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Extreme ^{15}N Depletion in Seagrasses

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Abstract Seagrass beds form an important part of the coastal ecosystem in many parts of the world but are very sensitive to anthropogenic nutrient increases. In the last decades, stable isotopes have been used as tracers of anthropogenic nutrient sources and to distinguish these impacts from natural environmental change, as well as in the identification of food sources in isotopic food web reconstruction. Thus, it is important to establish the extent of natural variations on the stable isotope composition of seagrass, validating their ability to act as both tracers of nutrients and food sources. Around the world, depending on the seagrass species and ecosystem, values of seagrass N normally vary from 0 to 8‰ δ^{15}N. In this study, highly unusual seagrass N isotope values were observed on the east coast of Qatar, with significant spatial variation over a scale of a few metres, and with δ^{15}N values ranging from +2.95 to −12.39‰ within a single bay during March 2012. This pattern of variation was consistent over a period of a year although there was a seasonal effect on the seagrass δ^{15}N values. Seagrass, water column and sediment nutrient profiles were not correlated with seagrass δ^{15}N values and neither were longer-term indicators of nutrient limitation such as seagrass biomass and height. Sediment δ^{15}N values were correlated with *Halodule uninervis* δ^{15}N values and this, together with the small spatial scale of variation, suggest that localised sediment processes may be responsible for the extreme isotopic values. Consistent differences in sediment to plant ^{15}N discrimination between seagrass species also suggest that species-specific nutrient uptake mechanisms contribute to the observed δ^{15}N values. This study reports some of the most extreme, negative δ^{15}N values ever noted for seagrass (as low as −12.4‰) and some of the most highly spatially variable (values varied over 15.4‰ in a relatively small area of only 655 ha). These results are widely relevant, as they demonstrate the need for adequate spatial and temporal sampling when working with N stable isotopes to identify food sources in food web studies or as tracers of anthropogenic nutrients.

Keywords Nutrients · Eutrophication · Seagrass health · Seagrass biomass · Redfield ratio · Stable isotopes

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Introduction

Although only 0.1 to 0.2 % of the ocean seabed is occupied by seagrass, it forms a highly productive ecosystem that provides a wide variety of very important functions, such as: protecting shorelines against erosion; trapping and cycling nutrients; providing food for coastal food webs and, creating habitats for an extensive range of wildlife, including commercially important species of fish and crustaceans (Thresher et al., 1992;

Loneragan et al., 1998; Nagelkerken, 2009; Kennedy et al., 2010). The sensitivity of seagrass species to environmental conditions means they can be used as indicators of ecological processes. For example, elemental stoichiometry and spatial patterns in elemental content of phytoplankton and higher plants (Redfield ratio) have been used to investigate their nutrient status and this approach can also be applied in seagrass (Duarte, 1990) to generate hypotheses about the nature of spatial and temporal variation in ecosystem processes (Fourqurean et al. 1992; Fourqurean and Zieman 2002). Stable isotopes are also being increasingly used as tools to investigate ecosystem processes, such as determining sediment source, nutrient cycling and carbon limitation, as well as their use as indicators of environmental threats to, and stresses on, seagrass ecosystems (Barrón et al., 2004; Kennedy et al., 2004, 2005; Papadimitriou et al., 2005a, b, 2006; Frederiksen et al., 2006; Walton et al., 2014). Research investigating natural and anthropogenic environmental threats has focused on establishing the extent of natural variations in the elemental (C, N, S) and stable isotope composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) of seagrass, validating its ability to act as a tracer of anthropogenic nutrient sources and in determining the limits of our ability to recognise anthropogenic impacts from natural environmental change (e.g. Udy et al., 1999; Papadimitriou et al., 2006). However, the success of this type of approach is strongly dependent on understanding the underlying processes that affect stable isotope values. The interpretation of stable isotope values is often limited by a lack of knowledge with regard to either the abiotic/biotic factors that define the incorporation of stable isotopes into their biogeochemical repositories or the range of biogeochemical processes that can subsequently alter any isotopic signature.

Seagrasses are able to absorb nutrients from the water column through their leaves and from the sediments through their roots (Lepoint et al., 2004), and so, the $\delta^{15}N$ value of seagrass is influenced by the $\delta^{15}N$ values of these sources; hence, their use in monitoring environmental change. Although plants are generally more depleted in ^{15}N than the nutrient source, the discrimination against the heavier ^{15}N isotope and preferential incorporation of ^{14}N in plant tissue can only occur when the supply of N is in excess of the plant's demand (Evans, 2001). This can lead to seasonal variation, with more positive $\delta^{15}N$ values in the summer when growth increases demand for N, becoming more negative in the winter (Fourqurean et al., 2005). Seagrass growth requires a range of elements, but N and P are the nutrients that commonly restrict growth (Duarte, 1990; Lee et al., 2007). P limitation increases the supply of N relative to demand providing greater opportunity for ^{15}N discrimination, and also affects seagrass growth, biomass and size (Udy and Dennison, 1997; Lee et al., 2007). Conversely, increased P supply can boost canopy height and biomass while reducing opportunity for ^{15}N discrimination (Udy and Dennison, 1997). Positive $\delta^{15}N$ values can also

result from sewage inputs, fertiliser runoff, atmospheric deposition (Yamamuro et al., 2003) and denitrification (Cline and Kaplan, 1975). Values around 0‰ occur as a result of N fixation (Yamamuro et al., 2003). Negative $\delta^{15}N$ values can occur as a result of remineralisation of organic matter that can produce sediments with a $\delta^{15}N$ value of ~−2‰ (Möbius, 2013) and nitrification that can lead to porewater nitrates with −5‰ $\delta^{15}N$ values (Yu et al., 2015). Even greater depletion in ^{15}N has been observed in plants with mycorrhizal associations, leading to values of −8 to −11‰ $\delta^{15}N$ (Hobbie et al., 2005), and in marine phytoplankton and bacteria (Liu et al., 2013) and terrestrial plants (Yoneyama et al., 1991) that utilise ammonium as a nitrogen source.

As part of a recent meta-analysis of the N isotopic values of seagrass species from around the world, Christiaen et al. (2014) reported $\delta^{15}N$ values ranged from +17.8 to −3.2‰, with temperate genera, including *Zostera*, *Posidonia* and *Ruppia*, being more enriched in ^{15}N, whereas tropical genera were impoverished (*Halodule* spp., *Halophila* spp. and *Syringodium* spp. being amongst the most depleted). Recently, in a discussion paper, Fourqurean et al. (2015) reported that the global range of $\delta^{15}N$ values has increased greatly with the discovery of seagrass beds in Bermuda that had $\delta^{15}N$ values that ranged from 8.8 to −10.6‰. However, mean values (±1 SD) were all positive (e.g. *Thalassia testudinum* 2.2±3.7‰, *Halodule* sp. 1.6±3.5‰) and much more negative means with similar variability were previously reported from *Halodule uninervis* beds in two bays on the east coast of Qatar −3.9±3.2‰ ($n = 129$) and Al Dhakira Bay −5.2±2.1‰ ($n = 3$) (Walton et al., 2014), suggesting that these extreme negative values are more common than previously thought. However, the cause for these depleted ^{15}N values remains unexplained.

Extensive seagrass meadows have been reported along the Qatar coast, with greatest abundances at water depths of between 3 and 8 m, but extending down to around 20 m below sea level (Sheppard et al., 1992). The extreme environment of the gulf (large seasonal variation in seawater temperatures (18 to 32 °C) and high salinity (36 and 45 ppt) on the east coast (Al-Maslamani et al., 2007), but with more extreme values off the south east and west coasts) exceeds the limits of most seagrass species. Only three species are found in the waters around Qatar: *H. uninervis* is most widely distributed (Phillips, 2003); less common is *Halophila stipulacea*; and least frequent is *Halophila ovalis* (Erftemeijer and Shuail, 2012). Although these three seagrass species may be well-adapted to these environmental conditions, they are likely near the threshold of their tolerance.

The current study examines the spatial and temporal variation in the $\delta^{15}N$ values of seagrasses, and investigates the likely factors that cause the variation. Systems associated with tropical carbonate sediments are often P limited, as phosphate is bound to the sediments making it less bioavailable (Short,

1987). Hence, the potential effect of nutrient limitation on seagrass δ^{15}N values is examined in detail in this study. As nutrient analysis of seagrass may not reveal longer-term nutrient limitation (Udy and Dennison, 1997), the current study also surveyed the differences in plant characteristics (biomass, plant height, leaf width, leaves per shoot and percentage cover) and compared them with other regions. Finally, this study investigates the biogeochemical factors (sediment and water column nutrients, sediment δ^{15}N values, sediment depth and sediment composition) as possible influences or causes of the highly variable and unusually depleted δ^{15}N values in the seagrass leaves, reported previously (Al-Maslamani et al., 2012; Walton et al., 2014).

Methods

Site

This study was carried out in Al Khor bay, Qatar (lat/long: 25.691, 51.523). The bay is bounded to the west by Al Khor city, to the east by mangrove forest, and with a narrow opening to the sea in the south (Fig. 1). The bay itself contains 420 ha of seagrass, mostly *H. uninervis*, with some *H. stipulacea* and much less *H. ovalis* (Al-Maslamani et al., 2012, 2013; Walton et al., 2014). There is limited freshwater input from an annual rainfall of 75 mm (UN, 1997). Maximal tidal range is 1.6 m and average daily air temperature ranges between 10 and 45 °C, resulting in coastal sea temperatures of 17 to 32 °C and salinities of 41 to 44 ppt (Al-Maslamani, 2006).

Sample Collection and Preparation

Isotopic Variation in Seagrass

Large-Scale Spatial and Seasonal Variation in Isotopic δ^{15}N Values in Seagrass Three seasonal surveys of seagrass in Al Khor Bay were conducted in June 2011 (peak growth period), October 2011 (end of growth) and March 2012 (beginning of growth) to determine the consistency of the depleted ^{15}N isotopic values both spatially and temporally. Spatial variation over the three seasonal surveys was investigated by sampling over a large-scale grid of 37 stations evenly spread across the bay, approximately 500 m apart (Fig. 1).

During each survey, samples of all seagrass species present (*H. uninervis*, *H. stipulacea* and *H. ovalis*) were collected at each station by hand and chilled on site before being transferred to the laboratories at the Environmental Science Center, Qatar University. In the laboratory, seagrass leaves (c. 10 leaves per sample) were scraped with a scalpel to remove epiphytes and washed in distilled water before being dried at 60 °C for 48 h.

Small-Scale Spatial Variation in Isotopic δ^{15}N Values in Seagrass In October 2011, to investigate small-scale spatial variation in isotopic values of seagrass, two grids of stations spaced 50 m apart (with a few additional stations separated by only 10 m), were overlaid on areas with a previously observed wide ranges of δ^{15}N values. A 5×5 station grid was sampled in the eastern end of the bay and a 3×9 station grid at the western end (see Figs. 1 and 5). At all stations, seagrass was sampled as previously described. In March, only the eastern small-grid stations were resampled to examine consistency of the negative δ^{15}N values.

Isotopic Partitioning Within and Between Species In March 2012, to investigate differences in isotopic discrimination between different parts of the three seagrass species, whole plants of each species were hand dug from the sediment and gently washed in seawater, before being placed on ice. In the laboratory, sand grains were hand-picked from the roots and rhizomes and leaves scraped, then washed in distilled water and separated into leaves, rhizomes and roots, before being dried as described previously.

Fig. 1 Location of sampling points for seagrass and sediments. Small-scale grid stations were sampled in October 2011; large spatial scale grid stations were sampled seasonally
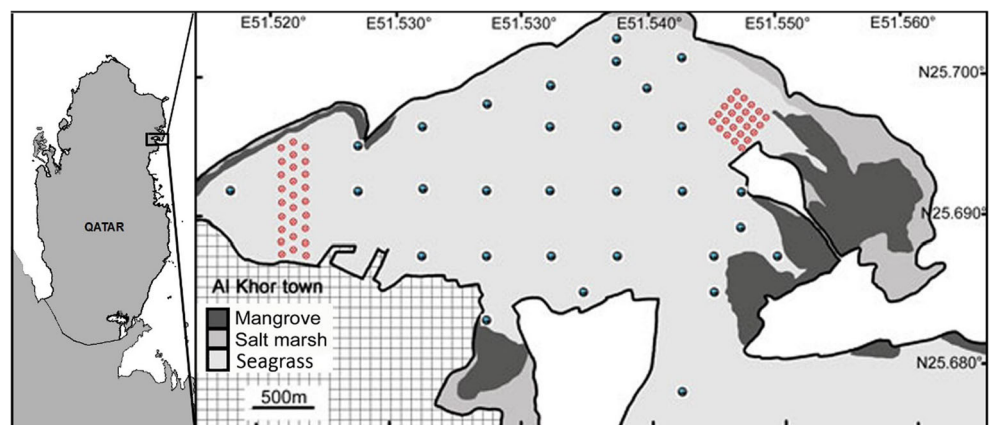
Plant Characteristics

Seagrass percentage cover was measured in situ per species (the sum for all species being the total seagrass cover) for all sites in the whole-bay sampling grid in October 2011 (Fig. 1). Seagrass cover and species composition was determined from photographs taken by underwater digital camera of 1×1 m quadrats, each divided into four equal sections of 25×25 cm.

Seagrass biomass cores were taken in October 2011 from the whole-bay large-scale sampling grid ($n = 37$) with a perspex corer (10-cm diameter) to as great a depth as possible. At each sample site, three cores were taken, separated by at least 2 m. Cores were wrapped in aluminium foil on ice until arrival at the laboratory where they were stored frozen until processed. Above-ground material was removed by slicing through the top of the core using a sharp knife. Above-ground living seagrass tissue, was measured to obtain plant height, a ~10 % sub-sample was then used for estimating leaves per shoot and leaf width, before being returned to the sample and then washed, dried and weighed. Below-ground material was separated into five 2-cm-deep sections and a final section (if present) of greater than 10-cm depth. Each section was then washed onto a fine sieve and living seagrass tissue was separated by floatation and rinsing through sieves. All obtained material was dried and weighed separately for each depth section.

Investigation of Environmental Factors Possibly Influencing Seagrass Isotopic Values

Water Column Sub-surface sea water samples in October and March were collected in 1-l acid-washed flasks, particulate organic matter (POM) removed by vacuum filtering through GF/F filters and filtrate preserved by freezing until analysis. The filters were acidified following Kennedy et al. (2004) and analysed for stable isotopes as described earlier. The filtrate was used for seawater nutrients (phosphate, ammonia, nitrite and nitrate) and quantified using a continuous flow nutrient analyser (Skalar). Temperature and salinity were also recorded in situ.

Sediment In June, samples of sediment were taken at each station using a cut-off 60-ml syringe pushed into the sediment up to the 50-ml mark. Water was removed from the surface of the sample by pushing up from the bottom of the syringe with a plunger. The sample was then placed on ice and frozen upon returning to the laboratory, before being prepared for isotopic analysis. Sediment depth was also measured at three points chosen haphazardly for each station and at least 2 m apart using a graduated 1.4-m-long metal pole c. 1 cm in diameter driven down into the substrate until it would go no further (using body weight to force it down). Sediment depth was measured to the nearest 1 cm until sediment depth exceeded 1.3 m.

For the October and March sampling, two extra sediment samples were obtained for porewater analysis from the whole-bay large-scale sampling grid ($n = 37$) in the same way as for isotopic analysis described earlier. In the laboratory, porewater was extracted through centrifuging, filtered and frozen until analysis.

Sediment particle size was determined using a Malvern Mastersizer laser diffraction particle analyser. Adsorbed (or available) phosphorus that is surface bound or carbonate-associated in sediments was determined by inductively coupled plasma mass spectrometry (ICP-MS) following extraction in 0.11 M acetic acid, following methods described by Spivakov et al. (1999). Sediment porewater nutrients (phosphate, ammonia, nitrite and nitrate) were quantified using a discrete analyser (Easychem).

Seagrass Nutrient Profile In October and March, extra samples of seagrass were collected at each station in both large- and small-scale grids and processed as for isotopic analysis described in the following section, before being frozen for subsequent nutrient analysis. Total phosphorus in plant tissue was determined by ICP-MS following extraction with 1.0 M hydrochloric acid, and nitrogen and carbon content was obtained from the stable isotope analysis.

Isotopic Analysis

Prior to stable isotope analysis, sediment samples were sequentially acidified with 0.1 M HCl and oven dried repeatedly until addition of HCl resulted in no bubbling, indicating all carbonates had been removed (Kennedy et al., 2004). Plant material was collected and treated as described previously. All samples were oven dried for 48 h before being homogenised and weighed into tin cups (Elemental Microanalysis Ltd.) and subsequently analysed for ^{13}C and ^{15}N isotopes using a PDZ Europa ANCA-GSL elemental analyser linked to a PDZ Europa 20:20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Data Analysis

Multiple correlation analysis was used to determine which variables were correlated, and only those that exhibited a significant relationship were analysed further. Data assumptions such as normality and homogeneity of variance were tested for prior to further analysis by regression or analysis of variance using Minitab 14. Data is presented as mean ± standard deviation. The correlations between measured plant health and environmental variables on the δ^{15}N values of seagrass were the first to be assessed. Secondly, the nutrients and nutrient ratios in seagrass, sediment and water were assessed for nutrient limitation based on deviations from the Redfield ratio. The

C/N ratios vs N and C/P ratios vs P were compared, as well as the relationships between plant and sediment δ^{15}N values and plant C/N ratios, C/P ratios and N/P ratios.

Results

Isotopic Variation in Seagrass

Large Spatial Scale and Seasonal Variations in Isotopic δ15N Values in Seagrass

The initial survey of the seagrass beds in June 2011 indicated high spatial variability in *H. uninervis* δ^{15}N values across the bay (+3.6 to −9.9‰), which was consistent across the subsequent seasonal samplings in October 2011 (+3.3 to −9.2‰) and March 2012 (+3.0 to −9.2‰, Fig. 2). Spatially, values, over all three seagrass species ranged from slightly enriched stations close to Al Khor City (+3.7‰) to highly depleted values (−10.3‰) at some stations at the eastern end of the bay. Analysis of variance of the *H. uninervis* leaf δ^{15}N values between seasons at each station in the large-scale grid suggested that there were significant differences ($F = 5.31$, $p = 0.007$) and a Tukey's test indicated that seagrass samples from stations in October (δ^{15}N $= -4.08 \pm 3.17$‰) were significantly more depleted than those from June (−1.86 ± 1.74‰) (ANOVA, $p < 0.05$) but not different from March values (−3.50 ± 2.80‰).

The temporal consistency of the spatial distribution of δ^{15}N values was tested by regression of values observed at each station between seasons (Figs. 3 and 4). The relationship between the months was in both cases linear, but did not fall on a 1:1 line between site and season. The slope of the regression line of June and October values is close to 1 (0.97) indicating that there was consistency in the spatial pattern of δ^{15}N values between these months, with intercept indicating the mean (±1 SD) depletion between June and October at each station being −2.5 ± 2.3‰. Comparing between October and March, the slope of the linear relationship (0.6) indicates that those stations with lower δ^{15}N values in October exhibited a further depletion over the winter, while those with higher δ^{15}N values exhibited little or no depletion or enrichment.

Small-Scale Spatial Variation in Isotopic δ15N Values in Seagrass

The range in δ^{15}N values of seagrass in the small-scale grids overlapped with that observed in the large-scale grid (west grid +3.7 to −5.5‰ and east grid −1.9 to −10.7‰). Variance in the October δ^{15}N (‰) values for *H. uninervis* leaves within the large-scale and each of the small-scale grids was homogenous (Bartlett's test statistic = 4.24, $p = 0.12$), indicating that there was no significant difference in the variability in δ^{15}N (‰) values over both large- (500-m interval) and small-scale (50- to 10-m intervals) sampling grids (Fig. 5). However, analysis of variance indicated there were significant differences in mean values between small-scale grids ($F = 23.3$, $p < 0.001$). Tukey's pairwise comparisons suggested that the west grid had significantly less-depleted values (mean δ^{15}N ± SD = −1.9 ± 1.7‰) than the east grid (mean δ^{15}N ± SD = −6.5 ± 4.7‰). In March, only a few leaf samples were obtained from the small grids due to the winter leaf fall and δ^{15}N values ranged from −2.5 to −12.4‰ ($n = 7$).

#### Inter- and Intra-Species Variability in δ15N in Seagrasses

Differences were found between the isotopic signature of leaves, roots and rhizomes of the three seagrass species. In March 2012 a significant correlation ($p < 0.05$) was seen in δ^{15}N values of *H. uninervis* leaves, rhizomes and roots, that deviated from a 1:1 relationship, and regression analysis suggested that as both leaf and rhizome became more depleted in ^{15}N the isotopic values were similar at −9‰, with the rhizome becoming more consistently depleted than the leaf when δ^{15}N values increase (Fig. 6). This was indicative of some isotopic partitioning between different parts of the plant but only when δ^{15}N values are high. Only six root samples were of sufficient weight to provide accurate isotope analysis, preventing regression analysis. However, no significant difference in δ^{15}N values ($p \geq 0.05$) was observed between roots and leaves or rhizomes.

The co-occurrence of both *H. uninervis* and *H. stipulacea* at a number of stations permitted direct comparison of leaf δ^{15}N values in the two species (Fig. 7). In June ($F = 7.12$, $p = 0.024$) and October ($F = 15.61$, $p = 0.002$), there was a significant linear relationship, between leaf δ^{15}N values of the two species. At each station, *H. uninervis* was depleted in ^{15}N compared to *H. stipulacea* by, on average, 2.5 ± 1.4 and 5.5 ± 1.8‰ during June and October, respectively. Comparisons with values from March and with *H. ovalis* were not possible due to the small number of co-occurring samples.

Seagrass Characteristics, Biomass and Distribution

A detailed study of seagrass biomass, leaf length, shoot number and cover was performed in October 2011. *H. uninervis* was the dominant seagrass present at almost all stations and at greater densities than *H. stipulacea* or *H. ovalis*. Greatest seagrass coverage between 65 and 95 % was noted in the deepest parts of the bay, with sparse seagrass in shallower areas and biomass varying from 5 to 900 g DW m^{-2} (mean ± 1 SD = 198 ± 229) (Supplement 1). Canopy height for *H. uninervis* varied from 3 cm in some marginal areas to 10.4 cm in the central areas, with the number of leaves per shoot following a similar pattern to that of canopy height and

Fig. 2 Variation in *Halodule uninervis* leaf δ^{15}N values (‰) in Al Khor Bay at stations visited during June and October 2011 and March 2012. *Black bubbles* are positive and *white bubbles* are negative. The *size of the bubble* represents the distance of numerical value from zero
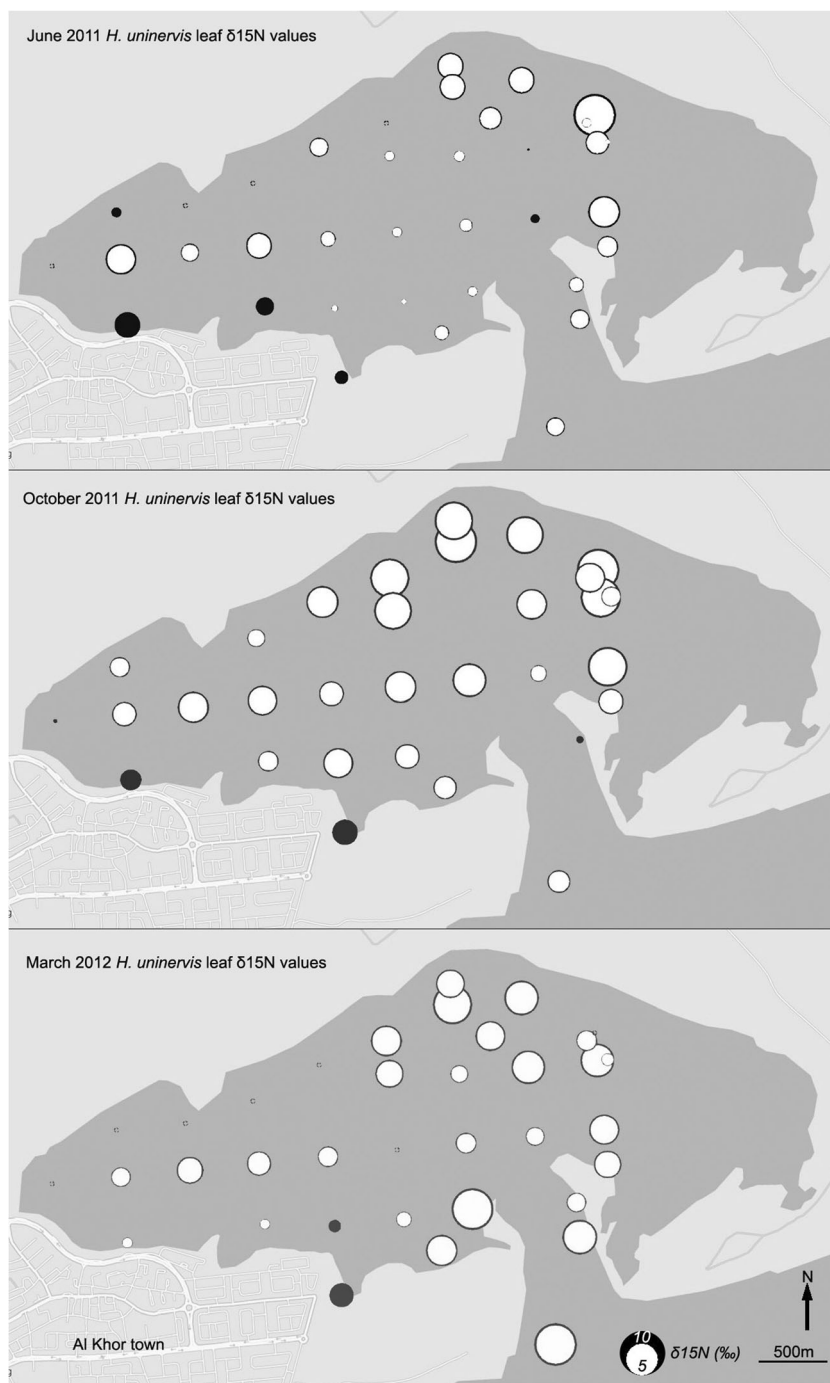

varied between 2.3 and 6.6 leaves per shoot. *H. uninervis* leaf width varied from 0.05 to 0.11 cm with a mean of 0.5 cm. *H. stipulacea* is mostly only present in the deeper western end of the bay where it has three to six leaves per shoot and with a canopy height of 3.1 to 4.6 cm (see Supplement 2 for more detail). Although not captured in the biomass sampling, there was a seasonal loss of leaves during the winter months, especially in the shallow and intertidal areas. None of the seagrass characteristics correlated with leaf δ^{15}N values.

Investigation of Environmental Factors Possibly Influencing Seagrass Isotopic Values

Sediments and Water Column

Sediment depth averaged 61 cm and ranged from 3.3 to >140 cm, with the shallowest sediments closest to the shore and deeper sediments in the deeper central bay area. The mean sediment composition was 62:35:3 sand/silt/clay. Neither

Fig. 3 Comparison of δ15N values for *Halodule uninervis* leaves at each station on the large-scale spatial grid between June and October
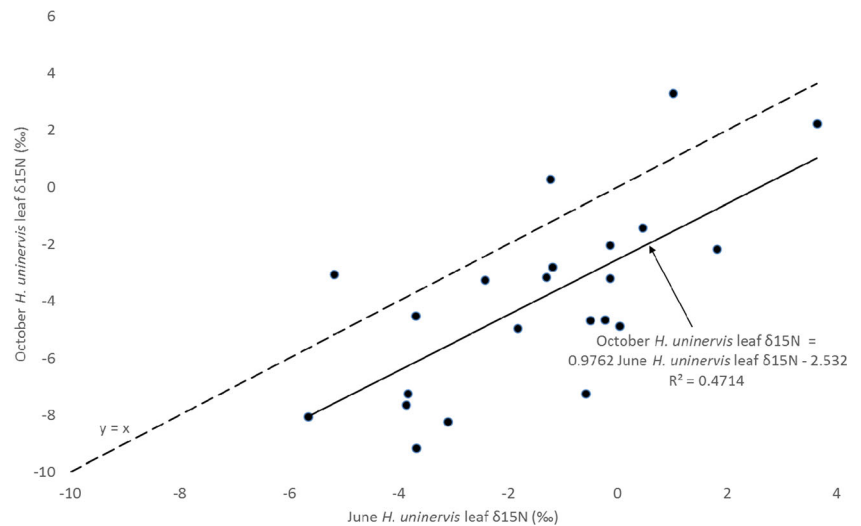
sediment depth nor composition correlated with sediment or seagrass $\delta^{15}N$ values.

The $\delta^{15}N$ of suspended POM was enriched, and showed neither significant seasonal variation ($t = -0.31$, $p = 0.9$) (October 3.1 ± 0.5 ‰, March 3.1 ± 0.9 ‰) nor consistent spatial variation. The $\delta^{15}N$ of sediment organic matter was also enriched, but variable; ranging from 0.13 to 4.8 ‰ over the study period. Data from October (0.13–4.8 ‰) and June (0.7–3.3 ‰) sampling dates were highly correlated ($F = 7.26$, $p < 0.001$), but neither dataset was correlated with March (0.5–1.9 ‰). The average increase in $\delta^{15}N$ values of 0.22 ‰ at each station indicated that October sediments were enriched in ^{15}N compared with June, although these differences in $\delta^{15}N$ values was not found to be significant ($p > 0.05$).

In June and October, regression analysis indicated a weak ($r^2 = 39.7$ % and $r^2 = 33.8$ %, respectively) but significant relationship between $\delta^{15}N$ values of *H. uninervis* leaves and those of sediments (June: $F = 18.63$, $p < 0.001$ and October: $F = 28.08$, $p < 0.001$). The average ^{15}N depletion of *H. uninervis* compared

to sediment at each station was 3.1 ± 2.2 and 5.2 ± 2.7 ‰ in June and October, respectively (Fig. 8). However, by March, the leaves were depleted by an average of -4.6 ± 2.3 ‰ and there was no significant correlation between $\delta^{15}N$ values of *H. uninervis* leaves and sediments. In October, in the western small-scale grid, discrimination between sediment and *H. uninervis* leaves was 3.9 ± 1.4 ‰ but was much greater in the eastern small-scale grid (7.4 ± 3.5 ‰). In no cases were there significant correlations between $\delta^{15}N$ values of either *H. stipulacea* or *H. ovalis* and those of sediments.

Nutrient Content of Seawater, Sediment, Porewater and Seagrass

Water column total N was low (0.7 to 7.5 µM), commensurate with the oligotrophic setting of the study site. Porewater nutrients showed no spatial patterns, but appeared to be seasonally reduced in March with the N/P ratio indicating that N

Fig. 4 Comparison of $\delta^{15}N$ values for *Halodule uninervis* leaves at each station on the large-scale spatial grid between March and October
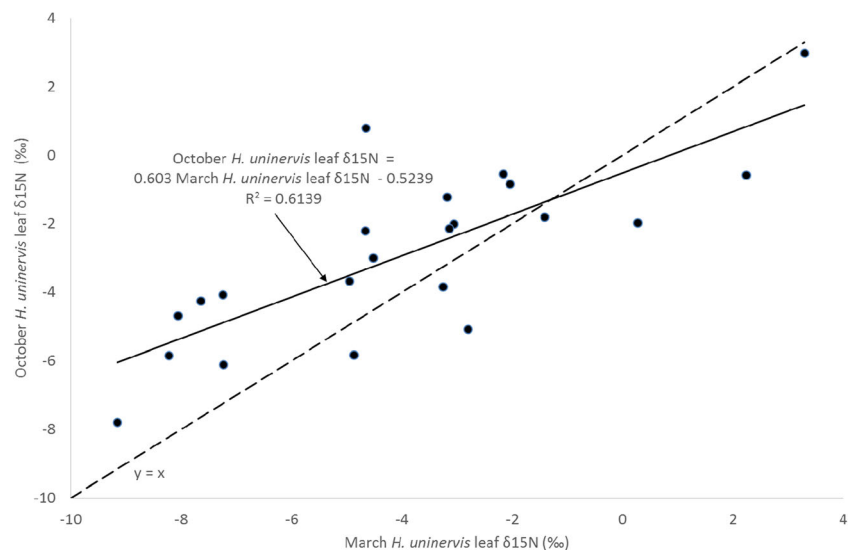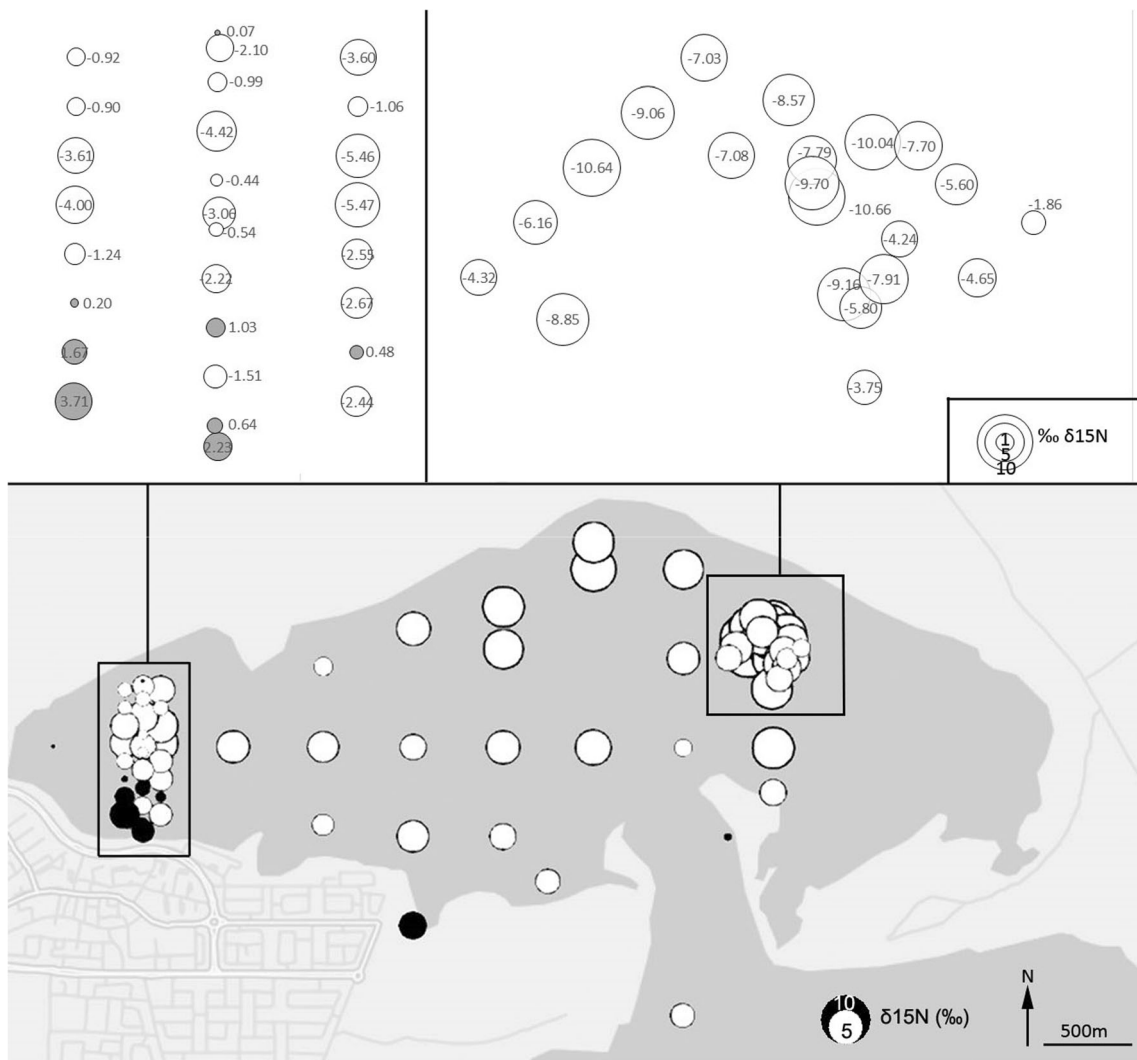
Fig. 5 Variation in *Halodule uninervis* δ¹⁵N in Al Khor Bay at stations visited during October 2011 sampling session with inserts of small-scale sampling grids. *Black/grey bubbles* are positive and *white bubbles* are negative

Fig. 6 Comparison of δ¹⁵N values in seagrass leaf and rhizome *in H. uninervis* in March 2012

H. uninervis rhizome δ15N = 0.6944 *H. uninervis* leaf δ15N − 2.7013
R² = 0.6063
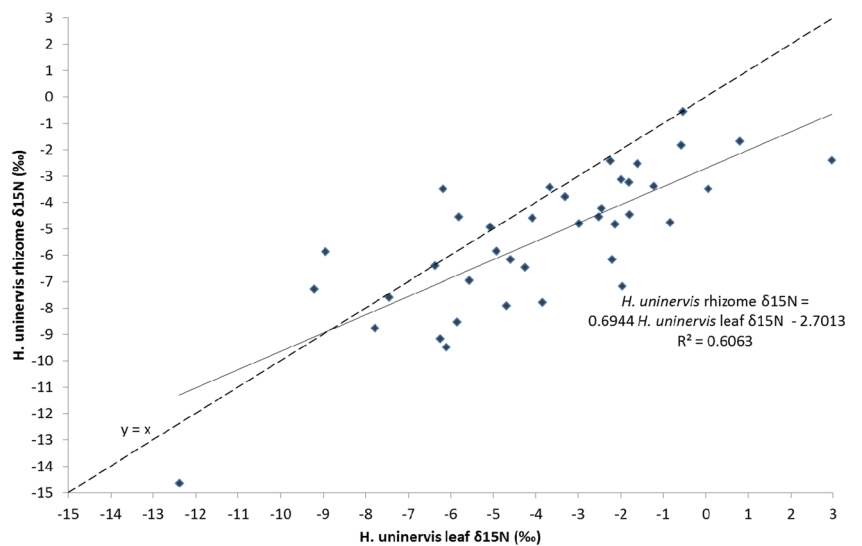
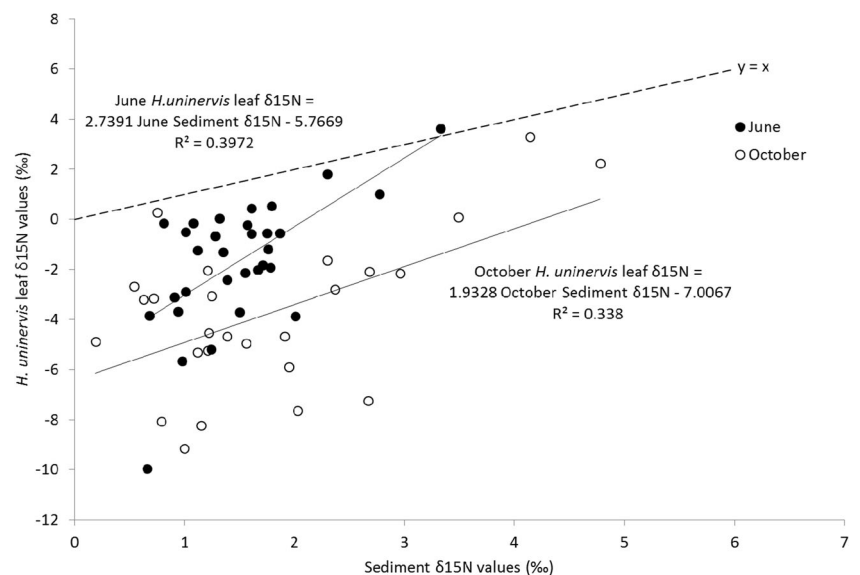**Fig. 7** Seasonal variation in the relationship between $\delta^{15}N$ values of *H. uninervis* and *H. stipulacea* samples from the same stations
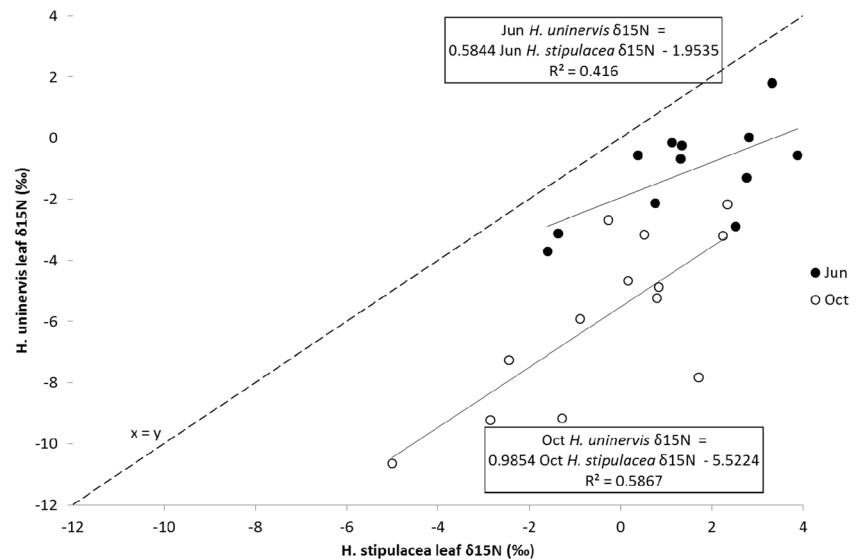
depletion was more severe than P (Table 1). Sediment adsorbed P showed no spatial or seasonal patterns. Neither the P and N values, nor the N/P ratio of the seagrass leaves showed any significant spatial or temporal patterns of variation. However, the variance of *H. uninervis* leaf N, P and N/P ratio was significantly greater in March than in October. Neither sediment nor any of porewater nutrients were significantly correlated with the *H uninervis* leaf $\delta^{15}N$ values. Moreover, *H uninervis* leaf $\delta^{15}N$ values had no significant relationship with leaf nutrient content or ratios in October and March in the large sampling grid (no nutrient samples were taken in June). Some weak correlations were found between nutrient content and $\delta^{15}N$ values of *H. uninervis* leaves when all data points were incorporated, including those in the small grids; however, the significance was reliant on a small number (less than four) of outlying data points that exerted a strong influence on the result.

Discussion

The current study reports some of the most extreme negative $\delta^{15}N$ values ever reported for seagrass (as low as −12.4‰) and some of the most highly spatially variable (values varied over 15.4‰ in a relatively small area of only 655 ha). The global variation in seagrass $\delta^{15}N$ values reported to date is from +17.8 to −10.6‰ (Christiaen et al., 2014; Fourqurean et al., 2015) with *Halodule* spp. and *Halophila* spp. (the two genera found in Qatar) being amongst the most depleted (Christiaen et al., 2014). Unusually low $\delta^{15}N$ values may be more of a response to location than a trait of certain genera. Fourqurean et al. (2015) recently reported *T. testudinum*, a genera not previously associated with depleted ^{15}N values, to have leaves with lower $\delta^{15}N$ values of 8.0 to −10.1‰ than *Halodule* sp. at the same location, which varied from 5.6 to −8.9‰ and *Halophila decipiens*, which was much less depleted. These negative values from

Fig. 8 Seasonal variation in the relationship between the $\delta^{15}N$ values of *H. uninervis* and sediment, sampled from the same stations (March data are not included as correlation was not significant)

Table 1 Sediment, pore water and water column nutrients and leaf P and N. Means are reported ±1 SD

Nutrients	October	March
Sediment adsorbed P (μmol g^{-1})	0.71 ± 0.2	0.70 ± 0.2
Sediment porewater P (μM)	0.05 ± 0.05	0.007 ± 0.004
Sediment porewater total N (μM)	1.13 ± 0.1	0.20 ± 0.07
Sediment porewater molar N:P	52.5 ± 59.7	37.2 ± 23.0
Water column total N (μM)	3.9 ± 2	
H. uninervis leaf P (μmol g^{-1})	48.7 ± 8.8	48.7 ± 18.5
H. uninervis leaf N (μmol g^{-1})	1998.2 ± 153.5	2220.7 ± 557.0
H. uninervis leaf molar N/P	42.2 ± 6.8	56.0 ± 41.5

the middle of the Bermuda platform were suggested to be the result of unspecified benthic processes and ammonification, although this could not be confirmed as there were no sediment chemistry data. In the current study, there was a slight localized but significant enrichment in ^{15}N in seagrass samples in the western end of the bay, resulting in some positive δ^{15}N values, which suggests that anthropogenic inputs of nitrogen are entering the bay from Al Khor city. This is likely to be the result of ground water or the overflow of tertiary-treated wastewater during the winter months, when evaporation does not exceed supply (personal communication, Dr. Abdel-Moati 2014). Indeed, seagrass δ^{15}N has previously been used to identify and trace the input of sewage N from its enriched ^{15}N value (McClelland and Valiela, 1998; Lepoint et al., 2004; Lassauque et al., 2010). One possible influence could be the mangrove forest at the eastern end of the bay, where senescent mangrove leaf δ^{15}N values are known to average −2‰ (Walton et al., 2014). However, application of isotopic mixing models (Walton et al., 2014) shows that there is almost no outwelling of mangrove material beyond the immediate boundary of mangrove area, indicating there is unlikely to be any influence from the mangrove on the ^{15}N content of sediments or seagrasses across the bay.

Temporal and Spatial Variation

The consistency of the variance at both large (500 m) and small (50 m) sampling scales indicates that the cause of the negative δ^{15}N values is operating at a scale of less than 50 m, and observed differences in δ^{15}N values of 3‰ in samples less than 10 m apart indicate that the influencing factor differed over the scale of just a few metres. At the smaller scale, correlation between the δ^{15}N values of different seagrass species from the same sample station (even though *H. stipulacea* was significantly less depleted in ^{15}N than *H. uninervis*) shows the factors influencing these values were similar over the scale of a metre.

Sampling during the three growth periods indicated that δ^{15}N values of *H. uninervis* are influenced by season, being

significantly less depleted in June than October or March. Cline and Kaplan (1975) report that metabolic rates are inversely related to isotopic discrimination, not only for plants but also for denitrifying bacteria. Therefore, in winter, when there is less demand for nitrogen, discrimination is likely to be greater. This is the most probable explanation behind the seasonality of the ^{15}N isotopic values in *H. uninervis* leaves in Al Khor bay, where reduced discrimination was observed during periods of high growth in June when demand for nitrogen is peaking, becoming increasingly depleted as growth stops during the winter period in October and before growth restarts in March. Despite these small seasonal changes, the consistency of the *H. uninervis* δ^{15}N values at any one sampling station throughout the year indicate the underlying cause of ^{15}N depletion is spatially consistent over the period studied.

Intra-/Inter- Specific Variation

The δ^{15}N values of *H. uninervis* leaves and rhizomes were significantly correlated ($R^2 = 60$ %) although some partitioning occurred, as rhizomes are generally less depleted in ^{15}N than leaves, becoming more similar at extreme ^{15}N depletion. In contrast, studies on *H. uninervis* in Indonesia found no significant difference between leaf and rhizome δ^{15}N values (Christianen et al., 2012). Seagrasses in general are often found in nutrient poor coastal waters where they are able to outcompete other macrophytes because of their relatively low demand for nutrients (Duarte, 1990), combined with their ability to maintain uptake rates of inorganic P and N even in these low nutrient conditions. Lepoint et al. (2002) list a number of strategies employed by seagrass to cope with low nutrient environments, including the storage of nutrients for future deployment, internal recycling, and re-absorption of nutrients from senescent leaves. A more positive δ^{15}N signal in rhizomes was also found in *Posidonia oceanica* and was reportedly due to the increased concentrations of ^{15}N-enriched amino acids used to store excess nitrogen in preparation for remobilization in times of nutrient limitation (Vizzini et al., 2003). The authors suggest that as amino acids are more enriched in ^{15}N than bulk material they can be used as an indicator of the changes in N reserves in different parts of the plant.

The relative depletion of ^{15}N in the leaves of *H. stipulacea* compared to those of *H. uninervis* and the lack of correlation between sediment and the leaf δ^{15}N values of *H. stipulacea* and *H. ovalis* (compared with *H. uninervis*) indicates these two seagrass species may have different N uptake mechanisms than *H. uninervis* (as suggested by Evans (2001)).

Seagrass Characteristics

Since discrimination against ^{15}N within the plant only occurs when supply of N is greater than assimilation rates (Evans, 2001), the nutrient profiles of seagrass and sediments and their

N/P and C/N ratios were checked to see if they correlated with seagrass leaf δ^{15}N values. It is thought that when there are low N concentrations or where C/N/P ratios indicate that N is limiting, then discrimination will be lower. Hence, N discrimination would be expected to increase with increasing N concentration, leading to seagrass being depleted in ^{15}N (Campbell and Fourqurean, 2009). The C/N values of the seagrass species present in Al Khor bay range from 16 to 22, which is approximately central in the range of values (mean 20) presented in the review of seagrass nutrient content by Duarte (1990). In contrast, N/P ratios of *H. uninervis* and *H. stipulacea* leaves in Al Khor bay were all >30, indicating P limitation (Duarte, 1990; Johnson et al., 2006), and with the exception of Tanzania, were much greater than ratios reported at any other locations globally (Table 2). P-limited systems are often associated with tropical carbonate sediments, such as those found in Qatar, where phosphate is bound to sediments making it less bioavailable (Short, 1987). However, in the current study, no significant relationship was detected between *H. uninervis* leaf δ^{15}N values and leaf N/P ratios. This is in agreement with the study by Mellors et al. (2005), where none of the nutrient parameters measured, including nutrient ratios, correlated with *H. uninervis* biomass. It should be noted that Ferdie and Fourqurean (2004) argue that seagrass leaf N/P ratio is a poor indicator of nutrient limitation, which can only be truly determined through sequential nutrient addition mesocosm experiments.

The seagrass community of Al Khor bay appears to be healthy, with *H. uninervis* coverage that ranged from 0 to 100 %, averaging almost 50 % and a mean above- and below-ground biomass of 198 and 663 g m^{-2}, respectively (Supplement 1). Similar values were obtained for above-ground biomass of *H. uninervis* (94–281 g m^{-2}) from three sites in Kuwait (Al-Bader et al., 2014). These values and that of the current study are much greater than the mean above- and below-ground biomass values of 27 and 60 g m^{-2} obtained from 25 studies around the world (Duarte and Chiscano, 1999). Canopy height appears to be fairly typical at 7 cm (range 3 to 12 cm) and similar to that of Udy and Dennison (1997) and Al-Bader et al. (2014). Elsewhere, reported leaf biomass has varied from 7 to 40 g m^{-2} for *H. uninervis* and *H. stipulacea* in the Red Sea (Wahbeh, 1988) and values of 13.8 g m^{-2} have been reported for *H. uninervis* in Indonesia (Christianen et al., 2012). This suggests that the high biomass found in Qatar is perhaps typical of *H. uninervis* growing in the western Arabian Gulf, which is much higher than values reported elsewhere. The ratios of above-/below-ground biomass (mean ± 1 SD: 0.63 ± 0.59) correlated with neither leaf nor sediment δ^{15}N values, nor with sediment nutrient concentrations, although at the eulittoral stations closest to Al Khor City the ratio averaged 1.44, possibly due to the increased nutrient concentrations noted around the urbanised area. Duarte and Chiscano (1999) suggested that above-ground

seagrass biomass is more responsive to nutrient increase, showing higher growth than below-ground biomass. The high biomass of the seagrass beds in Al Khor bay suggests *H. uninervis* is not stressed and perhaps explains why these proxies for seagrass health do not appear to correlate with the δ^{15}N values of *H. uninervis* leaves.

Influence of Water Column and Sediment Processes

Comparison of nutrient supply with other *H. uninervis* beds around the world indicates that water column total N in Al Khor (0.7 to 7.5 μM) is towards the lower end of the global range of 1.2 to 28.9 μM N (Daby, 2003; Christianen et al., 2012). Porewater N in Al Khor (0.2 to 1.13 μM N) was much lower than those reported from seagrass beds elsewhere, which range from 1.8 to 144.4 μM N globally (Udy and Dennison, 1997; Yamamuro et al., 2001; Mellors et al., 2005; Christianen et al., 2012). Porewater P in Al Khor (0.007 to 0.05 μM P) was also much lower than that reported from seagrass beds elsewhere, which range from 0.3 to 3.2 μM P globally (Udy and Dennison, 1997; Yamamuro et al., 2001; Mellors et al., 2005; Christianen et al., 2012). In contrast, adsorbed P in Al Khor sediments was 0.7 μmol g^{-1}; towards the higher end of the range reported by Mellors et al. (2005) that spanned from 0.02 to 0.82 μmol g^{-1}, indicating that much of the P is bound up in the sediments. These data suggest that although both nutrients are limited, the supply of N is in excess of demand relative to P, which could explain why the isotopic values are generally so depleted in ^{15}N. In a similar manner, when N has been experimentally supplied in excess of demand, the δ^{15}N values of the leaves of *H. uninervis* growing in those sediments was very depleted (see Table 2) (Udy and Dennison (1997) and Udy et al. (1999)).

The relative enrichment in ^{15}N of the POM, combined with the oligotrophic and homogeneous nature of the bay water precludes it as a major contributor to the observed heterogeneous and highly depleted seagrass δ^{15}N values. Seagrasses utilise both ammonia and nitrate, which are absorbed by both leaves and roots from the water column and sediment (Lepoint et al., 2004). The correlations between sediments and plant and the lack of correlation with the water column δ^{15}N values indicates that *H. uninervis* δ^{15}N values are linked to processes associated within the sediment. Seagrasses can uptake N in various forms, including NH_4^+, NO_3 urea and amino acids and these N sources are all generally found in higher concentrations in sediment pore waters. Given the anoxic status of most seagrass sediments, it is unlikely that processes that lead to depleted nitrate values, such as partial nitrification can be the cause of the observed depletions. In Al Khor, NH_4^+ accounts for 87 % of the total inorganic N in pore waters, and in common with other seagrass species NH_4^+ uptake rates are up to two orders of magnitude greater than for NO_3^{2-}, in both *H. uninervis* and *H. stipulacea* roots (Vonk et al., 2008; Alexandre et al., 2014). Experimental studies in a range of

Table 2 The percentage content of nitrogen and phosphorus by dry weight, molar N/P ratio and sample number of *Halodule uninervis*, *Halophila stipulacea* and *Halophila ovalis* from various locations around the world and from the current study (large grid)

Species	Location	% N	% P	N/P molar	Number	δ15N (‰)	Source
H. uninervis	Green Island, N. Qld, Aust.	2.40	0.26	20:1	3	1.7[a]	Udy et al. (1999)
H. uninervis	Green Island, N. Qld, Aust.	3.0	0.3	22:1	3	−2.0[b]	Udy et al. (1999)
H. uninervis	Moreton Bay, S.E. Qld, Aust.	2.40	0.24	22:1	3	2.4[a]	Udy and Dennison (1997)
H. uninervis	Moreton Bay, S.E. Qld, Aust.	3.2	0.24	22:1	3	−8.8[b]	Udy and Dennison (1997)
H. uninervis	Magnetic Island, N. Qld, Aust.	0.92	0.15	14:1	3	–	Birch (1975)
H. uninervis	Global average	2.40	0.19	27:1	15	–	Duarte (1990)
H. uninervis	Lugger Bay, N. Qld, Aust.	4.64 ± 0.27	0.28 ± 0.01	37:1	3	–	Mellors et al. (2005)
H. uninervis	Meunga Ck, N. Qld, Aust.	6.30 ± 0.25	0.45 ± 0.07	32:1	3	–	Mellors, et al. (2005)
H. uninervis	Cape Pallarenda, N. Qld, Aust	3.32 ± 0.08	0.32 ± 0.01	23:1	3	–	Mellors, et al. (2005)
H. uninervis	Geoffrey Bay, N. Qld, Aust.	4.45 ± 0.18	0.28 ± 0.02	35:1	3	–	Mellors, et al. (2005)
H. uninervis	Horseshoe Bay, N. Qld, Aust.	4.19 ± 0.14	0.21 ± 0.05	44:1	3	–	Mellors, et al. (2005)
H. uninervis	Long Bay, N. Qld, Aust.	3.47	0.23	33:1	3	–	Mellors, et al. (2005)
H. uninervis	Port Dennison, N. Qld, Aust.	3.02 ± 0.46	0.18 ± 0.04	38:1	3	–	Mellors, et al. (2005)
H. uninervis	Green Island, Aust.	2.09 ± 0.23	0.3 ± 0.01	16:1	3	2.68	Yamamuro et al. (2001)
H. uninervis	Dravuni Island, Fiji	1.55 ± 0.13	0.2 ± 0.1	17:1	2	−0.06	Yamamuro et al. (2001)
H. uninervis	Aqaba, Red Sea, Jordan	2.37 ± 0.07	–	–	36	–	Wahbeh (1988)
H. uninervis	W. A., Aust.	–	–	18:1	36	–	Atkinson and Smith (1983)
H. uninervis	Shark Bay, W. A., Aust.	1.78	0.12	34:1 [c]	161	–	Burkholder et al. (2013)
H. uninervis	Banten Bay, Indonesia	1.49 ± 0.21	0.19 ± 0.03	17:1	16	–	Kiswara et al. (2009)
H. uninervis	E. Kalimantan, Indonesia	2.21 ± 0.02	0.18 ± 0.01	13:1	10	1.63[d]	Christianen et al. (2012)
H. uninervis	Asian Average (5 sites)	1.50 ± 0.06	0.27 ± 0.01	12:1	17	–	Terrados, et al. (1999)
H. uninervis	Tanzania	1.64	0.05	73:1	–	–	(Hemminga et al. (1999))
H. uninervis (Oct)	Al Khor, Qatar	2.76 ± 0.19	0.15 ± 0.02	42:1	76	−4.08	This study
H. uninervis (Mar)	Al Khor, Qatar	3.14 ± 0.73	0.14 ± 0.06	56:1	41	−3.50	This study
H. uninervis (Oct)	Al Dhakira, Qatar	2.73 ± 0.25	0.17 ± 0.07	36:1	3	0.06	Walton (unpublished data)
H. uninervis (Mar)	Al Dhakira, Qatar	3.37 ± 0.17	0.18 ± 0.01	41:1	3	−1.4	Walton (unpublished data)
H. ovalis	Cockle Bay, N. Qld, Aust	0.72	0.16	10:1	2	–	Birch (1975)
H. ovalis	Global average	0.70	0.18	9:1	2	–	Duarte (1990)
H. ovalis	Swan-Canning Est, W.A., Aust.	–	–	8:1	–	–	Connell and Walker (2001)
H. ovalis	Picnic Bay, N. Qld, Aust.	2.36 ± 0.02	0.36 ± 0.19	14:1	3	–	Mellors et al. (2005)
H. ovalis	W. A., Aust	–	–	13:1	29	–	Atkinson and Smith (1983)
H. ovalis	Laem Yong Lam, S.W. Thailand	1.30	0.13	22:1	–	2.32	Yamamuro and Chirapart (2005)
H. ovalis	Laem Yong Lam, S.W. Thailand	1.98	0.22	20:1	1	3.21	Yamamuro et al. (2001)
H. ovalis	Fiji	2.50	0.33	17:1	–	−1.70	Yamamuro et al. (2001)
H. ovalis	Green Island, Aust.	2.50 ± 0.03	0.43 ± 0.1	15:1	2	2.00	Yamamuro et al. (2001)
H. ovalis	Dravuni Island, Fiji	2.51 ± 0.10	0.44 ± 0.01	14:1	2	1.41	Yamamuro et al. (2001)
H. ovalis	Asian Average (5 sites)	1.89 ± 0.08	0.37 ± 0.02	11:1	17	–	Terrados et al. (1999)
H. ovalis (Oct)	Al Khor, Qatar	2.18	–	–	1	−3.73	This study
H. ovalis (Mar)	Al Khor, Qatar	–	0.04 ± 0.01	–	4	–	This study
H. stipulacea	Shark Bay, W. A., Aust.	1.38	0.12	24:1	51	–	Burkholder, et al. (2013)
H. stipulacea	Aqaba, Red Sea, Jordan	1.33 ± 0.11	–		36	–	Wahbeh (1988)
H. stipulacea (Oct)	Al Khor, Qatar	1.96 ± 0.30	0.10 ± 0.03	49:1	17	0.44	This study
H. stipulacea (Mar)	Al Khor, Qatar	2.45 ± 0.30	0.16 ± 0.11	41:1	5	0.15	This study

[a] Ambient values from fertilisation experiments

[b] Enriched with N in fertilisation experiments

[c] Median values

[d] Unfertilized values

terrestrial plants demonstrate strong fractionation of $\delta^{15}N$ when N is supplied as NH_4^+, with plant tissue $\delta^{15}N$ values being up to 7 to 16‰ lighter than the supplied source (Yoneyama et al., 1991; Emmerton et al., 2001; Ariz et al., 2011). In seagrass, the fertilisation experiment of Udy and Dennison (1997) concur with these studies, but the results may not be applicable to our study site as much higher concentrations of NH_4^+ were available than are present in pore waters in Al Khor. Emmerton et al, (2001) showed that as the proportion of NH_4^+ taken up by plants increases the difference between source and plant $\delta^{15}N$ diminishes, reaching equivalence when all N added is absorbed. They induced differences

in ^{15}N abundance between source and plants of up to −8 ‰ in *Eriophorum vaginatum*, equivalent to the difference between sediment and plant ^{15}N for *H. uninervis* in Al Khor, but only when less than 20 % of supplied N had been taken up. While NH_4^+ concentrations in pore waters in Al Khor are low, this does not confirm low N supply; plant nutrient ratios did not indicate N, but P limitation, and seagrass biomass was at the high end of the range of observed values globally. Significant loss from the porewater NH_4^+/NH_3 pool through diffusion to the overlying water column from sediments is likely. Thus, the high degree of ^{15}N fractionation observed could be consistent with uptake of a relatively small proportion of the overall NH_4^+ supply.

In shallow carbonate environments, especially in subtropical areas such as the Arabian Gulf, the variably depleted δ^{15}N values for seagrass biomass relative to observed, less variable, N sources of SPOM and sediments across the bay indicate benthic N cycling as the most likely cause of negative ^{15}N values and ^{15}N fractionation during NH_4^+ uptake. The spatial correlation of δ^{15}N values between *H. uninervis* and *H. stipulacea* suggests consistent differences in ^{15}N fractionation between the species, as has been observed in experimental studies of co-existing terrestrial plants (Emmerton et al., 2001). The high discrimination seen in this study could be due to a highly depleted N source, resulting from one of a number of processes. For example, recent research discovered a number of fungi and microbial endophytes that inhabit seagrass root systems (Shoemaker and Wyllie-Echeverria, 2013 and references therein). In low nutrient environments, terrestrial plants with fungal symbionts tend to rely on the symbiont for the supply of N, which is normally very depleted and fractionated by −8 to −10 ‰ (Hobbie et al., 2005). However, the impact of these fungal associations on δ^{15}N values in seagrass is unknown.

In conclusion, we have observed consistently highly depleted δ^{15}N values in the bay studied here, which appear to be caused by processes occurring over very small spatial scales (a few metres). The high biomass present suggests that nutrient limitation does not appear to play a role in affecting *H. uninervis* leaf δ^{15}N values, even though N/P ratios indicate that P is the limiting nutrient in Al Khor bay. The consistently divergent δ^{15}N values for three species of seagrass growing in the same relatively ^{15}N-enriched sediment N pool suggest that these differences may be due to benthic processing and/or N uptake mechanisms. Similar areas of low, depleted ^{15}N in seagrasses have been recorded elsewhere in the world (Christiaen et al., 2014). Ammonium demand relative to supply, extent of ammonium dissociation, and P limitation have been identified as the most likely causes of the observed ^{15}N depletions. Mycorrhizal associations offer another possible explanation for the magnitude of ^{15}N depletion recorded in the seagrass leaves in Al Khor. The conditions causing the strongest depletions observed to date occur in geographically distinct locations in Bermuda (Fourqurean et al 2015) and Qatar (present study) and both also display very strong gradients in ^{15}N values across short distances. The paradoxically high biomass of seagrass found in these nutrient limited areas emphasises the need for further research to understand the common factors that support good growth and result in these highly depleted δ^{15}N signals as they are likely present at other locations globally. In the wider context, the current study observed some of the most extreme, negative and highly spatially variable δ^{15}N values ever reported for seagrass. These results demonstrate the need for adequate spatial and temporal sampling when working with nitrogen stable isotopes in either as sources in food web studies or as tracers of anthropogenic nutrients.

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