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Variability in δ15N of intertidal brown algae along a salinity gradient: Differential impact of nitrogen sources

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HIGHLIGHTS

- Variability of Fucacean $\delta^{15}N$ indicates N sources along a salinity gradient.
- $\delta^{15}N$ of Fucaceae and seawater are not correlated at short time scales.
- Isotopic fractionation in macroalgal tissue varies at seasonal and at local scale.
- Fucacean species are suitable for monitoring chronic N loadings.

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ABSTRACT

While it is generally agreed that $\delta^{15}N$ of brown macroalgae can discriminate between anthropogenic and natural sources of nitrogen, this study provides new insights on net fractionation processes occurring in some of these species. The contribution of continental and marine sources of nitrogen to benthic macroalgae in the estuary-ria system of A Coruña (NW Spain) was investigated by analyzing the temporal (at a monthly and annual basis) and spatial (up to 10 km) variability of $\delta^{15}N$ in the macroalgae *Ascophyllum nodosum* and three species of the genus *Fucus* (*F. serratus*, *F. spiralis* and *F. vesiculosus*). Total nitrate and ammonium concentrations and $\delta^{15}N$-DIN, along with salinity and temperature in seawater were also studied to address the sources of such variability. Macroalgal $\delta^{15}N$ and nutrient concentrations decreased from estuarine to marine waters, suggesting larger dominance of anthropogenic nitrogen sources in the estuary. However, $\delta^{15}N$ values of macroalgae were generally higher than those of ambient nitrogen at all temporal and spatial scales considered. This suggests that the isotopic composition of these macroalgae is strongly affected by fractionation during uptake, assimilation or release of nitrogen. The absence of correlation between macroalgal and water samples suggests that the $\delta^{15}N$ of the species considered cannot be used for monitoring short-term changes. But their long lifespan and slow turnover rates make them suitable to determine the impact of the different nitrogen sources integrated over long-time periods.

1. Introduction

Coastal areas receive nitrogen inputs from continental and marine sources. The growing anthropogenic pressure in these areas has increased dissolved inorganic nitrogen (DIN) concentrations in continental sources in comparison with seawater. Nitrogen concentrations could also be affected by other nitrogen inputs, as atmospheric deposition and upwelled seawater, and by processes as nitrogen uptake by primary producers, regeneration and movement of dissolved organic or inorganic nitrogen across the sediment–water interface (Tyler and McGlathery, 2006). Therefore, monitoring nutrient concentrations is not enough to identify the origin of DIN in coastal areas (Sebilo et al., 2006). To overcome this constrain, the ratio of nitrogen stable isotopes ($\delta^{15}N$) has been increasingly used as a marker of anthropogenic nutrient loading in the last years (Ahad et al., 2006; Deutsch and Voss, 2006; McClelland and Valiela, 1998; Schubert et al., 2013).

The direct measurement of stable isotopes on DIN has been widely used and characteristic isotopic signatures were identified for different nitrogen origins (Ahad et al., 2006; Heaton, 1986; Raimonet et al., 2013; Viana and Bode, 2013). Human and animal wastewaters are enriched in ^{15}N relative to seawater because of strong isotopic fractionation during nitrification and volatilization in the case of NH_4^+, or denitrification in the case of NO_3^- (Mariotti et al., 1981). In contrast, synthetic fertilizers are depleted in ^{15}N due to the atmospheric origin of the fixed nitrogen (Heaton, 1986). Consequently, $\delta^{15}N$ values of marine organisms show large variability in human impacted sites (Bode et al., 2014; Fry et al., 2003; McClelland and Valiela, 1998).

Macroalgae have been traditionally employed as biomonitors of eutrophication because their growth is rapidly enhanced by nutrient inputs

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(McClelland and Valiela, 1998; Piñón-Gimate et al., 2009). The $\delta^{15}N$ of the different macroalgal species shows a large variation, from 0.2 to 50.1‰ (Dailer et al., 2010). Besides the generalized use of macroalgae for monitoring nitrogen sources, only recent studies have addressed the influence of intrinsic or external factors on the variability of the isotopic values. The main intrinsic factors affecting variability of macroalgal $\delta^{15}N$ are the preferential mobilization of light isotopes (isotopic fractionation) during uptake, excretion and metabolic reactions (Teichberg et al., 2008) and intra-frond variability (Carballeira et al., 2014; Raimonet et al., 2013; Savage and Elmgren, 2004; Viana et al., in review). External factors are littoral position (Kim et al., 2013) or light (Dudley et al., 2010).

Fucoid species are long-lived species appropriate for detecting chronic nitrogen loadings and to obtain long-time integrative measures (Bode et al., 2011; Carballeira et al., 2013; Savage and Elmgren, 2004; Viana and Bode, 2013). As they are perennial, and they live in the intertidal area of a wide variety of environments, they are subject to a wide spatial and seasonal variability of abiotic factors and nitrogen sources. This variability might significantly influence the $\delta^{15}N$ in their tissues, but it is not known until what extent the variability observed in some studies is due to the sources or to the nitrogen metabolism in macroalgae (i.e. net fractionation of all processes occurring within macroalgal tissues). Simultaneous measures of both macroalgal and water $\delta^{15}N$ are thus required to detect the sources of this variability (Deutsch and Voss, 2006; Raimonet et al., 2013; Viana and Bode, 2013).

The coast of Galicia (NW Spain) is characterized by the presence of numerous estuaries and rias (tidal inlets) that are dually influenced by oceanic and terrestrial inputs (Viana and Bode, 2013). Oceanic inputs are characterized by spring-summer upwelling processes. This phenomenon naturally fertilizes the surface water from rias, and the initial inputs are amplified by remineralization of organic matter in the shelf and subsequent import with estuarine circulation (Álvarez-Salgado et al., 1996). Terrestrial inputs are represented by independent river basins draining in each ria. Riverine nutrient concentrations are generally higher than oceanic waters, but the low river flows compared to the water volume exchange by tides result in a small effect of the river water in the rias (Álvarez-Salgado et al., 1996; Bode et al., 2011). Nutrient concentrations of these continental inputs may be increased by the presence of urban nuclei and anthropogenic activities, as agriculture or cattle breeding. This region is therefore well suited to study the variability of stable isotopes due to the different nutrient sources present in the area (Bode et al., 2006, 2011, 2014; Carballeira et al., 2013; Viana and Bode, 2013). The variability of the nitrogen content of any effluent entering coastal waters is of considerable importance to management since it controls the level of primary production (Valiela, 1995) producing an imbalance between denitrification processes and inputs.

As brown macroalgae can discriminate between anthropogenic and natural nitrogen sources, variable N isotopic values were observed along impact sites (Carballeira et al., 2013; Gartner et al., 2002). The aim of this study is to determine if this variability of isotopic values is due to the differential impact of nitrogen sources or also to local or intrinsic factors of macroalgae. The impact of different nitrogen sources on the $\delta^{15}N$ of intertidal Fucaceae, *Ascophyllum nodosum* and the genus *Fucus* (*F. serratus*, *F. spiralis* and *F. vesiculosus*) along a salinity gradient was studied for the application of these species as biomonitors of nitrogen sources. As they are long-living species and show slow growth rates, long temporal scales (monthly and annual basis) were taken into account. To assess the possible origin of the isotopic variability in macroalgae, $\delta^{15}N–NO_3^- + NO_2^-$ and $\delta^{15}N–NH_4^+$ were also determined, along with nutrient concentrations, temperature and salinity of the seawater.

2. Material and methods

2.1. Study site

The study was conducted at Ría de A Coruña, NW Spain (Fig. 1), which can be divided into two areas. The inner part of the ria, called Ría do Burgo, has a steep salinity gradient (~9 to 33) and estuarine characteristics, with a mean depth of 10 m. The outer bay is 6 km long and has a width in the mouth of about 3 km, constituting a total area of about 24 km^2 (Varela et al., 1994). The bay includes a harbor area and a seawall, and it has a high influence of marine waters (Cabanas et al., 1987). The river Mero drains in the area, but the inorganic nutrient inputs of the river are considered to be less important than the fertilization by the upwelling regimes, especially in the bay. Dense population nuclei can be found at both sides of the ria, especially around the estuarine zone and in the western part (left bank) of the bay, the latter occupied by the city of A Coruña (~240,000 inhabitants). In contrast, the northern and eastern margins of the bay are characterized by mostly rural and residential landscapes. Several fucoid species are well distributed in the rocky intertidal areas of this ria from semi-exposed to wave protected areas (Bárbara et al., 1995). Only in the inner and middle estuary, sedimentation and accumulation of mud are noticeable, and other species such as vascular plants (as *Juncus* sp. or *Spartina* sp.) or *Zostera noltii* are present (Bárbara et al., 1995).

2.2. Sampling

To determine the impact of the different nitrogen sources on macroalgal and surface water nitrogen isotopic values and on nutrient concentrations, sampling was carried out at various spatial and seasonal scales. Fucoid species were selected and sampled according to their availability along the ria: *A. nodosum*, and one of several species of the genus *Fucus* (*F. serratus*, *F. spiralis* and *F. vesiculosus*).

The seasonal variability of isotopic values in macroalgae and DIN was investigated in samples collected at two sites: one in the estuarine area and one in the outer bay (Fig. 1). The estuarine site was located at Ría do Burgo (43°20′N, 8°22′W) in the central part of the estuary and affected by large salinity fluctuations. The oceanic influenced site was located at Mera (43°22′N, 8°20′W), which is a rocky semi-exposed beach near the outer limit of the bay. Sampling included the collection of water samples on a monthly basis from November 2010 to January 2012 ($n = 13$). Macroalgal samples were also collected with monthly frequency from October 2010 to November 2011 ($n = 13$). *A. nodosum* was only found at Ría do Burgo, while *F. vesiculosus* was sampled at both sites.

Interannual changes of macroalgal isotopic values were studied at the same sites that for seasonal variability, Ría do Burgo and Mera (Fig. 1). Samples collected previously along with new samples from this study were used. *A. nodosum* and *F. vesiculosus* were used for comparisons at Ría do Burgo. As no seasonal variability in $\delta^{15}N$ was observed at this site, averaged values of different periods were used for both species: Oct–Dec 2010 ($n = 3$), Jan–Nov 2011 ($n = 10$), and July 2013 ($n = 1$). At Mera, only *F. vesiculosus* samples collected in July 2011 and 2013 were used for the comparison. Additional samples of *F. vesiculosus* collected in July 2006 at this site for a previous study were also used.

The spatial variability and the impact of the different nitrogen sources were investigated in water and macroalgal samples collected along the Ría de A Coruña (Fig. 1). An arbitrary reference point was established at the river discharge point to compute relative spatial distances between sampling sites along the estuary. Macroalgae were collected at 27 sites on both banks of the ria in July 2013 (Fig. 1), while water samples were obtained from 5 sites (Fig. 1) between July 2009 and January 2012 ($n = 14$) in order to adequately represent water variability. *A. nodosum* and *F. vesiculosus* were sampled at the same site where available, but when the latter species was not present, any of the two other species of the genus *Fucus* (*F. serratus* and *F. spiralis*) were selected to complete the *F. vesiculosus* gradient as no differences in $\delta^{15}N$ were found among them in previous studies (Carballeira et al., 2013; Viana et al., 2011). Comparisons between values measured in water and macroalgae were made after classification of the sampling points in 0.5-km intervals according to their relative distance from the reference point.