# Latitudinal Patterns in European Seagrass Carbon Reserves: Influence of Seasonal Fluctuations versus Short-Term Stress and Disturbance Events

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Seagrass meadows form highly productive and valuable ecosystems in the marine environment. Throughout the year, seagrass meadows are exposed to abiotic and biotic variations linked to (i) seasonal fluctuations, (ii) short-term stress events such as, e.g., local nutrient enrichment, and (iii) small-scale disturbances such as, e.g., biomass removal by grazing. We hypothesized that short-term stress events and small-scale disturbances may affect seagrass chance for survival in temperate latitudes. To test this hypothesis we focused on seagrass carbon reserves in the form of starch stored seasonally in rhizomes, as these have been defined as a good indicator for winter survival. Twelve Zostera noltei meadows were monitored along a latitudinal gradient in Western Europe to firstly assess the seasonal change of their rhizomal starch content. Secondly, we tested the effects of nutrient enrichment and/or biomass removal on the corresponding starch content by using a short-term manipulative field experiment at a single latitude in the Netherlands. At the end of the growing season, we observed a weak but significant linear increase of starch content along the latitudinal gradient from south to north. This agrees with the contention that such

reserves are essential for regrowth after winter, which is more severe in the north. In addition, we also observed a weak but significant positive relationship between starch content at the beginning of the growing season and past winter temperatures. This implies a lower regrowth potential after severe winters, due to diminished starch content at the beginning of the growing season. Short-term stress and disturbances may intensify these patterns, because our manipulative experiments show that when nutrient enrichment and biomass loss co-occurred at the end of the growing season, *Z. noltei* starch content declined. In temperate zones, the capacity of seagrasses to accumulate carbon reserves is expected to determine carbon-based regrowth after winter. Therefore, processes affecting those reserves might affect seagrass resilience. With increasing human pressure on coastal systems, short- and small-scale stress events are expected to become more frequent, threatening the resilience of seagrass ecosystems, particularly at higher latitudes, where populations tend to have an annual cycle highly dependent on their storage capacity.

## INTRODUCTION

Seagrasses are flowering plants, adapted to the marine environment (Les et al., 1997), forming extensive and highly productive meadows worldwide (Short et al., 2007). Throughout the year, temperate seagrass meadows are submitted to various abiotic and biotic variations: (i) seasonal fluctuations related to variations in light and temperature controlling their presence and seasonal growth (Dennison, 1987; Duarte, 1991; Olesen and Sand-Jensen, 1993; Ochieng et al., 2010; Marbà et al., 2012); (ii) short-term stress events such as local nutrient enrichment leading to eutrophication; and (iii) small-scale disturbances such as biomass removal by grazing (e.g., by birds or sea turtles), jointly affecting their resilience and survival (Burkholder et al., 2007; Macreadie et al., 2014).

The capacity of seagrasses to respond and recover from stresses and disturbances (Charpentier et al., 2005; Godet et al., 2008) depends on their clonal growth strategy (i.e., potential rhizome elongation rate; Macreadie et al., 2014), their seasonal growth (Sordo et al., 2011; Soissons et al., 2016), their seed production (Van Tussenbroek et al., 2016), their high productivity (Plus et al., 2005; Ribaudo et al., 2016), and their ecosystem engineering capacity (McGlathery et al., 2012). Seagrasses can acclimate their morphological, physiological, and mechanical traits to local conditions (Peralta et al., 2005, 2006; Cabaço et al., 2009; de los Santos et al., 2010, 2013; La Nafie et al., 2013; Soissons et al., 2018). This plasticity improves their resilience under threats. However, in the current context of climate change and concomitant increasing anthropogenic pressure on coastal ecosystems, it can be assumed that environmental stressors are going to increase, on types and/or intensity. Stressor combinations can generate synergistic, additive, or antagonistic effects on seagrass populations, deteriorating environmental conditions for the resistance and resilience of seagrass ecosystems. The decline of seagrass ecosystems has been described worldwide (Orth et al., 2006;

Waycott et al., 2009). If changes in environmental conditions result in a decrease of seagrass resilience, their very valuable ecosystem services may not only decrease but also disappear (Scheffer et al., 2001, 2009; Carr et al., 2012).

Seagrasses overcome periods of low-light availability like the winter months or short-term light deprivation events (Burke et al., 1996; Govers et al., 2015) using their carbon reserve, in the form of non-structural carbohydrates (i.e., starch and/or soluble sugars) gained during their growing season (Madsen, 1991; Alcoverro et al., 1999; Lee et al., 2007; Olivé et al., 2007). Non-structural carbohydrates are usually stored when photosynthesis exceeds the carbon demand for growth and respiration (Madsen, 1991). The magnitude of the carbon reserve needed for seagrass survival depends on abiotic factors such as, i.e., temperature and light availability, but also on internal factors affecting the carbon balance, such as respiration and growth (Madsen, 1991; Alcoverro et al., 2001; Govers et al., 2015). Survival after winter thus depends on the plants' capacity to build up their carbon reserves, particularly in the form of starch, during their growing season (Govers et al., 2015).

Studies have shown that seagrass carbon balance, and therefore conditions to favor storage of non-structural carbohydrates depend on light irradiance, daylight length (photoperiod), temperature, weather, and hydrodynamic conditions (Marsh et al., 1986; Burke et al., 1996; Alcoverro et al., 2001; Olivé et al., 2007; Govers et al., 2015). To our knowledge, previous studies did not include a spatial dimension, such as latitudinal patterns, to understand carbon storage in seagrasses. Latitudinal gradients affect variables like day length, temperature, and winter intensity, which are important in defining the carbon balance of seagrasses (Alcoverro et al., 2001). Hence, the capacity of a seagrass species to store non-structural carbohydrates such as starch and constitute a carbon reserve may be influenced by its distribution along latitudinal gradients.

Short-term stress events and small-scale disturbances have also very high potential in affecting seagrass carbon reserves,

particularly when occurring during their growing season. Short-term stress events may be linked to high turbidity pulses or excess nutrient enrichment [leading to nitrate or ammonium toxicity (van Katwijk et al., 1997; Brun et al., 2002)]. Small-scale disturbances may reduce seagrass abundance and biomass, by, e.g., grazing, trampling, or digging. Such short-term stress and disturbance events may occur separately or act simultaneously, increasing the risk of carbon reserve depletion, in severe cases, or modifying the plant's capacity to store non-structural carbohydrates such as starch. However, these effects are not only expected to depend on the magnitude and type of events, but also on the timing of occurrence. It could thus be hypothesized that the consequences of short-term stress or disturbance events may be different if they would happen at the beginning or at the end of the seagrass growing season.

To clarify these questions, (i) we used the dwarf eelgrass *Zostera noltei* Hornem. as a model species to test how its carbon reserves (i.e., starch content) might differ depending on its distribution along a latitudinal gradient at key stages of the growing cycle (i.e., at the beginning, the peak, and the end of the growing season). With this, we aimed to evaluate the influence of seasonal fluctuations (i.e., air/water temperatures during the growing season and from the previous winter period, daylight length) on *Z. noltei* starch content. Additionally, (ii) we assessed, through a manipulative experiment on two *Z. noltei* meadows located at a single latitude in the Netherlands, the effect of short-term stress event (i.e., local nutrient enrichment), small-scale disturbance (i.e., above-ground removal), and their combination on their capacity to store carbon in the form of starch along their growing season. From this, (iii) we discussed how short-term stress and disturbance events might affect the plant's seasonal carbon reserve (i.e., starch content) and thus their capacity to withstand seasonal fluctuations, as well as their long-term resilience and survival depending on their distribution along a latitudinal gradient.

## MATERIALS AND METHODS

### Study Sites

In order to cover a latitudinal gradient, seagrasses were sampled at 12 sites along the European coastline, at three different times over the growing season: at the beginning, at the peak, and at the end of the growing season (**Table 1**). Sites were selected following a latitudinal gradient from south (warmer) to north (colder), being: E1 Cadiz (Spain); E2 and E3 Mondego estuary (Portugal); E4 Santander (Spain); E5 Bidasoa estuary (France); E6 and E7 Arcachon Bay (France); E8 Noirmoutier (France); E9 St-Jacut-de-la-mer (France); E10 and E11 The Oosterschelde (Netherlands); and E12 Sylt (Germany) (**Figure 1** and **Table 1**). The samples were always on healthy and well-developed *Zostera noltei* meadow found in intertidal areas (as determined by local experts). Sampling dates were not identical between locations, as the beginning and duration of the growing season are dependent on local conditions such as temperature and light availability, which are latitude-dependent. To get a comparable set of data for the different

stages in the growing season (i.e., beginning, peak, and end), the exact sampling dates were determined by local experts (**Table 1**).

### Experimental and Sampling Design
#### The Influence of Seasonal Fluctuations and Latitude on Carbon Reserves

For each sampling date and at each site (**Table 1**), seagrass samples ($n = 5$) were collected by using 10 cm diameter PVC cores inserted into the sediment. The seagrass samples were briefly washed *in situ* and stored in wet tissues for preservation during transportation to the NIOZ (Royal Netherlands Institute for Sea Research) in Yerseke, Netherlands. In the laboratory, the samples were carefully washed a second time with freshwater to remove all remaining sediment, algae, and epiphytes (scraped with a razor blade). Then, on every sample, rhizomes were carefully separated from the roots and leaves, and subsequently freeze-dried for carbon reserves (i.e., starch content) analyses (rhizomes only).

Water temperature was continuously monitored over the study period at every sites. Water temperature was measured using two HOBO Pendant Temperature loggers (64k – UA-002-64, ONSET) at a frequency of 1 measurement every 30 min. For every study site, two loggers were placed within the study area. Air temperature and daylight length in hours from the past winter months and during the experimental period were obtained from local weather stations and available climate and weather databases online (i.e., www.aemet.es for Spain; www.ipma.pt for Portugal; www.meteofrance.fr for France, www.buienradar.nl for Netherlands; www.dwd.de for Germany; and www.timeanddate.com for all). For each site, past winter temperature and daylight length were averaged from December 2013 until February 2014. Daylight length (hours expressed in decimals) was averaged over 1 month around the sampling date (**Table 1**).
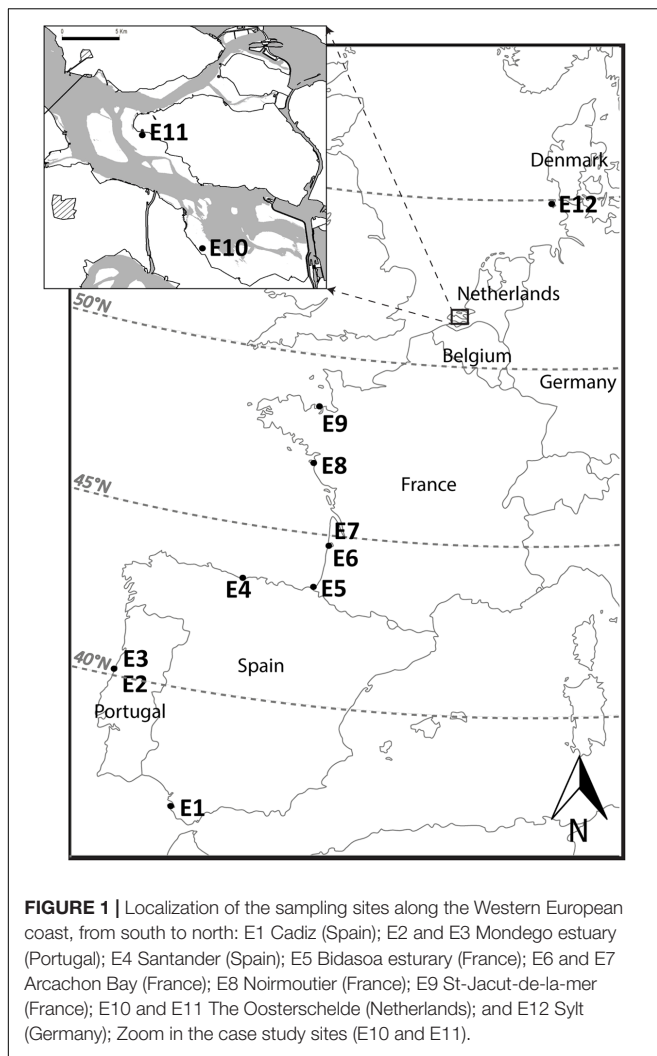
#### The Influence of Short-Term Nutrient Enrichment and Small-Scale Disturbances on Carbon Reserves

Seagrass carbon reserves' response to short-term stress (nutrient enrichment) and small-scale disturbance (above-ground biomass removal) was evaluated only at two sites located at the same latitude (Oosterschelde, Netherlands; Sites E10 and E11; **Figure 1** and **Table 1**). Sites E10 and E11 both display similar average exposure characteristics (Suykerbuyk et al., 2016) but experience different wind orientation and condition making them visibly different during the experimental period. At E10, the sediment particle median diameter (D50) was 78.1 ± 43.5 µm with 43.5 ± 0.6% of fine particles (<63 µm); whereas at E11 the D50 was 94.7 ± 13.8 µm with 22.9 ± 1.4% of fine particles (<63 µm) and the presence of marked sand ripples (personal observation). The study was designed as a factorial experiment with four treatments ($n = 5$ for each treatment): Control, i.e., undisturbed-no nutrient (C); undisturbed with nutrient (CN); disturbed-no nutrient (D); disturbed with nutrient (DN). The same experimental design was implemented at both sites three times over the growing season (at the beginning: 14 May–12 June; the peak: 22 July–20 August and the end: 27 August–26 September 2014).

**TABLE 1 |** Site characteristics and sampling dates.

| Site number and names | Coordinates | Start–end experimental dates | | | Temperature (°C) | | | | Daylight length (hours in decimals) | | | | Ever green Y/N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Beginning | Peak | End | Past winter | Beginning | Peak | End | Past winter | Beginning | Peak | End | |
| E1 | Cadiz Bay, Spain | N 36 30' W 6 10' | 2 July | 9 September | 6 November | 13.45 | – | 26.1 ± 0.08 | 20.6 ± 0.08 | 9.71 | 14.67 | 12.67 | 10.5 | Y |
| E2 | Upstream – Mondego estuary, Portugal | N 40 8' W 8 50' | 28 May | 13 August | 10 November | 10.0 | 17.6 ± 0.1 | 20.8 ± 0.09 | 18.8 ± 0.07 | 10.03 | 14.67 | 13.83 | 10.17 | Y |
| E3 | Downstream – Mondego estuary, Portugal | N 40 8' W 8 50' | 28 May | 13 August | 10 November | 10.0 | 17.3 ± 0.1 | 20.6 ± 0.1 | 18.7 ± 0.08 | 10.03 | 14.67 | 13.83 | 10.17 | Y |
| E4 | Santander, Spain | N 43 25' W 003 48' | 13 June | 11 August | 8 October | 11.5 | 17.2 ± 0.06 | 22.6 ± 0.03 | 19.9 ± 0.03 | 9.03 | 13.83 | 14.2 | 11.47 | Y |
| E5 | Bidasoa estuary, France | N 43 21' W 001 46' | 29 May | 7 August | 11 October | 9.7 | – | 23.3 ± 0.1 | 20.6 ± 0.1 | 9.02 | 15.08 | 14.37 | 10.5 | Y |
| E6 | Germanan – Arcachon Bay, France | N 44 42' W 001 8' | 27 May | 13 August | 27 October | 8.7 | 17.1 ± 0.09 | 23.2 ± 0.07 | 18.9 ± 0.08 | 8.87 | 15.17 | 14.21 | 10.5 | Y |
| E7 | Hautebelle – Arcachon Bay, France | N 44 43' W 001 09' | 27 May | 13 August | 27 October | 8.7 | 17.1 ± 0.09 | 23.2 ± 0.07 | 19.0 ± 0.08 | 8.87 | 15.17 | 14.21 | 10.5 | Y |
| E8 | Noirmoutier, France | N 46 98' W 002 21' | 27 June | 25 July | 11 September | 9.5 | 20.4 ± 0.09 | 21.3 ± 0.09 | 21.1 ± 0.07 | 8.55 | 15.92 | 15.25 | 12.88 | N |
| E9 | St-Jacut-de-la-mer, France | N 48 36' W 002 11' | 25 June | 29 July | 20 August | 7.8 | 17.3 ± 0.1 | 20.4 ± 0.1 | 20.1 ± 0.2 | 8.35 | 16.15 | 15.25 | 14.18 | N |
| E10 | Oostdijk – Oosterschelde, Netherlands | N 51 26' E 004 05' | 11 June | 19 August | 26 September | 6.8 | 19.2 ± 0.1 | 20.3 ± 0.1 | 19.0 ± 0.1 | 7.9 | 16.53 | 14.5 | 12.03 | N |
| E11 | Dortsman – Oosterschelde, Netherlands | N 51 34' E 003 59' | 12 June | 20 August | 25 September | 6.8 | 18.0 ± 0.1 | 20.2 ± 0.08 | 18.3 ± 0.07 | 7.9 | 16.53 | 14.5 | 12.03 | N |
| E12 | Sylt, Germany | N 54 54' E 008 19' | – | 30 July | 12 September | 3.3 | – | 22.2 ± 0.1 | 17.9 ± 0.09 | 7.4 | – | 16.05 | 12.98 | N |

*The past winter temperatures and daylight length (in hours) are averaged over the winter months 2013–2014. The water temperatures are averaged (±SE) from measurements taken by the HOBO-pendant loggers over 1 month around the sampling dates at all sites (data collected in 2014).*

**FIGURE 1 |** Localization of the sampling sites along the Western European coast, from south to north: E1 Cadiz (Spain); E2 and E3 Mondego estuary (Portugal); E4 Santander (Spain); E5 Bidasoa esturary (France); E6 and E7 Arcachon Bay (France); E8 Noirmoutier (France); E9 St-Jacut-de-la-mer (France); E10 and E11 The Oosterschelde (Netherlands); and E12 Sylt (Germany); Zoom in the case study sites (E10 and E11).

*Plot installation*
To minimize edge effects, the experimental plots at both sites were randomly allocated in the middle of the seagrass meadows, providing a minimum distance of at least 5 m between plots to avoid an experimental nutrient release overlap. Every experimental plot was delimited by two bamboo sticks placed 1 m apart and included an inner circle of 30 cm diameter for the treatments, delimited by 10 metal sticks. To avoid contamination from previous experimental setups, the experimental areas, at each site, were different and independent for each of the three experimental periods.

*Short-term nutrient enrichment*
Nutrient enrichment was simulated by placing small bags of slow release fertilizers (Osmocote, N:P:K = 15:9:12) in the upper layer of sediment around the (CN) and (DN) 30 cm diameter inner circles by using the metal sticks as anchors. The fertilizer bags were made of panty hoses, containing 10 g of slow release fertilizer each. Experimental plots with nutrient treatments [(CN): $n = 5$ and (DN): $n = 5$ plots per experiment]

had a total of 10 bags per plot, receiving in total 100 g of slow-release fertilizer per plot (i.e., 1.4 kg m$^{-2}$ slow release fertilizer), which corresponds to a high and potentially toxic enrichment (Govers et al., 2014).

*Small-scale disturbances*
Disturbances of above-ground biomass were created by clipping the leaves, leaving the below-ground and sheaths in place inside the (D) and (DN) ($n = 10$) 30 cm diameter inner circles. This type of disturbance was chosen to mimic the effect of over-grazing creating gaps in seagrass meadows and allowing direct regrowth measurements within the 4 weeks long experiments. All seagrass material removed at gap creation was kept in individual bags for biomass measurements. In order to minimize seagrass regrowth based on carbon reserves outside the experimental 30 cm inner circles (i.e., lateral carbon transfer), rhizomes around the inner circles were initially cut around the 30 cm inner circles (including control plots).

*Seagrass samples*
At the start of each experiment (at sites E10 and E11 only), initial seagrass samples ($n = 5$) were collected close to the experimental plots by using 10 cm diameter PVC cores inserted into the sediment. At the end of each experimental period, all plots were harvested by using the same 10 cm diameter PVC cores inserted into the sediment randomly within the inner 30 cm diameter inner circle. Seagrass samples were washed and processed in the same way as for the samples collected along the latitudinal gradient (see description above).

## Measuring Starch Content in Rhizomes for Seagrass Carbon Reserves

Seagrass rhizomes store most of the non-structural carbon reserves in the form of starch (Burke et al., 1996; Zimmerman and Alberte, 1996; Olivé et al., 2007; Govers et al., 2015). Those reserves were evaluated in rhizomes from control samples for all pan-European sites, to investigate the potential of each site to store carbon reserves over their growing season. Carbon reserves were measured in freeze-dried and ground rhizomes of the control samples from the beginning, peak, and end of the growing season of all 12 field sites. In addition, the carbon reserves of the rhizomes samples from all treatments at the Oosterschelde sites (site E10 and E11) were also analyzed to investigate their response to treatments.

To evaluate seagrass carbon reserves, we measured the starch content of seagrass rhizomes. Soluble sugars – glucose, fructose, and sucrose – were extracted out of the plant material by using an 80% ethanol solution. Those ethanol soluble sugars were not used for our analysis. The residue of the extraction was hydrolyzed with diluted hydrochloric acid (3% HCl) to convert starch into glucose. After hydrolysis, the residue was boiled at 100°C for 30 min. Rhizomal starch content was estimated by anthrone assay standardized to D-glucose (Yemm and Willis, 1954) and converted to milligram starch per gram dry plant material. All samples were measured in duplicate and a new calibration curve was prepared for every series of measurements.

## Statistical Analysis

Variations in water temperature and daylight length were tested using a one-way ANOVA followed by Tukey's *post hoc* tests when significant, considering as variation source the factor "experimental period" (i.e., the growing season stages: beginning, peak, and end). Differences in starch content and above-ground biomass were assessed with a two-way ANOVA using as variation sources the factors "experimental period" and "site".

For the latitudinal gradient, variations in water temperature and daylight length during the growing season and for the past winter were evaluated with linear regressions, on data split per experimental period. The effect of seasonal fluctuations on starch content was tested by evaluating the existence of linear relationships between past winter temperatures, daylight length, or latitude with starch content at the beginning and the end of the growing season. The linear regressions were separately performed for the two stages of the growing season. For daylight length, an additional linear regression was performed at the peak of the growing season. The relationships between temperature (i.e., water temperature, as measured from the loggers over the experimental period, and winter temperature, collected from weather stations or databases) with latitude were tested with a general linear model.

The effect of treatments (i.e., short-term nutrient enrichment and above-ground removal) on rhizomal starch content for the two treated sites E10 and E11 was tested using a mixed effects model considering "sites" (E10 and E11), "treatment" (C, N, D, ND), and "experimental period" (beginning, peak, and end) as fixed factors and replicates ($n = 5$) as a random factor. Differences between treated plots (N, D, and ND) and control (C) were obtained with *post hoc* Tukey tests. Data normality were tested prior to analysis using the Shapiro–Wilk test. All statistical analyses were realized with R version 3.1.3 (R Core Team, 2013).

## RESULTS

As expected water temperature was highest at the peak of the growing season (ANOVA: $F = 24.28$, $p < 0.001$; Tukey: peak > end > beginning, $p < 0.001$) with a temperature range from $20.2 \pm 0.1°C$ at site E11 up to $26.1 \pm 0.1°C$ at site E1 (**Table 1**). Daylight length significantly decreased at the end of the growing season (ANOVA: $F = 48.99$, $p < 0.001$; Tukey: beginning = peak, $p = 0.52$; beginning > end, $p < 0.001$; peak > end, $p < 0.001$). Daylight length presented a linear decrease from North to South at the beginning and at the end of the season (**Table 2**). Daylight length at the peak of the season and water temperatures along the season (i.e., for all experimental periods) did not show any significant linear relationships with latitude (**Table 2**). However, past winter temperature and daylight length were significantly higher in southern latitudes and lower in northern latitudes (**Tables 1**, **2**).

## Seasonal Variations in Carbon Reserves (i.e., Starch Content): Effects of Winter Intensity, Daylight Length, and Latitude

Overall, starch content in *Z. noltei* rhizomes significantly increased over the growing season (**Figure 2**). This was observed at most sites, except at site E1 where no significant changes were detected. At the end of the growing season, starch content in rhizomes reached values between 200 up to 600 mg g $DW^{-1}$ (**Figure 2**), showing a high variability along the latitudinal gradient.

*Zostera noltei* starch content at the beginning of the growing season was weakly but positively related to the averaged past winter temperature ($F = 13.46$, $R^2 = 0.1219$, $df = 97$, $p < 0.001$) (**Figure 3A**). At the end of the growing season, starch content was weakly but positively related to latitude ($F = 15.02$, $R^2 = 0.1403$, $df = 92$, $p < 0.001$) (**Figure 3B**). In other words, this suggests that *Z. noltei* populations had greater carbon reserves before winter at the northern sites than at the southern ones. Daylight length positively correlated to starch content at the peak of the growing season ($R^2 = 0.137$, $F = 7.152$, $p = 0.001$) but did not show any clear effect on starch content at the beginning or at the end of the growing season.

## Effects of Nutrient Enrichment and Small-Scale Disturbance on Carbon Reserves

To investigate the effects of short-term stress events such as nutrient enrichment, small-scale disturbances, such as aboveground biomass removal, and their combination on carbon

**TABLE 2** | Results from the linear regressions between latitude and temperature (water for beginning, peak, and end; air for past winter) or daylight length at the different stages of the growing season (beginning, peak, and end) and for the past winter.

| | Temperature | | | | Daylight length | | | |
|---|---|---|---|---|---|---|---|---|
| | $R^2$ | $F$ | *p*-value | Equation when sig. | $R^2$ | $F$ | *p*-value | Equation when sig. |
| Beginning | 0.20 | 1.77 | 0.22 | | 0.7 | 20.98 | 0.001* | $y = 8.46 + 0.15x$ |
| Peak | 0.26 | 3.58 | 0.09 | | 0.02 | 0.14 | 0.71 | |
| End | 0.17 | 2.10 | 0.18 | | 0.53 | 11.48 | 0.007* | $y = 3.3 + 0.18x$ |
| Past winter | 0.84 | 53.75 | <0.001* | $y = 28.7 - 0.44x$ | 0.93 | 126.11 | <0.001* | $y = 15.6 - 0.15x$ |

*Equations are given only when the linear relationship is significant with: y representing the tested variable (temperature and daylight length) and x representing latitude in all cases. Asterisks (*) highlight significant linear regressions.*
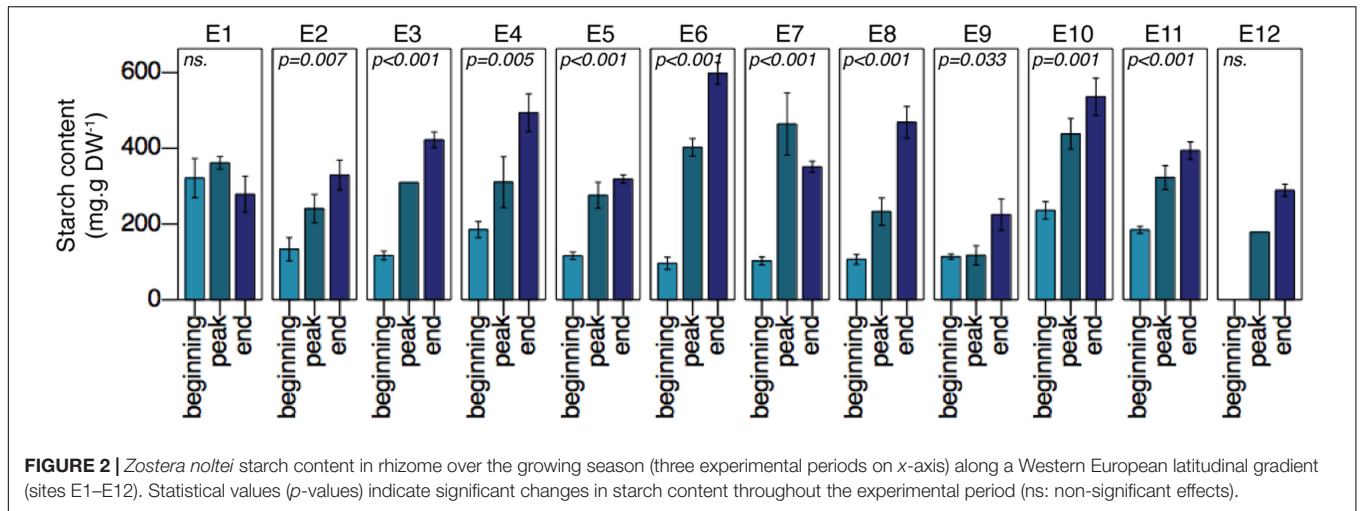
**FIGURE 2** | *Zostera noltei* starch content in rhizome over the growing season (three experimental periods on *x*-axis) along a Western European latitudinal gradient (sites E1–E12). Statistical values (*p*-values) indicate significant changes in starch content throughout the experimental period (ns: non-significant effects).
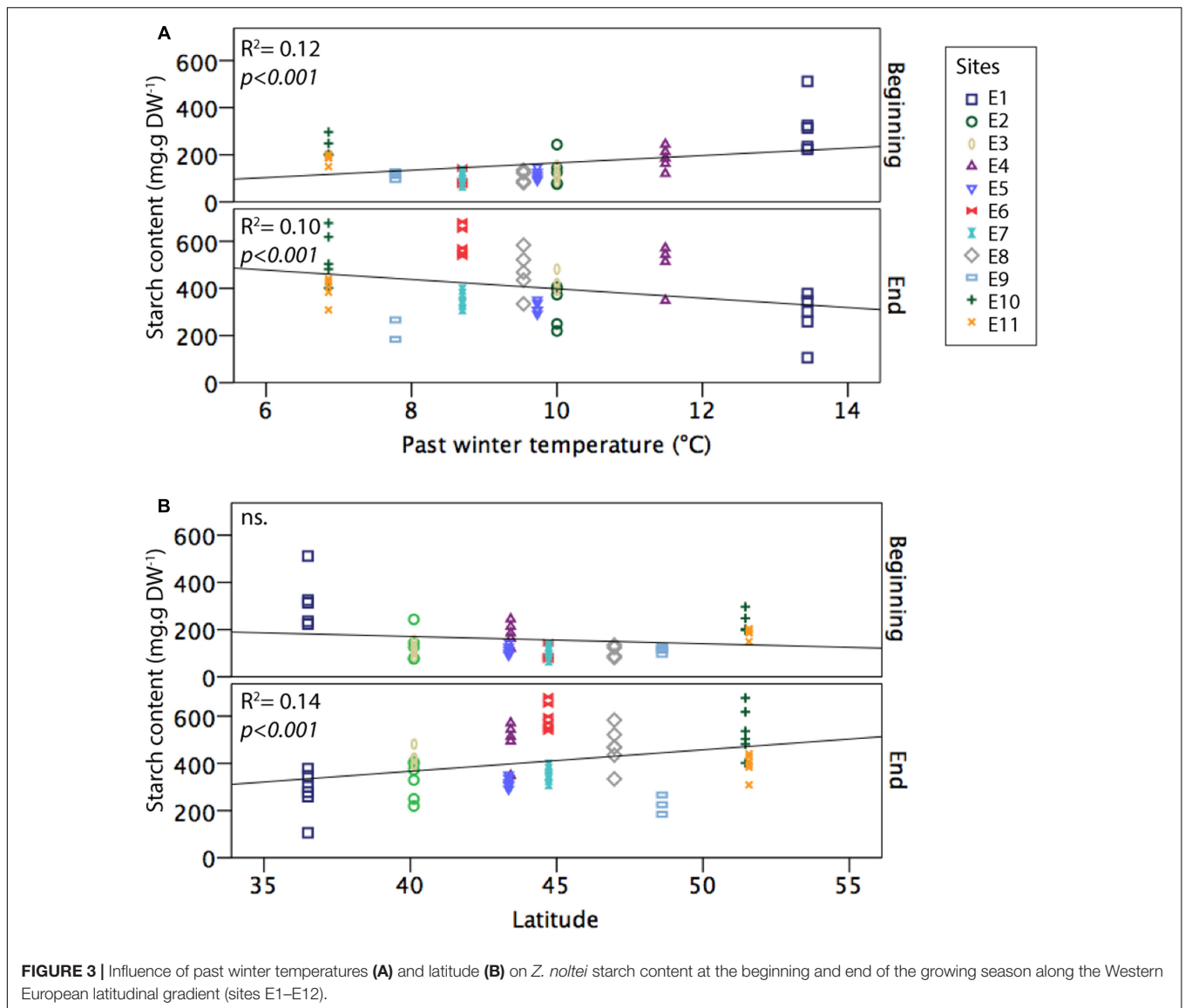


**FIGURE 3** | Influence of past winter temperatures **(A)** and latitude **(B)** on *Z. noltei* starch content at the beginning and end of the growing season along the Western European latitudinal gradient (sites E1–E12).

reserves, two sites located at the same latitude (sites E10 and E11) were subjected to manipulative *in situ* experiments. Treatments and experimental period had different effects at both sites (interaction effects, **Table 3** and **Figure 4**). In controls, starch content was significantly higher at site E10 as compared to site E11 (**Figure 4**). When applied alone, nutrient enrichment had no significant effect on starch content at either sites [Treatment (CN), **Figure 4**], while aboveground

biomass removal significantly decreased starch content at site E10 at the beginning and peak of the season [Treatment (D), **Figure 4**]. Aboveground biomass removal alone [Treatment (D)] did not result in a significant change in starch content at site E11 (**Figure 4**). However, when combined, nutrient enrichment and aboveground biomass removal significantly decreased starch content at site E10 at the peak of growth and at the end of the growing season [Treatment (DN), **Figure 4**]. Additionally, the combination of stress and disturbances tended ($p < 0.1$) to reduce starch content at both sites and all times, when compared to control situations [Treatment (DN), **Figure 4**].

**TABLE 3 |** Local effects of short-term stress and small-scale disturbances on *Z. noltei* starch content.

|  | % TSS | F | p-value |
|---|---|---|---|
| Experimental period | 8 | 111.807 | <0.001* |
| Treatment | 2 | 19.081 | <0.001* |
| Site | 0.008 | 0.244 | 0.623 |
| Experimental period * Treatment | 0.5 | 2.487 | 0.029* |
| Experimental period * Site | 0.3 | 4.395 | 0.015* |
| Treatment * Site | 1 | 9.461 | <0.001* |
| Experimental period * Treatment * Site | 0.4 | 2.899 | 0.06 |

*Sources of variation include three factors: Experimental period (three levels: beginning, peak, and end of the growing season); Treatment (three levels: nutrient enrichment, disturbance, and nutrient enrichment × disturbance); and Site (two levels: E10 and E11). Statistical analysis includes the effects of individual factor and their interactions. The column "%TSS" represents the total sum of squares as percentage (Type III) accounted by each factor. Asterisks (\*) highlight factors with significant effects (i.e., p < 0.05).*

## DISCUSSION

As dynamic systems, seagrass meadows experience variations in their abiotic and biotic conditions throughout the year. Some are related to seasonal fluctuations and geographical settings; but others are rather local such as short-term nutrient enrichment or small-scale disturbances. In this study, we observed the variation of seagrass carbon reserves (i.e., starch content) in rhizomes throughout the growing season along a latitudinal gradient and how this variation is related to the geographical distribution of seagrasses and seasonal fluctuations. We also had some indications on the effect of short-term local events, suggesting that short-term nutrient enrichment and
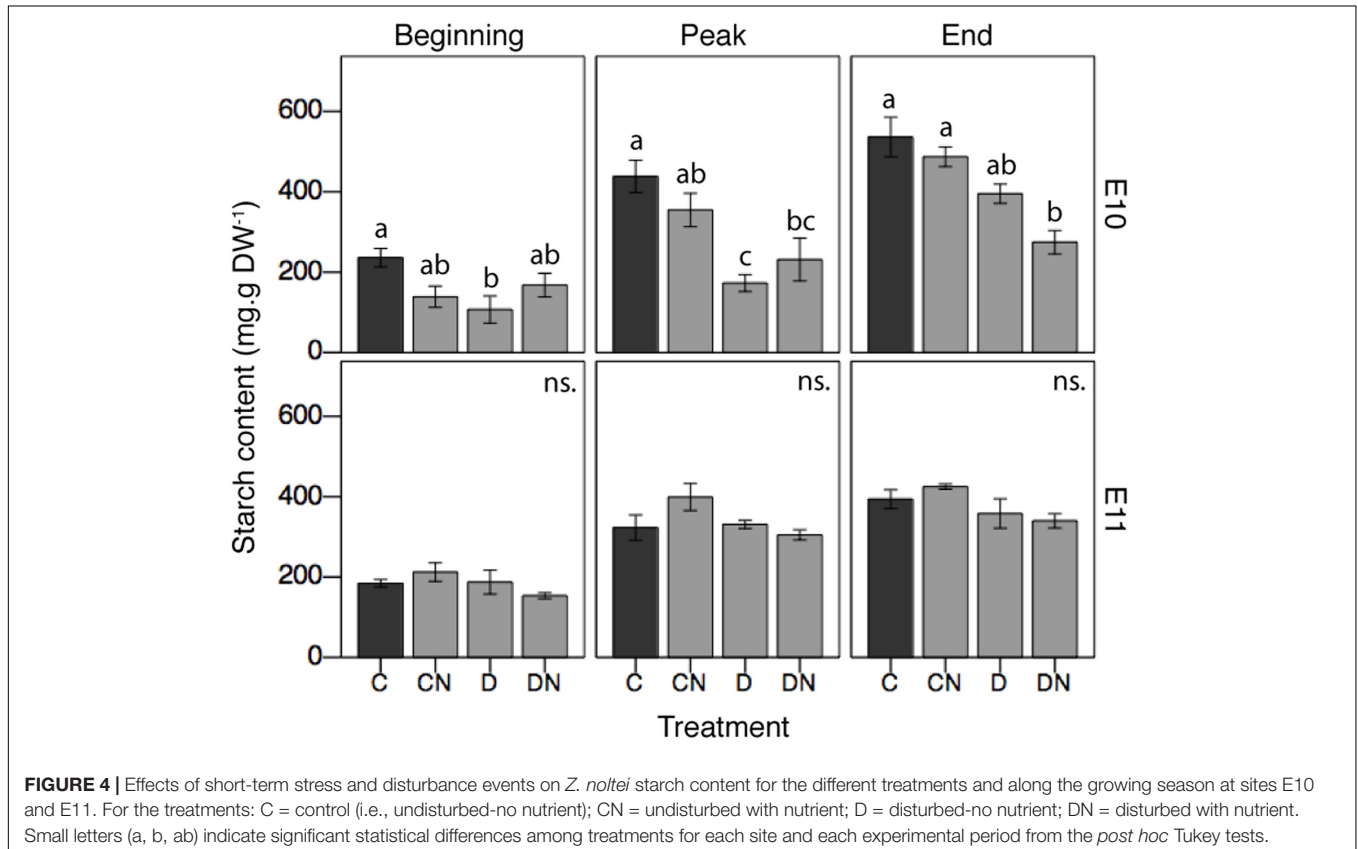


**FIGURE 4 |** Effects of short-term stress and disturbance events on *Z. noltei* starch content for the different treatments and along the growing season at sites E10 and E11. For the treatments: C = control (i.e., undisturbed-no nutrient); CN = undisturbed with nutrient; D = disturbed-no nutrient; DN = disturbed with nutrient. Small letters (a, b, ab) indicate significant statistical differences among treatments for each site and each experimental period from the *post hoc* Tukey tests.

aboveground biomass removal could reduce the storage of carbon reserves, particularly when combined. Potential implications of these latitudinal and local effects on seagrass long-term survival and resilience will also be discussed in the next sections.

## The Influence of Seasonal Fluctuations

Our study reveals that seasonal fluctuations such as winter intensity or latitude may affect seagrass rhizome carbon reserves, indicating that seagrass carbon reserves at the end of the season are positively related to latitude. In other words, northern seagrass populations accumulate more reserves over their growing season. However, this pattern is opposite at the beginning of the growing season, suggesting that northern seagrass populations have lower carbon reserves at the beginning of the growing season than southern populations. These cross patterns suggest that northern seagrass populations are more dependent on their carbon reserves during winter, but also build-up more reserve during the growing season than southern populations.

In southern Europe, winter temperatures are milder and days lengthier with longer photoperiod and higher daily doses of light (less clouds), allowing a higher photosynthetic production than in northern Europe (Touchette and Burkholder, 2000; Olivé et al., 2007). Those southern seagrass population usually form evergreen meadows whereas, for *Z. noltei* meadows in northern temperate areas in winter, only the below ground biomass, some sparse shoots, and seed banks remain until spring, when new shoots grow again and seedlings emerge (Pérez-Lloréns and Niell, 1993; Auby and Labourg, 1996; Vermaat and Verhagen, 1996; Plus et al., 2003). This likely explains the latitudinal pattern of consumption and larger depletion of carbon reserves (i.e., starch content) observed in the north as compared to the south since, in the north, seagrasses rely on these reserves for maintenance, rather than in photosynthesis to sustain the plant during winter. In a similar way, latitudinal differences and seasonal fluctuations can explain the differences in carbon storage during the growing season because, in summer, temperatures are milder while daily doses of light are longer in northern Europe as compared to southern locations. This hence provides an environment with larger light resources and minor temperature stress for the northern seagrass populations. This is supported partly by our results showing a weak but significant relationship between carbon reserves (i.e., starch content) and daylight length at the peak of the growing season, and by other studies where the combination of mild temperature and high daylight doses (duration of irradiance) has been described as an optimum situation to stimulate a positive growth–respiration balance (Madsen, 1991; Alcoverro et al., 1999; Brun et al., 2003). Additionally, southern seagrass populations are more susceptible of suffering from environmental stress linked to heat waves (Grilo et al., 2011) or excessive daily light irradiance causing photo-inhibition (Jimenez et al., 1987; Schubert et al., 2015). These summer stresses may explain the lack of carbon reserves (i.e., starch content) increase for the most southern site during the growing season. In

northern latitudes, carbon reserves are critical for winter survival. However, in southern latitudes, higher winter light availability (daily doses) and milder winter temperatures may allow southern seagrass populations to maintain a positive primary production that favors growth and survival overwinter (Madsen, 1991; Peralta et al., 2005). Winter seagrass growth in southern latitudes also explains the slightly higher carbon reserves (i.e., starch content) at the beginning of the next growing season observed in this study. In summary, seagrass populations in northern latitudes may benefit from the milder summer conditions (high daily light doses, limited stress caused by extreme temperatures or light inhibition) while southern seagrass populations benefit from milder winter conditions, favoring continuous growth, thereby sustaining evergreen populations.

These patterns of higher carbon reserves build-up in northern populations during summer, and stronger depletion during winter, also agree with seagrass reproductive patterns at northern latitudes, where populations tend to be deciduous and invest in higher reproductive effort (Van Tussenbroek et al., 2016). Thus, northern seagrass populations may be considered to be in a perpetual colonizing phase (Peralta et al., 2005). Yearly population survival partly depends on the carbon reserves (i.e., starch content) stored in dormant rhizomes to survive the cold winter conditions, and partly on seed production (Van Tussenbroek et al., 2016). In contrast, southern (evergreen) seagrass population may be considered as dependent on their constant clonal growth and on their carbon reserves only when external resources are limiting the positive balance of their net primary production (Coyer et al., 2004; Zipperle et al., 2011; Ribaudo et al., 2016).

## The Influence of Unpredictable Short-Term Stress Events

Short-term stress and small-scale disturbance events can occur at any time and severely affect seagrass meadows, independently from their geographical distribution. Short-term stress events range from waste water discharge (Cabaço et al., 2007), or algal cover (Han et al., 2016), while small-scale disturbances are related mainly to intense herbivory (Christianen et al., 2014), trampling (Eckrich and Holmquist, 2000), collection of fauna (Cabaço et al., 2005). Our study showed that combined short-term stress and small-scale disturbances, i.e., nutrient enrichment and above-ground removal, may generate a decrease in carbon reserves, i.e., starch content (effect detected at site E10). However, positive effects may sometimes compensate negative effects and environmental differences as seen for instance in Peralta et al. (2006) where a moderate increase in hydrodynamics can stimulate seagrass primary production. This may partly explain the lack of starch response at site E11, more exposed to winds and hydrodynamics (personal observations, Soissons LM) and where carbon reserves could be replenished fast after a disturbance due to an increased productivity (Peralta et al., 2006) and nutrients could be assimilated faster [positive effect of nutrient enrichment; (Brun et al., 2002)].

Despite a significant decrease of their carbon reserve, *Z. noltei* plants from site E10 had higher starch content throughout the growing season than those from site E11. It is therefore also plausible that plants from E10 used their carbon reserve to overcome the combined short-term stress and small-scale disturbance to maintain a vigorous growth. In contrast, the plants from site E11 (experiencing different local conditions) may be able to afford carbon storage with a lower biomass and growth. Working on natural systems implies to cope with additional effects associated with natural variability that are difficult to identify, but also provides a much more realistic frame for treatment responses. Divergences in the plants' response between sites E10 and E11 reflect differences in environmental conditions and/or among populations. However, our results still support that the combination of short-term stress and disturbance may potentially deplete seagrass carbon reserves.

Our results agree with responses found for other temperate seagrass species such as *Posidonia oceanica*, which carbon reserves decreased after stress events (Genot et al., 1994; Ruiz and Romero, 2003). Previous studies stated that soluble carbohydrate concentrations such as starch may not only be a valid indicator for seagrass growing success after long-time stress periods (Govers et al., 2015), but also for seagrass resilience (i.e., response to short-term stresses and disturbances). Carbon reserve pools and clonal growth rates are recognized as key elements for seagrass recovery (Hemminga and Duarte, 2000; Borum et al., 2004). However, both may vary widely among seagrass species (Ralph et al., 2007). Therefore, the amount of starch needed to overcome a short-term stresses and/or small-scale disturbance must also be species dependent.

## Potential Implications for Seagrass Meadows in a Changing Environment

Integrating the results of the latitudinal gradient with those of the stress-disturbance experiments suggest that northern seagrass populations might be more sensitive to short-term stress and disturbance events, as seagrass cope with this type of events by using their carbon reserves, which are fundamental to withstand colder winters at high latitudes. Present findings have implications for seagrass meadows under changing conditions, where unpredictable (stochastic) events due to climate change (Short and Neckles, 1999; Easterling et al., 2000; Thomson et al., 2015) or the increase of anthropogenic stressors on coastal ecosystems (Halpern et al., 2008; Duarte, 2014) are expected to become more frequent and of higher intensity. Indeed, although seagrasses have the capacity to acclimate to seasonal and latitudinal fluctuations (Peralta et al., 2005; Cabaço et al., 2009; Staehr and Borum, 2011; this study), our study shows that the combination of short-term stress and disturbance events may reduce seagrass carbon reserves, needed in general for overwintering. This may be particularly important as high carbon reserves are central to overwintering at northern latitudes (Govers et al., 2015).

## REFERENCES

Alcoverro, T., Manzanera, M., and Romero, J. (2001). Annual metabolic carbon balance of the seagrass *Posidonia oceanica*: the importance of carbohydrate reserves. *Mar. Ecol. Prog. Ser.* 211, 105–116. doi: 10.3354/meps211105

Alcoverro, T., Zimmerman, R. C., Kohrs, D. G., and Alberte, R. S. (1999). Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*. *Mar. Ecol. Prog. Ser.* 187, 121–131. doi: 10.3354/meps187121

Auby, I., and Labourg, P.-J. (1996). Seasonal dynamics of *Zostera noltii* Hornem. in the bay of Arcachon (France). *J. Sea Res.* 35, 269–277. doi: 10.1016/S1385-1101(96)90754-6

Borum, J., Duarte, C. M., Krause-Jensen, D., and Greve, T. M. (2004). "The four european seagrass species," in *European Seagrasses: an Introduction to Monitoring and Management*, eds J. Borum, C. M. Duarte, D. Krause-Jensen, and T. M. Greve [Hillerød: EU Project Monitoring and Managing of European Seagrasses (M&MS)].

Brun, F. G., Hernández, I., Vergara, J. J., Peralta, G., and Pérez-lloréns, J. L. (2002). Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*. *Mar. Ecol. Prog. Ser.* 225, 177–187. doi: 10.3354/meps225177

Brun, F. G., Hernández, I., Vergara, J. J., and Pérez-Lloréns, J. L. (2003). Growth, carbon allocation and proteolytic activity in the seagrass *Zostera noltii* shaded by Ulva canopies. *Funct. Plant Biol.* 30, 551–560. doi: 10.1071/FP03010

Burke, M. K., Dennison, W. C., and Moore, K. A. (1996). Non-structural carbohydrate reserves of eelgrass *Zostera marina*. *Mar. Ecol. Prog. Ser.* 137, 195–201. doi: 10.3354/meps137195

Burkholder, J. M., Tomasko, D. A., and Touchette, B. W. (2007). Seagrasses and eutrophication. *J. Exp. Mar. Biol. Ecol.* 350, 46–72. doi: 10.1016/j.jembe.2007.06.024

Cabaço, S., Alexandre, A., and Santos, R. (2005). Population-level effects of clam harvesting on the seagrass *Zostera noltii*. *Mar. Ecol. Prog. Ser.* 298, 123–129. doi: 10.3354/meps298123

Cabaço, S., Machás, R., and Santos, R. (2009). Individual and population plasticity of the seagrass *Zostera noltii* along a vertical intertidal gradient. *Estuar. Coast. Shelf Sci.* 82, 301–308. doi: 10.1016/j.ecss.2009.01.020

Cabaço, S., Machas, R., Vieira, V., and Santos, R. (2007). Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*). *Estuar. Coast. Shelf Sci.* 78, 1–13. doi: 10.1016/j.ecss.2007.11.005

Carr, J. A., D'Odorico, P., McGlathery, K. J., and Wiberg, P. L. (2012). Modeling the effects of climate change on eelgrass stability and resilience: future

scenarios and leading indicators of collapse. *Mar. Ecol. Prog. Ser.* 448, 289–301. doi: 10.3354/meps09556

Charpentier, A., Grillas, P., Lescuyer, F., Coulet, E., and Auby, I. (2005). Spatio-temporal dynamics of a *Zostera noltii* dominated community over a period of fluctuating salinity in a shallow lagoon, Southern France. *Estuar. Coast. Shelf Sci.* 64, 307–315. doi: 10.1016/j.ecss.2005.02.024

Christianen, M. J. A., Herman, P. M. J., Bouma, T. J., Lamers, L. P. M., van Katwijk, M. M., van der Heide, T., et al. (2014). Habitat collapse due to overgrazing threatens turtle conservation in marine protected areas. *Proc. R. Soc.* 281:20132890. doi: 10.1098/rspb.2013.2890

Coyer, J., Diekmann, O. E., Serrão, E. A., Procaccini, G., Milchakova, N., Pearson, G. A., et al. (2004). Population genetics of dwarf eelgrass *Zostera noltii* throughout its biogeographic range. *Mar. Ecol. Prog. Ser.* 281, 51–62. doi: 10.3354/meps281051

de los Santos, C. B., Brun, F. G., Bouma, T. J., Vergara, J. J., and Pérez-lloréns, J. L. (2010). Acclimation of seagrass *Zostera noltii* to co-occurring hydrodynamic and light stresses. *Mar. Ecol. Prog. Ser.* 398, 127–135. doi: 10.3354/meps08343

de los Santos, C. B., Brun, F. G., Vergara, J. J., and Pérez-Lloréns, J. L. (2013). New aspect in seagrass acclimation: leaf mechanical properties vary spatially and seasonally in the temperate species *Cymodocea nodosa* Ucria (Ascherson). *Mar. Biol.* 160, 1083–1093. doi: 10.1007/s00227-012-2159-3

Dennison, W. C. (1987). Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquat. Bot.* 27, 15–26. doi: 10.1016/0304-3770(87)90083-0

Duarte, C. M. (1991). Seagrass depth limits. *Aquat. Bot.* 40, 363–377. doi: 10.1016/0304-3770(91)90081-F

Duarte, C. M. (2014). Global change and the future ocean: a grand challenge for marine sciences. *Front. Mar. Sci* 1:63. doi: 10.3389/fmars.2014.00063

Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R., and Mearns, L. O. (2000). Climate extremes: observations, modeling, and impacts. *Science* 289, 2068–2074. doi: 10.1126/science.289.5487.2068

Eckrich, C. E., and Holmquist, J. G. (2000). Trampling in a seagrass assemblage: direct effects, response of associated fauna, and the role of substrate characteristics. *Mar. Ecol. Prog. Ser.* 201, 199–209. doi: 10.3354/meps201199

Genot, I., Caye, G., Meinesz, A., and Orlandini, M. (1994). Role of chlorophyll and carbohydrate contents in survival of *Posidonia oceanica* cuttings transplanted to different depths. *Mar. Biol.* 119, 23–29. doi: 10.1007/BF00350102

Godet, L., Fournier, J., Van Katwijk, M. M., Olivier, F., Le Mao, P., and Retière, C. (2008). Before and after wasting disease in common eelgrass *Zostera marina* along the French Atlantic coasts: a general overview and first accurate mapping. *Dis. Aquat. Organ.* 79, 249–255. doi: 10.3354/dao01897

Govers, L. L., de Brouwer, J. H. F., Suykerbuyk, W., Bouma, T. J., Lamers, L. P. M., Smolders, A. J. P., et al. (2014). Toxic effects of increased sediment nutrient and organic matter loading on the seagrass *zostera noltii. Aquat. Toxicol.* 155, 253–260. doi: 10.1016/j.aquatox.2014.07.005

Govers, L. L., Suykerbuyk, W., Hoppenreijs, J. H. T., Giesen, K., Bouma, T. J., and van Katwijk, M. M. (2015). Rhizome starch as indicator for temperate seagrass winter survival. *Ecol. Indic.* 49, 53–60. doi: 10.1016/j.ecolind.2014.10.002

Grilo, T. F., Cardoso, P. G., Dolbeth, M., Bordalo, M. D., and Pardal, M. A. (2011). Effects of extreme climate events on the macrobenthic communities' structure and functioning of a temperate estuary. *Mar. Pollut. Bull.* 62, 303–311. doi: 10.1016/j.marpolbul.2010.10.010

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., et al. (2008). A global map of human impact on marine ecosystems. *Science* 319, 948–952. doi: 10.1126/science.1149345

Han, Q., Soissons, L. M., Bouma, T. J., van Katwijk, M. M., and Liu, D. (2016). Combined nutrient and macroalgae loads lead to response in seagrass indicator properties. *Mar. Pollut. Bull.* 106, 174–182. doi: 10.1016/j.marpolbul.2016.03.004

Hemminga, M. A., and Duarte, C. M. (2000). *Seagrass Ecology*. Cambridge: Cambridge university Press.

Jimenez, C., Niell, F. X., and Algarra, P. (1987). Photosynthetic adaptation of *Zostera noltii* Hornem. *Aquat. Bot.* 29, 217–226. doi: 10.1016/0304-3770(87)90017-9

La Nafie, Y. A., de los Santos, C. B., Brun, F. G., Mashoreng, S., van Katwijk, M. M., and Bouma, T. J. (2013). Biomechanical response of two fast-growing tropical seagrass species subjected to in situ shading and sediment fertilization. *J. Exp. Mar. Biol. Ecol.* 446, 186–193. doi: 10.1016/j.jembe.2013.05.020

Lee, K.-S., Park, S. R., and Kim, Y. K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *J. Exp. Mar. Biol. Ecol.* 350, 144–175. doi: 10.1016/j.jembe.2007.06.016

Les, D. H., Cleland, M. A., and Waycott, M. (1997). Phylogenetic studies in Alismatidae, II: evolution of Marine Angiosperms (Seagrasses) and Hydrophily. *Syst. Bot.* 22, 443–463. doi: 10.2307/2419820

Macreadie, P. I., York, P. H., and Sherman, C. D. H. (2014). Resilience of *Zostera muelleri* seagrass to small-scale disturbances: the relative importance of asexual versus sexual recovery. *Ecol. Evol.* 4, 450–461. doi: 10.1002/ece3.933

Madsen, J. D. (1991). Resource allocation at the individual plant level. *Aquat. Bot.* 41, 67–86. doi: 10.1016/0304-3770(91)90039-8

Marbà, N., Krause-Jensen, D., Alcoverro, T., Birk, S., Pedersen, A., Neto, J. M., et al. (2012). Diversity of European seagrass indicators: patterns within and across regions. *Hydrobiologia* 704, 265–278. doi: 10.1007/s10750-012-1403-7

Marsh, J. A., Dennison, W. C., and Alberte, R. S. (1986). Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *J. Exp. Mar. Biol. Ecol.* 101, 257–267. doi: 10.1016/0022-0981(86)90267-4

McGlathery, K. J., Reynolds, L. K., Cole, L. W., Orth, R. J., Marion, S. R., and Schwarzschild, A. (2012). Recovery trajectories during state change from bare sediment to eelgrass dominance. *Mar. Ecol. Prog. Ser.* 448, 209–221. doi: 10.3354/meps09574

Ochieng, C. A., Short, F. T., and Walker, D. I. (2010). Photosynthetic and morphological responses of eelgrass (*Zostera marina* L.) to a gradient of light conditions. *J. Exp. Mar. Biol. Ecol.* 382, 117–124. doi: 10.1016/j.jembe.2009.11.007

Olesen, B., and Sand-Jensen, K. (1993). Seasonal acclimatization of eelgrass *Zostera marina* growth to light. *Mar. Ecol. Prog. Ser.* 94, 91–99. doi: 10.3354/meps094091

Olivé, I., Brun, F. G., Vergara, J. J., and Pérez-Lloréns, J. L. (2007). Effects of light and biomass partitioning on growth, photosynthesis and carbohydrate content of the seagrass *Zostera noltii* Hornem. *J. Exp. Mar. Biol. Ecol.* 345, 90–100. doi: 10.1016/j.jembe.2007.02.008

Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L. Jr., et al. (2006). A global crisis for seagrass ecosystems. *Bioscience* 56, 987–996. doi: 10.1641/0006-3568200656

Peralta, G., Brun, F. G., Hernández, I., Vergara, J., and Perezllorens, J. (2005). Morphometric variations as acclimation mechanisms in beds. *Estuar. Coast. Shelf Sci.* 64, 347–356. doi: 10.1016/j.ecss.2005.02.027

Peralta, G., Brun, F. G., Pérez-lloréns, J. L., and Bouma, T. J. (2006). Direct effects of current velocity on the growth, morphometry and architecture of seagrasses: a case study on *Zostera noltii. Mar. Ecol. Prog. Ser.* 327, 135–142. doi: 10.3354/meps327135

Pérez-Lloréns, J. L., and Niell, F. X. (1993). Seasonal dynamics of biomass and nutrient content in the intertidal seagrass *Zostera noltii* Hornem. from Palmones River estuary, Spain. *Aquat. Bot.* 46, 49–66. doi: 10.1016/0304-3770(93)90064-4

Plus, M., Auby, I., Verlaque, M., and Levavasseur, G. (2005). Seasonal variations in photosynthetic irradiance response curves of macrophytes from a Mediterranean coastal lagoon. *Aquat. Bot.* 81, 157–173. doi: 10.1016/j.aquabot.2004.10.004

Plus, M., Deslous-Paoli, J.-M., and Dagault, F. (2003). Seagrass (*Zostera marina* L.) bed recolonisation after anoxia-induced full mortality. *Aquat. Bot.* 77, 121–134. doi: 10.1016/S0304-3770(03)00089-5

R Core Team (2013). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Ralph, P. J., Durako, M. J., Enríquez, S., Collier, C. J., and Doblin, M. A. (2007). Impact of light limitation on seagrasses. *J. Exp. Mar. Biol. Ecol.* 350, 176–193. doi: 10.1016/j.jembe.2007.06.017

Ribaudo, C., Plus, M., Ganthy, F., and Auby, I. (2016). Carbon sequestration loss following *Zostera noltei* decline in the Arcachon Bay (France). *Estuar. Coast. Shelf Sci.* 179, 4–11. doi: 10.1016/j.ecss.2016.01.024

Ruiz, J. M., and Romero, J. (2003). Effects of disturbances caused by coastal constructions on spatial structure, growth dynamics and photosynthesis of the seagrass *Posidonia oceanica. Mar. Pollut. Bull.* 46, 1523–1533. doi: 10.1016/j.marpolbul.2003.08.021

Scheffer, M., Bascompte, J., Brock, W. A., Brovkin, V., Carpenter, S. R., Dakos, V., et al. (2009). Early-warning signals for critical transitions. *Nature* 461, 53–59. doi: 10.1038/nature08227

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., and Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature* 413, 591–596. doi: 10.1038/35098000

Schubert, N., Colombo-Pallota, M. F., and Enriquez, S. (2015). Leaf and canopy scale characterization of the photoprotective response to high-light stress of the seagrass *Thalassia testudinum*. *Limnol. Oceanogr.* 60, 286–302. doi: 10.1002/lno.10024

Short, F., Carruthers, T., Dennison, W., and Waycott, M. (2007). Global seagrass distribution and diversity: a bioregional model. *J. Exp. Mar. Biol. Ecol.* 350, 3–20. doi: 10.1016/j.jembe.2007.06.012

Short, F. T., and Neckles, H. A. (1999). The effects of global climate change on seagrasses. *Aquat. Bot.* 63, 169–196. doi: 10.1016/S0304-3770(98)00117-X

Soissons, L. M., Li, B., Han, Q., van Katwijk, M. M., Ysebaert, T., Herman, P. M. J., et al. (2016). Understanding seagrass resilience in temperate systems: the importance of timing of the disturbance. *Ecol. Indic.* 66, 190–198. doi: 10.1016/j.ecolind.2016.01.030

Soissons, L. M., van Katwijk, M. M., Peralta, G., Brun, F. G., Cardoso, P. G., Grilo, T. F., et al. (2018). Seasonal and latitudinal variation in seagrass mechanical traits across Europe: the influence of local nutrient status and morphometric plasticity. *Limnol. Oceanogr.* 63, 37–46. doi: 10.1002/lno.10611

Sordo, L., Fournier, J., De Oliveira, V. M., Gern, F., De Castro Panizza, A., and da Cunha Lana, P. (2011). Temporal variations in morphology and biomass of vulnerable *Halodule wrightii* meadows at their southernmost distribution limit in the southwestern Atlantic. *Bot. Mar.* 54, 13–21. doi: 10.1515/BOT.2011.007

Staehr, P. A., and Borum, J. (2011). Seasonal acclimation in metabolism reduces light requirements of eelgrass (*Zostera marina*). *J. Exp. Mar. Biol. Ecol.* 407, 139–146. doi: 10.1016/j.jembe.2011.05.031

Suykerbuyk, W., Govers, L. L., Bouma, T. J., Giesen, W. B. J. T., de Jong, D. J., van de Voort, R., et al. (2016). Unpredictability in seagrass restoration: analysing the role of positive feedback and environmental stress on *Zostera noltii* transplants. *J. Appl. Ecol.* 53, 774–784. doi: 10.1111/1365-2664.12614

Thomson, J. A., Burkholder, D. A., Heithaus, M. R., Fourqurean, J. W., Fraser, M. W., Statton, J., et al. (2015). Extreme temperatures, foundation species, and abrupt ecosystem change: an example from an iconic seagrass ecosystem. *Glob. Chang Biol.* 21, 1463–1474. doi: 10.1111/gcb.12694

Touchette, B. W., and Burkholder, J. M. (2000). Overview of the physiological ecology of carbon metabolism in seagrasses. *J. Exp. Mar. Biol. Ecol.* 250, 169–205. doi: 10.1016/S0022-0981(00)00196-9

van Katwijk, M. M., Vergeer, L. H. T., Schmitz, G. H. W., and Roelofs, J. G. M. (1997). Ammonium toxicity in eelgrass *Zostera marina*. *Mar. Ecol. Prog. Ser.* 157, 159–173. doi: 10.3354/meps157159

Van Tussenbroek, B. I., Soissons, L. M., Bouma, T. J., Asmus, R., Auby, I., Brun, F. G., et al. (2016). Pollen limitation may be a common Allee effect in marine hydrophilous plants: implications for decline and recovery in seagrasses. *Oecologia* 182, 595–609. doi: 10.1007/s00442-016-3665-7

Vermaat, J. E., and Verhagen, F. C. A. (1996). Seasonal variation in the intertidal seagrass *Zostera noltii* Hornem.: coupling demographic and physiological patterns. *Aquat. Bot.* 52, 259–281. doi: 10.1016/0304-3770(95)00510-2

Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., et al. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12377–12381. doi: 10.1073/pnas.0905620106

Yemm, E. W., and Willis, A. J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* 57, 508–514.

Zimmerman, R. C., and Alberte, R. S. (1996). Effect of light/dark transition on carbon translocation in eelgrass *Zostera marina* seedlings. *Mar. Ecol. Prog. Ser.* 136, 305–309. doi: 10.3354/meps136305

Zipperle, A. M., Coyer, J. A., Reise, K., Stam, W. T., and Olsen, J. L. (2011). An evaluation of small-scale genetic diversity and the mating system in *Zostera noltii* on an intertidal sandflat in the Wadden Sea. *Ann. Bot.* 107, 127–133. doi: 10.1093/aob/mcq214