

Detecting water quality improvement along the Catalan coast (Spain) using stress-specific biochemical seagrass indicators



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ABSTRACT

Evaluating the efficacy of management actions to improve environmental quality is often difficult because there may be considerable lags before ecosystem management actions translate into measurable indicator responses. These delays make it difficult to justify often-expensive remedial actions to prevent eutrophication. Therefore, it is critical to identify reliable, rapid and sensitive indicators to detect degradation and environmental quality improvement. We evaluate the efficacy of a set of indicators based on the seagrass *Posidonia oceanica* to reliably and quickly detect ecosystem improvements using a 7-year (2003–2010) dataset of 10 stations along the Catalan coast (north-western Mediterranean Sea). In the Catalan region, environmental agencies have invested heavily on wastewater treatment, resulting in significant reductions (ca. 75%) in the BOD₅ discharged to coastal waters from 2003 to 2010. These improvements were clearly reflected at the regional level (i.e. for all the stations averaged) in six biochemical seagrass indicators from our dataset. These indicators were directly related to eutrophication (nitrogen, δ¹⁵N, phosphorus and total non-structural carbohydrates content in rhizomes, δ³⁴S and δ¹³C in seagrass rhizomes and N content in epiphytes). In contrast, seagrass structural indicators, related to seagrass abundance or meadow structure (density, cover) did not show any sign of overall recovery during the monitored period. These results confirm that biochemical seagrass indicators are the most sensitive to water quality improvements within management time-scales (7–10 years) for slow-growing species like *P. oceanica*. Given the budgetary restrictions under which most management actions operate, the availability of decision-support tools that function at appropriate time-scales is crucial to help managers validate the relative success of their remedial efforts. Our results indicate that low inertia, biochemical seagrass indicators fit this task, and can be a robust set of tools to include in monitoring programmes.

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1. Introduction

More than half the world's human population lives within 100 km of the coast, a figure expected to increase by 25% over the next two decades. The steady growth in the occupation and the development of industrial and agricultural activities in the coastal

zone has led to increased nutrient loads in most coastal marine waters (Artioli et al., 2008; Kelly, 2008). By the middle of the last century, most seas in the developed world had seen increases of nutrient concentrations that sometimes reached eutrophic states (Nixon, 1995), and resulted in important changes in ecosystems (Artioli et al., 2008).

Recognizing the dire state of coastal waters, many European countries have, over the last decade, invested heavily in programmes that tackle eutrophication, targeting the improvement of wastewater treatment systems and the reduction of non-point source agricultural inputs (e.g. Nitrates Directive, Urban Waste Water Treatment Directive, Water Framework Directive, Common Agricultural Policy, among others). These efforts have already

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resulted in a directly observed reduction in nutrient concentrations in some areas of the Northern Sea (Artioli et al., 2008; McQuatters-Gollop et al., 2009) and the Mediterranean (Collos et al., 2009). However, the high spatial and temporal variability in nutrient concentrations often makes direct determinations of nutrient reductions/increases in the water column difficult at regional levels (Arhonditsis et al., 2000). Additionally, changes in nutrient conditions are not always linearly related to an improvement/degradation of coastal ecosystems (Krause-Jensen et al., 2005). To address this, the EU Water Framework Directive-WFD (2000/60/EC) has, over the past decade, been urging its member states to evaluate the quality of their aquatic ecosystems using reliable indirect indicators, which can be integrated to assess ecological water conditions across space and time. In response to that mandate, several European countries have developed their own set of tools to classify the ecological status of their waters, based on biological elements from different ecosystems (i.e. phytoplankton, macrophytes, seagrasses, zoobenthos; see (Ballesteros et al., 2007; Devlin et al., 2007; Martínez-Crego et al., 2010a; Oliva et al., 2012; Simboura and Argyrou, 2010). To ensure that these indicators are adequate, they have to be validated against an established gradient of anthropogenic pressures (Lopez y Royo et al., 2011; Martínez-Crego et al., 2008; Pinedo et al., 2013; Simboura and Reizopoulou, 2008).

A crucial, albeit generally neglected element of this programme has been to determine if these indicators, clearly sensitive to environmental deterioration, were also sensitive to water quality improvement. This is of particular relevance given the huge investments being made in reducing the effects of wastewater disposal (Balmer and Mattsson, 1994) as well as to fulfil the WFD mandate of achieving “good ecological status” of all water bodies by 2015. Observing the consequences of reducing nutrient and organic matter discharges, is however difficult because of the non-linearities inherent in the dynamics of ecosystem degradation and recovery. Many ecosystems show threshold responses to disturbances, shifting abruptly from one state to another beyond a tipping point (Scheffer et al., 2001; Veraart et al., 2010). Ecosystem improvement trajectories are similarly complex (Carstensen et al., 2011). For instance, an improvement in nutrient conditions does not always result in direct improvements in coastal communities (Andersen et al., 2009; Carstensen et al., 2011; Duarte et al., 2009). This uncoupling can have serious consequences for bioindicators that rely on communities or populations within ecosystems to assess the efficacy of long-term management actions. The inadequacy of indicators to accurately and rapidly track ecosystem improvements can make it difficult to justify sustaining these management interventions, particularly when they are expensive to maintain. The mismatch between what is known of how bioindicators behave when water quality deteriorates and how they respond during environmental improvement is a serious shortcoming. It limits the use and appeal of bioindicators for environmental managers who are often called upon to convincingly demonstrate that their management investments are producing the desired effect.

Seagrasses are common biological quality elements used in several environmental monitoring programmes, including those related to the Water Framework Directive as well as in other contexts (Marbà et al., 2012). Seagrasses are widespread in shallow coastal waters (Cullen-Unsworth and Unsworth, 2013) and show an extraordinary sensitivity to changes in water quality (e.g. nutrients, organic matter, turbidity) and to other human induced disturbances (Krause-Jensen et al., 2005; Lopez y Royo et al., 2010; Martínez-Crego et al., 2008; Short and Wyllie-Echevarria, 1996). *Posidonia oceanica* (L.) Delile is the most important and abundant seagrass in the Mediterranean Sea where it forms extensive meadows from the surface down to >40 m depth (Boudouresque et al., 2006). *P. oceanica* is an engineering species (Wright and Jones,

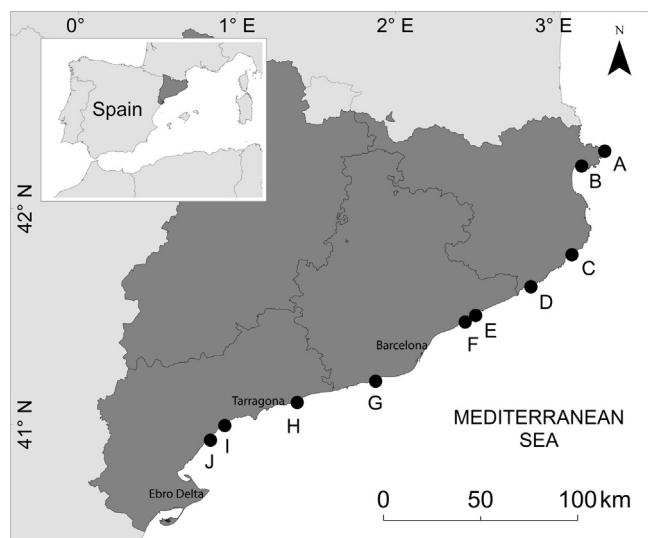


Fig. 1. Distribution of the monitoring sites along the Catalan coast.

2006) that performs important ecological and hydrodynamic functions in the ecosystem and, like other seagrass species, is also extremely sensitive to declining water quality. Whilst a large number of indicators have been described for this species thus far (Pergent-Martini et al., 2005), there are few reported examples of their ability to detect improvements in water quality (but see Roca et al., 2014).

This paper aims to fill this gap, by assessing the capacity of common *P. oceanica* indicators to detect improvements in water quality within time scales adequate for management. We present a case study of a zone (the Catalan coast) that has seen a large investment in the amelioration or deployment of sewage treatment plants since 2003. We analyse the temporal response of different seagrass (*P. oceanica*) indicators related to meadow structure, biochemical traits and indicators of the community (epiphytes), in 10 different meadows along the same stretch of coast over the same period. Our objective was to identify which indicators, if any, were sensitive in detecting management actions associated to water quality improvement over the 7 years of monitoring.

2. Materials and methods

2.1. Study area

The study was conducted along the coast of Catalonia (ca. 700 km of coastline) in NE Spain (42°19' N, 3°19' E to 41°02' N, 1°00' E, Fig. 1). The Catalan coast is densely populated, with approximately 4.5 million people living in coastal municipalities, and is subject to high seasonal tourism pressure, with more than 20 million tourists visiting the country every year. Human pressures are unevenly distributed along this 700-km coastal stretch. Large tourism facilities, big harbours, large cities and main industrial areas (Barcelona and Tarragona) are concentrated along its central regions, whilst other anthropogenic pressures such as agricultural land use occur along its central and southern coasts. Catalonia is a semi-arid region, where run-off takes place through small and/or temporal rivers, with the exception of the perennial River Ebro, in the southernmost extreme. Discharges from wastewater treatment plants (WWTP) play an essential role in continental runoff, accounting for 50–100% of freshwater riverine inputs, depending on the season, with the exception, once again, of the Ebro river (Martí et al., 2010). The region of Catalonia has, over the last decades, invested heavily in programmes to improve

the quality of the watersheds, including coastal waters, involving the closure of small and uncontrolled water discharges, the construction of numerous new water treatment plants and the implementation of better technologies in others.

2.2. Sampling design and data acquisition

This study uses a dataset of several indicators (structural, biochemical, and community measures) from the seagrass *P. oceanica*, obtained within the monitoring programme of the WFD in the Catalan region over the last decade. Temporal trends in these data are examined in the light of the development of wastewater treatment plants and their operation in the Catalan watersheds.

From the WFD seagrass monitoring programme (Martínez-Crego et al., 2008; Romero et al., 2007), we selected 10 sites with the longest temporal data set available, in order to maximize the detection of temporal trends (2003–2010, 10 sites: A–J, Fig. 1). These sites were distributed evenly across the studied coastal stretch and represent a full range of ecological status (Romero et al., 2007). At each site we analysed a set of indicators that included structural indicators (shoot density and meadow cover), biochemical indicators (nitrogen, phosphorus, and total non-structural carbohydrates (TNC) content in rhizomes, isotopic signatures ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$) in rhizomes and nitrogen content in epiphytes) and community indicators (epiphyte biomass). All of these are known to be sensitive to habitat deterioration and, specifically, to the effects of eutrophication (see Martínez-Crego et al., 2008 and references therein). The data corresponds to almost yearly, late summer visits to each site during a 7-year period (2003, 2005, 2006, 2007, 2008, 2010), except for shoot density and meadow cover that were estimated at five occasions over the 7-year period. For each visit and site, replicate samples/data (between 5 and 12, depending on the indicator) were obtained. Details of the sampling design and analytical methodologies are described in Romero et al. (2007). To document improvements in wastewater treatment, we obtained data on the temporal evolution in WWTP number (from 1980 to 2010, but largely for the period 2003–2010). We also obtained data on water Biological Oxygen Demand (BOD_5) discharged annually (from 2003 to 2010) into coastal waters for most of the Catalan watersheds, representing >98% of total wastewater discharges in the area. These data were provided by the Agència Catalana de l'Aigua, the official water authority in Catalonia.

2.3. Data analysis

We conducted a trend analysis for each seagrass indicator (dependent variable) for the 7-year period using linear mixed models, with BOD_5 and year as independent variables (one model for each). We included site as a random factor (random intercept *sensu*, Zuur et al., 2009). As some temporal trends appeared to be nonlinear, we also tested quadratic models, and compared them against linear models using Akaike Information Criteria (AIC *sensu*, Alfaro and Huelsenbeck, 2006), in each case, selecting the model with the lowest AIC. For each indicator, we averaged the values of all replicates for each site and sampling time while fitting the models, in order not to artificially increase sample sizes (n), thus reducing the probability of type I errors. To assess temporal trends within each site we used the same methodology, without averaging replicates and using only time as an independent variable (with the indicators used as dependent variables). We examined possible covariation between biochemical parameters (dependent factors) and shoot density (independent factor) using a linear model, with site as a random factor.

All models were run using the `lme` function from `nlme` package of R statistical software (Pinheiro et al., 2013; Rcoreteam, 2012). Homogeneity of variances and normality assumptions

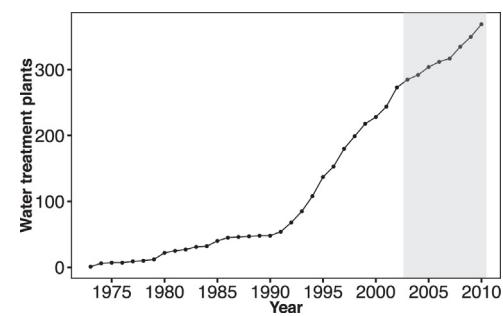


Fig. 2. Temporal evolution over the last decades in the number of functional wastewater treatment plants in the Catalan watersheds. The period encompassed by this study (2003–2010) appears shaded in the figure.

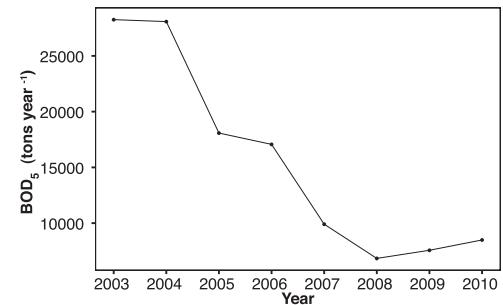


Fig. 3. Evolution of BOD_5 (tonnes year^{-1}) in wastewater discharges to Catalan watersheds. The data include ca. 98% of total discharges.

were checked using Levene's and Shapiro-Wilk's tests. When homoscedasticity and/or normality was not achieved (indicated in the tables), no transformations were attempted as sampling size was large enough (see Tables 1, 2, S1 and S2) to consider the *F* statistic robust against assumption violations (Underwood, 1981). The variables that did not meet these assumptions are indicated in the tables.

3. Results

Over the last three decades, the number of WWTP has increased ninefold, from 50 to 450 (Fig. 2). This increase was maintained over the period of interest (i.e. the seagrass monitoring period, from 2003 to 2010), in which 99 new WWTP were commissioned, increasing the total number from 351 to 450. Moreover, during this period (2003–2010), 36 plants upgraded their treatments techniques or expanded their treatment capacity (information provided by ACA). Concomitantly, the BOD_5 discharged into coastal waters reduced substantially, from ca. 28,000 tonnes year^{-1} at the start of the monitoring (2003–2004) to 6000–8000 tonnes year^{-1} at the end (2008–2010, Fig. 3), representing a reduction of ca. 75%. The central section of the Catalan coast (i.e. south of Barcelona) is where the greatest efforts to improve water quality have taken place, resulting in reductions of BOD_5 from ca. 23,000 tonnes year^{-1} to 4500–60,000 tonnes year^{-1} , i.e. concentrating close to the 75% of the total amount eliminated from all Catalan watersheds.

For the majority of seagrass indicators we examined, the analysis of temporal trends showed a tendency for improvement (Table 1, Figs. 4 and 5), correlated with BOD_5 reduction (Table 2, Fig. 6). Out of the 10 indicators considered, 7 showed significant temporal trends that either increased or decreased depending on the indicator, suggesting overall water quality improvements (Martínez-Crego et al., 2008). Specifically, nitrogen, $\delta^{15}\text{N}$ and phosphorus content in rhizomes showed, on average, a significant decrease of 30%, 8% and 20% respectively (Figs. 4a,b and 5a), while total non-structural carbohydrates increased 58% between 2003

Table 1

Results of the linear models fitted to assess temporal trends of each of the 10 seagrass indicators (dependent variables) for the monitoring period, with time as an independent variable and site as a random factor (indicator ~ year, random = site).

Indicator	df	F-ratio	p	R ^{2*}	Slope
Nitrogen content in rhizomes (N) ^a					
I(year ²)	48	5.9828	0.018	0.546	–
I(year)	48	27.936	0.001	–	–
N isotopic signature in rhizomes ($\delta^{15}\text{N}$) ^c	49	25.054	0.000	0.880	-0.085
Phosphorus content in rhizomes (P)	49	7.321	0.009	0.419	-0.014
Total nonstructural carbohydrates in rhizomes (TNC) ^b	49	34.215	0.000	0.582	0.874
C isotopic signature in rhizomes ($\delta^{13}\text{C}$)	49	13.507	0.001	0.615	0.024
S isotopic signature in rhizomes ($\delta^{34}\text{S}$)	49	27.574	0.000	0.747	0.470
N content in epiphytes ^{b,c}	49	9.653	0.003	0.388	-0.043
Epiphyte biomass ^b	49	29.673	0.000	0.435	0.051
Shoot density	39	0.002	0.966	0.785	-0.075
Meadow cover	39	0.718	0.402	0.747	0.309

df: degrees of freedom; p: probability of error when rejecting the null hypothesis of zero-slope; R^{2*}: adjusted conditional r²; slope: slope of fitted regression.

^a Indicators for which the quadratic model (indicator ~ I(year²) + I(year), random = site, see Table S5 for model selection) was retained.

^b Indicators not conforming to homogeneity of variances.

^c Indicators not conforming to normality.

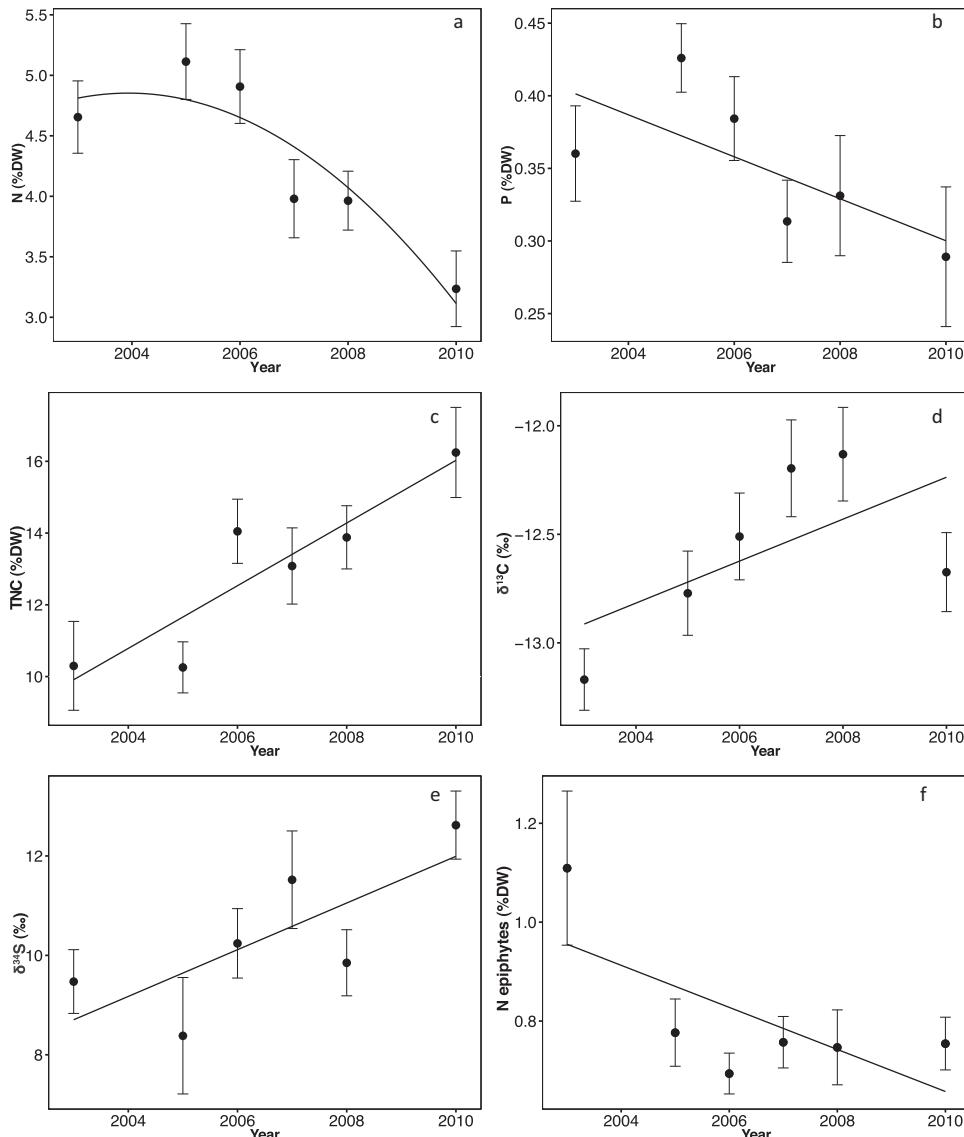


Fig. 4. Evolution of seagrass indicators in the Catalan coast during the monitoring period (2003–2010), including the quadratic or linear models fitted (see Table 1). (a) N: nitrogen content in rhizomes; (b) P: phosphorus content in rhizomes; (c) TNC: total non-structural carbohydrates in rhizomes; (d) $\delta^{13}\text{C}$: C isotopic signature in rhizomes; (e) $\delta^{34}\text{S}$: S isotopic signature in rhizomes; (f) N epiphytes: N content in leaf epiphytes.

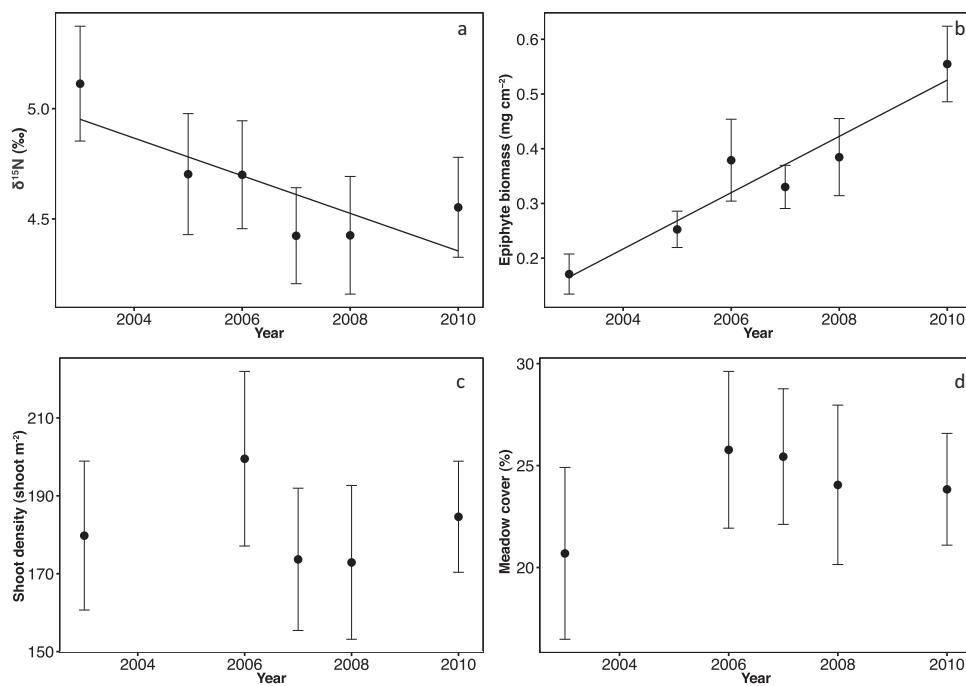


Fig. 5. Evolution of seagrass indicators in the Catalan coast over the monitoring period (2003–2010), including the linear models fitted (only when they explained a significant part of the variability, see Table 1). (a) $\delta^{15}\text{N}$: N isotopic signature in rhizomes; (b) epiphyte biomass (leaf epiphytes); (c) shoot density; (d) meadow cover.

Table 2

Results of the linear models fitted to assess the relationship between each one of the 10 seagrass indicators (dependent variables) and BOD_5 for the monitoring period, with BOD_5 as the independent variable and site as a random factor (indicator $\sim \text{BOD}_5$, random = site).

Indicator	df	F-ratio	p	R^2*	Slope
Nitrogen content in rhizomes (N) ^a					
I(BOD_5^2)	48	12.949	0.000	0.514	–
I(BOD_5)	48	15.238	0.000	–	–
N isotopic signature in rhizomes ($\delta^{15}\text{N}$) ^c	49	4.854	0.032	0.907	0.000004
Phosphorus content in rhizomes (P) ^a					
I(BOD_5^2)	48	3.35	0.073	0.445	–
I(BOD_5)	48	7.477	0.008	–	–
Total nonstructural carbohydrates in rhizomes (TNC) ^b	49	36.306	0.000	0.487	0.000008
C isotopic signature in rhizomes ($\delta^{13}\text{C}$)	49	12.568	0.001	0.719	–0.000107
S isotopic signature in rhizomes ($\delta^{34}\text{S}$)	49	19.347	0.000	0.684	–0.000219
N content in epiphytes ^{b,c}	49	13.502	0.001	0.427	0.000015
Epiphyte biomass ^b	49	16.772	0.000	0.314	–0.000013
Shoot density	39	0.333	0.567	0.787	0.000300
Meadow cover	39	1.254	0.270	0.750	–0.000119

df: degrees of freedom; p: probability of error when rejecting the null hypothesis of zero-slope; R^2* : adjusted conditional r^2 ; slope: slope of fitted regression.

^a Indicators for which the quadratic model (indicator $\sim I(\text{BOD}_5^2) + I(\text{BOD}_5)$, random = site, see Table S5 for model selection) was retained. Note that the quadratic model for phosphorus was marginally significant.

^b Indicators not conforming to homogeneity of variances.

^c Indicators not conforming to normality.

and 2010 (Fig. 4c). Similarly, isotope signatures $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$ also increased significantly (Fig. 4e and d), as did N content in epiphytes that presented very high values at most sites in 2003 (Fig. 4f). Epiphyte biomass increased through time, contrary to what we would generally expect if water quality improved (Fig. 5b, Table 1). In contrast, meadow cover and shoot density did not show any temporal trend (Fig. 5c and d).

Site-specific differences were also observed across indicators. While some indicators showed an almost generalized response across sites responding in at least 70% of sites (i.e. $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$, Table 3), others only responded at few sites (i.e. N content in epiphytes). Still, most indicators showed a response that occurred in more than 50% of the sites (N, P and total non-structural carbohydrates content). In contrast, meadow cover only showed temporal trends in two sites, Garraf and Torredembarra (sites G and H, Table 3) and shoot density only increased in Garraf (site G, see also

Table S2). Both sites (G and H) that recorded structural improvements also had very low density and cover in 2003 (Tables S3 and S4 and Fig. 7). No significant covariation was found between any of the 7 responding biochemical indicators and shoot density in the data set (Table S1). Garraf, a few km south of Barcelona city, was the site where the improvement in most indicators was the clearest. Garraf showed the strongest temporal trends (as indicated by the steepest slopes) in total non-structural carbohydrates (with increases of 257%) and also showed great reductions of N and P content in rhizomes, 59% and 52% respectively (Fig. 7a–c), from 2003 to 2010. Stable isotope signatures ($\delta^{13}\text{C}$, $\delta^{34}\text{S}$, Fig. 7d and e) increased 64% and 7% respectively and N content in epiphytes showed reductions of 51% (Fig. 7f), while epiphyte biomass increased 430% (Fig. 7h). Shoot density rose significantly up to 300% and meadow cover increased above 1000% (Fig. 7i and j, Tables S2–S4) at this site.

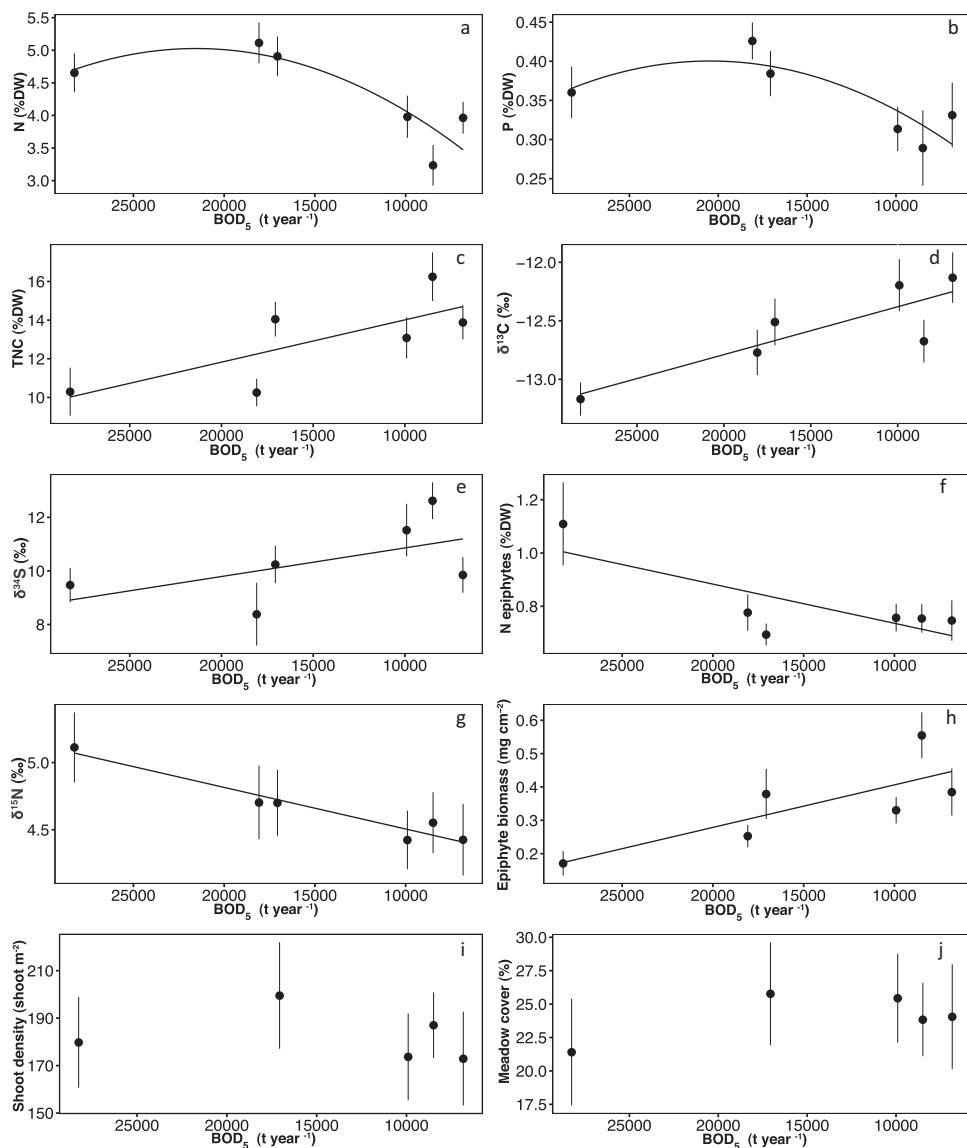


Fig. 6. Relationship between seagrass indicators on the Catalan coast over the monitoring period (2003–2010) and BOD₅, including the quadratic or linear models fitted (only when they explained a significant part of the variability, see Table 2). (a) N: nitrogen content in rhizomes; (b) P: phosphorus content in rhizomes; (c) TNC: total non-structural carbohydrates in rhizomes; (d) δ¹³C: C isotopic signature in rhizomes; (e) δ³⁴S: S isotopic signature in rhizomes; (f) N epiphytes: N content in leaf epiphytes. (g) δ¹⁵N: N isotopic signature in rhizomes; (h) epiphyte biomass (leaf epiphytes); (i) shoot density; (j) meadow cover.

Table 3
Summary of the significance and direction of the temporal trends of the 10 seagrass indicators (dependent variables) for the monitoring period, with time as an independent variable, as derived from fitting linear models (independent: time; dependent: each indicator) for each site separately.

Indicators	A	B	C	D	E	F	G	H	I	J
Nitrogen content in rhizomes (N)	NS	—	—	—	NS	NS	—	—	—	NS
N isotopic signature in rhizomes (δ¹⁵N)	NS	NS	NS	NS	—	—	NS	—	—	—
Phosphorus content in rhizomes (P)	NS	NS	—	—	+	NS	—	—	—	NS
Total nonstructural carbohydrates in rhizomes (TNC)	NS	NS	NS	+	+	+	+	+	+	NS
C isotopic signature in rhizomes (δ¹³C)	+	+	+	+	NS	+	+	NS	+	NS
S isotopic signature in rhizomes (δ³⁴S)	+	+	+	+	+	NS	+	+	+	NS
N content in epiphytes	NS	NS	NS	NS	NS	NS	—	—	—	NS
Epiphyte biomass	NS	NS	NS	NS	NS	+	+	+	+	+
Shoot density	NS	NS	NS	NS	NS	NS	+	NS	NS	NS
Meadow cover	NS	NS	NS	NS	NS	NS	+	+	NS	NS

In bold we indicate the indicators showing overall significant temporal trends based on the analysis reported in Table 1. NS, non significant; +, significant (increase with time); —, significant (decrease with time). The significance level was set at $p=0.05$, except in variables not conforming to normality and/or homoscedasticity, in which case $p=0.01$. The results of these analyses are detailed in Table S2.

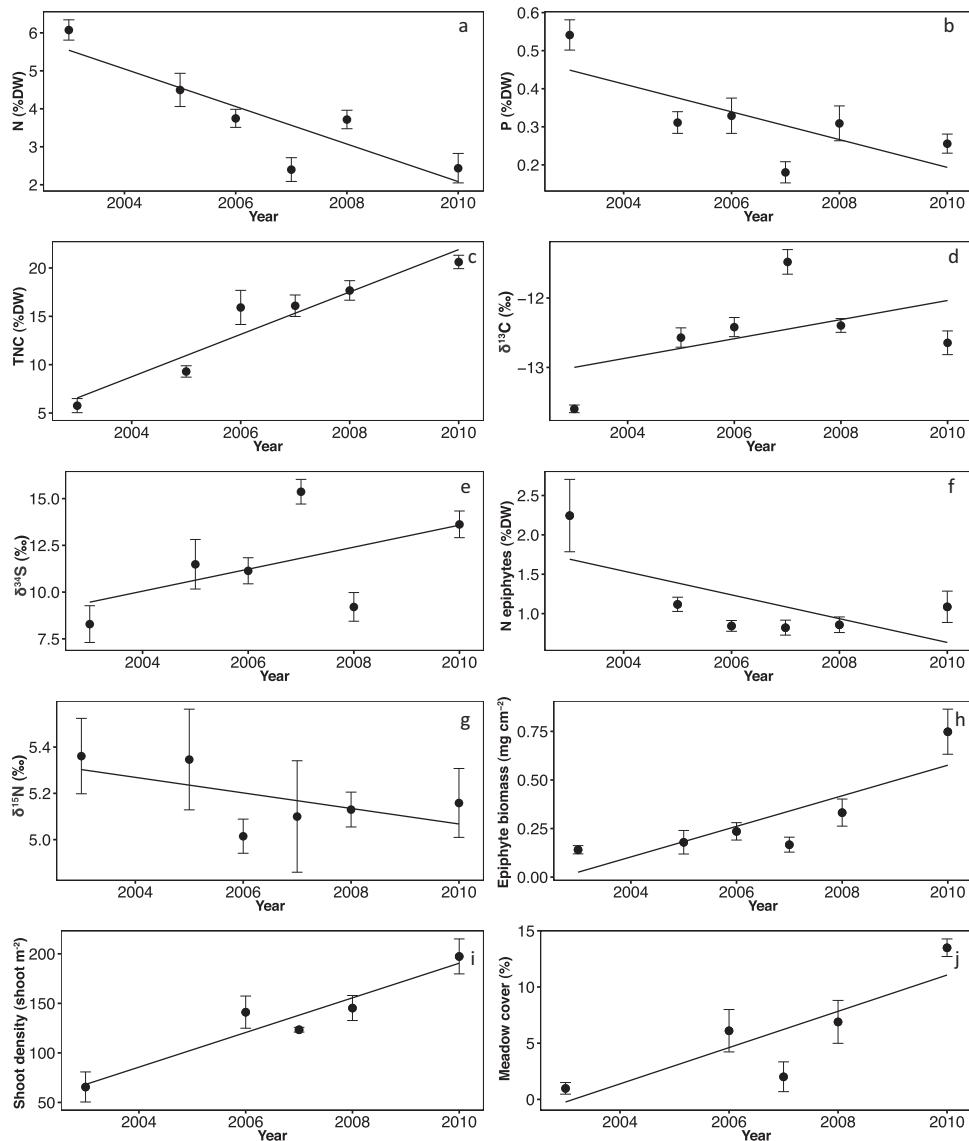


Fig. 7. Evolution of seagrass indicators in site G over the monitoring period, including the linear models fitted (see Table S2). (a) N: nitrogen content in rhizomes; (b) P: phosphorus content in rhizomes; (c) TNC: total non-structural carbohydrates; (d) $\delta^{13}\text{C}$: C isotopic signature in rhizomes; (e) $\delta^{34}\text{S}$: S isotopic signature in rhizomes; (f) N epiphytes: N content in leaf epiphytes; (g) $\delta^{15}\text{N}$: N isotopic signature in rhizomes; (h) epiphyte biomass (leaf epiphytes); (i) shoot density; (j) meadow cover.

4. Discussion

Our analyses indicate that most biochemical *P. oceanica* indicators are highly sensitive to improving conditions, and recorded an overall concurrent change at several locations along the Catalan coast, providing strong evidence of a generalized amelioration of coastal waters over the period 2003–2010. This change coincided with an intensification of wastewater treatment in the region, in which BOD_5 discharged to coastal waters decreased by 75%. These biochemical indicators were also sensitive to positive changes at the site level, suggesting that they may also be effective tools to assess improvements at local scales. Additionally, the most sensitive indicators were all measures of eutrophic conditions (light, nutrients, organic matter), confirming that regional investments in wastewater treatments are having a clear, measurable and beneficial effect. In contrast, the most standardly used indicators (shoot density, meadow cover) did not show the same clarity of response and were not able to detect water quality improvements at a decadal time scale. While they are highly integrative ecosystem-level measures, they may have considerable lags

before they register measurable changes and, on their own, may be insufficiently sensitive to rapidly evaluate the effectiveness of management interventions. These findings significantly bolster the few studies that focus on the efficacy of biological indicators in reflecting improvements in environmental conditions.

Ecosystems are often governed by sets of complex stabilizing feedbacks and thresholds that can introduce considerable non-linearity in their responses to external drivers, such as human pressures. While there is an increasing ecological understanding of how these discontinuities function, they make the design and interpretation of biological indicators based on ecological condition particularly difficult. Much of the effort thus far has focused on identifying indicators of environmental decline with relatively few studies that evaluate their effectiveness in reflecting improving conditions. This may be due to the fact that, from a point of decline, ecosystem recovery trajectories are often considerably slower, and it may be a long while before changes in environmental conditions will be reflected in improved ecosystem functioning. This makes it all the more critical to develop sensitive and reliable indicators of environmental recovery that can help managers justify that their

interventions are having a clear effect, even if they are not yet reflected in the most integrative ecosystem-level variables.

As in many other locations across Europe, the region of Catalonia has, over the last two decades, seen a marked increase in wastewater treatment (volume of water treated and treatment efficiency), spurred by a large investment in improving water quality of catchments and discharges. The target has been to reduce nutrient and organic matter in the water discharges through primary, secondary and tertiary treatments (ACA, 2010), with the overall objective of improving coastal water quality for fishing, recreational and conservation purposes. Our results indicate that biochemical seagrass indicators were very good in detecting an overall improvement in water quality as has been demonstrated earlier using macroalgae-based indicators (Pinedo et al., 2013). The fact that these indicators were all markers of eutrophication, allows us to unequivocally link these changes with a very specific intervention, in this case an amelioration in wastewater treatment. This region of the coast has not seen decreases in human population density, nor have agricultural or industrial activities reduced substantially over the last decade (IDESCAT, 2014), further strengthening the causal link between the clean water initiatives undertaken and the improvement of seagrass indicators along the coast.

Eutrophic conditions in coastal waters are typically characterized by an increase in both organic matter in the water column (eventually reaching the sediment) and nutrient concentrations. Moreover, the proliferation of planktonic, and/or benthic algae can increase the organic matter levels and can significantly reduce light availability for benthic vegetation (Brun et al., 2002; Short et al., 1995). With improving conditions of nutrients and organic matter, we would expect the water column to recover in turn, eventually improving ecosystem quality (Boesch, 2002), triggering reversals in indicator trends. Most of the indicators that responded to environmental improvement were directly linked to eutrophic detection, and closely tracked our expectations of how they would behave (increase or decrease) as eutrophic conditions improved. The observed decrease in nutrient content in plant tissues (both N and P) and in epiphytes (only N was measured) is indicative of decreased nutrient concentrations in the water column, and eventually in the sediment, as repeatedly demonstrated in previous studies (Alcoverro et al., 1997; Burkholder et al., 1992, 1994; Fourqurean et al., 1992; Martínez-Crego et al., 2006; Romero et al., 2006). The observed increases in S isotopic signature ($\delta^{34}\text{S}$) are indicative of a decrease in organic matter in the sediment, and thus, indirectly, of organic matter deposition (Frederiksen et al., 2006). The mechanism of increase in $\delta^{34}\text{S}$ is related to a decrease of anoxic conditions in the sediment linked to a reduction of sulphide intrusion in plants (Frederiksen et al., 2008). The observed increase in carbohydrates content in rhizomes is indicative of an increase in light reaching plants, and thus of an increase in water transparency with nutrient and organic matter reduction. The observed variability in $\delta^{13}\text{C}$ in seagrass tissues can be attributed to a variety of causes linked to the balance between photosynthetic inorganic carbon demand and inorganic carbon supply (see, for example, Enríquez et al., 2006) These includes water turbulence, carbon limitation and carbon concentration mechanisms, among others (Hemminga and Mateo, 1996; Hu et al., 2012). In our study, however, the most plausible explanation for the generalized $\delta^{13}\text{C}$ increase with water quality improvement is an increase in light reaching seagrass canopies, causing an increase in photosynthetic C demand and, consequently, relative enrichment in ^{13}C (Durako and Hall, 1992; Vizzini and Mazzola, 2003). This explanation concords with the expected increase in carbohydrate concentration we observed in our study (Collier et al., 2009; McMahon et al., 2011).

Unexpectedly, the nitrogen isotopic signature ($\delta^{15}\text{N}$), an indicator usually linked to anthropogenic nitrogen sources (McClelland et al., 1997), did not always follow the pattern observed in nitrogen

content. WWTP effluents are normally enriched in $\delta^{15}\text{N}$ (Kendall et al., 2008; Peipoch et al., 2012) and, we expected that a reduction in N in wastewater discharges would be associated with a reduction in $\delta^{15}\text{N}$. Overall, this did take place and $\delta^{15}\text{N}$ showed, a slight decreasing trend (Table 1). However, at 5 of the 10 sites there were no clear reductions in $\delta^{15}\text{N}$ indicating a trajectory that probably needs more unpacking. The observed uncoupling between N isotopic composition and N content in rhizomes responses (see Table 2) could be the result of internal N balance mechanisms. *P. oceanica* is known to meet its N demands by internal recycling, i.e. by reclaiming N from old leaves to supply new growing ones, that can influence the N ratio. Moreover, *P. oceanica* can store N uptaken during periods of high availability, in its massive rhizomes that can retranslocate to new tissues, delaying the response in N isotopic composition (Alcoverro et al., 1997; Lepoint et al., 2002; Prado et al., 2008). Additionally the use of N in new shoots can enhance a decrease in N content in seagrass tissues (dilution effect) contributing to further uncouple of N content and $\delta^{15}\text{N}$ trends (Marbà et al., 2002).

Seagrass abundance and meadow structure (shoot density and meadow cover), the most standard indicators used in monitoring programmes (Marbà et al., 2012), did not change at most sites over the monitoring period despite the generalized improvement detected in the rest of the indicators. These findings agree with previous works where no improvement was found in shoot density 2 years after cessation of impact (Roca et al., 2014), with changes in shoot density accruing only 8–10 years later (Guillén et al., 2013). *P. oceanica* is a slow-growing species with very low shoot recruitment and spreading rates, and seedling success is rare (Duarte, 1991; Marbà and Duarte, 1998; Procaccini et al., 1996). Expecting to detect meadow structural recovery by tracking changes in shoot density and meadow cover within reasonable management time scales is perhaps improbable, except in cases with very low initial shoot numbers and/or very large improvements in environmental conditions. That was probably the case in Garraf (site G, shoot density and meadow cover) and Torredembarra (site H, only meadow cover). These two sites were characterized by the most structurally deteriorated seagrass meadows (in terms of cover and density) of the sites included in this study (Romero et al., 2007). Under these conditions, even relatively small increases in shoot density become much more apparent than at sites with higher initial densities. Additionally it has also to be noted that the Garraf site is where the largest improvements of water quality have been observed, potentially contributing to the observed improvement. However, the shoot densities and cover at both meadows are still far from the conditions expected from a natural, undisturbed meadow (normal natural densities, 246–470 shoots m^{-2} , Pergent et al. 1995), indicating that the observed improvements in the environmental conditions will need to be sustained much longer for a full recovery at these sites.

The indicator that deviated the most from our initial expectation was epiphyte biomass, which actually showed clear increases with improving water quality. It has been classically assumed that an increase in nutrient availability would trigger the development of dense epiphytic communities (Larkum and West, 1990; Wear et al., 1999). However, this response in *P. oceanica* is far from clear, as nutrient increases do not always cause epiphyte biomass increases (Martínez-Crego et al., 2010b; Prado et al., 2010) and poor light conditions can contribute to reduce epiphyte biomass (Serrano et al., 2011). Moreover, epiphytic biomass can also be linked to differential leaf lifespan, particularly important in long-lived species, in turn dependent on environmental conditions. In addition, epiphyte species can be very diverse in terms of biomass. Thus, changes in epiphyte species composition can also affect epiphyte biomass (Martínez-Crego et al., 2010b; Prado et al., 2010). Until further ecological studies can determine the functioning of these

complex interactions, the use of epiphyte biomass as an indicator of improving conditions in *P. oceanica* meadows should be viewed with caution, at least in the temporal, environmental and spatial scales used in this work.

The decision to maintain, modify, reinforce or abandon remedial actions is central to adaptive environmental management. Sound scientific evidence can be a powerful enabler for managers to objectively evaluate and rationalize their management interventions. This is made all the more urgent given the increasing call for economic triage in an environment of financial crisis, declining budgets and increasing coastal pressures. Ecosystem managers are increasingly being called to be accountable to the considerable public investments made in improving environmental condition. Assessing improvements to water quality is particularly difficult given the high variability in nutrient concentrations (and other physico-chemical variables) in the water column (Alkhatab et al., 2012; Marty et al., 2002). Tracking these changes directly in the water column would require a sampling procedure that would be unrealistically complex (both in space and time) and prohibitively expensive to implement. While biological indicators can often serve as very good proxies of these changes, it is critical to clearly understand the underlying mechanism of their actions in order to interpret indicator trends. Not all indicators are equally reliable or sensitive to changes, and it is seldom easy finding the right suite of measures that can be linked directly to management interventions. Our analyses indicate that seagrass-based biochemical indicators meet these criteria well, and can be a powerful, cost-efficient tool to monitor water quality improvement.

5. Summary and conclusions

A range of unspecific ecosystem indicators such as shoot density and meadow cover has been shown to effectively track declining water quality. These structural indicators are often used in monitoring programmes across Europe within the WFD and others (Marbà et al., 2012) because they also link directly to ecosystem function, integrate responses from a variety of environmental stressors, and have been well tested against well-defined disturbance gradients. However, if the objective of management actions in Europe is not merely to detect but reduce eutrophication and ensure that water quality improves, we need an additional set of tools that can effectively track not just degradation but recovery as well. Given the large amount of public resources invested in these positive management actions, the ability to show that these are working will be central to directing future management. Our analysis shows that structural indicators, good as they are in signalling environmental decline, may not be equally fast and useful in tracking the recovery of ecosystems as water quality improves, at least in the short or medium term. This indicates that trajectories of seagrass degradation and recovery may follow radically different paths (Carstensen et al., 2011; Scheffer et al., 2001). These hysteretic properties may be inherent to the system and call for the development of a suite of indicators that respond to these trajectories in management-adequate time scales. The biochemical plant attributes we identified in our dataset seem particularly suited to this task, and sign improvements at regional as well as more local levels. In addition, these indicators are very pressure-specific, and allow for a clear linkage between management intervention and environmental response. Adding these biochemical indicators to existing monitoring programmes will make for a powerful tool that managers can use to evaluate the effectiveness of their interventions, plan course corrections if necessary and convince an increasingly concerned public that coastal water quality is improving as a result of their actions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2015.02.031>.

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