Seagrass epiphytic assemblages are strong indicators of agricultural discharge but weak indicators of host features

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ABSTRACT

Wastewater pulses from rice agriculture are persistently discharged into the northern shore of the Alfacs Bay (Ebro Delta, NW Mediterranean) from April to November. The bay also receives water from coastal lagoons which are subjected to freshwater inputs from the Ebro River mixed to an unknown extent with agricultural wastewater during the same period. This paper compares epiphyte assemblages growing on leaves of *Cymodocea nodosa* in sites exposed to agricultural drainage channels, lagoon connection channels, and control sites in the Ebro Delta Natural Park (southern shore of the bay). Leaf epiphytic assemblages of *Zostera noltii* patches in the northern shore of the bay were also compared with those of adjacent beds of *C. nodosa*. Drainage channel sites had consistently distinctive assemblages (higher species richness, biomass load, and taxa composition) than control sites. Assemblages from lagoon channel sites were more variable, with three sites showing particularly high covers of epiphytic algae and two sites more similar to controls. Epiphyte patterns clearly matched in situ measures of nutrient availability, and were consistent with decreased shoot densities in discharge sites. In contrast, differences in epiphyte assemblages between seagrass species were minor, and mostly a result of higher epiphytic loads on *C. nodosa* than on *Z. noltii*, which features thinner leaves. Further research is needed to investigate the consequences of these plant and epiphyte alterations in important ecosystem processes such as decomposition and export rates, as well as overall effects of nutrients and salinity in secondary producers such as associated macroinvertebrate assemblages supporting locally important marine fisheries.

Keywords: eutrophication; plant architecture; *Cymodocea nodosa*; *Zostera nolti*; Mediterranean; coastal lagoons

1. Introduction

Seagrasses usually colonize soft bottom areas, and their leaves provide the main hard substrate available for the growth of epiphytic organisms, which are continuously renewing and constitute a central component of the ecosystem (Borowitzka et al., 2006). Epiphyte communities may account for over the 50% of the standing stock of seagrass meadows and reach contributions of up to ca. 40-60% of the primary production of these ecosystems (Wear et al., 1999; Borowitzka et al., 2006). They have short generation times, and are able to remove large quantities of nutrients from the water column (Cornelisen and Thomas 2002), often leading to seagrass shading (Dalla Via et al., 1998). Certain groups such as incrusting coralline algae also have an important role in the formation of calcareous sediments (Frankovich and Zieman, 1994), whereas cyanobacterial epiphytes can supply a significant proportion of the nitrogen required for primary production (review by Welsh 2000). In addition, they represent a significant part of the biodiversity within seagrass meadows, and make a significant contribution to food webs and habitat supply for animal communities (Mazzella et al., 1992; Jernakoff et al., 1996).

The relative importance of their ecological roles depends on the pattern of epiphyte accrual along the leaf-life span, because of the dynamic succession of species and the increase in diversity and biomass from young to old leaves (Casola et al., 1987; Reyes et al., 1998). Additionally, variations in the number of epiphytic species within seagrass leaves are mostly constrained by the local availability of propagules for algal settlement (Borum et al., 1984). For instance, Reyes and Sansón (1997) studied the epiphytic flora of the leaves of *Cymodocea nodosa* across the annual cycle and found three distinctive groups of species based on their patterns of permanent, seasonal, or occasional presence. In *Posidonia oceanica*, Ballesteros (1987) reported assemblages dominated by incrusting coralline algae and bryozoans during the fall and winter, the typical seasonal community of brown and red algae in spring, and fast-growing opportunistic species during the summer. Hence, coexisting seagrass species exposed to the same sources of propagules should present a similar pool of species, but might differ in total richness and abundances due to variable leaf life-span accrual and possible effects of plant architecture. Although these hypotheses have been seldom tested, Lavery and Vanderklift (2002) evaluated the influence of influence of co-occurring host seagrass type on the patterns of epiphytic assemblages and confirmed that persistent stems of *Amphibolis griffithii* allowed this seagrass to support higher species richness than *Posidonia coriacea*.

Among environmental stressors influencing aquatic communities, eutrophication is the most common factor associated with the global decline of seagrass communities during the last decades (see reviews by Romero et al., 2006; Burkholder et al., 2007). Human activities such as terrestrial runoff due to overuse of land or deforestation, domestic sewage, or fish farms have greatly increased nutrient inputs to coastal waters (Peierls et al., 1991; Turner and Rabalais, 1991). The negative effects of eutrophication include nuisance algal blooms, including phytoplankton, free-floating macroalgae, and algal epiphytes, which reduce light availability to seagrass leaves and depress primary production (e.g., Cambridge et al., 1986; Sfriso et al., 2003). Such changes in epiphyte biomass are thought to be directly related to the importance of anthropogenic eutrophication, but often reflect the development of few species (Balata et al., 2008; Prado et al., 2008). In contrast, other community descriptors such as diversity and richness of small species might be initially increased by nutrient availability, because the effect of natural limitation is offset and the epiphytic faunal composition may be also favored (Prado et al., 2007; 2008). Yet, according to experimental experience, the strong seasonal dynamics of epiphyte communities cannot be easily disrupted by nutrient availability and negative effects are mostly restricted to spring and summer, when fast growing filamentous and fleshy macroalgae typically develop (Prado et al., 2008).

In the Ebro Delta (Southern Catalonia, NW Mediterranean) ca. 70 % of the total land area is devoted to rice cultivation and agricultural wastewater pulses (ca. 275 10^6 m^3 yr^{-1}) are discharged directly into the northern shore of the Alfacs Bay between April and October (Garcés et al., 1999). In addition, the Alfacs Bay is connected to coastal lagoons through natural and human made channels that receive seasonal freshwater inputs from the Ebro River (with lower organic matter and nutrients) mixed to an unknown extent with wastewater inputs from rice fields during the same period. The semi-enclosed estuarine water body of the Alfacs Bay plays, however, a very important role in the economy of the region due to its fish and shellfish aquaculture, particularly oysters and mussels (Solé et al., 2009). The shallow submerged bottom (<2 m) is dominated by dense patches of the seagrass *C. nodosa* (Marbà and Duarte, 1995) with some patches of the seagrass *Zostera noltii* also occurring along the northern shore (Procaccini et al., 2003) adjacent to points of agricultural discharge. In contrast, the southern shore (the Banya Sandspit) was included in the Ebro Delta Natural Park in 1986 and is also part of the Natura 2000 network of the European Union because of its importance for both *C. nodosa* and the presence of a very large population of *Pinna nobilis* (Ibáñez 1997; Prado et al., 2014).

Since both *C. nodosa* and *Z. noltii* grow together in some northern sites of the Alfacs Bay and seagrass epiphytes have been shown to be an effective indicator of the overall ecosystem integrity (e.g., Martínez-Crego et al., 2010), this scenario provided the opportunity to test for the effects of host type in epiphytic assemblages and compare these effects with those of enrichment. I tested the hypothesis that changes in community structure (species richness, diversity, biomass, and the species composition) could reflect seagrass host features and be a good indicator of environmental impact by seasonal pulses of agricultural wastewater, both directly through discharge canals that are intermittently opened according to agricultural needs, and indirectly through connection canals with coastal lagoons that are permanently opened. Besides, patterns of epiphytic assemblages were compared with measures of seagrass meadow density, as an integrative variable of seagrass vitality (Romero et al., 2007).

2. Materials and Methods

2.1. Study site and sampling design

The study was conducted in the Alfacs Bay (Ebro Delta, Catalonia, NW Mediterranean), a semi-confined estuarine area of ca. 49 km^2 with an average depth of 3.13 m. It is bordered by rice fields to the north and connected to the Banya Sandspit through a long sand bar known as the Trabucador that stretches along the eastern side of the bay (Fig. 1). During rice growth and some post-harvest period (April to November), the northern shore of the Alfacs Bay receives pulses of agricultural wastewater through multiple drainage channels that are not permanently opened but bring in significant amounts of nutrients and organic matter (Delgado, 1987). In addition, the northern shore is permanently connected at various points with the Encanyissada and Tancada lagoons (see Fig. 1) allowing the entrance of brackish water. Although these lagoons should be naturally hypersaline in summer, their current seasonal salinity ranges between 12.5 and 27.1 in the Encanyissada and between 18.6 and 28.1 in the Tancada, with the lowest values also being attained during the rice growth period, due to freshwater additions from the River and rice fields by the Ebro Delta Natural Park (see Prado et al., 2013 for further details). As a result, the overall salinity of the north shore may decrease to ranges between 30 and 35 (Garcés et al., 1999). *Cymodocea nodosa* is the dominant seagrass in the area, but in summer is often overgrown by fast growing macroalgae (*Chaetomorpha linum* and *Ulva* spp. P. Prado, personal observation), and some areas with *Zostera nolti* do also occur. The salinity along the southern shore of the bay is more similar to the open sea (Table 1) and dominant macrophytes are *C. nodosa* with sparse patches of the benthic macroalgae, *Caulerpa prolifera*.

Study sites along the northern shore were selected in front of a drainage channel (N= 5), or in front of a connection channel with a lagoon (N= 5), within the *C. nodosa* seagrass bed, at a distance of about 50 m from the discharge point. Along the southern shore, control sites with no water input (N= 5) were randomly selected at similar depths within the *C. nodosa* seagrass bed for comparative purposes. Where the seagrass *Z. nolti* was available (growing in patches adjacent to those of *C. nodosa* (N= 5)), samples were collected for comparison between epiphyte communities growing on both species. This occurred in one drainage channel site (D4) and in four lagoon sites (L1, L2, L4, and L5; see Table. 1). At each site, 20 shoots of *C. nodosa* (and *Z. nolti* where available) were collected in late May 2015 (period when seagrass feature its characteristic leaf epiphytic community; Ballesteros 1987) at 0.5-1 m depth and preserved in a 4% formaldehyde-seawater solution. Later in the lab, three shoots featuring an oldest leaf with a similar length were selected among available shoots in order to ensure standardization of the epiphytic community in relation to leaf age and size (Reyes et al. 1998; Prado et al. 2008). Prior to the detachment of epiphytes from the surface of the oldest leaf, the abundance of encrusting and more abundant taxa (e.g. *Hydrolithon* spp. and bryozoans) were determined qualitatively through visual estimation under the microscope. Then, epiphytes were carefully removed with a razor blade, and the determination of covers conducted as in Prado et al. (2008) in which rare and small taxa found only once per leaf were assigned 0.1%, and the remaining taxa were given values from 0.2% by comparison with previous abundances (i.e., taxa present only once). Macroalgae and most groups of sessile epifauna (ascidians, bryozoans, hydrozoans, and actinians) were identified to species level, except for polychaeta which were determined at the family level and foraminifera at the phylum level.

Species richness was estimated as the total number of taxa recorded growing on the oldest leaf of each shoot, and the alpha diversity was calculated with the Shannon index:

$$\mathrm{H'} = -\sum_{\mathrm{i}=1}^{\mathrm{s}} \mathrm{p_i} \log \mathrm{p_i}$$

where s is the total number of species and pi is the proportion of species *i* observed in the sample.

The three selected shoots per sample were also used to determine the number of leaves per shoot, leaf length and width, and the presence of bite marks (fish or sea urchin) or broken apices. After identification, epiphytes on the oldest leaf of each shoot were dried at 60°C for 48 h, and weighed for biomass estimation (mg DW).

2.2. Environmental and seagrass variables

To evaluate differences in water quality characteristics among study sites water samples and abiotic measures were taken directly in front of each drainage channel and lagoon connection, next to the area of collection of seagrass samples. At each study site, one sample of dissolved nutrients (PO_4, NH_4, NO_2, and NO_x (NO_3 and NO_2)) was collected with 100 ml water bottles and kept frozen at -20 °C until determination following the Koroleff method (Koroleff, 1977). Water pH and salinity were measured within a two-hour range with an YSI 6660 multiparametric probe (equipped with a 650 MDS data logger). Alkalinity (TA) was also quantified once per site with a Hanna Checker alkalinity meter HI 755 (±5 ppm accuracy at 25 °C). Finally, seagrass shoot density was measured in each site and for each seagrass species as an indicator of plant vitality and ecological status (Romero et al., 2007) using a 20 x 20 quadrat (N= 3) and results expressed as numbers per m^{-2}.

2.3. Data analysis

Patterns of nutrient concentrations, pH, salinity, and alkalinity among water discharge treatments (fixed factor: drainage channels, lagoon channels, and controls without channels) were investigated with a one-way ANOVA.

Differences in shoot density of *C. nodosa*, and epiphyte species richness, alpha diversity, and biomass across water discharge treatments (fixed factor, 3 levels), and sites (fixed factor) were investigated with a 2-way nested ANOVA. Differences in species richness, alpha diversity, and epiphyte biomass between seagrass species (fixed factor, 2 levels: *C. nodosa* and *Z. nolti*) and among sites (fixed factor) were investigated with a 2-way factorial ANOVA.

ANOVA assumptions of homogeneity of variances and normality were tested by Cochran's test and Kolmogorov-Smirnov distribution-fitting test of the residuals, respectively. NOx, and NO_2 data were double square root transformed and epiphytic biomass Asen x transformed for statistical analysis, because homoscedasticity and normality were clearly improved and balanced ANOVA is robust against violations of these assumptions (Sokal and Rohlf, 1995). For all ANOVA analyses, the critical level of significance was fixed at $p < 0.05$. Student-Newman-Keuls (SNK) post hoc comparisons were used to identify significant differences between treatments at each month of sampling.

Patterns of spatial variation in epiphytic assemblages were investigated using the PRIMER software package from Plymouth Marine Laboratory (UK). Leaf cover values for each species were log (x +1) transformed prior to analysis and then used to build a Bray-

Curtis (BC) dissimilarity matrix. Non-metric multidimensional scaling (n-MDS) was applied to examine the influence of discharge type and site, and that of seagrass species and site in the structure of epiphyte assemblages. Analysis of similarities (ANOSIM) was also conducted to test whether differences in species composition and abundance observed in n-MDS results were significant. A 2-way nested design was used for discharge type comparisons and a 2-way factorial design for seagrass species comparisons. In addition, SIMPER analyses were used to investigate which taxa had the highest contributions to both similarities and dissimilarities within and among factor groups, respectively.

3. Results

3.1. Environmental variables

Nitrate and nitrite (NOx) concentrations in the water column were significantly higher in drainage channel sites (D: 933.5 ± 542.3 $\mu g \cdot L^{-1}$) than in lagoon and control sites which were not significantly different (L: 490.8 ± 247.6; and C: 48.4 ± 9.4 $\mu g \cdot L^{-1}$; $F_{2,12}$= 4.67, p= 0.031). Yet, lagoon sites L3 to L5 also showed very high nutrient levels within the same range as drainage sites (see Table 1). NO_2 alone also showed significant differences across all levels of discharge treatments ($F_{2,12}$= 6.84, p= 0.010), with higher values in lagoon channels (L: 28.1 ± 17 $\mu g \cdot L^{-1}$), intermediate in drainage channels (D: 15.2 ± 4.1 $\mu g \cdot L^{-1}$) and lowest in controls (C: 1.55 ± 0.5 $\mu g \cdot L^{-1}$). In contrast, the levels of NH_4^+ and PO_4^{-3} tended to attain higher values at drainage and lagoons channels (Table 1) but showed non-significant effects (NH_4^+: $F_{2,12}$= 1.87, p= 0.195; PO_4^{-3}: $F_{2,12}$= 2.74, p= 0.105). Control sites showed higher salinities (32.8 ± 1.9) than drainage and lagoon channel sites (20.52 ± 1.9, and 23.7 ± 7, respectively), although differences were non-significant ($F_{2,12}$= 2.13, p= 0.161) as were those for pH ($F_{2,12}$= 0.78, p= 0.476). In contrast, alkalinity was found to be significantly higher in control and drainage sites (C: 104.4 ± 6.3; D: 85 ± 10.7) than in lagoon sites (L: 74 ± 9.2; $F_{2, 12}$: 4.067, p= 0.044; Table 1).

Shoot density of *C. nodosa* was significantly higher in control sites, than in lagoon and drainage channels (1483.3 ± 140, 1155 ± 53, and 833 ± 33 shoots · m^{-2}, respectively; $F_{2, 38}$= 8.54, p= 0.0008) with no differences among sites nested with drainage type. Compared to *C. nodosa*, shoot density of *Z. noltii* was ca. 3.8 times higher (3578 ± 178 shoots · m^{-2}; $F_{1, 24}$= 188.93, p= 0.0000).

3.2. Seagrass leaf features and epiphytic taxa

The oldest leaf used for assessment of the epiphytic community (specifically selected for similar size) featured an average length of 15.3 ± 0.2 cm by 0.31 ± 0.006 cm width in *C. nodosa*, and an average length of 22 ± 0.5 cm by 0.15 ± 0.0006 cm width in *Z. nolti*. This resulted on a higher leaf area indexes (LAI) in *C. nodosa* (4.8 ± 0.1 cm^2) and compared to *Z. nolti* (3.3 ± 0.1 cm^2).

A total of 45 taxa were found across all types of discharge sites and seagrass species (see percent cover values in supplementary Annex 1). The following taxa were identified: Bacillariophyceae (1 taxa), Rodophyta (11 taxa), Chlorophyta (8 taxa), Hydrozoans (6 taxa), Actiinidae (1 taxa), Bryozoans (2 taxa), Polychaeta (3 taxa), Ascidians (1 taxa), Foraminifera, and invertebrate eggs. Dominant taxa, which varied among sites, were: the Corallinaceae *Hydrolithon farinosum* (0 to 40%) and *Titanoderma pustulatum* (0 to 25%), the Rodophyta *Chondria marei* (0 to 7%), *Ceramium diaphanum* (0 to 60%), and *Polysiphonia* sp. (4 periaxial cells; 0 to 15%), the Chlorophyta *Chaetomorpha mediterranea* (0 to 5%), the Hydrozoans *Agaophenia harpago* (0 to 12%) and *Kirchenpaueria pinnata* (0 to 12%), the anemone *Paranemonia cinerea* (0 to 8%), the three Polychaeta families (Spirorbidae, Nereididae, and Ampharetidae; 0 to 20%), the Bryozoan *Mymosella gracillis*

(0 to 10%), and the Ascidian *Botryllus schlosseri* (0 to 15%). The rest, constituted a long tail of small and rare species which were of lesser importance to differences among discharge conditions and between species (Annex 1).

3.3. Univariate analyses

3.3.1. Discharge conditions

Results from ANOVA showed that both discharge type and site had significant effects on the number of epiphytic species growing on the oldest leaf of *C. nodosa* (Table 2a). Drainage channels sites showed more than double the species richness than control sites (8.5 ± 0.5, and 4 ± 0.3 species leaf^{-1}, respectively), and were also significantly higher than lagoon channel sites (4.8 ± 0.2 species leaf^{-1}) (Fig. 2a).

Patterns for the Shannon index were similar to those of species richness, with significant effects in both investigated factors (Table 2a). Drainage channels showed higher alpha diversity (1.7 ± 0.1) than control and lagoon channel sites which featured similar values (1.2 ± 0.1) (Fig. 2b).

There were significant differences in the values of epiphyte biomass across discharge types and sites (Table 2a). The highest biomasses were attained at some lagoon channels sites followed by drainage channels sites and control sites (54.4 ± 13, 21.9 ± 3.2, and 3.16 ± 0.7 mg DW leaf^{-1}, respectively; Fig. 2c).

3.3.2. Species comparisons

There were significant effects of site and a Species x Site interaction, but not species (5.6 ± 0.5, and 5.3 ± 0.4 species leaf^{-1}, respectively for *C. nodosa* and *Z. noltii*). Significant differences were exclusively due to the D4 site, which showed higher number of epiphytic species in *C. nodosa* than in *Z. noltii* (Table 2b, Fig. 2d).

For the Shannon index, both factors (species and site) and their interaction displayed significant effects (Table 2a). Values were slightly higher in *Z. noltii* than in *C. nodosa* (1.5 ± 0.1, and 1.3 ± 0.1, respectively), which more mostly due to higher values in the L2 and L4 sites (Fig. 2e).

Epiphyte biomass showed significant differences in between species, sites, and a marginally significant interaction (Table 2b). *C. nodosa* showed overall higher values than *Z. noltii* (48.4 ± 13.5, and 22.3 ± 6 mg DW leaf^{-1}, respectively), and highest values were recorded in L4 and L5 sites (Fig. 2f).

3.4. Multivariate analyses

For the discharge type design, nMDS analyses evidenced distinctive groupings among discharge treatments. Samples of drainage channel sites were clustered together on the middle of the plot, a second cluster was formed by lagoon sites 3 to 5, and the remaining lagoon sites (L1 and L2) were mixed with control sites without freshwater inputs (Fig. 3a). Further ANOSIM results confirmed the significance of observed differences among discharge types and, to a lesser extent, by variability among sites (2-way nested ANOSIM, R= 0.889, p= 0.001; and R= 0.519, p= 0.001 for discharge and site effects, respectively).

SIMPER analyses also indicated important differences among discharge treatments, ranging from ca. 79 to 83% (see Table 3a). Drainage channel sites showed higher cover of coralline algae (30% of dissimilarities), and polychaetes of the families Nereididae (17.3%) and Spirorbidae (5.1%), but lower amounts of invertebrate egg masses (6.7%) and of the ascidian *Botryllus schlosseri* (5.2%) than control sites. Lagoon channel sites L3 to L5 showed lower cover of coralline algae (13.7% of dissimilarities) than control sites (and also lower amounts of invertebrate egg masses and the bryozoans *Aglaophenia harpago*; 9.5 and 5.1% of dissimilarities, respectively), and higher amounts of filamentous algae (the

Rhodophyta *Ceramium diaphanum* (15.4%), *Chondria marei* (11.9%), *Polisiphonia* sp. (6.8%), and the Chlorophyta *Cladophora dalmatica* (9.3%)), and of the anemone *Paranemonia cinerea* (5.6%). Yet, two of the lagoon sites (L1 and L2) were more similar to control sites and showed higher covers of coralline algae and the absence of *C. diaphanum*.

For species comparisons, nMDS analyses showed similar assemblages in both seagrasses, with no clear grouping of samples (Fig. 3b). ANOSIM results showed significant R ratios for both factors, although comparatively lower for Species (2-way crossed ANOSIM, R= 0.419, p= 0.001 and R= 0.985, p= 0.001, respectively for Species and Site). SIMPER results also evidenced low percentage of variability between species compared to dissimilarities among sites (Table 3b). *C. nodosa* showed higher covers of coralline algae (*Hydrolithon farinosum*) (18.5% of dissimilarities), filamentous algae (the Rhodophyta *Chondria marei* (10.6%) and *Polisiphonia* sp. (5%), and the Chlorophyta *Cladophora dalmatica* (13.2%)), and invertebrate eggs masses (10.1%). In contrast, *Z. noltii* showed higher abundance of the polychaete family Nereididae (10.4%), and of the filamentous red algae *Anotrichium furcellatum* (7.6%) and *C. diaphanum* (6.8%).

4. Discussion

Epiphyte assemblages of *Cymodocea nodosa* mirrored local conditions of water discharge, whereas differences with assemblages growing on adjacent shoots of *Zostera noltii* were very weak. Sites subjected to pulses of agricultural wastewater discharge consistently displayed distinctive epiphyte assemblages (higher species richness, biomass load, and taxa composition) compared to control sites (see also Balata et al., 2008; Prado et al., 2008) and showed the highest availability of nutrients, particularly nitrate, and nitrite. In contrast, assemblages close to lagoon connection channels were more variable, with two sites featuring especially high covers of fast growing epiphytic algae and two more comparable to control sites, concurring with lower nutrient conditions. Differences between coexisting seagrass species were restricted to higher epiphytic loads and total number of taxa growing on *C. nodosa* vs. *Z. noltii*, as expected by higher leaf area indexes (Vermaat et al., 1993). Overall, the results show that both direct (drainage channels) and indirect (connection channels with coastal lagoons) wastewater discharges from rice field agriculture into the Alfacs Bay are driving alterations in the abundance and composition of epiphytic assemblages. Given the central role of epiphytic assemblages in the functioning of seagrass beds (Borowitzka et al., 2006), the combined effects of nutrients and epiphytic loads may trigger a sequence of flow-on effects that range from altered plant growth and herbivory pressure (Prado et al., 2010) to changes in the mobile epifauna and secondary production of the system (Bologna and Heck, 1999). Evidence of such undergoing changes is also provided by considerably lower shoot densities in drainage and lagoon sites than in control sites (by ca. 22.1 to 40.5%), suggesting long-term eutrophication of the northern shore of the Alfacs Bay.

Seagrass epiphyte assemblages are regarded as sensitive to eutrophication and positive relationships between epiphyte biomass and nutrient concentrations are commonly reported (Borum et al., 1984; Wear et al., 1999). During the study, the highest covers of epiphytic algae (by ca. 30 times) were evidenced in lagoon sites L4 and L5 connecting the Western basin of the Tancada lagoon with the Alfacs Bay (mostly the Rodophytes *Ceramium diaphanum*, and *Chondria marei*, and the Chlorophyta *Cladophora dalmatica*). Although strong variability in nutrient conditions resulted on non-significant effects for lagoon channels, those two sites had ca. 22 times higher availability of NOx than controls. This region of the Tancada lagoon has been indicated to remain mostly unvegetated, and to feature high turbidity due to destabilized sediments and historical nutrient loading

(Prado et al., 2013), which are steadily discharged into the Alfacs Bay. In addition, given the particular location of the sites, opposite from the mouth of the bay, more reduced water exchange rates might have additionally favored the development of epiphytic algae. Drainage water sites, also displayed 7 times higher epiphytic loads than control sites, suggestive of discharge activity from rice fields during the study period (Comín et al., 1991). Mean nitrate and nitrite concentrations in drainage sites were ca. 10 to 20 times higher than control sites, although with strong peak at the D3 site (also for PO_4^{-3}), evidencing a pulse of agricultural discharge at the moment of sampling. Patterns of nutrient availability may have also triggered growth responses of encrusting corallines (see Burkepile and Hay, 2009; Smith et al., 2001) and partly account for observed differences in leaf cover across discharge treatments (>20% in drainage channels, 0 to > 20% in lagoon channels, and < 3% in control sites). Alternatively, the abundance of coralline algae could be also connected to local inputs of freshwater from the Ebro River with higher concentration of calcium carbonate than marine waters (Bouza-Deaño et al., 2008), although only lagoon sites displayed significantly higher alkalinities than control sites (by ca. 30%). Unexpectedly, none of the study treatments displayed species of Phaeophyta, including those typically found growing on seagrass leaves during the spring period (e.g., *Castagnea* spp., *Giraudia sphacelarioides,* or *Myrionema magnusii*, among others; Ballesteros 1987), the reasons for this result being unclear.

The number of epiphyte species (Annex 1) per leaf was two-fold higher in drainage water sites, whereas values in lagoon sites were only slightly (but significantly) higher than controls. These results suggest that under enhanced spring light conditions, nutrient availability first acts triggering the development of a multiplicity of macroalgae that have positive effects on the overall diversity. Later in the summer, the community usually becomes dominated by few species of fast-growing algae, and diversity decreases despite higher number of small and occasional species can be observed (see Prado et al., (2008) for similar results). In addition, higher cover of epiphytic fauna in drainage channel sites may have contributed to diversity patterns. Higher ratios of epifauna vs. epiphytic algae have been related to degraded seagrass meadow conditions (Prado et al., 2007; Martínez-Crego et al., 2010) since enhanced turbidity and reduced light availability can interfere with the photosynthetic activity of primary producers. For instance, polychaetes (families Nereididae and Spirorbidae) were more abundant in drainage channels sites and could be indicative of enhanced inputs of organic matter and lower size of sediment particles (Giangrande et al., 2005; Dauvin and Ruellet, 2007).

Recurrent runoff conditions in the northern shore of the bay also resulted in decreased shoot densities of *C. nodosa* in drainage and lagoon channel sites (1155 and 883 shoots · m^{-2}, respectively) compared to control sites (1483 shoots · m^{-2}), an effect typically attributed to enduring eutrophication in seagrass beds (Romero et al., 2007). Pérez et al. (1994) found that high nutrients concentrations may result in enhanced shoot mortality due to limitation in light availability and/ or oxygen supply to belowground tissues. In addition, leaf shading by large loads of fleshy epiphytes during the summer period (P. Prado, personal observation) causes light limitation and may result in lower plant growth and declined meadow density (Silberstein et al., 1986). Enhanced tolerance to low salinities in *Z. noltii* (see Fernández-Torquemada and Sánchez-Lizaso, 2006) may also account for its distribution along the northern shore of the bay, particularly in lagoon channels sites subjected to longer historical discharge. Low salinity may also be responsible for alterations in the local abundance of macrograzers such as the sea urchin *Paracentrotus lividus*, which is totally absent from sites subjected to agricultural discharge (P Prado, personal observation) thus favoring the accumulation of epiphyte loads.

The influence of seagrass host type in epiphyte assemblages was lower than that caused by water discharge conditions. Both seagrass species featured similar mean numbers of epiphyte species (5.6 and 5.3 species per leaf, respectively for *C. nodosa* and *Z. noltii*, respectively), and alpha diversity (1.3 and 1.5, also respectively for *C. nodosa* and *Z. noltii*), and evidenced stronger compositional differences among sites than between species. According to Pérez and Romero (1994), the longevity of *C. nodosa* leaves during the month of May is around 90 days, and that reported for the *Zostera* genus also has a similar mean (see Hemminga et al., 1999). In addition, leaf and plant architecture is comparable in the two species, which altogether may account for similar patterns of epiphytic accrual compared to other species with more contrasting structure and patterns of leaf development (e.g., *Amphibolis griffithii* vs. *Posidonia coriacea*; Lavery and Vanderklift, 2002). Although results of this study were limited to a single month, epiphyte assemblages usually feature higher number of taxa in early spring than in summer, when few species of fast-growing algae tend to dominate the communities (e.g., Reyes and Sanson, 1997; Prado et al., 2008). Yet, the total number of species found on leaves of *C. nodosa* was higher than that found on *Z. noltii* (25 vs. 15, respectively) mostly due to higher numbers of occasional macroalgae. Also, epiphytic biomass on *C. nodosa* attained ca. double values than those found growing on *Z. noltii*, accordingly to 1.5 higher values of leaf area index as also found in other studies (Vermaat et al., 1993). Differences in epiphyte biomass between species may increase in summer when few fast growing species become dominant, particularly in sites subjected to recurrent nutrient discharge (Prado et al., 2008).

5. Conclusions

Agricultural run-off, rather than host plant features was found to be an effective driver of epiphyte community structure. Seasonal agricultural discharges through drainage channels and persistent arrival of nutrients from areas featuring historical eutrophication (Western basin of the Tancada lagoon) could be evaluated through measures of epiphyte species richness, biomass loads, and community structure as well as seagrass shoot density and species composition (see also Prado et al., 2008). Some lagoon channels investigated, although permanently open, are connected with restored areas of the Encanyissada lagoon or subjected to lower historical discharge (Forés et al., 2002; Prado et al., 2013) and present communities that are more similar to control sites. For species comparisons, differences in epiphyte community structure were mostly restricted to enhanced biomass loads in *C. nodosa* than *Z. noltii*, possibly associated with higher leaf area index. Given that *Z. noltii* tolerates hyposalinity conditions much better than *C. nodosa* (Fernández-Torquemada and Sánchez-Lizaso, 2006), and that enhanced nutrient availability appear to cause a decline in the shoot density of *C. nodosa*, agricultural discharges are not only altering epiphytic communities (both directly by promoting algal growth and indirectly by possibly affecting macrograzer assemblages), but also the seagrass species composition and meadow density of Alfacs bay seagrass habitats. These changes in plant-epiphyte composition and productivity, coupled with alterations in salinity may further alter central ecosystem processes such as decomposition, and export rates (Mateo et al., 2006), impacting associated macroinvertebrate assemblages (Bologna and Heck, 1999) that support locally important marine fisheries.

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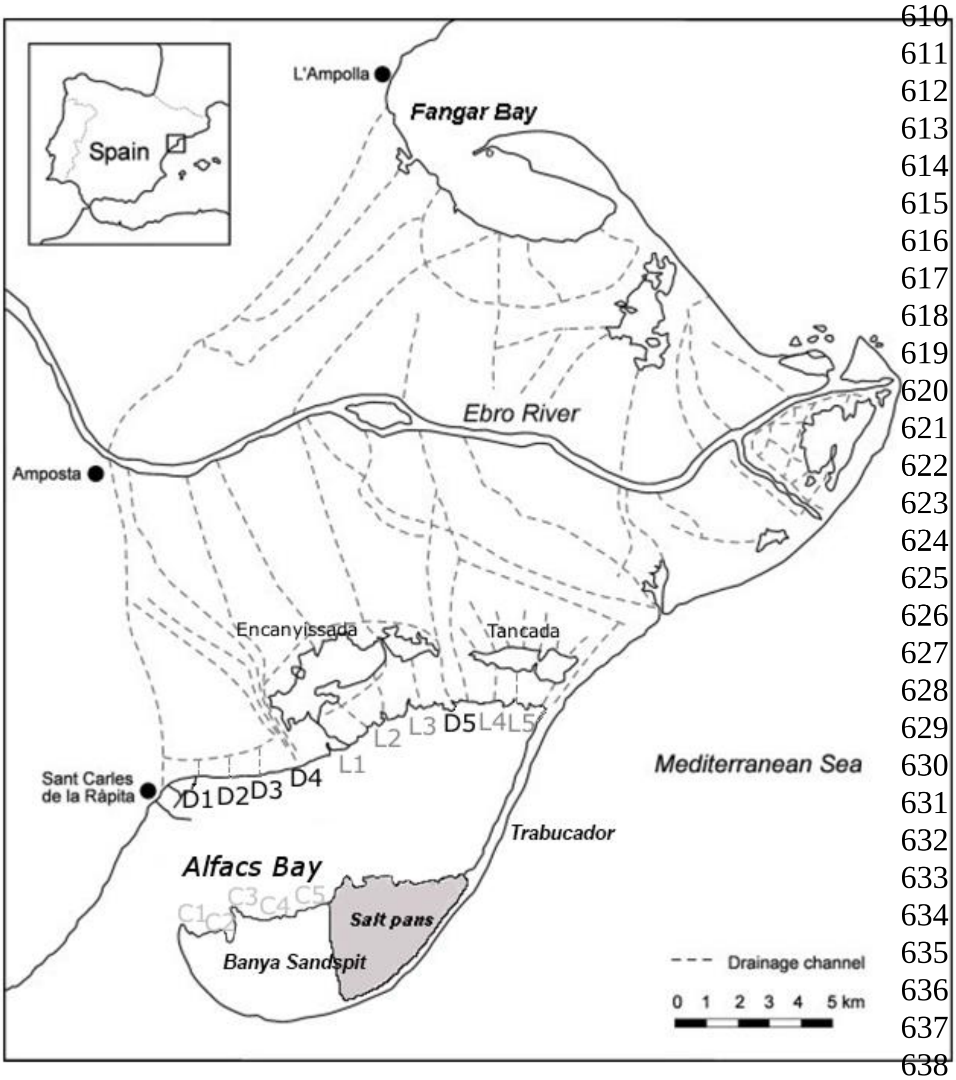

Fig. 1. Map of the Ebro Delta showing the network of channels draining water to the Alfacs Bay. The situation of study sites is showed with stars. Control sites (C) along the Banya Sandspit are indicated in light grey, lagoon sites (L) in dark grey, and drainage (D) channels in black.

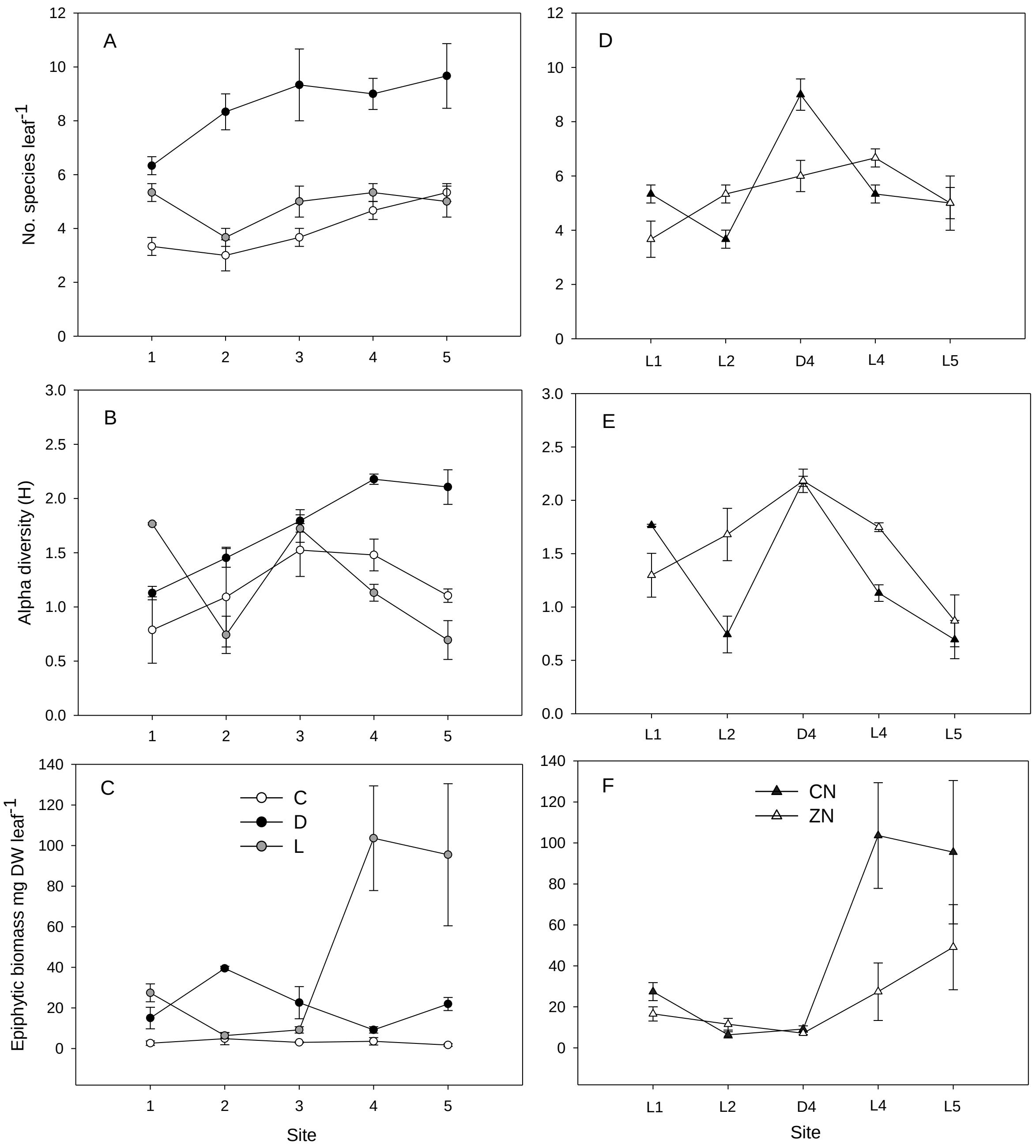

Fig. 2. Univariate meassures (species richness, alpha diversity, and epiphytic biomass) recorded in the oldest leaf in: (1) sites featuring water discharge from canals and lagoons and in control sites with no impact (A, B, and C); and (2) sites including both *C. nodosa* and *Z. noltii* (D, E, and F). C: Control, D: Drainage, L: Lagoon channel sites.

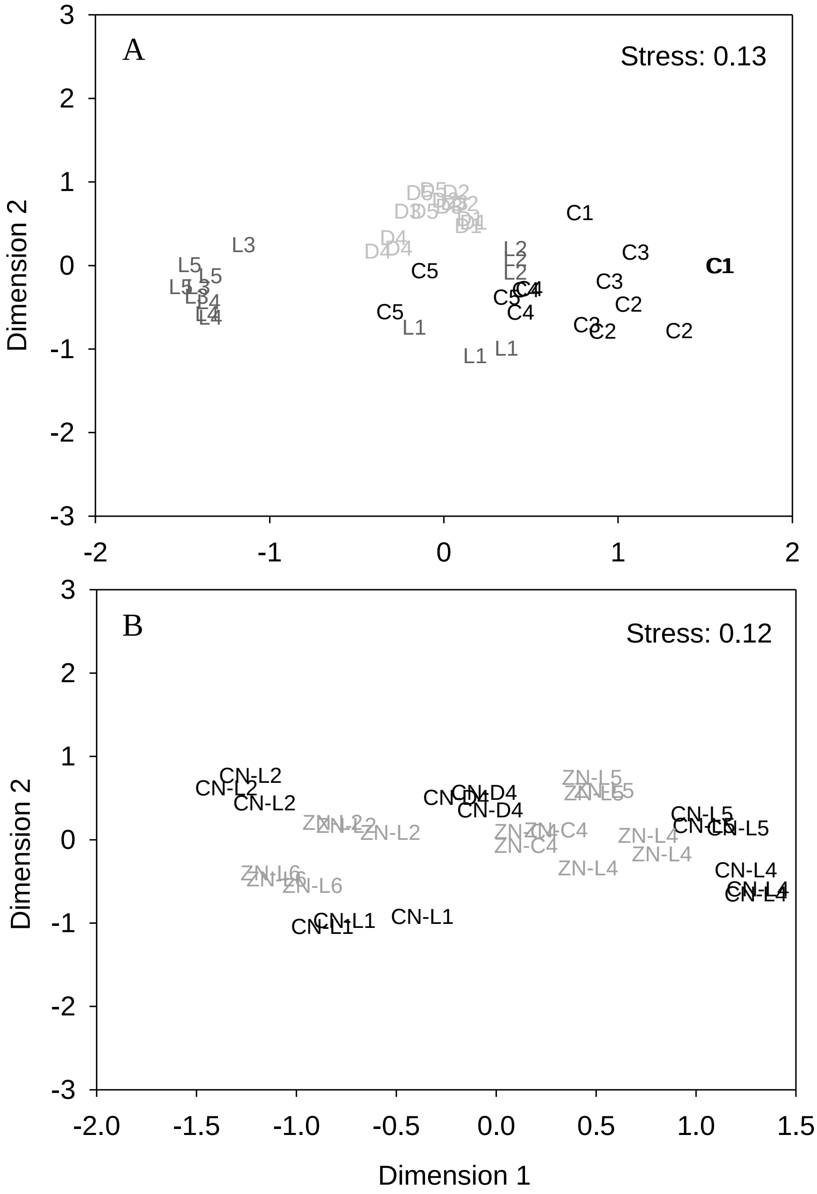

Fig. 3. nMDS showing patterns of epiphytic assemblages: A) Among sites featuring different types of water discharge (D= drainage channel, L= lagoon, C= control sites with no water input); and B) Among sites with *Cymodocea nodosa* (CN) and *Zostera noltii* (ZN). Data were Log (x + 1) transformed.

Site	Shoot density CN No. m^{-2}	Shoot density ZN No. m^{-2}	pH	Alkalinity (ppm)	Salinity	NH_4^+ (µg L^{-1})	NO_3^- + NO_2^- (µg L^{-1})	NO_2^- (µg L^{-1})	PO_4^{-3} (µg L^{-1})
D1	1458 ± 139		7.44	107	33.79	468.54	220.39	9.59	23.65
D2	1633 ± 68		7.5	107	36.56	619.21	320.43	7.86	25.86
D3	792 ± 88		7.45	70	6.71	359.35	3089.45	29.84	110.94
D4	933 ± 8.3	3692 ± 365	8	52	6.26	493.07	463.93	18.86	33.56
D5	958 ± 30		7.33	89	35.14	718.54	573.12	10.08	154.12
L1	933 ± 60	3908 ± 566	8.01	59	17.01	375.27	42.74	5.65	<20.65
L2	817 ± 36	3408 ± 248	7.45	78	27.31	261.4	38.2	3.02	<20.65
L3	1317 ± 36		8.01	82	19.89	472.8	186.23	20.06	27.16
L4	683 ± 60	3008 ± 271	7.88	81	21.59	619.33	1123.35	95.09	52.46
L5	667 ± 44	3875 ± 485	7.82	70	16.82	218.73	1063.74	16.71	27.32
C1	1867 ± 44		6.75	127	33.25	308.69	36.6	2.02	<20.65
C2	2008 ± 162		8.2	104	35.86	308.1	67.31	0.91	<20.65
C3	883 ± 8		8.15	91	25.32	684.27	69.33	3.33	<20.65
C4	1808 ± 8		7.93	107	34.81	191.96	19.62	0.95	<20.65
C5	850 ± 66		7.98	93	34.69	69.49	49.36	0.52	<20.65

Table 1. Plant and environmental variables measured during the study (N= 3). D= Drainage channel, L= Lagoon channel, and C= Control site. CN= *Cymodocea nodosa* and ZN= *Zostera nolti*. Errors are SE.

A) Among discharge types				B) Between *C. nodosa* and *Z. noltii*			
	R	*p (%)*	Sim./ Diss (%)		*R*	*p (%)*	Sim./ Diss (%)
Discharge	0.889	**0.1**		Species	0.419	**0.1**	
D-L	0.468	**1.6**	81.34	CN-ZN			46.77
D-C	0.82	**0.8**	79.27	CN			79.10
L-C	0.38	5.6	83.51	ZN			78.83
D			59.01				
L			29.04				
C			33.19				
Site	0.519	**0.1**		Site	0.985	**0.1**	
				L1-L2	1	**1**	64.65
				L1-D4	1	**1**	72.68
				L1-L4	1	**1**	86.64
				L1-L5	1	**1**	84.60
				L2-D4	1	**1**	56.13
				L2-L4	1	**1**	88.13
				L2-L5	1	**1**	81.82
				D4-L4	0.852	**1**	55.20
				D4-L5	1	**1**	55.45
				L4-L5	0.926	**1**	49.85
				D4			79.36
				L1			76.24
				L2			78.08
				L4			77.55
				L5			83.61

Table 2. Results of 2-way ANOSIM (global effects and pair-wise comparisons) and SIMPER analyses (Similarities/ dissimilarities) based on dissimilarity matrices derived from abundance of epiphyte species (log (x + 1) transformation). A) Effects among discharge types (only one factor indicated for the SIMPER analysis due to constrictions imposed by the nested design), and B) Effects between species and among sites (factorial design). *R*: ANOSIM statistic; *p*: significance level of sample statistic (%). In SIMPER results, pair-wise comparisons indicate dissimilarities and single groups similarities.

ANOVAs		Species richness			Alpha diversity			Biomass (Asen x)		
A) Discharge	df	MS	*F*	*p*	MS	F	*p*	MS	*F*	*p*
Discharge= D	2	86.87	73.75	**0.0000**	1.392	13.29	**0.0000**	25.96	70.88	**0.0000**
Site= Si(D)	12	3.18	2.70	**0.014**	0.557	5.32	**0.0000**	1.63	4.45	**0.0000**
Error	30	1.179			0.1047			0.366		
B) Species	df	MS	*F*	*p*	MS	F	*p*	MS	*F*	*p*
Species= S	1	0.833	0.93	0.347	0.482	6.57	**0.019**	1.779	5.81	**0.026**
Site= Si	4	9.75	10.83	**0.0000**	1.562	21.27	**0.0000**	16.70	16.70	**0.0000**
S x Si	4	5.917	6.57	**0.002**	0.447	0.447	**0.002**	2.91	2.91	**0.047**
Error	20	0.900			0.073			0.306		

Table 3. 2-way ANOVA results for testing for overall variability in species richness, alpha diversity, and biomass of epiphytes A) among sites subjected to distinctive water discharges, and B) between *Cymodocea nodosa* and *Zostera noltii*. Epiphytic biomasses were Asen (x) transformed to meet ANOVA assumptions. Statistically significant results are indicated in **Bold.**