Chapter 3: Seagrass sensitivity to collapse along a hydrodynamic gradient: evidence from a pristine subtropical intertidal ecosystem

Chapitre 3. SUSCEPTIBILITÉ D’HERBIERS MARINS À L’EFFONDREMENT : PREUVES APPORTÉES PAR UN ÉCOYSTÈME INTERTIDAL SUBTROPICAL PRÉSERVÉ

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ملخص

التشبع الغذائي الناتج عن الاستخدام المفرط للأسمدة الزراعية سبب انهيار وتدمير العديد من مسطحات الأعشاب البحرية حول العالم، ومن المتوقع أن يتباين تأثير الزيادة المفروطة على الأعشاب البحرية الواقعة على تدرج في القوى المائية الدافعة على استقرار هذه المروج ومحتواها من المغذيات وكذلك ردة فعلها. أتowards the gradient in driving forces for these communities and the responses.

لمسطحات العشبة المكسوة بالأعشاب البحرية في حوض آرغين من أجل وصف طبعة تشكلها (توزيع الكتلة الحيوية، مساحات ومساحة الأوراق، طول ونسبة الأوراق، طول ونسبة الأوراق، طول ونسبة الأوراق، طول ونسبة الأوراق) ومحتواها من المغذيات (الكربون، النتروجين، الفسفور، الحديد، الالمونيوم) والتناظر المغذي (الكربون والنترتشوف، النترتشوف) على طول تدرج كبير في القوى المائية الدافعة ومن ثم قمنا بدراسة تأثير الفصول السنوية على استقرار هذه المسطحات وتدفق المغذيات وكذلك ردة فعل ثلاث مسطحات عشبية لتجربة تسخين مفروطة (نترتشوف، الفوسفور، نترتشوف، وفوسفور) في هذه المسطحات الثلاثة اختيرت لتمثل ثلاثة مستويات من القوى الهيدروdinamique (قوية، معتدلة، وضعيفة).

المسمح والتقييم الميداني الواسع النطاق كشف لنا عن وجود علاقة سلبية بين محتوى الأعشاب من النترتشوف والفوسفور من جهة والقوى المائية الواقعة من جهة أخرى: كلما زادت قوى الأمواج والتيارات البحرية كلما قلص مستوى المسطحات من المغذيات. الانخفاض العام في مستويات النترتشوف ونسبة النترتشوف على الفوسفور يشير إلى أن المنطقة تعاني أساسا من نقص في النترتشوف. التغيير الموسمي في عدد ووزن الكتلة العشبية أظهر أن المناطق التي تخضع لتيارات وأموجات قوية أكثر استقرارا من المناطق التي تخضع لقوى هادية وديناميكية ضعيفة.

الغزير الموسمي في مؤشر نظير النترتشوف دل على تجربة الصدمة في مصادر النترتشوف في المنطقة المعرضة لتيارات القوية، أما نتيجة تجربة التسميد فقد أظهرت أن الإضافة المفروطة لعنصر النترتشوف أدى إلى موت الأعشاب البحرية في المنطقة خصبة لأموجات قوية في حين تبين أن الأعشاب الواقعة في المنطقة...
Résumé

L’eutrophisation est une cause de pertes immenses d’herbiers marins dans le monde. Les effets de la charge en nutriments varient au long de gradients environnementaux, et il est attendu que la force des vagues, en particulier, affecte la stabilité des prairies, le statut des nutriments ainsi que les réponses à l’approvisionnement en nutriments. Ici, nous avons réalisé un relevé du système d’herbiers intertidal subtropical préservé du Banc d’Arguin, en Mauritanie, pour caractériser *Zostera noltii* en termes de morphologie (allocation de la biomasse, longueur et aire foliaires, distance entre-nœuds des rhizomes), de teneur en nutriments (carbone : C, azote : N, phosphore : P, fer : Fe, aluminium : Al) et de signatures δ¹³C et δ¹⁵N, au long d’un gradient ample de conditions hydrodynamiques. Nous avons ensuite évalué la variabilité temporelle de la stabilité des herbiers et des flux de nutriments, ainsi que les réponses à des évènements de fertilisation expérimentale (impulsions de +N, +P, +N+P) entrepris sur trois prairies représentant différents degrés de forces de vagues (prairie exposée, intermédiaire et abritée). L’étude à grande échelle a révélé une augmentation marquée de la limitation en N et en P à mesure que l’énergie des vagues augmente. Les valeurs généralement basses de %N foliaire (1.74 ± 0.04 ; moyenne ± erreur type) et du ratio N:P (8.67 ± 0.14) suggèrent que N est limitant dans la zone. La variation saisonnière en couvert végétal de l’herbier et en biomasse montre que le site exposé est le plus stable, et le site abrité le moins stable. La variation des signatures δ¹⁵N indique des transitions saisonnières en source de N, au niveau du site exposé uniquement. Les fertilisations avec +N et +N+P ont accru la mortalité de l’herbier au niveau du site exposé, alors que sur le site abrité l’herbier a été dégradé par +P. Dans l’ensemble, nos résultats indiquent qu’avec l’accroissement de la force des vagues, la stabilité des
herbiers augmente, mais la limitation en nutriments et la vulnérabilité face à l’eutrophisation augmentent également.

Abstract

Eutrophication drives seagrass losses across the globe. However, as effects of nutrient overloads are expected to vary along environmental gradients it is important to understand how wave-forces affect the nutrient-status, stability, and the response of seagrass to eutrophication. Here, we used the pristine subtropical intertidal seagrass system of Banc d’Arguin, Mauritania to characterise the morphology (biomass allocation, leaf length & area, rhizome internode length), nutrients (carbon: C, nitrogen: N, phosphorus: P, iron: Fe, aluminium: Al) content, and δ¹³C and δ¹⁵N isoscapes of Zostera noltii across a wide hydrodynamic gradient. We then assessed temporal variability in seagrass stability and nutrient fluxes as well as its response to experimental fertilisation (pulses of +N, +P, +N+P) on three meadows representing different wave-forces (exposed, intermediate, and sheltered). The large-scale survey revealed a marked increase in N and P limitation with increasing wave energy. The overall low leaf %N (1.74 ± 0.04; mean ± se) and N:P ratio (8.67 ± 0.14) also suggests that the area is N-limited. Seasonal variation in seagrass cover and biomass showed the exposed site to be the most stable meadow and the sheltered site the least stable. Variation in δ¹⁵N signatures indicates a seasonal shift in N sources at the exposed site only. Fertilisation showed that +N and +N+P enrichments to induce seagrass mortality at the exposed site, while at the sheltered site it was +P that degraded seagrass. Collectively, our results indicate that nutrient limitation, seagrass stability and vulnerability to eutrophication to increase with increasing wave-forces.

Introduction

Human increasing use and exploitation of coastal systems have impaired the majority of seagrass beds around the world (Short & Wyllie-Echeverria, 1996; Orth et al.,
2006; Waycott et al., 2009). Of all human-related disturbances, eutrophication-induced mortality is considered the most destructive factor for seagrasses (Burkholder et al., 2007). Eutrophication (mainly excess nitrogen) stimulates the growth of phytoplankton, epiphytes, and the ephemeral macroalgae (Duarte, 1995; Viaroli et al., 2008), which kills seagrasses through shading and light limitation (Short et al., 1995). Eutrophication generally occurs at the landscape scale (Green et al., 2004) leading to mass mortality in seagrass beds and subsequently provoking sediment suspension (Maxwell et al., 2017), which will further hinder their recovery (Folmer et al., 2012) and could triggers a regime shift (van der Heide et al., 2007). Potential responses of seagrass to eutrophication are usually assessed with nutrient addition experiments. Across sites meta-analyses on the responses of seagrass to experimental fertilisation studied at small scales (Leoni et al., 2008; Östman et al., 2016) show that these responses are not always uniform across systems (Jiménez-Ramos et al., 2017). Assessing the effect of nutrients enrichment on seagrasses along hydrodynamic gradients remains a major challenge (but see Armitage and others 2005, 2011). The paucity of ecosystems that are large and untouched enough limit the opportunities for addressing this question at landscape scales.

Hydrodynamics vary in space and time (Paul & Amos, 2011), creating environmental gradients at the landscape-scale. Intertidal seagrass communities are profoundly affected by hydrodynamic forces (Fonseca and Bell 1998; Turner and others 1999; van Katwijk and Hermus 2000; Cornelisen and Thomas 2004; Peralta and others 2006; Vacchi and others 2012). Wave-action and tidal flow not only impose physical stress on seagrass, but also spatially and temporally affect sediment dynamic and nutrient supplies (Morris et al., 2008, 2013; Malta et al., 2017). Global warming is expected to exacerbate the intensity and frequency of the extreme weather events such as floods, drought, and storms (Easterling et al., 2000; Jentsch et al., 2007; IPCC, 2012). Such extreme events together with the accelerating sea-level rise are likely to affect hydrodynamic regimes and sediment dynamics, and thus seagrass
resilience (Cardoso et al., 2008; Grilo et al., 2011; Jeanson et al., 2014; Malta et al., 2017).

The extensive intertidal flats of Parc National du Banc d’Arguin, Mauritania, West Africa, harbour one of the most pristine and extensive seagrass beds on Earth (Campredon, 2000; Folmer et al., 2012; de Fouw et al., 2016a). Giving its large-scale and clear gradient in wave exposure, the Banc d’Arguin provides an ideal system to experimentally investigate the separate and interactive effects of hydrodynamic and nutrients enrichment on the resilience of seagrass beds. The area has three potential external sources that could affect its nutrient budget: (1) the active upwelling zone of Cap Blanc (Steinfeldt et al., 2015), (2) dust of the Sahara (Neuer et al., 2004) and (3) the large number of breeding seabirds (Anderson & Polis, 1999). The extent to which these systems may affect the nutrient status of the seagrass beds of Banc d’Arguin is presently unclear.

Owing to its sensitivity to changes in nutrient conditions, seagrass morphology and stoichiometry are increasingly used as ecological indicators to infer coastal ecosystem processes, nutrient limitation as well as an early warning sentinels for regime shifts (Duarte, 1990; Grice et al., 1996; Fourquarean et al., 2007; Burkholder et al., 2013). It has been suggested that seagrass leaf %N and %P contents are indicators of nutrient limitation (Duarte, 1990) and nutrient loading (Govers et al., 2014b). Biomass allocation (Herbert & Fourquarean, 2009; Armitage et al., 2011) as well as leaf and rhizomes internode lengths (Marbà and Duarte 1995; Peralta and others 2000; Cabaço and Santos 2007; Balestri and Lardicci 2014) have been associated mostly with nutrient availability and sediment dynamics. Leaf isotopic ratios ($\delta^{13}C$ & $\delta^{15}N$), on the other hand, proven to be powerful tracers for the sources of nutrients in the system (Lepoint et al., 2004). For instance, abundances of $\delta^{15}N$ in the tissues have been widely used to infer the sources of N in the system (Lepoint et al., 2004). Values of $\delta^{15}N$ increase when it originates from an external source rather than being fixed in situ (Schrama et al., 2013). $\delta^{13}C$ values tend to decrease with tidal
elevation reflecting bicarbonate (HCO$_3^-$) signals lower on the gradient and atmospheric CO$_2$ signals higher on (Lepoint et al., 2003; Ruesink et al., 2015), a trend has been shown to occur to *Z. noltii* in our study area (Clavier et al., 2011). Finally, iron (Fe) and aluminium (Al) trace elements proved to be indicators for Saharan dust input into the adjacent marine systems (Kramer et al., 2004; Baker et al., 2006b; Castillo et al., 2008).

The objective of this study was to understand how differences in environmental setting occurring along hydrodynamic gradients affect seagrass stoichiometry and stability, and subsequently its sensitivity to collapse in response to nutrient pulses (i.e., artificial fertilisation). We combined a snapshot large spatial-scale survey, a two year detailed survey of 48 plots, and a field fertilisation experiments at three sites in the hydrodynamic gradient to: (1) characterise the effect of wave-energy gradient and distance to breeding birds on nine variables from seagrass morphology and stoichiometry as well as two sediment variables; (2) identify the temporal variability in seagrass morphology, stoichiometry, and stability on three sites representing different hydrodynamic regimes (exposed, intermediate, and sheltered); and (3) experimentally assess the responses of seagrass to nutrients addition on these sites.

**Materials and methods**

*Study system*

This study was conducted across the intertidal parts of the Parc National du Banc d’Arguin (PNBA), Mauritania (Fig. 3.1A). The intertidal system of PNBA contains the largest intertidal seagrass beds in Africa (500 km$^2$; Wolff and Smit 1990). The dwarf eelgrass *Zostera noltii* is the most dominant intertidal species and the main primary producer of the system (Wolff et al., 1993b). Higher on the intertidal gradient the opportunistic macro-algae *Vaucheria* seems to compete with *Z. noltii* for space and possibly resources, especially in areas with high sedimentation rates. In PNBA
the precipitation is very low (<100 mm, Ahmedou and others 2008) and the wind is abundant year-round, reaching on average 9 m s\(^{-1}\) in May-June and 6 m s\(^{-1}\) in November-December with predominately northern winds (ould Dedah, 1993).

**Hydrodynamic gradient and wave energy calculation**

The open source software Wave Exposure Model (WEMo, Malhotra & Fonseca 2007) was used as a proxy for chronic hydrodynamic forces at our study sites (263 stations). The model was developed by the National Oceanic and Atmospheric Administration (NOAA) and is implemented in ESRI ArcGIS 9.3 software. WEMo incorporates the surrounding water masses (GIS bathymetric map) and the strength and direction of the wind (separate data file) to calculate fetch length (calculated in 32 compass directions) and then 11 indices related to wave exposure. In order to conduct the exposure index, we first created a bathymetric map for the study area on a 30 m resolution (Fig. 3.1A) based on a Landsat 8 Satellite Imagery, scene of January 2016 (for description of the technique see, Jagalingam and others 2015). The bathymetric map was calibrated with field depth measurements by Jager (1993) plus extra ground trothing measurements around Iwik peninsula by Sidi Cheikh and others (Unpub. data). Wind data were obtained for the period 1980-2016 from MERRA product publicly available online (http://disc.sci.gsfc.nasa.gov/mdisc/data-holdings/merra/).

Spatial wave energy distribution (J m\(^{-1}\)) and the average wave height (m) were extrapolated to the core intertidal flats of Banc d’Arguin around the island of Tidra by IDW interpolation (Spatial Analyst Tools, ArcGIS) based on the 263 stations simulated by WEMo.

**Seagrass survey and fertilisation experiment**

The effect of hydrodynamic gradient on seagrass morphology and stoichiometry as well as sediment characteristics was determined at a spatial scale on 160 stations; 90 stations chosen along nine channels with different wave-exposure (Fig. 3.1A), and another 70 randomly selected stations. Then, a total of 48 permanent plots (1 m\(^2\),16
plots in 4 blocks per site) were established on three sites representing different wave-forces (Table S3.1) to investigate seagrass temporal stoichiometry, morphology, and stability as well as its responses to experimental fertilisation (Control: C, nitrogen addition: +N, phosphorus addition: +P, and +N+P).

Treatments were randomly assigned within each block. Fertilisation was applied in an even manner with the aid of a grid of 10 x 10 cm$^2$ by injecting the treatment beneath the seagrass shoots into the rhizosphere. For nitrogen treatment, small syringes (2 cm in diameter) with the tip cut off were used to inject 350 g/m$^2$ Osmocote® slow-release granules fertiliser (g:g:g ratio N:P:K 35:0:0) with a lifespan of 8-9 months. Phosphorus was applied by injecting (to a 10 cm depth) high dose of granule hydrated tri-calcium phosphate (“CaPO$_4$”) solution (400g in 2 L of seawater/m$^2$) with 70 mL syringes (see appendix, Christianen and others 2012). Nitrogen release was estimated as 41 g m$^2$ per month, a nutrient loading rate exceeding thresholds that cause eutrophication (Connell et al., 2017). Treatments were applied once at the start and the response of seagrass was assessed six month later.

**Sampling and measurements**

The measurements below were conducted in a similar manner for the survey’s stations and the fertilisation plots. Seagrass biomass was sampled in each plot by a cylindrical PVC core (15 cm diameter) up to a depth of 20 cm. The collected substrate was sieved over a 1-mm mesh sieve. Above- and belowground seagrass biomass was separated. Morphological parameters (leaf and internode length) were measured on three fresh intact shoot-rhizome samples from each core, and their first ten rhizome internodes were measured to the nearest 0.1 mm as well as the shoot length. Leaf area (LA) was estimated on photos taken from the intact shoots with ImageJ software (freely available online). Seagrass cover within each plot was visually estimated on 0-100% scale using a 10 x 10 cm grid. Diatoms densities ($\mu$g.cm$^{-2}$) were estimated with fluorescence-calibrated device, BenthoTorch (bbe-Moldaenke BenthoTorch, Germany). Other relevant abiotic factors were measured.
once for each sampling station, including sediment samples to determine median grain size (D50) and organic matter content (loss of ignition at 500 °C). Distance to seabird colonies was measured in Google Earth.

In the laboratory, the above- and belowground biomass was dried until constant weight at 70 °C for 48 h, weighed and then grinded for nutrients and stable isotope analyses. To assess seagrass nutrient status and isotopic signals, dried and grinded leaf material was used to determine the percentage of carbon (%C), nitrogen (%N), δ15N and δ13C with an elemental analyser (Type NA 1500 Carlo Erbo Termo Fisher Science, USA), coupled to a spectrometer (Thermo Finnigan Delta Plus, USA). Total phosphorus (%P), iron (‰ Fe), and aluminium (%Al) in leaf tissue was measured on an inductivity-coupled-plasma emission spectrophotometer (ICP) (Spectroflame, Spectro Inc), after digestion of dried material with nitric acid and hydrogen peroxide. Grain size was determined on freeze-dried and sieved (1 mm mesh) samples by laser diffraction on a Malvern particle size analyser (Master 2000).

Statistical analyses

We sought to characterise the environmental gradient related to hydrodynamic forces using ordination approaches. Detrended correspondence analysis (DCA) and principal component analysis (PCA) were considered to summarise the environmental gradient along the wave energy index and to identify the main variables that might affect the seagrass response to fertilisation. Gradient lengths from DCA were very short (< 0.1), suggesting that PCA analysis was more appropriate (Lepš & Šmilauer, 2003). Thus, only PCA results are presented. Seagrass morphology (leaf area (LA), leaf length, leaves density, below-to-aboveground biomass ratio), stoichiometry (%N, %P, %Fe), and isoscapes (δ15N, and δ13C) as well as sediment characteristics (D50, OM) from the 160 stations were used for the PCA. Prior PCA analysis, some variables were (log +1) transformed to ensure normality and homogeneity of each variable. The circle of equilibrium contribution was used to visualise the significance of the variables. This
circle defines a region beyond which variables can be interpreted with great confidence (Legendre & Legendre, 1998).

The spatial patterns in variables that might affect the response to fertilisation (seagrass stoichiometry, sediment OM, and grain size) were examined with linear regression to test for the ability of RWE to predict changes and trends in these variables. Furthermore, descriptive results of seagrass %N, %P, N:P, δ¹⁵N, sediment OM and grain size were presented as contour maps generated using a gridding interpolation (Kriging technique in Spatial Analyst Tools, ArcGIS) based on 160 stations to describe spatial trends at a system-scale.

The seasonal (winter vs. spring) changes of seagrass’ morphology, stoichiometry and stability along the exposure gradient (exposed, intermediate, and sheltered) were analysed with two-way analysis of variance (ANOVA). Parametric assumptions were checked on the residuals and data were transformed when necessary. To assess the seasonality without effect from the fertilisation treatments, only controls and samples from Jan 2013 (just before the application of the fertilisation) and May 2015 (two years later) were used for the analysis.

To compare the magnitude of differences in seagrass response to fertilisation across an exposure gradient, effect size analysis was estimated as the natural log of response ratios, $LRR = \log \left( \frac{\text{treatment}}{\text{control}} \right)$ calculated following Hedges and others (1999). The log response ratio was chosen over other methods because its high capacity to detect true effects and their robustness in small sample sizes (Lajeunesse & Forbes, 2003). Mean effect size ± CI of treatments were calculated using the R package “Metafor” (Viechtbauer, 2010) and were considered significant if the 95% CI did not overlap zero.

All statistical analyses were conducted with the statistical software R (version 3.4.3, R Development Core Team 2017, Vienna, Austria; available at: http://www.R-project.org).
Results

Wave exposure across the Banc d’Arguin and at the fertilisation sites

Relative wave energy (RWE) showed great variability across Banc d’Arguin (Fig. 3.1B) with an average ± se of $2681 ± 84 \text{ J m}^{-1}$. As expected, there was a strong north to south gradient, ranging from 23031 in the north to 0 J m$^{-1}$ at the isolated inner intertidal flats (Fig. 3.1B). The eastern-side of the island of Tidra showed a gradual south-ward decrease in wave energy (Fig. 3.1B), whereas the western side seemed to experience more heterogeneity in wave action due to the complexity of the mudflats and water bodies (Fig. 3.1A, C).

![Figure 3.1](image)

**Figure 3.1.** (A) Detailed bathymetric map of Banc d’Arguin, Mauritania, and the emerging mudflats at low tide (grey) based on Landsat 8 image. Black circles represent the landscape survey sampling stations. Abbreviations of long-term study sites: E = exposed; I = intermediate; and S = sheltered. (B) Spatial relative wave energy distribution (RWE, J m$^{-1}$) and (C) the average wave height (m) in the core intertidal flats of Banc d’Arguin, obtained by IDW interpolation (Spatial Analyst Tools, ArcGIS, based on 260 stations) of relative wave energy and wave height, respectively, simulated by wave exposure model (WEMo).
The selected sites for the fertilisation experiment had RWE’s of 3639 in the exposed site, 1629 for the intermediate, and 676 J m\(^{-1}\) for the sheltered site. Additional information on the initial conditions of these sites is summarised in Table S3.1, based on the spatial survey data (see below).

**Main environmental gradient characteristics in Banc d’Arguin**

PCA ordination revealed a subtle environmental gradient related to hydrodynamic forcing as indicated by the RWE and sediment characteristic such as median grain size (D50) and organic matter content (OM) along the first axis (Fig. 3.2) and another gradient seemingly related to tidal elevation represented by seagrass isotopic \(^{13}\)C-values (d\(^{13}\)C) and seagrass density (PCA2 in Fig. 3.2).

![Figure 3.2. Plot of the first two dimension of principal component analysis (PCA) for sediment properties, seagrass traits, and wave energy gradient. RWE = relative wave energy (J m\(^{-1}\)), D50 = sediment median grain size, B:A = seagrass belowground/aboveground biomass ratio, D to bird colonies = distance to the nearest birds’ breeding colonies or major roosting sites (m), d\(^{15}\)N= leaf nitrogen isotope, %P= leaf phosphorus content, seagrass density = leaves density (# m\(^{-2}\)), leaf length = average leaves length (mm), LA= average leaf area (cm\(^{2}\)), %N= leaf nitrogen content, %Fe= leaf iron content, % OM= sediment organic matter content and d\(^{13}\)C= leaf carbon isotope. Variables with heads outside of the grey equilibrium circle significantly contribute to the ordination diagram (Legendre & Legendre, 1998).](image-url)
All variables extend beyond the circle of equilibrium contribution, revealing that they all contributed significantly to the PCA (Fig. 3.2). Along the first axis (39%), RWE showed a strong positive correlation with both sediment grain size and DW below-to-aboveground ratio. These variables showed strong inverse correlation with sediment OM, leaf %N, leaf %P, leaf %Fe, leaf length and LA. The second PCA axis (13%) was associated to a gradient defined by seagrass density and isotopic values having a denser seagrass beds at one end and a more sparse/ algal dominated at the other (Fig. 3.2). Seagrass density correlated positively with leaf $\delta^{15}$N and distance to birds’ breeding colonies, and negatively with leaf $\delta^{13}$C (Fig. 3.2). The first two axes of the PCA accounted for 53% of the total variation in sediment and seagrass properties at the landscape scale.

Spatial variability across the wave-exposure gradient

Seagrass %P content decreased as RWE increased ($r^2 = 0.25, P < 0.001$, Fig. S3.1A). The northern part of the study area, situated north of the island of Tidra, had on average 0.18 ± 0.02 mg P g$^{-1}$ while the inner southern intertidal flats had 0.25 ± 0.03 mg P g$^{-1}$ (Fig. 3.3A). Leaf %N content also decreased as RWE increased ($r^2 = 0.37, P < 0.001$, Fig. S3.1B). On average (± se) the northern part of the study area had 1.5 ± 0.2 mg N g$^{-1}$ while the inner intertidal flats had 2.08 ± 0.3 mg N g$^{-1}$. The western part of the study site, situated behind the island of Tidra, had relatively high %N compared with sites on eastern side of Tidra (Fig. 3.3B). N:P ratios were very low and ranged from 3.7 to 16.4% (with an average of 8.7% ± 2.19) (Fig. 3.3C), indicating that the system is more N-limited than P-limited. Seagrass $\delta^{15}$N values showed more complex and patchy spatial patterns, with different pockets of high values spreading across the study area without any clear pattern along the wave energy gradient (Fig. 3.3D). Values of $\delta^{15}$N ranged from 0.02 to 5.73 (2.4 ± 0.9), suggesting the existence of multiple sources of N from different trophic levels. Sediment grain size and OM content were spatially decoupled, with coarser sediments containing less organic matter (Fig. 3.3E, F). Sediment OM decreased with
increasing RWE ($r^2 = 0.25$, $P < 0.001$, Fig. S3.1C), while grain size increased as REW increased ($r^2 = 0.31$, $P < 0.001$, Fig. S3.1D).

**Figure 3.3.** Spatial patterns of averaged seagrass stoichiometry and sediment condition across the Banc d’Arguin, Mauritania, using a gridding interpolation (Kriging technique in Spatial Analyst Tools, ArcGIS) based on data collected from 160 stations across its intertidal flats (see Fig. 3.1). (A) = Seagrass % Phosphorus (P) distribution with darker areas indicates higher P concentrations; (B) = leaf %Nitrogen (N); (C) = leaf N:P ratio; (D) = Seagrass $\delta^{15}$N values; (E) = Sediment median grain size (D50); and (F) = Sediment organic matter content (OM).
Seasonal variability along the wave-exposure gradient

Leaf macronutrient (N & P) contents were generally higher in winter than in spring, and the largest seasonal contrast was found in the most sheltered site with a strong peak in winter and a dip in spring (Table 3.1; Fig. 3.4A, B). Micronutrient (Fe & Al) leaf concentrations, on the other hand, were generally higher in spring compared to winter, and thus showed a strong opposite trend to macronutrients between seasons (Table 3.1; Fig. 3.4C, D).

Table 3.1. Results of the analysis of variance (two-way ANOVA) of the mean effects of site and season and their interactions on the morphology and stoichiometric of seagrass and its associated epiphytes. Bold characters highlight the statistical significant. B/A represents seagrass belowground/aboveground biomass. Significant difference levels, ns = P >0.05, *= P <0.05, ** = P <0.01, and *** = P <0.001.

| Source of variations | Season | Site | Season:site |
|----------------------|--------|------|-------------|
|                      | df | MS | F | df | MS | F | df | MS | F |
| Leaf total N (%)     | 1  | 2.7 | 28.6 *** | 2  | 1.82 | 19.2 *** | 2  | 0.94 | 9.9 *** |
| Leaf total P (%)     | 1  | 0.01 | 12.7 *** | 2  | 0.005 | 3.81 ** | 2  | 0.004 | 3.63 * |
| Leaf total Fe (%)    | 1  | 13.6 | 91.05 *** | 2  | 2.65 | 17.8 *** | 2  | 0.9 | 6 ** |
| Leaf total Al (%)    | 1  | 7.8 | 41.2 *** | 2  | 2.6 | 13.8 *** | 2  | 1.1 | 5.7 ** |
| Seagrass cover       | 1  | 0.06 | 0.3 ns | 2  | 1.8 | 10.1 *** | 2  | 0.4 | 3.3 ns |
| Vaucheria cover      | 1  | 0.45 | 9.4 ** | 2  | 1.14 | 24.1 *** | 2  | 0.15 | 3.15 * |
| B/A biomass ratio    | 1  | 42.1 | 57.9 *** | 2  | 4.5 | 6.17 ** | 2  | 1.82 | 2.5 ns |
| Diatom biomass       | 1  | 1.62 | 3.8 ns | 2  | 4.7 | 10.9 *** | 2  | 1.4 | 3.1 ns |
| Leaf δ^{13}C         | 1  | 0.82 | 1.8 ns | 2  | 5.9 | 12.9 *** | 2  | 1.4 | 2.9 * |
| Leaf δ^{15}N         | 1  | 0.24 | 3.1 * | 2  | 1.6 | 1.8 ns | 2  | 1.1 | 3.7 * |

Seagrass cover and stability (i.e., smaller variability in cover) increased with wave-exposure (Table 3.1; Fig. 3.4E). Although seagrass cover did not differ significantly between seasons, there was a marked increase in spring in the exposed and intermediate sites and a decrease in the sheltered site (Fig. 3.4E). Macro-algae *Vaucheria* cover, on the other hand, differed significantly between seasons, with an increase in winter compared to spring across the sheltered and intermediate sites (Table 3.1; Fig. 3.4F). During our survey *Vaucheria* was never found at the exposed site. Seagrass below-to-aboveground biomass ratios were significantly lower in
spring than in winter across sites, and the difference between the seasons seems to increase from the more exposed site to the more sheltered one (Table 3.1; Fig. 3.4G).

**Figure 3.4.** Bar-plots of the seasonal variations in seagrass (A) leaf %Nitrogen, (B) leaf %Phosphorus, (C) % leaf Fe, (D) % leaf Al, (E) seagrass cover, (F) *Vaucheria* cover, (G) belowground/aboveground biomass, and (H) associated epiphytic diatoms in the different hydrodynamic regime sites (Exp = exposed, Inter = intermediate, Shelt = sheltered). Different colours indicate different sampling period (winter vs. spring). All bars show mean ± SE; significant differences between seasons as well as sites are depicted by different lower-case letters (2-way ANOVA followed by post hoc Tukey tests, where $P < 0.05$ is significant).
Diatom biomasses showed a marginally significant seasonal trend in the most exposed site, with a decrease in biomass over winter compared to spring (Table 3.1; Fig. 3.4H). Leaf $\delta^{13}$C and $\delta^{15}$N signatures demonstrated a unique temporal pattern per site (Fig. 3.5). Nitrogen isotopes only significantly differed between seasons at the exposed site, suggesting the existence of a seasonal change in the source of N to this area (Table 3.1; Fig. 3.5). While carbon isotope signatures differed only in the intermediate site, indicating a seasonal shift in inorganic carbon sources (Table 3.1; Fig. 3.5). The two isotope signatures did not differ significantly in the most sheltered site (Table 3.1; Fig. 3.5). The exposed and sheltered sites had significantly different $\delta^{13}$C values throughout the survey (Table 3.1; Fig. 3.5), indicating that the two sites use different sources of inorganic carbon.

**Figure 3.5.** Seasonal variation in stable carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotopic values of *Zostera noltii* leaves sampled in sheltered (triangles), intermediate (squares), and exposed (circles) seagrass beds in Banc d’Arguin, Mauritania. Open symbols represent spring samples, while the filled symbols represent the winter ones. Values are means ± SE.

*Responses of seagrass to fertilisation along the wave-exposure gradient*

Overall, the magnitude responses of seagrass to nutrient treatments (+N, +P, & +N+P) differed per exposure site (Fig. 3.6). Seagrass cover showed a distinct
response to N additions (+N & +N+P) in the exposed site, where seagrass died in response to these treatments (Fig. 3.6A). In the sheltered site, however, it was +P that significantly decreased seagrass cover (Fig. 3.6A). The seagrass cover of the intermediate site was not significantly affected by any treatment, although +P led to a marginal increase in cover (Fig. 3.6A). Seagrass above- and belowground biomasses were significantly decreased by N additions (+N and +NP) in the exposed and intermediate sites while the treatments did not seem to affect the biomass of the sheltered site (Fig. 3.6B, C).

**Figure 3.6.** Responses of (A) seagrass cover, (B) aboveground biomass, (C) belowground biomass, and (D) diatom densities to nutrient addition by nitrogen (N), phosphorus (P), and both (NP) in three different sites (Exp = exposed, Interm = intermediate, Shelt = sheltered) representing different wave energy forces. The bars represent mean effect sizes (log response ratios, LRR) with 95% CI. The zero line indicates no effect, and the significance of mean effects is determined when the 95% confidence interval does not overlap zero.
Diatom biomass significantly increased in all nutrient treatments in the exposed site (Fig. 3.6D). Diatom biomass in the intermediate site was boosted only by +P, while only +N increased their biomass in the sheltered site (Fig. 3.6D).

**Discussion**

Using a natural wave-exposure gradient we quantify the spatial and temporal status of seagrass stoichiometry, morphology, and stability and how the setting along this gradient affects the response of *Zostera noltii* to fertilisation. We showed that: (1) hydrodynamic gradient had strong spatial and temporal effects on seagrass nutrient-status and stability, with increasing nutrient limitation and stability with increasing wave energy; (2) the magnitude effect of fertilisation on seagrass varied tremendously across the wave-exposure, with the most exposed site being most sensitive to biomass loss due to fertilisation and the sheltered one being least sensitive.

**Environment gradient characteristics**

Seagrass leaf nutrient content (N, P, Fe, Al), leaf morphology, biomass, sediment grain size, and sediment organic matter showed a strong direct correlation with wave-energy gradient (PCA1; Fig. 3.3). The observed increase in below-to-aboveground ratio with exposure indicates that seagrass increasingly invest in anchoring strength. In areas of low water velocity and high sediment deposition seagrass seems to invest more in their light-harvesting compartment, the leaves. These results are in agreement with reported observational and experimental findings along intertidal gradients elsewhere (Marbà and Duarte 1995; Peralta and others 2000, 2006; Cabaço and Santos 2007; Hovey and others 2012; Balestri and Lardicci 2014).

Greater investment in belowground system holds two benefits for seagrass in high velocity areas: the complex rooting system will ensure seagrass persistence (e.g., against erosion and leaf detachment) and nutrient acquisition from the generally
sandy and poor sediments. Simultaneously, investing more in aboveground biomass in low wave-energy areas also provides twofold benefits: seagrasses can thus cope with sediment deposition and win the competition with the opportunistic macro-algae *Vaucheria*, characteristic of sheltered areas (Fig 3.4F). Wave-action could also affect seagrass indirectly through setting the background sediment characteristics such as silt and OM contents. These characteristics have been shown to play an important role in the stability of seagrass in Banc d’Arguin through a negative feedback loop between seagrass density, grain size and OM content (Folmer et al., 2012).

**Spatial variability along the environment gradient**

Nutrient concentrations in leaves decreased with increasing wave energy. The vast majority of the measurements of leaf N content scored way below the 1.8 %N threshold suggested by Duarte (1990) to represent N-limitation. In addition, the spatial patterns of the relative importance of N to P (N:P ratio) in limiting productivity also indicate an N rather than P limitation across the Banc d’Arguin ecosystem. N:P ratios less than 25–30 are considered to reflect N-limitation as well (Duarte, 1990; Armitage et al., 2005; Fraser et al., 2012; Burkholder et al., 2013). In fact, seagrass %N found in our study site are among the lowest reported for *Z. noltii* compared with many other systems (Pérez-Lloréns and Niell 1993; Brun and others 2002, 2003; Marbà and others 2002; Peralta and others 2005; Cabaço and others 2009; Castro and others 2009; Holmer and others 2016). The transport of the rich fine-sediment from the more exposed to the more sheltered areas, bringing an additional nutrient input to the latter one and impoverishing the exposed sites, may well explain the relative decrease in nutrient concentrations with increasing wave-action.

**Seasonal variability along the environment gradient**

In the two years of monitoring, biomass allocation, leaf isotopic signals, and leaf nutrient contents strongly varied between sites (exposed, intermediate, sheltered) as
well as with season (winter vs. spring). Biomass seemed to follow the seasonal variations in the availability of macronutrients, while seagrass cover was mainly site-dependent and seemed to follow seasonal changes in the micronutrients (Fig. 3.4). The observed increase in seagrass cover in spring at the exposed site is in agreement with the more general seasonal growth pattern of *Z. noltii* (Vermaat & Verhagen, 1996). The increase in cover in winter at the sheltered site (Fig. 3.4E) is different from areas further north, where *Z. noltii* is known to lose most of its aboveground biomass in the course of winter (Pérez-Lloréns & Niell, 1993; Pergent-Martini et al., 2005). The observed decoupled seasonal leaf macronutrient and micronutrient contents as well as the seasonal seagrass cover change (Fig. 3.4) indicate seasonal nutrient-cycle-driven phenomena, and could reflect changes in atmospheric (dust) and/or oceanographic forces (upwelling). Banc d’Arguin lies between year-round very active upwelling zone (Gabric et al., 1993), and the Sahara that is known to supply large amount of dust to adjacent systems (Goudie & Middleton, 2001). The process of energy flow from the upwelling zone to Banc d’Arguin inner intertidal system, however, lacks large scale empirical evidence and both systems seem not to exchange water masses (Sevrin-Reyssac, 1993). Nevertheless, an episodic flow of nutrient might occur during the intense storms driven from the north-west and affect the northern most exposed part of the area (Carlier et al., 2015). The episodic Saharan dust storms, on the other hand, are known to affect nutrient budgets of the recipient systems (Neuer et al., 2004). Thus, given the seasonal influence of dust and upwelling forcing on nutrient fluxes, strong temporal and seasonal changes in seagrass morphology and stoichiometry in Banc d’Arguin are expected. Our leaf isotopic signature data (Fig. 3.5), as well as trace elemental content (Fe, Al), support the idea of connectivity between Banc d’Arguin exposed flats to an external source of energy that is affecting its temporal and spatial nutrient status. Fluxes of nutrients associated with episodic dust storms and/or upwelling intense events may increase in the future, with climate change projections suggesting that the intensity and frequency of the extreme weather events such as floods, drought, and storms will increase (Easterling
et al., 2000; Jentsch et al., 2007; IPCC, 2012). Thus, predicting the response of seagrass beds to increased episodic nutrient fluxes along environmental gradient should be a research priority.

**Responses of seagrass to fertilisation along the environment gradient**

The responses of *Z. noltii* to N and P fertilisation exhibited strong site-element-specific variation: with the strongest negative response (mortality) to N and N+P addition at the most exposed site (Fig. 3.6). This is surprising as this area showed strong signals of N-limitation (leaf %N < 1.5). This means that where N-limitation is severe, sensitivity to high nutrient load is also high. In contrast, seagrass growing in the more sheltered site only responded to P addition by biomass loss. The mortality in response to N-addition at the exposed site may be due to a combination of excessive N-loads and efficiency in nutrient uptake. Excess N-loads are known to induce seagrass mortality through ammonium (NH$_4^+$) toxicity (Santamaría et al., 1994; van Katwijk et al., 1997; Brun et al., 2002; Govers et al., 2014a). Exposed plots treated with +N might have received high NH$_4^+$ levels at once due to high diffusion rate across sediment-water interface in the coarser sediment (Huettel, 2001; Burdige, 2006). Furthermore, seagrasses in high velocity conditions have high ammonium uptake efficiency owing to a reduction in the thickness of the diffusion boundary-layer of the leaves (Koch, 1994; Hurd et al., 1996; Cornelisen & Thomas, 2006). Thus, high leaves nutrient uptake efficiency and high sediment-surface exchange rate at the exposed site may have led to an excessive toxic NH$_4^+$ accumulation in the leaves (van der Heide et al., 2008).

The lack of porewater ammonium measurement regretfully restricted our ability to exactly pin down the main cause of the die-offs in the exposed site. In contrast, high P-loads do not generate toxic effects in seagrasses, nor in any other plants (Touchette & Burkholder, 2000; Marschner, 2012). Negative effects of P-fertilisation on seagrass biomass can generally be attributed to a secondary response: P-stimulated algal and epiphyte blooms that may outcompete seagrasses (Burkholder 62
et al., 2007). We indeed found an increase in diatom biomass in the exposed site (Fig. 3.6D). Surprisingly, we did not find a similar algal response in the P-fertilisation treatment in the sheltered site. Thus, the observed biomass decrease as a result of our treatment may be due to other causes that we could not identify.

The intermediate site showed inconsistency morphologic and stoichiometric responses to fertilisation suggesting the presence of a factor other than artificial fertilisation affecting the nutrient availability for seagrasses in the plots. The nature of this factor is perhaps suggested by the intermediate site being close to the island of Nair, a breeding site for hundreds of seabirds that could bring in extra nutrients to the site. Seabirds are known to supply large amount of nutrients, notably P, to the neighbouring areas (Post et al., 2008).

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Appendix 3

Table S3. 1. Initial environmental settings for the sites chosen along the wave energy gradient to conduct the fertilization experiment in Banc d’Arguin. Data are presented as mean ± SE. Significant differences between sites are depicted by different lower-case letters (one-way ANOVA followed by post hoc Tukey tests, where $P < 0.05$ is significant).

| Source of variation                          | Exposed       | Intermediate | Sheltered    | $F$  |
|---------------------------------------------|---------------|--------------|--------------|------|
| Aboveground biomass (g DW m$^{-2}$)         | 0.56$^{a}$ ± 0.08 | 0.85$^{ab}$ ± 0.1 | 1.12$^{b}$ ± 0.16 | 2.51$^*$ |
| Belowground biomass (g DW m$^{-2}$)         | 2.27$^{a}$ ± 0.22 | 1.99$^{a}$ ± 0.24 | 1.17$^{b}$ ± 0.11 | 8.10$^{***}$ |
| Leaf length (cm)                            | 10.87$^{a}$ ± 0.6 | 15.07$^{b}$ ± 0.58 | 19.19$^{a}$ ± 1.37 | 19.92$^{****}$ |
| Leaf area index                             | 2.18$^{a}$ ± 0.17 | 3.77$^{a}$ ± 0.19 | 6.43$^{a}$ ± 0.67 | 27.49$^{***}$ |
| Rhizome internode length (mm)               | 3.53$^{a}$ ± 0.42 | 5.99$^{b}$ ± 0.45 | 7.68$^{a}$ ± 0.39 | 24.53$^{***}$ |
| Leaf total N (%)                            | 1.48$^{a}$ ± 0.04 | 1.84$^{b}$ ± 0.07 | 2.02$^{b}$ ± 0.07 | 13.38$^{***}$ |
| Leaf total P (%)                            | 0.16$^{a}$ ± 0.01 | 0.18$^{ab}$ ± 0.01 | 0.20$^{b}$ ± 0.01 | 4.59$^*$ |
| Leaf total C (%)                            | 28.39$^{a}$ ± 0.49 | 26.84$^{a}$ ± 0.34 | 27.03$^{a}$ ± 0.59 | 2.27$^{ns}$ |
| Leaf C:N                                    | 20$^{a}$ ± 0.85 | 15$^{b}$ ± 0.51 | 14$^{b}$ ± 0.51 | 25.45$^{***}$ |
| Leaf total Fe (%)                           | 1.17$^{a}$ ± 0.10 | 1.48$^{a}$ ± 0.06 | 2.42$^{b}$ ± 0.17 | 31.0$^{***}$ |
| Leaf total Al (ppm %)                       | 0.09$^{a}$ ± 0.01 | 0.10$^{a}$ ± 0.00 | 0.18$^{b}$ ± 0.01 | 36.42$^{***}$ |
| Z. noltii cover (%)                         | 80$^{a}$ ± 5 | 60$^{a}$ ± 19 | 66$^{b}$ ± 8 | 1.45$^{ns}$ |
| Vaucheria cover (%)                         | 0$^{a}$ | 3$^{a}$ ± 1.12 | 24$^{b}$ ± 7 | 9.5$^{***}$ |
| Sediment % N                                | 0.23$^{a}$ ± 0.003 | 0.24$^{a}$ ± 0.04 | 0.29$^{b}$ ± 0.05 | 6.7$^{**}$ |
| Sediment % P                                | 0.04$^{a}$ ± 0.003 | 0.04$^{a}$ ± 0.004 | 0.004$^{a}$ ± 0.006 | 0.3$^{ns}$ |
| Sediment organic matter (%)                 | 8.87$^{a}$ ± 0.41 | 7.08$^{b}$ ± 0.51 | 9.14$^{a}$ ± 0.49 | 5.68$^{**}$ |
| Sediment CaCO$_3$                           | 6.24$^{a}$ ± 0.25 | 4.6$^{b}$ ± 0.28 | 5.5$^{b}$ ± 0.24 | 9.77$^{***}$ |
| Silt content (%)                            | 74$^{a}$ ± 1.39 | 66$^{b}$ ± 1.62 | 71$^{b}$ ± 1.87 | 5.87$^{**}$ |
| Redox potential (mv)                        | -53.93$^{a}$ ± 0.01 | -53.90$^{b}$ ± 0.02 | -53.84$^{a}$ ± 0.03 | 4.5$^{*}$ |
| Relative wave energy (j m$^{-1}$)           | 3639 | 1629 | 676 |
| Near-bed shear stress (Kg m$^{-1}$ s$^{-2}$) | 0.071 | 0.17 | 0.19 |
| Water temperature (°C)                      | 23.81 ± 1.57 | 24.79 ± 3 | 25 ± 2.74 |
| Tidal elevation (m)                         | 1 ± 0.58 | 1.26 ± 0.52 | 1.24 ± 0.59 |

Notes: Data are presented as mean ± 1 se. Significant difference levels,ns$P > 0.05$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. 

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Figure S3. 1. Linear regression relationships between relative wave energy and (A) leaf total P (%), (B) leaf total N (%), (C) sediment organic matter content (%), and (D) sediment median grain size from samples collected at the landscape-scale in Banc d’Arguin, Mauritania. Fitted regression lines, $r^2$ values, and model equations are given for $P$-values below the 5% significance level.