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EDITED BY

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REVIEWED BY

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Sevilla University (Spain) and
Mohammed V University
(Morocco), Spain
Joanne Irene Ellis,
University of Waikato, New Zealand

*CORRESPONDENCE

Vasco M. N. C. S. Vieira
vasco.vieira@tecnico.ulisboa.pt

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Seagrasses benefit from mild anthropogenic nutrient additions

Vasco M. N. C. S. Vieira^{1*}, Jorge Lobo-Arteaga^{2,3},
Rafael Santos^{2,4}, David Leitão-Silva^{2,4}, Arthur Veronez^{5,6},
Joana M. Neves^{2,3}, Marta Nogueira²,
Joel C. Creed⁷, Chiara M. Bertelli⁸, Jimena Samper-Villarreal⁹
and Mats R. S. Pettersen¹

¹Marine Environment and Technology Center, Instituto Superior Técnico, Universidade Técnica de Lisboa, Lisbon, Portugal, ²Portuguese Institute for Sea and Atmosphere, Algés, Portugal, ³Marine and Environmental Sciences Centre, Universidade Nova de Lisboa, Caparica, Portugal, ⁴Barreiro School of Technology, Polytechnic Institute of Setúbal, Lavradio, Portugal, ⁵Centre for Functional Ecology, Universidade de Coimbra, Coimbra, Portugal, ⁶Centro de Ciências do Mar (CCMAR) - Universidade do Algarve, Faro, Portugal, ⁷Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil, ⁸Department of Biosciences, Swansea University, Swansea, United Kingdom, ⁹Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), Universidad de Costa Rica, San José, Costa Rica

Seagrasses are declining globally, in large part due to increased anthropogenic coastal nutrient loads that enhance smothering by macroalgae, attenuate light, and are toxic when in excessive concentrations of inorganic nitrogen and phosphorus. However, as sanitation is improved many seagrass meadows have been observed to recover, with a few studies suggesting that they may even benefit from mild anthropogenic nutrient additions. Monitoring seagrass demography and health has faced difficulties in establishing the adequate variables and metrics. Such uncertainty in the methods has caused uncertainty of the significance of results presented and compromised extrapolations to other seasons, areas, or species. One solution has come from within the plant self-thinning theories. During the 1980s, an interspecific boundary line (IBL) was determined as the upper limit of the combination of plant density and above-ground biomass for any stand on Earth, setting their maximum possible efficiency in space occupation. Recently, two meta-analyses to determine specific IBLs for algae and for seagrasses have been performed. The recently updated seagrass dataset comprises 5,052 observations from 78 studies on 18 species. These IBLs opened new perspectives for monitoring: the observed distance of a stand to the respective IBL (i.e., each stand's relative efficiency of space occupation) was demonstrated to be a valuable indicator of a population's health. Thus, this metric can be used to determine the impact of nutrients and pollutants on algae and seagrass populations. Furthermore, because the IBLs are common to all species, they may be used to compare all species from any location worldwide. This novel approach showed that *Halodule wrightii*, *Halodule beaudettei*, *Halophila baillonii*, *Zostera marina*, and *Zostera noltei* meadows benefit from anthropogenic additions of nitrogen and phosphorus, as long as

these additions are moderate. In fact, the healthier *Z. noltei* meadows in Portugal (and among the healthiest meadows worldwide) were the ones exposed to effluents from wastewater treatment plants (WWTP) and a food factory. We conclude that those effluents are providing water with enough quality and that their optimal management should coordinate the technological solutions of the WWTP with the natural potential of seagrass meadows as water purifiers and biomass producers.

KEYWORDS

seagrass, water quality, indicator, coastal, eutrophication, monitoring, mitigation, valuation

Introduction

Seagrass meadows are one of the most common marine coastal biotopes, representing hotspots for biodiversity and productivity (Morrison et al., 2014; Hyman et al., 2019; McHenry et al., 2021; Surugiu et al., 2021). Seagrasses are keystone species in these biotopes, providing many services to both ecosystems and human populations. The most common are nursery and shelter for fish, bivalves, arthropods, and marine invertebrates—some of them commercially exploited—food, and water purification (Morrison et al., 2014; Nordlund et al., 2016). Recently, seagrass meadows have been revealed as an important carbon sink (Bedulli et al., 2020; Ricart et al., 2020) as well as a sustainable source of renewable raw material and energy for construction and industry (Ntalos and Sideras, 2014; Plis et al., 2016; Hamdaoui et al., 2018; Jedidi and Abrough, 2020; Rammou et al., 2021), and soilless agriculture (Parente et al., 2014).

Seagrasses have declined globally during the 20th century due to increased anthropogenic impacts, especially those affecting water quality. The most common negative impact is increased nutrient load to coastal systems originating from agricultural runoff and urban effluents. In many cases, this has led to eutrophication, resulting in smothering by drift or epiphytic macroalgae, light attenuation due to increased phytoplankton and turbidity, and the toxic effects of excessive concentrations of inorganic nitrogen (N) and phosphorus (P) (Burkholder et al., 2007; Lee et al., 2007). Other negative impacts include clam harvesting (Cabaço et al., 2005; Garmendia et al., 2017), herbicides (McMahon et al., 2005; Waycott et al., 2005; Negri et al., 2015; Espel et al., 2019), pesticides (Bester, 2000; Waycott et al., 2005; Espel et al., 2019), and industrial contamination (Waycott et al., 2005; Fraser and Kendrick, 2017; Espel et al., 2019; Lafratta et al., 2019). However, evidence is accumulating on the recovery of some seagrass beds worldwide (Greening et al., 2014; Bertelli et al., 2018; Burdick et al., 2020; Orth et al., 2020). This trend is particularly true for European seagrasses, for which a reversal was determined from the 2000s onward (de los

Santos et al., 2019). This improvement was likely associated with the significant European-wide efforts that took place through the latter decades to improve sanitation and protect coastal habitats. A few studies have suggested that seagrass stands may even benefit from mild anthropogenic nutrient additions (Cabaço et al., 2013; Vieira et al., 2018).

Inference about the demography and health of seagrass stands is complex as we face difficulties in finding adequate variables, metrics, and models. Often, studies have been performed exclusively on a single species, comprising only a narrow band of its geographical distribution, and sometimes over only part of the seasonal cycle (Cabaço et al., 2007, 2008; Romero et al., 2007; García-Marín et al., 2013; Jones and Unsworth, 2016; Jones et al., 2018). This has brought great uncertainty on the significance of the results presented and compromised extrapolations to other seasons, geographical areas, or species. Broader studies still face problems with numerical methods. Examples of this are the debate between Cabaço et al. (2013) and Vieira et al. (2015), or inadequate analyses such as flawed PCA applications lacking criteria to screen the meaningful principal components and the variables significantly contributing to each of these components. Concomitantly, the efficiency of space occupation and its related phalanx and guerrilla clonal growth forms were used as indicators of the health of seagrass meadows (Sintes et al., 2005) as well as of meadows of other clonal plants in wetlands and their response to environmental stressors (Chen et al., 2011, 2014, 2017; Geremew et al., 2018). Healthier meadows adopt the phalanx strategy by simultaneously growing new shoots and increasing their biomass. Weak or unhealthy stands adopt the guerilla strategy by simultaneously losing shoots and decreasing their biomass. One aspect that has been at the core of monitoring of seagrass systems has been the widespread use of seagrass population parameters as indicators of health. Following on from that has been the attempt to establish a biomass–density relationship for seagrasses and place it on the core of the metrics and models inferring the health of seagrass stands (Cabaço et al., 2007, 2008, 2013; Romero et al., 2007;

García-Marín et al., 2013; Jones and Unsworth, 2016, 2019; Jones et al., 2018; Vieira et al., 2018).

Biomass–density relationships started to be investigated in the plant kingdom from the 1960s onward (Yoda et al., 1963; White and Harper, 1970). Some patterns emerged about how biomass and density interact through intraspecific competition and plant growth which led to some generalizations. The most outstanding aspects were the “Law of Constant Final Yield” and the theory related to self-thinning, the concurrent increase in biomass with reduction in density due to mortality through intraspecific competition. The dynamic self-thinning trajectory of plant stands, also known as the “-3/2 power law” (Hutchings, 1979; Weller, 1989), was at the time considered the only theory in ecology worthy of being named a “law” (Harper quotea in Hutchings, 1983). Alongside this, an interspecific boundary line (IBL) was determined as the upper limit for any plant stand on Earth, thus setting the maximum for efficient packing of biomass in space in plant stands (Weller, 1989; Scrosati, 2000). Recently, revised IBLs have been determined for algae (Creed et al., 2019) and for seagrasses (Vieira et al., 2018, 2019). Both the self-thinning theory and IBL determination rely on the stand biomass (B) vs. density (D) plotted on log scales, i.e., $\log_{10}B = a + b \times \log_{10}D$. In the case of seagrasses, “density” refers to shoots·m⁻² and its original IBL had constants $a = 4.569$ and $b = -0.438$ (Vieira et al., 2018). The determination of IBL specific of algae and of seagrasses allowed updated analytical tools offering new perspectives in the research and monitoring of these taxa as well as their communities and ecosystems. Given that the IBL sets the maximum space occupation efficiency, each stand’s distance to the IBL has been used as a robust indicator of how good/healthy (small distance) or how bad/impacted (large distance) the stand is. Following this rationale, the distance to the IBL has been shown as a valuable tool to access the effects of nutrients, pollutants, seasonality, light, temperature, and depth in algae (Creed et al., 2019) and seagrass (Vieira et al., 2018; Creed et al., 2019). Furthermore, because the seagrass IBL is common to all seagrass species, using the distance to the IBL (d_{grass}) allows different seagrass species to be analyzed together.

Here, we use the d_{grass} metric as an indicator in order to examine the health of seagrass stands subject to varying concentrations of nitrogen and phosphorus as a consequence of anthropogenic additions to natural background levels. We reanalyze data from previous publications of *Zostera noltei* Hornemann, 1832, *Zostera marina* Linnaeus, 1753, *Halodule wrightii* Asch., *Halodule beaudettei* Hartog, *Halophila baillonii* Ascherson, 1874, and *Cymodocea nodosa* (Ucria) Asch and analyze our own new data from *Z. noltei* meadows in the Tagus and Sado estuaries, Portugal.

Methods

This study includes results of monitoring of *Z. noltei* in Portugal, *Z. noltei* and *C. nodosa* in Italy, *Z. marina* in the British Isles, *H. wrightii* in Brazil, and *H. beaudettei* and *H. baillonii* in

Costa Rica. The monitoring of *Z. noltei* in the Tagus and Sado estuaries, Portugal, was an unpublished experiment performed during the summer of 2021. Therefore, below we present the methods in detail. The remaining studies with *Z. noltei*, *Z. marina*, *H. wrightii*, *H. beaudettei*, and *H. baillonii* have already been published. Therefore, we present only the fundamental aspects of those studies. Further details about their field sampling and laboratory procedures are provided in their respective publications.

Z. noltei was monitored in the Tagus and Sado estuaries, Portugal (Figure 1A), during the summer of 2021. Sampling took place during July because that is when meadows peak their biomass, density, efficiency of space occupation, and health (Vieira et al., 2018). The rivers Tagus and Sado have meso-tidal estuaries, with a mean tidal amplitude of 2.4 m. Detailed characterizations are available of the geography and hydrodynamics of the Tagus (Dias et al., 2013) and Sado (Biguino et al., 2021) estuaries. Roughly 3 million people live in the region, mainly around the Tagus estuary, where the Portuguese capital, Lisbon, is located. Natural sources of nutrients to these systems are riverine discharge and tidal transport of nutrients upwelled in the coastal ocean during the summer upwelling season. The main anthropogenic sources of nutrients are urban effluents and agriculture, the latter being most intense in livestock and rice fields. The Tagus monitoring included nine stations (Figure 1B). Stations “T.AL1” and “T.AL2” were inside the Tagus Estuary Natural Reserve and thus expected to be the most pristine sites. Station “T.AL3” was along the Alcochete city margin. Stations “T.Sam1,” “T.Sam2,” and “T.Sam3” were next to the Samouco locality. Station “T.Sex” was located at the Seixalinho wastewater treatment plant (WWTP) runoff. Station “T.Rib1” was located at the Ribeirvalves fish factory runoff, and “T.Rib2” was approximately 300 m away. All stations were located at mid intertidal heights. The Sado monitoring included six stations (Figure 1C). Station “S.ST” was along the main channel, closest to the estuary inlet and next to the “Sol Troia” resort. It was the only station located at the lower intertidal limit, only emerged during spring tides. In the Sado estuary, stations “S.Com1,” “S.Com2,” and “S.Com3” were located along the Comporta channel whereas stations “S.Cr1” and “S.Cr2” were located in the main channel next to the Carrasqueira village. All these stations were located at mid intertidal heights. The area next to the Comporta and Carrasqueira villages, and landward for tens of km along the main channel is intensively occupied by rice fields. In each location, three cores (11 cm diameter, 20 cm length) were taken from the center of the seagrass patch. Hence, the three distinct d_{grass} observations corresponded to the three cores taken at each location. While immersed, each location was sampled for water properties (temperature, N, P, and Si) three times: soon after immersion, during peak high tide, and soon before emersion. The data from these three time instances were averaged, hence the nutrient concentrations common to all three data points from each location. In the laboratory, the seagrasses were cleaned from sediment and measured for counts of shoots and nodes. Above-ground and below-ground biomasses were separated, dried in the

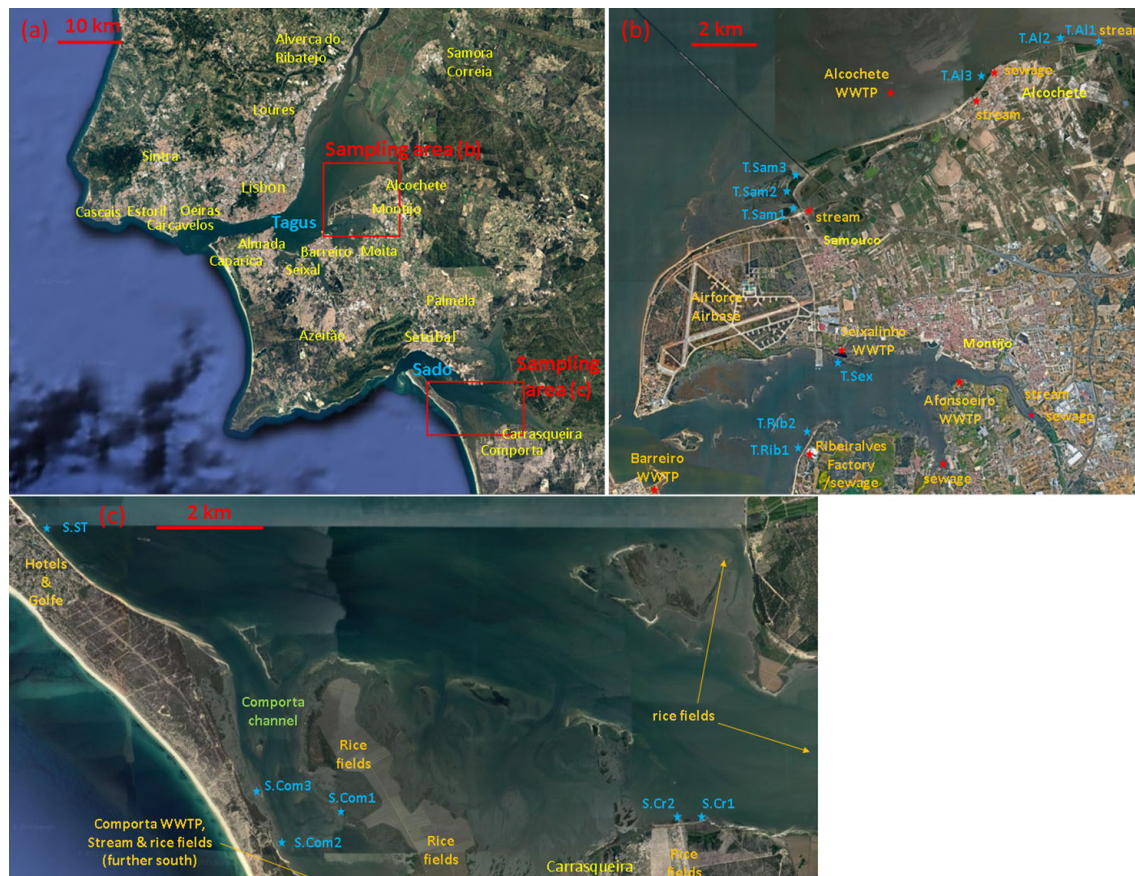


FIGURE 1

Region of study with the Tagus and Sado estuaries, urban areas, locations of sampling sites, and anthropogenic sources of nutrient enrichment. Aerial photos obtained from Google Maps. (A) Lisbon and Setúbal metropolitan areas with Tagus and Sado estuaries, (B) sampling locations in the Tagus estuary, and (C) sampling locations in the Sado estuary.

oven for 48 h at 60°C, and weighed. Samples for nutrients in the water column were filtered through a 0.45- μm membrane (Whatman cellulose acetate) to 25-ml plastic bottles and frozen until analysis in the laboratory. Ammonium and phosphates were analyzed by colorimetry in a Skalar autoanalyzer SAN+ (Hansen and Koroleff, 1999).

Z. noltei was monitored in Ria Formosa, south Portugal, during 2001 and 2002, along a distance gradient from Montenegro's WWTP (Cabaço et al., 2008). One census per season was performed in the stations S1, S2, S3, and S4, located 270, 520, 610, and 1,500 m away from WWTP, respectively. Monitoring included the above- and below-ground biomass, shoot density, and morphometrics of *Z. noltei*, as well as concentrations of ammonium and phosphates.

Z. marina was monitored at 11 locations along the British Isles (Jones and Unsworth, 2016; Jones et al., 2018). The monitoring during the summer season (May to August 2013) certified that the meadows were sampled during the seasonal peak of biomass, density, efficiency of space occupation, and health (Vieira et al.,

2018). Monitoring included the above- and below-ground biomass and shoot density of *Z. marina*, as well as above-ground $\delta^{15}\text{N}$ isotopic ratios. The seagrass biomass and density, presented by (Jones and Unsworth, 2016; Jones et al., 2018), were relative to quadrat area (0.25 m²). In this work, as well as in previous ones, we present the data standardized to unit area (1 m²).

C. nodosa and *Z. noltei* were monitored in an intermixed meadow at 5-m depth in a protected embayment adjacent to the Aragonese Castle on the island of Ischia in the Gulf of Naples, Italy (Kraemer and Mazella, 1999). Monitorization took place from October 1994 to October 1995. Three quadrats were sampled each month. Ammonium concentrations were measured in the water and in the sediment.

H. wrightii was monitored along southeast Brazil (Bertelli et al., 2020). The ecological and environmental characteristics were quantified in April 2017 in 10 seagrass meadows along the coast of Rio de Janeiro and São Paulo states, Brazil. At each site, single transect lines (50 -10 m depending on the size of the meadow) running parallel to the shore were placed through the

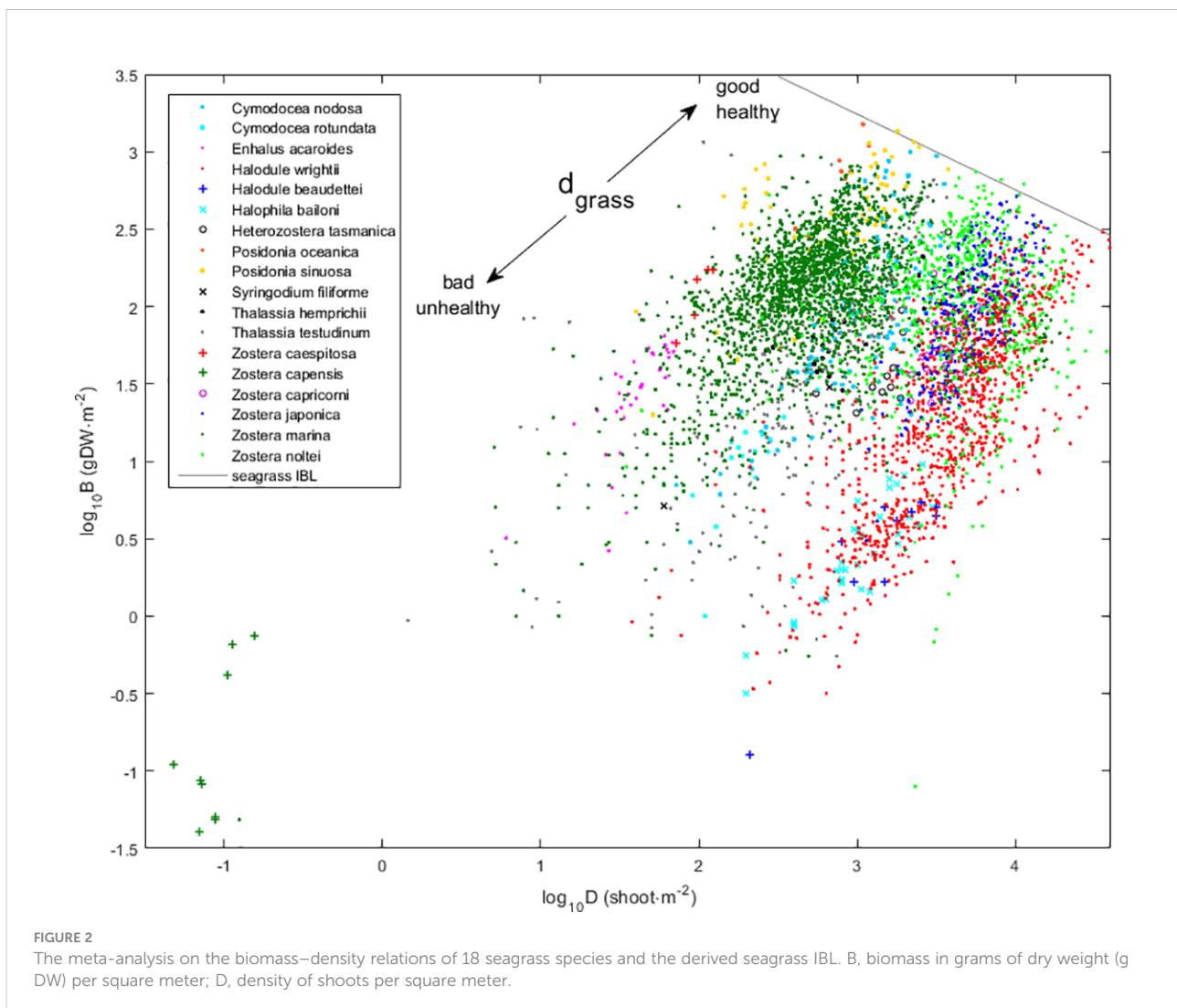
middle of the seagrass meadow and along the outer (deep) edge of the meadow. Monitoring included above- and below-ground biomass and shoot density of *H. wrightii*, as well as above-ground concentrations of N and of the $\delta^{15}\text{N}$ isotopic ratios.

H. beaudettei and *H. baillonii* were monitored along the Pacific coast of Costa Rica at Potrero during February 2016 (Samper-Villarreal et al., 2018), at Playa Refugio Animal, Playa Colibrí, Golfito, and Puerto Jiménez during May 2016 (Samper-Villarreal and Cortés, 2020); at Potrero, Jobo, and Matapalito during August 2017, December 2017, and May 2018 (Samper-Villarreal et al., 2020); and at Sámara Bay during August 2018 and March 2019 (Samper-Villarreal et al., 2022). Measured seagrass variables included, among others, above- and below-ground biomass, shoot density, and above- and below-ground concentrations of N as well as $\delta^{15}\text{N}$ isotopic ratios.

Seagrasses couple their growth in biomass and density (Figure 2, Supplementary Material Figure S1, Cabaço et al., 2013, 2019; Vieira et al., 2018), thus allowing the use of the distance to the seagrass IBL (d_{grass}) as a proxy for their health (Vieira et al., 2018).

Adopting the phalanx strategy, healthier meadows approach the IBL (and d_{grass} approaches zero) by simultaneously growing new shoots and increasing their biomass. Adopting the guerilla strategy, weak or unhealthy stands depart the IBL (and d_{grass} departs from zero) by simultaneously losing shoots and decreasing their biomass. The $d_{\text{grass}} = (\log_{10}\hat{B} - \log_{10}B) \cdot \cos\theta$. Here, \hat{B} is the theoretical maximum biomass given an observed density. It is estimated from the IBL as $\log_{10}\hat{B} = a + b \cdot \log_{10}D$. Coefficients are $a = 4.708$, $b = -0.489$, $\theta = \arctg(|b|) = 0.455$, and thus, $\cos\theta = 0.898$. The seagrass data compiled to estimate this IBL were here updated with 26 more studies providing 1,221 more observations. The current full data set, with 5,052 observations from 78 studies on 18 seagrass species, confirmed the seagrass clonal growth form (Figures 2, S1) but led to a re-estimation of the seagrass IBL with slightly different coefficients.

The d_{grass} indicator was compared with the concentrations of ammonium, phosphate, and the $\delta^{15}\text{N}$ isotope. In the studies by (Jones and Unsworth, 2016; Jones et al., 2018), Bertelli et al. (2020); Samper-Villarreal and Cortés (2020), and Samper-Villarreal et al.



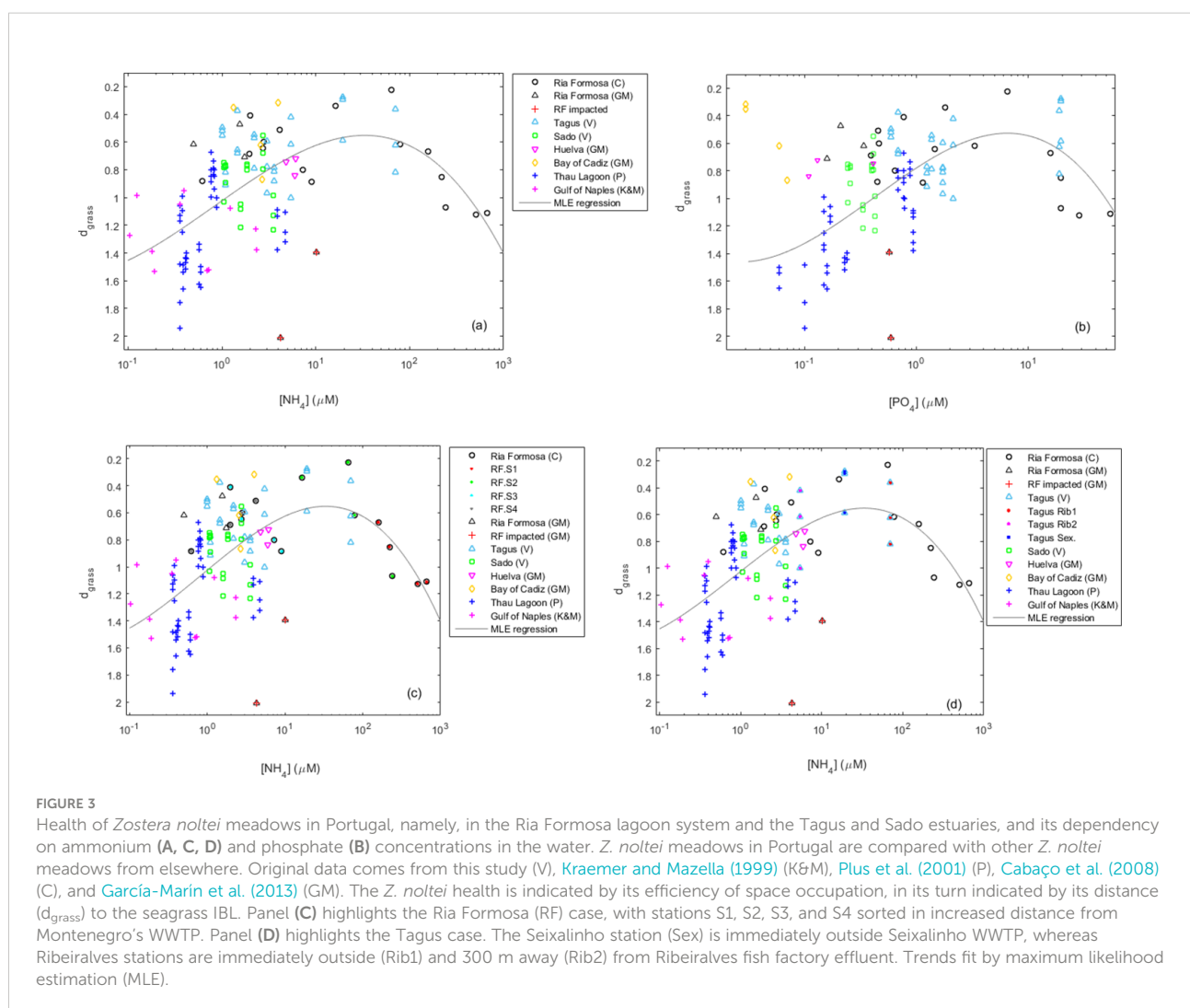
(2018, 2020, 2022), the proportion of the $\delta^{15}\text{N}$ isotope to the total N in the leaf content was considered to be an indicator of the N provenience: lower proportions (below 4 ppt) usually correspond to N coming from natural sources, whereas higher proportions (above 4 ppt) usually correspond to N coming from anthropogenic sources, namely, urban sewage and livestock effluents (following Macko and Ostrom, 1994; McClelland et al., 1997; McClelland and Valiela, 1998; Kjonaas and Wright, 2007; Bruland and MacKenzie, 2010; Román et al., 2019).

Third-order polynomials were fit to the non-linear relations between d_{grass} (response) and nutrients (predictor). Distinct fitting methods were applied, namely, ordinary least squares (OLS), weighted least squares (WLS), iterative reweighted least squares (IRLS), linear-in-the-parameters oblique least squares (LOLS), and maximum likelihood estimation (MLE). A detailed presentation of these methods and their comparison is presented by Vieira et al. (2016). Their application was done using the Matlab software developed by Vieira et al. (2016). The best fits should be those presenting the highest R^2 and the lowest root mean square deviation

(RMSD, also known as root mean square error: RMSE). However, the data distribution was strongly biased (asymmetrical), with many more observations on the side of lower nutrient concentrations. Consequently, the highest R^2 and the lowest RMSD optimized the fit on the side of lower nutrient concentrations but at the cost of poor fits on the side of higher nutrient concentrations. This problem was overcome by attributing relative weights to the data on each side and applying an MLE using the Newton–Raphson method (Vieira et al., 2016).

Results

The *Z. noltei* meadows monitored in south and central Portugal, namely, in Ria Formosa and in the Tagus and Sado estuaries, had smaller d_{grass} (indicative of healthier meadows) under moderate nutrient concentrations in the water (Figures 3A, B). The healthier Ria Formosa meadow was S2 located 510 m away from the WWTP (Figure 3C). The only

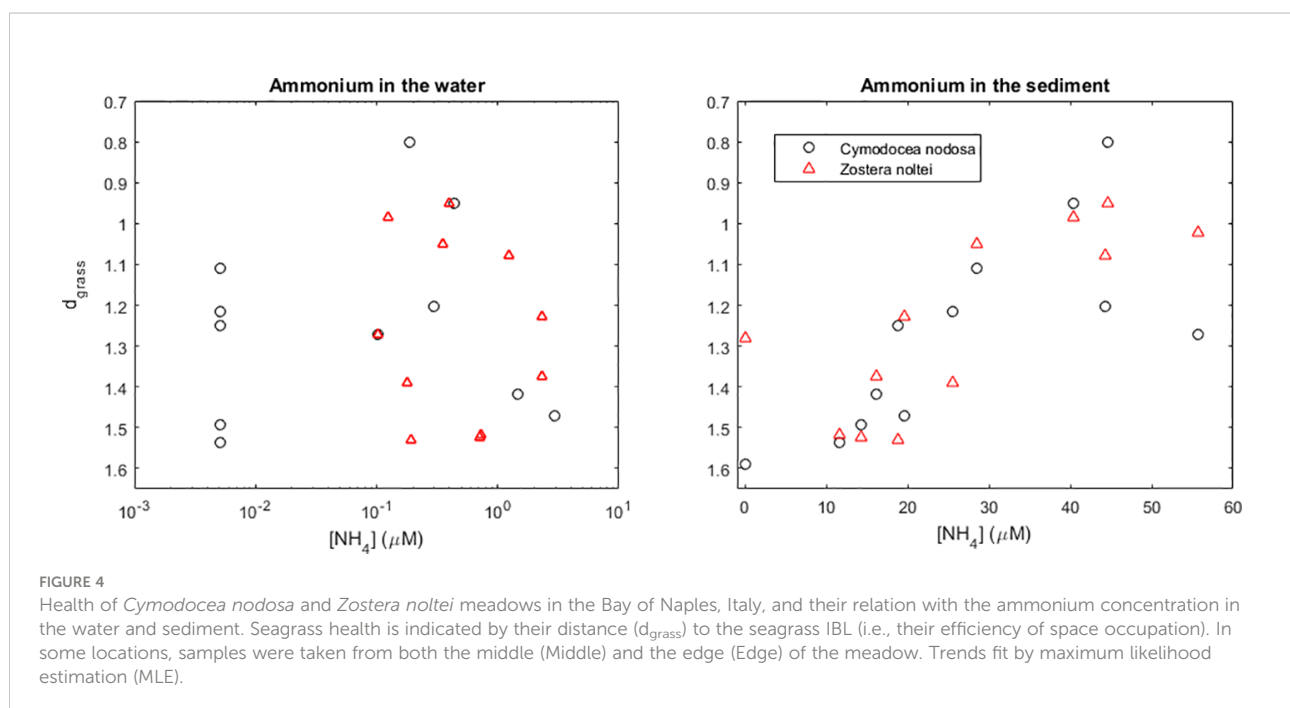


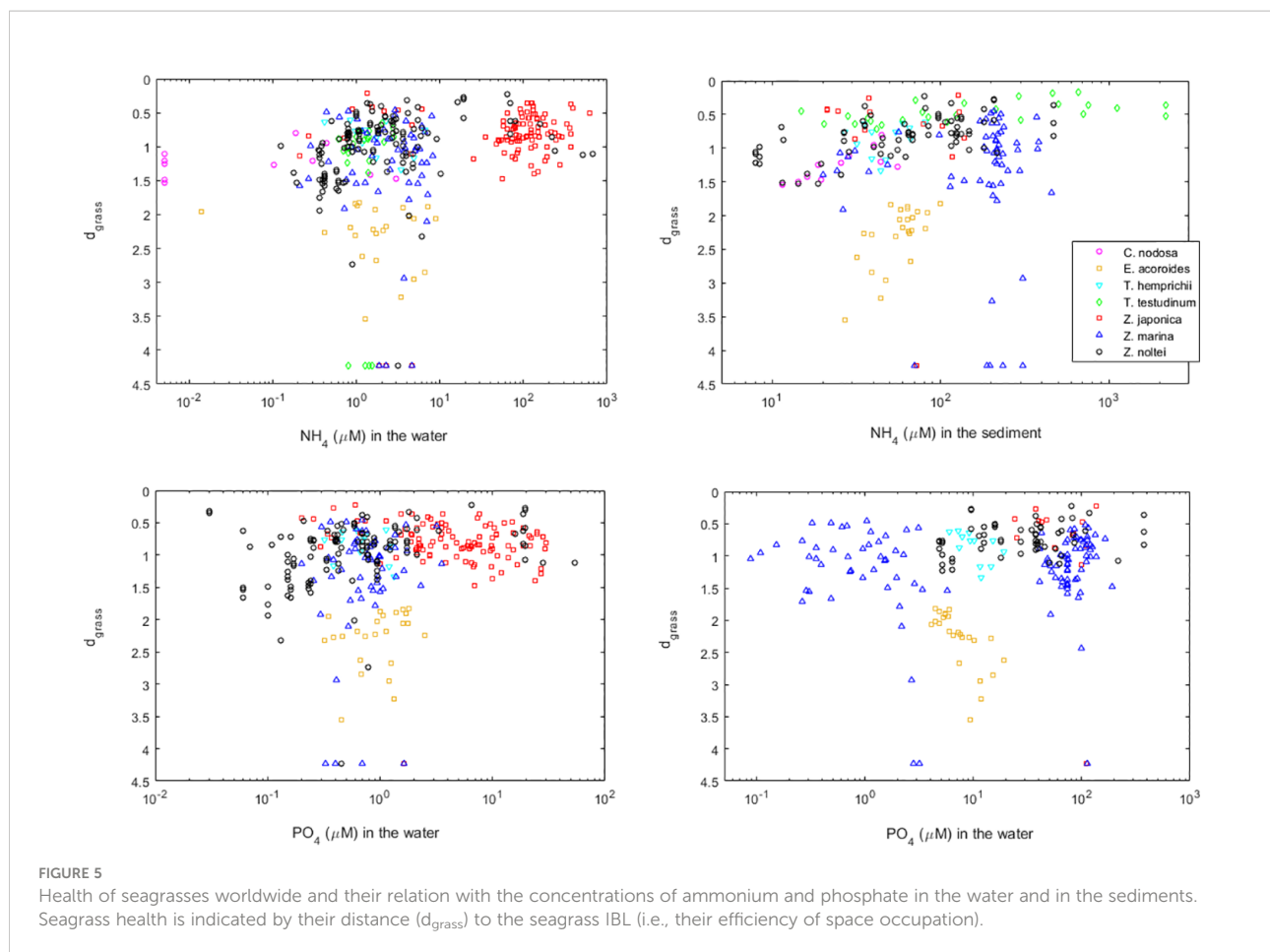
time S2 was in a poor condition was during winter under a peak of excess ammonium. In the Tagus estuary, the healthier meadows were located exactly next to the Seixalinho WWTP and the Ribeiraves factory effluent (Figure 3D), where the ammonium concentrations were similar to those observed for the Ria Formosa meadows S2 and S3 located at intermediate distances from the Montenegro WWTP (Figures 3C, D). Under lower nutrient loads, the d_{grass} of *Z. noltei* meadows increased (i.e., departed the IBL), suggesting that they were under nutrient deprivation. These lower loads occurred in the Ria Formosa and Tagus meadows furthest away from the WWTPs, and in the Sado meadows, which were all far from WWTP (Figures 3C, D). Furthermore, the Sado meadows were even less healthy than the Tagus and Ria Formosa meadows under equivalently low ammonium loads (Figure 3A), suggesting that the lack of phosphate worsened its condition (Figure 3B). Under the highest nutrient loads, *Z. noltei* meadows also increased d_{grass} (i.e., departed the IBL), suggesting that they were suffering from toxicity (Figures 3A, B). This happened in the Ria Formosa S1 meadow located closer to the Montenegro WWTP. Comparison with *Z. noltei* meadows from other locations and reported in other studies shows that the meadows from Ria Formosa and the Tagus estuary under moderate anthropogenic nutrient additions were among the healthier ever reported (Figure 3).

The *C. nodosa* and *Z. noltei* meadows monitored in the Bay of Naples, Italy, had smaller d_{grass} (indicative of healthier meadows) under intermediate nutrient concentrations in the water and in the sediment (Figure 4). It is noteworthy that both species exhibited very similar responses.

In order to have a global perspective, we plotted all data showing how the health of seagrass meadows may depend on the concentrations of ammonium or phosphate, in the water or in the sediment (Figure 5). Overall, seagrasses improved their efficiency of space occupation (and thus, their health) as nutrient concentrations increased, with the healthier meadows occurring under moderate nutrient concentrations. Only under the most extreme nutrient concentrations observed in this dataset were the seagrass meadows less healthy. From all these cases, the healthier meadows, occupying almost all the space available (i.e., d_{grass} close to zero), were located in coastal systems subject to anthropogenic pressure, as were the cases of *Z. noltei* in Ria Formosa (from Cabaço et al., 2008), in the Tagus estuary (from this study), and in the Bay of Cadiz (from García-Marín et al., 2013), and of *Z. japonica* in Dadae Bay (from Lee et al., 2006), in Koje Bay (from Kim et al., 2018), and in Willapa Bay (from Ruesink et al., 2009). It was also the case of *T. testudinum* in Laguna Madre and in Corpus Christy Bay, for both the control and the ammonium-enriched meadows (from Lee and Dunton, 2000).

The *Z. marina* meadows in the British Isles showing smaller d_{grass} (thus in better health) were in Priory Bay, Isle of Wight, Mannin Bay, Ireland, and Kircubbin Bay, Northern Ireland (Figure 6). With $\delta^{15}\text{N}$ within 5 to 11 ppt, these meadows were clearly under anthropogenic nutrient input. They were particularly better than the meadow sampled in the pristine Isles of Sicily (with $\delta^{15}\text{N} \leq 5$ ppt), or the heavily impacted meadows sampled in Studland Bay (with $\delta^{15}\text{N}$ within 11 to 13 ppt) or in Southend-On-Sea (with $\delta^{15}\text{N} > 15$ ppt). Nevertheless, there were also meadows showing $\delta^{15}\text{N}$ signals suggesting moderate-to-large anthropogenic sources of N (5 ppt < $\delta^{15}\text{N}$





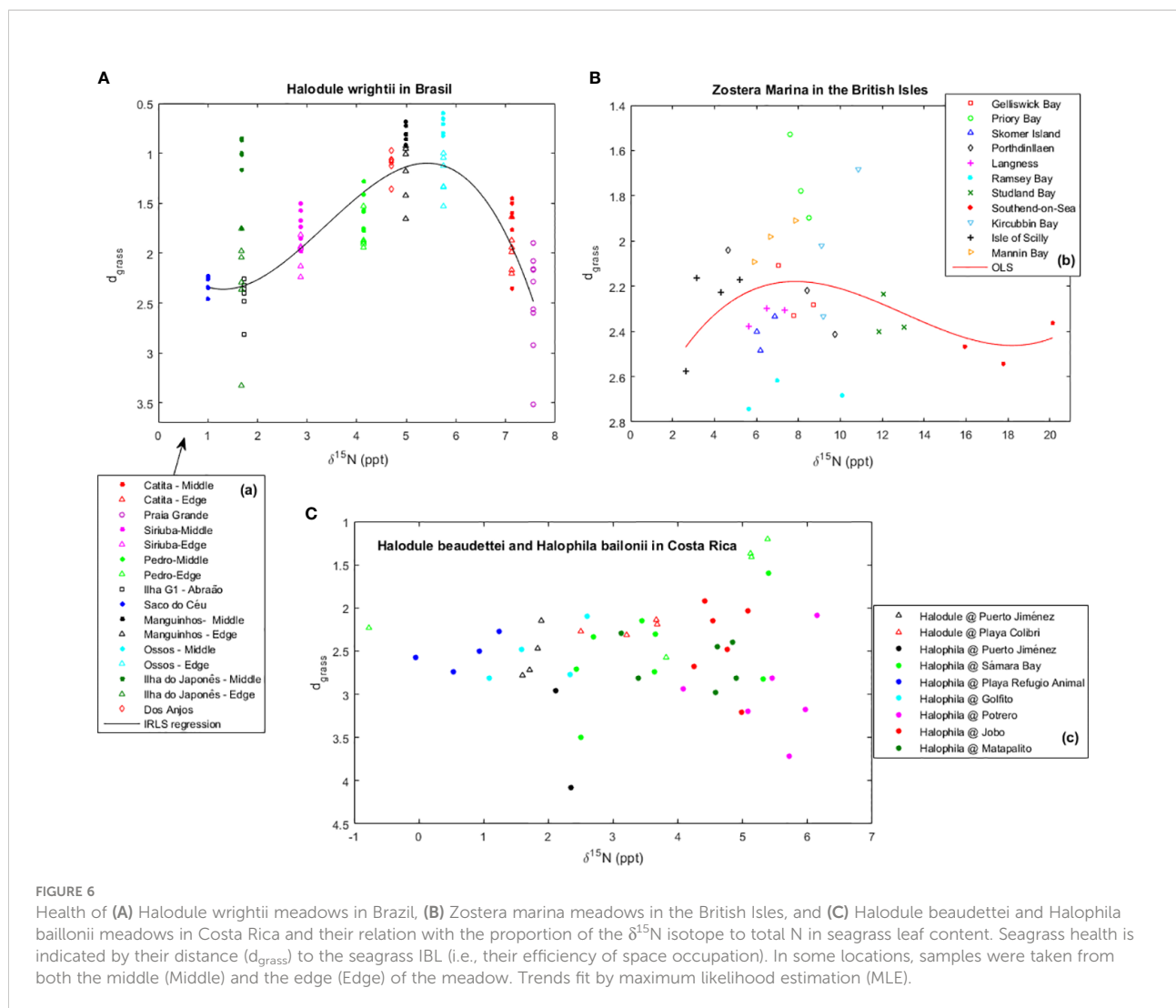
<10 ppt) for which the d_{grass} was large, thus indicating unhealthy stands. Therefore, there was something else in addition to the source of N that severely affected the health of *Z. marina* meadows in the British Isles.

The *H. wrightii* in Brazil, as well as the *H. beaudettei* and *H. baillonii* in Costa Rica, benefited from moderate nutrient enrichment (Figure 6). The meadows showing smaller d_{grass} were those with a $\delta^{15}\text{N}$ proportion within 4 to 6 ppt, indicating moderate anthropogenic N enrichment. For the *H. wrightii* in Brazil, these meadows were in Ossos, Manguinhos, dos Anjos, and Pedro. The meadows showing $\delta^{15}\text{N}$ proportions either above (Catita and Praia Grande) or below (Siriuba, Ilha do Japonês, Ilha G1 - Abraão and Saco do Céu) these bounds also showed larger d_{grass} . Furthermore, the samples taken from the middle of the meadow systematically indicated better health than the samples taken from the edge. For *H. beaudettei* and *H. baillonii* in Costa Rica, within each location sampled at different time instances, the respective meadow was generally healthier (smaller d_{grass}) as the proportion of $\delta^{15}\text{N}$ in above-ground biomass increased. In order to have a global perspective, we plotted all data showing how the health of seagrass meadows may depend on anthropogenic nutrient additions as inferred

from the proportion of the $\delta^{15}\text{N}$ isotope (Figure 7). Overall, seagrasses were healthier under moderate nutrient addition. For the tropical seagrasses, the optimal $\delta^{15}\text{N}$ was roughly around 5 to 6 ppt, whereas for temperate seagrasses, the optimal $\delta^{15}\text{N}$ was slightly higher, around 8 ppt.

Discussion

The monitoring of *Z. noltei* in Portugal showed that the nutrient concentrations immediately next to a WWTP and a food factory in the heavily populated Tagus estuary (outside the Natural Reserve) during the summer of 2021 were only as moderate as the nutrient concentrations 500 and 600 m away from a WWTP inside the Ria Formosa Natural Reserve during 2001 and 2002. Under these moderate nutrient concentrations were found the healthier *Z. noltei* meadows ever reported (considering their efficiency of space occupation). The main determinant of their health seems to have been the concentration of ammonium in the water. Concentrations too high were toxic for *Z. noltei* whereas concentrations too low were limiting for their growth. Nevertheless, under similarly



low ammonium concentrations, the Sado meadows were seemingly less healthy than the Tagus and Ria Formosa meadows. The reason for this may have been the significantly lower phosphate concentrations in the Sado estuary. The increased vulnerability to ammonium and the light environment under phosphate deficiency had already been reported for *Z. noltei* in the Bay of Cadiz, Spain (Brun et al., 2008). The present contrast between Sado and the other sites, particularly regarding the phosphate concentrations, may result from the distinct types of anthropogenic inputs. Rice crops are abundant both in the Sado River and in the Tagus river and its Sorraia tributary. Rice is not cultivated anywhere within the Ria Formosa basin. However, in the Sado estuary rice crops are the main source of anthropogenic inputs, with crops abounding in the estuary and right next to the sampling sites. In the Tagus estuary, urban effluents and livestock gain preponderance, with many being located right next to the sampling sites, whereas rice

crops are located many kms further upstream. Rice (*Oryza* sp.) growth and production have a high demand and uptake of P, with crops generally being limited by P availability and consumption (Beyrouy et al., 1994; Akinrinde and Gaizer, 2006; Julia et al., 2016; Rajaona et al., 2017; Jiang et al., 2021). It is therefore feasible that the P concentrations observed in the Sado estuary lower than the observed in the Tagus estuary and Ria Formosa are driven by P uptake by rice crops and that this relative P deficiency drives the relatively less healthy status of the *Z. noltei* meadows in the Sado estuary. The monitoring of *Z. marina* in the British Isles, of *H. wrightii* in Brazil, and of *H. beaudettei* and *H. baillonii* in Costa Rica showed that the meadows in better conditions were found in locations subject to mild or moderate nutrient additions to the natural background. In the cases of the tropical *H. wrightii*, *H. beaudettei*, and *H. baillonii*, the optimal proportion of the $\delta^{15}\text{N}$ isotope to the total N in leaf content was roughly within

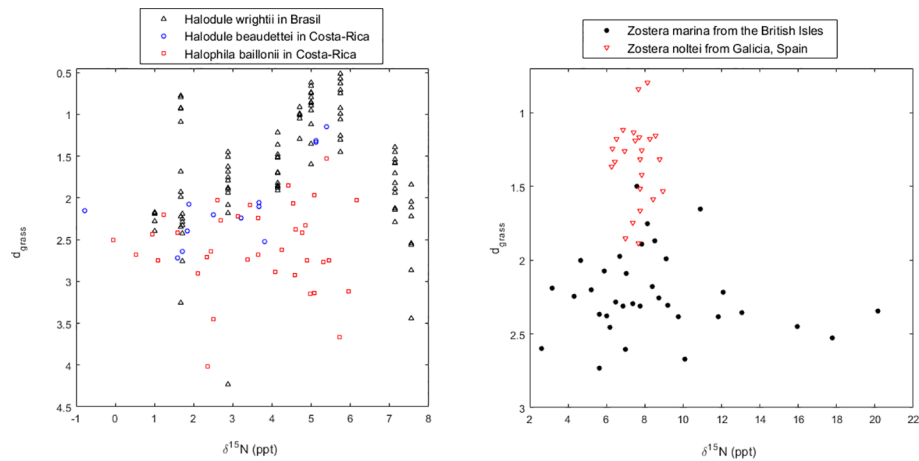


FIGURE 7

Health of seagrasses worldwide and their relation with the proportion of the $\delta^{15}\text{N}$ isotope to total N in seagrass leaf content. Seagrass health is indicated by their distance (d_{grass}) to the seagrass IBL (i.e., their efficiency of space occupation).

4 to 5 ppt. In the case of the temperate *Z. marina* and *Z. noltei*, the optimal range placed slightly higher around 8 ppt. The meadows under lower nitrogen enrichment were less healthy, presumably due to nitrogen deficiency, whereas meadows under higher nitrogen enrichment were also less healthy, presumably due to nitrogen toxicity. Overall, our results suggest that *H. wrightii* meadows in Brazil, *H. beaudettei* and *H. baillonii* in Costa Rica, *Z. noltei* meadows in Portugal, and *Z. marina* meadows in the British Isles may benefit from anthropogenic nitrogen additions, as long as these additions are moderate, and that such meadows can in fact do better than the meadows in pristine environments.

Our findings match previous studies on the physiological and demographic responses of seagrass beds to nutrient concentrations. The review by Touchette and Burkholder (2000) also determined that many seagrass species respond favorably to mild or moderate nutrient enrichment and that only when enrichment is too high do the seagrasses respond unfavorably with decreasing survival and/or growth. In this case, the negative response can be a direct physiological response to N or P toxicity or an indirect effect of eutrophication due to stimulation of algal overgrowth or smothering and associated light reduction. In the review by Waycott et al. (2005) of four seagrass species in the Great Barrier Reef, nutrients were only reported as limiting growth due to their weak supply. N or P toxicity was never found. Reported pollutants with negative impacts were only herbicides, pesticides, and other chemical contaminants. Our findings, together with the concordant previous findings reported above, contradict the former generalized negative perception that anthropogenic nutrient additions, however small these additions may be, are always harmful to seagrass beds.

A large contribution to this biased perception may have come from the lack of an adequate indicator for the health of seagrass meadows. This handicap has been solved by the distance to the seagrass IBL (d_{grass}), reflecting the efficiency of space occupation by the respective stand. The application of this new indicator to several species scattered worldwide showed that all monitored species benefit from moderate anthropogenic nutrient additions, which explains the recent recovery of seagrass meadows in populated coastlines where the improved management of wastewaters reduced but not eradicated the anthropogenic input of nutrients (Greening et al., 2014; Bertelli et al., 2018; de los Santos et al., 2019; Burdick et al., 2020; Orth et al., 2020). Furthermore, our results suggest that, in situations where WWTP are the main source of anthropogenic nutrient enrichment (i.e., agricultural runoff is reduced), modern wastewater treatment cleans the wastewater well enough to improve the health of nearby seagrass meadows, and their level of reduced nutrient inputs may even promote seagrass growth.

Seagrasses provide many ecosystem services, including CO_2 trapping, as well as shelter and food for commercially exploited species (Nordlund et al., 2016; de los Santos et al., 2020). Furthermore, the restoration of formerly deteriorated seagrass habitat leads to rapid recovery of coastal ecosystem services (Orth et al., 2020). Here, we determined that moderate anthropogenic nutrient additions have the potential to enhance these ecosystem services in seagrass meadows. Instead of trying to minimize the nutrient load in effluents from human origin as much as possible, society might better divert its focus to identifying maximal (beneficial) nutrient loads at specific sites for different species as well as improving strategies to maximize nutrient dilution when discharged to the sea, so that the

threshold concentrations for inorganic nitrogen and phosphorus to become toxic are not exceeded.

In addition to the detrimental concentrations of inorganic N and P, seagrass meadows are subject to a multitude of other stressors, acting alone or in consonance. Large nutrient inputs result in eutrophication, which, in estuarine and lagoon systems, take the form of extensive algal blooms often smothering seagrass meadows (Brun et al., 2003; Burkholder et al., 2007; Pergent et al., 2008; Cognat et al., 2018), or epiphytes and phytoplankton reducing light availability (Brun et al., 2003; Plus et al., 2003). Such was the case for *Z. noltei* in the Tagus estuary, with particular incidence in Samouco, where an extensive mat of mainly *Ulva* sp. but also some *Enteromorpha* sp. and *Sargassum* sp. covered almost all of the seafloor. During the summer of 2021 monitoring, patches with *Z. noltei* were sparse, and the healthy but sparse Samouco patches still showed some algae coverage (Supplementary Figure S2). During a subsequent monitoring in the summer of 2022, patches with *Z. noltei* in Samouco were even harder to find among the many tons of green algae, but the few patches left showed some of the best efficiencies of space occupation (d_{grass}) reported in the data set of seagrasses worldwide. This sparse coverage of extremely healthy *Z. noltei* patches mingled among an extensive green algae bloom suggests that moderate anthropogenic nutrient additions may lead seagrass meadows to their best health provided that the collateral effects of eutrophication are avoided. Otherwise, the formation of extensive algal mats covering the seagrasses, epiphytes, and/or phytoplankton becomes largely detrimental to seagrass meadows (Brun et al., 2003; Plus et al., 2003; Burkholder et al., 2007; Pergent et al., 2008; Cognat et al., 2018; and present study). Also, temperature affects and interacts with other factors that determine seagrass performance. Temperature affects the photosynthetic efficiency (P-I) curve of seagrasses (Plus et al., 2005; Lee et al., 2007; Vieira et al., 2018), their nutrient uptake and metabolic rates (Plus et al., 2001, 2003; Lee et al., 2007), and the toxicity of inorganic N and P, herbicides, and trace metals (Gamain et al., 2018). In the Tagus and Sado estuaries, during the summer, water temperature was always above 24°C and in their landward sections often above 30°C, which is considered too warm for temperate seagrasses (Lee et al., 2007). However, the largest stressor to *Z. noltei* in the Tagus and Sado estuaries was by far clam harvesting. During the July 2021 monitoring, the *Z. noltei* meadows in the Ribeirinhas stations were composed of a few healthy patches interspersed within the bare sediment, with the daily pressure of tens of clandestine clam harvesters plucking their shoots and breaking their roots. By August 2021, these meadows had entirely disappeared, never to reappear thus far. The same happened in many other stations and locations in the Tagus and Sado estuaries. As examples, the *Z. noltei* meadow in the original Comporta 2 station had also disappeared by July 2022. The beach front along Alcochete is abundant in clandestine recreational clam harvesters daily destroying *Z. noltei* patches dispersed within the bare sediment (Supplementary Figure S3).

Data availability statement

The original contributions presented in the study are provided online at <http://dx.doi.org/10.13140/RG.2.2.32343.14240>.

Author contributions

VV contributed to the conceptualization, design, field sampling, model and software development, data analysis, and writing of the manuscript. RS, DL-S, JC, CB, and JS-V contributed to the field sampling. JL-A, RS, DL-S, AV, JN, MN, JC, CB, and MP contributed to the laboratorial work. JL-A, MN, JC, CB, and JS-V contributed to reviewing the manuscript. All authors contributed to the work and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.960249/full#supplementary-material>

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