

## Article

# The Ecological Role of *Ruppia cirrhosa* (Petagna) Grande in a Choked Lagoon

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**Abstract:** We studied the ecological and functional aspects (such as role in sediment characteristics and plant–animal interactions) of a *Ruppia cirrhosa* (Petagna) Grande meadow during its vegetative season in the choked Valle Campo lagoon, a sub-basin of the wider Valli di Comacchio, on the Northern Adriatic coast. Sampling campaigns were carried out with a roughly fortnightly frequency in 2017 at two sites, one with the *Ruppia* meadow and one with bare sediment. Sediment parameters analyzed were microphytobenthic chlorophyll-*a*, protein, carbohydrate, and lipid content, and total organic matter. The macrobenthos was identified at the lowest possible taxonomic level. Chlorophyll *a*, as a surrogate of microphytobenthos, showed differences between the two sites, probably mainly related to light intensity; thus, it is expected that the absence of seagrass canopy results in the higher production of microphytobenthos. At both sites, proteins were the dominant class of labile compounds, suggesting that detrital organic matter present at both study sites is of high nutritional quality. The high protein/carbohydrate ratio also suggests the presence of non-aged organic matter. We recorded a total of 18 macroinvertebrate taxa. The *Ruppia* meadow showed a positive influence on macrofauna abundance, diversity, species richness, and composition of trophic groups. Only the infaunal taxa *Capitella capitata* and *Chironomus salinarius* exhibited higher abundance at the bare site. The ecological quality status measured by the M-AMBI index was unsatisfactory everywhere. However, the presence of the *Ruppia* meadow resulted in index values being consistently higher. The role of this minor seagrass has been proved to be of great importance, improving the nutritional quality of the organic matter in the sediments and, above all, providing new habitats and new niches for a number of benthic macrofauna species.

**Keywords:** *Ruppia cirrhosa*; sediment nutritional quality; macrobenthos; ecological status; Valli di Comacchio; Northern Adriatic Sea



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## 1. Introduction

Seagrasses play an essential role in a number of key coastal processes, such as productivity, carbon sequestration, and the implementation of food webs [1,2]. Furthermore, seagrass meadows provide food and habitat for numerous faunal organisms, including a wide range of macroinvertebrates [1]. It is known that seagrass meadows, compared to bare non-vegetated beds, constitute areas of high biodiversity: the leaf canopy and the root–rhizome system create a three-dimensional habitat of relatively high structural complexity and provide spatial niches for a variety of organisms [3]. The complexity of the habitat and the creation of spatial refugia in areas otherwise devoid of vegetation make seagrass meadows important for maintaining the biodiversity of the shallow littoral ecosystems in which they occur [1].

Since the 1980s, there has been a reduction in seagrass meadows in many lagoons of the Mediterranean basin, where aquatic angiosperms decreased significantly due to increasing eutrophication [4,5]. However, the environmental policies implemented by the European Community and by various states have led to an improvement in environmental conditions in many lagoons, suggesting that, at selected sites, the seagrass meadows, once present, can be restored [6,7].

In this perspective, the LIFE TRANSFER project funded by the European Community, which began in 2020, aims to improve the ecological status of some Mediterranean lagoons through the reintroduction of aquatic angiosperms. The project involves the removal of sods or rhizomes from a donor site and reimplantation at a recipient site. Among the various seagrasses living in the northern Adriatic, *Ruppia cirrhosa* may be a candidate for replanting in lagoon areas where there are no particular hydrodynamics. As a matter of fact, the Valli di Comacchio hosted luxuriant *R. cirrhosa* meadows until the end of the 1970s [8], when hypereutrophication eliminated them from most of the basins [9]. Currently, *Ruppia* meadows are present in Valle Campo, a choked sub-basin, covering approximately 600 hectares.

The genus *Ruppia*, a cosmopolitan aquatic plant complex, is generally restricted to shallow waters such as coastal lagoons and brackish habitats characterized by fine sediments and high fluctuations; in particular, *R. cirrhosa* tolerates a wide range of water temperature (mainly between 5 and 30 °C) and salinity (1.5–60PSU) [10,11]. *Ruppia* forms monospecific beds and can be regarded as a “continental seagrass” because it grows in sheltered shallow nontidal estuaries and lagoons as well as in coastal or inland water bodies. Its meadows are inhabited by an abundant but species-poor fauna [12], and the value of *Ruppia* seeds and habitat-associated invertebrates as food for birds is especially known for coots, wigeons, and flamingos [13]. In the Mediterranean Sea, most studies of seagrass meadows focus mainly on *Posidonia oceanica* and *Cymodocea nodosa* [14,15], while a few studies have dealt with *R. cirrhosa*, which nevertheless remains one of the least studied seagrasses [16]. The presence of *P. oceanica* or *C. nodosa* meadows increases the abundance of organisms by increasing the amount of physical structure usable as living space, the number of microhabitats, sediment deposition and stabilization, food resources, and protection from predators [1–3]. Compared to these seagrasses, there is a lack of quantitative data focusing on *Ruppia* ecological and functional aspects, such as plant–animal interactions and its role in sediment characteristics. The aim of this study was to study the ecological role of a *Ruppia* meadow during its vegetative season in Valle Campo, a lagoon which, despite presenting extreme environmental conditions due to limited extension, confinement, and a lack of tidal flow or freshwater inlet, maintains the presence of the seagrass meadow.

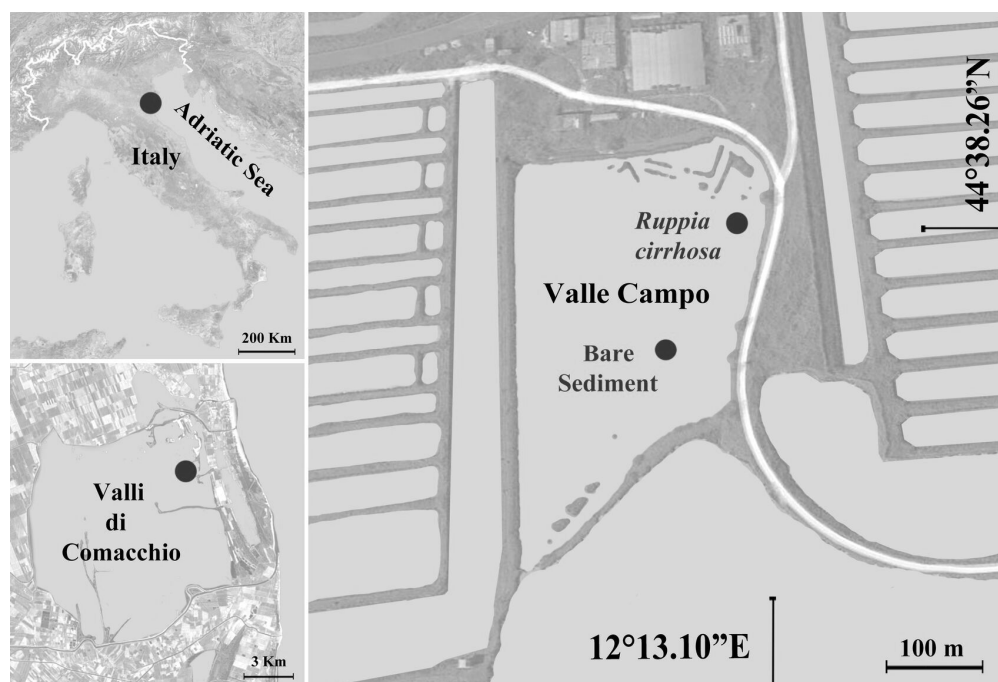
## 2. Materials and Methods

The samplings were carried out from June to December 2017 at Valle Campo with a roughly fortnightly frequency. Two sites were considered, one with *Ruppia* meadow (44°38.257' N; 12°13.075' E) and one with bare sediment (44°38.216' N; 12°13.056' E). The two sites were approximately 200 m apart (Figure 1). Valle Campo is a choked basin, which is not affected by tidal excursions. The study area has a rough basin shape, shallower at the edges and deeper in the center. The *Ruppia* site, closest to the edge, has a depth of between 20 and 30 cm, while the bare sediment site, more towards the center of the basin, has a depth of about 50 cm. The sediments consist of silty–clayey sand.

Water parameters (temperature, salinity, dissolved oxygen) were measured in the field with an OxyGuard® Handy Gamma probe (OxyGuard International, Farum, Denmark). Sediment cores (diameter 5 cm) were taken from both sites in triplicate; the superficial 3 cm were cut off and immediately frozen for subsequent analyses. The *Ruppia* meadow and the bare sediment were sampled by means of a Van Veen grab (4 L volume) in triplicate. All the sampled material was sieved at 0.5 mm and preserved in formaldehyde.

In the laboratory, microphytobenthic chlorophyll-a was extracted (12 h at 4 °C, in the dark) from the superficial (0–1 cm) sediment samples, using 90% (vol/vol) acetone as

the extractant. The extracts were analyzed fluorometrically to estimate chlorophyll-a [17]. The nutritional quality of the sediment was evaluated by considering its protein (PRT), carbohydrate (CHO), and lipid (LIP) content. Protein, carbohydrate, and lipid analyses were carried out on the sediment samples using photometric protocols [18], converted to C equivalents using the conversion factors 0.49, 0.40, and 0.75 mg of C per milligram, respectively, and their sum was referred to as the biopolymeric carbon, BPC [18]. Total organic content was estimated by combustion and incineration at 500°. Possible differences in the nutritional quality of the sediment between the *Ruppia* and bare sediment sites were investigated by analysis of variance (ANOVA).



**Figure 1.** Study site location.

Sorting of the macrofauna was carried out under a Nikon SMZ-745T stereomicroscope and organisms were identified at the lowest possible taxonomic level. The biomass (as ash-free dry weight, AFDW) of *Ruppia* from grab samples was estimated by combustion and incineration at 500°.

The distribution of sedimentary and macrofaunal assemblage variables was examined through multivariate analyses using PRIMER7 [19]. Non-metric multidimensional scaling (nMDS) of distances among centroids based on a Euclidean resemblance matrix (log-transformed and normalized data) was used to visualize multivariate sedimentary patterns (4999 permutations). We visualized multivariate patterns of the macrofauna using an nMDS of distances among centroids based on Bray–Curtis resemblance matrices of macrofauna assemblages (4th-root transformed). The macrobenthic taxa that mostly contributed to the similarity among sites were identified using SIMPER analysis. To define the amount of overall change (i.e., variance partition) in macrobenthic composition between *Ruppia* and bare sediment sites that can be explained by sedimentary features, distance-based linear models (DistLM) were performed (4999 permutations). Bray–Curtis (taxa-specific abundance) and Euclidean (sediment parameters) similarity matrices were constructed after transforming the data. DistLM were fitted using the stepwise selection procedure and  $R^2$  criteria. Distance-based redundancy analysis (dbRDA) was applied to visualize the position of the sites according to the macrobenthic assemblages fitted to the significant sediment predictor variables.

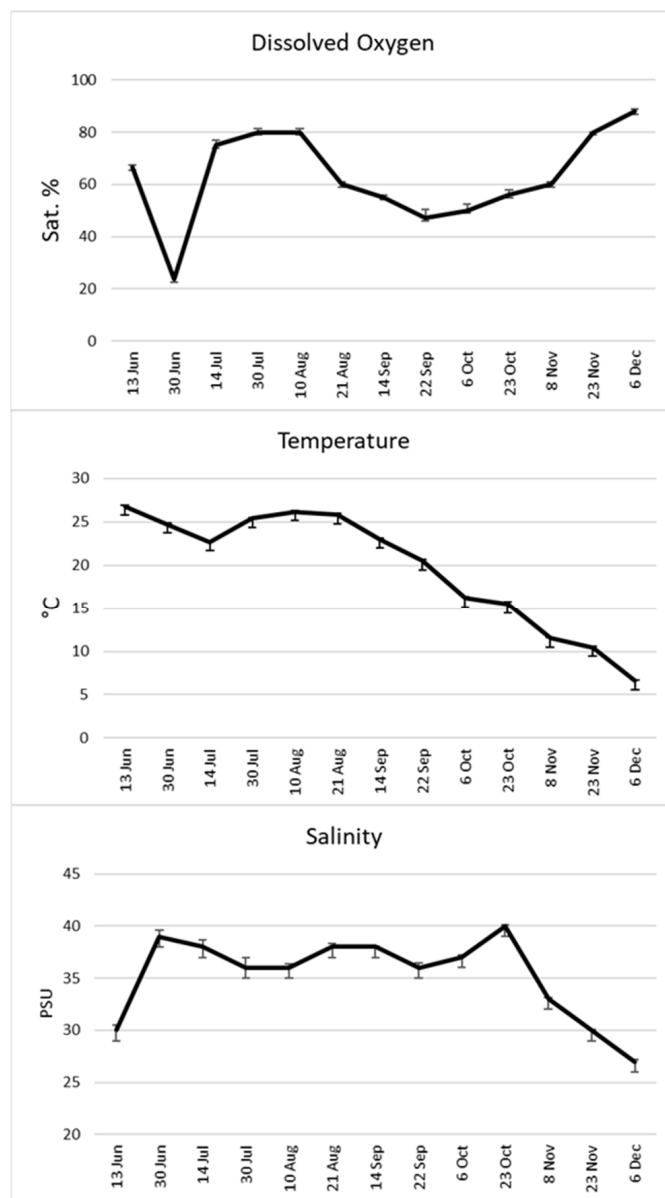
The ecological quality status at both sites was assessed by the macrobenthic community by applying the M-AMBI [20] index. Valle Campo is a choked and non-tidal sub-basin;

therefore, the reference values for the status “High” are AMBI = 1.85,  $H'$  = 3.3,  $S$  = 28 (as defined by the Italian Ministerial Decree D.M.260/10).

### 3. Results

#### 3.1. Water and Sediment Parameters

Water parameters showed a clear seasonal trend and were obviously identical at the two sites, given the short distance. Temperature varied between 27.8 and 6.6 °C. Dissolved oxygen saturation varied between 88% and 23.6%. Salinity varied between 40 and 27 PSU. Figure 2 shows the values of water parameters.



**Figure 2.** Trend of the values of water parameters measured on multiple sampling dates at Valle Campo (bars are standard deviation, S.D.; Jun: June, Jul: July, etc.).

Table 1 shows the values of sediment parameters. Microphytobenthic chlorophyll-a concentration was higher at the bare sediment site (mean value:  $16.2 \pm 4.7 \mu\text{g/g}$ ) than at the *Ruppia* site (mean value:  $13.1 \pm 4.5 \mu\text{g/g}$ ). At both sites, proteins (PRT) were the dominant class of labile compounds, with yearly averages of 75% and 76% at the *Ruppia* and bare sites, respectively, followed by carbohydrates (CHO: 13% and 9%, respectively)

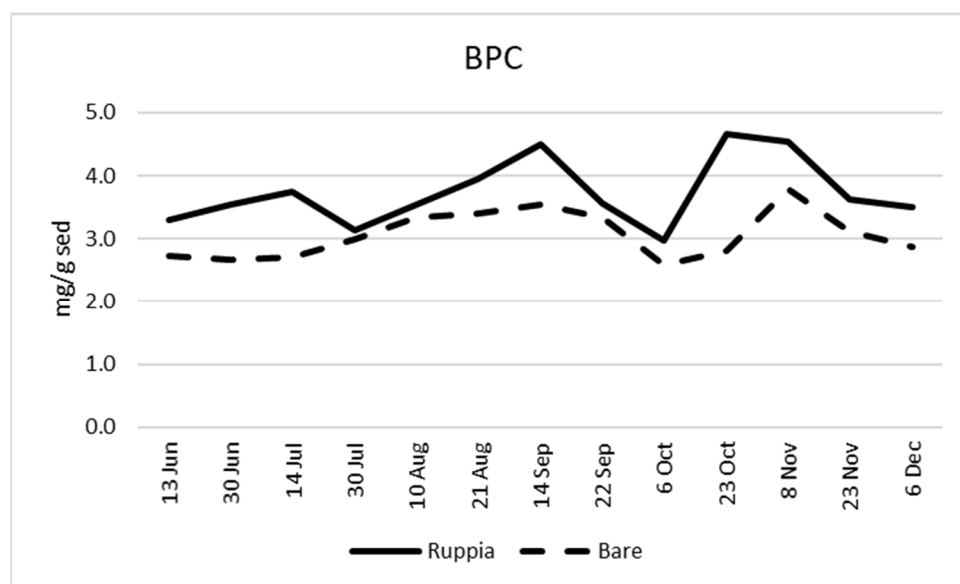
and lipids (LIP: 12% and 13%, respectively). The mean PRT/CHO ratio at the *Ruppia* site was slightly lower than that at the bare site, with mean values of 6.1 ( $\pm 1.1$  SD) and 8.2 ( $\pm 2.0$  SD), respectively. Two-way ANOVA (Table 2) indicated that significant differences were detected between sites and among dates in the content of chlorophyll-a and CHO, with the *Ruppia* site always showing a higher content of CHO (Tukey HSD test  $p < 0.01$ ). On average, the *Ruppia* site always exhibited a higher content of biopolymeric C (Figure 3).

**Table 1.** Sediment parameters ( $\pm$ standard error, S.E.) at *Ruppia* and bare sediment sites (O.M.: %; Chl-a:  $\mu\text{g/g}$ ; CHO:  $\text{mg/g}$ ; PRT:  $\text{mg/g}$ ; LIP:  $\text{mg/g}$ ).

	13 June	30 June	14 July	30 July	10 August	21 August	14 September	22 September	6 October	23 October	8 November	23 November	6 December
<i>Ruppia</i>													
O.M.	10.4	16.9	11.7	26.4	14.8	12.2	8.2	5.7	7.6	22.1	6.8	6.3	7.0
Chl-a	16.15	17.40	16.92	19.08	3.11	17.73	14.29	14.06	11.66	10.74	10.94	8.19	10.00
$\pm$ S.E.	3.40	1.89	1.13	2.98	0.22	2.82	1.99	2.47	1.21	0.56	0.32	0.40	1.07
CHO	0.66	0.91	0.80	0.83	0.98	1.24	1.23	1.07	1.15	1.28	1.16	1.08	1.10
$\pm$ S.E.	0.05	0.17	0.04	0.12	0.13	0.05	0.18	0.05	0.09	0.07	0.06	0.07	0.10
PRT	5.65	5.91	6.40	5.50	5.83	6.38	7.43	6.41	4.57	7.69	7.58	5.90	5.63
$\pm$ S.E.	1.60	0.73	0.32	0.22	0.64	0.09	1.33	1.91	0.24	0.32	0.02	0.56	0.16
LIP	0.94	0.99	1.07	0.72	0.97	1.06	1.24	0.63	0.76	1.28	1.26	0.98	0.94
$\pm$ S.E.	0.27	0.12	0.05	0.04	0.11	0.01	0.22	0.32	0.04	0.05	0.00	0.09	0.03
<i>Bare</i>													
O.M.	13.6	18.6	10.1	8.6	11.3	12.1	8.3	8.5	17.8	11.6	12.5	8.5	8.7
Chl-a	12.13	13.83	13.76	16.11	22.74	21.97	15.72	23.44	20.49	13.90	16.45	11.28	8.39
$\pm$ S.E.	4.53	3.86	5.49	2.48	11.48	1.27	3.47	3.09	2.00	1.46	3.80	2.23	1.35
CHO	0.49	0.75	0.48	0.57	0.89	0.82	0.52	0.84	0.82	0.52	0.77	0.50	0.59
$\pm$ S.E.	0.09	0.26	0.16	0.11	0.29	0.07	0.17	0.13	0.13	0.10	0.12	0.10	0.02
PRT	4.69	4.12	4.43	5.12	5.50	5.68	6.20	5.51	4.12	4.84	6.43	5.42	4.88
$\pm$ S.E.	1.54	0.46	0.50	0.36	1.03	0.25	0.12	1.36	1.03	0.36	1.36	0.27	0.53
LIP	0.81	0.88	0.94	0.88	0.95	0.98	1.07	0.95	0.71	0.83	1.11	0.93	0.84
$\pm$ S.E.	0.27	0.08	0.09	0.06	0.18	0.04	0.02	0.23	0.18	0.06	0.23	0.05	0.09

**Table 2.** Results of two-way ANOVA (factors: site and date) on content of chlorophyll-a (CHL) and carbohydrates (CHO). Significant  $p$  values are in italics.

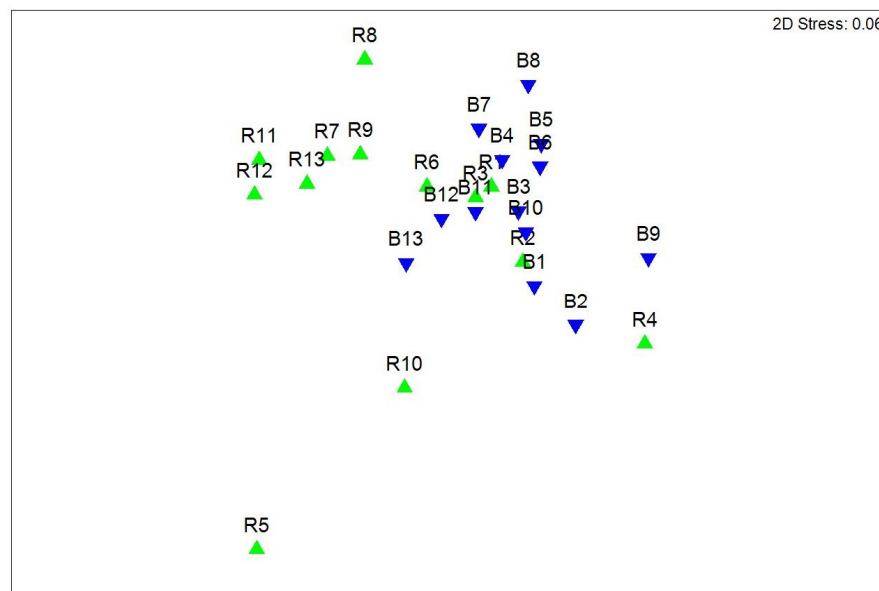
Factor	CHL				CHO		
	df	MS	F	$p$	MS	F	$p$
Date	12	0.055	2.124	<i>0.031</i>	0.007	2.123	<i>0.031</i>
Site	1	0.185	7.112	<i>0.010</i>	0.166	51.644	<i>0.001</i>
Date $\times$ Site	12	0.125	4.799	<i>0.001</i>	0.003	1.072	0.402
Error	52	0.026			0.003		



**Figure 3.** Sediment content of biopolymeric C at the *Ruppia* and bare sites (Jun: June, Jul: July, etc.).

The nMDS ordination of the sedimentary characteristics indicated a clear separation of the different sites, with the site points almost entirely segregating into two separate clouds (Figure 4). Only on the first dates (June) did the *Ruppia* site seem undifferentiated

from the bare site, as they are interspersed in the plot. Then, over time, the sedimentary characteristics between the R and B site differed and site points segregated separately in the ordination plot.



**Figure 4.** Non-metric multidimensional scaling (nMDS) of distances among centroids based on the Euclidean measure of the sedimentary variables at *Ruppia* (R) and bare sediment (B) sites during the monitoring period (1: 13 June; 2: 30 June; 3: 14 July; etc.).

### 3.2. Macrofaunal Community Biodiversity across a Vegetation Gradient

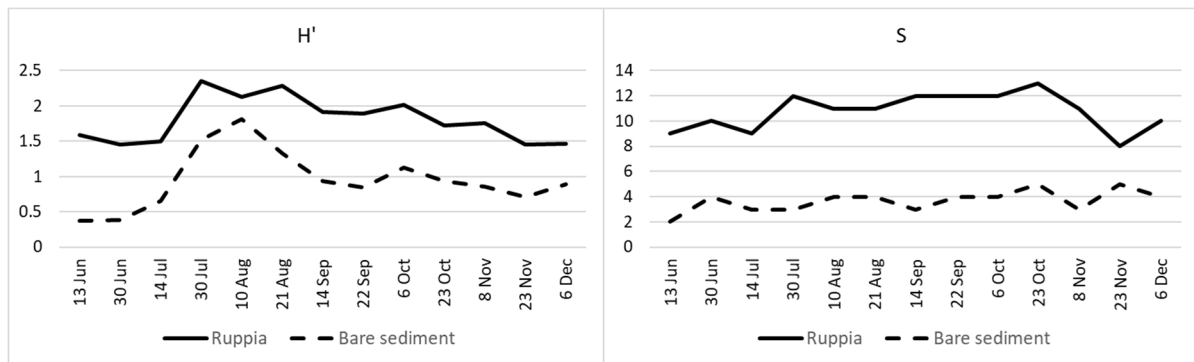
We recorded a total of 18 macroinvertebrate taxa (7 molluscs, 5 annelids, and 4 crustaceans), and 2 other taxa belonging to other groups. The macrobenthic community was found to be different between the two sites (Table 3), despite the short distance.

**Table 3.** Faunistic inventory and mean abundance at the two study sites (abundance in ind m<sup>-2</sup>; \*: 0–100; \*\*: 101–1000; \*\*\*: 1001–10,000; \*\*\*\*: >10,000).

	<i>Ruppia</i>	Bare
<i>Actiniaria</i> sp.	*	
<i>Hydrobia acuta</i>	***	**
<i>Haminaea hydatis</i>	**	*
<i>Cyclope neritea</i>	*	*
<i>Littorina obtusata</i>	**	
<i>Bittium reticulatum</i>	*	
<i>Cerastoderma glaucum</i>	*	
<i>Abra ovata</i>	*	*
<i>Flabelligera</i> sp.	*	
<i>Hydroides dianthus</i>	*	
<i>Alitta succinea</i>	**	*
<i>Capitella capitata</i>	***	****
<i>Oligochaeta</i>	***	***
<i>Palaemon elegans</i>	*	
<i>Idotea baltica</i>	**	
<i>Gammarus insensibilis</i>	**	
<i>Monocorophium insidiosum</i>	**	
<i>Chironomus salinarus</i>	***	****

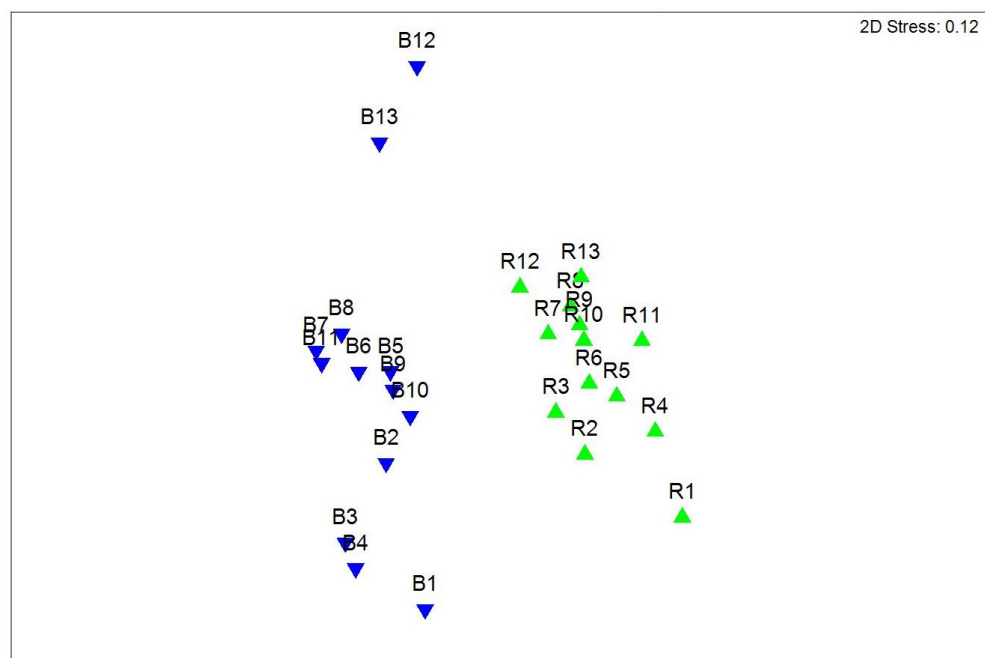
In general, the *Ruppia* site showed the highest macrofauna abundance and exhibited higher values of H' diversity and species richness than the bare sediment site (Figure 5). However, the infaunal taxa *Capitella capitata* and *Chironomus salinarius* exhibited higher

abundance at the bare site. At the *Ruppia* site, the benthic community was more diversified also in composition of trophic groups, being present herbivores such as the Gastropods *Hydrobia acuta*, *Cyclope neritea*, and *Littorina obtusata* and the Amphipods *Gammarus aequicauda* and *Idotea baltica*; filter feeders such as the Bivalves *Cerastoderma glaucum*, *Abra ovata*, and the Polychaete *Hydroides dianthus*; detritivores such as the Polychaete *Alitta succinea* and the Amphipod *Monocorophium insidiosum*; and limivores such as the Polychaete *C. capitata*, and the Diptera *C. salinarius*. Meanwhile, at the bare sediment site, only the limivores (*C. capitata* and *C. salinarius*) were numerically dominant.



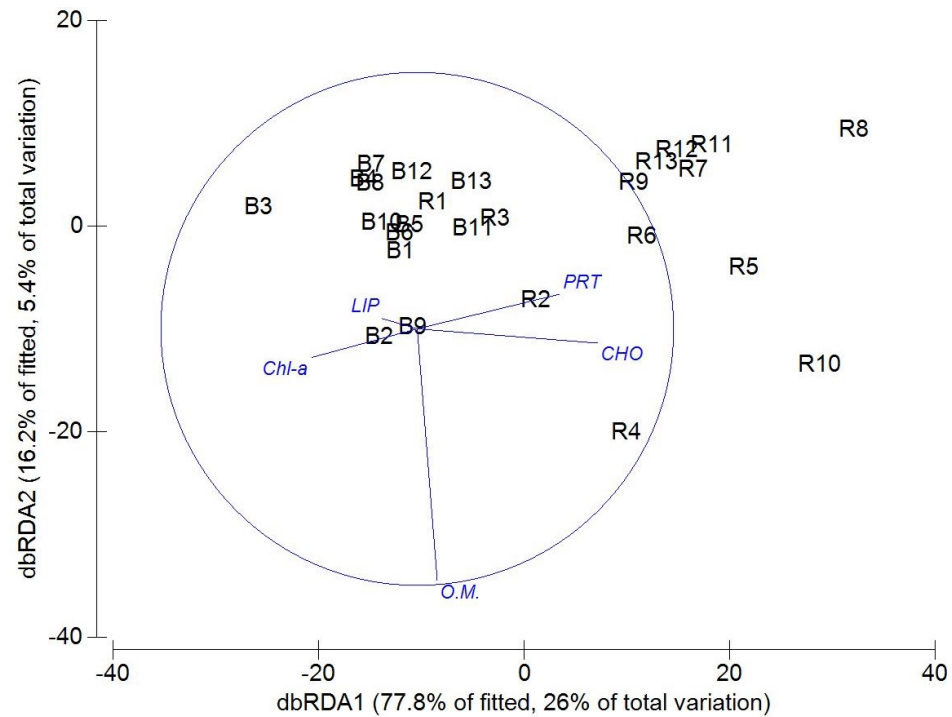
**Figure 5.** Shannon–Wiener’s diversity ( $H'$ ) and species richness ( $S$ ) at the *Ruppia* and bare sediment sites (Jun: June, Jul: July, etc.).

The nMDS ordination of the macrofauna assemblages indicated a clear separation of the different sites along the vegetation gradient (Figure 6). SIMPER analysis identified the species that typified each site (70% cut-off): five taxa contributed the most to the similarity (avg sim = 72.8%) at the *Ruppia* site (*C. salinarius*, Oligochaeta, *H. acuta*, *C. capitata*, *A. succinea*) and only two (*C. salinarius*, *C. capitata*) at the bare site (avg sim = 63.5%). Taxa dissimilarities (avg dissim = 49%) among sites were mostly related to the presence of seven taxa (*C. capitata*, Oligochaeta, *C. salinarius*, *A. succinea*, *C. insidiosum*, *H. acuta*, *I. baltica*).



**Figure 6.** nMDS of distances among centroids based on the Bray–Curtis measure of the macrofauna community abundance at *Ruppia* (R) and bare sediment (B) sites during the monitoring period (1: 13 June; 2: 30 June; 3: 14 July; etc.).

Only a relative part of the observed variation in the macrofauna assemblages was explained by sediment variables, particularly by CHO and PRT (DistLM model explained 18.5% and 11.5% variation, respectively). The first two dbRDA axes captured nearly 31% of the total variation in the data cloud (Figure 7)—the plot showed quite a clear separation of bare and *Ruppia* sites, the latter being associated with higher values of CHO and PRT.



**Figure 7.** dbRDA ordination for the main sediment predictors and the macrofauna assemblages at *Ruppia* (R) and bare sediment (B) sites during the monitoring period (1: 13 June; 2: 30 June; 3: 14 July; etc.).

### 3.3. Ecological Quality Status

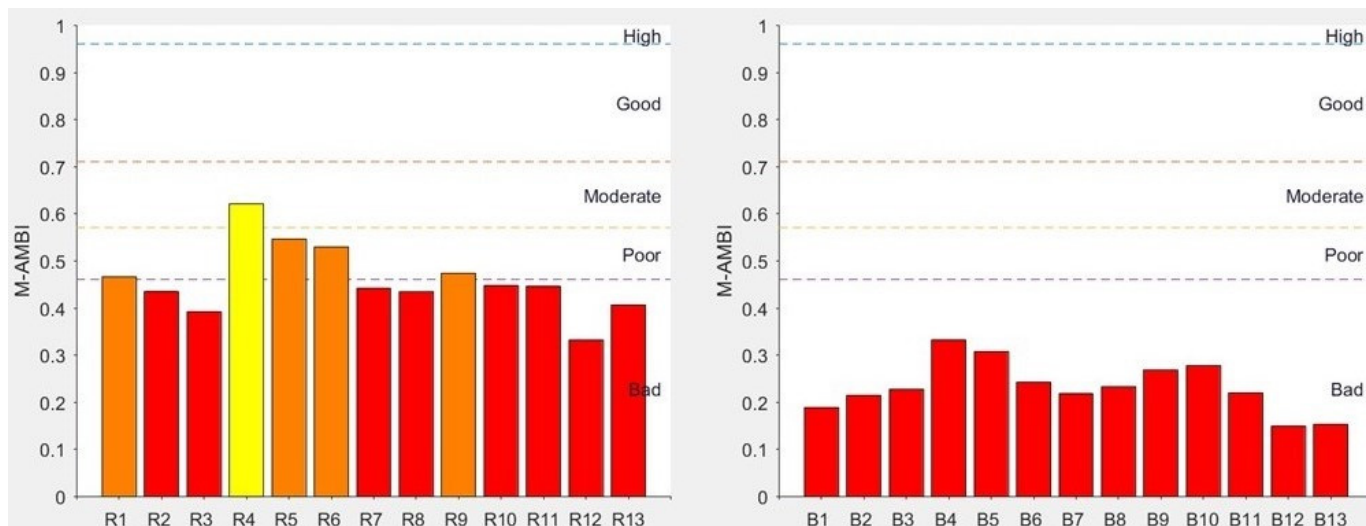
The dominant ecological groups (EG) were, at both sites, first- and second-order opportunistic species (EGIV and EGV). However, at the *Ruppia* site, tolerant species (EGIII) were also found in discrete abundance (up to 44% on 10 August), and even indifferent (EGII) and sensitive (EGI) species, with cumulative (EGI + EGII) abundances up to 25% (10 August). Table 4 reports the composition of EGs on various dates.

**Table 4.** Composition of ecological groups of the macrobenthic community at bare sediment and *Ruppia* sites on the various monitoring dates.

	Ecological Groups									
	Bare					<i>Ruppia</i>				
	I (%)	II (%)	III (%)	IV (%)	V (%)	I (%)	II (%)	III (%)	IV (%)	V (%)
13 June	0	0	7.3	92.7	0	5.5	2.2	41.1	51.2	0
30 June	0	0	5.3	93.7	1	7.3	3	9.8	73.7	6.2
14 July	0	0	8.3	87.5	4.2	0.2	4.7	8	67.9	19.2
30 July	0	0	40	40	20	3.9	20.8	40.6	31.9	2.8
10 August	0	0	11	37.8	51.2	0.6	11.2	43.8	32.1	12.4
21 August	0	0	1.3	44.2	54.5	0.1	9.6	29.3	35.4	25.6
14 September	0	0	0	72.4	27.6	0.2	0.6	9.1	40	50.2
22 September	0	0	0.6	78.9	20.5	1.5	0.9	4.1	37.5	56
6 October	0	0	4.8	76.5	18.7	1.1	1.8	9.7	51.2	36.1
23 October	0	0.7	7.3	81.7	10.2	0.3	0.7	8.4	52.7	37.9
8 November	0	0	0	79.8	20.2	0.9	2.4	13.4	58.8	24.6
23 November	1.1	0.6	0.6	0	97.8	0	0.2	5.2	47.8	46.9
6 December	0.8	0	1.6	0	97.6	2.6	0.3	9.2	72.2	15.7

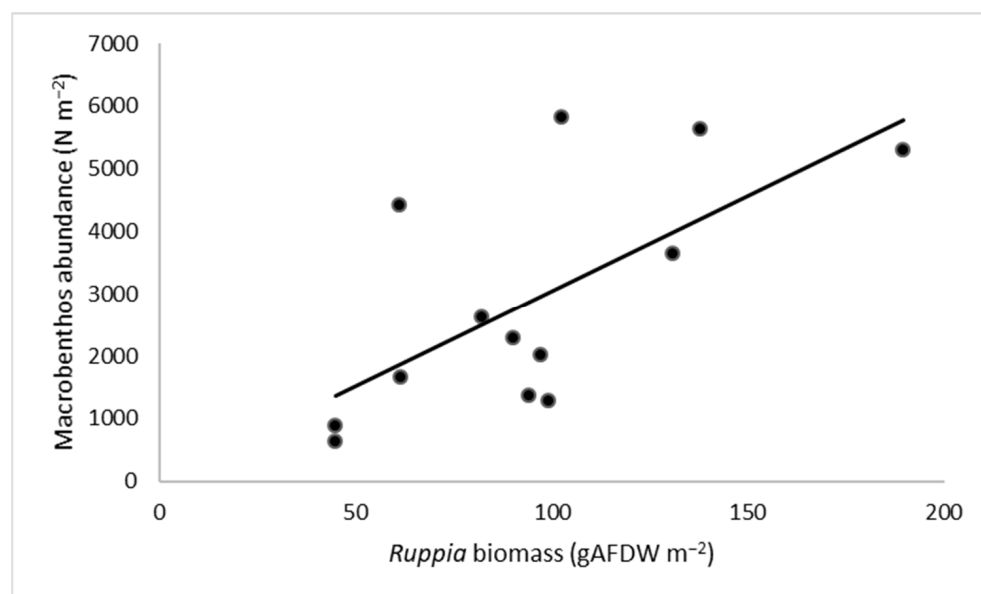


The ecological quality status measured by M-AMBI was unsatisfactory everywhere. However, the presence of the *Ruppia* meadow resulted in M-AMBI values being consistently higher than those obtained at the bare sediment site. Figure 8 shows the M-AMBI values at the two sites during the monitoring period.



**Figure 8.** Ecological quality status measured through M-AMBI at *Ruppia* (R) and bare sediment (B) sites during the monitoring period (1: 13 June; 2: 30 June; 3: 14 July; etc.).

*Ruppia* biomass oscillated between 189.5 (13 June) and 44.7 (6 December) gAFDW  $m^{-2}$ . At the *Ruppia* site, the abundance of macrobenthos oscillated between 5647.7 (10 August) and 638.3 (23 November) individuals  $m^{-2}$ . There was a significant relationship (regression ANOVA;  $r = 0.66$ ,  $p < 0.05$ ) between the amount of *Ruppia* biomass and the abundance of macrobenthos (Figure 9).



**Figure 9.** Relationship between *Ruppia* biomass and macrobenthos abundance.

#### 4. Discussion

The phenology and autoecology of *Ruppia cirrhosa* thriving in Mediterranean brackish lagoons have been recently assessed [11]: the biomass is highly variable during the year and lies mainly in the above-ground portion, while the root apparatus penetrates only

a few centimeters into the sediments, with the photosynthetic portion that occupies the water column [16]. *Ruppia* may also play a role in the biogeochemical cycles of the lagoon, having the capacity to influence nitrogen cycling by incorporating large DIN (dissolved inorganic nitrogen) pools in its biomass [21]. In the choked Valle Campo, the *Ruppia* meadow is concentrated along the edges, while the center of the basin has bare bottoms. Due to water stagnation, most of the biomass of *Ruppia* produced and associated epiphytes are not exported and diluted in the marine environment, but cause the accumulation of organic matter inside the basin. Despite the limited size of the basin, and the proximity of the two study sites, significant differences were found in the substrate biogeochemical characteristics. It is well known [22] that sediment biogeochemical characteristic is an important factor governing the types of species that occupy the substrate, and consequently, sediment variables explained a proportion of the macrofauna variability. Chlorophyll *a*, as a surrogate of microphytobenthos, showed differences between *Ruppia* and bare sediment sites probably mainly related to light intensity; thus, it is expected that the absence of seagrass canopy, even if relatively sparse like that of *Ruppia*, results in higher production of microphytobenthos [23]. At both study sites, the three classes of organic compounds analyzed (PRT, CHO, LIP) showed concentrations among the highest reported in the literature [24]. Their composition showed a dominance of PRT, followed by CHO and LIP. Organic nitrogen (i.e., protein) is widely considered the major limiting factor for deposit feeder organisms [25]. PRT concentrations in sediments of both sites were high, even when compared with very productive areas such as the Mediterranean Marsala Lagoon [24]. PRT accounted for over 60% of sedimentary biopolymeric carbon, highlighting how the amount of detrital organic matter present at both Valle Campo study sites is of high nutritional quality. A high PRT/CHO ratio suggests the presence of non-aged organic matter, and that PRT content is not a limiting factor for benthic consumers [25].

Regardless of the organic content of the sediment, the addition of structures in the form of leaves and rhizomes altered the abundance and species richness patterns from what would be predictable based on substrate biogeochemical characteristics alone. As a matter of fact, a direct correlation between the biomass of *Ruppia* and the abundance of macrofauna was highlighted in this study. This last observation may not be due merely to the fact that as the biomass of *Ruppia* increases, there is more leaf substrate available to the epifauna, since other factors may also play a role in determining the abundance of particular associated species to the habitat of *Ruppia*. The vulnerability of epifaunal prey items in seagrass beds may be related to the ecological characteristics of individual prey species: epifaunal isopods and amphipods, for example, are less likely to be preyed upon by fish within the seagrass canopy [26,27].

*Ruppia* meadow showed a positive influence on macrofauna abundance and diversity, in line with previous observations [28]. Macrophytic characteristics are important explanatory factors for macrofauna communities associated with seagrasses. In our study, the great difference in the faunal community is mainly explained by the presence of *Ruppia* itself, which, with its rhizomes and leaf canopy, provides additional niche spaces that are occupied by organisms that otherwise would not be present (or would be present at much lower densities). The faunal assemblage at the *Ruppia* site consisted of groups of animals with many different life forms and ecological characteristics: species living on leaves, including mobile walking (gastropods) and swimming (amphipods and shrimps) epifauna, and infaunal species, including burrowers and tube dwellers (bivalves, annelids, and chironomids), as well as those animals creeping or crawling at the sediment–water interface (gastropods). In contrast, the bare sediment site's assemblage is poorer and included only burrowers. More generally, these findings could be relevant for the management and conservation of lagoon ecosystems, since understanding the importance of *Ruppia* meadows and their associated fauna can inform conservation strategies, habitat restoration efforts, and even the design of marine protected areas. As a matter of fact, a recent study showed the enhancement of macrofauna diversity across an increasing gradient of seagrass complexity, and the dominance of the turnover component, suggesting that devoting con-

servation efforts to many different types of meadows, including the less diverse, should be a priority for coastal habitat management [29].

A non-negligible aspect in the management of any European lagoon concerns the state of the ecological conditions, sensu European Water Framework Directive (WFD) 2000/60/EC. The objective of the WFD was to achieve “good status” of water bodies by 2015. If the objectives were not achieved, member states will have to achieve them by the end of the third (2027) management cycle [30]. At both sites, the ecological quality status sensu WFD was found to be unsatisfactory, albeit slightly better at the *Ruppia* site. The M-AMBI index relies on the Pearson and Rosenberg paradigm [31]. With the aim of determining anthropogenic stress, it is related to the concept of stress-tolerant species, i.e., just the species (tolerant of natural stressors) which constitute the majority of the benthic fauna in lagoons [32]. The presence of *Ruppia* meadow, with respect to bare sediment, improves the EG composition of the benthic community, for the reasons already discussed above. Unfortunately, this is not sufficient to obtain a “good ecological status”. The well-known term “estuary quality paradox” [33] finds full application here: lagoons, including Valle Campo, are structurally eutrophic habitat islands in the coastal landscape, characterized by low benthic diversity. Due to these natural characteristics, indices such as M-AMBI could probably lose some of their effectiveness. The WFD is implemented in Italy by Legislative Decree 260/10. As far as the marine environment is concerned, the Decree requires the use of biotic indices for macrobenthos, macroalgae, and angiosperms (*Posidonia oceanica*) to assess the ecological status. For the lagoon environment, indices are envisaged only for macrobenthos and macroalgae. The presence of angiosperms plays an important role also in the lagoon environment, as our (and many others) study shows, so the hypothesis of implementing the number of biotic indices including angiosperms also in the lagoon environment should be taken into consideration.

Finally, since the duration of our monitoring period refers to a single growing season of the *Ruppia* meadow, it may be necessary to acknowledge the limitations of our study considering the sources of uncertainty that could theoretically have somehow influenced our results. In our opinion, the main confounding factor can be given by the sudden changes in some climatic aspects, such as the rainfall regime and extreme temperatures—in fact, the summer 2003 heatwave heavily impacted a *Ruppia* meadow in the Comacchio Saltwork, not far from our study site [34]. The suggestion of possible avenues for future research in order to draw more definitive conclusions is to undertake long-term autoecological and synecological studies (more prosaically being able to have research funds for an extended period on a topic that, at least in our country, does not seem to enjoy priority), which can take into consideration the responses of the meadow and its associated organisms to those extreme events which are increasingly common in this Mediterranean area [35].

## 5. Conclusions

In many lagoons around the world, we have witnessed changes in the composition and structure of vegetation due to the loss of seagrass meadows, and this is one of the main consequences of environmental degradation. Seagrasses were rapidly replaced by nuisance macroalgae (Ulvaceae, Gracilariaceae, and Cladophoraceae) and, under extreme conditions, by algal blooms [36,37]. However, a trend reversal is possible [6], as reported for other European seagrass meadows and in the Venice Lagoon, where seagrass has been transplanted and meadows have expanded into lagoon areas where they have not been present for a long time [7]. The role of *Ruppia* meadows in transitional coastal ecosystems has so far been little considered, but the results of this study indicate that *Ruppia* plays an important role in enhancing macrobenthic biodiversity, increasing their abundance and functional diversity. The *Ruppia* meadow of Valle Campo operates as an almost intact complex food web containing key functional groups such as herbivores and predators. The role of this minor seagrass has proved to be of great importance, improving the nutritional quality of the organic matter in the sediments, especially for the carbohydrate component, and, above all, providing new habitats and new niches for a number of benthic macrofauna

species. The ecological quality status was not particularly high despite the presence of the meadow, but this paradox can be ascribed to the normal stress conditions to which choked lagoons, such as Valle Campo, are physiologically subjected.

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