., 2001. Predicting the consequences of anthropogenic disturbance: large-scale effects of loss of canopy algae on rocky shores. *Mar. Ecol. Prog. Ser.* 214, 137–150.
- Bertocci, I., Araújo, R., Oliveira, P., Sousa-Pinto, I., 2015. Potential effects of kelp species on local fisheries. *J. Appl. Ecol.* 52, 1216–1226.
- Bevilacqua, S., Savonitto, G., Lipizer, M., Mancuso, P., Ciriaco, S., Srijemsi, M., Falace, A., 2019. Climatic anomalies may create a long-lasting ecological phase shift by altering the reproduction of a foundation species. *Ecology* 100, e02838.
- Bevilacqua, S., Airoldi, L., Ballesteros, E., Benedetti-Cecchi, L., Boero, F., Bulleri, F., Cebrian, E., Cerrano, C., Claudet, J., Colloca, F., Coppari, M., Di Franco, A., Frascchetti, S., Garrabou, J., Guarnieri, G., Guidetti, P., Guerranti, C., Halpern, B.J., Katsanevakis, S., Mangano, M.C., Micheli, F., Milazzo, M., Pusceddu, A., Renzi, M.,



- Rilov, G., Sarà, G., Terlizzi, A., 2021. Mediterranean rocky reefs in the Anthropocene: present status and future concerns. *Adv. Mar. Biol.* 89, 1–51.
- Bianchelli, S., Danovaro, R., 2020. Impairment of microbial and meiofaunal ecosystem functions linked to algal forest loss. *Sci. Rep.* 10, 19970.
- Blanfuné, A., Boudouresque, C.F., Verlaque, M., Thibaut, T., 2016. The fate of *Cystoseira crinita*, a forest-forming fucales (phaeophyceae, stramenopiles), in France (north western Mediterranean Sea). *Estuar. Coast Shelf Sci.* 181, 196–208.
- Blonder, B., 2017a. Hypervolume concepts in niche- and trait-based ecology. *Ecography* 40, 1–13.
- Blonder, B., Lamanna, C., Violle, C., Enquist, B., 2014. The *n*-dimensional hypervolume. *Global Ecol. Biogeogr.* 23, 595–609.
- Blonder, B., Morrow, C.B., Maitner, B., Harris, D.J., Lamanna, C., Violle, C., Enquist, B., Kerckhoff, A.J., 2017. New approaches for delineating *n*-dimensional hypervolumes. *Methods Ecol. Evol.* 9, 305–319.
- Blonder, B., Morrow, C.B., Harris, D.J., Brown, S., Butruille, G., Laini, A., Chen, D., 2022. Hypervolume: High Dimensional Geometry, Set Operations, Projection, and Inference Using Kernel Density Estimation, Support Vector Machines, and Convex Hulls. R package version 3.0.3. <https://CRAN.R-project.org/package=hypervolume>.
- Brey, T., Müller-Wiegmann, C., Zittler, Z.M.C., Hagen, W., 2010. Body composition in aquatic organisms - a global data bank of relationships between mass, elemental composition and energy content. *J. Sea Res.* 64, 334–340.
- Cannarozzi, L., Bevilacqua, S., Alongi, G., Asnaghi, V., Chiantore, M., Pagnotta, A., Paoli, C., Rigo, I., Vassallo, P., Falace, A., 2023. Assessing the effect of full protection on the biomass of *Ericaria amantacea* and understory assemblages: evidence from two mediterranean marine protected areas. *Diversity* 15, 89.
- Cappelletti, L., Mauffrey, A.R.L., Griffin, J.N., 2019. Applying continuous functional traits to large brown macroalgae: variation across tidal emersion and wave exposure gradients. *Mar. Biol.* 166, 145.
- Cebrian, E., Tamburello, L., Verdura, J., Guarnieri, G., Medrano, A., Linares, C., Hereu, B., Garrabou, J., Cerrano, C., Galobart, C., Fraschetti, S., 2021. A roadmap for the restoration of mediterranean macroalgal forests. *Front. Mar. Sci.* 8, 709219.
- Celis-Plá, P.S.M., Martínez, B., Quintano, E., García-Sánchez, M., Pedersen, A., Navarro, N., Copertino, M., Mangiyarkarasi, N., Mariath, R., Figueroa, F.L., Korbee, N., 2014a. Short-term ecophysiological and biochemical responses of *Cystoseira tamariscifolia* and *Ellisolandia elongata* to environmental changes. *Aquat. Bot.* 222, 227–243.
- Celis-Plá, P.S.M., Korbee, N., Gómez-Garreta, A., Figueroa, F.L., 2014b. Seasonal photoacclimation patterns in the intertidal macroalga *Cystoseira tamariscifolia* (Ochrophyta). *Sci. Mar.* 78, 377–388.
- Celis-Plá, P.S.M., Hall-Spencer, J.M., Horta, P.A., Milazzo, M., Korbee, N., Cornwall, C.E., Figueroa, F.L., 2015. Macroalgal responses to ocean acidification depend on nutrient and light levels. *Front. Mar. Sci.* 2, 26.
- Celis-Plá, P.S.M., Bouzon, Z.L., Hall-Spencer, J.M., Schmidt, E.C., Korbee, N., Figueroa, F.L., 2016. Seasonal biochemical and photophysiological responses in the intertidal macroalga *Cystoseira tamariscifolia* (Ochrophyta). *Mar. Environ. Res.* 115, 89–97.
- Cheminée, A., Sala, E., Pastor, J., Bodilis, P., Thiriet, P., Mangialajo, L., Cottalorda, J.M., Francour, P., 2013. Nursery value of *Cystoseira* forests for Mediterranean rocky reef fishes. *J. Exp. Mar. Biol. Ecol.* 442, 70–79.
- Clausing, R.J., De La Fuente, G., Falace, A., Chiantore, M., 2023. Accounting for environmental stress in restoration of intertidal foundation species. *J. Appl. Ecol.* 60, 305–318.
- Dal Bello, M., Leclerc, J.-C., Benedetti-Cecchi, L., De Lucia, G.A., Arvanitidis, C., Van Avesaath, P., Bachelet, G., Bojanic, N., Como, S., Coppa, S., Coughlan, J., Crowe, T., Degraer, S., Espinosa, F., Foulwetter, S., Frost, M., Guinda, X., Jankowska, E., Jourde, J., De La Pena, J.A.J., Kerckhof, F., Kotta, J., Lavesque, N., Magni, P., De Matos, V., Orav-Kotta, H., Pavlouli, C., Pedrotti, M.L., Peleg, O., Pérez-Ruzafa, A., Puente, A., Ribeiro, P., Rigaut-Jalabert, F., Rilov, G., Rousou, M., Rubal, M., Ruginis, T., Silva, T., Simon, N., Sousa-Pinto, I., Troncoso, J., Warzocha, J., Weslawski, J.M., Hummel, H., 2017. Consistent patterns of spatial variability between NE Atlantic and Mediterranean rocky shores. *J. Mar. Biol. Assoc. U. K.* 97, 539–547.
- De La Fuente, G., Chiantore, M., Asnaghi, V., Kaleb, S., Falace, A., 2019. First ex situ outplanting of the habitat-forming seaweed *Cystoseira amantacea* var. *stricta* from a restoration perspective. *PeerJ* 7, e7290.
- de Vries, J., Monteiro, F., Wheeler, G., Poulton, A., Godrijan, J., Cerino, F., Malinverno, E., Langer, G., Brownlee, C., 2021. Haplo-diplontic life cycle expands coccolithophore niche. *Biogeosciences* 18, 1161–1184.
- Delgado, O., Ballesteros, E., Vidal, M., 1994. Seasonal variation in tissue nitrogen and phosphorus of *Cystoseira mediterranea* Sauvageau (fucales, phaeophyceae) in the northwestern Mediterranean Sea. *Bot. Mar.* 37, 1–9.
- Duarte, C., Losada, I., Hendriks, I., Mazarrasa, I., Marbà, N., 2013. The role of coastal plant communities for climate change mitigation and adaptation. *Nat. Clim. Change* 3, 961–968.
- Duarte, C.M., Gattuso, J.-P., Hancke, K., Gundersen, H., Filbee-Dexter, K., Pedersen, M. F., Middelburg, J.J., Burrows, M.T., Krumhansl, K.A., Wernberg, T., Moore, P., Pessarrodona, A., Ørberg, S.B., Sousa-Pinto, I., Assis, J., Queirós, A.M., Smale, D.A., Bekkby, T., Serrão, E.A., Krause-Jensen, D., 2022. Global estimates of the extent and production of macroalgal forests. *Global Ecol. Biogeogr.* 31, 1422–1439.
- Fabbrizzi, E., Scardi, M., Ballesteros, E., Benedetti-Cecchi, L., Cebrian, E., Ceccherelli, G., De Leo, F., Deidun, A., Guarnieri, G., Falace, A., Fraissinet, S., Giommi, C., Maciç, V., Mangialajo, L., Mannino, A.M., Piazzali, L., Ramdani, M., Rilov, G., Rindi, L., Rizzo, L., Sarà, G., Souissi, J.B., Taskin, E., Fraschetti, S., 2020. Modeling macroalgal forest distribution at mediterranean scale: present status, drivers of changes and insights for conservation and management. *Front. Mar. Sci.* 7, 20.
- Falace, A., Zanelli, E., Bressan, G., 2005. Morphological and reproductive phenology of *Cystoseira compressa* (esper) Gerloff & Nizamuddin (fucales, fucophyceae) in the gulf of Trieste. *Ann. Hist. Sci. Soc.* 15, 71–78.
- Falace, A., Marletta, G., Savonitto, G., Candotto Carniel, F., Srijemsi, M., Bevilacqua, S., Tretiach, M., Alongi, G., 2021. Is the South-mediterranean canopy-forming *Ericaria giacomiei* (= *Cystoseira hyblaea*) a loser from ocean warming? *Front. Mar. Sci.* 8, 760637.
- Filbee-Dexter, K., Feehan, C.J., Smale, D.A., Krumhansl, K.A., Augustine, S., de Bettignies, F., Burrows, M.T., Byrnes, J.E.K., Campbell, J., Davout, D., Dunton, K.H., Franco, J.N., Garrido, I., Grace, S.P., Hancke, K., Johnson, L.E., Konar, B., Moore, P. J., Norderhaug, K.M., O'Dell, A., Pedersen, M.F., Salomon, A.K., Sousa-Pinto, I., Tiegs, S., Yiu, D., Wernberg, T., 2022. Kelp carbon sink potential decreases with warming due to accelerating decomposition. *PLoS Biol.* 20, e3001702.
- Fraschetti, S., Terlizzi, A., Benedetti-Cecchi, L., 2005. Patterns of distribution of marine assemblages from rocky shores: evidence of relevant scales of variation. *Mar. Ecol. Prog. Ser.* 296, 13–29.
- Gennaro, P., Piazzali, L., Persia, E., Porrello, S., 2019. Responses of macroalgal assemblages dominated by three Mediterranean brown macroalgae with different life strategies to nutrient enrichment. *Eur. J. Phycol.* 54, 432–446.
- Graham, M.H., 2004. Effects of local deforestation on the diversity and structure of southern California giant kelp forest food webs. *Ecosystems* 7, 341–357.
- Graham, M.H., 2008. Diversity and dynamics of Californian subtidal kelp forests. In: Branch, G.M., McClanahan, T.R. (Eds.), *Food Webs and the Dynamics of Marine Reefs*. Oxford Univ. Press, UK, pp. 103–134.
- Guidetti, P., 2006. Marine reserves reestablish lost predatory interactions and cause community changes in rocky reefs. *Ecol. Appl.* 16, 963–976.
- Hereu, B., Zabala, M., Sala, E., 2008. Multiple controls of community structure and dynamics in a sublittoral marine environment. *Ecology* 89, 3423–3435.
- Hill, R., Bellgrove, A., Macreadie, P.I., Petrou, K., Beardall, J., Steven, A., Ralph, P.J., 2015. Can macroalgae contribute to blue carbon? An Australian perspective. *Limnol. Oceanogr.* 60, 1689–1706.
- Kaleb, S., Sánchez de Pedro, R., Bañares-España, E., Alboresi, A., Savonitto, G., Natale, S., Bevilacqua, S., Falace, A., 2023. Cultivation of *Gongolaria barbata* (Fucales, Phaeophyceae) with a seaweed-derived biostimulant in order to improve photophysiological fitness and promote fertility to advance the restoration of marine macroalgal forests. *J. Appl. Phycol.* (in press).
- Koenigs, C., Miller, R.J., Page, & H.M., 2015. Top predators rely on carbon derived from giant kelp *Macrocystis pyrifera*. *Mar. Ecol. Prog. Ser.* 537, 1–8.
- Krause-Jensen, D., Lavery, P., Serrano, O., Marbà, N., Masque, P., Duarte, C.M., 2018. Sequestration of macroalgal carbon: the elephant in the Blue Carbon room. *Biol. Lett.* 14, 20180236.
- Lamanna, C., Blonder, B., Violle, C., Kraft, N.J.B., Sandel, B., Šimová, I., Donoghue, J.S., Svenning, J.-C., McGill, B.J., Boyle, B., Buzzard, V., Dolins, S., Jørgensen, P.M., Marcuse-Kubitza, A., Moureta-Holme, N., Peet, R.K., Piel, W.H., Regetz, J., Schildhauer, M., Spencer, N., Thiers, B., Wiser, S.K., Enquist, B.J., 2014. Functional trait space and the latitudinal diversity gradient. *Proceedings of the National Academy of Science USA* 111, 13745–13750.
- Mancuso, F.P., Strain, E.M.A., Piccioni, E., De Clerck, O., Sarà, G., Airoldi, L., 2018. Status of vulnerable *Cystoseira* populations along the Italian infralittoral fringe, and relationships with environmental and anthropogenic variables. *Mar. Pollut. Bull.* 129, 762–771.
- Mancuso, F.P., Messina, C.M., Santulli, A., Laudicella, V.A., Giommi, C., Sarà, G., Airoldi, L., 2019. Influence of ambient temperature on the photosynthetic activity and phenolic content of the intertidal *Cystoseira compressa* along the Italian coastline. *J. Appl. Phycol.* 3 (1), 3069–3076.
- Mancuso, F.P., Milazzo, M., Chemello, R., 2021. Decreasing in patch-size of *Cystoseira* forests reduces the diversity of their associated molluscan assemblages in Mediterranean rocky reefs. *Estuar. Coast Shelf Sci.* 250, 107163.
- Mauffrey, A.R.L., Cappelletti, L., Griffin, J.N., 2020. Seaweed functional diversity revisited: confronting traditional groups with quantitative traits. *J. Ecol.* 108, 2390–2405.
- Milazzo, M., Badalamenti, F., Riggio, S., Chemello, R., 2004. Patterns of algal recovery and small-scale effects of canopy removal as a result of human trampling on a Mediterranean rocky shallow community. *Biol. Conserv.* 117, 191–202.
- Mineur, F., Arenas, F., Assis, J., Davies, A.J., Engelen, A.H., Fernandes, F., Malta, E., Thibaut, T., Van Nguyen, T., Vaz-Pinto, F., Vranken, S., Serrão, E.A., De Clerck, O., 2015. European seaweeds under pressure: consequences for communities and ecosystem functioning. *J. Sea Res.* 98, 91–108.
- Molinari-Novoa, E.A., Guiry, M.D., 2020. Reinstatement of the genera *Gongolaria* boehmer and *Ericaria* stackhouse (sargassaceae, phaeophyceae). *Notulae Algarum* 172, 1–10.
- Nygård, C. A. & Dring, M. J. Influence of salinity, temperature, dissolved inorganic carbon and nutrient concentration on the photosynthesis and growth of *Fucus vesiculosus* from the Baltic and Irish Seas. *Eur. J. Phycol.*, 43, 253–262.
- Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Antoniazzi Evangelista, H.B., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlenn, D., Ouellette, M.-H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J., 2022. In: *Vegan: Community Ecology Package*. R Package Version 2.6-2. <https://CRAN.R-project.org/package=vegan>.
- Orlando-Bonaca, M., Savonitto, G., Asnaghi, V., Trkov, D., Pitacco, V., Šisko, M., Makovec, T., Slavinec, P., Lokovšek, A., Ciriaco, S., Chiantore, M., Kaleb, S., Descourvières, E.P., Srijemsi, M., Falace, A., 2022. Where and how - new insight for brown algal forest restoration in the Adriatic. *Front. Mar. Sci.* 9, 988584.

- Peleg, O., Guy-Haim, T., Yeruham, E., Silverman, J., Rilov, G., 2020. Tropicalization may invert trophic state and carbon budget of shallow temperate rocky reefs. *J. Ecol.* 108, 844–854.
- Pergent-Martini, C., Pergent, G., Monnier, B., Boudouresque, C.F., Mori, C., Valette-Sansevin, A., 2021. Contribution of *Posidonia oceanica* meadows in the context of climate change mitigation in the Mediterranean Sea. *Mar. Environ. Res.* 165, 105236.
- Pessarodona, A., Assis, J., Filbee-Dexter, K., Burrows, M.T., Gattuso, J.-P., Duarte, C., Frause-Jensen, D., Moore, J.P., Smale, D.A., Wernberg, T., 2022. Global seaweed productivity. *Sci. Adv.* 8, eabn2465.
- Phillips, J.C., Hurd, C.L., 2004. Kinetics of nitrate, ammonium, and urea uptake by four seaweeds from New Zealand. *J. Phycol.* 40, 534–545.
- Sala, E., Boudouresque, C.F., Harmelin-Vivien, M., 1998. Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. *Oikos* 82, 425–439.
- Sales, M., Ballesteros, E., 2012. Seasonal dynamics and annual production of *Cystoseira crinita* (Fucales: ochrophyta) - dominated assemblages from the Northwestern Mediterranean. *Sci. Mar.* 76, 391–401.
- Sales, M., Cebrian, E., Tomas, F., Ballesteros, E., 2011. Pollution impacts and recovery potential in three species of the genus *Cystoseira* (Fucales, Heterokontophyta). *Estuar. Coast Shelf Sci.* 92, 347–357.
- Sánchez de Pedro, R., Fernández, A.N., Melero-Jiménez, I.J., García-Sánchez, M.J., Flores-Moya, A., Bañares-España, E., 2023. Temporal and spatial variability in population traits of an intertidal furoid reveals local-scale climatic refugia. *Mar. Environ. Res.*, 106006.
- Sandrini-Neto, L., Camargo, M.G., 2022. GAD: an R package for ANOVA designs from general principles. Available on CRAN.
- Sant, N., Ballesteros, E., 2020. Photosynthetic activity of macroalgae along a bathymetric gradient: interspecific and seasonal variability. *Sci. Mar.* 84, 7–16.
- Sant, N., Ballesteros, E., 2021. Depth distribution of canopy-forming algae of the order Fucales along a bathymetric gradient is related to their photosynthetic features. *Mar. Ecol.* 42, e12651.
- Searle, S.R., Casella, G., McCulloch, & C.E., 1992. Chapter 4. *Variance Components*. John Wiley & Sons Inc., pp. 112–167.
- Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience* 57, 573–583.
- Stengel, D.B., Conde-Alvarez, R., Connan, S., Nitschke, U., Arenas, F., Abreu, H., Bonomi Barufi, J., Chow, F., Robledo, D., Malta, E.J., Mata, M., Konotchick, T., Nassar, C., Pérez-Ruzafa, Á., López, D., Marquardt, R., Vaz-Pinto, F., Celis-Plá, P.S.M., Hermoso, M., Ruiz, E., Ordoñez, G., Flores, P., Zanolla, M., Bañares-España, E., Altamirano, M., Korbee, N., Bischof, K., Figueroa, F.L., 2014. Short-term effects of CO<sub>2</sub>, nutrients and temperature on three marine macroalgae under solar radiation. *Aquat. Biol.* 22, 159–176.
- Strain, E.M.A., Thomson, R.J., Micheli, F., Mancuso, F.P., Airoldi, L., 2014. Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biol.* 20, 3300–3312.
- Tamburello, L., Chiarore, A., Fabbrizzi, E., Colletti, A., Franzitta, G., Grech, D., Rindi, F., Rizzo, L., Savinelli, B., Fraschetti, S., 2022. Can we preserve and restore overlooked macroalgal forests? *Sci. Total Environ.* 806, 150855.
- Terlizzi, A., Anderson, M.J., Fraschetti, S., Benedetti-Cecchi, L., 2007. Scales of spatial variation in Mediterranean subtidal sessile assemblages at different depths. *Mar. Ecol. Prog. Ser.* 332, 25–39.
- Teruzzi, A., Di Biagio, V., Feudale, L., Bolzon, G., Lazzari, P., Salon, S., Coidessa, G., Cossarini, G., 2021. Mediterranean Sea biogeochemical reanalysis (CMEMS MED-biogechemistry. DOI (Product). In: MedBFM3 System) (Version 1) Data Set. Copernicus Monitoring Environment Marine Service (CMEMS), 10.25423/cmcc/medsea\_multiyear\_bgc.006.008.medbfm3.
- Umanzor, L.L., Calderon-Aguilera, L.E., Zertuche-González, J.A., 2019. Testing the relative importance of intertidal seaweeds as ecosystem engineers across tidal heights. *J. Exp. Mar. Biol. Ecol.* 511, 100–107.
- Underwood, A.J., 1997. *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variances*. Cambridge University Press, UK, p. 504.
- Verdura, J., Sales, M., Ballesteros, E., Cefali, M.E., Cebrian, E., 2018. Restoration of a canopy-forming alga based on recruitment enhancement: methods and long-term success assessment. *Front. Plant Sci.* 9, 1832.
- Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier, E., 2007. Let the concept of trait be functional. *Oikos* 116, 882–892.
- Vranken, S., Robuchon, M., Dekeyser, S., Bárbara, I., Bartsch, I., Blanfuné, A., Boudouresque, C.-F., Decock, W., Destombe, C., de Reviers, B., Díaz-Tapia, P., Herbst, A., Julliard, R., Karez, R., Kersen, P., Krueger-Hadfield, S.A., Kuhlenkamp, R., Peters, A.F., Peña, V., Piñeiro-Corbeira, C., Rindi, F., Rousseau, F., Rueness, J., Schubert, H., Sjøtun, K., Sansón, M., Smale, D., Thibaut, T., Valero, M., Vandepitte, L., Vanhoorne, B., Vergés, A., Verlaque, M., Vieira, C., Le Gall, L., Leliaert, F., De Clerck, O., 2023. *AlgaeTraits: a trait database for (European) seaweeds*. *Earth Syst. Sci. Data* 15, 2711–2754.
- Wallentinus, I., 1984. Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. *Mar. Biol.* 80, 215–225.