



Isotopic signatures of eelgrass (*Zostera marina* L.) as bioindicator of anthropogenic nutrient input in the western Baltic Sea

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ABSTRACT

Eutrophication is a global environmental problem. Better management of this threat requires more accurate assessments of anthropogenic nitrogen (N) inputs to coastal systems than can be obtained with traditional measures. Recently, primary producer N isotopic signatures have emerged as useful proxy of such inputs. Here, we demonstrated for the first time the applicability of this method using the widespread eelgrass (*Zostera marina*) in the highly eutrophic Baltic Sea. Spatial availability of sewage N across a bay with one major sewage outflow predicted by eelgrass $\delta^{15}\text{N}$ was high near and downstream of the outflow compared to upstream, but returned to upstream levels within 4 km downstream from the outfall. General conclusions were corroborated by traditional eutrophication measures, but in contrast to these measures were fully quantitative. Eelgrass N isotope ratios therefore show high potential for coastal screens of eutrophication in the Baltic Sea, and in other areas with eelgrass meadows.

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1. Introduction

Eutrophication, the anthropogenic input of otherwise limiting nutrients to aquatic ecosystems, is among the largest human-induced global scale environmental alterations (Vitousek et al., 1997). Due to their vicinity to human activity on land, coastal ecosystems are notably affected. Of particular concern is the additional influx of nitrogen species, as primary production in coastal waters is mainly limited by N supply (e.g. Valiela et al., 1997). The three major sources of anthropogenic N in coastal waters are human sewage (wastewater), agricultural fertilizer, and atmospheric depositions (Cole et al., 2006), and differentiation between these sources is crucial for water-quality management. For example, sewage outfalls represent point source discharges that are easier to manage on a local scale than diffuse or atmospheric sources as long as information about the magnitude and spatial extent of effects are available. Increased inputs of sewage-derived N to estuaries and coastal areas have been shown to increase primary production and substantially alter pathways of N cycling (e.g. Bowen and Valiela, 2001; McClelland and Valiela, 1998).

The Baltic Sea is particularly sensitive to eutrophication due to its large and densely populated catchment area, its relatively small water body (Ranft et al., 2011), and limited water exchange with the North Sea (Fennel, 1995). Owing to these hydrographic characteristics, excess anthropogenic N has already led to a variety of ad-

verse effects on coastal ecosystems of the Baltic Sea including changes in primary production, loss of biodiversity, and alterations of food-web dynamics (HELCOM, 2009).

Assessment and reduction of N inputs are therefore focal points in intergovernmental programs for the protection and management of the marine environment of the Baltic Sea, including the Helsinki Commission Baltic Sea Action Plan (Backer et al., 2010) and the Marine Strategy Framework Directive of the European Union (MSFD, 2008). However, the integration of nutrient input over time from concentration measurements is difficult, due to high variability driven by rainfall patterns on the one hand (e.g. Fourqurean et al., 1997), and organismal consumption on the other (Costanzo et al., 2001).

A promising new tool has been the use of primary producers, which naturally integrate available nutrients via tissue growth. In particular, nitrogen stable isotope composition ($^{15}\text{N}/^{14}\text{N}$) of algae or plant tissue (Costanzo et al., 2005; McClelland et al., 1997; Savage, 2005) has been proven to be an effective indicator of anthropogenic N inputs and specifically sewage, which is enriched in ^{15}N isotopes due to volatilization of ^{14}N -rich ammonia during the process of nitrification and denitrification (Heaton, 1986; Macko and Ostrom, 1994). Water derived from sewage typically has $\delta^{15}\text{N}$ values of +10 to +20‰, while water influenced by atmospheric deposition has values of +2 to +8‰, and water loaded with fertilizer features values between −3 to +3‰ (Cole et al., 2006). Primary producers will integrate available nutrients over time, depending on their growth rate and longevity, and reflect those signatures. Moreover, primary producer isotopic signatures

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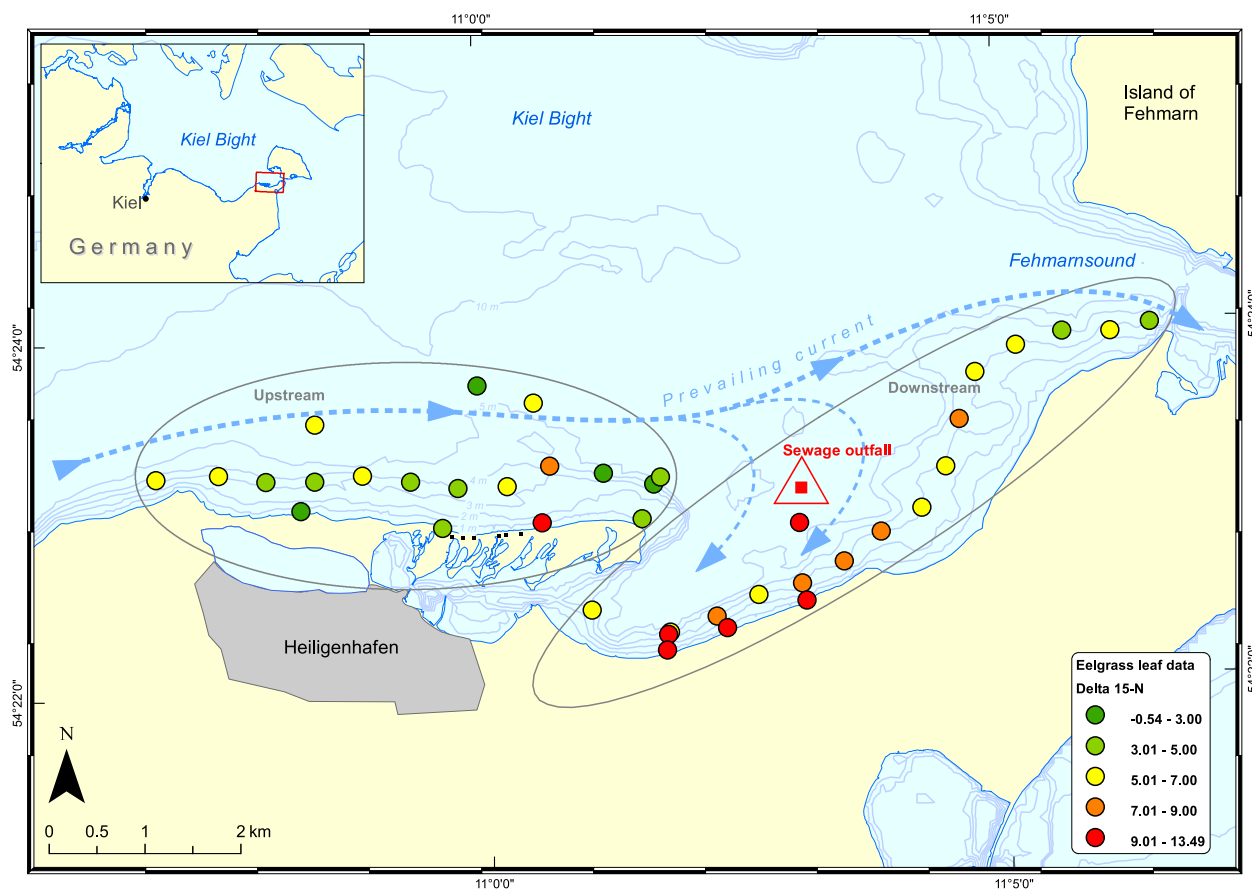


Fig. 1. Map of the study area and of sampling locations across Heiligenhafen Bay ($n = 40$), and eelgrass $\delta^{15}\text{N}$ ratios obtained in this study as proxy for sewage Nitrogen availability. Grey ellipses: samples considered upstream and downstream in respect to the sewage outfall, red triangle: location of the sewage outfall, colored circles: eelgrass leaf $\delta^{15}\text{N}$ values, blue dotted line: prevailing current direction, black squares: location of mentioned houses on the spit, insert: overview of western Baltic Sea, study site marked with red rectangle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

indicate the presence of anthropogenic nutrients long before major ecological changes can be observed in the field (Lepoint et al., 2004; McClelland et al., 1997). Considering the advantages outlined above, the use of bioindicators may be an ideal complement to traditional eutrophication measures, such as depth limits or epiphyte load of macrophytes, both employed in the Baltic Sea for the European Union Water Framework Directive (WFD, 2000).

In the western Baltic Sea, the seagrass species *Zostera marina* (eelgrass) is the most widespread, common, and abundant plant species (HELCOM, 1998), which in principle makes it the ideal indicator species for the large-scale assessment of anthropogenic nutrient inputs in this area. However, seagrasses have rarely been applied as “biosamplers” to date (but see Carruthers et al., 2005; Fernandes et al., 2009; McClelland et al., 1997), and never in the Baltic. Here, we test the validity of eelgrass as nitrogen indicator in the heavily nutrient affected south-western Baltic Sea. Heiligenhafen Bay (Fig. 1), which is characterized by a strong point source of anthropogenic N, served as model system. The general approach was a comparison of the expected distribution of sewage-derived N (based on the known sewage point source and prevailing current pattern) and the spatial pattern in eelgrass $\delta^{15}\text{N}$ ratios. Specifically, we tried to answer the following questions:

1. Can $\delta^{15}\text{N}$ ratios of eelgrass leaves in the Baltic Sea serve as proxy of time integrated sewage-derived N availability?
2. Provided they do, what do our data suggest about eutrophication in Heiligenhafen Bay and about patterns of eutrophication from point sources in general?

3. How do $\delta^{15}\text{N}$ ratios of eelgrass compare with other biological parameters?
4. How well do $\delta^{15}\text{N}$ ratios of eelgrass as proxy of sewage N availability fit with traditional indicators used to assess water quality with regard to eutrophication?

2. Methods

2.1. Study site

Heiligenhafen Bay is a small, shallow bay of Kiel Bight in the western Baltic Sea, northern Germany (area ca. 4.6 km^2 , $54^\circ 23' \text{N}$, $11^\circ 03' \text{E}$, Fig. 1). It is partly separated from Kiel Bight by a large sand bar (“Graswarder”). The spit is a nature reserve and marine protected area due to its importance for migrating birds. The bay and surrounding coasts are densely populated by eelgrass *Zostera marina* (own unpubl. data). Water depth at our study site ranged from 0.8 to 7.6 m. Salinity in the region varies between 8 and 18 psu depending on the inflow of fully saline North Sea water. Tides are negligible, but wind driven water level changes are common. The climate regime is cold temperate with water temperatures ranging from 2°C in February to 18°C in August. One wastewater treatment plant (WWTP), Luetjenbrode-Nord, discharges sewage from the city of Heiligenhafen and its surroundings (approx. 24,300 inhabitants) via a pipeline into the center of the bay, ca. 1100 m offshore (Fig. 1). It provides state-of-the-art tertiary treatment prior to disposal. The annual total effluent from Luetjenbrode-Nord is approx. $2,000,000 \text{ m}^3$, annual total N input

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