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Seagrass space occupation efficiency is key for their role as ecosystem engineers and ecological indicators

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Studies for the preservation of seagrass beds biotopes have met difficulties in establishing appropriate methods assessing their health. We tested the efficiency of space occupation by seagrasses scattered worldwide (d_{grass} index), which proved to be dependent on clonal growth form and morphometric plasticity. d_{grass} correlated with the above-ground to below-ground biomass ratio. However, the latter was misleading when high ratios resulted from low below-ground biomass. Nutrient Posphate-limitations were revealed in situations of theoretical Nitrogen-limitation. Enhanced nutrient supply benefitted seagrasses only up to a threshold after which it became detrimental. Better nurtured, healthier meadows with denser canopies increased the organic matter in the sediment and had associated greater abundances of benthic macrofauna. Hence, seagrass biotopes could benefit from moderate anthropogenic nutrient additions. However, organic matter above ≈6% and/or reduced riverine discharges (dams upstream and climate-change-related droughts) were detrimental to healthy meadows, jeopardizing ecosystem services such as macrofauna abundances and carbon sinks.

Seagrass meadows are one of the most common marine coastal biotopes, and hotspots for biodiversity[1–6] and productivity[2,3,7]. Additionally, seagrasses provide many important services to both ecosystems and humankind. Such services include functioning as nursery habitat and shelter for fish, bivalves, crustaceans, and other marine invertebrates, providing food, their role in water purification[2,3,8–10] and carbon storage[9,11–16], and their potential role as a sustainable source of renewable raw material and energy for construction and industry[17–21], and soilless agriculture[22].

Seagrass meadows are useful ecological indicators of the quality of coastal waters as well as of the health status of their biotopes[23,24]. Using multivariate analysis, associations among a wide array of seagrass variables have been tested as indicators, namely populational, morphometrical, physiological and biochemical[25–33]. These evaluations have systematically used seagrass biomass, shoot density and a possible existence of a biomass–density relationship. However, several factors have deterred more robust findings. The simultaneous use of large amounts of variables resulted in unfavourable signal-to-noise ratios. Most studies monitored single species in relatively restricted geographical areas, undermining extrapolations to other species and/or locations. The searches for associations among seagrass variables were exclusively empirical/statistical, thus disregarding mechanistic explanations grounded in ecological theory. This was particularly true for the earlier research on the biomass–density relationships in seagrasses, which neglected the biomass–density theories well-established for terrestrial plants, namely the self-thinning law and the determination of an Interspecific Boundary Line (IBL) representing the maximum possible space occupation efficiency on Earth by terrestrial plants[34–39]. This gap was

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filled by Vieira et al. [40–42]. First, an IBL which was specific for seagrasses was determined from 5052 observations of 18 species scattered worldwide and reported in 79 studies[40,42]; thus, grounding its generalization to seagrasses globally (Fig. 1). Concomitantly, an algae-specific IBL was also determined[43]. The estimated perpendicular distances of monitored algae stands (d_{algae}) or seagrass meadows (d_{grass}) to their respective IBLs (Fig. 1), representing the free space left available and thus, their efficiencies of space occupation, were demonstrated as biological indicators of their health, among other aspects of their biology and ecology[40,43].

The biomass-density relation in seagrasses is mediated by their modular construction. The establishment and growth of new meadows are primarily done by the elongation of the rhizome system through the iteration of new shoots, leading to either of two clonal growth forms, namely the "guerilla" (sparse meadows with low shoot densities) or the "phalanx" (compact meadows with high shoot densities)[44–48]. Due to its modular construction and clonal growth, healthy seagrass meadows approach the IBL adopting a phalanx clonal growth form where they simultaneously increase their shoot density and their above-ground biomass. On the contrary, less healthy meadows depart the IBL, thereby adopting a guerilla clonal growth form where they simultaneously decrease their shoot density and their above-ground biomass[40,41] (Fig. 1).

Grounded on the theory and index presented above about seagrass ecology and demography, the healthier seagrass stands were found to be those benefiting from mild anthropogenic nutrient additions to the natural background, as long as these additions were not excessive to the point of nitrogen (N, namely ammonium, nitrate and nitrite) or phosphate (P) becoming toxic[42,49]. This finding matched former analyses showing that seagrasses in pristine environments are nutrient-limited[50–60] and benefited from experimental nutrient additions to the natural background[53,55,57]. Additionally, these benefits cascaded to the biotope and the ecosystem services provided by seagrasses[61,62]. Hodgson and Bucher[61] found that moderate levels of nutrient inputs from properly managed sewered catchments may increase not only seagrass abundances but also fish harvests, and by association, production of estuary-dependent fisheries. The increasing awareness by society about environmental protection, the improved technological solutions for waste-water management, and the evidence that seagrasses may benefit from mild anthropogenic nutrient additions to the natural background, support the accumulating evidence on the recovery of seagrass beds worldwide[14,62–68]. Recent seagrass recovery is particularly true for European seagrasses, for which a reversal from its 20th century decline was determined from the 2000s onward[68].

The objective of this work was to determine whether d_{grass}, besides being an indicator of the health of seagrass meadows, may be extrapolated to their biotopes and ecosystem services. That requires knowing how, and to what extent, the efficiency of space occupation by seagrasses may influence their role as ecosystem engineers. We formulate a hypothesis about cascading effects, with the availability of nutrients setting the efficiency of space occupation, which, in its turn, regulates sediment properties and macrofauna biodiversity. For such inference, we monitored *Zostera noltei* Hornemann, 1832 meadows from the Tagus and Sado estuaries, Portugal (Fig. 2), and analysed them together with data from *Z. noltei* monitored elsewhere, as well as data from other species and systems (data compiled in WorldSeagrassBiomassDensity.xlsx[69]). The approach of analysing many seagrass species together has been successfully implemented before[7,27,68,70,71] and argued as the only way to unveil the intricate dynamics of seagrasses and their interactions with the local environment[71]. While implementing this approach, the Results and Discussion were organized in sections dedicated to specific topics.

In Section 2.1 'Environmental properties', we focus on water temperature and availability of ammonium, dissolved inorganic nitrogen (DIN), and phosphate, in the water column as well as in the sediment pore water. The availability of nutrients was significantly different among estuaries. The environmental molar N:P was usually lower than the molar N:P in seagrass tissues, thus suggesting that seagrass growth was usually limited by N.

In section 2.2 'd_{grass} *vs.* nutrients', we show how the availability of nutrients affects the efficiency of space occupation by seagrasses. Seagrasses peaked their efficiencies under moderate nutrient concentrations. Under low nutrient concentrations, seagrasses were always limited, showing reduced efficiencies. Excess nutrient concentrations were harmful to

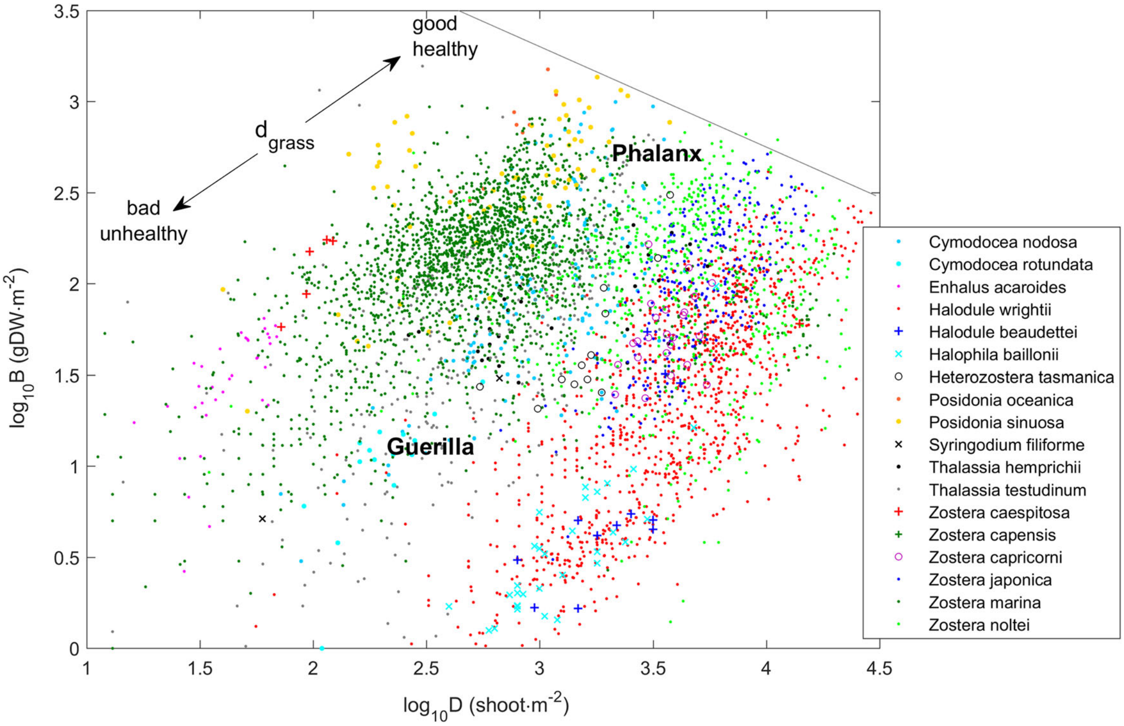

Fig. 1 | The biomass-density relations of seagrasses. The Interspecific Boundary Line (IBL) and the d_{grass} metric inferring each stand's efficiency of space occupation. Data from seagrasses worldwide compiled in Vieira et al.[69] and used in Vieira et al.[40–42].

Fig. 2 | General overview of the study area. The metropolitan areas of Lisbon and Setúbal in the Tagus and Sado estuaries (**a**), and sampling stations from the Tagus (**b**) and the Sado (**c**) estuaries. The Tagus stations were "Al1" and "Al2", inside the Tagus Estuary Natural Reserve and thus expected to be the most pristine sites, "Al3" and "Al4" along the Alcochete city beach, "Sam1", "Sam2", "Sam3" and "Sam4" next to the Samouco locality, "Sex" was located at the Seixalinho wastewater treatment plant (WWTP) runoff, "Rib1" was located at the Riberalves fish factory runoff, and "Rib2" and "Rib3" approximately 300 m and 600 m away, respectively. The Sado stations were "ST" along the main channel, closer to the estuary inlet and next to the SolTroia tourist and residential development area, "Com1", "Com2", "Com3" and "Com4" located along the Comporta secondary channel, and "Cr1", "Cr2" and "Cr3" in the main channel next to the Carrasqueira village. Aerial photos obtained from Google Maps. Imagery ©2024 TerraMetrics, Map data ©2024 Google, Inst. Geogr. Nacional.

seagrasses, thus also leading to reduced efficiencies. The d_{grass} dependency on the proportion of the $\delta^{15}N$ isotope corroborated that seagrasses benefit from mild anthropogenic nutrient additions to the natural background.

In section 2.3 'd_{grass} *vs.* environmental N:P ratios', we show how the d_{grass} indicator was able to detect P-limitations for seagrass growth despite the generalized theoretical N-limitations.

In section 2.4 'd_{grass} *vs.* seagrass morphometry', we show how the morphometric plasticity of seagrasses affects their efficiency of space occupation. This is achieved by increasing their rhizomes' branching, node density, number of shoots per node and leaf length as they shift from a guerilla to a phalanx clonal growth form.

In section 2.5 'd_{grass} *vs.* above:below ground biomass', we show that the above:below ground biomass ratio as an indicator of seagrass health, per se, is misleading when high ratios result from low below-ground biomass. The best option is to use d_{grass} as an indicator of health and the above:below ground biomass ratio as an indicator of the recent trend.

In section 2.6 'd_{grass} *vs.* sediment properties', we show the relations among the efficiency of space occupation, granulometry and the % of organic matter in the sediment. Denser meadows retained more organic matter, but only until a ≈ 6% OM threshold, above which too much OM became detrimental. The role of seagrass meadows as carbon sinks is dependent on the availability of resources to sustain their carbon incorporation.

In section 2.7 'd_{grass} *vs.* the benthic macrofauna', we show that denser meadows accommodated larger abundances of benthic macrofauna. However, the number of species was not significantly affected. High content of OM and/or reduced riverine discharges also had negative effects on the abundances of benthic macrofauna.

In section 2.8 'd_{grass} *vs.* alternative coastal ecosystem indicators' we compare the use of seagrass-based indicators with the widely used AMBI and M-AMBI based on benthic macrofauna biodiversity. Both options broadly agreed about the general good quality of the seagrass biotopes in the Sado and Tagus estuaries. However, the AMBI and M-AMBI failed to detect the clear differences between these two systems resulting from the reduced riverine discharges to the Sado estuary, whereas the seagrass indicators promptly detected these differences.

Results and discussion

Environmental properties

The sampling in the Tagus and Sado estuaries took place during the peak summer season of 2021 and 2022. Therefore, the temperatures of the water immediately over the meadows were very high (Table 1), ranging from 22 °C

Table 1 | Sampled environmental variables

Estuary	Site	year	Temp Water °C	NH_4 Water µM	NH_4 Sed. µM	DIN Water µM	DIN Sed. µM	PO_4 Water µM	PO_4 Sed. µM
Tagus	Al 1	2021	23.8	2.2	132.2	9.5	135.8	1.4	41.3
Tagus	Al 1	2022	29.9	9.3	na	20	na	na	na
Tagus	Al 2	2021	23.7	1.1	123.6	9.2	128.7	1.2	37.5
Tagus	Al 2	2022	28.3	9.5	na	13.4	na	na	na
Tagus	Al 3	2021	23.3	3.6	68.7	21.7	74.3	1.7	39.2
Tagus	Al 3	2022	28	6.8	na	12	na	na	na
Tagus	Al 4	2022	27.3	8.7	na	12	na	na	na
Tagus	Sam 1	2021	22.7	1.5	35.6	9.7	38.5	0.7	37.1
Tagus	Sam 1	2022	27.3	5.8	na	9.2	na	na	na
Tagus	Sam 2	2021	23.3	3.0	116.1	20.9	122.0	1.7	27.0
Tagus	Sam 2	2022	26.9	5.4	na	7.8	na	na	na
Tagus	Sam 3	2021	21.7	1.0	96.8	9.0	100.6	0.6	15.8
Tagus	Sam 3	2022	27.2	4.4	na	6.9	na	na	na
Tagus	Sam 4	2022	28.8	4.5	na	7.9	na	na	na
Tagus	Rib 1	2021	21.9	70.1	468.2	74.3	469.2	18.8	373.8
Tagus	Rib 2	2021	22.6	5.4	181.4	9.9	182.8	2.1	64.4
Tagus	Rib 3	2022	29.4	39.7	na	45.3	na	na	na
Tagus	Sex	2021	22.5	19.2	209	47.5	210.7	19.6	9.6
Tagus	Sex	2022	26.6	11.1	na	16.4	na	na	na
Sado	Com 1	2021	23.6	1.9	8	4.5	9.2	0.3	6.4
Sado	Com 2	2021	25.4	2.7	125.0	5.5	125	0.4	12.4
Sado	Com 2	2022	25.4	3.1	na	5.4	na	na	na
Sado	Com 3	2021	23	3.6	8.4	6.2	9.0	0.4	5.2
Sado	Com 4	2021	22.9	3.1	na	5.8	na	na	na
Sado	Cr 1	2021	26.1	1.1	26.8	3.5	29.8	0.3	4.8
Sado	Cr 1	2022	25.2	4.9	na	19.1	na	na	na
Sado	Cr 2	2021	26.3	1.8	64.6	4.4	67.0	0.4	5.2
Sado	Cr 2	2022	25.6	3.5	na	6.4	na	na	na
Sado	ST	2021	22.1	1.1	148.0	3.7	152.2	0.2	17.8
Sado	ST	2022	22.8	2.5	na	5.1	na	na	na

Temperature (Temp Water) and nutrients (Ammonium—NH_4, Dissolved Inorganic Nitrogen—DIN and Phosphate—PO4) in the water column and sediment (Sed.) pore water of the Tagus and Sado estuaries. Values are the averages of the replicates taken over time during low tide (sediment) and high tide (water). na sediments were not sampled during 2022. The Tagus stations were "Al1" and "Al2", inside the Tagus Estuary Natural Reserve and thus expected to be the most pristine sites, "Al3" and "Al4" along the Alcochete city beach, "Sam1", "Sam2", "Sam3" and "Sam4" next to the Samouco locality, "Sex" was located at the Seixalinho wastewater treatment plant (WWTP) runoff, "Rib1" was located at the Riberalves fish factory runoff, and "Rib2" and "Rib3" approximately 300 m and 600 m away, respectively. The Sado stations were "ST" along the main channel, closer to the estuary inlet and next to the SolTroia tourist and residential development area, "Com1", "Com2", "Com3" and "Com4" located along the Comporta secondary channel, and "Cr1", "Cr2" and "Cr3" in the main channel next to the Carrasqueira village.

closer to the estuaries' mouths to over 31 °C in the inner estuaries during ebb tide. Such variation in water temperature has been reported in these systems due to tidal-driven circulation moving warmer inner estuary waters and cooler upwelled coastal ocean waters[72].

The comparisons with data from the Tagus and Sado estuaries, Dadae Bay[73], Barang Lompo and Gusung Tallang[74] (only these sites were compared because they were the only ones that had measurements for all substrates and nutrients) showed that nutrient concentrations were much higher in the sediment pore water than in the water column ($p \leq 0.0001$), often by about 1-2 orders of magnitude (Table 1 and Fig. 3). Differences between sites were significant ($p = 0.0001$), with the Tagus and Dadae Bay meadows showing higher nutrient concentrations than the Sado (post hoc $p = 0.0016$ and $p = 0.0032$), Barang Lompo (post hoc $p = 0.0004$ and $p = 0.0005$) and Gusung Tallang (post hoc $p = 0.0667$ and $p = 0.0462$) meadows. The Sado river (southwestern Portugal), being much smaller than the Tagus (largest river in the Iberian Peninsula, crossing Portugal and Spain), delivers less riverine inputs to its estuary; which is aggravated by dams upstream and climate change-related successive droughts in southern Portugal during recent years having led to significant decreases in the Sado riverine discharges[75]. Dadae Bay is in a heavily populated area in South Korea whereas Barang Lompo and Gusung Tallang are respectively a mud flat and a Coral reef kms off-shore the populated Jenerebang river estuary, South Sulawesi.

d_{grass} *vs.* nutrients

Seagrasses have declined globally during the 20th century[3,30,67,68,76]. The most common cause was increased nutrient load to coastal systems from agricultural runoff and urban effluents. This has often led to eutrophication, which in turn led to smothering by drifting or epiphytic macroalgae, light attenuation due to enhanced phytoplankton growth and turbidity[76–78], and the toxic effects of excessive concentrations of inorganic nitrogen (N) and phosphorus (P)[76,79–84]. Other anthropogenic negative impacts included clam harvesting[85,86], herbicides[87–90], pesticides[88,90–92], and industrial contamination[88,90,92–94]. However, recent seagrass recovery has been observed scattered throughout the globe[13,62–70]. Furthermore, new evidence emerged about this recovery being due to seagrasses benefiting from

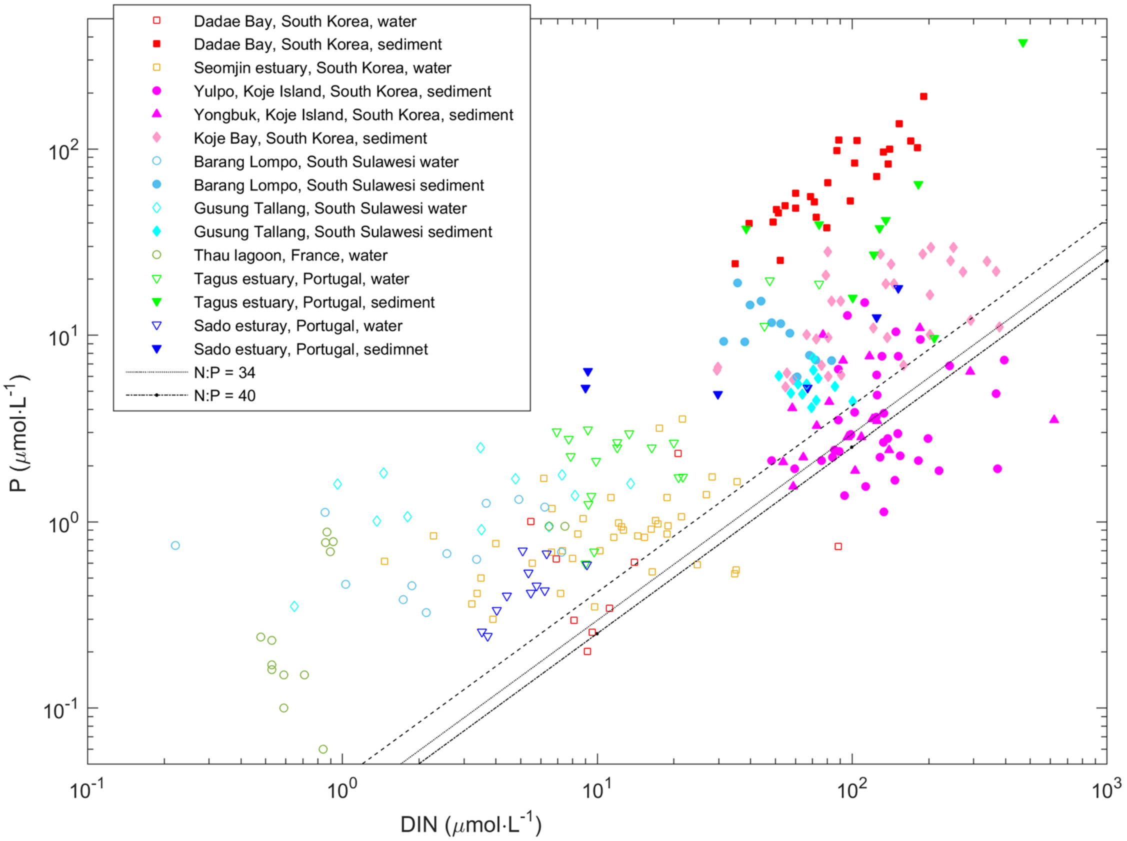

Fig. 3 | Dissolved Inorganic Nitrogen (DIN) and Phosphate (P) in the water and in the sediment of seagrass meadows. Measured concentrations are compared with N:P thresholds for N or P limitation following Duarte[6] (here converted to mol:mol) and Fourqurean et al. [59]. Data from seagrasses worldwide compiled in Vieira et al. [69]. and used in Vieira et al.[40–42].

anthropogenic nutrient additions to the natural background as long as these additions are mild, do not lead to excessive N and P concentrations surpassing healthy thresholds, and impacts from clam harvesting are avoided[11,41,48,62]. The application of the d_{grass} indicator was a valuable tool to demonstrate that these findings hold for *Zostera marina* in the British Isles, *Halodule wrightii* Ascherson, 1868 in Brazil, *Halodule beaudettei* (Hartog) Hartog, and *Halophila baillonii* Ascherson, 1874 in Costa Rica and *Zostera noltei* in Portugal[42]. Furthermore, the application of the d_{grass} showed that the healthier *Z. noltei* meadows in the Tagus and Sado estuaries, peaking their space occupation efficiency, were precisely the meadows next to Waste Water Treatment Plants (WWTP) and a food factory effluent (stations 'Sex', 'Rib1' and 'Rib2' in this work and in Vieira et al.[42,69]).

Here, we focus on the beneficial and detrimental effects of N and P on seagrasses. N is taken up both as ammonium and nitrate, ammonium being the preferred form[71,95–103]. P is taken up as phosphate[12,54–59]. Seagrasses in pristine locations are often nutrient-limited[50–59]. On the other hand, excessive nutrients lead to plant necrosis[79–84]. The literature is unclear on the levels and thresholds for positive (growth) *vs* negative (mortality) effects. Hindering their determination, there is a strong interaction with other factors such as light, temperature, salinity or pH[79–84]. Even the own plant stands' densities interfere with these thresholds as the presence of more plants leads to more nutrient uptake and incorporation in plant tissue, and consequently, lowered concentrations in the water. Hence, there is beneficial feedback where increased plant densities raise the threshold for nutrients to become toxic[84]. Here, we applied polynomial quantile regressions estimating the d_{grass} limitations by ammonium, dissolved inorganic nitrogen (DIN) and phosphate (see Methods section 4.5.2) to highlight the signal in the data and cast aside the noise. These regressions determined the maximum efficiency of space occupation (i.e., minimum d_{grass}) possible under the given predictor value (in these cases, the $\log_{10}$ nutrient concentrations). This was the desired signal. Lower efficiencies of space occupation (i.e., higher d_{grass} values) corresponded to situations where some other unaccounted factor was limiting seagrass health and growth. Such cases corresponded to the noise that the regressions fit to extreme quantiles managed to overcome.

The non-linear quantile regressions simultaneously applied to seven species scattered worldwide confirmed that seagrass meadows, irrespective of the species, peak their efficiencies of space occupation (i.e., were healthier) under intermediate concentrations of N and P in the water and sediment (Fig. 4). Lower concentrations of N and P were detrimental for seagrasses presumably due to nutrient deprivation, whereas larger concentrations of N and P were detrimental presumably due to toxicity and eutrophication. These results clarified the concentration thresholds for nutrients shifting from beneficial to detrimental, which, so far have been far from consensual. Our meta-analysis showed that optimal phosphate concentrations were around 1–10 μmol·L^{-1} when in the water, and around 10–100 μmol·L^{-1} when in the sediment. Optimal ammonium and DIN were around 10 μmol·L^{-1} when in the water, and around 100 μmol·L^{-1} in sediment. These results match the work by Lee and Dunton[53] proposing the 100 μM of ammonium in the sediment as a threshold limiting seagrass growth. Other relevant generalizations are that seagrasses tolerate nutrient concentrations in the sediments higher than in the water, as well as tolerate N concentrations higher than P concentrations.

A comparison was carried out only among *Z. noltei* meadows. The Sado meadows occupied space less efficiently (i.e., were less healthy) than the Tagus meadows under similar ammonium concentrations. Possibly, lack of nitrate and/or phosphate were limiting the growth and health of the Sado meadows. Likely due to lesser inputs, natural and/or anthropogenic, the concentrations of these nutrients in the Sado were lower than in the

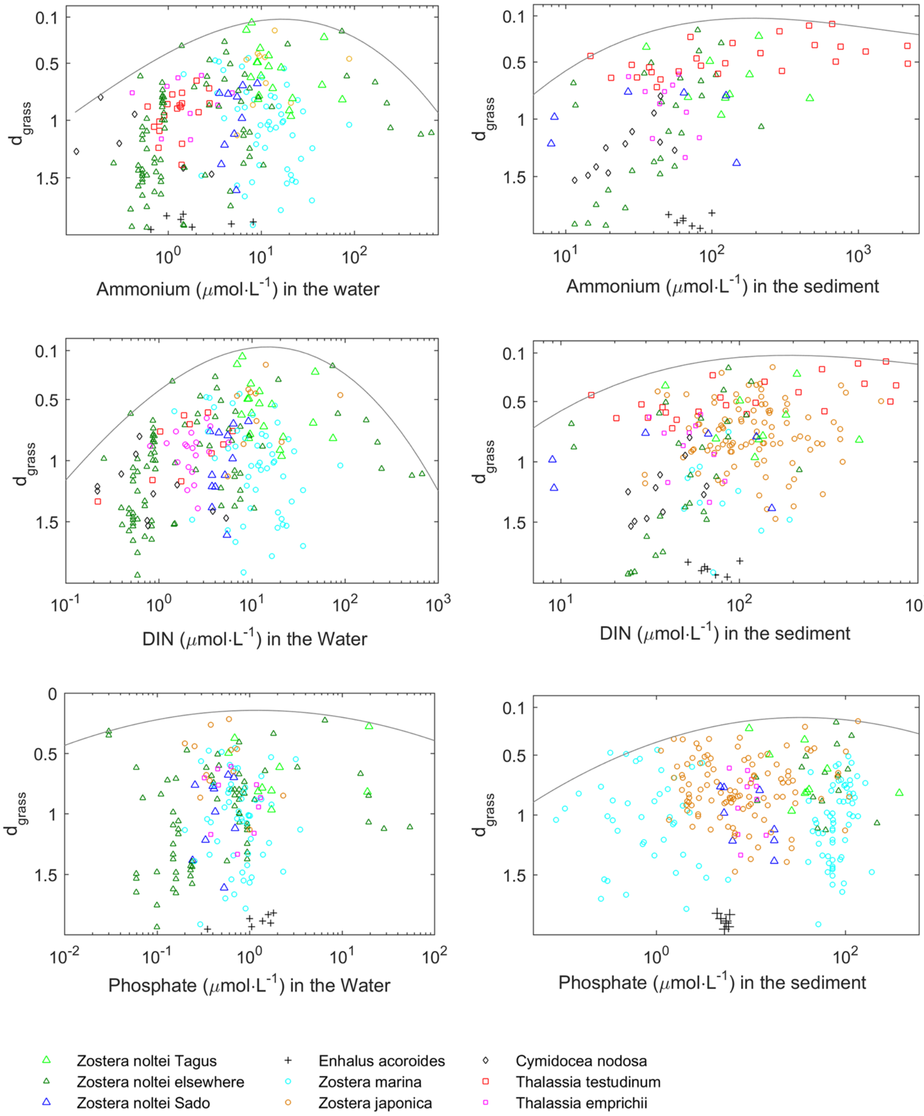

Fig. 4 | Dependency of d_{grass} from nutrient concentrations. Smaller d_{grass} represents less free space left to be occupied, indicative of healthier meadows occupying space more efficiently. In this study, *Zostera noltei* meadows were sampled in the Tagus and Sado estuaries, Portugal. *Zostera noltei* meadows elsewhere were sampled in Ria Formosa, Portugal[25,28], Cadiz and Huelva, Spain[28], Gulf of Naples, Italy[194], and Thau Lagoon, France[98]. *Zostera marina* was sampled in Dadae Bay[73] and Seomjin estuary[113], South Korea. *Zostera japonica* was sampled in Dadae Bay[73], Koje Bay[114], and Yongbuk and Yulpo[115], all in South Korea. *Cymodocea nodosa* was sampled in Gulf of Naples, Italy[194]. *Enhalus acoroides* was sampled in Barang Lompo and Gusung Tallang, South Sulawesi[74]. *Thalassia testudinum* was sampled in Corpus Christi Bay and Laguna Madre, USA[53]. *Thalassia hemprichi* was sampled in Barang Lompo, South Sulawesi[74].

Tagus. This suggests that moderate nutrient surplus to the natural background promotes seagrass health and growth.

The *Z. noltei* meadows sampled in the Tagus estuary near effluents from WWTP and the Riberalves food factory were among the meadows occupying space more efficiently and thus, presumably healthier. These effluents discharge into large channels with strong hydrodynamic mixing. The remaining meadows with better efficiencies of space occupation were sampled in the summers of 2009–2011 (13–15 years ago) in Ria Formosa at intermediate distances from a WWTP[25]. On the other hand, the *Z. noltei* meadows in worst conditions were sampled in the summers of 2009–2011 immediately next to the same WWTP[25], and in Huelva, south Spain, also immediately next to a WWTP[28]. These effluents discharge into narrower channels with weak hydrodynamic mixing. A water quality monitoring in the Ria Formosa areas affected by WWTP during 2018-2019 concluded that the hydrodynamic mixing was the fundamental factor determining the wastewater effluents' footprint[104]. Accordingly, despite the strong input of wastewater into Bahía Almirante (Caribbean Panama shore), its signature was not perceived when sampling *T. testudinum*[105]. It may be concluded that efficient modern wastewater treatment facilities discharging into well-mixed water bodies provide water with enough quality for seagrass meadows and may even promote their growth.

The d_{grass} was compared against the δ^{15}N isotope to strengthen the inference about seagrasses benefiting from moderate nutrient additions. The proportion of the δ^{15}N isotope to the total N is considered an indicator of N origin, with $\delta^{15}N > 4$ppt generally indicating anthropogenic N sources[31,106–110]. From the data compiled about seagrasses worldwide[69], five studies were retrieved on six seagrass species enabling the estimation of d_{grass} and its comparison to the proportion of the δ^{15}N isotope (Fig. 5). The quantile regressions applied to these data showed that the healthier seagrass meadows showing better efficiencies of space occupation (i.e., smaller d_{grass}) were those with a δ^{15}N proportion within 0 to 8 ppt (Fig. 5). Furthermore, in each experiment the best d_{grass} was obtained under intermediate δ^{15}N. With the exception of *Posidonia sinuosa*, for all other seagrass species, healthier meadows were found when δ^{15}N was between 4 and 8 ppt. Hence, these results corroborate that seagrasses may thrive under moderate anthropogenic N enrichment, with N concentrations typical of pristine conditions limiting growth and excessive anthropogenic N additions being detrimental, presumably due to toxicity and eutrophication. For the tropical *Halodule* spp. and *Halophila* sp., the best d_{grass} were obtained with $\delta^{15}N \approx 6$ ppt, whereas for the temperate *Zostera* spp. the best d_{grass} were obtained with $\delta^{15}N \approx 8$ ppt.This may suggest that seagrasses in temperate climates (cooler waters) tolerate anthropogenic nutrient additions better than tropical seagrasses (warmer waters). It has been well established that the toxic effects of too much nutrients are positively correlated with water temperature[79,81].

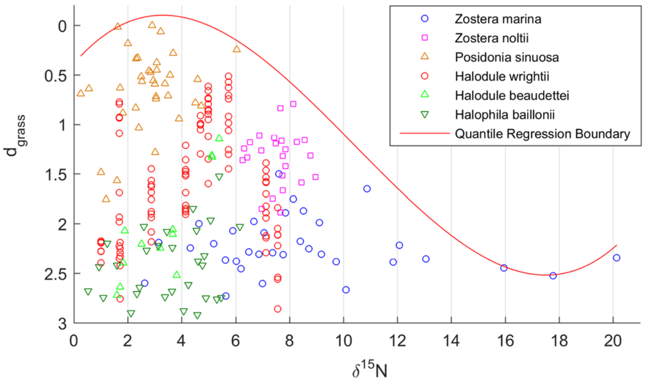

Fig. 5 | Dependency of d_{grass} from the $\delta^{15}N$ isotope. Smaller d_{grass} represents less free space left to be occupied, indicative of healthier meadows occupying space more efficiently[40–43]. The proportion of the $\delta^{15}N$ to total $N > 4$ is an indicator of anthropogenic N sources[31,106–110]. The *Zostera noltei* meadows were sampled in the Ria de Vigo, Spain[66]. *Zostera marina* meadows were sampled in the British Isles[31]. *Posidonia sinuosa* meadows were sampled in Cockburn Sound, Western Australia[93]. *Halodule wrightii* meadows were sampled in Brazil[32]. *Halodule beaudettei* and *Halophila baillonii* meadows were sampled in Costa Rica[132,133].

d_{grass} *vs.* environmental N:P ratio

The foundational work by Atkinson and Smith[70] determined that the molar N:P ratio in seagrass tissues was ≈30. Fourqurean et al.[59] determined that the molar N:P ratios in the tissues of four seagrass species in Bermuda varied within 34 to 40. Duarte[7] compiled data from 27 species, concluding that the average N:P was 24. Based on these ratios, seagrasses are considered commonly limited by the availability of N. Nevertheless, situations typical of P-limitation are known, namely seagrass meadows inhabiting carbonate sediments[50,51]. This P-limitation is a consequence of phosphate adsorption to the grains' surface and thus dependent on the grain size due to its surface:volume ratio[111,112]. Additionally, nutrient sources to the coastal system, location and season are other factors potentially driving a P-limitation[52,54,57–60]. Finally, it has been proposed that P availability determines which primary producers (seaweeds or seagrasses) dominate the community structure, whereas N availability determines their growth[58].

In the present study, considering the N:P in the water and in the sediment, seagrasses should have been usually limited by N (Fig. 3). Only occasionally should seagrasses have been limited by P, namely the *Z. noltei* meadows sampled in Huelva and Cadiz[28] as well as the *Z. marina* and *Z. japonica* sampled in South Korea[73,113–115]. On the opposite side, the Tagus and Sado estuaries showed low N:P corresponding to strong N limitation. However, under similarly moderate and healthy N concentrations, the Tagus meadows tended to occupy space more efficiently than the meadows elsewhere, and particularly than the meadows in the Sado estuary (Permutation Test $p = 0.0005$) (Fig. 4). Furthermore, considering only these meadows under healthy moderate N concentrations, the Tagus meadows showed higher d_{grass} associated to higher P concentrations. This suggests that the Tagus meadows benefited from larger P concentrations despite the theoretical generalized N-limitation following the consensual N:P thresholds[7,59,70]. These findings match earlier studies showing that nutrient limitation for seagrass growth cannot be solely inferred from the environmental N:P as the uptake of nutrients by seagrasses is a rather complex process[57,112,116].

d_{grass} *vs.* seagrass morphometry

Seagrasses have a modular construction. As the rhizomes spread belowground, new nodes are formed at intervals named 'internode length'. From each node, roots extend downward and shoots (commonly one to three) extend upward. The underground part of the shoot is the sheath. Aboveground, several leaves emerge from the sheath and extend vertically[44–48,53,85,117,118]. The iteration of new nodes and shoots – and the resulting morphometrics – in a response to the suitability of local environmental conditions, follows a clonal growth form[40,41,44–48]: Where the environment is favourable, seagrasses adopt a phalanx strategy. In this case, to keep competitors out, seagrass meadows are as compact as possible by maximizing their shoot densities and sizes. In contrast, where the environment is unfavourable, seagrasses adopt a guerilla strategy. In this case, being competitively inferior, seagrass meadows become sparse, spreading their rhizomes by widening their internode lengths and/or reducing their branching to maximize horizontal elongation in search for better conditions in the vicinities. Growth of the rhizomes and root system is fundamental for the establishment in newly colonized locations, as well as for the persistence of established meadows[44–48,53,85,117,118]. According to Sintes et al.[47,48] the colonization of new areas starts by a guerilla growth form that may take long to spread. Only once the rhizome system is fully spread does the meadow proceed to a second growth phase where it attempts to pack shoots as much as possible in a phalanx strategy. For the clonal growth dynamics of seagrasses and their space occupation (i.e., packing of the shoots), traits such as rhizome elongation rate, rhizome branching rate and angle, internode length, shoot mortality and leaf elongation rate have been considered fundamental[44–48,117]. The phenotypic plasticity of such traits has been demonstrated as being fundamental for the response and recovery from disturbances of *Z. noltei*[118].

The species here analysed placed separately on the parameter space, corresponding to different morphologies that result from conditional differentiation (see Vieira et al.[40] for generalized conditional differentiation among seagrasses of the same genus). Despite their conditional differentiation, analysing several species together helped seeing the bigger picture: the present analysis confirmed that, irrespective of the species, seagrass meadows from the Zostera genus increase their biomass and their space occupation by simultaneously increasing the shoot density (D) (i.e., producing new shoots) and increasing the size of the older ones (Fig. 6a)[41,44]. The same results were obtained when analysing the *Z. noltei* meadows in the Tagus and Sado estuaries separately (Fig. 6b). Shoot size was increased by growing leaves that were only longer and not wider. In fact, the variable "leaf width" was uncorrelated with d_{grass} ($r = -0.045$, $p = 0.824$) and unable to improve its regression against the morphometric variables. The shoot density was enhanced by increasing the node density and the number of shoots per node (Fig. 6c). The node density has been considered mainly determined by branching[44–48,117]. Given the absence of data on branch count, we tested the density of apical shoots as its proxy. In sparser meadows apical shoots usually represented less than 5% of all shoots, whereas in denser meadows they could represent up to 26%. Therefore, the count of apical shoots was well correlated with the node count ($r = 0.258$ and $p = 0.051$ for the Tagus, and $r = 0.45$ and $p = 0.0097$ for the Sado). Furthermore, this relation was significantly different between the Tagus and Sado meadows ($p \leq 0.0001$) (Fig. 6d). These results corroborate that the iteration of new secondary, tertiary and quaternary branches, and the consequent increase in apical shoots, is fundamental for the development of seagrass meadows and their adaptation to the local environment[46–48]. The internode length (i.e., the distance between consecutive nodes) was also relevant for the node density ($r = -0.23$, $p = 0.032$), with smaller internode lengths associated with greater node densities (Fig. 6e). The slopes of this correlation were not significantly different between the Tagus and Sado meadows ($p = 0.82$). However, their intercepts were significantly different ($p = 0.024$), with the Sado meadows tending to have slightly larger internode lengths. The average shoot per node being substantially below one (Fig. 6c) resulted from the elevated count of nodes without shoots, presumably due to shoot decay and mortality. The frequent existence of nodes without shoots, the high variability of the shoots:node (Fig. 6c) and the high variability in the number of apical shoots (Fig. 6d) suggest that, similarly to other species[113,119–122], the shoot turnover rate is a fundamental factor in the dynamics of *Z. noltei* meadows and their adaptation to current local conditions. In conclusion, the morphometric plasticity of *Z. noltei*, allowing its adaptation to local conditions, should be mainly dependent on the branching of new rhizome sections, internode length, shoot turnover rate, and vertical elongation of leaves.

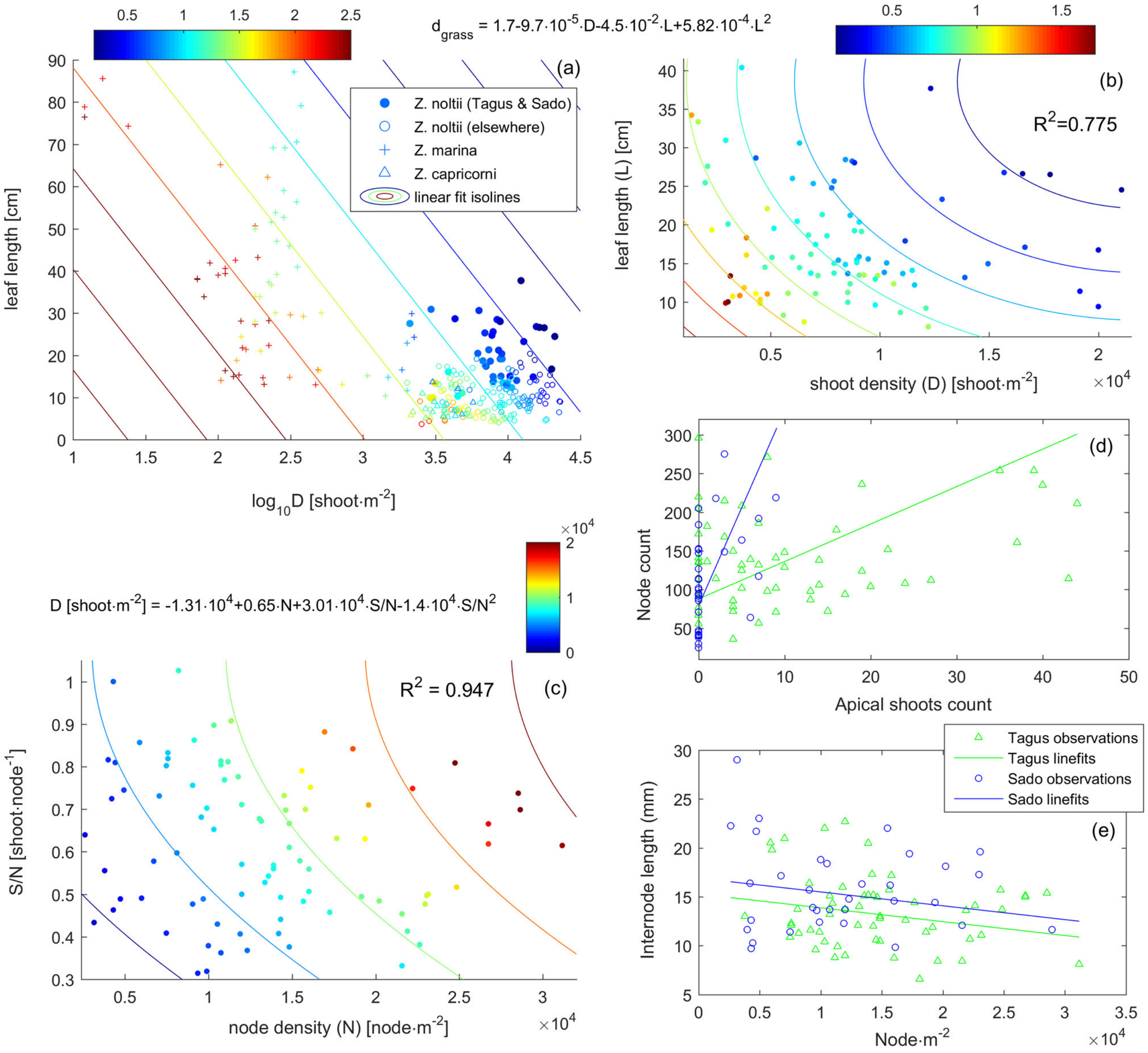

Fig. 6 | Seagrass morphometric responses to maximize the efficiency of space occupation. Seagrass meadows occupying space more efficiently were closer to the seagrass IBL and consequently showed d_{grass} values (the colourbar) closer to 0. For that, seagrass increased their shoot density (D) and leaf length (L) (panel a). This was particularly the case with *Z. noltei* (panel b). *Zostera noltei* shoot density was increased by increasing the node density (N) and the number of shoots per node (S/N) (panel c). Circles represent *Z. noltei* stands observed in the Tagus and Sado estuaries. Coloured isolines are given by the model fit shown atop the respective panel. R^2 is the goodness-of-fit of the equations atop the respective panels. Data from seagrasses worldwide compiled in Vieira et al.[69] and used in Vieira et al.[40–42]. The node density was dependent on the development of new branches (apical shoots as proxy for branching) (**d**) as well as on the internode length (**e**).

d_{grass} *vs.* above:below ground biomass

Like with many terrestrial angiosperms, the below-ground biomass (BB) of seagrasses (i.e., their rhizome and roots) are the perennial structures essential for their long-term survival. The preservation of the below-ground biomass guarantees the preservation of the meadow. However, if severely damaged, the meadow will have difficulty recovering due to the relatively slower growth of the rhizomes[47,48]. Hence, the below-ground biomass is resistant yet has limited resilience to perturbations. On the contrary, the above-ground biomass (AB) typically has an annual dynamic[40,41]. In fact, individually, each shoot has a very limited time-span[113,119–122]. Seagrass meadows' most immediate response to the environment is through phenotypic plasticity of its canopy. Consequently, the above-ground biomass has little resistance yet is resilient to perturbations. The contrast between the dynamics of AB and BB made the AB:BB ratio a potential indicator of the ecological status of a meadow[55,73,123]. Healthier meadows exhibit larger AB:BB whereas meadows in poor shape exhibit smaller AB:BB (Fig. 7). However, this should be framed in the context of each species and its environment.

Confirming that both are indicators of seagrass meadow state, the log(AB:BB) and d_{grass} were correlated (Fig. 7a, b). This correlation was more striking in *Z. noltei* ($r = -0.76$, $p = 0.0001$) than in the remaining species ($r = -0.19$, $p = 0.0001$). Still, the AB:BB was misleading about the good state of the respective meadow when large AB:BB resulted from abnormally low BB. Such was the case of *Z. marina* in the Hanko Peninsula, Baltic Sea[124], where AB:BB ranged between 4 and 10 (the best in our records) as a result of extremely low BB ≈10gDW·m^{-2} (the worst in our records) which is indicative of meadows in very poor state. Clarifying their true degraded status were their poor d_{grass} which ranged between 1.0 and 2. Another example comes from the *Z. noltei* meadow in the Tagus Al1 site sampled during the summer 2022. Its AB:BB = 2.14 was among the best in our monitoring. In normal conditions, this would be indicative of a meadow in very good state. However, this value was due to a very low BB = 25 gDW·m^{-2}; the worst in

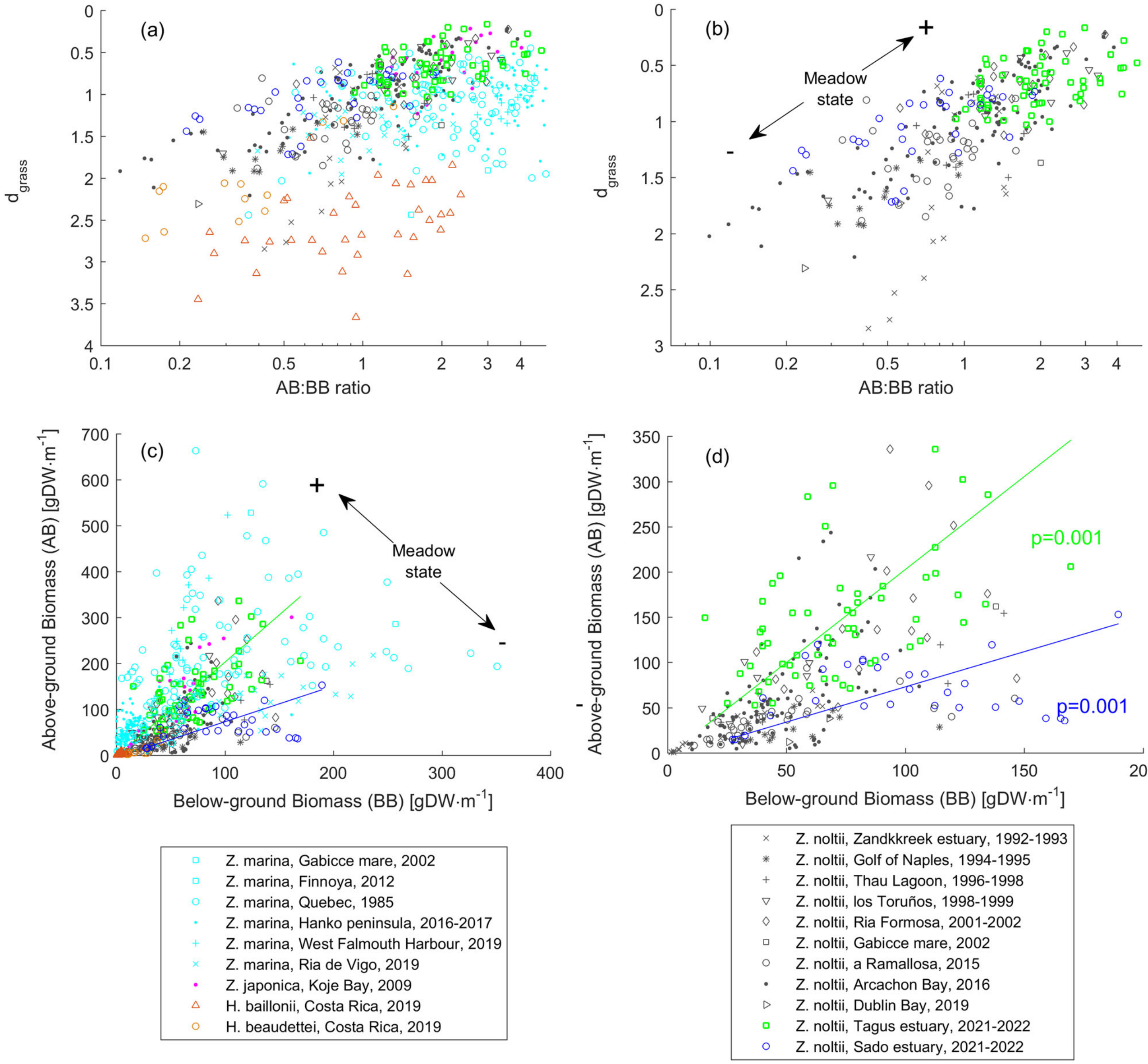

Fig. 7 | Relations between seagrass biomass-based metrics and efficiency of space occupation. The relations between the Above-ground Biomass to Below-ground Biomass (AB:BB) ratio and the efficiency of space occupation of seagrasses (d_{grass}) for seagrasses worldwide (**a**) and specifically for *Zostera noltii* (panel b). The relations between Above-ground Biomass and Below-ground Biomass for seagrasses worldwide (panel c) and specifically for *Zostera noltii* (panel d). Seagrass meadows occupying space more efficiently were closer to the seagrass IBL and consequently showed d_{grass} values closer to 0. Data from seagrasses worldwide compiled in Vieira et al.[69] and used in Vieira et al.[40–42].

our *Z. noltei* monitoring. Clarifying its true status as a degraded meadow, its $d_{grass} = 0.99$ was the worst from all Tagus meadows and among the worse in our monitoring. Dunton[123] provide insights on extreme losses of BB. Alternatively, these could be recent colonisations with a rhizome system still poorly developed. In any case, the large AB relative to the BB suggests that recent environmental conditions have been more favourable than earlier conditions, thus leading to recent gains in AB that BB had yet to catch-up with. Nonetheless, the fact that BB was low was undeniable, which made it at least arguable that the meadow itself was effectively in a good state. In contrast, d_{grass} was immune to this biomass ratio bias. We conclude that the most insightful analyses should include both indices simultaneously. The d_{grass} is an indicator of the state of the meadow when compared to a global standard, whereas the AB:BB is an indicator of the recent trend without needing to have sampled over time. In the case of the Tagus Al1 meadow, although the d_{grass} showed that it was the meadow in worst conditions from all the Tagus, the AB:BB suggested that, being summer, the state of the meadow improved relative to its status in the recent winter.

Given similar AB:BB, *Z. noltei* generally occupied space more efficiently (i.e., approximated the seagrass IBL better) than the other species (Fig. 7a). It is particularly interesting that *Z. noltei* occupied space substantially more efficiently than *Z. marina* with less investment in both BB and AB (Fig. 7a, c). The key to this was *Z. noltei* producing more shoots with the same amount of AB, because its shoots are of smaller sizes compared to *Z. marina* shoots. Moreover, Brun et al.[46] had already found that *Z. noltei* was able to maximize its shoot density while simultaneously minimizing its BB.

The *Z. noltei* meadows in the Tagus estuary were clearly healthier than the *Z. noltei* meadows in the Sado estuary (Fig. 7b, d). This was demonstrated by the larger AB, larger AB:BB and better efficiency of space occupation (i.e., smaller d_{grass}). In fact, the *Z. noltei* meadows in the Tagus estuary

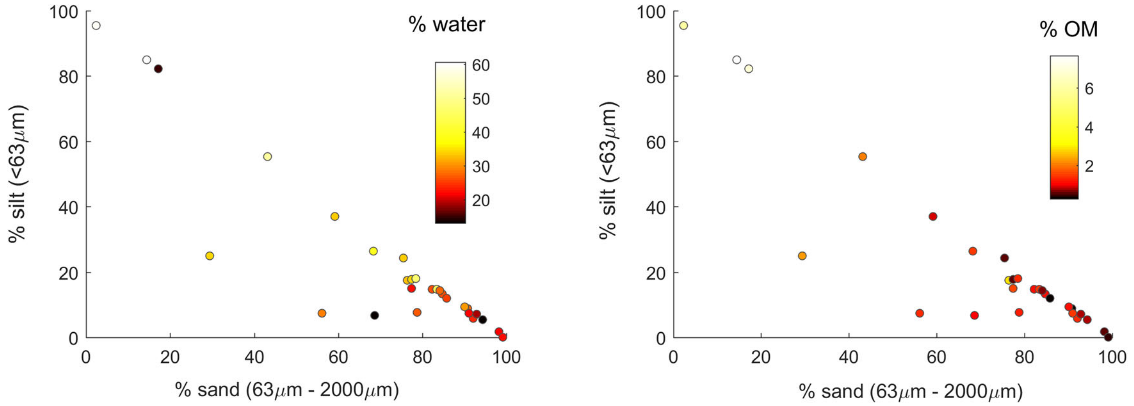

Fig. 8 | Sediment characteristics in the Tagus and Sado estuaries. Percentages of sand, silt, pore water retained during low tide and Organic Matter (OM).

subject to mild anthropogenic nutrient additions were among the ones showing both better efficiencies of space occupation and larger AB:BB.

d_{grass} *vs.* sediment properties

The organic matter (OM) content in sediments from estuaries, lagoons, and lakes typically ranges between 0% and 10%[114,124–133]. Its origin is the deposition of particulate OM that can either be allochthonous (e.g. terrestrial detritus, mangroves) or autochthonous (e.g. dead phytoplankton and seagrass leaves, particle resuspension)[7,126,127,129,131,134]. Seagrass canopies promote the sedimentation of suspended particles and inhibit their resuspension by protecting the seabed from current- and wave-driven turbulence. This effect is stronger when seagrass canopies are denser[7,126,131,134,135]. The carbon in the deposited OM either accumulates or enters the food web. Thus, seagrass meadows are seen as a carbon sink and a significant part of the Blue Carbon sector[8–15,129,131]. However, fine sediments and high OM content can have a negative impact on seagrasses by decreasing pore water exchange, inducing hypoxia, anoxia and accumulation of toxic sulphides[131,136–138].

The dominant sediment types in the Tagus and Sado meadows were silt and sand (Fig. 8). Naturally, their fractions were inversely proportional (r = -0.91, $p \leq 0.0001$). The percentage of pore water during low tide and of organic matter (OM) were larger in finer sediments ($r = 0.62$, $p = 0.0015$, and $r = 0.89$, $p \leq 0.0001$, respectively for water and OM), matching other estuarine systems[125,128,135]. A few simple explanations may be advanced: (i) lower hydrodynamics leading to more deposition of finer sediments and OM, and less water drainage[131,139], (ii) finer sediments exerting more resistance than coarser sediments to water draining during low tide[131] and (iii) greater net adsorption of OM onto finer grains with higher surface areas[140]. Next, we test whether the presence and density of *Z. noltei* meadows affected the percentage of pore water during low tide and of OM in the sediments.

The percentage of pore water retained during low tide varied between 13.2% and 60.6%. The efficiency of space occupation by *Z. noltei* meadows was mildly correlated with it (Fig. 9a) and the correlation improved when the AB:BB was used instead (Fig. 9b). Two effects may influence this correlation: (i) seagrass effect on pore water, where denser meadows help retain the water by obstructing its drainage and evaporation, and (ii) porewater effect on seagrasses, where roots and rhizomes of intertidal *Z. noltei* benefit from sediments retaining water while exposed during low tide, thus avoiding desiccation. Both effects may coexist and even interact positively[141,142].

The percentages of OM in the sediments of the Tagus and Sado estuaries, varying from 0.3% to 8%, matched the OM concentrations found elsewhere around the globe[114,124–133]. It is particularly interesting that it matched the 0.74 to 11.43% OM found in *Z. noltei* meadows in the Banc d'Arguin, Mauritania[131]. The %OM in the sediment had a clear non-linear relation with the efficiency of space occupation by the *Z. noltei* meadows in the Tagus and Sado estuaries (Fig. 9c), as well as with their AB:BB (Fig. 9d). The relation was positive until a ≈ 4–5%OM threshold, shifting to negative thereafter. This matched exactly the dynamic and the 5% OM threshold found in the review by Koch[143], as well as for *Z. noltei* in the Banc d'Arguin[131], at the exception that, in the latter case, the OM threshold was slightly higher (≈7%). These results confirm the local fishermen knowledge that "seagrasses don't like and decline where the sediment is too dark and muddy". The positive relation under low %OM results from denser canopies better protecting the seabed from hydrodynamic turbulence, thus promoting greater sedimentation of suspended particles and greater resuspension inhibition[7,8,12,126,131,134,135]. However, above the given thresholds, the high OM content becomes detrimental for seagrasses due to hypoxia, anoxia, and the accumulation of toxic sulphides[131,136–138,143]. This creates a negative feedback loop where increased canopy densities cause increased OM in the sediments, which in their turn cause decreased canopy densities[131,143]. Our results support the existence of such negative feedback loop. Still, this may be avoided or delayed if the OM in the sediment is absorbed by seagrasses in quantities large enough to significantly reduce it. Seagrasses may uptake OM from the sediment[131,143–145]. On the other hand, seagrass productivity may be a source of labile OM to the sediment[12]. In the experiments by Armitage and Fourqurean[12] and by Folmer et al.[131], the carbon in the sediment decreased with experimental nutrient additions. Accordingly, in our work, the most degraded *Z. noltei* meadows in the Sado estuary, with an OM above the 5% threshold, were the Com1 and Com3, which also had some of the lowest nutrient concentrations, particularly within the sediment (Table 1). These evidences support the hypothesis that nutrients promote seagrass growth, which in its turn promotes net OM absorption from the sediments by seagrass roots. An alternative for seagrasses coping with extreme %OM in the sediment is the symbiosis between seagrasses, lucinid bivalves and their gill-inhabiting bacteria. This three-party symbiosis nurtures all symbionts, decreases sulphides and increases oxygen[146,147]. The experiment by van der Heide[146] was performed with *Z. noltei*. However, in our study, lucinids were nowhere to be found. In conclusion, for *Z. noltei* meadows to act as efficient sustainable carbon sinks they need sufficient nutrient inputs to sustain healthy meadows and can benefit from the presence of lucinid bivalves counteracting the harmful effects of anoxia and sulphides.

d_{grass} *vs.* the benthic macrofauna

Seagrass meadows generally act as a buffer stabilizing environmental conditions and protecting the seafloor[148]. Consequently, despite their complex interactions with biotopes[148–151], seagrass meadows substantially enhance the abundance and diversity of benthic macrofauna when compared to bare sediments[124,152–154]. Seagrasses also enhance the ecosystem metabolism, namely by gross primary production and community respiration[124]. The increasing size and complexity of the above- and below-ground structures of seagrasses, besides promoting sediment deposition and retention (see sections above and references therein), also promote macrofauna abundance and diversity[124,128,139,148,150,151,155–158]. In fact, in the review by Heck Jr and Orth[147] the variation in the physical structure and complexity of seagrass meadows were considered the fundamental factors determining the

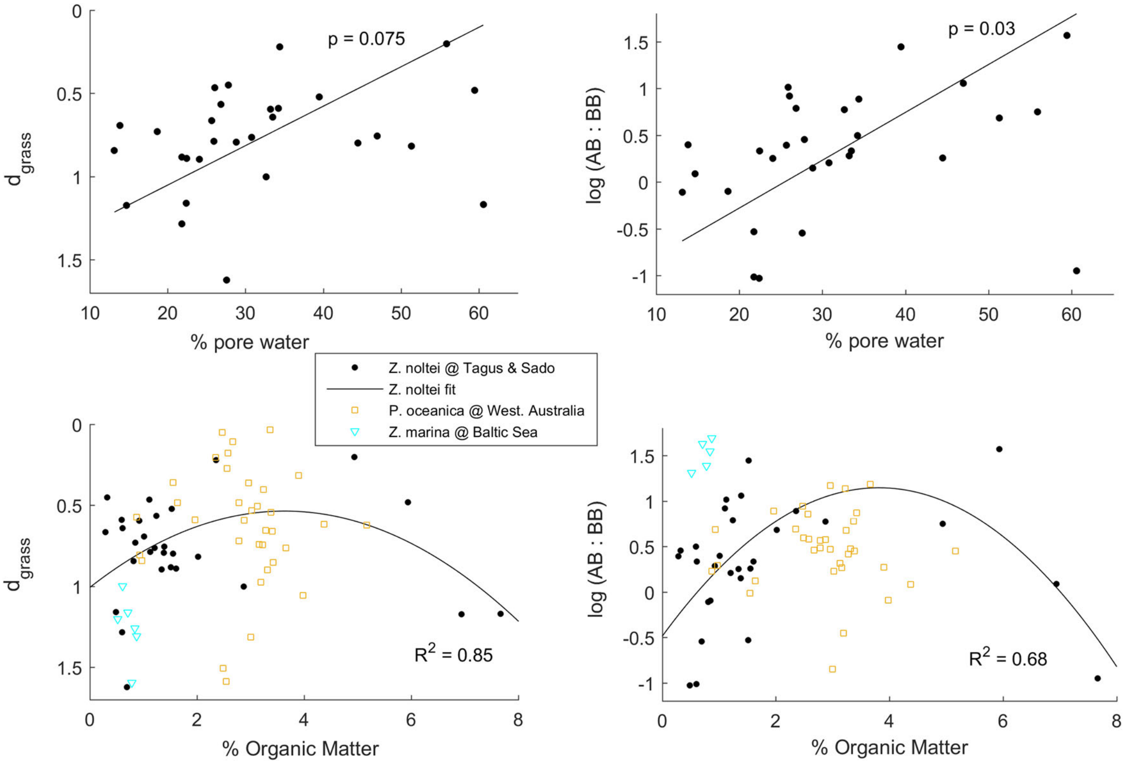

Fig. 9 | Relation between the compaction of *Z. noltei* meadows in the Tagus and Sado estuaries and the sediment properties. Other species from other locations are shown for comparison. The metrics used for seagrass meadows were the efficiency of space occupation (d_{grass}) and the ratio of above-ground biomass to below-ground biomass (AB:BB). Because the AB:BB is a ratio, its logarithm should be used in regressions against other variables, otherwise, the ratios < 1 are underrated relative to the ratios > 1[165]. Linear regressions were fit by Reduced Major Axis (RMA). This is a model 2 regression adequate under the assumed inexistence of a hierarchical predictor-response relation[195–197]. Non-linear regressions were fit by 2nd order polynomial regressions.

composition of the associated fauna and their trophic interactions (e.g., providing prey with shelter from predators). Here, we studied the relation between the efficiency of space occupation by seagrasses and their associated benthic macrofauna, using data from the *Z. noltei* meadows that we sampled in the Tagus and Sado estuaries, and *Z. marina* meadows from the Hanko peninsula in the Baltic Sea[124]. Our results broadly fit the previous findings. In particular, our results showed that meadows occupying space more efficiently (i.e., smaller d_{grass}) had associated larger abundances of benthic macrofauna, thus matching previous reports that the increasing size and complexity of seagrass structures promotes the abundance and diversity of benthic macrofauna[124,128,139,148,150,151,155–158].

The benthic macrofauna was more abundant (ind·m^{-2}) in denser meadows occupying space more efficiently ($r = 0.457$, $p = 0.016$) (Fig. 10), and thus, much more abundant in the Tagus meadows than in the Sado and Baltic meadows ($p = 0.0009$). The macrofauna abundances were independent of the pore water retention ($r = 0.05$, $p = 0.241$). On the contrary, the OM had a clear non-linear effect. OM between 2% – 6% seems to be optimal for benthic macrofauna abundance. With OM below ≈ 2% the macrofauna abundances declined, possibly due to shortage of detritus to scavenge upon. This decline was particularly relevant in the Sado estuary and the Baltic Sea, due to an outstanding deficit of the gastropod *Peringia ulvae* (Pennant, 1777). With OM above ≈ 6% the macrofauna abundances declined, possibly due to anoxic conditions and the toxic effects of sulphides[131,143]. Also, in Moulay Bousselham, the macrobenthos abundances, species richness and biodiversity indexes worsened significantly in stations with OM roughly above 6%[125].

The benthic macrofauna species richness (i.e., the number of species) was not significantly dependent on the efficiency of space occupation by *Z. noltei* meadows ($r = 0.15$, $p = 0.49$), pore water ($r = 0.005$, $p = 0.98$), or OM ($r = 0.178$, $p = 0.33$), nor was it significantly different between the Tagus meadows and the Sado meadows ($p = 0.34$) (Fig. 10). The species evenness was better in seagrass meadows occupying space less efficiently (d_{grass}-Simpson D's $r = -0.631$, $p = 0.0003$, d_{grass}-Shannon-Weiner's $r = -0.628$, $p = 0.0001$). The Tagus meadows showed significantly more uneven macrofauna abundances than the Sado and Baltic Sea meadows (differences in Simpson D's $p = 0.0001$ and differences in Shannon-Weiner's $p = 0.0001$), meaning that the dominant species were more dominant in the Tagus meadows. This negative correlation between species evenness and abundance of benthic macrofauna matches previous reports about seagrass meadows[159] as well as about the generalized recovery of European freshwater systems during the last half-century[160].

The fundamental factor for this macrobenthos biodiversity pattern and contrast was the gastropod *Peringia ulvae*, previously named *Hydrobia ulvae*. This small marine snail tolerant to brackish water showed a correlation $r = -0.45$ with log(d_{grass}). *Peringia ulvae* was of lesser relevance where seagrasses were less efficient in space occupation. In the Sado and Baltic Sea it was often absent or represented smaller abundances and fractions of the benthic macrofauna. Nevertheless, in the Carrasqueira stations (along the Sado main channel) and the Baltic during the summer 2017, it represented between 37% and 62% of the benthic macrofauna abundance. In Tagus meadows, which were much more efficient occupying space, *P. ulvae* was abundant with up to 10000ind·m^{-2} or even 30000 ind·m^{-2}, representing at least 50% of the benthic macrofauna community, and often above 90%. This contrast between the Tagus and the Sado meadows is likely to be explained by:

(i) the differences between the Tagus and Sado in riverine inputs and brackish water, a consequence of the Sado estuary having been exposed for long from drastic reductions in riverine discharges. Accordingly, for *Z. noltei* and *Z. marina* meadows in the Bay of Santander, riverine freshwater input was also a fundamental factor[128]. Seagrasses were also in better condition in the more riverine locations, showing larger densities and biomasses (which corresponds to better efficiencies of

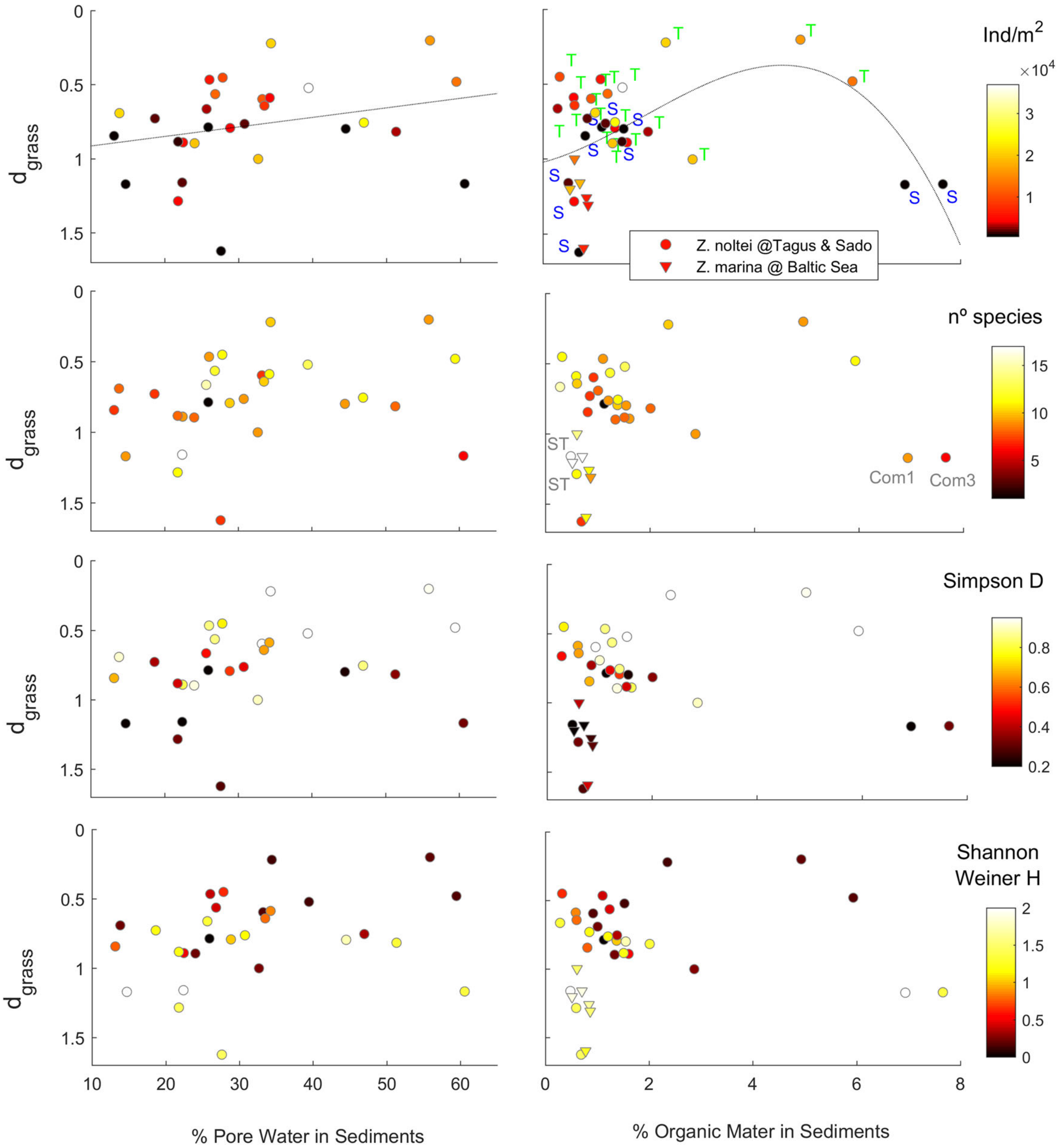

Fig. 10 | Relation between the efficiency of spare occupation by seagrasses, sediment properties and macrobenthic biodiversity. *Zostera noltei* samples taken from the Tagus (T) and Sado (S) estuaries. *Zostera marina* samples taken from the Hanko peninsula in the Baltic Sea[124]. The d_{grass} closer to 0 means more efficiency of space occupation and less free space available for macrophyte competitors. Sediments characterized by their percentage of organic matter and percentage pore water at low tide. Black dotted lines are a linear regression and a 3rd order polynomial regression. Biodiversity characterized by abundance (individuals/m^2), number of species in a sample, Simpson D and Shannon-Weiner (H) indexes.

space occupation). Benthic macrofauna was also more abundant near riverine locations but more diverse near marine locations, and *P. ulvae* (*Hydrobia ulvae* per Ondiviela et al.[128]) was also a key factor influencing this pattern.

(ii) the differences in the protection provided by denser seagrass meadows, preserving moisture better, protecting from desiccation, shading UV-radiation and sheltering from hydrodynamic stress. Such sources of stress and protective effects of denser meadows are known for intertidal seagrasses[158,161,162] as well as intertidal algae[163–167]. Furthermore, seagrass cover also protects from predation by fishes[148] as well as predation from above, namely crabs and aquatic birds foraging for small invertebrates in intertidal meadows[149,168–170]. Our results support these theories, showing that denser seagrass mats, harder to scout, have more abundant benthic macrofauna communities. However, protection from predators can have complex trophic cascading effects. Many of the sheltered macroinvertebrates are grazers. Their increase results in decreased benthic microalgae that they feed upon, and consequently, may contribute to decreased %OM[149]. This effect may oppose the accumulation of OM by recycling it and incorporating the carbon into the food web. The end result is highly unpredictable, depending on the environmental properties, ecosystem pathways and identity and number of epibenthic consumers.

The results showed a very close match between the *Z. noltei* meadows in Portugal and the *Z. marina* meadows in the Baltic, despite their distance, different environments, and different seagrass and macrofauna species (Fig. 10). Our results also matched a 14-year survey of *Zostera marina* meadows along 500 km of Britany coastline (France) showing that, as the sediment became muddier the diversity of benthic macrofauna increased but their abundances decreased strongly[150]. The patterns of benthic macrofauna biodiversity observed in our study were broadly similar to those reported for *Z. noltei* meadows in the Bay of Santander (a lagoon system in Spain)[128], reported for *Z. noltei* and *Ruppia cirrhosa* (Petagna) Grande, 1918 meadows in Moulay Bousselham, (a lagoon system in Morocco)[125], and reported for other *Z. marina* meadows in the Baltic Sea[153]. Together, they support the notion that riverine inputs, intermediate concentrations of organic matter, and substantial seagrass cover are of great importance for benthic macrofauna communities.

d_{grass} *vs.* alternative coastal ecosystem indicators

Coastal ecosystems are broader than seagrasses and their associated benthic macrofauna. Besides the seagrass meadows, there is also the bare substrate and saltmarshes. Besides benthic macrofauna there is also benthic flora, and pelagic and microbial communities. The AMBI-AZTI Marine Biotic Index[171] and the M-AMBI[172,173] are widely used alternative tools for the assessment of the ecological status of coastal waters focusing on the composition of the soft-bottom benthic macro-invertebrate communities. One step further, the integration of several ecosystem components, together with processes such as community respiration, gross and net production, and ecosystem services provided to humankind, help to provide a global perspective. An example of such broader indices is the ECOAdS[174]. In this context, the d_{grass} may facilitate the application of such wider indices. As an example, the d_{grass} can substitute at least 15 of the seagrass metrics used in the ECOAdS application to the Adriatic[174]; some of which are rather ambiguous, as are the cases of the biomass and density of reproductive shoots and flowering frequency, to which a multitude of driving factors and dynamics have been attributed[175–181], not necessarily related with the condition of the respective meadows.

Here, we compare d_{grass} against the AMBI and M-AMBI, as those are some of the simpler indices applicable to the set of variables that were sampled. Both the AMBI and M-AMBI agree with the d_{grass} in that Tagus seagrass biotopes were generally in good status as were some of the Sado seagrass biotopes (Fig. 11). This was particularly evident when comparing their d_{grass} with estuaries worldwide subject to elevated human occupation and pressures (Section 2.2, Fig. 4 and Section 2.5, Fig. 7). However, the AMBI and M-AMBI considered that the other Sado seagrass biotopes and the Baltic seagrass biotopes were also in good condition, which was clearly contradicted by the seagrass indicators, namely the d_{grass}, above-ground, below-ground, and total biomass (Fig. 11). At a finer resolution, the AMBI and M-AMBI completely misfit the seagrass indicators, in the sense that they were either uncorrelated or negatively correlated. The negative correlation between the efficiency of space occupation and the M-AMBI was particularly conspicuous and relevant. This resulted from the M-AMBI giving large preponderance to a species evenness index under the assumption that more even abundances report to healthier communities[172,173]. However, this has been contradicted by recent evidence, namely our work, a meta-analysis on seagrass biotopes[159], and a meta-analysis on the generalized recovery of European freshwater systems during the last half-century[160]. In all cases, it was found that healthier systems were characterized by increased benthic macrofauna abundance associated with decreased species evenness. Also misaligned with the seagrass indicators, the AMBI and M-AMBI were insensitive to nutrients, percentage pore water and organic matter in the sediment (Fig. 12). More surprisingly, despite being based on benthic macrofauna, AMBI and M-AMBI were insensitive to the fundamental factor in the benthic macrofauna data set: the changes in *P. ulvae* (see previous section 2.7), likely because this species belongs to ecological group III. Consequently, and unlike the seagrass indicators, the AMBI and M-AMBI were unhelpful for differentiating between the Tagus, Sado, and Baltic seagrass biotopes (Figs. 11 and 12) or to identify the factors driving their differences (see previous sections).

The Com1, Com3, and ST stations in Sado are an illustrative case of the limitations of simple indexes that only evaluate one system component, as are d_{grass}, AMBI and M-AMBI. The ST station was the most different from the others, being located close to the estuary's mouth and a summer resort, lower in the intertidal, subject to the strongest currents, with the lowest OM% in the Sado stations, and under intense human impacts from beachgoers, boating and recreational clam harvesting. On the opposite extreme, the Com1 and Com3 were located deep in the inner estuary, in the Comporta secondary channel, hardly accessible on foot, on mid intertidal mudflats with the largest OM% observed in the entire monitoring study (Figs. 2, 10 and 12, and Table 1), and maybe the largest ever reported in seagrass studies. Alone, the seagrass indicators were only able to identify Com1, Com3, and ST among the most degraded *Z. noltei* meadows. However, analysed together with more information, some of the distinct drives for their poor performance were clearly identified (namely tidal height, nutrient concentrations and % OM). And yet, contradicting all this, the AMBI and M-AMBI erroneously considered Com1, Com3 and ST among the stations with best ecological quality, particularly ST and Com1 (Fig. 12). Contributing to this biased assessment was the favourable species evenness (as per the Shannon-Weiner H), which was misleading as (i) it reported to a larger number of species but with very low abundances (Fig. 10) and, (ii) the other Tagus and Sado stations showed less favourable species evenness due to the strong dominance of *P. ulvae*; yet this was likely a consequence of brackish water influence, and not of poorer ecological status.

Conclusions

The availability of free space to be occupied is the ultimate factor limiting the growth and size of terrestrial plants[34–39], algae[43], and seagrasses[40–42]. Here, we showed that the efficiency by seagrass meadows in occupying that space (i.e., the compaction of their canopies) was achieved by increasing their node density (increasing the branching of their rhizomes and decreasing the internode length), number of shoots per node, and leaf length. Based on this efficiency of space occupation, the d_{grass} was developed as an indicator of seagrass condition[40–42]. Here, we showed that d_{grass} correlated well with other seagrass morphometric indicators such as the above-ground to below-ground biomass ratio. However, the latter was misleading when high ratios resulted from low below-ground biomass. The d_{grass} metric, being immune to this ratio bias, was a more reliable indicator.

The d_{grass} enabled P-limitation detection in situations of theoretical N-limitation. It also revealed the thresholds for low nutrient concentrations to limit seagrass growth and for high nutrient concentrations to become harmful. Also, d_{grass} showed that seagrasses are more sensitive to inorganic nitrogen than to phosphate, and to nutrients in the water than to nutrients in the sediment. This metric showed that moderately enhanced nutrient concentrations benefited seagrass meadows, even when these nutrient additions came from anthropogenic sources such as WWTP and a food factory. This benefit cascaded through the biotope. The compaction of the canopies thereby promoted organic matter (OM) deposition in the sediment and the abundance of benthic macrofauna by providing food and shelter.

We highlight that healthier meadows with increased benthic macrofauna abundances were associated with decreased species evenness. This same macrofauna abundances-evenness pattern has characterized the benthic community of seagrass biotopes[159] and the generalized recovery of European freshwater systems during the last half-century[160]; thus, contradicting the predominant perception that healthier benthic communities are characterized by more even species abundances.

Although meadows in better condition promoted OM deposition, sediment OM above 6% was associated with more degraded meadows, likely due to anoxia, hypoxia, and sulphides damaging their rhizomes and root system[131,136–138,143]. Our results support the hypothesis by Folmer et al.[131] on direct negative feedback between seagrasses and sediments. When canopies become very dense, they enhance the deposition of organic matter and fine sediments. When a threshold is surpassed, the excessive organic matter content and reduced pore water renovation create hypoxic conditions harmful for the rhizomes of seagrasses. This negative feedback poses a limitation to the role of seagrass beds as carbon sinks.

Our results showed that anthropogenic sources of nutrients, which have generally been perceived as harmful to seagrass systems, may

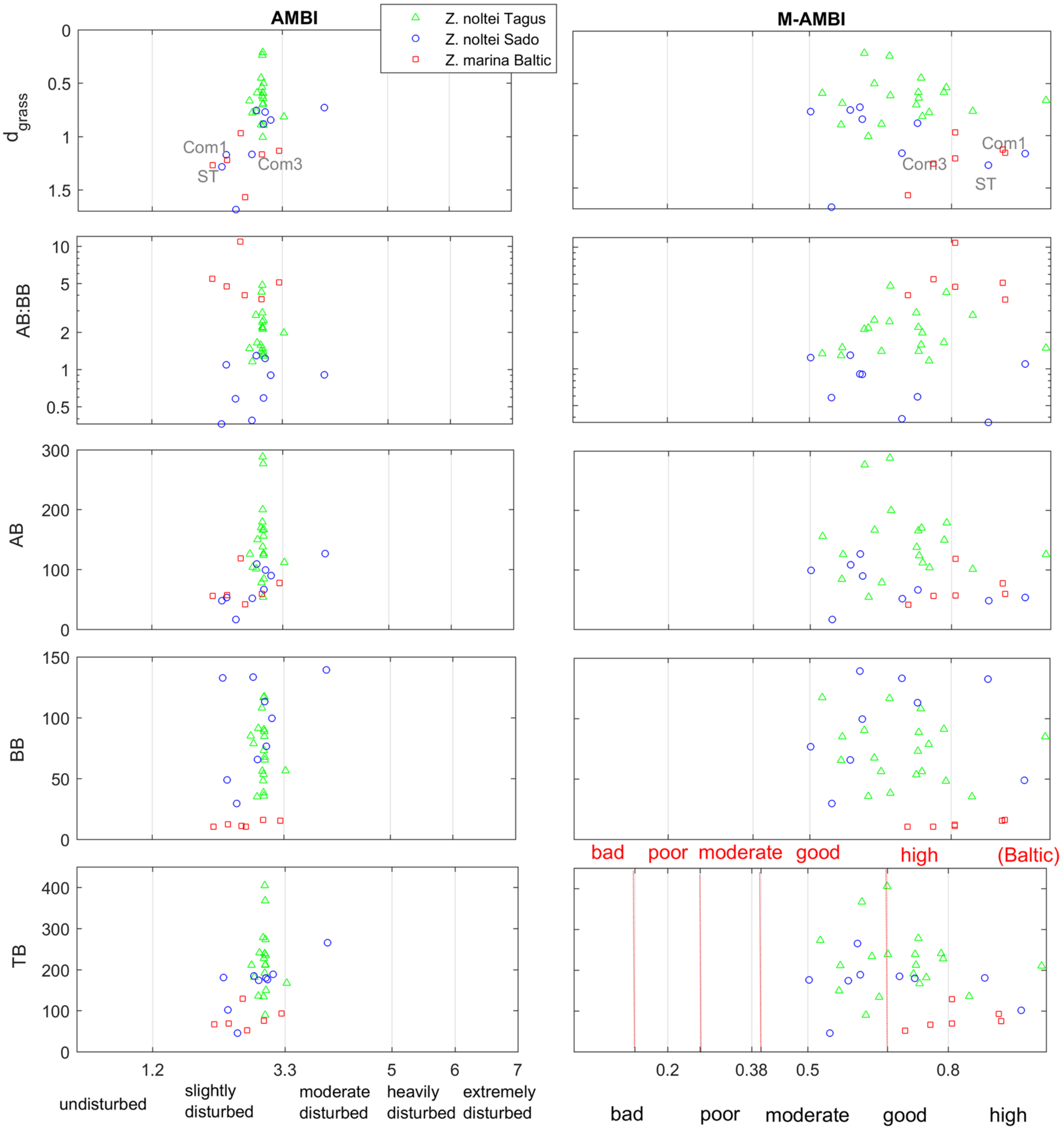

Fig. 11 | Environmental evaluation of *Z. noltei* meadows from seagrass indicators and benthic macrofauna community. Seagrass indicators are the efficiency of space occupation (d_{grass} closer to 0 means more efficiency of space occupation and less free space available for macrophyte competitors), Above-ground Biomass (AB), Below-ground Biomass (BB), Total Biomass (TB) and AB:BB ratio (AB:BB). Benthic macrofauna indicators are the AMBI and M-AMBI. Samples taken from the Tagus (green triangles) and Sado (blue circles) estuaries.

contribute to a good seagrass condition, the provision of ecosystem services, and enhance their role as carbon sinks. This is provided anthropogenic nutrient input is moderate, well-managed, and coordinated with the natural role of seagrass as water purifiers. Such benefits to seagrass systems brought-about by anthropogenic nutrient additions and their cascading effects have been previously reported. Hodgson and Bucher[61] found that moderate levels of nutrient inputs from properly managed sewered catchments beneficed seagrass meadows, their productivities, and ultimately the production of estuary-dependent fisheries.

In our case, the health of *Z. noltei* meadows and their cascading effects contrasted between the Tagus and Sado estuaries. The Sado had less nutrients, sparser meadows, highly muddy sediments, and less abundant benthic macrofauna. Together with the drastic reduction in the abundance of *Peringia ulvae* —a small marine gastropod tolerant to brackish water— these conditions suggest that the Sado estuary is being affected by reduced riverine discharges, consequence of the dams upstream, freshwater capture for agriculture, and successive droughts that have been affecting south Portugal in recent decades. Deterioration of estuarine and lagoon systems due to decreased riverine discharges has been reported elsewhere (Texas Department of Water Resources 1983 in Lee and Dunton[53,128],). The 21st century may become a new era of seagrass decline, associated biotopes, and ecosystem services due to decreased riverine discharges consequence of dams upstream, farming overexploitation of water resources, and climate-change induced droughts.

Methods

Study areas

Monitoring took place in the Tagus and Sado estuaries, Portugal (Fig. 2a) during the summers of 2021 and 2022. These are meso-tidal estuaries, with a mean tidal amplitude of 2.4 m. Detailed characterizations of the geography and hydrodynamics are available for both the Tagus[182] and Sado[75]. Approximately 3 million people live in the region, mainly around the Tagus estuary, where the Portuguese capital, Lisbon, is located (Fig. 2a). Natural sources of nutrients to these estuaries are riverine discharge, runoff, and tidal

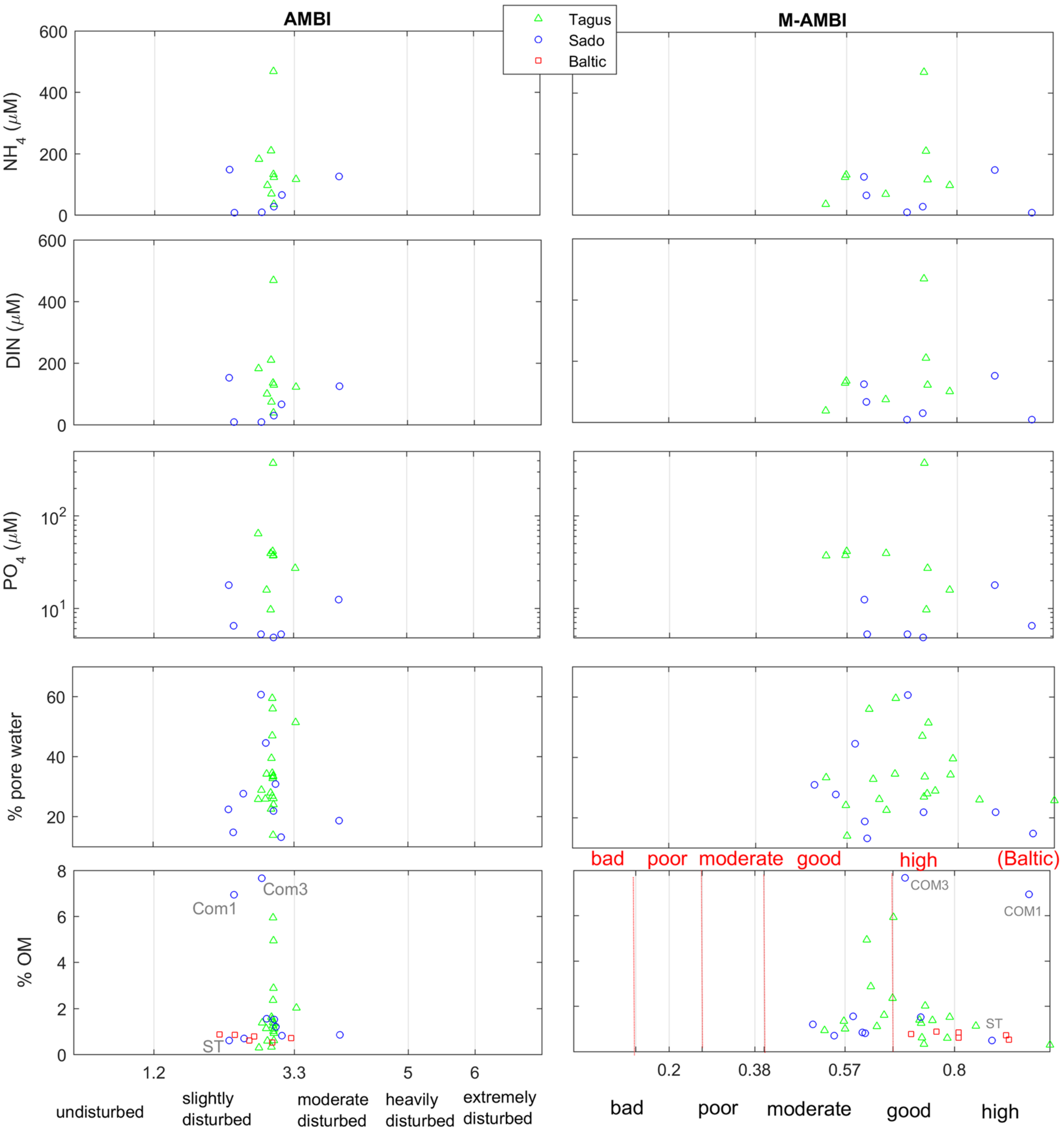

Fig. 12 | Environmental evaluation of *Z. noltei* meadows from sediment properties and benthic macrofauna community. Sediments characterized by ammonium, DIN, phosphate, % of organic matter and % pore water at low tide. Benthic macrofauna indicators are the AMBI and M-AMBI. Samples taken from the Tagus and Sado estuaries (our monitoring), and the Baltic Sea[124].

transport of nutrients upwelled in the coastal ocean during the summer upwelling season. The main anthropogenic sources are urban effluents, agriculture, livestock, and rice fields. In the Sado estuary, the area next to the Comporta and Carrasqueira villages, and landward for tens of km along the river, is intensively occupied by rice fields. However, the gates are opened only once per year to let the rice-field water outflow. The Sado estuary has been severely affected by decreasing riverine discharges, consequence of the successive droughts that have been affecting south Portugal in recent decades, concomitantly with water retention in dams upstream[75,183].

The stations were selected based on the logistical and safety conditions to accessing the *Z. noltei* meadows. Stations were also selected to be at least ~ 200-300 m away from each other. Larger and denser meadows were prioritized as these were considered more likely to be fully developed, thus minimizing a potential bias from lower density and biomass from meadows potentially still in initial stages of development. Monitoring at Tagus included twelve stations (Fig. 2b and Table 1). Stations "Al1" and "Al2" were inside the Tagus Estuary Natural Reserve and thus expected to be the most pristine sites. Stations "Al3" and "Al4" were along the Alcochete city beach. Al3 was next by the Alcochete village centre and with a small sewer discharge. Stations "Sam1", "Sam2", "Sam3" and "Sam4" were next to the Samouco locality, along a distance gradient from a small stream with agricultural run-off. Station "Sex" was located at the Seixalinho wastewater treatment plant (WWTP) runoff. Station "Rib1" was located at the Riberalves fish factory runoff, whereas "Rib2" and "Rib3" were approximately 300 m and 600 m away, respectively. All stations were located at the mid intertidal. The Sado estuary monitoring included eight stations (Fig. 2c). Station "ST" was along the main channel, closer to the estuary inlet and next to the SolTroia tourist and residential development area. It was the only station located at the lower intertidal limit, only exposed during spring tides. Stations "Com1", "Com2", "Com3" and "Com4" were located along the Comporta secondary channel whereas stations "Cr1", "Cr2" and "Cr3" were in the main channel next to the Carrasqueira village. All these stations were located at the mid intertidal. Not all of these stations were sampled during both summers. Some *Z. noltei* meadows monitored in 2021 were subsequently destroyed by intensive clam harvesting, being unavailable for sampling in 2022 (Rib1, Rib2, and Com2), or we had difficulties returning there (Com1 and Com4). A few new stations were included in the sampling campaign of 2022 (Al4, Sam4 and Rib3) to compensate with alternative locations.

Field sampling and laboratorial analysis

Sampling occurred in July and August, the period when *Zostera noltei* meadows peak in biomass, density, efficiency of space occupation, and meadow condition[40,41]. At each location, 6 cores were taken from the centre of the meadow (or patch), away from the edges to avoid edge effects on the seagrass variables: three cores for seagrasses and associated benthic macrofauna, two cores for nutrients in the sediment porewater and one core for sediment granulometry and organic matter (OM). All cores were taken within 1 m^2 without interfering with each other. Additionally, three water samples were taken for nutrient content.

Each PVC core for seagrasses (11 cm diameter, 20 cm depth) was placed over the meadow and a small piece of wire was used to pull all the leaves belonging to the shoots inside it. Then, the core was push through the sediment. In the laboratory, each core was washed using a 0.5 mm filter and seagrasses removed. Later, the benthic macroinvertebrates were sorted, isolated, and preserved for further morphological identification to the lowest possible taxonomic level using a stereomicroscope and identification keys. The *Z. noltei*, shoot and node density, shoots per node, internode length, shoot length, sheath length, leaf length, leaf diameter and counts of apical shoots were estimated for each core with the aid of a magnifying glass and a calliper. Finally, above-ground and below-ground biomass were separated, dried in an oven for 48 h at 60 °C, and weighed (whole sample and not individual-wise). Data about *Z. noltei* meadows from other locations, as well as from other species, were also used for comparison (data from WorldSeagrassBiomassDensity.xlsx[69]). Nutrients (ammonium, nitrites, nitrates, phosphate and silica) in the sediment porewater were sampled at each location while exposed during low tide. One core 5 cm wide and 10 cm deep was taken soon after exposure and another core prior to the immersion. While the meadows were immersed, each location was sampled for water properties (temperature, ammonium, nitrites, nitrates, phosphate, and silica) three times: soon after immersion, during peak high tide, and soon before exposure. Temperature was recorded with a digital thermometer to the decimal place. In the laboratory, samples were filtered through a 0.45 μm membrane (Whatman cellulose acetate) to 25 ml plastic bottles and frozen until analysis. Nutrients were determined by colorimetry in a Skalar autoanalyzer SAN +[184]. Data from subsequent samplings was averaged.

Another core 5 cm wide and 10 cm deep was taken for grain size and organic content. Grain size analysis was carried out by dry sieving, using the protocol described in Gaudêncio et al.[185]. Sediment type was identified using the percentage of fines and gravel according to Folk's classification[186]. Total OM from sediment samples was estimated by mass loss on ignition in a muffle furnace. Samples were oven-dried at 100 °C and weighed to estimate the total weight of grains plus OM (W_T). Subsequently, the samples were mashed up and then ignited at 450 °C to constant weight. They were weighed again after cooling in a desiccator at room temperature (W_f). The total OM content was calculated by the difference between the weight of the oven-dried samples and the weight of the combusted ones ($OM = W_T - W_f$). Later, it was converted to percentage organic matter (OM%) in the sample ($OM/\ W_T \times 100$).

Seagrass biological indicator

The plant Interspecific Boundary Line (IBL) is the upper limit for the biomass-density relation observed for any terrestrial plant on the planet, thus representing the maximum possible efficiency of space occupation by terrestrial plants[38,39]. Conversely, IBLs specific for algae[43] as well as seagrasses[40] were found (Fig. 1). The seagrasses specific IBL has been updated as more biomass-density data from seagrasses worldwide have been gathered and analysed[42,69]. This updated seagrass IBL is given by $\log_{10}B = a + b\cdot\log_{10}D$, where B is above-ground biomass in grams of dry weight (g DW), D is shoot density ($shoots\cdot m^{-2}$), $a = 4.708$ and $b = -0.489$. Thirty years ago, Olesen and Sand-Jensen[187] first tested the biomass-density relations in seagrasses gathering *Zostera marina* Linnaeus, 1753 data from 29 studies in Europe, America, and Japan. However, they focused on the -3/2 power law—also known as the self-thinning law—driven by intraspecific competition and the resulting density-dependent mortality[34–39]. According to this law, environmental constraints induce competition, whose strength is reflected on the slope and intercept of the negative correlation log(Biomass) = a + b·log(Density) obtained from time series of monospecific plant stands. The basis is that the mortality of the weaker opens space for the growth of the stronger. However, because seagrasses have modular construction—a form of clonal growth—with shoots sharing the same rhizome and root system, shoots do not compete but cooperate instead. Thus, the self-thinning law (and intraspecific competition) does not apply to seagrasses[40,187] nor to other clonal macrophytes[39,43]. Nevertheless, the Interspecific Boundary Line (IBL) -i.e., the maximum possible space occupation—still applies to clonal macrophytes, as much as it does to non-clonal (Fig. 1)[39,40,43]. The distance of a macrophyte stand to its IBL was demonstrated as a robust biological/ecological indicator[40–43]. Clonal macrophytes approach and depart this IBL in trajectories approximately perpendicular to it, i.e. couple their biomass and density dynamics by simultaneously increasing or decreasing them (Fig. 1)[40,41]. Curiously, this pattern was originally reported by Olesen and Sand-Jensen188 (their Figs. 2, 3 and 4).

The actual efficiency of space occupation by a monitored seagrass meadow is given by its observed distance (d_{grass}) to the seagrass IBL (Fig. 1)[40–42]. This distance corresponds to the space still free/available to be occupied. As meadows grow approaching the IBL, this d_{grass} free space tends to zero. To estimate d_{grass}, the observed shoot density (D) is first used to estimate its theoretically correspondent maximum possible biomass ($\dot{B}$) from $\log_{10}\dot{B} = a + b\cdot\log_{10}D$. Then, the perpendicular distance between the observation and the IBL is given by $d_{grass} = (\log_{10}\dot{B} - \log_{10}B)\cdot\cos\theta$, with $\theta = \mathrm{arctg}(\,|\,b\,|\,) = 0.455$, and thus, $\cos\theta = 0.898$.

Biodiversity indicators

The benthic macrofauna was characterized by several indicators, namely:

(i) the density, or total abundance standardized to m^2 (i.e., $individuals\cdot m^{-2}$).

(ii) the species richness (i.e., the number of species in the three cores).

(iii) the Simpson D index is a measure of species evenness. It estimates the probability that two individuals consecutively randomly picked from the sample belong to the same type. The Simpson D ranges between 0 and 1, with 0 representing the maximum species evenness.

(iv) the Shannon-Weiner (H) index is a measure of species evenness. It estimates the uncertainty in predicting the type of the next randomly chosen individual. The Shannon-Weiner (H) index ranges between 0 and ∞, with 0 representing the least species evenness and total certainty in predicting the next randomly chosen individual because there is only one species present.

(v) The AMBI index estimates the ecological status of a biotope by accounting the biodiversity of species and their abundances, as well as their perceived sensibility to environmental factors[171].

(vi) The M-AMBI (Multivariate AMBI) is a multimetric index based on the macroinvertebrate communities and designed to meet the needs of the WFD. It uses a multivariate approach integrating the response of three selected metrics, i.e. species richness, Shannon's diversity and AMBI[172,173].

Permutation tests inferring statistical significances

A 3-way permutation test, with factors 'substrate', 'estuary' and 'nutrients', was performed comparing among the nutrients in the Tagus and Sado estuaries, Dadae Bay[73], Barang Lompo and Gusung Tallang[74]. Only these sites were compared because they were the only ones that had measurements for all substrates and nutrients. The data was log-transformed for homoscedasticity.

In all analyses, statistical significances were estimated by permutation tests[188]. These are non-parametric alternatives to the traditional parametric tests (e.g. ANOVA) where the null hypothesis of no differences between groups is simulated by randomly permuting the observations among all groups. Then, to simulate the null hypothesis, the observations were randomly permuted among the treatments of each factor. The null hypothesis was simulated 9999 times. Adding the observed h_a to the 9999 simulations of h_0 gives 10000 iterations. The significance of h_a was given by the proportion of times that h_0 was larger than h_a.

Quantile regressions

Usually, regressions aim at the central tendency of the bivariate (in simple regressions) or multivariate (in multiple regressions) distributions, in linear or non-linear, model 1 (minimizing the error in y) or model 2 (simultaneously minimizing the error in x and y) regressions. Such are the cases of Ordinary Least Squares (OLS), Weighted Least Squares (WLS), Iterative Re-weighted Least Squares (IRLS), Principal Components Analysis (PCA), Reduced Major Axis (RMA) and Errors-in-Variables, to name a few. Often, these have closed form analytical solutions, which are often given by the Least Squares method.

Quantile Regression is a particular type of regression that aims not at the central tendency but at any given quantile of the bivariate distribution[189]. The coefficients yielding the i^{th} quantile line are found by optimization/search algorithms minimizing the quantile loss function. Quantile Regressions can be fit to linear[40–43,189] and non-linear forms[190]. Linear Quantile Regressions have been used to find the Interspecific Boundary Lines (IBL) of plants[189], algae[43], and seagrasses[40,42]. In these cases, the upper boundary has been estimated by extreme upper quantiles (e.g., the 99.5% quantile). Here, 2nd and 3rd order polynomial Quantile Regressions were used to estimate the d_{grass} dependency on nutrients. Because the objective was to find the boundary to the minimum d_{grass}—corresponding to the minimum space left free/available consequence of the maximum efficiency-of-space-occupation—the 0.5% quantile was generally used. Notice on the respective figures that the d_{grass} vertical axis is inverted. Quantile Regressions are numerically unstable when there is large scatter and/or the bivariate distribution is severely skewed. The stabilizing solution applied here and elsewhere[40,42,43] was to select only the n observations closer to the desired boundary. For that, here, x was aggregated into intervals and from each interval were selected the n observations with lower d_{grass}. A preliminary curve was estimated, from each were selected the N observations closer to it. From these N observations was estimated the definite curve. Quantile Regressions are also numerically unstable when very extreme quantiles are inferred from small data sets. Therefore, when the data selection procedure yielded small sets, the Quantile Regressions were applied estimating more moderate quantiles (1-2%).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The original contributions presented in the study are provided online at https://doi.org/10.5281/zenodo.13880753.

Code availability

All analysis was made in Matlab ® Mathworks. Regression fitting and its statistical inference from Monte Carlo methods were done from scripts developed from Vieira and Creed[191–193], currently available at https://doi.org/10.13140/RG.2.2.28182.36169.

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References

1. Duffy, J. E. Biodiversity and the functioning of seagrass ecosystems. *Mar. Ecol. Prog. Ser.* **311**, 233–250 (2006).
2. Morrison, M. A. et al. *"Seagrass Meadows as Biodiversity and Productivity Hotspots," in New Zealand Aquatic Environment and Biodiversity Report No 137.* https://docs.niwa.co.nz/library/public/NZAEBR-137.pdf (2014).
3. Unsworth, R. & Cullen-Unsworth, L. Biodiversity, ecosystem services, and the conservation of seagrass meadows. *In Coastal Conservation*. (eds. Maslo, B. & Lockwood, J. L.) 95–130 (Cambridge University Press, 2014).
4. Hyman, A. C., Frazer, T. K., Jacoby, C. A., Frost, J. R. & Kowalewski, M. Long-term persistence of structured habitats: seagrass meadows as enduring hotspots of biodiversity and faunal stability. *Proc. R. Soc. B.* **286**, 1912 (2019).
5. McHenry, J. et al. Modelling the biodiversity enhancement value of seagrass beds. *Divers. Distrib.* **27**, 2036–2049 (2021).
6. Surugiu, V., Adrian, T., Ilie, S. & Quijón, P. A. A hotspot in the Romanian black Sea: Eelgrass beds drive local biodiversity in surrounding bare sediments. *Front. Mar. Sci*. https://doi.org/10.3389/fmars.2021.745137 (2021).
7. Duarte, C. M. Seagrass nutrient content. *Mar. Ecol. Prog. Ser.* **67**, 201–207 (1990).
8. Gacia, E., Granata, T. C. & Duarte, C. M. An approach to measurement of particle flux and sediment retention within seagrass (*Posidonia oceanica*) meadows. *Aquat. Bot.* **65**, 255–268 (1999).
9. Boer, W. F. Seagrass–sediment interactions, positive feedbacks and critical thresholds for occurrence: a review. *Hydrobiologia* **591**, 5–24 (2007).
10. Nordlund, L. M., Koch, E. W., Barbier, E. B. & Creed, J. C. Seagrass ecosystem services and their variability across genera and geographical regions. *PloS One* **11**, e0163091 (2016). Ntalos, G., and Sideras, A.The usage of *Posidoniaa oceanica* as raw material for wood composite and thermal energy production. *Mater. Methods Technol.* **8**, 605–611 (2014).
11. Duarte, C. M. et al. Seagrass community metabolism: assessing the carbon sink capacity of seagrass meadows. *Glob. Biogeochem. Cycles* **24**, GB4032 (2010).
12. Armitage, A. R. & Fourqurean, J. W. Carbon storage in seagrass soils: long-term nutrient history exceeds the effects of near-term nutrient enrichment. *Biogeosciences* **13**, 313–321 (2016). pp.
13. Reynolds, L., Waycott, M., McGlathery, K. & Orth, R. Ecosystem services returned through seagrass restoration: restoration of ecosystem services. *Restor. Ecol*. **24**, 583–588 (2016).
14. Sousa, A. I., da Silva, J. F., Azevedo, A. & Lillibo, A. I. Blue carbon stock in *Zostera noltei* meadows at Ria de Aveiro coastal lagoon (Portugal) over a decade. *Sci. Rep.* **9**, 14387 (2019).
15. Bedulli, C., Lavery, P. S., Harvey, M., Duarte, C. M. & Serrano, O. Contribution of seagrass blue carbon toward carbon neutral policies in a touristic and environmentally-friendly island. *Front. Mar. Sci*. https://doi.org/10.3389/fmars.2020.00001 (2020).
16. Ricart, A. M. et al. High variability of blue carbon storage in seagrass meadows at the estuary scale. *Sci. Rep.* **10**, 5865 (2020).
17. Ntalos, G. & Sideras, A. The usage of *Posidonia oceanica* as a raw material for wood composite and thermal energy production. *Mater. Methods Technol.* **8**, 605–611 (2014).
18. Plis, A., Lasek, J. A., Zuwała, J., Yu, C.-C. & Iluk, A. Combustion performance evaluation of *Posidoniaa oceanica* using TGA and bubbling fluidized bed combustor (batch reactor). *J. Sustain. Min.* **15**, 181–190 (2016).
19. Hamdaoui, O., Ibos, L., Mazioud, A., Safi, M. & Limam, O. Thermophysical characterization of *Posidoniaa oceanica* marine fibers intended to be used as an insulation material in mediterranean buildings. *Constr. Build. Mater.* **180**, 68–76 (2018).

20. Jedidi, M. & Abroug, A. Valorization of *Posidoniaa oceanica* balls for the manufacture of an insulating and ecological material. *Jordan J. Civ. Eng.* **14**, 417–430 (2020).
21. Rammou, E. et al. The potential use of seaweed (*Posidonia oceanica*) as an alternative lignocellulosic raw material for wood composites manufacture. *Coatings* **11**, 2079–6412 (2021).
22. Parente, A., Serio, F., Montesano, F. F., Mininni, C. & Santamaria, P. The compost of *Posidoniaa oceanica* residues: a short review on a new component for soiless growing media. *Acta Hortic.* **1034**, 291–298 (2014).
23. McMahon, K., Collier, C. & Lavery, P. S. Identifying robust bioindicators of light stress in seagrasses: a meta-analysis. *Ecol. Indic.* **30**, 7–15 (2013).
24. European Community. Directive 2000/60/EC of the European parliament and of thec ouncil of 23 october 2000 establishing a framework for Community action in the field of water policy. *Off J. Eur. Commun.* **327**, 1–73 (2000).
25. Cabaço, S., Machás, R. & Santos, R. Biomass–density relationships of the seagrass *Zostera noltii*: A tool for monitoring anthropogenic nutrient disturbance. *Estuar. Coast. Shelf. Sci.* **74**, 557–564 (2007).
26. Cabaço, S., Machás, R., Vieira, V. & Santos, R. Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*). *Estuar. Coast. Shelf. Sci.* **78**, 1–13 (2008).
27. Cabaço, S. et al. Effects of nutrient enrichment on seagrass population dynamics: evidence and synthesis from the biomass–density relationships. *J. Ecol.* **101**, 1552–1562 (2013).
28. Romero, J., Martínez-Crego, B., Alcoverro, T. & Perez, M. A. A multivariate index based on the seagrass *Posidonia oceanica* (POMI) to access the ecological status of coastal waters under the water framework directive (WFD). *Mar. Pollut. Bull.* **55**, 196–204 (2007).
29. Garcıa-Marín, P. et al. Multimetric index based on the seagrass *Zostera noltii* (ZoNI) for ecological quality assessment of coastal and estuarine in SW Iberian peninsula. *Mar. Pollut. Bull.* **68**, 46–54 (2013).
30. Jones, B. L. & Unsworth, R. K. F. The perilous state of seagrass in the British Isles. *R. Soc. Open Sci*. **3**, 150596 (2016).
31. Jones, B. L., Cullen-Unsworth, L. C. & Unsworth, R. F. K. Tracking nitrogen source using d15N reveals human and agricultural drivers of seagrass degradation across the British Isles. *Front. Plant Sci*. https://doi.org/10.3389/fpls.2018.00133 (2018).
32. Bertelli C. M., Creed J. C., Nuuttila H. K. & Unsworth R. K. F. The response of the seagrass *Halodule wrightii* Ascherson to environmental stressors. *Estuar. Coast. Shelf Sci.* **238**, 106693 (2020).
33. Bertelli, C. M., Bull, J. C., Cullen-Unsworth, L. C. & Unsworth, R. K. F. Unravelling the spatial and temporal plasticity of Eelgrass meadows. *Front Plant Sci.* **12**, 664523 (2021).
34. Yoda, K., Kira, T., Ogawa, H. & Hozumi, K. Self-thinning in overcrowded pure stands under cultivated and natural conditions intraspecific competition among higher plants. *J. Biol. Osaka. City. Univ.* **14**, 107–129 (1963).
35. White, J. & Harper, J. L. Correlated change in plant size and number in plant populations. *J. Ecol.* **58**, 467–485 (1970).
36. Hutchings, M. J. Weight–density relationships in ramet populations of clonal perennial herbs, with special reference to the 3/2 power law. *J. Ecol.* **67**, 21–33 (1979).
37. Westoby M. The self-thinning rule. *Adv. Ecol. Res*. **14**,167–225 (1984).
38. Weller, D. E. The interspecific size-density relationship among crowded plant stands and its implications for the -3/2 power rule of self-thinning. *Am. Nat.* **133**, 20–41 (1989).
39. Scrosati, R. A. The interspecific biomass–density relationship for terrestrial plants: where do clonal red seaweeds stand and why? *Ecol. Lett.* **3**, 191–197 (2000).
40. Vieira, V. M. N. C. S., Lopes, I. E. & Creed, J. C. The biomass–density relationship in seagrasses and its use as an ecological indicator. *BMC Ecol.* **18**, 44 (2018).
41. Vieira, V. M. N. C. S., Lopes, I. E. & Creed, J. C. A model for the biomass-density dynamics of seagrasses developed and calibrated on global data. *BMC Ecol.* **19**, 4 (2019).
42. Vieira, V. M. N. C. S. et al. Seagrasses benefit from mild anthropogenic nutrient additions. *Front. Mar. Sci.* **9**, 960249 (2022).
43. Creed, J., Vieira, V. M. N. C. S., Norton, T. A. & Caetano, D. A meta-analysis shows that seaweeds surpass plants, setting life-on-earth's limit for biomass packing. *BMC Ecol.* **19**, 1–11 (2019).
44. Brun, F. G. et al. Shoot organization in the seagrass *Zostera noltii*: implications for space occupation and plant architecture. *Helgol. Mar. Res.* **60**, 59–69 (2006).
45. Brun, F. G. et al. Clonal building, simple growth rules and phylloclimate as key steps to develop functional–structural seagrass models. *Mar. Ecol. Prog. Ser.* **323**, 133–148 (2006).
46. Brun, F. G., Cummaudo, F., Olivé, I., Vergara, J. J. & Pérez-Lloréns, J. L. Clonal extent, apical dominance and networking features in the phalanx angiosperm *Zostera noltii* Hornem. *Mar. Biol. 2007* **151**, 1917–1927 (2007).
47. Sintes, T., Marbà, N., Duarte, C. M. & Kendrick, G. A. Nonlinear processes in seagrass colonisation explained by simple clonal growth rules. *Oikos 2005* **108**, 165–175 (2005).
48. Sintes, T., Marbà, N. & Duarte, C. M. Modeling nonlinear seagrass clonal growth: assessing the efficiency of space occupation across the seagrass flora. *Estuar. Coast. J. ERF* **29**, 72–80 (2006).
49. Andrade, M. F. et al. Assessing the influence of sewage outfalls on seagrass meadows using nitrogen isotopes. *Mar. Pollut. Bull.* **196**, 115578 (2023).
50. Short, F. T., Dennison, W. C. & Capone, D. G. Phosphorus-limited growth of the tropical seagrass syringodium filiforme in carbonate sediments. *Mar. Ecol. Prog. Ser.* **62**, 169–174 (1990).
51. Fourqurean, J. W., Zieman, J. C. & Powell, G. V. N. Phosphorus limitation of primary production in Florida Bay: Evidence from C: N: P ratios of the dominant seagrass Thalassia testudinum. *Limnol. Oceanogr.* **37**, 162–171 (1992).
52. Udy, J. W., Dennison, W. C., Long, W. J. L., Len, J. & McKenzie, L. J. Responses of seagrass to nutrients in the great barrier reef, Australia. *Mar. Ecol. Prog. Ser.* **185**, 257–271 (1999).
53. Lee, K.-S. & Dunton, K. H. Effects of nitrogen enrichment on biomass allocation, growth, and leaf morphology of the seagrass Thalassia testudinum. *Mar. Ecol. Prog. Ser.* **196**, 39–48 (2000).
54. Fourqurean, J. W. & Zieman, J. C. Nutrient content of the seagrass Thalassia testudinum reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida keys USA. *Biogeochemistry* **61**, 229–245 (2002).
55. Armitage, A. R. et al. Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. *Estuaries* **28**, 422–434 (2005).
56. Johnson, M. W., Heck, K. L. & Fourqurean, J. W. Nutrient content of seagrasses and epiphytes in the northern Gulf of Mexico: evidence of phosphorus and nitrogen limitation. *Aquat. Bot.* **85**, 103–111 (2006).
57. Armitage, A. R. & Fourqurean, J. W. Stable isotopes reveal complex changes in trophic relationships following nutrient addition in a coastal marine ecosystem. *Estuar. Coast.* **32**, 1152–1164 (2009).
58. Allgeier, J. E., Rosemond, A. D. & Layman, C. A. Variation in nutrient limitation and seagrass nutrient content in Bahamian tidal creek ecosystems. *J. Exp. Mar. Biol. Ecol.* **407**, 330–336 (2011).
59. Fourqurean, J. W., Manuel, S. A., Coates, K. A., Kenworthy, W. J. & Boyer, J. N. Water quality, isoscapes and stoichioscapes of seagrasses indicate general P limitation and unique N cycling in shallow water benthos of Bermuda. *Biogeosciences* **12**, 6235–6249 (2015).
60. Allgeier, J. E. et al. Anthropogenic versus fish-derived nutrient effects on seagrass community structure and function. *Ecol. (Durh.)* **99**, 1792–1801 (2018).

61. Hodgson, B. R. & Bucher, D. J. Biological processes in seagrass beds of coastal lagoons to maintain estuary-dependent marine fisheries. *Mar. Environ. Res.* **189**, 106033 (2023).
62. Orth, R. J. et al. Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. *Sci. Adv.* **6**, 41 (2020).
63. Greening, H., Janicki, A., Sherwood, E. T., Pribble, R. & Johansson, J. O. R. Ecosystem responses to long-term nutrient management in an urban estuary: Tampa Bay, Florida, USA. *Estuar. Coast. Shelf. Sci.* **151**, A1–A16 (2014).
64. Bertelli, C. M., Robinson, M. T., Mendzil, A. F., Pratt, L. R. & Unsworth, R. K. F. Finding some seagrass optimism in Wales, the case of *Zostera noltii*. *Mar. pollut. Bull.* **134**, 216–222 (2018).
65. Burdick, D. M. et al. A case for restoration and recovery of zostera marina l. in the great bay estuary. *PREP Rep. Publ.* **441**, 1–19 (2020).
66. Román, M., Fernández, E., Zamborain-Mason, J., Martínez, L. & Méndez, G. Decadal changes in the spatial coverage of *Zostera noltei* in two seagrass meadows (Ría de Vigo; NW Spain). *Reg. Stud. Mar. Sci.* **36**, 101264 (2020).
67. Dunic, J. C., Brown, C. J., Connolly, R. M., Turschwell, M. P. & Côté, I. M. Long-term declines and recovery of meadow area across the world's seagrass Bioregions. *Glob. Change Biol.* **27**, 4096–4109 (2021).
68. de los Santos, C. B. et al. Recent trend reversal for declining European seagrass meadows. *Nat. Commun.* **10**, 3356 (2019).
69. Vieira, V. M. N. C. S. et al. World seagrass biomass density. *Original Res.* https://doi.org/10.13140/RG.2.2.32343.14240 (2022).
70. Atkinson, M. J. & Smith, S. V. C:N:P ratios of benthic marine plants. *Limnol. Oceanogr.* **28**, 568–574 (1983).
71. Touchette, B. W. & Burkholder, J. M. Review of nitrogen and phosphorus metabolism in seagrasses. *J. Exp. Mar. Biol. Ecol.* **250**, 133–167 (2000).
72. Nascimento, Â. et al. Tidal variability of water quality parameters in a mesotidal estuary (Sado Estuary, Portugal). *Sci. Rep.* **11**, 23112 (2021).
73. Lee, S. Y., Kim, J. B. & Lee, S. M. Temporal dynamics of subtidal *Zostera marina* and intertidal *Zostera japonica* on the southern coast of Korea. *Mar. Ecol.* **27**, 133–144 (2006).
74. Erftemeijer, P. L. A. & Herman, P. M. J. Seasonal changes in environmental variables, biomass, production and nutrient contents in two contrasting tropical intertidal seagrass beds in South Sulawesi, Indonesia. *Oecologia* **99**, 45–59 (1994).
75. Biguino, B., Sousa, F. & Brito, A. C. Variability of currents and water column structure in a temperate estuarine system (Sado estuary, Portugal). *Water* **13**, 187 (2021).
76. Burkholder, J. M., Tomasko, D. A. & Touchette, B. W. Seagrasses and eutrophication. *J. Exp. Mar. Biol. Ecol.* **350**, 46–72 (2007).
77. Lee, K.-S., Park, S. R. & Kim, Y. K. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *J. Exp. Mar. Biol. Ecol.* **350**, 144–175 (2007).
78. Han, Q. & Liu, D. Macroalgae blooms and their effects on seagrass ecosystems. *J. Ocean Univ. China* **13**, 791–798 (2014).
79. Burkholder, J. M., Mason, K. M. & Glasgow, J. H. B. Water-column nitrate enrichment promotes decline of eelgrass Zostera marina: evidence from seasonal mesocosm experiments. *Mar. Ecol. Prog. Ser.* **81**, 163–178 (1992).
80. van Katwijk, M. M., Schmitz, G. H. W., Gasseling, A. P. & van Avesaath, P. H. Effects of salinity and nutrient load and their interaction on Zostera marina. *Mar. Ecol. Prog. Ser.* **190**, 155–165 (1999).
81. van Katwijk, M. M., Vergeer, L. H. T., Schmitz, G. H. W. & Roelofs, J. G. M. Ammonium toxicity in eelgrass Zostera marina. *Mar. Ecol. Prog. Ser.* **157**, 159–173 (1997).
82. Bird, K. T., Johnson, J. R. & Jewett-Smith, J. In vitro culture of the seagrass Halophila decipiens. *Aquat. Bot.* **60**, 377–387 (1998).
83. Brun, F. G., Hernandez, I., Vergara, J. J., Peralta, G. & Perez-Llorens, J. L. Assessing the toxicity of ammonium pulses to the survival and growth of Zostera noltii. *Mar. Ecol. Prog. Ser.* **225**, 177–187 (2002).
84. van der Heide, T. et al. Toxicity of reduced nitrogen in eelgrass (Zostera marina) is highly dependent on shoot density and pH. *Oecologia* **158**, 411–419 (2008).
85. Cabaço, S., Alexandre, A. & Santos, R. Population-level effects of clam harvesting on the seagrass Zostera noltii. *Mar. Ecol. Prog. Ser.* **298**, 123–129 (2005).
86. Garmendia, J. M. et al. Effect of trampling and digging from shellfishing on Zostera noltei (Zosteraceae) intertidal seagrass beds. *Sci. Mar.* **81**, 121–128 (2017).
87. McMahon, K. et al. Herbicide contamination and the potential impact to seagrass meadows in Hervey Bay, Queensland, Australia. *Mar. Pollut. Bull.* **51**, 325–334 (2005).
88. Waycott, M., Longstaff, B. J. & Mellors, J. Seagrass population dynamics and water quality in the great barrier reef region: a review and future research directions. *Mar. pollut. Bull.* **51**, 343–350 (2005).
89. Negri, A. P., Flores, F., Mercurio, P., Mueller, J. F. & Collier, C. J. Lethal and sub-lethal chronic effects of the herbicide diuron on seagrass. *Aquat. Toxicol.* **165**, 73–83 (2015).
90. Espel, D. et al. Dynamics of the seagrass Zostera noltei in a shallow Mediterranean lagoon exposed to chemical contamination and other stressors. *Est. Coast. Shelf Sci.* **222**, 1–12 (2019).
91. Bester, K. Effects of pesticides on seagrass beds. *Helgol. Mar. Res.* **54**, 95–98 (2000).
92. Gamain, P. et al. Can pesticides, copper and seasonal water temperature explain the seagrass zostera noltei decline in the arcachon bay? *Mar. Poll. Bull.* **134**, 66–74 (2018).
93. Fraser, M. W. & Kendrick, G. A. Belowground stressors and long-term seagrass declines in a historically degraded seagrass ecosystem after improved water quality. *Sci. Rep.* **7**, 2045–2322 (2017).
94. Lafratta, A. et al. Seagrass soil archives reveal centennial-scale metal smelter contamination while acting as natural filters. *Sci. Tot. Environ.* **649**, 1381–1392 (2019).
95. Short, F. T. & McRoy, C. P. Nitrogen uptake by leaves and roots of the seagrass Zostera marina L. *Botanica Mar.* **27**, 547–556 (1984).
96. Paling, E. I. & McComb, A. J. Nitrogen and phosphorus uptake in seedlings of the seagrass Amphibolis Antarctica in Western Australia. *Hydrobiologia* **294**, 1–4 (1994).
97. Lee, K.-S. & Dunton, K. H. Inorganic nitrogen acquisition in the seagrass Thalassia testudinum: development of a whole-plant nitrogen budget. *Limnol. Oceanogr.* **44**, 1204–1215 (1999).
98. Plus, M., Deslous-Paoli, J.-M. & Dagault, F. Factors influencing primary production of seagrass beds (Zostera noltii hornem) in the Thau lagoon (French Mediterranean coast). *J. Exp. Mar. Biol. Ecol.* **259**, 63–84 (2001).
99. Plus, M., Chapelle, A., Ménesguen, A., Deslous-Paoli, J.-M. & Auby, I. Modelling seasonal dynamics of biomasses and nitrogen contents in a seagrass meadow (Zostera noltii hornem): application to the thau lagoon (French Mediterranean coast). *Ecol. Model.* **161**, 213–238 (2003).
100. Kaldy, J. E. Effect of temperature and nutrient manipulations on eelgrass Zostera marina L from the Pacific Northwest, USA. *J. Exp. Mar. Biol. Ecol.* **453**, 108–115 (2014).
101. Nayar, S., Loo, M. G. K., Tanner, J. E., Longmore, A. R. & Jenkins, G. P. Nitrogen acquisition and resource allocation strategies in temperate seagrass Zostera nigricaulis: Uptake, assimilation and translocation processes. *Sci. Rep.* **8**, 17151 (2018).
102. Viana, I. G., Saavedra-Hortúa, D. A., Mtolera, M. & Teichberg, M. Different strategies of nitrogen acquisition in two tropical seagrasses under nitrogen enrichment. *N. Phytol.* **223**, 1217–1229 (2019).
103. Han Q. et al. Effect of DIN and DON sources on the nitrogen uptake of the seagrass Zostera japonica and the macroalgae Ulva pertusa

previously grown in different light levels. *Front. Mar. Sci.* https://doi.org/10.3389/fmars.2023.1015323 (2023).

104. Cravo, A. et al. Unravelling the effects of treated wastewater discharges on the water quality in a coastal lagoon system (Ria Formosa, South Portugal): relevance of hydrodynamic conditions. *Mar. Pollut. Bull.* **174**, 113296 (2022).
105. Gaubert-Boussarie, J., Altieri, A. H., Duffy, J. E. & Campbell, J. E. Seagrass structural and elemental indicators reveal high nutrient availability within a tropical lagoon in Panama. *PeerJ* **9**, e11308 (2021).
106. Macko, S. A., and Ostrom, N. E. Pollution studies using stable isotopes. In *Stable Isotopes in Ecology and Environmental Science*. (eds. Lajtha, K & Michener, R.H.) 45–62 (Oxford: Blackwell Scientific Publications, 1994).
107. McClelland, J. W., Valiela, I. & Michener, R. H. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. *Limnol. Oceanogr.* **42**, 930–937 (1997).
108. McClelland, J. W. & Valiela, I. Linking nitrogen in estuarine producers to land-derived sources. *Limnol. Oceanogr.* **43**, 577–585 (1998).
109. Kjønaas, O. J. & Wright, R. F. Use of 15N-labelled nitrogen deposition to quantify the source of nitrogen in runoff at a coniferous-forested catchment at gårdsjön, Sweden. *Environ. Pollut.* **147**, 791–799 (2007).
110. Román, M., Emilio, F., Zamborain-Mason, J. & Martınez, G. Anthropogenic impact on Zostera noltei seagrass meadows (NW Iberian Peninsula) assessed by carbon and nitrogen stable isotopic signatures. *Estuar. Coast* **42**, 987–1000 (2019).
111. Erftemeijer, P. L. A. & Middelburg, J. J. Sediment-nutrient interactions in tropical seagrass beds: a comparison between a terrigenous and a carbonate sedimentary environment in South Sulawesi (Indonesia). *Mar. Ecol. Prog. Ser.* **102**, 187–198 (1993).
112. Erftemeijer, P. L. A., Stapel, J., Smekens, M. J. E. & Drossaert, W. M. E. The limited effect of in situ phosphorus and nitrogen additions to seagrass beds on carbonate and terrigenous sediments in South Sulawesi, Indonesia. *J. Exp. Mar. Biol. Ecol.* **182**, 123–140 (1994).
113. Kim, J. B. et al. Growth dynamics of eelgrass, Zostera marina, in the intertidal zone of Seomjin Estuary, Korea. *Ocean Sci. J.* **48**, 239–250 (2013).
114. Kim, J.-H., Kim, S. H., Kim, Y. K., Park, J. L. & Lee, K. S. Growth dynamics of the seagrass Zostera japonica at its upper and lower distributional limits in the intertidal zone. *Estuar. Coast. Shelf Sci.* **175**, 1–9 (2016).
115. Suonan, Z., Kim, S. H., Qin, L.-Z. & Lee, K. S. Reproductive strategy of the intertidal seagrass Zostera japonica under different levels of disturbance and tidal inundation. *Estuar. Coast. Shelf Sci.* **197**, 185–193 (2017).
116. Fraser, M. W. et al. Nutrient status of seagrasses cannot be inferred from system-scale distribution of phosphorus in Shark Bay, Western Australia. *Mar. Freshw. Res.* **63**, 1015–1026 (2012).
117. Llabres, E., Blanco-Magadán, A., Sales, M. & Sintes, T. The effect of global warming on Western Mediterranean seagrasses: a preliminary agent-based modelling approach. *Mar. Ecol. Prog. Series*. **710**, 43–56 (2023).
118. Peralta, G., Brun, F., Hernadez, I., Vergara, J. & Pérez-Lloréns, J. Morphometric variations as acclimation mechanisms in Zostera noltii beds. *Estuar. Coast. Shelf Sci.* **64**, 356 (2005).
119. Duarte, C. M. & Sand-Jensen, K. Seagrass colonization: Biomass development and shoot demography in Cymodocea nodosa patches. *Mar. Ecol. Prog. Ser.* **67**, 97–103 (1990).
120. Collier, C. J., Lavery, P. S., Masini, R. J. & Ralph, P. J. Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth related gradient of light availability. *Mar. Ecol. Prog. Ser.* **337**, 103–115 (2007).
121. Cunha, A. H. & Duarte, C. M. Biomass and leaf dynamics of *Cymodocea nodosa* in the Ria Formosa lagoon, South Portugal. *Botanica Mar.* **50**, 1–7 (2007).
122. Pergent, G., Boudouresque, C. F., Dumay, O., Pergent-Martini, C. & Wyllie-Echeverria, S. Competition between the invasive macrophyte *Caulerpa taxifolia* and the seagrass *Posidonia oceanica*: contrasting strategies. *BMC Ecol.* **8**, 20 (2008).
123. Dunton, K. H. Seasonal growth and biomass of the subtropical seagrass *Halodule wrightii* in relation to continuous measurements of underwater irradiance. *Mar. Biol.* **120**, 479–489 (1994).
124. Rodil, I. F., Attard, K. M., Gustafsson, C. & Norkko, A. Variable contributions of seafloor communities to ecosystem metabolism across a gradient of habitat-forming species. *Mar. Environ. Res* **167**, 105321 (2021).
125. Boutoumit, S. et al. Seasonal trends in benthic macrofauna communities and their relationship with environmental factors in an Atlantic lagoonal system. *Biologia* **77**, 1039–1055 (2022).
126. Gacia, E. & Duarte, C. M. Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. *Estuar. Coast. Shelf Sci.* **52**, 505–514 (2001).
127. Gao, H. et al. The sources of sedimentary organic matter traced by carbon and nitrogen isotopes and environmental effects during the past 60 years in a shallow Steppe lake in Northern China. *Water* **15**, 2224 (2023).
128. Ondiviela, B., Fernández, L., Puente, A., García-Castrillo, G. & Juanes, J. A. Characterization of a resilient seagrass meadow during a decline period. *Sci. Mar.* **82**, 67–75 (2018).
129. Rahayu, P. et al. Sedimentary seagrass carbon stock and sources of organic carbon across contrasting seagrass meadows in Indonesia. *Environ. Sci. Pollut. Res* **30**, 97754–97764 (2023).
130. Tahir, I. et al. Variation of carbon content in sediments of seagrass ecosystems based on the presence of seagrass species on Mare Island, Indonesia. *AACL Bioflux.* **16**, 887–898 (2023).
131. Folmer, E. O. et al. Seagrass–sediment feedback: an exploration using a non-recursive structural equation model. *Ecosystems* **15**, 1380–1393 (2012).
132. Samper-Villarreal, J., Moya-Ramírez, J. & Cortés, J. First characterization of seagrasses at Samara Bay, pacific coast of Costa Rica. *Aquat. Bot.* **178**, 103486 (2022).
133. Samper-Villarreal, J., Bolaños, R. C., Heidemeyer, M., Vargas, M. M. & Vargas, T. M. Characterization of seagrasses at two new locations in the Eastern Tropical Pacific (El Jobo and Matapalito, Costa Rica). *Aquat. Bot.* **165**, 103237 (2020).
134. Barcelona, A., Oldham, C., Colomer, J., Garcia-Orellana, J. & Serra, T. Particle capture by seagrass canopies under an oscillatory flow. *Coast. Eng.* **169**, 103972 (2021).
135. Bijak, A. L., Reynolds, L. K. & Smyth, A. R. Seagrass meadow stability and composition influence carbon storage. *Landsc. Ecol.* **38**, 4419–4437 (2023).
136. Larkum, A. W. D., Orth, R. J., Duarte, C. M. *Seagrasses: Biology, Ecology, and Conservation* 1st edn, Vol. 676 (Springer, Berlin, 2006).
137. Calleja, M. L., Marba, N. & Duarte, C. M. The relationship between seagrass (*Posidonia oceanica*) decline and sulfide porewater concentration in carbonate sediments. *Estuar. Coast. Shelf Sci.* **73**, 583 (2007).
138. Koch, M. S., Schopmeyer, S., Kyhn-Hansen, C. & Madden, C. J. Synergistic effects of high temperature and sulfide on tropical seagrass. *J. Exp. Mar. Biol. Ecol.* **341**, 91 (2007).
139. Al-Wedaei, K., Naser, H., Al-Sayed, H. & Khamis, A. Assemblages of macro-fauna associated with two seagrass beds in Kingdom of Bahrain: implications for conservation. *J. Assoc. Arab Univ. Basic Appl. Sci.* **10**, 1–7 (2011).
140. Mayer, L. M. Surface area control of organic carbon accumulation in continental shelf sediments. *Geochim. Cosmochim. Ac.* **58**, 1271–1284 (1994).

141. Powell, G. V. N. & Schaffner, F. C. Water trapping by seagrasses occupying bank habitats in Florida Bay. *Estuar. Coast Shelf Sci.* **32**, 43–60 (1991).
142. Boese, B. L., Robbins, B. D. & Thursby, G. Desiccation is a limiting factor for eelgrass (*Zostera marina* L.) distribution in the intertidal zone of a northeastern Pacific (USA) estuary. *Bot. Mar.* **48**, 274–283 (2005).
143. Koch, E. Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. *Estuaries* **24**, 1–17 (2001).
144. Vonk, J., Middelburg, J., Stapel, J. Bouma, T. Dissolved organic nitrogen uptake by seagrasses. *Limnol. Oceanogr.* **53**, 542–548 (2008).
145. Alexandre, A., Hill, P. W., Jones, D. L. & Santos, R. Dissolved organic nitrogen: a relevant, complementary source of nitrogen for the seagrass Zostera marina. *Limnol. Oceanogr.* **60**, 1477–1483 (2015).
146. van der Heide, T. et al. A three-stage symbiosis forms the foundation of seagrass ecosystems. *Science* **336**, 1432–1434 (2012).
147. Chin, D. W. et al. Facilitation of a tropical seagrass by a chemosymbiotic bivalve increases with environmental stress. *J. Ecol*. **109**, 204–217 (2020)
148. Heck, K. L. Jr. & Orth, R. J. Seagrass habitats: The roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. **1980**, 449–464(1980).
149. Canuel, E. A., Amanda, L., Spivak, C., Waterson, E. J. & Duffy, J. E. Biodiversity and food web structure influence short-term accumulation of sediment organic matter in an experimental seagrass system. *Limnol. Oceanogr.* **52**, 590–602 (2007). 2007.
150. Millot, J., Grall, J., Toumi, C., Maguer, M. & Boyé, A. Quantifying the direct and indirect relationships linking the environment, seagrass, and their associated fauna. *Ecosphere* **15**, e4708 (2024).
151. Lawrence, C. Quantifying direct and indirect linkages between seagrasses, environment and associated macrofauna in a temperate lagoon. *Mar. Ecol.* **45**, e12804 (2024).
152. Boström, C., O'Brien, K., Roos, C. & Ekebom, J. Environmental variables explaining structural and functional diversity of seagrass macrofauna in an archipelago landscape. *J. Exp. Mar. Biol. Ecol.* **335**, 52–73 (2006).
153. Boström, C. & Bonsdorff, E. Community structure and spatial variation of benthic invertebrates associated with Zostera marina (L.) beds in the northern Baltic Sea. *J. Mar. Res.* **37**, 153–166 (1997).
154. González-Ortiz, V., Alcazar, P., Vergara, J. J., Pérez-Lloréns, J. L. & Brun, F. G. Effects of two antagonistic ecosystem engineers on infaunal diversity. *Estuar., Coast. Shelf Sci.* **139**, 20–26 (2014).
155. Hovel, K. A., Fonseca, M. S., Myer, D. L., Kenworthy, W. J. & Whitfield, P. E. Effects of seagrass landscape structure, structural complexity and hydrodynamic regime on macrofaunal densities in North Carolina seagrass beds. *Mar. Ecol. Prog. Ser.* **243**, 11–24 (2002).
156. González-Ortiz, V. et al. Submerged vegetation complexity modifies benthic infauna communities: the hidden role of the belowground system. *Mar. Ecol.* **37**, 543–552 (2016).
157. Vonk, J. A., Christianen, M. J. A. & Stapel, J. Abundance, edge effect, and seasonality of fauna in mixed-species seagrass meadows in southwest Sulawesi, Indonesia. *Mar. Biol. Res.* **6**, 282–291 (2010).
158. Avila, E., Yáñez, B. & Vazquez, L. Influence of habitat structure and environmental regime on spatial distribution patterns of macroinvertebrate assemblages associated with seagrass beds in a southern Gulf of Mexico coastal lagoon. *Mar. Biol. Res*. **11**, 755–764 (2015).
159. Barnes, R. Distribution patterns of macrobenthic biodiversity in the intertidal seagrass beds of an estuarine system, and their conservation significance. *Biodivers. Conserv.* **22**, 357–372 (2013).
160. Haase, P. et al. The recovery of European freshwater biodiversity has come to a halt. *Nature* **620**, 582–588 (2023).
161. Unsworth, R. K. F., Rasheed, M. A., Chartrand, K. M. & Roelofs, A. J. Solar radiation and tidal xeposure as environmental drivers of enhalus acoroides dominated seagrass meadows. *PLoS ONE* **7**, e34133 (2012).
162. Ruiz-Montoya, L. et al. Ecophysiological responses and self-protective canopy effects of surfgrass (Phyllospadix torreyi) in the intertidal. *Mar. Environ. Res.* **172**, 105501 (2021).
163. Scrosati, R. & DeWreede, R. E. The impact of frond crowding on frond bleaching in the clonal intertidal alga Mazzaella cornucopiae (Rhodophyta, Gigartinaceae) from British Columbia, Canada. *J. Phycol.* **34**, 228–232 (1998).
164. Vieira, V. M. N. C. S., Engelen, A. H., Huanel, O. R. & Guillemin, M.-L. Haploid females in the isomorphic biphasic life-cycle of Gracilaria chilensis excel in survival. *BMC Evol. Biol.* **18**, 174 (2018).
165. Vieira, V. M. N. C. S., Engelen, A. H., Huanel, O. & Guillemin, M.-L. Differentiation of haploid and diploid fertilities in Gracilaria chilensis affect ploidy ratio. *BMC Evol. Biol.* **18**, 183 (2018).
166. Vieira, V. M. N. C. S., Engelen, A. H., Huanel, O. & Guillemin, M.-L. Differential frond growth in the isomorphic haploid-diploid red seaweed Agarophyton chilense by long-term in situ monitoring. *J. Phycol.* **57**, 592–605 (2021).
167. Vieira, V. M. N. C. S., Engelen, A. H., Huanel, O. R. & Guillemin, M.-L. An individual-based model of the red alga agarophyton chilense unravels the complex demography of its intertidal stands. *Front. Ecol. Evol.* **10**, 797350 (2022).
168. Blundon, J. A. & Kennedy, V. S. Refuges for infaunal bivalves from blue crab, Callinectes sapidus (Rathbun), predation in Chesapeake Bay. *J. Exp. Mar. Biol. Ecol.* **65**, 67–81 (1982).
169. Gagnon, K. et al. Facilitating foundation species: the potential for plant–bivalve interactions to improve habitat restoration success. *J. Appl Ecol.* **57**, 1161–1179 (2020).
170. Galván, C. & Puente, A. Exploring the newly emerging effects of native seagrasses on survival and growth of non-native juvenile clams. *Front. Mar. Sci*. https://doi.org/10.3389/fmars.2023.1160734 (2023).
171. Borja, A., Franco, J. & Pérez, V. A marine biotic index to establish the ecological quality of soft bottom benthos within European estuarine and coastal environments. *Mar. Pollut. Bull.* **40**, 1100–1114 (2000).
172. Borja, A., Mader, J., Muxika, I., Rodríguez, J. G. & Bald, J. Using M-AMBI in assessing benthic quality within the water framework directive: some remarks and recommendations. *Mar. Pollut. Bull.* **56**, 1377–1379 (2008).
173. Carletti A., Heiskanen A. S. Water framework directive intercalibration technical report. Part 3: coastal and transitional waters. *JRC Sci. Tech. Rep*. https:doi.org/10.2788/19561 (2009).
174. Manea E. et al. An ecosystem-based system of variables to enhance marine species and habitat monitoring and conservation: the adriatic natura 2000 case study. *Front. Mar. Sci.* https://doi.org/10.3389/fmars.2022.920366 (2022).
175. Ankel, M., Rubal, M., Veiga, P., Sampaio, L. & Guerrero-Meseguer, L. Reproductive cycle of the seagrass Zostera noltei in the Ria de Aveiro Lagoon. *Plants (Basel)* **10**, 2286 (2021). Oct 26.
176. Cabaço, S., Machás, R. & Santos, R. Individual and population plasticity of the seagrass Zostera noltii along a vertical intertidal gradient. *Estuar. Coast. Shelf Sci.* **82**, 301–308 (2009).
177. Cabaço, S., Santos, R. & Sprung, M. Population dynamics and production of the seagrass Zostera noltii in colonizing versus established meadows. *Mar. Ecol.* **33**, 280–289 (2012).
178. Guerrero-Meseguer, L., Veiga, P., Sampaio, L. & Rubal, M. Sediment characteristics determine the flowering effort of Zostera noltei meadows inhabiting a human-dominated lagoon. *Plants (Basel)* **10**, 1387 (2021).
179. Auby, I. & Labourg, P. J. Seasonal dynamics of Zostera noltii Hornem. in the Bay of Arcachon (France). *J. Sea Res.* **35**, 269–277 (1996).

180. Alexandre, A., Santos, R. & Serrão, E. Effects of clam harvesting on sexual reproduction of the seagrass Zostera noltii. *Mar. Ecol. Prog. Ser.* **298**, 115–122 (2005).
181. Cabaço, S. & Santos, R. Seagrass reproductive effort as an ecological indicator of disturbance. *Ecol. Indic.* **23**, 116–122 (2012).
182. Dias, J. M., Valentim, J. M. & Sousa, M. C. A numerical study of local variations in tidal regime of Tagus Estuary, Portugal. *PLoS ONE* **8**, e80450 (2013).
183. Brito, A. C. et al. Increased oyster aquaculture in the Sado Estuary (Portugal): how to ensure ecosystem sustainability? *Sci. Total Environ.* **855**, 158898 (2023).
184. Hansen, H. & Koroleff, F. "Determination of nutrients." In, *Methods of seawater Analysis.* (eds. Grasshoff, K. Kremling, K. & M. Ehrhardt, M.) 159–226 (Weinheim: Wiley-VCH Verlag, (1999).
185. Gaudêncio, M. J., Guerra, M. T., & Glémarec, M. Recherches biosedimentaires sur la zone maritime de l'estuaire du Tage, Portugal: donnees sedimentaires preliminaires. In *Estuaries and Coasts: Spatial and Temporal Intercomparisons*, (eds. Elliot, M. & Ducrotoy, J. C.) 11–16 (Olsen and Olsen, Fredensborg, 1991).
186. Folk, R. L. The distinction between grain size and mineral composition in sedimentary-rock nomenclature. *J. Geol.* **62**, 344–359 (1954).
187. Olesen, B. & Sand-Jensen, K. Biomass–density patterns in the temperate seagrass Zostera marina. *Mar. Ecol. Prog. Ser.* **109**, 283–291 (1994).
188. Manly, B. J. F. *Randomization and Monte Carlo Methods in Biology* 3rd edn, Vol. 480 (Chapman & Hall, London, UK 1991).
189. Zhang, L., Bi, H., Gove, J. H. & Heath, L. S. A comparison of alternative methods for estimating the self-thinning boundary line. *Can. J. Res* **35**, 1507–1514 (2005).
190. Geraci, M. Modelling and estimation of nonlinear quantile regression with clustered data. *Comput. Stat. Data Anal.* **136**, 30–46 (2019).
191. Vieira, V. M. N. C. S. & Creed, J. Estimating significances of differences between slopes: a new methodology and software. *Comput. Ecol. Softw.* **3**, 44–52 (2013a).
192. Vieira, V. M. N. C. S. & Creed, J. 2013b. Significances of differences between slopes: an upgrade for replicated time series. *Comput. Ecol. Softw.* **3**, 102–109. (2013b).
193. Vieira, V. M. N. C. S. *Regression Fitting, Splines and ANCOVA Scripts.* https://www.diva-portal.org (2024).
194. Kraemer, G. P. & Mazella, L. Nitrogen acquisition, storage, and use by the co-occurring Mediterranean seagrasses cymodocea nodosa and zostera noltii. *Mar. Ecol. Prog. Ser.* **183**, 95–103 (1999).
195. Draper, N. R. Straight line regression when both variables are subject to error. *Conf. Appl. Stat. Ag.* **1992**, 1–18 (1991).
196. Smith, R. J. Use and misuse of reduced major axis for line-fitting. *Am. J. Phys. Anthropol.* **140**, 476–486 (2009).
197. Vieira, V. M. N. C. S. et al. On the choice of linear regression algorithms. *Annu. Res. Rev. Biol.* **10**, 1–9 (2016).

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