Estuarine, Coastal and Shelf Science xxx (2014) 1–9

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Dissolved inorganic and organic nitrogen uptake in the coastal North Sea: A seasonal study

Alessia Moneta [a, *], Bart Veuger [a], Pieter van Rijswijk [a], Filip Meysman [a, b], Karline Soetaert [a], Jack J. Middelburg [a, c]

[a] Department of Ecosystem Studies, Royal Netherlands Institute for Sea Research NIOZ-Yerseke, PO Box 140, 4400 AC Yerseke, The Netherlands
[b] Laboratory of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[c] Faculty of Geosciences, Utrecht University, PO Box 80021, 3508 TA Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 1 August 2013
Accepted 25 May 2014
Available online xxx

Keywords:
nitrogen uptake
phytoplankton
bacteria
dissolved organic nitrogen
stable isotopes and biomarkers
D-alanine
The Netherlands
Wadden sea
Marsdiep tidal inlet (53.001833° N, 4.789201° E)

ABSTRACT

Nitrogen incorporation into total particulate suspended matter, hydrolysable amino acids and bacterial biomarker D-Alanine was assessed seasonally in the coastal North Sea using ^{15}N-labeled ammonium, nitrate, nitrite and ^{15}N- and ^{13}C-labeled urea, glycine, leucine, phenylalanine, and two complex pools of dissolved organic matter (DOM) derived from algal and bacterial cultures (A-DOM, B-DOM). We investigated: 1) uptake rates for the various substrates and their contribution to total N uptake; 2) microbial preferences for the different N sources; 3) the coupling of C and N uptake from organic substrates; 4) the contribution of bacteria to the total microbial uptake of these substrates, and 5) the role of a complex pool of organic matter for plankton nutrition. Seasonality in the preferences for N substrates was observed, with A-DOM and B-DOM being preferred in autumn and winter whereas NH_4^+ was preferentially taken up in spring and summer. C and N uptake was coupled for all the organic substrates, except urea that was mainly used as a nitrogen source in summer and spring. Bacterial contribution to the uptake of A-DOM and B-DOM was, on an annual average, the lowest among the N-substrates. This suggests an important role for phytoplankton in the incorporation of complex organic matter and the importance of DOM for phytoplankton nutrition.

1. Introduction

Nitrogen (N) is an essential element for growth of phytoplankton and heterotrophic bacteria and is often limiting in marine and coastal waters. It can be present in dissolved inorganic forms (DIN: NH_4^+, NO_3^-, NO_2^-) or as dissolved organic nitrogen (DON), a complex pool whose chemical composition varies spatially and temporally (Benner, 2002). The chemical composition of this heterogeneous DON pool is difficult to characterize and only a small fraction (<20%) of the individual compounds can be typically identified (Benner, 2002). This fraction includes urea, amino acids, amino sugars, humic and fulvic substances, as well as nucleic acids. DON typically accounts for 10–20% of the total dissolved nitrogen in coastal waters and ~80% in open ocean systems, but its role in phytoplankton nutrition was considered negligible (Antia et al., 1991; Benner, 2002; Berman and Bronk, 2003).

However, more than fifty years ago the ability of phytoplankton to take up DON was demonstrated by Hattori (Antia et al., 1991). Moreover, there is increasing evidence that natural communities of phytoplankton and bacteria can rely on a much greater range of N substrates than traditionally believed (Andersson et al., 2006; Bradley et al., 2010a; Van Engeland et al., 2013). The DON contribution to the total N uptake range from ~20% to up to 80% and phytoplankton can rely more on organic N when competition with bacteria for DIN is high. However, these studies have used a limited range of simple organic substrates, mainly urea and amino acids, whereas a more complex and heterogeneous pool of DON is available in marine ecosystems. Few studies have examined whether natural planktonic microbial communities can utilize this large, complex pool (Bronk and Glibert, 1993; Veuger et al., 2004; Van Engeland et al., 2013).

Another important aspect that remained understudied is the relative importance of phytoplankton versus bacteria in total microbial N assimilation. Early works from the 1970's (Goering et al., 1970; Eppley et al., 1977) already suggested bacterial use of DIN showing that heterotrophs were important in the total uptake of

* Corresponding author.
E-mail address: alessia.moneta@nioz.nl (A. Moneta).

http://dx.doi.org/10.1016/j.ecss.2014.05.022
0272-7714/

DIN in different systems. More recent studies showed that a large fraction of the bacterial production is supported by NH_4^+ and that bacteria could switch to NH_4^+ and NO_3^- when the supply of dissolved free amino acids is insufficient. Moreover, bacteria appear able to outcompete phytoplankton in the uptake of NH_4^+ at low concentrations (Hoch and Kirchman, 1995; Middelboe et al., 1995; Kirchman, 2000; Jørgensen, 2006). The assessment of the relative contribution of phytoplankton versus bacteria to N uptake, remains a challenging issue. Methods applied so far are size fractionation, flow-cytometric sorting, the use of specific metabolic inhibitors, or a combination of these (Veuger et al., 2004; Bradley et al., 2010b; Trottet et al., 2011). However, each suffers from limitations such as overlapping sizes fractions and limited efficiency and specificity of inhibitors (Glibert et al., 1991; Bradley et al., 2010b; Trottet et al., 2011). Recently, the use of stable isotope tracers in combination with biomarkers, has enabled better discrimination between the algal and bacterial contribution to N and C uptake in natural systems. The use of the hydrolysable amino acid D-alanine, a structural component of bacteria, has proven a powerful tool to estimate the bacterial contribution to the uptake of N by benthic microorganisms (Veuger et al., 2005; 2007).

Another aspect of microbial N-assimilation is the use of N versus C from organic substrates. While organic N is primarily used to support growth, the coupled organic C may either be used for growth (assimilation) or be used as a source of energy (respiration). Few studies have studied the relative use of C and N from organic substrates by natural communities (Bronk and Glibert, 1993; Fan and Glibert, 2005; Andersson et al., 2006; Veuger and Middelburg, 2007). These have demonstrated that DON and DIN uptake by natural marine communities, as well as competition for different substrates, vary both spatially and temporally, highlighting the need for further investigations (Middelburg & Nieuwenhuize, 2000; Veuger et al., 2004; Fouilland et al., 2007; Bradley et al., 2010a; Bronk et al., 2007).

To the best of our knowledge, in this study, we are the first one investigating monthly over a whole year the uptake of DIN, urea, dissolved free amino acids (DFAA) and two complex DON pools by phytoplankton and bacteria in the water column of the coastal North Sea. The use of ^{15}N and ^{13}C labeled organic substrates and stable isotope analysis of bulk suspended particulate matter (SPM) and of hydrolysable amino acids (HAA) therein allowed us to investigate 1) the importance of various DIN and DON substrates in total microbial N assimilation, 2) microbial preferences for the various N substrates, 3) the coupling between N and C from organic N-substrates, 4) the role of algae versus bacteria in total microbial N assimilation, and 5) the potential importance of uncharacterized DON as source of N for microbial growth.

2. Materials and methods

2.1. Study site and sampling

Water was sampled from the Marsdiep from the pier of the Royal Netherlands Institute for Sea Research on the island of Texel (53.001833° N, 4.789201° E), starting in October 2009 until October 2010. The Marsdiep is the most southwestern tidal inlet of the Wadden Sea and is connecting the Western Wadden Sea to the Southern North Sea. Even though it is an outflow channel, at high tide it receives coastal North Sea water (Cadée and Hegeman, 1993). Samples were always collected at high tide, meaning that we sampled coastal North Sea water. Due to strong tidal currents, these waters are fully mixed (i.e. sampled water represents an average for the whole water column).

A pump and hose apparatus was used to collect surface water in 10 L white plastic containers, one for each treatment described below, and the ^{15}N- and ^{13}C-containing substrates were added immediately after sampling. Due to time, budget and other logistic constraints, replicates were unfortunately not performed.

2.2. Incubations

NH_4^+ ($^{15}NH_4Cl$, 99%), NO_3^- ($Na^{15}NO_3$, 98%) and NO_2^- ($Na^{15}NO_2$, 98%) were used as inorganic substrates, whereas urea (^{13}C, 99%; $^{15}N_2$, 98%), L-glycine ($U^{13}C_2$, 98%; ^{15}N, 98%), L-leucine ($U^{13}C_6$, 98%; ^{15}N, 98%) and L-phenylalanine ($U^{13}C_9$, 98%; ^{15}N, 98%) (all from Cambridge Isotope Laboratories) were used as simple, well-defined organic substrates with different structural complexity. In addition, two pools of complex DOM were derived from an axenic algal culture and a bacterial culture grown on ^{15}N and ^{13}C labeled substrates. For the algae derived DOM (referred to as A-DOM), an axenic culture of the diatom *Skeletonema costatum* was grown in artificial sea water containing F2 medium using 30% $NaH^{13}CO_3$ (^{13}C, 99%) and 15% $Na^{15}NO_3$ (^{15}N, 98%). For the bacteria derived DOM (B-DOM), a bacterial sample isolated from waters of the Eastern Scheldt (a marine coastal bay in the southwest of the Netherlands) was grown on the modified medium M63 (Miller, 1978) with 15% NH_4Cl (^{15}N, 99%) and 15% D-glucose ($U^{13}C_6$, 99%). After approximately 3 weeks of incubation, algal and bacterial material was harvested through filtration and suspended in Milli-Q water inside centrifuge tubes. To eliminate all the DIN, Devarda's Alloy (to reduce NO_x to NH_4^+) and MgO (to convert all NH_4^+ to NH_3) were added to the tubes, which were then shaken for 48 h to remove all gaseous NH_3 from the water. This DIN removal procedure has been tested extensively as reported in Veuger et al. (2004). Following three consecutive steps of centrifugation and collection of the supernatant, organic material was filtered onto 0.2 μm polycarbonate filters (Millipore) to isolate only the dissolved fraction. Final isotope enrichments of A-DOM and B-DOM were 12.4% and 16.7%, respectively for N (^{15}N atom %) and 5.8% and 14.7%, respectively for C (^{13}C atom %).

^{15}N enriched substrates were targeted to be added at a tracer level of 10% of ambient concentrations. The latter were based on data from previous years, which were retrieved from the DONAR database (Rijkswaterstaat, 2009) via the Waterbase website (http://live.waterbase.nl). Added ^{15}N in the urea and, on some occasions, in the DIN incubations was higher than 10% of the ambient concentrations, but we will discuss in more detail the implications of this in the sections 3.2 and 4.1. DFAA concentrations were always very low (<0.1 μM) which made labeling at true trace level very difficult. For this reason labeled DFAA were added at concentrations of 0.1 μmol L^{-1}. Finally the unknown chemical composition of the natural DON pool made it not possible to estimate percentages of ^{15}N addition for our complex DOM tracers. Final concentrations of ^{15}N in the incubations, C:N ratios, mean percentages of label addition and the incubation time for each tracer, are summarized in Table 1.

Sample containers were mixed manually at three times: immediately after addition of the tracers, after approximately 2 h of incubation, and immediately before water filtration. Incubations were performed in a water bath at ambient light and temperature and lasted 4–6 h (see Table 1). Incubations were ended by filtration on pre-combusted (450 °C for 4 h) GF/F filters which were rinsed with filtered sea water and then immediately frozen and stored at −20 °C until further analysis. Approximately 2 L of water was filtered (GF/F) for extraction of hydrolysable amino acids from suspended particulate matter, while about 1 L was filtered on pre-weighed GF/F filters for bulk SPM analysis.

2.3. Analytical methods

Concentrations and isotopic ratios of particulate organic C and N were measured using a Thermo EA 1112 elemental analyzer

Please cite this article in press as: Moneta, A., et al., Dissolved inorganic and organic nitrogen uptake in the coastal North Sea: A seasonal study, Estuarine, Coastal and Shelf Science (2014), http://dx.doi.org/10.1016/j.ecss.2014.05.022