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Posted Date: 23 November 2023

doi: 10.20944/preprints202311.1485.v1

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Article

# Conserving Marine Forests: Assessing the Effectiveness of a Marine Protected Area for *Cystoseira sensu lato* Populations in the Central Mediterranean Sea

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**Abstract:** Marine Protected Areas (MPAs) are vital for biodiversity conservation, yet their effectiveness in preserving foundation seaweeds remains understudied. This study investigates the diversity and distribution of *Cystoseira sensu lato* (including *Cystoseira*, *Ericaria* and *Gongolaria*) populations in the an MPA located in the central Mediterranean Sea, comparing them with those in two unprotected control sites. We hypothesize that MPA *Cystoseira s.l.* populations will display higher diversity and structure compared to outside control sites. Results reveal a total of 19 *Cystoseira s.l.* species at depths of 0-20 m, with the MPA exhibiting a higher diversity than control sites. Thus, MPAs can play a crucial role in fostering the diversity of *Cystoseira s.l.* populations. However, no significant differences were observed among the MPA's protection zones, raising questions about the zoning effectiveness. Additionally, our survey uncovered a substantial presence of non-indigenous seaweeds within the MPA. In conclusion, while MPAs improve *Cystoseira s.l.* diversity compared to unprotected sites, the varying efficacy of protection within MPA zones suggests a necessity for site-specific conservation strategies. The presence of non-indigenous seaweeds emphasizes ongoing challenges. This study provides a baseline for understanding *Cystoseira s.l.* population dynamics, crucial for future monitoring and conservation efforts in the face of climate change.

**Keywords:** macroalgal distribution; *Cystoseira sensu lato*; *Cystoseira*; *Ericaria*; *Gongolaria*; Biodiversity conservation; Marine Protected Areas; Mediterranean Sea

## 1. Introduction

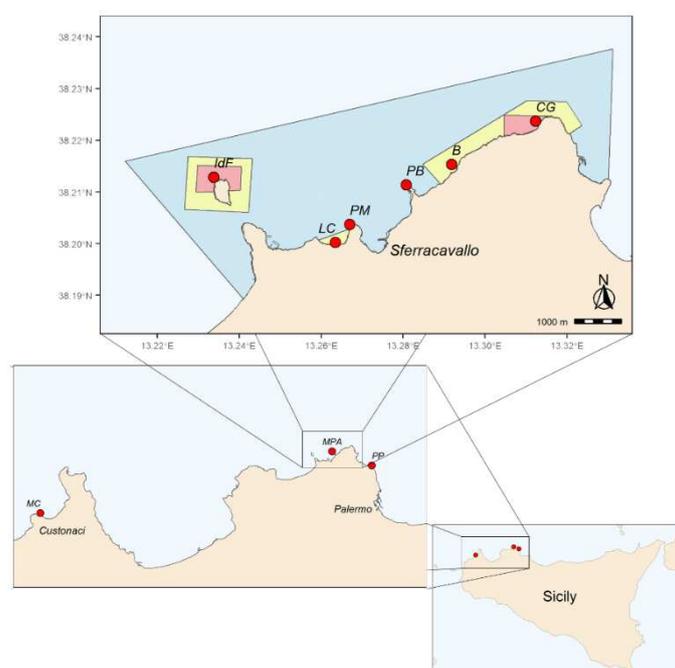
Marine forest seaweeds, mainly brown algae of the orders Fucales and Laminariales, form some of the most productive and diverse habitats on the world's cold-water and temperate rocky reefs [1–4]. In the Mediterranean Sea, these foundation seaweeds mainly comprise species of the genus *Cystoseira* C. Agardh (Fucales, Phaeophyceae), recently divided into three genera: *Cystoseira*, *Gongolaria* Boehmer and *Ericaria* Stackhouse [5,6] and hereafter referred to as *Cystoseira s.l.* (*sensu lato*). *Cystoseira s.l.* forests provide a wide range of ecosystem services, supporting a rich associated biota, including fish, invertebrates, and other algae [2,7,8]. They are essential foundation species, significantly enhancing the structural complexity and productivity of coastal communities from the surface down to the upper circalittoral zone [9–12]. Additionally, they are considered useful indicators of water and ecosystem quality according to the Water Framework Directive (2000/60/EC) and the Marine Strategy Framework Directive (2008/56/EC).

Like other coastal marine ecosystems, these long-lived primary producers are highly impacted, particularly close to urban areas, due to combined effects of anthropogenic impacts such as pollution, urbanization, the introduction of non-native species, overfishing, coastal aquaculture, and climate-change [2,13–19]. Consequently, the decline or loss of *Cystoseira s.l.* populations have been observed

on many rocky coasts, leading to a shift from complex and productive benthic communities to less structured assemblages dominated by turf-forming algae, mussels, or sea urchin barrens [1,2,13,15,18,20–23]. This shift results in a decrease in essential ecosystem services [2,24] and economic value of coastal areas [25].

Marine Protected Areas (MPAs) have become highly important tools for biodiversity conservation and management worldwide [26]. In the Mediterranean Sea, a large part of the research concerning the effectiveness of MPAs has focused on fish assemblages [27], while few studies focused on foundation seaweeds [14,28,29]. MPAs can play a critical role in the conservation of *Cystoseira s.l.* forests, as they guarantee protection from various anthropogenic impacts [28]. Healthy *Cystoseira s.l.* forests in MPAs may also represent an important source of propagules for the restoration of lost or degraded forests outside MPA borders, a crucial aspect due to the low dispersal capacity of most of these species [14,21,30,31].

In this study, we investigate the diversity and distribution of *Cystoseira s.l.* populations within the “Capo Gallo - Isola delle Femmine” MPA in the central Mediterranean Sea. Moreover, we tested the effectiveness of the MPA for *Cystoseira s.l.* conservation by comparing these populations within the MPA to those in two unprotected control sites (Figure 1). We hypothesize that *Cystoseira s.l.* populations will be more diverse and structured within the MPA compared to outside control sites, and that the areas within the MPA characterized by higher levels of protection will host highly diverse populations.



**Figure 1.** Study sites (red dots) along the northwestern rocky-shore of Sicily: MPA = MPA of “Capo Gallo-Isola delle Femmine”, MC = Monte Cofano, PP = Punta Priola. The top panel displays information about the sites investigated within the MPA: IdF = Isola, LC = La Cala, PM = Punta Matese, PB = Punta Barcarello, B = Barcarello, CG = Capo Gallo. Within the MPA, red, yellow, and blue areas denote no-take, general, and limited protection zones, respectively.

## 2. Results

A total of 19 *Cystoseira s.l.* species were found between 0 to 20 m of depth in the surveyed sites (Tables 1 and 2). The highest number (17 species) was found within the “Capo Gallo – Isola delle Femmine” MPA, while Monte Cofano and Punta Barcarello hosted 9 and 5 species, respectively (Table 1).

**Table 1.** List of the recorded *Cystoseira s.l.* species. Taxonomy and nomenclature were updated according to the AlgaeBase [32] database.

Species	MPA zones			Control sites	
	A	B	C	white	urban
<i>Cystoseira compressa</i> (Esper) Gerloff & Nizamuddin 1975	+	+	+	+	+
<i>Cystoseira humilis</i> var. <i>myriophylloides</i> (Sauvageau) J.H.Price & D.M.John	+	+	+		
<i>Cystoseira compressa</i> f. <i>rosetta</i> (Ercegovic) Cormaci, G.Furnari, Giaccone, B.Scammacca & Serio	+		+		
<i>Cystoseira humilis</i> Schousboe ex Kützing		+	+	+	
<i>Cystoseira foeniculacea</i> f. <i>tenuiramosa</i> (Ercegovic) A.Gómez Garreta, M.C.Barceló, M.A.Ribera & J.Rull Lluch		+	+		
<i>Cystoseira foeniculacea</i> (Linnaeus) Greville		+			+
<i>Cystoseira foeniculacea</i> f. <i>latiramosa</i> (Ercegovic) A.Gómez Garreta, M.C.Barceló, M.A.Ribera & J.R.Lluch 2001		+	+		
<i>Cystoseira dubia</i> Valiante		+	+		
<i>Ericaria amentacea</i> (C.Agardh) Molinari & Guiry	+	+	+	+	
<i>Ericaria brachycarpa</i> (J.Agardh) Molinari & Guiry	+	+	+	+	+
<i>Ericaria crinita</i> (Duby) Molinari & Guiry	+	+	+		
<i>Ericaria brachycarpa</i> var. <i>claudiae</i> Boudouresque, Perret-Boudouresque & Blanfuné	+	+			+
<i>Ericaria funkii</i> (Schiffner ex Gerloff & Nizamuddin) Molinari & Guiry		+	+		
<i>Ericaria mediterranea</i> (Sauvageau) Molinari & Guiry					+
<i>Gongolaria montagnei</i> (J.Agardh) Kuntze	+		+	+	+
<i>Gongolaria montagnei</i> var. <i>compressa</i> (Ercegovic) Verlaque, Blanfuné, Boudouresque & Thibaut	+	+	+	+	
<i>Gongolaria squarrosa</i> (De Notaris) Kuntze	+				
<i>Gongolaria montagnei</i> var. <i>tenuior</i> (Ercegovic) Molinari & Guiry		+	+	+	
<i>Gongolaria sauvageauana</i> (Hamel) Molinari & Guiry					+

**Table 2.** Average abundance (N), frequency of occurrence (Fr %) and dominance (D %) of the *Cystoseira* sl. species identified on each site.

Depth range	Species	MPA zones									Control sites					
		A			B			C			white			urban		
		N	F%	D %	N	F%	D %	N	F%	D %	N	F%	D %	N	F %	D %
	<i>Cystoseira compressa</i> (Esper)	3.5	41.	3.8	8.3	50.	4.7	5.4	37.	5.1	7.9	50.	6.7	11.	50.	51.
	Gerloff & Nizamudd in 1975		7			0			5			0		7	0	9
	<i>Cystoseira compressa</i> f. <i>rosetta</i> (Ercegovic)	1.0	12.	1.1				0.4	4.2	0.4						
	Cormaci, G.Furnari, Giaccone, B.Scammacca & Serio		5													
0-5 m	<i>Cystoseira humilis</i> Schousboe ex Kützing				15.	54.	8.6	4.4	25.	4.1	13.	50.	11.			
					2	2			0		3	0	3			
	<i>Ericaria amentacea</i> (C.Agardh)	50.	100	54.	82.	100	46.	58.	100	54.	60.	100	50.			
	Molinari & Guiry	8	.0	5	3	.0	3	1	.0	8	0	.0	7			
	<i>Ericaria brachycarpa</i> (J.Agardh)	37.	95.	40.	71.	100	40.	37.	87.	35.	31.	83.	26.	10.	50.	48.
	Molinari & Guiry	9	8	6	9	.0	4	7	5	6	7	3	8	8	0	1
	<i>Ericaria mediterranea</i> (Sauvagea u) Molinari & Guiry										5.4	25.	4.6			
												0				
	<i>Cystoseira humilis</i> var. <i>myriophylloides</i> (Sauvagea u) J.H.Price & D.M.John	3.1	12.	11.	8.3	20.	8.0	4.4	16.	6.3						
			5	0		8			7							
5-10 m	<i>Cystoseira humilis</i> Schousboe ex Kützing				9.0	37.	8.6	6.0	29.	8.7	3.3	25.	7.3			
						5			2			0				
	<i>Cystoseira foeniculacea</i> (Ercegovic)				8.1	37.	7.8	7.5	29.	10.						
	A.Gómez Garreta,					5			2	8						



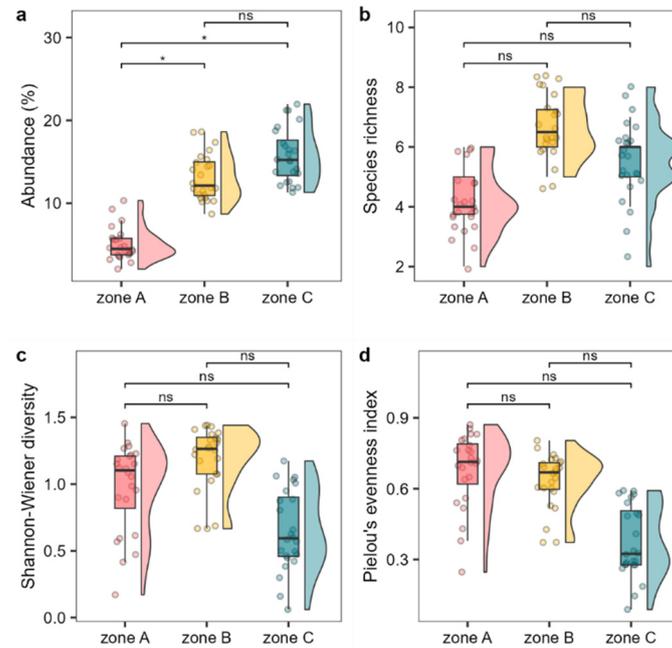


	Nizamuddin)															
	Molinari & Guiry															
<i>Gongolaria montagnei</i>	(J.Agardh) Kuntze	15.6	41.7	41.0				10.0	41.7	37.5	8.3	33.3	52.6	5.4	33.3	100.0
<i>Gongolaria montagnei</i>	(Ercegovic) Verlaque, var. <i>compressa</i>	14.8	70.8	38.8	5.0	16.7	7.5	3.5	20.8	13.3	4.2	33.3	26.3			
	Bouduresque & Thibaut															
<i>Gongolaria squarrosa</i>	(De Notaris) Kuntze	7.7	29.2	20.2												
<i>Gongolaria montagnei</i>	(Ercegovic) Molinari & Guiry				22.9	54.2	34.5	3.5	12.5	13.3						
var. <i>tenuior</i>																
<i>Gongolaria sauvageana</i>	(Hamel) Molinari & Guiry										3.3	16.7	21.3			

### 2.1. *Cystoseira s.l.* assemblage within MPA

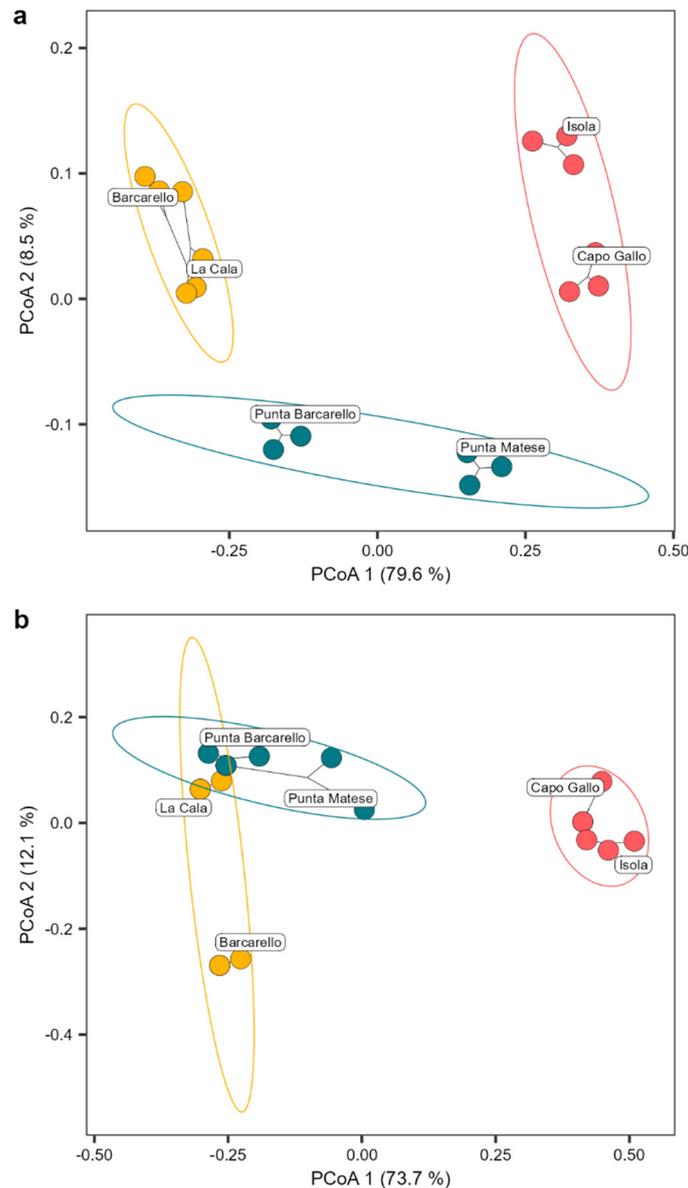
Within the MPA, 10 *Cystoseira s.l.* species were found into zone A, while 14 were found in zone B and C (Table 1), with 6 species (*Cystoseira compressa*, *Cystoseira humilis* var. *myriophylloides*, *Ericaria amentacea*, *Ericaria brachycarpa*, *Ericaria crinita*, and *Gongolaria montagnei* var. *compressa*) consistently found across different levels of protection.

Whitin the MPA, zone A exhibited lower abundances (average cover percentage) and a lower number of *Cystoseira s.l.* species compared to the other two levels of protection (B and C) (Figure 2 a-b). The values for these two metrics differed between zone B and C, with zone B showing slightly lower average abundance but higher number of species compared to zone C (Figure 2 a-b). Shannon diversity and Pielou's evenness were higher in zone A and B compared to zone C (Figure 2 c-d).



**Figure 2.** Abundance (a, average cover percentage), species richness (b), Shannon-Wiener diversity (c), and Pielou's evenness index (d) of the *Cystoseira s.l.* assemblage among the different MPA protection levels. Boxplots show extreme and lower whisker (vertical black line), lower and upper quartile (box), and median (horizontal black line). Density plot are shown beside each boxplot. Dots are raw data (n = 24). Signif. codes: \*  $p < 0.01$ , .  $p < 0.05$ , ns  $p > 0.05$ .

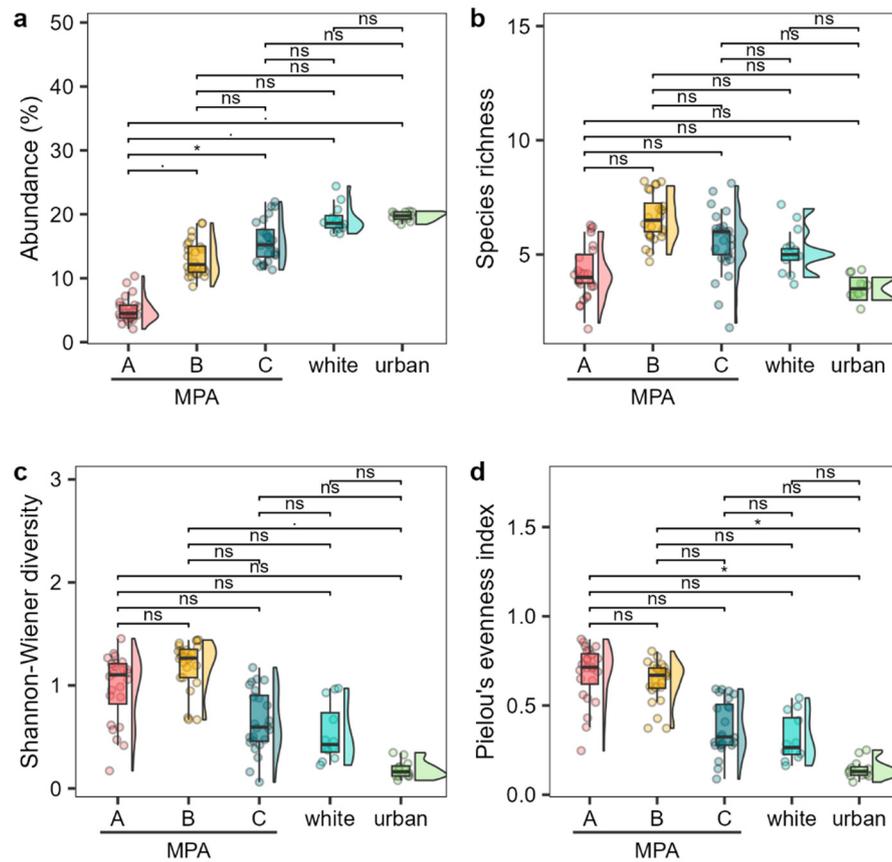
The *Cystoseira s.l.* assemblage differed significantly among the different MPA zones (PERMANOVA results model:  $F_{2,17} = 55.027$  and  $40.394$  for structure and composition, respectively,  $p < 0.001$ ; Table S1), explaining a substantial amount of the total variation ( $R^2 = 70.73\%$  and  $64.27\%$  for structure and composition, respectively). Additionally, there was a significant difference among sites within MPA zones (PERMANOVA results model:  $F_{3,17} = 10.706$  and  $10.711$  for structure and composition, respectively,  $p < 0.001$ ; Table S1), accounting for a moderate amount of the total variation ( $R^2 = 20.64\%$  and  $25.56\%$  for structure and composition, respectively; Table S1). No significant difference was found for transects within sites and MPA zones (Table S1). The Principal Coordinate Analysis (PCoA) ordination plot clearly separated the structure of the *Cystoseira s.l.* assemblage among MPA zones, with zone A at a far distance from zone B, while sites in zone C placed among the other two protection zones, with Punta Barcarello being closer to sites in zone B and Punta Matese to zone A (Figure 3 a). *Cystoseira s.l.* composition, instead, clearly separated zone A from B and C, with partial overlap of the *Cystoseira s.l.* composition of the site of La Cala (within zone B) with sites of zone B (Figure 3 b).



**Figure 3.** Structure (a) and composition (b) of the *Cystoseira s.l.* assemblage founded. Circles show the 95% confidence of interval for each MPA zone (red = zone A, yellow = zone B and blue = zone C). Principal coordinate analysis plot (PCoA) based on Bray–Curtis measure of square-root transformed *Cystoseira s.l.* percentage cover (structure) or Jaccard measure (composition).

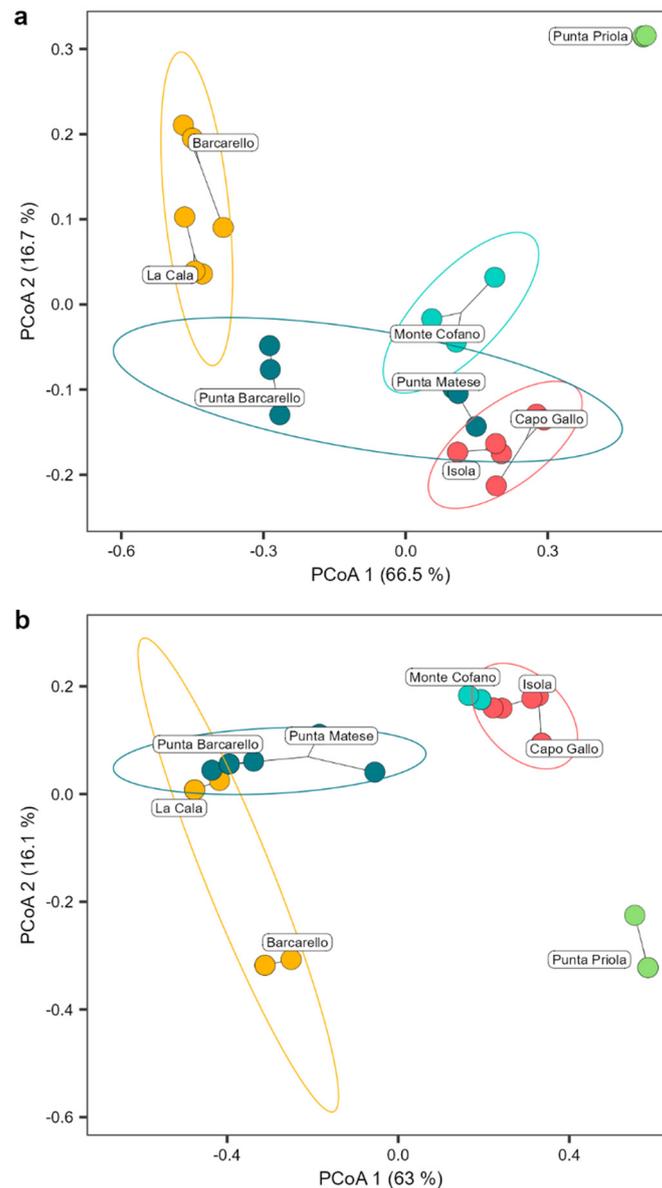
## 2.2. Comparison of *Cystoseira s.l.* assemblage between MPA and control sites

When comparing the MPA with control sites, urban and white sites exhibited higher average *Cystoseira s.l.* abundance, comparable to zones C and B within the MPA rather than zone A (Figure 4 a). The species richness of the white and urban sites was comparable to that within the MPA, with the white site being closer to zone C, while the urban site showed lower values more comparable to those of zone A (Figure 4 b). Shannon diversity and Pielou's evenness of the white site were comparable to those of zone C, while those of the urban site showed lower values, although no significant differences were detected (Figure 4 c-d).



**Figure 4.** Abundance (a, average cover percentage), species richness (b), Shannon-Wiener diversity (c), and Pielou's evenness index (d) of the *Cystoseira s.l.* assemblage among the different MPA zones and outside control sites. Boxplots show extreme and lower whisker (vertical black line), lower and upper quartile (box), and median (horizontal black line). Density plots are shown beside each boxplot. Dots are raw data ( $n = 12 - 24$ ). Signif. codes: \*  $p < 0.01$ , ·  $p < 0.05$ , ns  $p > 0.05$ .

When comparing the *Cystoseira s.l.* assemblage between the MPA zone and control sites (Table S2), it was revealed that there were significant differences at the urban site compared to both the MPA's and white sites (Figure 5, a-b), with an average dissimilarity of 71% and 60% for structure and composition, respectively (Table S3). On the other hand, the *Cystoseira s.l.* assemblage of the white site was more similar to the MPA's sites (Figure 5 a-b) with an average similarity of 55% and 59% for structure and composition, respectively. It was closer to the Punta Matese site (zone C) with an average similarity of 64% and 67% for structure and composition, respectively (Table S3).



**Figure 5.** Structure (a) and composition (b) of MPA and outside control sites *Cystoseira s.l.* assemblage. Circles show the 95% confidence of interval for each MPA zone (red = zone A, yellow = zone B, blue = zone C, light-blue = white site and green = urban site). Principal coordinate analysis plot (PCoA) based on Bray–Curtis measure of square-root transformed *Cystoseira s.l.* percentage cover (structure) or Jaccard measure (composition).

Here, we provide an overall description of *Cystoseira s.l.* populations across the investigated sites. Of the 19 species discovered, *Cystoseira humilis* showed the large depth adaptability (range depth = 0-20 m) followed by four species (*Cystoseira foeniculacea* f. *tenuiramosa*, *Gongolaria montagnei*, *Gongolaria montagnei* var. *tenuior* and *Gongolaria sauvageauana*) found between 5 and 20 meters depth (Table 2). At all investigated sites, *E. amentacea* grows on the outer margin of vermetid where forms dense and continuous belts across the MPA's sites and the Monte Cofano (white) site; however, this species was not found at Punta Priola (urban) site. Sparse thalli of *Cystoseira compressa* f. *rosetta* were also found at the sites of Isola and Punta Barcarello, while *C. compressa* was present within both the MPA and the two control sites. *Cystoseira compressa* formed either small dense patches with thalli of about 10 cm high (without aerocystis) or individual thalli up to 40 cm high with branches provided by aerocystis. Between 1 and 5 meters depth, *E. brachycarpa* forms large and extended forests within the MPA at the sites of Punta Barcarello, La Cala, Isola, whereas at Punta Matese, Barcarello, and

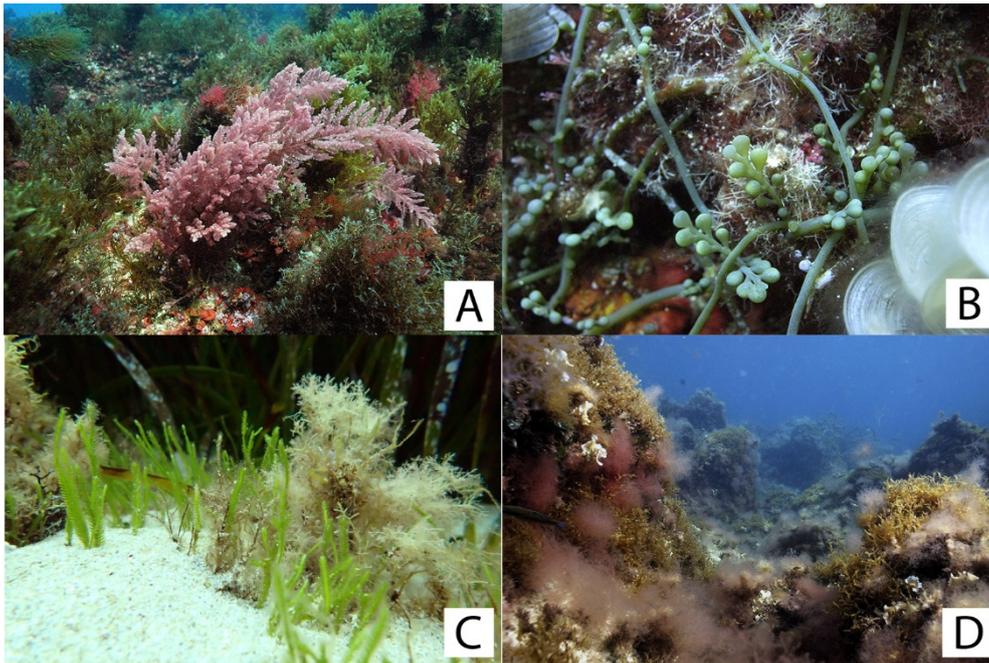
Capo Gallo, despite being dense, the populations assume a discontinuous pattern due to the presence of large rocky boulders placed on rocky carbonate platforms. Dense and continuous populations of *E. brachycarpa* were also found at Monte Cofano, whereas at Punta Priola *E. brachycarpa* stands were patchy and less dense, intercalated by thalli of *Dictyopteris polypodioides* (De Candolle) J. V. Lamouroux. *Ericaria crinita* was the second most common species discovered between 1 and 5 meters depth. At the sites of Barcarello, Punta Barcarello and Capo Gallo, this species forms dense patches covering big boulders or, as in the other sites, its thalli are widely spread on the substrates and surrounded by other macroalgae. *Cystoseira humilis* was found at the site of La Cala and Monte Cofano as individual sparse thalli or dense patches. Finally, only at the site of Monte Cofano sparse thalli of *E. mediterranea* were found.

Between 5 and 10 meters depth, *G. montagnei* var. *tenuior* was the most abundant which was found within the MPA at the sites of La Cala, Punta Matese, Punta Barcarello and Barcarello, and Monte Cofano. This species forms extensive dense forests, which can be seen in particular in La Cala and Punta Barcarello. In the other sites, *G. montagnei* var. *tenuior* grows in small patches or as individual thalli surrounded by other macroalgae. *Cystoseira humilis* var. *myriophylloides* on the other hand, was only found within the MPA at Isola, La Cala, Punta Matese, and Barcarello as isolated or groups of few thalli surrounded by other seaweeds, whereas *C. foeniculacea* was found only at the sites of Barcarello and Punta Priola.

The larger part of *Cystoseira s.l.* species (7 species) was found between 10 and 20 meters depth (Table 2). *Ericaria funkii* was only found within the MPA at the sites of La Cala, Punta Matese, Punta Barcarello and Barcarello. This species has a green iridescence and was found as groups of 2-3 individuals so close to be perceived as a single big individual. *Cystoseira foeniculacea* f. *latiramosa* and *C. foeniculacea* f. *tenuiramosa* were found as scattered individual thalli within the MPA, the first one at the sites of La Cala, Punta Matese, and Barcarello, and the second at La Cala, Punta Matese, Punta Barcarello and Barcarello. *Cystoseira dubia* was discovered in small patches within the MPA (Isola, La Cala and Punta Barcarello) in areas with high sedimentation, with flattened primary branches of light brown color emerging from the sediment. Finally, *E. brachycarpa* var. *claudiae* was found as isolated individual thalli within the MPA (Punta Barcarello, Barcarello and Capo Gallo) and at the controlled site of Punta Priola (Table 2).

### 2.3. Non-indigenous seaweeds

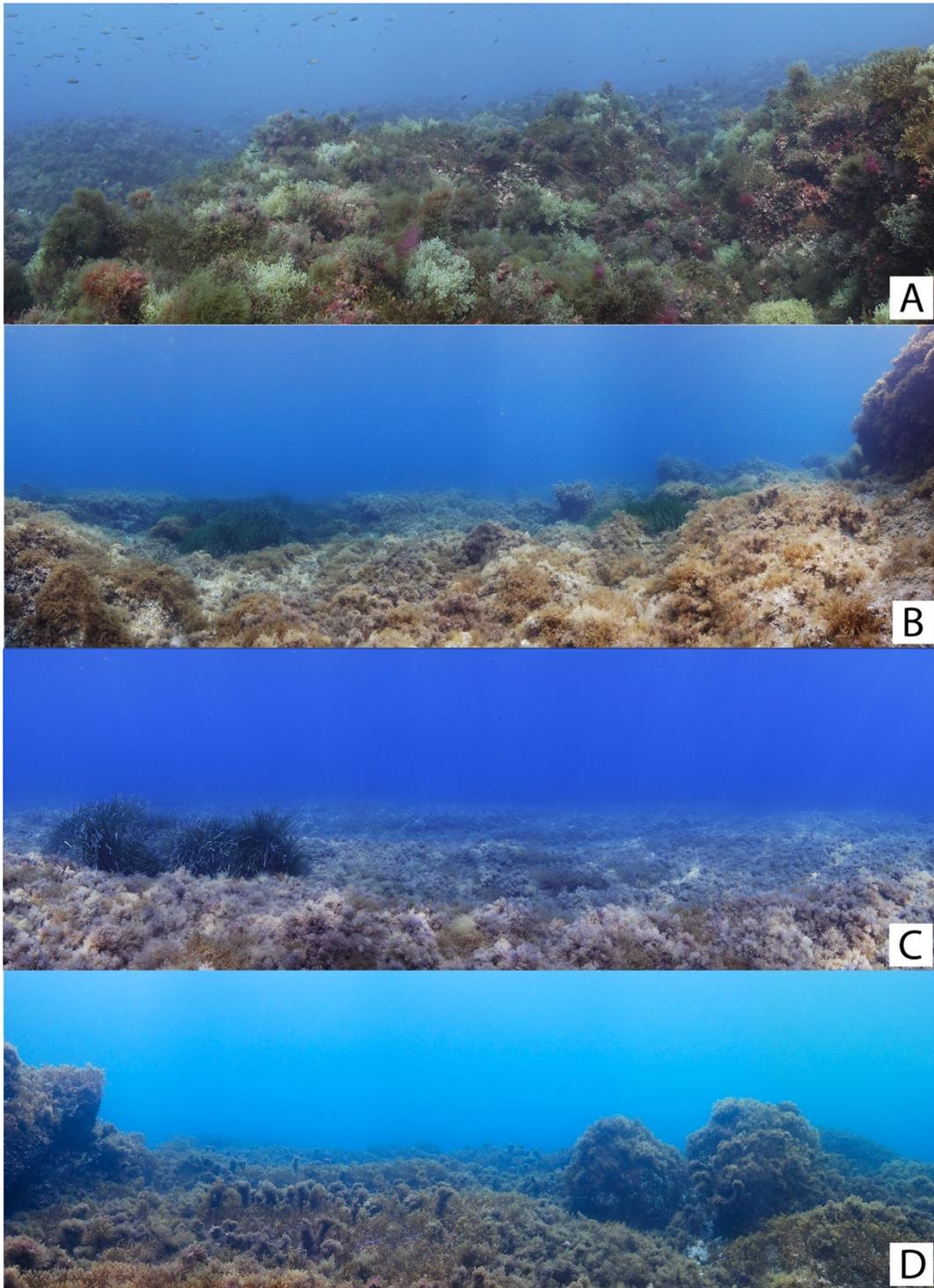
During data collection within both the MPA and control sites, the presence of non indigenous seaweeds was observed, regardless of the level of protection. In particular, four species were discovered: *Asparagopsis taxiformis* (Delile) Trevisan, *Caulerpa cylindracea* Sonder 1845, *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini and *Lophocladia lallemandii* (Montagne) F. Schmitz (Figure 6). Thalli of *A. taxiformis* (Figure 6A) can be found across the entire "Capo Gallo - Isola delle Femmine" MPA, regardless of the protection zone and depth range. The species was discovered from 1m to 20 m depth with a particularly high density in the Isola delle Femmine site. Moreover, *A. taxiformis* was found as epiphyte on *Cystoseira s.l.* species, especially *G. montagnei* var. *tenuior*. *Caulerpa cylindracea* (Figure 6B) was also observed across the MPA and does not appear to have a preferred depth. It can be found from the mediolittoral zone (intertidal rocky pools, vermetid reef cuvettes) down to the 20 m. While stolons can attain enormous densities, the vertical frond of the alga is not always apparent. *Caulerpa taxifolia* var. *distichophylla* (Figure 6C) was found between 0 and 10 m at Punta Barcarello and Barcarello, particularly on rocky substrate covered with sediment. Finally, blooms of *L. lallemandii* (Figure 6D) were detected over the MPA during the summer, capable of completely covering vast areas of substrate and all the seaweeds inhabiting them. This species has also been found to epiphyte *G. montagnei* var. *tenuior*.



**Figure 6.** Invasive species found within the MPA of Capo Gallo – Isola delle Femmine. A = *Asparagopsis taxiformis*; B = *Caulerpa cylindracea*; C = *Caulerpa taxifolia* var. *distichophylla*; D = *Lophocladia lallemandii*. Photos by Francesco Paolo Mancuso.

### 3. Discussion

According to our surveys, the MPA had more diverse *Cystoseira s.l.* populations in terms of number of species (Figure 7) than outside control sites (Figure 8), confirming the hypothesis that MPAs can be an effective strategy for preserving and restoring these important foundation seaweeds [14,28,29]. Although this observation remains true when comparing *Cystoseira s.l.* populations across the MPA and external sites, our findings demonstrate no variations among the various degrees of protection within the MPA. Then, our initial hypothesis posited that the effects of protection within the MPA would lead to significant differences in the patterns of structuring and abundance of these communities between areas with total protection and those with less protection and control, was thus unfounded.



**Figure 7.** Examples of benthic habitats with *Cystoseira s.l.* populations within the MPA of “Capo Gallo – Isola delle Femmine”: A = Isola (zone A); B = Barcarello (zone B); C = La Cala (zone B); D = Punta Barcarello (zone D). Photos by Francesco Paolo Mancuso.



**Figure 8.** Examples of benthic habitats at the two control sites. A = Monte Cofano characterized by the presence of well stated *Cystoseira s.l.* populations; B = Punta Priola, dominated by *Dictyopteris polypodioides*. Photos by Francesco Paolo Mancuso.

Studies have shown that some Mediterranean MPAs are unable to protect or restore Fucalean algal forests, implying that some of them are merely “paper parks” where regulations are not enforced [29,33]. We believe that the lack of efficacy in protection observed within the MPA is due to the fact that the different zones within the MPA were designed primarily to protect fish stocks rather than Fucalean algal forests. Moreover, the observed differences between the zones can be explained by variations in seabed geomorphic features across MPA sites. In fact, our observations suggest that when the seabed consists of a gently sloping rocky carbonate platform, as seen at the MPA sites of La Cala and Punta Barcarello, we find more continuous and dense *Cystoseira s.l.* populations compared to other sites, such as Capo Gallo and Barcarello, where the presence of large scattered rocky boulders (Riggio and Raimondo, 1991; Lucido et al., 1992) creates a discontinuous environment with heterogeneous light conditions, where small patches and individual thalli of *Cystoseira s.l.* are most common.

Data on *Cystoseira s.l.* species distribution within the “Capo Gallo – Isola delle Femmine” MPA are scarce and date back at least thirty years, making them only partially helpful for comparison. Giaccone and Sortino (1964) reported the presence of *C. compressa*, *E. mediterranea*, *E. crinita* and *Gongolaria barbata* on the seabed of Isola. Of these, *E. mediterranea* and *G. barbata* were not found in our surveys. In 1985, data from *G. barbata* e *G. montagnei* were reported at Capo Gallo and Isola [36], while more recent data reported *E. amentacea*, *Ericaria crinita* and *G. montagnei* at the sites of Punta Barcarello and Capo Gallo [37,38]. The lack of historical data on *Cystoseira s.l.* species distribution within the MPA emphasizes the significance of our study as a baseline for understanding how these populations will change in the future.

The comparison with unprotected control sites revealed differences that were more evident when comparing the MPA sites with the Punta Priola site than with the site of Monte Cofano. The vegetation in Punta Priola was mainly characterized by high sedimentation rates, with *Dictyopteris*

*polypodioides* being the main macroalgal species covering large part of the rocky substrate, which appears to inhibit the growth of other seaweeds (Figure 9B). The absence of *E. amentacea*, one of the most important *Cystoseira s.l.* species used to measure water quality, further revealed the site's impacted state [39]. In contrast, our findings demonstrate that *Cystoseira s.l.* populations are comparable between the control site of Monte Cofano and the sites within the "Capo Gallo - Isola delle Femmine" MPA (Figure 9A). This indicates that anthropogenic disturbance, such as trampling, harvesting, pollution, and overgrazing, are fairly limited at Monte Cofano, and confirm that healthy dense forests can still be found in non-protected, naturally isolated, and lightly disturbed locations [29].

In addition to the above observations, it is important to mention the presence of four non-indigenous seaweeds (*A. taxiformis*, *C. cylindracea*, *C. taxifolia* var. *distichophylla*, and *L. lallemandii*) within the MPA and at the control sites. Non-indigenous species are one of the major threats to the Mediterranean Sea [40,41] *Asparagopsis taxiformis* has been named one of the top 100 invasive seaweeds in the Mediterranean Sea [41]. The presence of *A. taxiformis* need further investigation because it has negative effects on *Cystoseira s.l.* populations, eroding biomass of primary producers and the associated biodiversity [42]. In accordance with other research [43–45], *C. cylindracea* was largely observed within the MPA area across different habitats and types of substrate, regardless of the levels of protection. It was found across all the investigated depth range, but thalli were also found at depths of 35 m (Mancuso's personal observations). This suggest that although MPAs are a useful management tool for the protection of biodiversity, they are still vulnerable to non-indigenous seaweeds [45,46]. The presence of *Caulerpa cylindracea* can have a negative impact on native seaweeds assemblages, also facilitating the subsequent invasion of a trophic specialist that takes advantage of niche opportunities created by the alga [47–49]. Finally, *L. lallemandii* can affect the structure of macrofauna associated with habitat forming seaweeds of *Cystoseira s.l.* [46], or cause the mortality of seagrasses [50].

#### 4. Materials and Methods

The study was performed on the shallow rocky substrate (0 to 20 m depth) within the "Capo Gallo-Isola delle Femmine" MPA (Lat: 38.213961, Long: 13.277121) and two control sites, Monte Cofano (Lat: 38.114429, Long: 12.677827) and Punta Priola (Lat: 38.192074, Long: 13.358161), located in the northwestern coast of Sicily, Italy (Figure 1), which are not subjected to marine protection.

##### 4.1. The "Capo Gallo – Isola delle Femmine" MPA

The "Capo Gallo-Isola delle Femmine" MPA, established in 2002 by the Italian Ministry of Environment and Protection of Land and Sea, affects the sea stretch between the towns of Palermo and Isola delle Femmine. Covering approximately 22 km<sup>2</sup> of sea area and a coastline of about 16 km, it is bounded to the east by the gulf of Mondello and to the west by the bay of Carini. An imposing calcareous dolomitic mountain crest (Capo Gallo, 562 m a.s.l.) defines the coastal strip, resulting in a steep and rocky coastal morphology. Due to the limestone nature, flowing waters generate karst phenomena, leading to caves of high naturalistic value (Grotta dell'Olio and Grotta della Mazzara). Only towards the western part, the rocky coast assumes a flat conformation, enlivened by the presence, about 300 meters from the mainland, of the Isola delle Femmine (also known as Isola di Fuori), an isolated vestige of the aforementioned calcareous ridge.

The MPA is divided into three main zones (A, B and C), each with its own level of environmental protection (Figure 1). There are two no-take/no-access zones (zone A, total area of 1 km<sup>2</sup>, Figure 1), one in the north sector of Isola delle Femmine and the other in the stretch of sea at the west of Capo Gallo promontory, between the Puntazza and the Capo Gallo lighthouse. Zones B and C are buffer zones where human use restrictions, including fishing, become progressively lower. In particular, there are three general protection zones (zone B, total area of 2 km<sup>2</sup>, Figure 1), while the remaining sea within the MPA's border includes a partial protection area (zone C, total area of 19 km<sup>2</sup>, Figure 1). The MPA area is also identified as Site of Community Importance (SCI, ITA020047 – Fondali di Isola delle Femmine Capo Gallo).

#### 4.2. Control sites

To analyze the efficiency of MPA protection, we chose two unprotected marine regions: one natural and less impacted site named Monte Cofano and one highly impacted site close to the urban center called Punta Priola (Figure 1). The Monte Cofano site is located in front of the coast of the natural terrestrial reserve of the Monte Cofano promontory, near Custonaci and San Vito Lo Capo. Although it is not a marine reserve, its status as a terrestrial reserve offers some level of protection by restricting access from the land. Furthermore, there are no large urban areas nearby, and pollution is virtually absent. However, there are no restrictions on marine activities (like fishing or harvesting) in the area. The Monte Cofano site can then be considered an unaltered control site based on its characteristics (white site). The site of Punta Priola was located between Mondello and Palermo. This site is clearer influenced by several anthropogenic stressors (urban site). The shoreline is densely developed, with small untreated outfalls (particularly in summer), and the little Rousvelt harbor located approximately 300 meters west of the site. Moreover, the site attracts bathers who pour onto the seashore, resulting in an increase in stressors from trampling and harvesting activities.

#### 4.3. Survey of *Cystoseira s.l.* populations

Scuba diving surveys were conducted at 6 sites within the “Capo Gallo - Isola delle Femmine” MPA, representing a large part of the MPA and the 3 levels of protection (2 sites for each level of protection), while surveys were carried out at 1 site in the control sites. The sites were mostly exposed to northwest winds and had a similar seabed environment with carbonate platforms and rocky substrates. At each site, 3 belt transects [51] from 0 to 20 meters depth were used to determine the distribution of *Cystoseira s.l.* species. Transect length changed according to seabed degradation, while width was 6 m (3 m left and right the transect). For each bathymetric range (0-5 m, 5-10 m, 10-15 m and 15-20 m), cover percentage of *Cystoseira s.l.* species were estimated in 4 quadrats (50 x 50 cm) haphazardly selected. During each dive, the water visibility was at least 10 meters, allowing easily identification of *Cystoseira s.l.* thalli. The depth range was chosen to allow safe scuba diving (diving constraints such as decompression schedules and air consumption normally limit depths to less than 20 m) and to give access to the majority of *Cystoseira s.l.* species.

All surveys were carried out in May, when the thalli of *Cystoseira s.l.* species in this area reach their maximum development [52]. Furthermore, pictures of the landscape were acquired to describe the status of the *Cystoseira s.l.* populations. Collection of thalli was limited to species that were difficult to identify in the field. Sampled thalli were deposited in the algological laboratory (Department STEBICEF - University of Palermo).

#### 4.4. Data analysis

For each investigated depth range, the abundance (N, average percentage cover), frequency (F%, the percentage of samples in which a particular species is present), and dominance index (D%, the percentage ratio of individuals of a particular species to the total number of individuals within the sample) for each *Cystoseira s.l.* species were estimated [53]. Additionally, for each area investigated (MPA's zone and control sites), *Cystoseira s.l.* species were characterized based on total abundance (N; expressed as cover percentage), rarefied species richness (S), Shannon-Wiener diversity index (H'), and Pielou's Evenness index (J).

Analyses of variance (ANOVAs) were used to test: i) differences in the *Cystoseira s.l.* indices (N, S, H', J) among the three protection zones (fixed factor with 3 levels: zone A, zone B and zone C) within the MPA; ii) differences among zones within the MPA and external control sites (fixed factor with 5 levels: zone A, zone B, zone C, white and urban). Besides the main factor in each analysis, ANOVAs included the factors site (random factor nested within zone) and transect (random factor nested within site and zone).

Louvain community detection [54] was performed to detect the *Cystoseira s.l.* set for each considered depth range. Differences in the *Cystoseira s.l.* structure (which takes into account species identity and relative abundance) and composition (presence/absence, which only takes into account

species identity) among sites were assessed by Permutational Multivariate Analysis of Variance (PERMANOVA). The analyses were based on a Bray-Curtis distance matrix of square-root transformed cover percentage of *Cystoseira s.l.* using 9999 permutations [55]. A principal coordinate analysis (PCoA) plot was generated to visualize the variation in *Cystoseira s.l.* assemblage structure (based on a Bray-Curtis distance matrix) and composition (based on Jaccard distance matrix).

Statistical analyses were carried out in R open access statistical software version 4.1.2 [56].

## 5. Conclusions

Our findings emphasize the importance of the “Capo Gallo - Isola delle Femmine” MPA as a valuable tool for Fucalean forests of the genera *Cystoseira*, *Ericaria* and *Gongolaria*, as well as a good reference for monitoring the temporal evolution of these foundation seaweeds. Further research should be conducted to gain more insights into *Cystoseira s.l.* species present in the MPA, particularly at depths greater than 20 meters. Up until now, data on deeper *Cystoseira s.l.* species within the “Capo Gallo – Isola delle Femmine” MPA come from point-like observations. In particular, we have observed the presence of *Ericaria zosteroides* (C.Agardh) Molinari & Guiry and *G. montagnei* var. *compressa* were at a depth of 35 to 40 meters at the site of Isola and the presence of *G. barbata* (Stackhouse) Kuntze at a depth of around 1 meter in the port of Isola delle Femmine. This information remarks the MPA’s role in preserving diverse *Cystoseira s.l.* populations. It also emphasizes the importance of doing extensive habitat mapping of these key foundation seaweeds to monitor their range and health status. This is crucial in understanding how these valuable foundation species will respond to the effects of climate change.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org., Table S1: - PERMANOVAs results of the structure and composition of the *Cystoseira s.l.* assemblage among the MPA’s zones; Table S2: PERMANOVAs results of the structure and composition of the *Cystoseira s.l.* assemblage among all areas investigated (MPA’s zones and control sites); Table S3: Average similarity of the *Cystoseira s.l.* assemblage between and within the different zones investigated.

**Author Contributions:** Conceptualization, F.P.M. and A.M.; Methodology, F.P.M. and A.M.M.; Data curation, F.P.M.; Investigation, F.P.M. and A.M.M.; Visualization, F.P.M.; Laboratory Analyses, F.P.M. and A.M.M.; Writing – original draft, F.P.M. and A.M.M.; Writing – review & editing, F.P.M., A.M.M. and G.S.; Supervision, G.S. and A.M.M.; Funding acquisition, G.S. and A.M.M.

**Funding:** This research was funded by the Ministero dell’Ambiente e della Tutela del Territorio e del Mare through the project “AMPOCYS” (nota Prot. N. 29962 del 13/12/07), by the Ministero della transizione ecologica (MiTE) through the projects “Realizzazione di misure di conservazione del sito di interesse comunitario ITA 020047 “Fondali Isola delle Femmine – Capo Gallo” and “Valutazione dello stato di conservazione della vegetazione algale nell’AMP di Capo Gallo – Isola delle Femmine and Ministero delle politiche agricole alimentari, forestali e del turismo (Mipaaf) FEAMP PO 2014- 2020 Fondo europeo per gli affari marittimi e la pesca Misura: “1.40 - Protezione e ripristino della biodiversità e degli ecosistemi marini e dei regimi di compensazione nell’ambito di attività di pesca sostenibili.

**Data Availability Statement:** All data generated or analyzed during this study are included in this published article.

**Conflicts of 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.

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