The degree of urbanization across the globe is not reflected in the δ^{15}N of seagrass leaves

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ABSTRACT

Many studies show that seagrass δ^{15}N ratios increase with the amount of urbanization in coastal watersheds. However, there is little information on the relationship between urbanization and seagrass δ^{15}N ratios on a global scale. We performed a meta-analysis on seagrass samples from 79 independent locations to test if seagrass δ^{15}N ratios correlate with patterns of population density and fertilizer use within a radius of 10–200 km around the sample locations. Our results show that seagrass δ^{15}N ratios are more influenced by intergeneric and latitudinal differences than the degree of urbanization or the amount of fertilizer used in nearby watersheds. The positive correlation between seagrass δ^{15}N ratios and latitude hints at an underlying pattern in discrimination or a latitudinal gradient in the ^{15}N isotopic signature of nitrogen assimilated by the plants. The actual mechanisms responsible for the correlation between δ^{15}N and latitude remain unknown.

1. Introduction

Seagrass meadows are ranked among the most productive and valuable ecosystems in the biosphere (Costanza et al., 1997). They provide habitat for commercially important fish species (Beck et al., 2001), stabilize sediments with their roots and rhizomes (Hemminga and Duarte, 2000), and provide subsidies to adjacent ecosystems through export of organic matter (Heck et al., 2008). As such, they are important components of the food web in coastal marine ecosystems. Seagrass beds are threatened by human activities, both on local and global spatial scales (Orth et al., 2006; Waycott et al., 2009). Seagrasses are sensitive to changes in water clarity because they require relatively high light levels for maintaining primary production (Dennison et al., 1993). They are negatively impacted by dredging, trawling and algal overgrowth from nutrient enrichment (Burkholder et al., 2007).

Increased human development in coastal watersheds has led to increased inputs of nutrients to marine ecosystems in many regions of the world (Vitousek et al., 1997). As a consequence, identifying and managing sources of nutrient pollution has become an important challenge for seagrass conservation (Orth et al., 2006). During the last decade, there has been increasing interest in developing reliable indicators of nutrient pollution in seagrass beds (Jones et al., 2001; Ferrat et al., 2003; Lee et al., 2004; Burkholder et al., 2007). One possible indicator of nutrient pollution is the relative abundance of ^{15}N in tissues of marine organisms. Measuring δ^{15}N ratios in tissues of marine plants has become widespread since McClelland et al. (1997) linked ^{15}N isotopic signatures in marine organisms to anthropogenically derived nitrogen from watersheds in Waquoit Bay (MA). Elevated δ^{15}N values in tissues of marine plants are considered indicative of nitrogen from anthropogenic sources, because dissolved inorganic nitrogen (DIN) in sewage and manure is enriched in ^{15}N compared to DIN in marine ecosystems (Kendal et al., 2007). Inorganic fertilizer has a depleted ^{15}N isotopic signature because it is generated from atmospheric nitrogen through the Haber–Bosch reaction (Erisman et al., 2008). However, a large fraction of the added nitrogen to terrestrial ecosystems is denitrified in the watershed before it reaches the coast (Seitzinger et al., 2006). This increases the isotopic signature of the remaining nitrogen. Agricultural runoff from heavily fertilized watersheds can therefore be enriched in ^{15}N when it enters coastal waters (Fry et al., 2003).

A large number of studies have documented the relationship between δ^{15}N ratios of seagrass leaves and nutrient pollution. Most of them measure isotope ratios as a function of distance to known point sources, such as sewage outflows and aquaculture facilities (Costanzo et al., 2001; Dolenec et al., 2006; Perez et al., 2008;

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Lassauque et al., 2010). Others focus on the relationship between isotope ratios and the degree of urbanization for specific locations (Carruthers et al., 2005; Castro et al., 2007; Olsen et al., 2010). Only a few studies examine the relationship between land use and seagrass δ^{15}N ratios on regional scales. Yamamuro et al. (2003) documented that δ^{15}N ratios are correlated with DIN concentrations in bottom water, when comparing ^{15}N isotopic signatures in seagrass leaves from different coral reef ecosystems. Cole et al. (2004) measured δ^{15}N ratios in algae and macrophytes from estuaries with different characteristics and found a positive relation between ^{15}N signatures in macrophytes, DIN concentrations in the water column and the relative contribution of wastewater to total nutrient load. Fourqurean et al. (2005) documented a large scale pattern in seagrass δ^{15}N ratios throughout the Florida Keys, and Fourqurean et al. (2007) found a latitudinal gradient in the ^{15}N isotopic signature of *Posidonia oceanica* near the Balearic Islands. However, in these two studies there was no clear relationship between seagrass δ^{15}N ratios and potential sources of nutrient pollution.

Because there are only a few studies that investigate broad scale relationships between urbanization, fertilizer use, and seagrass δ^{15}N ratios, it remains unclear if δ^{15}N measurements can be used as indicator of nutrient pollution on regional and global spatial scales. To address this gap in knowledge, we compiled a database of seagrass δ^{15}N ratios from 79 independent locations, and tested if seagrass δ^{15}N ratios correlate with population densities or the amount of fertilizer used within a radius of 10–200 km around the sample sites. Although population size is no perfect indicator of wastewater pollution, large scale patterns should emerge, because watersheds with a large degree of urbanization have high nutrient loads and a high contribution of wastewater to the total nutrient load (Costanzo et al., 2001; Cole et al., 2004). More specifically we ask the following questions:

- Are there correlations between seagrass δ^{15}N ratios, the size of the human population and the amount of fertilizer applied within a radius of 10–200 km around the sample sites?
- Are seagrass δ^{15}N ratios different between open coasts and semi-enclosed locations, such as estuaries and bays?
- Do seagrass δ^{15}N ratios differ between genera?
- Are there global patterns in seagrass δ^{15}N ratios? If so, which factors could explain these patterns?

Answering these questions will allow us to assess the use of seagrass δ^{15}N ratios as indicator of nutrient pollution at regional and global spatial scales.

2. Methods

We created a database of δ^{15}N ratios in seagrass leaves based on a literature survey of published studies, reports and masters theses. Data for the Florida Keys was obtained from the websites of the Florida Coastal Everglades Long-Term Ecological Research Program (LTER, 2012) and the Florida Keys National Marine Sanctuary (FKNMS, 2012). Data sources were identified by a web-search with Google, Scopus and Google Scholar using different combinations of the terms: "seagrass", "nitrogen", "sewage", "food web" and "isotope". All studies where tissue samples were subjected to acidification or lipid extraction prior to analysis for δ^{15}N were rejected because these treatments have the potential to influence δ^{15}N ratios (Bunn et al., 1995). Stable isotope ratios were averaged per sample site and per seagrass species. Sample sites were identified based on spatial coordinates and maps. A limited number of studies contained data for mixtures of seagrass species. These values were entered as "tropical, mixed". The database contained 324 records of δ^{15}N ratios for 24 different species of seagrass, pooled over 253 different sample sites. These values were based on 3693 individual measurements of δ^{15}N in seagrass leaves, extracted from 76 different data sources. One record was removed as an outlier, because its δ^{15}N value was unrealistically high for marine macrophytes (17.8‰). The dataset and the references to all data sources are included as Supplementary material.

The population size within a radius of 10, 50, 100 and 200 km around each sample site was calculated with Arcmap 10 (ESRI, Redlands, CA). A base layer of population counts in a 30 arc-s grid was obtained from the Columbia University Center for International Earth Science Information Network (CIESIN 2011). Different size geodesic buffers were created around each sample site and the size of the population within each buffer was calculated with the model builder and zonal statistics tools. The same analysis was performed on a map of global N-fertilizer application (Potter et al., 2011) with a grid size of 0.5 arc-degrees (units: kg of N/Ha from 1994 to 2001). Because of the large grid size of the base layer, fertilizer application was only measured within a radius of 100 and 200 km around each sample site.

For the spatial analysis, all data from sample sites that were located in the same body of water (coastline, estuary or bay) were averaged. In water bodies with a strong gradient of urbanization in the surrounding watershed, the sample sites were averaged over two locations (high and low development), depending on the size of human population in a radius of 10 km around each individual sample site (the smallest scale for the spatial analysis). Averaging

Fig. 1. Locations of pooled data used in the spatial analysis of nitrogen isotope ratios in seagrass leaves.

was deemed necessary to avoid spatial autocorrelation. The result was a "pooled" dataset with 79 independent sample locations (Fig. 1). Locations along open coasts were classified as "open" ($n = 31$) and locations in estuaries, bays and lagoons were classified as "semi-enclosed" ($n = 48$), based on visual inspection with Google Earth. Using partial spearman rank correlations, both groups were analyzed to determine if there was a relationship between the average $\delta^{15}N$ ratios in seagrass leaves, the absolute value of the latitude, the average size of the human population in a radius of 10, 50, 100 and 200 km around the sample sites and the average amount of fertilizer applied within 100 and 200 km around each sample site. The partial spearman rank correlations were calculated with the ppcor package in R (package 2.15.2; www.r-project.org).

For the comparison among genera, values were averaged per location and per genus. *Heterozostera* and *Thalassodendron* were excluded from the analysis because of the limited number of measurements for each genus. The resulting "pooled" dataset contained 118 unique combinations of genus and location. A one-way ANOVA, with post hoc Tukey test, was used to determine potential differences between the mean $\delta^{15}N$ ratios per genus. Data were tested for normality using the Anderson – Darling test ($p = 0.233$) and for equality of variance using Levene's test ($p = 0.228$). The ANOVA, normality and equal variance tests were conducted in Minitab 14 (Minitab Inc).

3. Results

The database of $\delta^{15}N$ ratios in seagrass leaves contains samples from most regions that support seagrass beds. Yet, it is somewhat heterogeneous. Certain regions, such as the Florida Keys and the Mediterranean Sea are more intensively sampled than others. This is well illustrated by the composition of the dataset. *Thalassia*, *Zostera* and *Posidonia* are by far the most abundant genera. They represent 78% of all individual measurements, and are present in 73% of all pooled locations (Fig. 2). Site averaged $\delta^{15}N$ ratios in seagrass leaves range from −3.2‰ to 17.8‰, with a median of 3.64‰ and a mean of 4.14‰. The highest value in the dataset (17.8‰) came from a *Ruppia* bed in a highly eutrophic coastal lagoon (Dierking et al., 2012). This value was removed as outlier from subsequent analysis. The lowest values were usually from tropical seagrass beds associated with coral reef habitats (Yamamuro et al., 2003; Abreu, 2008; Lugendo et al., 2006; Marconi et al., 2011).
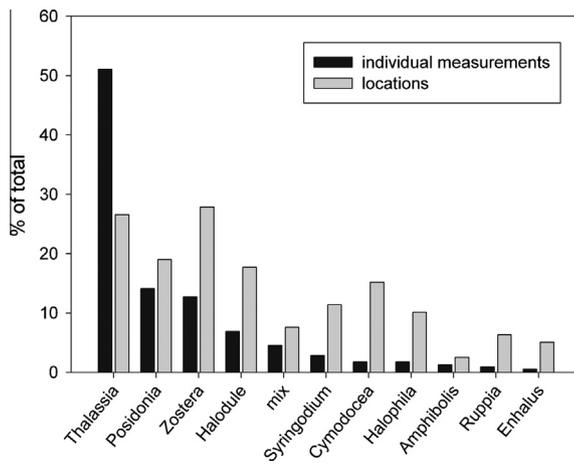
Fig. 2. Generic composition of the dataset. Black: proportion of individual $\delta^{15}N$ measurements per genus in the dataset ($n = 3693$). Grey: proportion of pooled locations per genus ($n = 79$). *Thalassia*, *Posidonia* and *Zostera* represent 78% of all individual measurements and are present in 73% of all locations sampled.

Table 1
Partial spearman rank correlation for open sites ($n = 31$). Significant correlations are indicated in bold.

(km)		Population size	Absolute value latitude	Fertilizer
10	$\delta^{15}N$	$\rho = -0.142$ ($p = 0.449$)	$\rho = 0.475$ ($p = 0.004$)	
	Population size		$\rho = 0.312$ ($p = 0.082$)	
50	$\delta^{15}N$	$\rho = 0.076$ ($p = 0.687$)	$\rho = 0.452$ ($p = 0.007$)	
	Population size		$\rho = 0.058$ ($p = 0.758$)	
100	$\delta^{15}N$	$\rho = 0.098$ ($p = 0.608$)	$\rho = 0.375$ ($p = 0.035$)	$\rho = 0.310$ ($p = 0.090$)
	Population size		$\rho = 0.058$ ($p = 0.761$)	$\rho = 0.277$ ($p = 0.135$)
	Absolute value latitude			$\rho = 0.099$ ($p = 0.604$)
200	$\delta^{15}N$	$\rho = -0.012$ ($p = 0.950$)	$\rho = 0.353$ ($p = 0.050$)	$\rho = 0.257$ ($p = 0.167$)
	Population size		$\rho = 0.063$ ($p = 0.744$)	$\rho = 0.506$ ($p = 0.002$)
	Absolute value latitude			$\rho = 0.182$ ($p = 0.336$)

There are no significant partial correlations between seagrass $\delta^{15}N$ ratios and the size of the population in a 10, 50, 100 or 200 km radius around the open (Table 1) and semi-enclosed (Table 2) sample locations. Seagrass $\delta^{15}N$ ratios are not correlated with the amount of fertilizer applied in a 100 or 200 km radius. However, seagrass $\delta^{15}N$ ratios are always positively correlated with the absolute value of the latitude (Fig. 3). The positive correlation between seagrass $\delta^{15}N$ ratios and latitude is reflected by the mean $\delta^{15}N$ ratios per genus (Fig. 4). Genera that can be classified as temperate, such as *Amphibolis*, *Posidonia* and *Zostera* have higher $\delta^{15}N$ ratios than tropical genera such as *Thalassia*, *Halophila* and *Syringodium* (Short et al., 2007). *Ruppia* and *Zostera*, genera that are mostly found in coastal lagoons and estuarine habitats (Kantrud, 1991; Moore et al., 2006), have among the highest $\delta^{15}N$ ratios in the entire dataset. The visual trend in Fig. 4 corresponds to the results from the one-way ANOVA ($p < 0.001$, R^2-adj = 36.28%). Post hoc Tukey tests indicate that the differences between

Table 2
Partial spearman rank correlation for semi-enclosed sites ($n = 48$). Significant correlations are indicated in bold.

| (km)		Population size	Absolute value latitude	Fertilizer
10	$\delta^{15}N$	$\rho = 0.185$ ($p = 0.208$)	$\rho = 0.513$ ($p < 0.001$)	
	Population size		$\rho = 0.024$ ($p = 0.870$)	
50	$\delta^{15}N$	$\rho = 0.191$ ($p = 0.189$)	$\rho = 0.539$ ($p < 0.001$)	
	Population size		$\rho = -0.137$ ($p = 0.352$)	
100	$\delta^{15}N$	$\rho = 0.163$ ($p = 0.272$)	$\rho = 0.469$ ($p < 0.001$)	$\rho = 0.148$ ($p = 0.320$)
	Population size		$\rho = -0.053$ ($p = 0.726$)	$\rho = 0.130$ ($p = 0.384$)
	Absolute value latitude			$\rho = 0.226$ ($p = 0.123$)
200	$\delta^{15}N$	$\rho = 0.027$ ($p = 0.858$)	$\rho = 0.412$ ($p = 0.003$)	$\rho = 0.240$ ($p = 0.101$)
	Population size		$\rho = 0.145$ ($p = 0.331$)	$\rho = 0.354$ ($p = 0.012$)
	Absolute value latitude			$\rho = 0.191$ ($p = 0.195$)
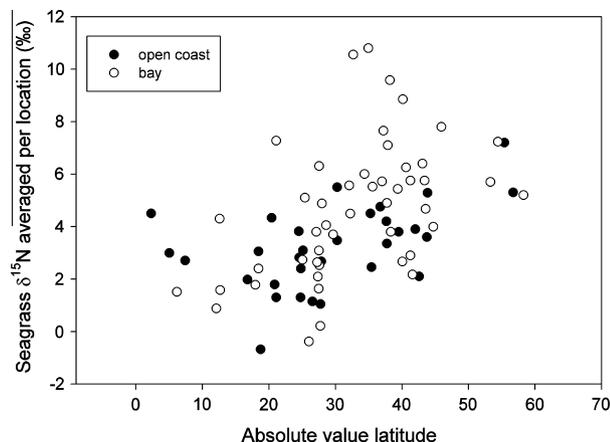
Fig. 3. Seagrass $\delta^{15}N$ ratios per pooled location versus absolute value of the latitude. Locations that are associated with bays and estuaries are indicated in white. Locations along open coasts are indicated in black. The highest recorded value in the dataset (17.8‰) was considered an outlier and removed from the analysis ($n = 79$).
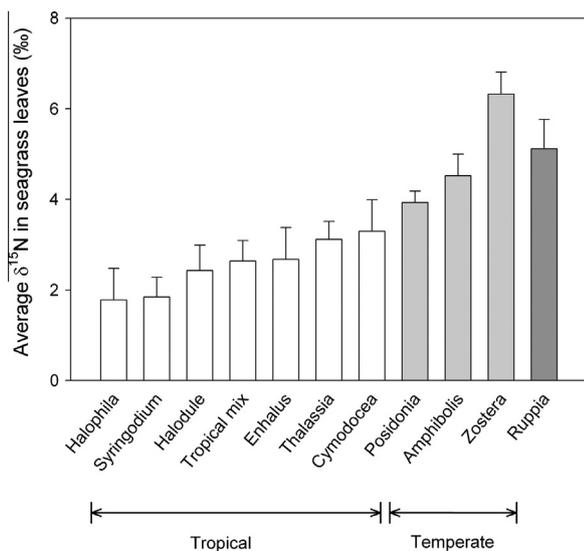

Fig. 4. $\delta^{15}N$ ratios per genus. Tropical genera are depicted in white, temperate genera in light grey and *Ruppia*, which has a global distribution, is depicted in dark grey. *Thalassodendron* and *Heterozostera* were excluded from the statistical analysis because of the limited number of measurements for each genus ($n = 118$).

the genera are mostly driven by *Zostera*. The mean $\delta^{15}N$ ratio in leaves of *Zostera* is significantly higher than those found in tropical genera (with $\alpha = 0.05$).

4. Discussion

While many studies have shown the influence of anthropogenic nitrogen on $\delta^{15}N$ ratios of plants, it remains challenging to use $\delta^{15}N$ ratios for source tracking of nutrient pollution. Several factors influence the $\delta^{15}N$ ratios of marine primary producers besides the isotopic signature of nitrogen from the surrounding watershed (Fourqurean et al., 1997). Plants can discriminate differently against ^{15}N depending on the ratio of nitrogen availability to plant nitrogen demand (Fourqurean et al., 2005). Microorganisms can increase or decrease the isotopic ratio of the source pool by denitrification or nitrogen fixation (Mariotti et al., 1981; Robinson, 2001). Plant ^{15}N signatures can vary with the geographic location:

$\delta^{15}N$ ratios from plants growing in sheltered sites are usually higher than those from open coastal areas (Viana et al., 2011; Viana and Bode, 2013). Also, in agricultural watersheds, denitrification (enriched $\delta^{15}N$) and fertilizer addition (depleted $\delta^{15}N$) have opposite effects on the ^{15}N isotopic signature of DIN. These factors complicate the interpretation of nitrogen stable isotope ratios in tissues of plants. However, through a meta-analysis and pooled sample approach, localized differences in tissue $\delta^{15}N$ ratios can be minimized and broad spatial patterns do emerge.

4.1. $\delta^{15}N$ ratios vs. anthropogenic nitrogen

In near-shore coastal ecosystems, there is often an inverse relationship between seagrass $\delta^{15}N$ ratios and the distance from point sources of sewage pollution (Costanzo et al., 2001; Lassauque et al., 2010) or the degree of urbanization in nearby watersheds (Cole et al., 2004; Carruthers et al., 2005; Castro et al., 2007; Olsen et al., 2010). However, on a regional scale seagrass $\delta^{15}N$ ratios do not increase with the degree of urbanization in the surrounding area (measured as the size of the human population within 10–200 km around the sample locations). There is also no correlation between seagrass $\delta^{15}N$ ratios and the amount of fertilizer applied in nearby watersheds. This is unexpected, especially for seagrass beds from semi-enclosed locations, which are more likely to be influenced by terrestrial ecosystems than seagrass beds along open coasts. There are several possible explanations for the lack of a correlation.

$\delta^{15}N$ ratios of marine primary producers are not only influenced by the isotopic signature of DIN sources, but also by fractionation of the DIN pool during nitrogen uptake. Macrophytes are able to discriminate against ^{15}N when the nutrient supply is greater than what is needed for growth (Campbell and Fourqurean, 2009). As a consequence, there can be seasonal variability in $\delta^{15}N$ ratios. Seagrass leaves are often ^{15}N enriched during the growing season, when plant nutrient demand is high, and ^{15}N depleted during winter, when nutrients are in ample supply (Fourqurean et al., 2005). By averaging per location, the effect of seasonal variability is dampened. However, 45 of the 79 locations have samples collected during 1 season only. This could account for enough variability to mask a potential relationship between seagrass $\delta^{15}N$ ratios and sources of anthropogenic nitrogen.

Another explanation for the lack of correlation between seagrass $\delta^{15}N$ ratios, urbanization and fertilizer use is that large scale patterns in fractionation overwhelm the effect of local sources of anthropogenic nitrogen. This implies that $\delta^{15}N$ ratios cannot be used as bio-indicators of anthropogenic nitrogen on regional spatial scales. If seagrass $\delta^{15}N$ ratios are to be used for estimating the impact of anthropogenic nitrogen pollution, high $\delta^{15}N$ ratios should be correlated with the presence of nearby pollution sources. However, $\delta^{15}N$ ratios appear to be more influenced by intergeneric or latitudinal differences than the degree of urbanization or the amount of fertilizer used in nearby watersheds.

4.2. Intergeneric differences in $\delta^{15}N$ ratios

The rate at which seagrasses discriminate against ^{15}N can vary between species, as different species have different nutrient requirements (Campbell and Fourqurean, 2009). There was a significant difference between the mean $\delta^{15}N$ ratios of seagrass genera in our dataset. However, intergeneric differences are not necessarily caused by physiological differences in nitrogen uptake and discrimination; they can also be caused by spatial variation in seagrass distribution (Campbell and Fourqurean, 2009). In our study, the two genera with the highest $\delta^{15}N$ ratios are *Zostera* and *Ruppia*. These genera are most commonly found in very shallow habitats (Kantrud, 1991; Moore et al., 2006). Coastal ecosys-

tems can receive substantial nutrient load from submarine ground-water discharge. Because DIN from groundwater is often enriched in [15]N (Tappin, 2002; McClelland et al., 1998), the higher δ^{15}N ratios in *Zostera* and *Ruppia* leaves could be caused by groundwater entering shallow coastal habitats.

4.3. Latitudinal gradient in seagrass δ^{15}N ratios

The analysis suggests that the intergeneric differences in sea-grass δ^{15}N ratios are somehow related to the latitude at which these plants occur, because leaves of tropical seagrass genera are ^{15}N depleted compared to genera from temperate latitudes (Fig. 4). The latitudinal gradient in seagrass δ^{15}N ratios hints either at an underlying pattern in discrimination against ^{15}N by seagrass-es on large spatial scales or a difference in the ^{15}N isotopic signa-ture of nitrogen assimilated by the plants.

It is possible that the correlation between seagrass δ^{15}N ratios and latitude could be the result of a large scale gradient in the ratio of nitrogen availability to nitrogen demand. If plants are not nitro-gen limited, they selectively discriminate against the heavier iso-tope, which results in their tissue δ^{15}N ratios being depleted compared to the δ^{15}N ratios in nutrient source pools. The positive correlation between seagrass δ^{15}N ratios and latitude implies that the ratio of nitrogen availability to nitrogen demand should de-crease with increasing latitude. In other words, the latitudinal gra-dient in δ^{15}N suggests that seagrasses from higher latitudes are more often limited by nitrogen than their (sub) tropical counter-parts. This is counter intuitive, because light availability is often considered to be the limiting factor for seagrass growth (Burkhold-er et al., 2007). Also, temperate waters are generally considered to have higher nutrient concentrations than tropical waters. How-ever, seagrasses can be limited by nutrients depending on nutrient concentrations in the overlying water column, and the type of sediment in which they grow (Touchette and Burkholder, 2000; Burkholder et al., 2007). In nutrient poor waters, seagrasses may be N-limited when they grow in sandy or organic sediments (Burkholder et al., 2007). In carbonate sediments, seagrasses are usually more P-limited than N-limited, because phosphorous can be bound in calcium phosphate complexes (Touchette and Burkholder, 2000). Since tropical seagrasses are more likely to grow in carbonate sediments than temperate seagrass species, there could be a latitudinal gradient in N- vs. P-limitation, which may contribute to the observed pattern in seagrass δ^{15}N ratios.

Another explanation for the latitudinal gradient in seagrass δ^{15}N ratios is a different isotopic baseline of source DIN between seagrass beds in tropical and temperate environments. Such a gra-dient could be caused by latitudinal differences in nitrogen fixa-tion, a process that can decrease the isotopic signature of the source DIN pool. Seagrass beds harbor significant populations of microbes capable of nitrogen fixation, such as cyanobacteria and sulfate reducers. These microbes can satisfy as much as 50% of sea-grass nitrogen demand (O'Donohue et al., 1991; Welsh, 2000). Be-cause the contribution of nitrogen fixation to seagrass nutrient demand is generally larger in tropical and subtropical seagrass beds compared to temperate seagrass beds (Welsh, 2000), higher rates of nitrogen fixation may be responsible for the lower δ^{15}N ra-tios in seagrass beds at lower latitudes. The pattern in seagrass δ^{15}N ratios could also be the result of a large scale gradient in sew-age processing efficiency. Tropical regions may have a lesser de-gree of wastewater treatment facilities than temperate regions. Because sewage treatment enriches the isotopic signature of the remaining DIN, sewage outflow at temperate latitudes could have a more enriched δ^{15}N signature than sewage outflow in the tropics. This could contribute to the positive correlation between seagrass δ^{15}N ratios and latitude.

Finally, one could argue that the latitudinal gradient in seagrass δ^{15}N ratios is caused by a spatial pattern in the characteristics of the sample locations, such as the proximity to upwelling zones or the degree of exposure (open vs. semi enclosed). δ^{15}N ratios of marine organisms are usually higher when they live near upwell-ing zones (Hill et al., 2008). Marine plants from estuaries and bays are often enriched compared to similar plants from off-shore loca-tions (Viana and Bode, 2013). It is unlikely that upwelling is responsible for the latitudinal gradient in seagrass δ^{15}N ratios, be-cause only a few of the 79 locations are close to large upwelling zones. The degree of exposure can also be ruled out, because sea-grass δ^{15}N ratios are positively correlated with latitude for both open and semi-enclosed sample locations (Fig. 3).

5. Conclusion

While the impact of urbanization on the functioning of coastal ecosystems is well documented, the influence of population den-sity within a radius of 10–200 km is not reflected in the δ^{15}N ratios of seagrass leaves from open or semi-enclosed locations. This indi-cates that δ^{15}N ratios of marine organisms cannot be used to assess the impact of urbanization on regional or global spatial scales. Measuring seagrass δ^{15}N ratios is likely more useful for source tracking of nutrient pollution on small spatial scales, where the im-pact of confounding factors is less pronounced. The positive corre-lation between seagrass δ^{15}N ratios and latitude hints at an underlying pattern in discrimination or a latitudinal gradient in the ^{15}N isotopic signature of nitrogen assimilated by the plants. The actual mechanisms responsible for the correlation between δ^{15}N and latitude remain unknown.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2013.06.024.

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