



# Article Assessing the Effect of Full Protection on the Biomass of Ericaria amentacea and Understory Assemblages: Evidence from Two Mediterranean Marine Protected Areas

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**Abstract:** *Cystoseira sensu lato* marine forests, which are among the most productive and diverse systems in rocky intertidal and subtidal habitats of the Mediterranean Sea, are experiencing a widespread decline throughout the basin due to increasing human pressures. Yet it is still unclear whether Marine Protected Areas (MPAs) may represent effective tools for conservation of these important habitat formers and their associated assemblages. Here, we compared the biomass of intertidal stands of *Ericaria amentacea* (C. Agardh) Molinari and Guiry and their understory assemblages between the no-take zone and control sites in two Mediterranean MPAs. We did not find evidence supporting a significant effect of full protection in increasing the biomass of *E. amentacea* stands and associated assemblages, except for macroalgae of the understory in one of the investigated MPAs, raising concerns on the potential effectiveness of MPAs in mitigating human impacts on these marine forests. Such findings call for major efforts to implement long-term monitoring programs of protected *Cystoseira s.l.* forests in order to inform an adaptive management of conservation measures within MPAs and eventually to set active interventions of restoration.

**Keywords:** community ecology; conservation; *Cystoseira sensu lato*; human impact; macrobenthos; rocky intertidal habitats

# 1. Introduction

Macroalgal forests are the largest and most productive components of coastal ecosystems worldwide [1]. In the Mediterranean Sea, brown algae such as *Cystoseira sensu lato* (including the genera *Cystoseira, Ericaria* and *Gongolaria*) are particularly important as habitat formers [2]. These fucoid algae dominate rocky intertidal and subtidal habitats, forming dense macroalgal stands that, due to their structure and high biomass, play a crucial role as ecosystem engineers by creating highly complex three-dimensional structures and providing secondary substrate which, in turn, support highly diverse understory and epiphytic assemblages [3]. Moreover, this complexity allows sustaining diversified trophic levels, enhancing primary and secondary productivity along with many other ecosystem functions [4,5]. Canopies of *Cystoseira s.l.* and associated macroalgae are spatially organized into four distinct layers: a basal layer consisting of the basal portions of *Cystoseira s.l.* thalli and of encrusting calcareous algae, a turf layer made of sciaphilic algae, a mid-canopy layer of arborescent and laminar algae, and an upper-canopy layer formed by *Cystoseira s.l.* fronds and their epiphytes [6]. Due to their structural features, *Cystoseira s.l.* canopies are inhabited by a number of different species belonging to several phyla, among which



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). are other algae, crustaceans, molluscs, and polychaetes dominate [7,8]. In Mediterranean shallow rocky bottoms, these macroalgal stands also harbour a very high fish species richness compared to other habitats, which are important nurseries for many species of commercial interest [2,9]. Due to their ecological importance, *Cystoseira s.l.* species are listed in several protocols for the protection of marine biodiversity, such as the SPAMI Protocol of the Barcelona Convention [10], and are recognized as privileged descriptors of the ecological status of Mediterranean coastal marine communities [11,12].

*Cystoseira s.l.* forests are experiencing widespread decline due to the detrimental effects of different anthropogenic stressors such as pollution, eutrophication, overfishing and climate change [13–20] which, in densely populated coastal zones, could combine with direct physical disturbance from human trampling [21] and/or destructive fishing practices [22]. As a result of cumulative human pressure, *Cystoseira s.l.* canopies may be subjected to the reduction in cover and biomass or local extinctions and may be replaced by less structured and opportunistic algal species, such as turf-forming algae, mussel beds or sea urchin barrens, leading to an overall simplification of the architectural and functional complexity of the habitat [23–25]. The loss of these fucoid algae in favour of turf algae or barren habitats results in the reduction in community respiration, gross and net primary productivity compared to turf algae or barren habitats [26], the decrease in biodiversity of associated assemblages [27], and the impairment of microbial degradation of organic matter and secondary production [28]. Since Cystoseira s.l. canopies extend over ~15% of Mediterranean coasts, although data on the presence of such species in large portion of the Mediterranean Sea are still lacking [29], the decline in these habitat formers are likely to have profound consequences on the functioning of the whole coastal ecosystem at a basin scale. Protecting these canopy-forming species is, therefore, a priority for the management of coastal ecosystems, as their decline may trigger cascading effects leading to loss of biodiversity and ecosystem functions and services [30,31].

Marine Protected Areas (MPAs) and especially the no-take zones can be effective tools for conservation of macroalgal forests and the associated marine biodiversity. First, MPAs could promote the persistence of large brown algae by ensuring their protection from different types of physical human disturbances (e.g., coastal development, destructive fishing) that can directly affect these species [32–34]. Moreover, the reduced level of cumulative human disturbance within their boundaries could confer to protected communities a greater resistance to environmental stress with respect to unprotected communities [35]. The role of MPAs in mitigating human pressure, nevertheless, may also rely on indirect effects of protection that, through the stabilization of community-wide regulative processes such as, for instance, top-down control of predator fish on grazers [36–38], may increase the overall abundance and stability of macroalgal stands and associated invertebrate assemblages [39,40]. However, evidence from several Mediterranean MPAs does not seem to support a consistent correlation among increased abundance of predator fish, reduced population size of the main grazers (i.e., sea urchins) and increased stands of canopy-forming brown algae in subtidal habitats [38]; idiosyncratic effects of protection on intertidal Cystoseira s.l. canopies were also detected [33,41,42].

The proportion of *Cystoseira s.l.* populations under different conservation regimes (including MPAs, marine parks, Natura 2000 sites, etc.) in the NW Mediterranean Sea has largely increased in the recent years [25], but it is still far from being clearly ascertained whether current protection strategies within MPAs are effective for the conservation and management of these habitat formers and their understory assemblages. In this study, we compared the canopy of *Ericaria amentacea* (C. Agardh) Molinari and Guiry in the intertidal fringe between the no-take zone and control sites under less restrictive protection measures in two Mediterranean MPAs to test the effects of full protection on the biomass of canopies, and of the two main components of the associated understory assemblages, namely macroalgae and invertebrates.

# 2. Material and Methods

## 2.1. Study Area and Sampling Design

The experiment was carried out in two MPAs, the Portofino MPA (Ligurian Sea) and the Isole Ciclopi MPA (NW Ionian Sea) (Figure 1). The Portofino MPA (PFN) extends over 346 ha and was established in 1998, whereas the Isole Ciclopi MPA (CIC) is larger (623 ha) and older, dating back to 1989. The two MPAs have a single no-take, no-access zone (18 and 35 ha for PFN and CIC, respectively) enclosed within a buffer area comprising zones of general and partial protection, where fishing and recreational human activities (e.g., diving, boating) are allowed.



**Figure 1.** Study areas (the MPAs of Portofino, PFN, and Isole Ciclopi, CIC) and sampling sites. P = no-take, no-access area; C1 = control site 1; C2 = control site 2. Limits of zones under different protection regimes within the MPAs were also reported. Example of *E. amentacea* midlittoral fringe at PFN (bottom left corner).

In each MPA, *E. amentacea* canopies and associated assemblages of the intertidal fringe were sampled on sub-horizontal rocky reefs at one site within the no-take, no-access zone (hereafter referred to as the protected site, P) and in two control sites (i.e., C1 and C2, collectively referred to as Cs) where most of human activities are allowed (Figure 1). Cs were characterized by environmental conditions comparable to those at P in terms of type and slope of rocky substrate, exposure to wave actions, currents and prevailing winds. In each site, five random samples of sessile assemblages were collected on two sampling occasions, namely October 2020 and July 2021. Sampling consisted of the complete removal of the *E. amentacea* thalli and the associated understory assemblage from the rocky substrate on a surface of approximately 160 cm<sup>2</sup> for each sampling unit. Samples were collected

with the help of a PVC cylinder placed upon the canopy and an inox blade, which was slid under the cylinder in order to cut the *E. amentacea* thalli, to scrape off all the other organisms, and to keep everything in the cylinder. A plastic bag was placed on the top of the cylinder to prevent loss of scraped material and vagile species. Care was taken to collect all scraped organisms from the substrate. Samples were preserved in formaldehyde (4%) and sea water solution and brought back to the laboratory for subsequent processing.

Samples were sieved with 1 mm mesh and soaked in sea water for 24 h to remove formaldehyde residues and were then sorted by separating organisms from inorganic debris and by detaching epiphytes (algae and invertebrates) from the *E. amentacea* thalli. Mesh size of 1 mm was chosen as it is widely recognized to allow an accurate quantification of biomass of macrobenthos (e.g., [43,44]). Macroalgae were separated from invertebrates and grouped altogether, whereas invertebrates were sorted under magnification and grouped into main taxa (phylum or class). A list of all taxa is provided as Supplementary Material (Table S1). As we focused on the effect of full protection on biomass rather than on its effect on species-level assemblage structure, we collapsed organisms into large taxonomic groups. Coarse taxonomic resolution facilitated estimations of biomass, avoiding the intrinsic difficulties of weighting the biomass of taxa accounting for very few (and often very small) individuals. The biomass of all groups of organisms was estimated as dry weight after desiccation at 80 °C for 48 h.

#### 2.2. Statistical Analysis

Analysis of Variance (ANOVA) was used to test for differences in the total biomass of *E. amentacea*, associated macroalgae and invertebrates between the protected site P and Cs. The design for the analysis was the same for both MPAs, and included two factors: Time (Ti), random, with two levels (T1, October 2020, and T2, July 2021), and Site (Si), with three levels, one protected site and two controls, with n = 5 replicates. The design for the analysis was asymmetrical [45] as it involved a single protected site to be compared against two control sites. The Site term was therefore partitioned into two portions: the 1-d.f. fixed contrast of P versus Cs (P-vs.-Cs) and the variability between Cs. This partitioning was applied to the other term in the analysis that includes the factor Site, that is the Ti  $\times$  Si interaction. Finally, the residual variation was partitioned in two portions, Res P and Res Cs, which is the residual variability of observations at P and Cs, respectively. The assumption of normality of the response variables was tested with the Shapiro–Wilk test. Cochran's C-test [45] was used to test the assumption of homogeneity of variances prior to analysis and data were log(x + 1) transformed to stabilize variance if required. For all response variables, the assumptions of normal distribution and variance homogeneity were met using raw data (or after data transformation), except macroalgae biomass at PFN. In this last case, non-normality and variance heterogeneity persisted after transformation. Although ANOVA is robust to slight deviations from non-normality, heterogeneous variances may increase the rate of Type I error, and thus results of tests for this variable were interpreted using a more conservative significance level of  $\alpha = 0.01$  [45].

Distance-based permutational multivariate analysis of variance (PERMANOVA) [46] was used to test for differences between P and Cs in the structure of invertebrate assemblages associated with *E. amentacea* canopies following the same design as for ANOVA. The analysis was based on Bray–Curtis dissimilarities of untransformed data and each term in the analysis was tested with 5000 random permutations.

A canonical analysis of principal coordinates (CAP) [47,48] was performed for the Ti × Si interaction term to depict temporal and spatial patterns of variation in invertebrate assemblages. Invertebrate taxa that might be responsible for any group differences seen in the CAP plot were investigated by calculating the product–moment correlations of original variables (taxa) with canonical axes [47]. Correlations of individual variables with the two canonical axes ( $r_1$  and  $r_2$ ) were represented as lines in a projection biplot. Taxa were included in the biplot only if exceeding an arbitrarily chosen value of correlation ( $=\sqrt{r_1^2 + r_2^2} \ge 0.3$ ).

# 3. Results

# 3.1. Portofino MPA

The total biomass of *E. amentacea* did not differ between P and Cs at PFN (Table 1). Visual inspection of the graph in Figure 2a suggested higher biomass of *E. amentacea* in T2, although ANOVA did not detect significant temporal variations (Table 1).

**Table 1.** Summary of asymmetrical ANOVA testing for differences between *P* and *Cs* in total biomass (dry weight) of *E. amentacea*, associated macroalgae, and invertebrates at PFN. The main effect of terms involved in higher-order interactions were not tested. Results of tests for the assumption of normality (Shapiro–Wilk test) and variance heterogeneity (Cochran's *C*-test) were also reported: NS = not significant, \* = p < 0.05, \*\*\* = p < 0.001.

|                                 |      | E. amentacea            |        |        | Macroalgae           |             |        | Invertebrates |       |        |  |
|---------------------------------|------|-------------------------|--------|--------|----------------------|-------------|--------|---------------|-------|--------|--|
| Source                          | d.f. | MS                      | F      | Р      | MS                   | F           | Р      | MS            | F     | Р      |  |
| Ti                              | 1    | 1957.60                 | 13.205 | 0.0681 | 9.64                 | 2.543       | 0.2518 | 537.51        | 2.931 |        |  |
| Si                              | 2    | 40.62                   | 0.274  | 0.7849 | 3.34                 | 0.880       | 0.5320 | 235.66        | 1.285 |        |  |
| P-v-Cs                          | 1    | 27.4                    | 0.182  | 0.7109 | 0.92                 | 0.674       | 0.4199 | 95.94         | 0.523 | 0.5447 |  |
| Cs                              | 1    | 54.21                   | 0.215  | 0.7238 | 5.75                 | 1.001       | 0.4998 | 375.38        | 1.086 | 0.4869 |  |
| $\mathrm{Ti} 	imes \mathrm{Si}$ | 2    | 148.24                  | 2.066  | 0.1487 | 3.79                 | 2.764       | 0.0831 | 183.42        | 4.322 | 0.0249 |  |
| $Ti \times P$ -v-Cs             | 1    | 43.90                   | 0.612  | 0.4418 | 1.84                 | 1.343       | 0.2579 | 21.01         | 0.061 | 0.8462 |  |
| $\mathrm{Ti} 	imes \mathrm{Cs}$ | 1    | 252.58                  | 3.131  | 0.0959 | 5.74                 | 2.867       | 0.1098 | 345.82        | 8.331 | 0.0107 |  |
| Res                             | 24   | 71.76                   |        |        | 1.37                 |             |        | 42.44         |       |        |  |
| Res P                           | 8    | 56.94                   |        |        | 0.11                 |             |        | 44.30         |       |        |  |
| Res Cs                          | 16   | 80.67                   |        |        | 2.00                 |             |        | 41.51         |       |        |  |
| Transformation                  |      | $\log(x+1)$             |        |        |                      | $\log(x+1)$ |        |               | None  |        |  |
| Shapiro–Wilk test               |      | W = 0.967 NS            |        |        | W = 0.913 *          |             |        | W = 0.969 NS  |       |        |  |
| Cochran's C-test                |      | C = 0.443 <sup>NS</sup> |        |        | <i>C</i> = 0.753 *** |             |        | C = 0.330  NS |       |        |  |



**Figure 2.** Mean  $\pm$  SE (n = 5) total biomass (dry weight) of (**a**) *E. amentacea*, (**b**) macroalgae, and (**c**) invertebrates at the fully protected site (P) and the two control sites (C1 and C2, dashed bars) of PFN in each sampling time. Time 1 (T1) = orange bars, Time 2 (T2) = green bars. Note that scales on y-axis are different.

For macroalgae of the understory, values of total biomass were similar between times of sampling and sites, except for the high value recorded in T1 at C2 (Figure 2b), and no significant spatial and temporal variations were detected, nor did the biomass of macroalgae significantly differ between P and Cs (Table 1).

Significant spatio-temporal variations instead characterized the total biomass of invertebrates (significant Ti  $\times$  Si interaction, Table 1), which showed a general increase in T2, though not consistent among sites (Figure 2c). However, the biomass of this component of the understory assemblage did not differ between P and Cs (Table 1). PERMANOVA did not detect significant differences between P and Cs in the multivariate structure of the understory invertebrate assemblages at PFN (Table 2). Assemblages significantly varied over time and among sites (significant Ti  $\times$  Si interaction, Table 2). Patterns of variation were portrayed in the CAP plot (Figure 3), showing that the invertebrate assemblages at C1 were mostly characterized by bivalves (Biv), polychaetes (Pol), colonial tunicates (Tun) and holothurians (Hol) in T2, whereas the remaining taxa characterized the assemblages at this site in T1, and at P and C2 in all sampling times.

**Table 2.** Summary of asymmetrical ANOVA testing for differences between P and Cs in total biomass (dry weight) of *E. amentacea*, associated macroalgae, and invertebrates at CIC. The main effect of terms involved in higher-order interactions were not tested. Results of tests for the assumption of normality (Shapiro–Wilk test) and variance heterogeneity (Cochran's C-test) were also reported: NS = not significant.

|                                 |      | E. amentacea |                         |        | Macroalgae |                         |        | Invertebrates |              |        |
|---------------------------------|------|--------------|-------------------------|--------|------------|-------------------------|--------|---------------|--------------|--------|
| Source                          | d.f. | MS           | F                       | Р      | MS         | F                       | Р      | MS            | F            | Р      |
| Ti                              | 1    | 2091.30      | 3.715                   |        | 330.61     | 4.873                   |        | 52.24         | 8.712        | 0.0982 |
| Si                              | 2    | 207.41       | 0.368                   |        | 66.84      | 0.985                   |        | 2.16          | 0.359        | 0.7356 |
| P-v-Cs                          | 1    | 161.37       | 0.287                   | 0.6459 | 129.91     | 1.915                   |        | 2.92          | 0.486        | 0.5577 |
| Cs                              | 1    | 253.46       | 0.519                   | 0.6025 | 3.76       | 0.568                   |        | 1.39          | 0.132        | 0.7784 |
| ${ m Ti} 	imes { m Si}$         | 2    | 562.88       | 11.906                  | 0.0003 | 67.85      | 12.517                  | 0.0002 | 6.00          | 2.052        | 0.1505 |
| $Ti \times P$ -v-Cs             | 1    | 637.72       | 1.307                   | 0.4576 | 129.07     | 19.493                  | 0.0002 | 1.40          | 0.480        | 0.4952 |
| $\mathrm{Ti} 	imes \mathrm{Cs}$ | 1    | 488.08       | 9.455                   | 0.0073 | 6.62       | 2.571                   | 0.1284 | 10.59         | 3.760        | 0.0703 |
| Res                             | 24   | 47.28        |                         |        | 5.42       |                         |        | 2.92          |              |        |
| Res P                           | 8    | 38.60        |                         |        | 11.11      |                         |        | 3.14          |              |        |
| Res Cs                          | 16   | 51.62        |                         |        | 2.58       |                         |        | 2.82          |              |        |
| Transformation                  |      |              | None                    |        |            | log(x + 1)              |        |               | $\log(x+1)$  |        |
| Shapiro–Wilk test               |      | I            | N = 0.955 N             | S      |            | W = 0.989 NS            | 3      |               | W = 0.981 N  | 5      |
| Cochran's C-test                |      | (            | C = 0.329 <sup>NS</sup> | 5      |            | C = 0.452 <sup>NS</sup> |        |               | C = 0.306 NS | 5      |



**Figure 3.** Canonical analysis of principal coordinates (CAP) for the factor  $Ti \times Si$  based on the distance matrix among sampling units for total biomass of invertebrates at PFN. Squares (dashed) = control site 1 (C1), triangles (dashed) = control site 2 (C2), circles = no-take zone (P). Orange symbols = Time 1 (T1), green symbols = Time 2 (T2).

### 3.2. Isole Ciclopi MPA

No differences in the total biomass of *E. amentacea* between P and Cs were detected at CIC (Table 2). The total biomass of *E. amentacea* inconsistently varied among sites and between sampling times (significant Ti  $\times$  Si interaction, Table 3). However, as for PFN, a general increase in biomass can be observed in T2 (Figure 4a).

**Table 3.** Results of asymmetrical PERMANOVA testing for differences between P and Cs in biomass of invertebrate assemblages associated to *E. amentacea* in both the investigated MPAs (PFN and CIC). The analysis is based on Bray–Curtis dissimilarities (untransformed data) and each test was performed using 5000 random permutations. Significant tests are provided in bold. Denominators for tests (MS<sub>DEN</sub>) were also indicated.

|                                 |      |         | PFN      |          |           | CIC      |          |                                 |
|---------------------------------|------|---------|----------|----------|-----------|----------|----------|---------------------------------|
| Source                          | d.f. | MS      | Pseudo-F | P (perm) | MS        | Pseudo-F | P (perm) | MS <sub>DEN</sub>               |
| Ti                              | 1    | 6962.60 | 1.640    |          | 14,908.00 | 2.444    |          |                                 |
| Si                              | 2    | 6715.40 | 1.582    |          | 3396.40   | 0.557    |          |                                 |
| P-v-Cs                          | 1    | 9595.80 | 2.261    | 0.1694   | 3382.10   | 0.554    | 0.6978   | $\mathrm{Ti} 	imes \mathrm{Si}$ |
| Cs                              | 1    | 3835.20 | 0.650    | 0.6415   | 3410.70   | 0.322    | 0.8175   | $\mathrm{Ti} 	imes \mathrm{Cs}$ |
| ${ m Ti} 	imes { m Si}$         | 2    | 4244.40 | 2.605    | 0.0168   | 6101.00   | 6.551    | 0.0002   | Res                             |
| $Ti \times P$ -v-Cs             | 1    | 2592.10 | 0.440    | 0.7678   | 1623.00   | 0.153    | 0.9332   | $\mathrm{Ti} 	imes \mathrm{Cs}$ |
| $\mathrm{Ti} 	imes \mathrm{Cs}$ | 1    | 5896.70 | 3.417    | 0.0158   | 10,579.00 | 11.109   | 0.0002   | Res Cs                          |
| Res                             | 24   | 1629.50 |          |          | 931.34    |          |          |                                 |
| Res P                           | 8    | 1437.25 |          |          | 889.38    |          |          |                                 |
| Res Cs                          | 16   | 1725.60 |          |          | 952.29    |          |          |                                 |



**Figure 4.** Mean  $\pm$  SE (n = 5) total biomass (dry weight) of (**a**) *E. amentacea*, (**b**) macroalgae, and (**c**) invertebrates at the fully protected site (P) and the two control sites (C1 and C2, dashed bars) of CIC in each sampling time. Time 1 (T1) = orange bars, Time 2 (T2) = green bars. Note that scales on y-axis are different.

The total biomass of macroalgae associated with *E. amentacea* canopies significantly differed between P and Cs, although this pattern was not consistent between the two sampling times (significant Ti  $\times$  P-v-Cs interaction, Table 3); at CIC, the biomass of macroalgae was significantly higher at P than at Cs in T1, whereas their total biomass was comparable among sites in T2, irrespective of protection level (Figure 4b).

No effects of full protection were detected for invertebrate biomass, and there were no statistically significant spatial and temporal variations (Table 3). A general increase in their biomass, nevertheless, seemed to characterize the understory assemblages in T2 (Figure 4c). The average multivariate structure of invertebrate assemblages significantly varied between times and among sites (significant Ti  $\times$  Si interaction), but no significant differences imputable to full protection were detected (Table 2). The CAP plot for CIC (Figure 5) showed that invertebrate assemblages in T1 were dominated by hydroids (Hyd) at P and C2, whereas sponges (Por) and bryozoans (Bry) characterized C1. In T2, invertebrate assemblages were quite distinct, showing an increase in biomass of different sessile taxa (bivalves (Biv), tunicates (Tun), and anthozoans (Ant)) and of vagile organisms (crustaceans (Cru), holothurians (Hol), polychaetes (Pol)) in all sites (Figure 5).



**Figure 5.** Canonical analysis of principal coordinates (CAP) for the factor  $Ti \times Si$  based on the distance matrix among sampling units for total biomass of invertebrates at CIC. Squares (dashed) = control site 1 (C1), triangles (dashed) = control site 2 (C2), circles = no-take zone (P). Orange symbols = Time 1 (T1), green symbols = Time 2 (T2).

# 4. Discussion

Assessing the effectiveness of MPAs is crucial to ensure the achievement of conservation goals and to refine conservation strategies for an adaptive management of protected communities in the face of changing environmental conditions [49,50]. In most cases, assessments of the ecological effects of MPAs, and particularly of full protection in no-take zones, have focused on fish documenting increased assemblage diversity, abundance and biomass of protected populations with respect to unprotected areas [51–55]. Macrobenthic assemblages received comparatively less attention, except for commercial or charismatic invertebrate species (e.g., [56–58]), and often responded to protection inconsistently (e.g., [33,41]). Moreover, if fish assemblages promptly respond to protection (e.g., [59]), the effects of protection on macrobenthos can take much more time to become evident. Differently, these effects may occur through an increased temporal stability/resilience of assemblages rather than through changes in their average structure [40,60,61], or the effects can vary in time or concerning only some components of the assemblages [41].

E. amentacea (as all Cystoseira s.l. species) naturally exhibits substantial phenological variations in its morphology/biomass and associated understory [62,63]. In winter, only the perennial basal cauloids remain in most species. Later, in spring, new branches sprout, and in summer thalli consist of main axes covered by many leaf-like structures. In late summer-autumn, the erect branches are shed and only the cauloid persists in a quiescent state during the following unfavorable, cold season. Such changes reflect on the associated assemblages [64], leading to the proliferation of photophilic algae in the understory during the resting season, alternating with an increase in vagile (e.g., polychaetes, crustaceans, holothurians) and epiphytic (e.g., hydroids) invertebrate abundance during the growing season [62,65]. These natural patterns clearly emerged in both study areas, with an overall average turnover in total biomass (including all assemblage components) between T1 and T2 equal to 1468  $\pm$  346 g·m<sup>-2</sup> and 794  $\pm$  335 g·m<sup>-2</sup>, at PFN and CIC respectively. Yet, for all the investigate variables, temporal variations were not consistent among sites, and changes in biomass of *E. amentacea* canopies, despite being quite evident on average, were not statistically significant. This is probably because *E. amentacea* biomass was not yet completely depleted in T1 (October 2021), as typically occurs during the resting period, thus smoothing out potential variations with respect to T2 (July 2022). Nevertheless, the interest here was not to confirm well-known cyclic patterns of change in E. amentacea, but rather to understand whether the effects of protection on these fucoid algae and their

associated assemblages, if any, were consistent through time or affected their temporal patterns of variation.

At PFN, our analysis did not detect significant differences between P and Cs in neither *E. amentacea* nor the associated macroalgae or the structure of invertebrate assemblages of the understory, suggesting no effects of full protection on intertidal assemblages, at least concerning the biomass of the investigated components. It could be argued that control sites fell under a regime of general protection, and that this could have contributed to preserve the integrity of *E. amentacea* stands also at Cs. General and partial regimes of protection, nevertheless, allow several human activities (e.g., recreational uses, tourism), which are generally not strictly regulated, potentially leading to detrimental effects on marine communities [66–68]. Evidence from adjacent areas highlighted that in the last decades, the effects of increasing cumulative human pressure have expanded, being likely responsible for detrimental changes in subtidal reef communities and plankton assemblages [69,70] despite the presence of conservation initiatives, and suggested the difficulty of local protection regimes to face widespread environmental degradation. Regardless, sea-based human activities (e.g., artisanal fishery, boating, anchoring) are likely to exert a limited influence on intertidal habitats, such as those investigated in this study, which can be more susceptible to land-based pollution (e.g., terrestrial runoffs) and direct onshore activities (e.g., trampling due to human frequentation for bathing or rod angling). The mainland at PFN, however, is poorly urbanized and the implementation of a terrestrial reserve since 1935 prevented the expansion of land-based (e.g., industries, intensive agriculture) sources of pollution in the area [71], largely limiting their potential effects on nearshore habitats. It is also worth noting that control sites at PFN, due to the local features of the whole coastline, are not easily accessible to human frequentation and that the MPA is strongly enforced [72]. Thus, the lack of significant effects of full protection were most likely due to the incidental reduction (or exclusion) of direct human impacts on *E. amentacea* stands at Cs to levels not significantly different from those experienced in the no-take, no-access zone P. Analogously to what occurred for PFN, no effect of full protection in modifying the biomass of *E. amentacea* or the total and multivariate biomass of associated invertebrate assemblages was detected at CIC. Unlike PFN, this MPA is located along a highly urbanized coastline (see also Figure 1) with high touristic frequentation during summer and beyond due to the favourable climate. In such an environmental context, the potential effects of protection on macrobenthic assemblages may be strongly reduced, or completely overcome, as a consequence of high anthropization (e.g., [40,69]), and the abundance of *E. amentacea* could eventually correlate with gradients of human pressure rather than the spatial arrangement of protection regimes [42]. In addition, CIC is a relatively small MPA with low enforcement [72], which makes it more vulnerable to neighbouring direct physical disturbance from human attendance and indirect effects of coastal human activities such as the exposure to land and sea-based chemical pollution and organic enrichment [73,74]. Interestingly, a significant effect of full protection was detected for understory macroalgae at CIC, which had a higher biomass in T1 at P than at Cs. Control sites at CIC are easily accessible and therefore more exposed to direct effects of human frequentation, and especially to mechanical disturbance of human trampling with respect to P where human access is forbidden. Since erect macroalgae are more vulnerable to trampling than turf algae [32], high trampling pressure could reduce the abundance of large, erect macroalgae favouring small, filamentous, turf-forming species, thus leading to an overall decrease in biomass in this component of the understory. We found no evidence of a significant decrease in biomass of *E. amentacea* at Cs, probably because it is quite resistant to trampling [75], unlike other Cystoseira s.l. species [32].

MPAs are the main global strategy to conserve and restore marine biodiversity and associated ecosystem services [76,77]. However, they cannot provide an exhaustive solution to the complexity of human-driven environmental issues affecting marine ecosystems [78,79], often leading to contrasting outcomes depending on reserve features, social and environmental contexts, and ecology and biology of species involved [32,42,80]. Our findings do not support the hypothesis of a significant effect of no-take zones in increasing the biomass of E. amentacea stands and associated assemblages with respect to control areas under lower levels of protection, although further investigations to increase the spatial and temporal extents of these assessments are needed. As *Cystoseira s.l.* forests are declining from local to large scale [29,81], these results reinforce concerns that human pressures affecting these habitat formers cannot be effectively mitigated within MPA boundaries [25,67,82–84]. Managing human pressures at a wider spatial scale is crucial to ensure the effectiveness of local-scale conservation initiatives in enhancing the resilience of *Cystoseira s.l.* forests to present and future stressors [17,19,85,86]. Wide gaps in information on fucoid algae in the Mediterranean MPAs also limit our understanding of the actual role of protection in preserving the integrity of these habitat formers [25], which urgently call for major efforts in long-term monitoring of their distribution and ecological status in MPA networks at a basin scale. This would also allow identifying critical cases within MPAs requiring active interventions of restoration, especially when the decline of *Cystoseira s.l.* originates from transient environmental deterioration, and natural recovery may be hampered by hysteresis. Finally, future research needs to provide a deeper insight into the functioning of *Cystoseira s.l.* forests, and the ensuing goods and services they provide in order to optimize conservation investments taking into account their actual contribution to the overall natural capital of coastal ecosystems.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15010089/s1, Table S1: Taxonomic list of taxa of the E. amentacea understory assemblages. For taxonomic and morphological groups, grouped species or taxa are reported.

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