Response of seagrass indicators to shifts in environmental stressors: a global review and management synthesis

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Abstract

Although seagrass-based indicators are widely used to assess coastal ecosystem status, there is little universality in their application. Matching the plethora of available indicators to specific management objectives requires a detailed knowledge of their species-specific sensitivities and their response time to environmental stressors. We conducted an extensive survey of experimental studies to determine the sensitivity and response time of seagrass indicators to ecosystem degradation and recovery. We identified seagrass size and indicator type (i.e. level of biological organization of the measure) as the main factors affecting indicator sensitivity and response time to degradation and recovery. While structural and demographic parameters (e.g. shoot density, biomass) show a high and unspecific sensitivity, biochemical/physiological indicators present more stressor-specific responses and are the most sensitive detecting early phases of environmental improvement. Based on these results we present a simple decision tree to assist ecosystem managers to match adequate and reliable indicators to specific management goals.

1. Introduction

The global decline of critical ecosystems to human pressures makes it increasingly urgent to effectively track ecosystem status, in order to detect, halt, and, where possible, reverse these losses (Millennium Ecosystem Assessment, 2005). Seagrass meadows are among the most threatened ecosystems, declining at an estimated 7% per year globally (Waycott et al., 2009). This is being driven by a range of anthropogenic disturbances related to eutrophication (e.g. organic matter and nutrient increases), shading, siltation from deforestation, shoreline modification, and physical removal by trawling and anchoring (Duarte, 2002). Because many seagrass species are also particularly sensitive to disturbance, they are ideal systems to assess environmental change (Marbà et al., 2012). Tracking changes to environmental quality and the ecosystem itself have become increasingly important mandates for ecosystem managers and scientists (Montefalcone, 2009). As a result, there has been a recent burgeoning of monitoring programmes based either directly or indirectly on seagrass responses to environmental change (Martínez-Crego et al., 2008).

In general, monitoring programs have evolved in response to three principal management goals: tracking general trends in ecosystem status, assessing environmental quality, and evaluating impacts of development projects or effectiveness of management actions. Monitoring of ecosystem status is typically linked to habitat management (for instance within Marine Protected Areas), where it primarily serves as an early-warning of change in response to a wide variety of potential stressors. In contrast, monitoring environmental quality (e.g. the European Water Framework Directive) aims at detecting if changes – degradation or amelioration – in water quality are reflected in ecosystem status. Impact assessment focuses instead on detecting if a set of specific, known

70 pressures, associated with a particular action (a coastal development or a management
71 intervention for instance), are affecting the ecosystem. Each of these management
72 objectives places a very different set of requirements in terms of the specificity and
73 expected response time of the indicators used. It is unlikely that a universal set of
74 indicators can be developed to suit all needs, and a more bespoke solution will require a
75 careful matching of management goals with the characteristics of available indicators.
76 These can vary strongly between target seagrass species, the time scale of disturbance
77 and post-disturbance processes, and the sensitivity of the chosen indicators to the
78 stressors of interest. One approach has been to develop multi-metric indices which
79 provide a synthetic measure of environmental or ecological quality based on a
80 combination of parameters (García-Marín et al., 2013; Gobert et al., 2009; Lopez y
81 Royo et al., 2010; Romero et al., 2007). While certainly powerful, there are currently
82 insufficient data to test these composite indices perform in terms of response or
83 recovery time when exposed to known events of environmental disturbance or recovery.
84 As a result, we have explicitly excluded multi-metric indices from this review.
85
86 In this review, we adopt the relatively broad definition of indicators proposed by Heink
87 and Kowarik (2010). By their definition, an indicator in ecology and environmental
88 planning is something used to depict or evaluate environmental conditions or changes or
89 to set environmental goals, where this something can be either a component or a
90 measure of environmentally relevant phenomena. This definition is useful since it
91 reflects the wide diversity of contexts within which indicators have been used. A large
92 number of indicators have been developed, based on different seagrass species, and
93 encompassing a broad spectrum of biochemical, physiological, organismal, population
94 and community level traits (Marbà et al., 2012; Martínez-Crego et al., 2008; Rees et al.,

95 2008). Choosing adequate sets of indicators from this plethora to meet management
96 objectives can be challenging. Indicators are not universally sensitive to changes in
97 ecosystem status or environmental conditions, and there are few objective means to
98 evaluate their appropriateness to specific mandates. Understanding how sensitivity and
99 response time vary between seagrass indicators is essential to rationalising the choice of
100 indicators and to designing monitoring and impact assessment programmes.
101
102 Response time is the time an indicator takes to register changes (degradation or
103 recovery) in ecosystem (or coastal) health (Contamin and Ellison, 2009), and helps
104 determine its potential either as an early warning indicator (sensitive to degradation) or
105 an improvement indicator (sensitive to recovery). Response times and sensitivity to
106 stressors of environmental change may vary with the type of indicator (biochemical,
107 physiological, growth, morphological, structural, community, etc), and intrinsic species
108 traits that constrain organism and population dynamics (e.g. size or growth and
109 demographic dynamics) (Collier et al., 2009). In fact McMahon et al. (2013) in a recent
110 review found important differences in the response time of indicators between those
111 responding to light stress. Moreover, response times may also differ during degradation
112 and recovery since ecosystem responses often display hysteresis, tracking very different
113 trajectories during decline and recovery phases (Andersen et al., 2009; Duarte et al.,
114 2013; Heide et al., 2007).
115
116 The relative sensitivity of indicators to specific stressors is also critical in the
117 assessment of seagrass indicators. Non-specific seagrass indicators that integrate
118 ecosystem health such as shoot density or cover, may be best suited to detect
119 unanticipated environmental or ecosystem changes not linked to a specific impact (e.g.

monitoring climate change or general environmental quality). More stressor-specific indicators may be more appropriate when a clearly identified stressor, such as light availability, excess of organic matter or nitrogen, is being monitored (McMahon et al., 2013; Pérez et al., 2008; van Lent et al., 1995). Stress-specific indicators are best suited to evaluating the effectiveness of mitigatory management actions (Roca et al., 2015). As a rule of thumb, indicator specificity tends to decrease with the level of biological organisation (*sensu,* Whitham et al., 2006), from more integrative, structural metrics to specific physiological and molecular indicators (Adams and Greeley, 2000). How this general rule holds between seagrass species is completely unknown.

We evaluate the utility of the most common seagrass-based indicators to objective-specific management. We identify a wide set of indicators currently employed in seagrass monitoring programs and, where possible, assess their sensitivity (percent of response) to increased/decreased stressors and their response time to degradation and recovery. We test how universal these responses are between species, level of biological organisation and type of stressors. We do this by conducting a comprehensive survey of published and unpublished experimental studies that report the time-response of seagrass parameters currently being used as indicators to a variety of stressors. We use this to develop a simple decision tree to help managers choose a set of seagrass indicators best suited to their specific mandate, be it monitoring general trends in ecosystem health, assessing environmental quality or evaluating the consequences of a known impact or mitigation measure.

2. Materials and Methods

2.1. Identifying and selecting relevant studies

We compiled an extensive database on the likelihood of response to increased or reduced stressors and the response time to degradation and recovery of different seagrass indicators from experimental, mesocosm or field studies (Table 1). Our approach in compiling this database was to focus on a suite of parameters that have been employed by indicator studies across the world, starting with a list initial reviewed by Marbà et al. (2012) and extending it based on more updated reviews (see Table 2). For this shortlist of parameters, we looked for studies that specifically tested their responses to gradients (or levels) of stressors, regardless of whether these studies were specifically designed to test the efficacy of these parameters as indicators. For the purposes of this review, we refer to these chosen parameters as indicators. The data was extracted from scientific reports of experiments from the laboratory, mesocosms or the field. The database was compiled by conducting an exhaustive literature survey on seagrass experiments published before March 2013 using the "Scopus" search engine. We used the search terms ("seagrassses" OR "eelgrass" OR "*Posidonia*" OR "*Zostera*" OR ...(i.e. all seagrass genera)) AND ("response" OR "recovery") AND ("light" OR "shade" OR "shading" OR "dredge" OR "dredging" OR "sediment" OR "burial" OR "organic matter" OR "salinity" OR "hypersalinity" OR "brine" OR "nutrients" OR "N" OR "P" OR "eutrophy" OR "mechanical removal" OR "anchoring"). In addition, to account for older references that may not have been available through "Scopus", the reference lists of each article was also scanned and the bibliographic sources checked for relevant additions to the database. We also updated the dataset with our own unpublished data from recent experiments. Decisions to include or exclude particular studies can have a large impact on the results of meta-analyses, particularly if the

168 number of studies is small (Englund et al., 1999; Gates, 2002; Hughes et al., 2004). To
169 avoid bias in the selection of studies we attempted to be as consistent as possible, only
170 extracting information from those experiments in which indicator responses were
171 estimated under clearly defined possible stressors (organic matter, nutrients, shading,
172 mechanical removal, burial, hypersalinity). For instance, we avoided all studies that
173 examined the effect of multiple stressors acting together since we would be unable to
174 attribute responses to a single stressor. In addition, we separated between three principal
175 factors associated with eutrophication (light, nutrient and organic matter) as they do not
176 always co-occur (Erftemeijer and Robin Lewis III, 2006; Roca et al., 2014). A *study*
177 was defined as every individual publication or experiment. A *case* was defined as every
178 single measurement of responses to increased/decreased stressors or response time to
179 degradation/recovery of a particular indicator taken from each study, carried out in a
180 particular *site*, for a single *species* under a certain *stressor* recorded and measured
181 *indicator*. Seagrass response to increased/decreased level of stressors as well as the
182 response time to degradation/recovery was recorded for each *case*.
183
184 The response time of each indicator to increased stress (henceforth, "indicator response
185 time to degradation") was identified as the time taken for the indicator to register a
186 statistically significant change when exposed to a specific stressor (e.g. increased
187 nutrient level, increased shading). Similarly, the response time of the indicator to the
188 removal of the stress (henceforth, "indicator response time to recovery") was identified
189 as the time before a statistically significant change was detected after the removal of the
190 stressor. Therefore, "degradation" and "recovery" refer to environmental quality and do
191 not necessarily imply seagrass degradation or recovery. This estimate is conservative
192 since significant effects could perhaps have been registered over a shorter time span and

193 we did not take into account variations in the responses of indicators to different
194 stressor intensities; there is no consistent way to compare stressor intensities between
195 studies and experiments, which are often also conducted in different seasons. In both
196 cases, if no significant change was registered, we recorded this as “no degradation/no
197 recovery”. The time intervals between sampling events can strongly influence the
198 precision of the estimates of indicator responses. We, therefore, discarded studies using
199 long sampling intervals, established as at least 1.5 times longer than the minimum
200 response time observed for the same indicator, stress and species in all the data sets, to
201 avoid biasing our estimates of indicator response time.
202
203 Indicators were classified into three broad types based on the level of biological
204 organization they addressed: physiological and biochemical, growth and morphological,
205 and structural and demographic (Fig. 1, Table 2). Physiological and biochemical
206 indicators included metabolic processes and chemical constituents of the plant. Growth
207 and morphological indicators included descriptors related to shoot/leaf morphometry or
208 production. Finally, structural and demographic indicators included parameters that
209 characterise the configuration of meadows such as cover, as well as population
210 parameters such as shoot density. We ignored indicators that employed meadow
211 community composition from the analysis because these indicators ranged widely in the
212 level of biological organisation or the species on which they relied. We additionally
213 classified indicators according to the environmental stressor their response was tested
214 against (shading, nutrients, burial, organic matter and hypersalinity). Finally, we also
215 classified seagrass species based on their rhizome diameter, considered one of the best
216 proxies of seagrass size (Duarte, 1991). We grouped seagrasses into small (rhizome

diameter ≤ 3.5mm) and large (rhizome diameter >3.5mm) species (Marbà and Duarte, 1998).

2.2. Data analysis

Indicator response to increased stressor levels

We used generalized linear mixed effect models (glmm) to examine the relationship between the two principal dependent variables, *Indicator response time to degradation* (in weeks) or *Indicator response to increased stressor* (yes/no) observed and the type of stressor, the plant size and level of biological organisation of the indicator. In the two models, seagrass size (rhizome diameter), level of organisation (structural/demographic, growth/morphological, physiological/biochemical) and stressor type (organic matter, nutrients, shading, burial, hypersalinity) were treated as fixed factors. The interaction between "study" and "species" was treated as a random factor to account for the influence of data from different indicators belonging to the same study (sample dependence). The variable *Response to increased stressor* was analysed using a binomial distribution due to the dichotomic nature of the data (response yes or no, i.e. a statistically significant change vs. no response in the absence of such changes). We used a Poisson distribution to model the variable *Indicator response time to degradation*. In addition, we used the same *Indicator response time to degradation* model with indicators instead of level of biological organisation to check the variance due to differences in response time among individual indicators. All models were performed using the Lme4 package in the statistical software, R (Bates, 2008, 2005; R core Team, 2013). To avoid the influence of stressors that cause immediate responses, the pressure 'mechanical removal' was extracted from the analysis because this stress involves, by definition, plant removal, and the response of structural indicators is self-evident. We

used Tukey's HSD post-hoc comparisons to check for differences between indicator types and stressors in both models using the MULTCOMP R package. In addition, we examined correlations of the variable *Indicator response time to degradation* with log-rhizome diameter for each level of biological organisation.

An indicator was considered robust when it showed a clear response (statistically significant change) to the stressor in question in at least 66% of independent cases. For most stressors, we evaluated robustness only for those indicators that had 5 or more independent assessments of response. For indicators with fewer than 5 independent assessments, we considered it to be potentially robust when it showed a consistent response in more than 75% of reported studies, highlighting that further assessments are needed to confirm its utility. We determined the specificity/generality of each indicator to an increased stressor level by assessing the proportion of studies that showed responses. Indicators were classified as general indicators when they responded to three or more stressors while specific indicators were those that responded to one independent stressor or two related stressors.

Indicator response to decreased stressor levels

Indicator response to decreased stressor levels (yes/no) and *Indicator response time to recovery* were tested using models similar to those described above. The dataset to test responses to decreased stressor levels (24 studies) was much smaller and less balanced than for responses to increased stressor levels (74 studies). In order to avoid potential biases due to this reduced sample, analyses of *Indicator response to decreased stressor levels* and *Indicator response time to recovery* were simplified to focus on three

267 separate, more balanced models. To test the variable *Indicator response to decreased*
268 *stressor levels* (yes/no) we first ran an analysis with the whole dataset to test for effects
269 of the level of biological organisation and species size (fixed factors). As the factor
270 "size" appeared to introduce some potentially confounding variability, we ran two
271 separate analyses for large seagrass species (12 studies, 42 cases) and small species (10
272 studies, 57 cases) to identify size-dependent differences among indicator types. All
273 three models were fitted to a binomial distribution. To test the variable *Indicator*
274 *response time to recovery* we included the effects of level of biological organisation and
275 species size (as fixed factors). The number of studies was relatively small for this model
276 (19 studies). Due to the lack of significant random effects, we ran response and time
277 response to decreased stressor levels models without random effects using the *glm*
278 function in the R stats package (R core Team, 2013).
279

280 **3. Results**

281 The compiled dataset included 25 of the 60 existing species of seagrasses (Green and
282 Short, 2003), with *Zostera marina, Posidonia oceanica*, *Cymodocea nodosa* and
283 *Thalassia testudinum* accounting for the highest records (Table 1). Most studies used
284 indicators to assess responses to environmental degradation (n=74) with far fewer
285 studies assessing recovery after the cessation of stress (n=24, Table 1). The studies
286 covered a wide geographic extent, including coastal areas in Australasia (Australia 10),
287 Asia (Korea 1, Philippines 1, India 1, Malaysia 1, Indonesia 1), Europe (Denmark 4,
288 Italy 1, Netherlands 5, Germany 1, Portugal 2, Spain 23, France 1, Italy 1), North
289 America (USA 16), Central America (Puerto Rico 1), South America (Brazil 1). In total
290 we identified 85 distinct indicators (Table 2). The vast majority were physiological and
291 biochemical indicators (61 unique measures), while growth/morphological and
292 structural/demographic indicators were much less common (13 and 10 respectively).
293
294 *Response to increased and decreased stressor levels*
295 The likelihood of responses to increased levels of stressors (n=668) differed
296 significantly between physiological/biochemical indicators (58%) and the other two
297 groups belonging to higher levels of biological organisation (Fig. 1 and Table 3).
298 Structural and demographic indicators showed the highest percentage of significant
299 responses (75%) followed by growth and morphological indicators (70%).While most
300 indicators recorded a high percentage of response to increased stressor levels (see Table
301 4), a few showed no significant response (C content in epiphytes, $\delta^{13}C$ in rhizomes, and
302 $\delta\ ^{34}S$ in leaves). However, the number of cases for these indicators was too low to
303 adequately evaluate their responses (n=1 or 2).
304

While structural and demographic indicators were very effective in detecting degradation, they were not as effective in signalling the cessation of stressing agents as other indicators at experimental time-scales. They showed responses in 60 % of recorded cases, whereas physiological/biochemical and growth/morphological indicators detected recovery processes in around 80% of the cases (Fig. 1). The proportion of responses to decreased stressor levels among indicators belonging to different biological organisation levels showed a mild difference between small and large species, although it was not significant (interaction between seagrass size and level of biological organisation, $p = 0.09$, Table 5). Indeed, the response of indicators to decreased stressors differed significantly between level of biological organisation in large species but not in small ones (Table 5).

Response time of indicators to degradation and recovery

The response time of seagrass indicators to degradation was dependent on seagrass size interacting with the level of biological organisation and showed a mild, though non-significant difference between stressors (Table 6). In fact, the response time of structural/demographic, growth/morphological and physiological/biochemical indicators to degradation increased with seagrass size (Fig. 2 and 4, Table 4), with structural/demographic parameters showing significantly longer response times for seagrasses with larger rhizome diameters (Seagrass size: level of biological organisation, $p= 0.01$) (Fig. 2, Table 6).

In general, indicators took longer to respond to recovery processes than to degradation conditions for all levels of biological organisation (Fig. 3). This was particularly true for structural indicators that did not recover within the experimental time frame of the

330 studies (Fig. 3). Unfortunately, the data from available studies were insufficient to
331 explore how recovery response times of indicators differed between stressors.
332
333 *General versus specific indicators*
334 Two structural parameters (density and aboveground biomass), one morphological
335 indicator (leaf growth) and one physiological indicator (sucrose concentration in
336 rhizomes) were found to be general indicators of a wide range of stressors for both
337 small and large seagrasses (>60% response, responding to at least 3 stressors) (Table 4).
338 Nitrogen concentration in leaves responded consistently across species, increasing with
339 shading and increasing nutrient availability. Likewise, decreased photosynthetic rates
340 responded to increased loads of organic matter inputs and hypersalinity. The structural
341 indicators shoot mortality and belowground biomass each showed robust responses to
342 two types of stressors; shoot density decreased in response to burial and hypersalinity
343 while below-ground biomass decreased in response to burial and nutrients (Table 4). In
344 contrast, most indicators were much more specific, responding to a single stressor.
345 Physiological/biochemical indicators were particularly good in detecting single stressors,
346 with more than 60% of positive responses. This was true for $\delta^{13}C$ in leaves, $\delta^{15}N$ in
347 leaves, and S concentrations in roots and rhizomes, which appeared to be clearly
348 stressor-specific (Table 4). However, while $\delta^{13}C$ decreased with shading, the time-scale
349 of response was longer than other physiological and biochemical indicators (see Fig. 4).
350 Nutrient addition in small plants resulted in decreased levels of $\delta^{15}N$ in leaves. An
351 important caveat, however, is that $\delta^{15}N$ response is not unidirectional and depends on
352 the $\delta^{15}N$ signal of the source. While the S content in roots and rhizomes of large
353 seagrass species was also a potentially robust indicator – its concentration increased

354 with organic matter loading – it requires independent validation from more studies
355 before it can be fully trusted (Table 4, Fig. 4).
356 Although chlorophyll content, tissue C/N ratios, necrosis in leaf tissues and dark
357 respiration rates showed higher percentages of response (>60%) for one stressor, they
358 cannot be considered stressor-specific since they also responded to other stressors with
359 lower percentages of positive responses (Table 4). Thus, chlorophyll content and tissue
360 C/N ratios while mainly decreasing with nutrient additions also responded to changes in
361 shading. Similarly, necrosis and dark respiration showed potential as indicators of
362 hypersalinity, increasing and decreasing with high salinity, respectively. However,
363 necrosis also increased with nutrient additions, whereas for dark respiration, there were
364 far too few cases available to assess its specificity (Table 4, Fig. 4).
365

366 **4. Discussion**

367 As developmental pressures increase in the coastal ocean, the need to keep track of this
368 change is becoming increasingly acute (Agardy et al., 2005; Carpenter et al., 2009;
369 Erftemeijer and Robin Lewis III, 2006; Martínez-Crego et al., 2008). Our review
370 reflects this growing urgency to document decline, with the vast majority of seagrass
371 indicators developed to measure ecosystem and environmental degradation rather than
372 improving conditions. This bias is perhaps also due to the difficulty of tracking seagrass
373 recovery after the removal of stresses, since recovery responses may take place over
374 considerably longer time scales than most studies allow (e.g. Heide *et al.* 2007; Duarte
375 *et al.* 2009, 2013, this study). Nonetheless, we were able to assess the performance of
376 34 indicators in relation to six of the most common and important drivers of seagrass
377 decline (shading, increased nutrient and organic inputs, burial and hypersaline effluents,
378 see Waycott *et al.* 2009). These are among the stressors of most concern for seagrass
379 managers. Indicators ranged from physiological and biochemical parameters to
380 ecosystem-level measures and included 25 species of seagrass from across the globe.
381 Indicators clearly varied widely in their sensitivity, specificity and response time while
382 tracking degradation and recovery.
383
384 Our meta-analysis shows that most indicators clearly differed in their ability to detect
385 degradation and recovery processes. Thus while more integrative structural and
386 demographic parameters (like shoot density or biomass) were very responsive to
387 degradation from multiple stressors, they were not as effective in reflecting
388 improvements at short management time-scales when these stressors reduced. In
389 contrast, physiological and biochemical indicators were much more effective in
390 documenting recovery processes, particularly for large seagrass species. The underlying

391 ecological processes of degradation and recovery are likely very different. Seagrasses
392 respond predictably to a range of stressors, often with noticeable declines in meadow
393 structure. However, the capacity for seagrasses to recover these structural losses when
394 conditions improve is driven by species-specific demographic rates, largely dependent
395 on plant size (Marbà and Duarte, 1998). It is therefore unsurprising that structural
396 indicators may be ineffective in tracking recovery of environmental conditions
397 (particularly for larger, slow-growing species), since it may often take several decades
398 before these changes are reflected at the level of the meadow (Badalamenti et al., 2011;
399 Meehan and West, 2002) (see later).
400
401 In tracking degradation, physiological/biochemical indicators showed considerable
402 variability in their response, due, at least in part, to their higher stressor specificity.
403 Thus, while highly integrative variables like seagrass shoot density and biomass
404 responded to increased stressor levels across the spectrum of examined stressors,
405 physiological/biochemical parameters like $\delta^{13}C$, $\delta^{15}N$ and S were linked to changes in
406 few or single stressing agents (shading, nutrients or organic matter inputs respectively)
407 (Table 4).
408
409 Most parameters in our review were very reliable indicators of generic or specific
410 stressors. For instance, robust indicators to light disturbances found here were quite
411 consistent with those previously identified by McMahon et al. (2013), with the
412 exception of some physiological and morphological measures, which we attribute to
413 differences in the studies reviewed. However, some measures showed rather limited
414 response for the stressors we tested here. For example, C content in epiphytes or $\delta^{34}S$ in
415 leaf tissue showed no significant responses to shading, nutrients, burial, organic matter

or hypersalinity. Though they may not be useful as indicators of these stressors, they may still respond to stressors not included in our study. For instance, $\delta^{34}S$ in leaf tissue has been shown, experimentally and in the field, to respond to warming (García et al., 2013), and % inorganic carbon in epiphytes may be a useful indicator of ocean acidification (Campbell and Fourqurean, 2014; Fabricius et al., 2011). As CO_2 emissions rise, warming and acidification are likely to increase, making seagrasses and their epiphytes potentially important sentinels of future climate change (Duarte, 2002; Koch et al., 2013).

The time scale of responses differed greatly between indicators, varying with level of biological organisation and plant size. Physiological/biochemical and growth/morphological indicators were generally able to detect degradation responses much faster than structural/demographic indicators, especially for large, slow-growing, seagrass species. This contrast likely reflects the strong hysteresis that operates in many coastal ecosystems as the mechanisms controlling the recovery of indicators differ from those controlling degradation (see Fig. 5- Heide et al. 2007; Duarte et al. 2009, 2013). This is particularly true for structural and demographic indicators in long-lived seagrass meadows (e.g., shoot density, above- and belowground biomass). These meadows are often characterised by positive feedbacks that buffer the structure of the habitat against even relatively high levels of environmental stress. For instance, larger plants have greater reserves, making them better able to resist short-term adverse conditions. Once a particular threshold is breached however, the effects of degradation can accrue very rapidly as the structural integrity of the meadow unravels. Recovery from this point can be protracted, with recovery rates often almost four or five times slower than degradation (Backman and Barilotti, 1976; Collier et al., 2009). As discussed earlier,

441 there is an important size-dependence in seagrass growth, tissue turnover and
442 demographic dynamics (Duarte, 1991) which determines response time of indicators.
443 The time lags imposed by species-specific intrinsic growth rates are further
444 compounded by shifts in ecosystem baselines that further impede or slow down natural
445 recovery (Duarte et al., 2009). In habitats dominated by large, slow-growing species
446 like *Posidonia oceanica*, this recovery may require several decades, if not centuries
447 (Duarte, 2002; González-Correa et al., 2005).
448
449 The natural hysteresis that characterises seagrass ecosystems has important implications
450 when choosing indicators to monitor ecosystem status. Structural and morphological
451 indicators, while responsive to a range of stressors, may, especially for large species,
452 detect impacts much too late for effective action to be taken (van Katwijk *et al.* 2010,
453 this study). Physiological and biochemical parameters are less influenced by hysteretic
454 properties, making them much better early-warning candidates to detect changes (both
455 decline and recovery) in environmental conditions over time-scales relevant for
456 management. However, these indicators, since their response is highly stress-specific,
457 need to be used as part of a set and may not be appropriate to be used on their own.
458
459 **Designing Fit-for-Purpose Seagrass Monitoring Programs**
460 From the discussion above, it is clear that no single indicator can satisfy every
461 management objective. The array of available indicators represents a valuable toolbox
462 from which to choose a set of indicators to match specific management goals. Given the
463 number of indicators available and their differences in specificity, sensitivity and
464 response times, it is unsurprising that selecting the appropriate set of indicators can be
465 perplexing. We provide a generic decision tree to assist this process, following the

potential life cycle of a monitoring programme, when there is no change with respect to reference conditions, and under conditions of change whose source is either known (in some cases even planned) or unknown (Figure 6). Each condition requires a design that employs a contingent set of indicators best suited to the task. In general, the scheme is designed to ensure that the resulting programme (i) provides early warning responses to degradation (Generic ecosystem monitoring strategy), (ii) can attribute changes in indicators to specific pressures (Stress screening strategy), and (iii), detect the onset of ecosystem recovery (Assessment strategy). We suggest sets of potential indicators to match these monitoring strategies used together as a multi-metric index or separately. These sets of indicators serve merely as a general heuristic that will require context-specific tailoring based on management goals, environmental conditions and the seagrass species present. While the objectives of management can vary widely, the figure indicates how this scheme could be employed for typical management scenarios: (i) assessing general trends in ecosystem health, (ii) assessing environmental quality and (iii) assessing impacts or remediation measures. The decision tree allows entry and exit at any point based on needs and circumstances.

Generic ecosystem monitoring strategy Tracking ecosystem health under normal conditions is important to detect unforeseen changes in overall condition and their causes, so that remedial actions can be taken to stop the decline. This is often an essential management mandate and chosen indicators need to be both generic, to detect responses from a wide variety of stresses, and respond rapidly, to serve as an early warning. Structural and demographic indicators have a large integrative capacity and are linked most directly to ecosystem structure and function, making them ideal generic indicators. Indicators such as shoot density, seagrass cover or meadow depth limit are

491 widely used in monitoring programmes (Marbà et al., 2012), and have proven excellent
492 in detecting generalized degradation responses, mostly linked to eutrophication
493 (Martínez-Crego et al., 2008). However, most of these variables respond very slowly.
494 With some exceptions, such as mechanical removal (which directly modifies structure
495 and demographics) changes in structural indicators are the result of changes in the
496 environment first reflected in plant physiology, which modifies seagrass growth and
497 morphology, finally triggering changes in meadow structure and demography (Fig. 5)
498 (Collier et al., 2012), and it can be fairly long before these changes are detectable. As a
499 result, ecosystem monitoring strategy benefit from incorporating early-warning
500 indicators together with these structural measures, especially for large species. Some
501 physiological/biochemical indicators such as sucrose or N respond to a range of
502 stressors and their inclusion can serve as early warnings of eutrophication processes
503 such as shading, nutrients, and organic matter.
504
505 **Stress screening strategy:** Often, when change is registered, for example through a
506 generic ecosystem monitoring, the drivers/stressors for these changes are difficult to
507 establish. Screening strategies help in identifying these drivers using stressor-specific
508 indicators. Many physiological and biochemical parameters are particularly useful here,
509 since they respond reliably to changes in single or few drivers. For instance, $\delta^{13}C$
510 responds only to changes in light availability (Serrano et al., 2011), and S content in
511 roots and rhizomes responds to intrusion of H_2S under organic inputs (although this
512 needs independent confirmation, but see Frederiksen et al., 2008, 2006; Pérez et al.,
513 2007) (Table 4, Fig. 4). While $\delta^{15}N$ mostly responds to variations in nitrogen inputs
514 (Christianen et al., 2012), it may also be influenced by changes in light conditions
515 (Lavery et al., 2009), and while it is a useful stress screening indicator, it needs to be

516 interpreted with caution. In addition, the elemental contents of rhizomes are very
517 reliable indicators of detecting metal variations (Fe, Cd, Pb, Ni, Cu) in the environment
518 (Richir et al., 2013; Roca et al., 2014). Because several of these measures respond
519 predictably to both increasing and decreasing drivers, they are also useful in monitoring
520 improvements in environmental quality. For instance, specific elemental indicators can
521 effectively track reductions in inputs of silver or lead into coastal waters, linked to the
522 advent of digital photography or unleaded fuel, respectively (Tovar-Sánchez et al.,
523 2010). While stressor-specific indicators are generally good at identifying drivers of
524 change, it is useful to include structural and demographic parameters in the monitoring
525 program; used together, these indicators can provide a more accurate assessment of
526 ecosystem function.
527 In addition, since stress specific indicators can respond to more than one driver (e.g.
528 $\delta^{15}N$ to nutrients and light (Lavery et al., 2009), it is advisable to include more than one
529 indicator that responds to the same driver in order to increase the reliability of
530 identifying the relevant stressor.
531 **Assessment strategy:** Assessment strategies are employed when the nature of the
532 stressors is well understood, and the interest of management is to assess impacts or the
533 efficacy of remedial actions. For instance, managers may want to test if stress-reducing
534 interventions are actually working (e.g. reducing nutrients from urban sewage), or may
535 need to evaluate the impact of coastal development projects such as harbour
536 constructions or beach replenishments. In order to detect these effects as early as
537 possible (within weeks or months), monitoring needs to be based on
538 physiological/biochemical indicators that respond rapidly and specifically to the drivers
539 in question (a subset of the screening set, see Fig. 6). These indicators are thus a
540 valuable tool in evidence-based management and can also help managers quickly adapt

541 their interventions based on measured efficacy. As with all strategies, these assessments
542 must also include the more integrative structural/demographic drivers to track potential
543 ecosystem-level effects.
544 In attempting to address these different needs, researchers have developed a suite of
545 synthetic and integrative multi-metric indices to measure ecological status or water
546 quality (García-Marín et al., 2013; Gobert et al., 2009; Lopez y Royo et al., 2010;
547 Romero et al., 2007). While very useful in summarizing ecosystem status, these multi-
548 metric indices still depend eventually on the behaviour and response of their individual
549 constituent indicators. Analysed individually, the detection of indicator trends in
550 environmental or ecological status may be less integrative, but allows for far greater
551 precision than multi-metric indices.
552 **5. Summary and conclusions**
553 Indicators based on seagrass parameters provide robust measures of change, which
554 explains their proliferation and use in monitoring programmes in recent decades. The
555 analyses performed here showed that the 34 indicators we evaluated ranged widely in
556 their responsiveness, relative specificity and response time, dependent largely on the
557 size of the plant and the level of biological organisation of the measured indicator.
558 Taken together, these indicators serve as an invaluable toolbox to address a range of
559 monitoring needs. Employing purpose-specific indicators to match management goals
560 enables the detection of change within weeks to months, allows managers to ascertain
561 the cause of these changes, and provides a means to evaluate recovery after the
562 particular stressor has been reduced. This review establishes objective criteria by which
563 the perplexingly large number of available indicators can be critically assessed and used
564 to monitor and manage globally threatened seagrass ecosystems.
565

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576 **Figure and table footnotes.**

577 **Fig.1.** Percentage of reviewed studies that documented significant responses of
578 indicators to degradation (increased stressor levels) and recovery (decreased stressor
579 levels), classified by indicator type (physiological/biochemical, growth/morphological
580 or structural/demographic). Post-hoc comparisons highlight significantly different
581 indicator types (a and b).

582

583 **Fig.2.** The relationship between response time to increased stress and rhizome diameter
584 for different indicator types (physiological/biochemical, growth/morphological or
585 structural/demographic). Solid lines represent the fitted log-log regression equations for
586 structural and demographic indicators ($R^2 = 0.225$, $P= 4*10^{-9}$), dashed lines represent
587 growth and morphological indicators ($R^2 =0.028$, $P= 0.041$) and dotted line represents
588 physiological and biochemical indicators ($R^2 =0.142$, $P= 5*10^{-7}$).

589

590 **Fig.3.** Mean indicator response time to increased stressor levels and recovery (decreased
591 stressor levels) for each level of biological organization. Error bars represent standard
592 errors. The asterisk indicates significant differences based on model results. Refer to
593 Methods and Results for details on datasets employed and model specifications.

594

595 **Fig.4.** Indicator response times of small and large seagrass species to common stressors.
596 Dots indicate mean response times and bars represent the minimum and maximum
597 observed response times reported in the literature. Black dots represent a negative
598 relationship (an increase in stressor levels results in decreased indicator values), white
599 dots represent a positive relationship (an increase in stressor levels result in increased
600 indicator values) and black and white dots represent situations when both positive and

601 negative relationships were reported. Rhiz suc = Sucrose in rhizomes, A. biomass =
602 Aboveground biomass, B. biomass = Belowground biomass, Dark resp = Dark
603 respiration, Photosyn rate = Photosynthetic rates.
604
605 **Fig. 5.** Degradation and recovery pathways in response to variations in environmental
606 stress. (a) Responses of structural and demographic indicators; small seagrass species
607 (blue dashed line) respond faster to environmental improvements than large species
608 (blue solid line). (b) Physiological and biochemical indicators are more quick to respond
609 to degradation and improvement of environmental conditions and show less hysteresis
610 than structural and demographic indicators.
611
612 **Fig. 6.** Designing a fit-for-purpose seagrass monitoring program. Above: Decision tree
613 to help choose monitoring strategies based on three common management objectives.
614 Below: Sets of suggested indicators corresponding to each management objective in the
615 decision tree above. A single asterisk (*) represents indicators not tested in our study
616 but widely used and accepted, a double asterisk (**) represents stressor-specific
617 indicators that require further testing. A. biomass = Aboveground biomass, B. biomass
618 = Belowground biomass, EIA: Environmental Impact Assessment.
619
620 **Table 1.** Number of cases (Nº cases) and sources for indicator response time to
621 degradation (increased stress levels) and recovery (decreased stress levels) for different
622 species. See table references.
623
624 **Table 2**. The 85 indicators compiled in the study classified in three different levels of
625 biological organization. N: number of cases. APA: Alkaline phosphatase, Ek= Light

626 saturation, Etr= Electron Transport Rate, Max and min fluorescence, Above.=
627 aboveground, Below.= belowground, Fv/Fm: chlorophyll fluorescence measurement,
628 LAI= leaf area index.
629
630 **Table 3.** Results of analyses of variance (Type III tests) of percentage of responses (%)
631 to increased stressor levels of seagrass indicators in relation to seagrass size (as
632 reflected by rhizome diameter). Biological organisation refers to either structural and
633 demographic, growth and morphological, or physiological and biochemical indicators.
634 Seagrass size:level of biological organization = Interaction between rhizome diameter
635 and level of biological organization. The percent response (%) was fitted to a binomial
636 distribution. DF (degrees of freedom), DenDF (denominator DF). For further details,
637 refer to Methods.
638
639 **Table 4.** List of robust and potentially robust indicators to degradation. Number of
640 cases, percentage of indicator response to increased stressor levels and associated
641 indicator response time (weeks) are shown only for the most robust indicators (%
642 response >60) and potential indicators for each driver. For example, we recorded 5
643 cases of Leaf N measured in shading experiments, of these 100% (all 5 cases)
644 responded with changes in Leaf N. In subsequent columns we indicate the minimum
645 and maximum response time recorded in these experiments for large and small seagrass
646 species. Level = level of biological organization, Physiological = physiological and
647 biochemical, Morphological = growth and morphological, Structural = structural and
648 demographic, A. Biomass = Aboveground biomass, B. Biomass = Belowground
649 biomass, References = references used in each line (see table1). Indicators marked with
650 an asterisk (*) are potentially robust indicators, but have limited sample cases.

651

652 **Table 5.** Results of analyses of variance (Type III tests) of indicator recovery response
653 (%) in relation to level of biological organization (structural and demographic, growth
654 and morphological, physiological and biochemical) for all species together, large
655 species and small species. All three models are fitted to a binomial distribution. The
656 analysis of all species also includes the effect of seagrass size (as reflected by rhizome
657 diameter). DF (degrees of freedom), LR Chi (likelihood ratio Chi squared test). For
658 further details, refer to Methods.

659

660 **Table 6.** Results of analyses of variance (Type III tests) on indicator response time (top)
661 and recovery time (bottom) in relation to seagrass size (as reflected by rhizome
662 diameter), level of biological organization (structural and demographic, growth and
663 morphological, physiological and biochemical) and type of environmental stressor.
664 Seagrass size: level of biological organization = Interaction between rhizome diameter
665 and level of biological organization. Response time was fitted to a Poisson distribution
666 and recovery time to a quasi-Poisson distribution with an overdispersion parameter
667 taken to be 29.3). DF (degrees of freedom), LR Chi (likelihood ratio Chi squared test).
668 For further details, refer to Methods.

669

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848

Table 1. Number of cases (Nº cases) and sources for indicator response time to degradation (increased stress levels) and recovery (decreased stress levels) for different species. (Size. rhiz. diam, cm): size of rhizome diameter in centimetres. See table references.

		Indicator degradation response		Indicator recovery response	
Species	**Size (rhiz. diam, cm)**	**Nº cases**	**References**	**Nº cases**	**References**
Amphibolis griffithi	2	28	1	31	1
Cymodocea nodosa	3	92	2, 3, 4, 5, 6, 7, 8	7	6, 5, 2
Cymodocea rotundata	2.4	3	9	0	
Cymodocea serrulata	2	17	10, 11, 12	0	
Enhalus acoroides	14.1	9	9	0	
Halophila engelmani	-	1	13		
Halophila johnsonii	-	3	14	0	
Halophila ovalis	1.5	43	12, 15, 16,17	15	15, 16
Halophila pinnifolia	1.5	6	18	0	
Halophila spinulosa	1	2	10	0	
Halophila tasmanica	1.74	4	19	0	
Halodule uninervis	1.4	39	10, 11, 20, 12	2	16
Halodule wrightii	1.6	9	21, 22, 23	2	23
Posidonia australis	7.2	5	24, 25, 26	2	25, 26
Posidonia oceanica	9.7	133	27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40	16	29, 41, 30, 42, 27, 39
Posidonia sinuosa	5.5	26	43, 44, 25, 45	12	43, 25, 45
Ruppia maritima	-	7	21, 22	0	
Syringodium isoetifolium	1.7	12	10, 46, 12	1	46
Thalassia hemprichii	3.6	17	9, 11	0	
Thalassia testudinum	6	53	47, 48, 49, 50, 51, 52-54	4	47
Zostera capricorni	1.4	10	10, 55	0	
Zostera japonica	1	2	56	2	56
Zostera marina	3.5	98	57, 58, 41 21, 59, 60, 61, 62, 63, 64, 65, 66, 67	6	41, 64, 65
Zostera muelleri	2	10	11, 68	0	
Zostera noltii	1.6	49	69, 70, 2, 71, 72, 73	3	72, 73

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Table 2. The 85 indicators compiled in the study classified in three different levels of biological organization. N: number of cases. APA: Alkaline phosphatase, Ek= Light saturation index, Etr= Electron Transport Rate, Max and min fluorescence, Above.= aboveground, Below.= belowground, Fv/Fm: chlorophyll fluorescence measurement, LAI= leaf area index.

Physiological and biochemical						**Morphological and growth**		**Structural and demographic**	
	N		N		N		N		N
Amino acid content	2	Dark respiration	4	P roots	1	Internode distance	2	Above. biomass	41
APA leaf	1	Ek	4	P total	1	LAI	4	Below. biomass	30
C rhizomes	1	Etr	2	Na	1	Leaf growth	72	Cover	10
C leaf	14	Fe rhizomes	2	Pb rhizomes	1	Leaf length	18	Depth limit	6
C/N aboveground	6	Fe leaf	2	Phenolics	1	Leaf necrosis	7	Leaf biomass	17
C/N belowground	11	Fv/Fm	3	Photosynthesis rates	16	Leaf number	23	Leaf density	78
C/N_leaf	6	K content	1	Quantum yield	2	Leaf thickness	4	Mortality	13
Ca	2	Max fluorescence	2	S leaf	2	Leaf width	15	Rhizome biomass	4
Carotenoids	2	Min fluorescence	2	S rhizomes	4	Mean canopy height		Root biomass	4
Cd rhizomes	1	Mg rhizomes	1	S roots	4	Plastochrone interval	1	Shoot biomass	14
Chlorophyla a	18	Mn rhizomes	1	Starch leaf	6	Rhizome elongation	1		
Chloroplast density	1	N leaf	23	Starch rhizomes	11	Root length	1		
Cu rhizomes	1	N rhizomes	18	Starch roots	6	Root/shoot ratio	1		
δ^{13}C leaf	12	N roots	2	Sucrose leaf	6	Shoot size			
δ^{13}C rhizomes	6	N total	1	Sucrose rhizomes	11				
δ^{13}C shoots	6	N/P aboveground	6	Sucrose roots	9				
δ^{15}N leaf	4	N/P belowground	3	Total carbohydrates	2				
δ^{15}N rhizomes	10	Ni rhizomes	1	Zn leaf	2				
δ^{34}S leaf	5	P rhizomes	2	Zn rhizomes	2				
δ^{34}S rhizomes	4	P leaf	13						
δ^{34}S roots	2	P rhizomes	6						

Table 3. Results of analyses of variance (Type III tests) of percentage of responses (%) to increased stressor levels of seagrass indicators in relation to seagrass size (as reflected by rhizome diameter). Biological organisation refers to either structural and demographic, growth and morphological, or physiological and biochemical indicators. Seagrass size:level of biological organization = Interaction between rhizome diameter and level of biological organization. The percent response (%) was fitted to a binomial distribution. DF (degrees of freedom), DenDF (denominator DF). For further details, refer to Methods.

Response %	DF	DenDF	F.value	P.value	
Level of biological organization	2	630	5.29	0.005	**
Stressor	4	93	0.79	0.537	
Seagrass size	1	1	68.2	0.23	
Seagrass size : Level of biological organization	2	630	1.20	0.303	

Significance level: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

					Indicator response time (weeks)				References
					Large species		Small species		
Stressor	**Level**	**Robust indicators**	**N**	**% Response**	**Min**	**Max**	**Min**	**Max**	
Shading	Physiological	Leaf N	5	100	8	24	2	8	29,44,48,55,63
	Physiological	Rhizome N	7	85	-	-	2	12	1,29,45,71
	Physiological	Rhizome sucrose	10	88	3	15	0.5	2	1,15,29,45,71
	Physiological	Leaf $\delta^{13}C$	7	100	28	28	4	11	10,18,32,45,55,
	Growth	Leaf growth	30	76	1	20	1	8	1,11,15,19,26,29,32, 43,45,48,55, 63,71
	Structural	Density	27	85	4	36	2	11	11,15,18,19,26,29,32, 43,44,45,48,55,58,63, 64
	Structural	A. biomass	17	88	6	29	1	15	1,11,15,45,48,58,
Nutrients	Physiological	Leaf N	16	75	4	24	1.4	14	4,9,20,21,37,38,48, 52,59,60,62
	Physiological	Rhizome N	7	85	32	32	8	20	9,20,21,34
	Physiological	Chlorophyll a	5	80	5	20	20	20	9,61,62
	Physiological	C/N	5	80	3	12	-	-	21,48
	Physiological	Rhizome sucrose*	4	100	14	24	-	-	21,34
	Physiological	Leaf $\delta^{15}N$ *	1	100	-	-	8	8	20
	Growth	Leaf growth	18	78	1	20	2	14	4,9,21,33,34,48,49, 50,59,60
	Structural	Density	15	73	5	12	4	24	4,9, 21,22,48,50, 61,62
	Structural	A. biomass	12	58	6	48	8	24	9,20,48, 50,52,58
	Structural	B. biomass*	4	100	6	48	8	8	20,48,52,58
Burial	Structural	Mortality	10	100	-	-	3	4	69,70
	Structural	Density	20	65	1	36	2	5	8,12,27,28,30,69
	Structural	A. biomass	13	85	-	-	4	15	4,5
	Structural	B. biomass	13	77	-	-	4	4	8,12
OM	Physiological	Rhizome sucrose	10	60	2	12	-	-	35,57
	Physiological	Photosyntesis*	3	100	1	1	-	-	57,66
	Physiological	Roots S*	2	100	12	12	-	-	35
	Physiological	Rhizome S*	2	100	12	12	-	-	35
	Growth	Leaf growth*	4	75	2	2	-	-	50,53,57
	Structural	A. biomass*	3	67	24	24	-	-	50
	Structural	Density*	3	67	12	12	-	-	35,50
Hypersalinity	Physiological	Photosyntesis rate	5	100	7	12	0.14	7	3,7,14,17,39,67
	Physiological	Dark respiration*	3	66	7	7	7	7	7,39
	Growth	Leaf growth	6	100	4	12	1	2	2,3,14,31,39,40, 54
	Growth	Necrosis*	3	66	7	7	-	-	7,39,40,67
	Structural	Mortality	7	71	12	12	1	2	2,3,7,14,39,40,67
	Structural	Density*	2	100	5	8	-	-	31,62

Table 4. List of robust and potentially robust indicators to increased stressor levels. Number of cases, percentage of indicator response to degradation and associated indicator response time (weeks) are shown only for the most robust indicators (% response >60) and potential indicators for each driver. For example, we recorded 5 cases of Leaf N measured in shading experiments, of these 100% (all 5 cases) responded with changes in Leaf N. In subsequent

columns we indicate the minimum and maximum response time recorded in these experiments for large and small seagrass species. Level = level of biological organization, Physiological = physiological and biochemical, Morphological = growth and morphological, Structural = structural and demographic, A. Biomass = Aboveground biomass, B. Biomass = Belowground biomass, References = references used in each line (see table1). Indicators marked with an asterisk (*) are potentially robust indicators, but have limited sample cases.

Table 5. Results of analyses of variance (Type III tests) of indicator recovery response (%) in relation to level of biological organization (structural and demographic, growth and morphological, physiological and biochemical) for all species together, large species and small species. All three models are fitted to a binomial distribution. The analysis of all species also includes the effect of seagrass size (as reflected by rhizome diameter). DF (degrees of freedom), LR Chi (likelihood ratio Chi squared test). For further details, refer to Methods.

Recovery % (all species)	LR Chi	DF	P.value	
Level of biological organization	0.1738	2	0.91676	
Seagrass size	0.5283	1	0.46733	.
Seagrass size: Level of biological organization	4.6562	2	0.09748	

Recovery % (large species)	DF	Deviance	P.value	
Level of biological organization	2	7.6594	0.021	*
Residuals	39	47.088		

Recovery % (small species)	DF	Deviance	P.value
Level of biological organization	2	1.98	0.371
Residuals	54	56.69	

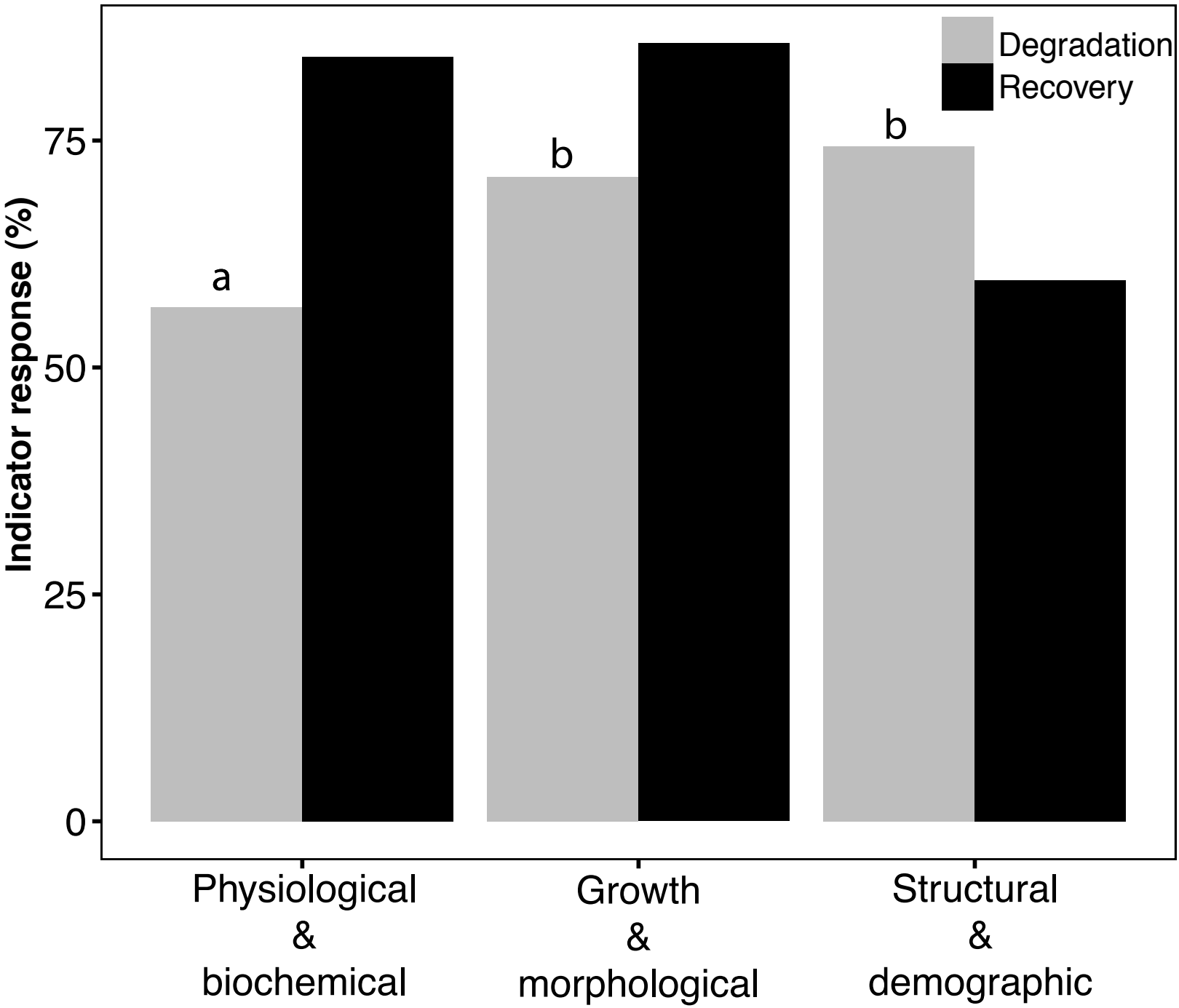
Significance level : '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

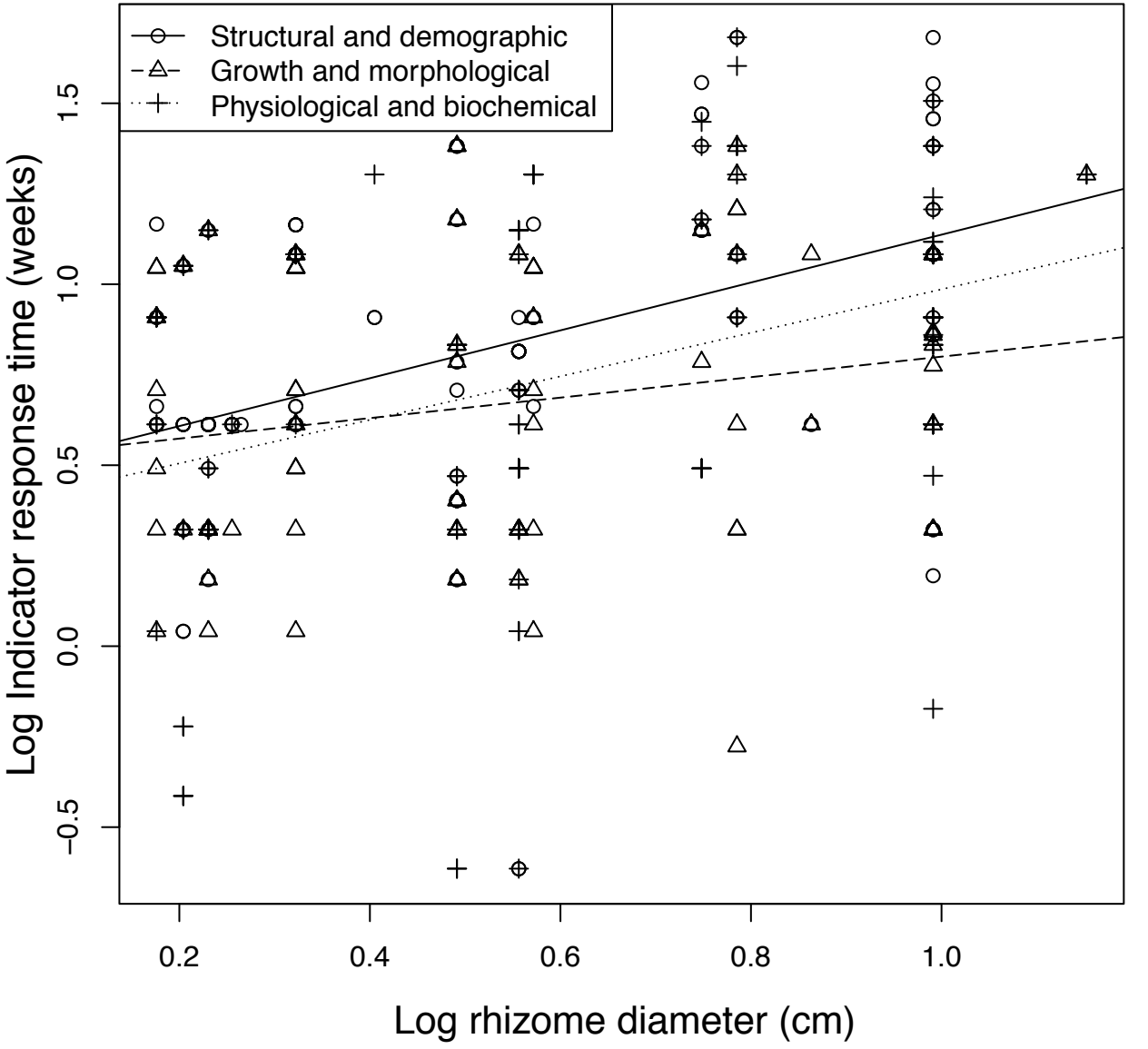
Table 6. Results of analyses of variance (Type III tests) on indicator response time (top) and recovery time (bottom) in relation to seagrass size (as reflected by rhizome diameter), level of biological organization (structural and demographic, growth and morphological, physiological and biochemical) and type of environmental stressor. Seagrass size: level of biological organization = Interaction between rhizome diameter and level of biological organization. Response time was fitted to a Poisson distribution and recovery time to a quasi-Poisson distribution with an overdispersion parameter taken to be 29.3). DF (degrees of freedom), LR Chi (likelihood ratio Chi squared test). For further details, refer to Methods.

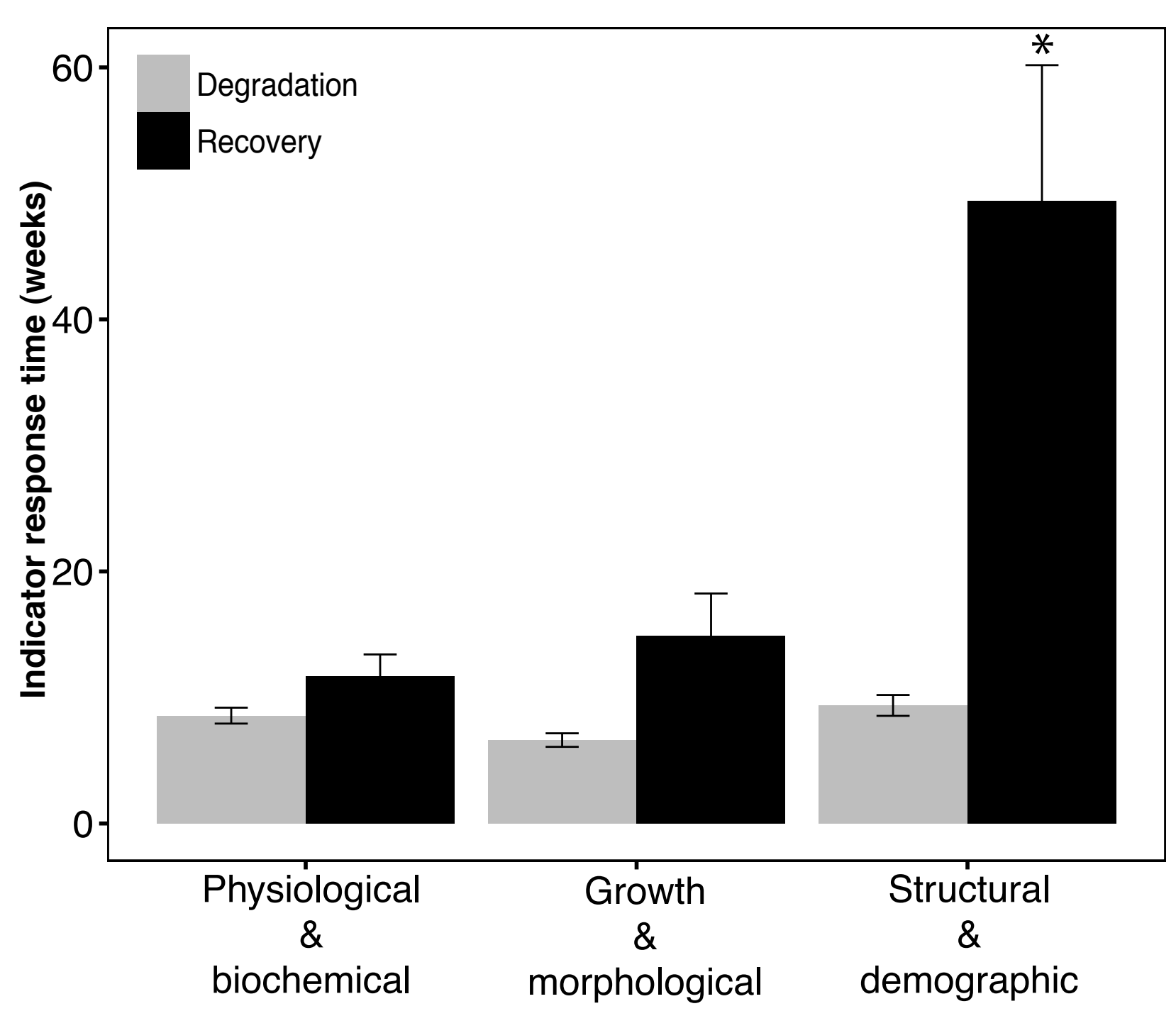
Response time	DF	DenDF	F.value	P.value	
Level of biological organization	2	346.7	0.09	0.91	
Stressor	4	80.7	2.36	0.06	.
Seagrass size	1	56.2	18.91	1.00E-04	***
Seagrass size : Level of biological organization	2	346.9	4.57	0.01	*

Recovery time	LR Chisq	Df	P.value	
Level of biological organization	16.8057	2	0.0002242	***
Seagrass size	2.2123	1	0.1369122	
Seagrass size: Level of biological organization	1.8116	2	0.4042195	

Significance level: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


Degradation
Recovery
Indicator response (%)
75
50
25
0
a
b
b
Physiological & biochemical
Growth & morphological
Structural & demographic


Structural and demographic
Growth and morphological
Physiological and biochemical
Log Indicator response time (weeks)
−0.5
0.0
0.5
1.0
1.5
0.2
0.4
0.6
0.8
1.0
Log rhizome diameter (cm)

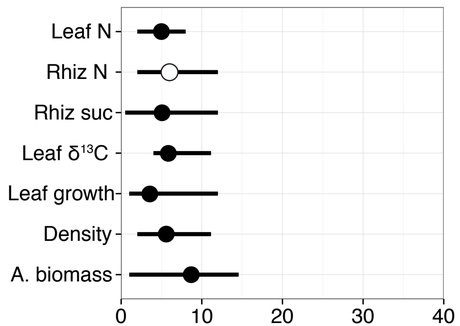

Degradation
Recovery
Indicator response time (weeks)
60
40
20
0
*
Physiological & biochemical
Growth & morphological
Structural & demographic

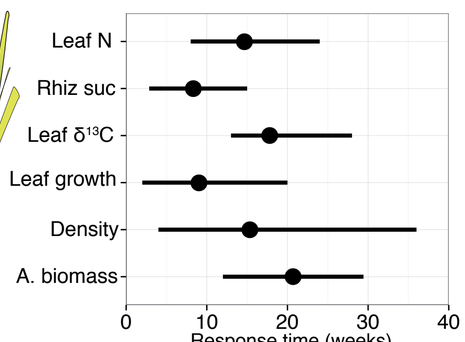
Indicator response time to stressors

Small

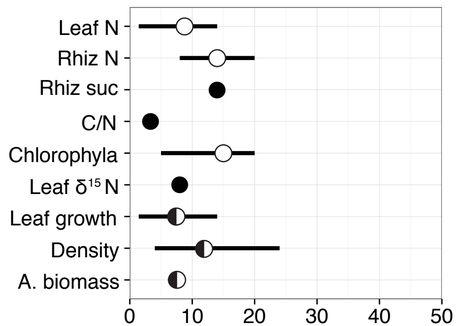
Large

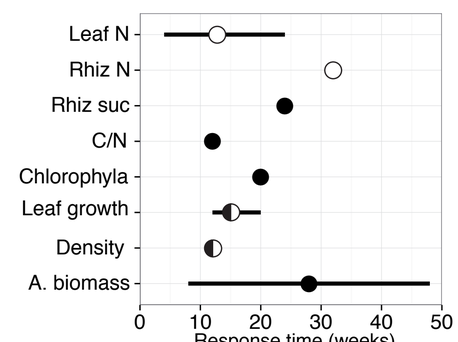
Shading



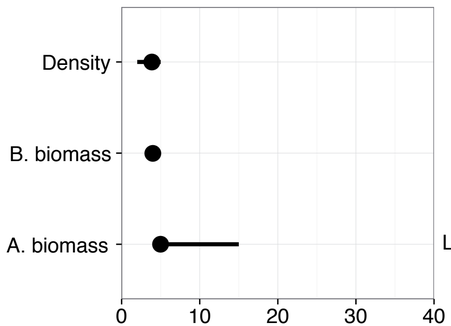


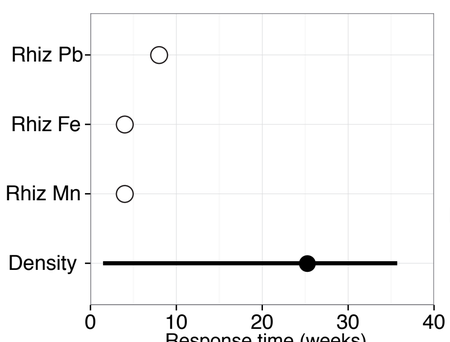
Nutrients



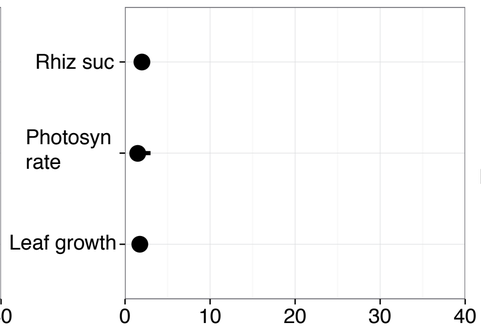


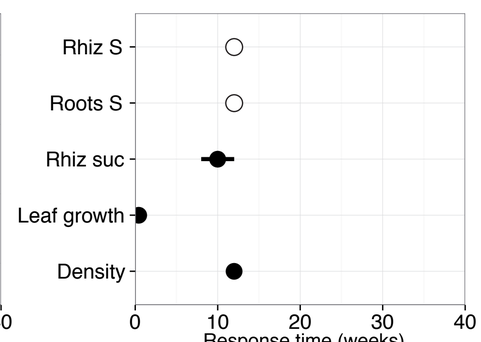
Burial



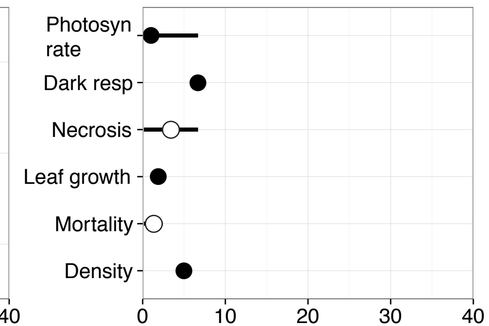


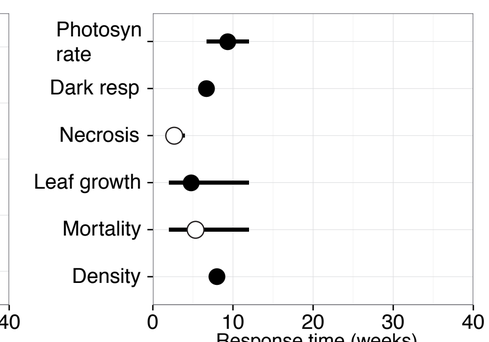
Organic Matter





Hypersalinity





○ Positive relationship ● Negative relationship ◐ Positive and negative relationships

a

Response time to degradation (months)

Large

Degradation

Small

Small

Recovery

Large

Response time to stress reduction for small species (months)

Response time to stress reduction for large species (years-decades)

Structural & demographic indicators

Environmental Stress

b

Early-response to degradation
(weeks)

Degradation

Recovery

Early-response
to ecosystem improvement

(weeks)

Physiological & biochemical indicators

Environmental Stress

Management Objective: Assess general trends in ecosystem status

Management Objective: Asses environmental quality

Management Objective: Assess impacts or evaluate management actions (eg EIA)

Generic Ecosystem Monitoring Strategy (1)

change

no change respect to reference conditions

change agent (stressor) unknown

Stress Screening Strategy (2)

change agent (stressor) known or identified

intervention

Assessment Strategy (3)

normal conditions established

Strategy	1 Generic Ecosystem Monitoring	2 Stress Screening	3 Assessment
Indicators	Structural indicators: - Density, cover, depth limit, A. and B. biomass + Generic early-warning indicators: - N or Sucrose	Stressor specific set - Eutrophication: N, C/ N, Chlorophyll δ^{15}N leaf - Shading: δ^{13}C leaf, Sucrose - Organic inputs: S rhizomes, δ^{34}S** - Hypersalination: Photosynthesis rate, Dark respiration - Burial: Rhizome elongation* - Metal pollution: Pb, Fe, Mn, Cd, Cu, Zn, Ni + Structural indicators: Density, cover, A. and B. biomass, depth limit	Stress related specific indicators from (2) + Structural indicators: - Density, cover, depth limit, A. biomass, B. biomass