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Benthic primary production and bacterial denitrification in a Mediterranean eutrophic coastal lagoon

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ABSTRACT

Microphytobenthos and macroalgal mats are simultaneously present in eutrophic lagoons and are expected to have different direct and indirect effects on nitrogen related processes through uptake and inhibition or stimulation of microbial activity. To assess the relative contribution of different primary producer communities and heterotrophic processes to benthic nitrogen cycling, we studied nitrogen uptake and bacterial denitrification in the eutrophic Sacca di Goro lagoon (northern Italy). Benthic fluxes of oxygen and dissolved inorganic nitrogen (DIN), and rates of nitrification-coupled (Dn) and water–nitrate (Dw) denitrification were measured every 30–45 days for one year at two shallow sites. Station Giralda is close to the main freshwater inlet, has turbid waters and muddy-organic and bioturbated sediments with microphytobenthos (MPB). Station Gorino is brackish with muddy-sand sediments which are covered by macroalgal mats of the genus *Ulva*. Here, sediment patches with and without macroalgae (MA) were simultaneously studied. Sediments with MPB were net heterotrophic and regenerated large amounts of ammonium to the water column. At Gorino, sediments with MA were net autotrophic through the year, and DIN fluxes were mainly controlled by macroalgal uptake. On an annual basis, denitrification rates were three fold higher at Giralda (2.27 ± 0.06 mol N m^{-2} yr^{-1}) than at Gorino (0.83 ± 0.01 mol N m^{-2} yr^{-1}), due to higher nitrate in the water column and nitrification in surface sediments. At Gorino, denitrification was one order of magnitude lower than DIN uptake by macroalgae (10.39 ± 1.30 mol N m^{-2} yr^{-1}). Nevertheless, the differences between denitrification rates in sediments with and without MA were unexpectedly negligible, showing that the denitrification capacity was not suppressed by macroalgal competition. Results from this study suggest that in eutrophic lagoons nitrogen cycling seems less affected by MPB compared to more oligotrophic coastal waters and that most of the available DIN flows through benthic macroalgae. However, *Ulva* is only a temporary N-sink and most of its nitrogen pool can be either rapidly recycled or exported by tidal currents to the open sea.

1. Introduction

Eutrophic coastal lagoons display high rates of both primary production and respiration; the balance between these opposite processes can be extremely variable over small spatial and temporal scales, with implications for oxygen availability and nutrient cycling (Diaz and Rosenberg, 2008; Risgaard-Petersen, 2003; Viaroli and Christian, 2003). Anthropogenic nitrogen loads to coastal areas are demonstrated to increase the rates of primary production and organic matter input to surface sediments and to alter the composition of the benthic vegetation (Galloway et al., 2004; Middelburg and Levin, 2009; Valiela et al., 1997). The relative biomass abundance and the activity of benthic vegetation, viz seagrasses and macroalgae, and microphytobenthos (Burkholder et al., 2007; McGlathery et al., 2007; Viaroli et al., 2008) can greatly influence the fluxes of nitrogen at the water–sediment interface (Bartoli et al., 2003; Dalsgaard, 2003; Sundbäck et al., 2000; Tyler et al., 2003; Welsh et al., 2000a). A large body of literature has explored nitrogen cycling within seagrass meadows, as rooted macrophytes are particularly vulnerable to the increase of reactive nitrogen (Burkholder et al., 2007; McGlathery et al., 2007). Healthy meadows are generally found in oligotrophic, well flushed coastal areas, with elevated light penetration, and are generally net autotrophic during the vegetative period (Welsh et al., 2000a). In areas with seagrass meadows, the main nitrogen pool is within sediments and nitrogen fluxes are mainly driven by root and leaf uptake (Bartoli et al., 2008; Risgaard-Petersen et al., 1998). While marine plants compete with denitrifiers for nitrogen and have scarce oxygen transport capacity to the roots, limiting subsurface nitrification, they stimulate bacterial nitrogen fixation, that contributes to a variable fraction of the plant N requirement (Ottosen et al., 1999; Welsh, 2000b).

The interactions between microphytobenthos (MPB) and benthic nitrogen cycling were also explored in detail. In autotrophic sediments

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with benthic microalgae, direct nutrient uptake in the MPB layer can retain inorganic nitrogen and significantly attenuate its regeneration. The MPB activity can either stimulate or depress nitrification and denitrification, depending upon N availability (Bartoli et al., 2003; Risgaard-Petersen, 2003; Sundbäck et al., 2000). Photosynthesis by MPB at the sediment–water interface can expand the oxic sediment horizon with a potential positive effect on nitrifiers (Revsbech et al., 1981). Conversely, MPB metabolism removes NH_4^+ and CO_2 from the porewater, occasionally resulting in pH levels of 9–10, which inhibit nitrification and, consequently, the coupled denitrification (Focht and Verstraete, 1977). Furthermore, oxygen production and consumption at the sediment–water interface regulate the thickness of the microoxic surficial layer, and as a consequence the pathway of nitrate from the water to the anoxic denitrification zone (Risgaard-Petersen et al., 1994).

By comparison, some studies were addressed to the interactions between macroalgal mats (MA) and benthic nutrient cycling (McGlathery et al., 2004, 2007; Sundbäck and Miles, 2002; Sundbäck et al., 2003; Tyler et al., 2003; Villares and Carballeira, 2003) while a few studies explored the interactions between the activity of macroalgae and denitrification (Dalsgaard, 2003; Krause-Jensen et al., 1999; LaMontagne et al., 2002; Trimmer et al., 2000a). Macroalgal blooms are primarily sustained by the DIN supply from the water column, although the nitrogen requirement can be recovered from sediments, via intense recycling of both ammonium and dissolved organic nitrogen, or from internal nitrogen pools (Naldi and Viaroli, 2002; Sundbäck et al., 2003; Trimmer et al., 2000b; Tyler et al., 2003). Dalsgaard (2003) demonstrated that even if limited to a short time lag (1–2 months), the transient accumulation of macroalgal mats in a shallow fjord had the potential to change the annual primary productivity and nutrient budgets. He demonstrated that macroalgal production can switch from a heterotrophic, net nitrogen releasing sediment into an autotrophic, net N-sink one. In eutrophic warm waters, MA may attain growth rates up to 0.3 d^{-1}, which are mainly sustained by their capacity to take up and store dissolved inorganic nitrogen (DIN) sources, especially nitrate (Naldi and Viaroli, 2002). At peak biomass, macroalgae such as *Ulva* sp. usually attain a standing stock of some hundred g m^{-2} as dry weight, with net nitrogen uptake rates ranging from 10 to 20 μmol g^{-1} h^{-1}. Under these circumstances, most of DIN flux is likely controlled by MA (Viaroli et al., 2005), with a probable suppression of nitrate loss via denitrification (Krause-Jensen et al., 1999; LaMontagne et al., 2002; Trimmer et al., 2000a). However, the MA biomass is only a temporary sink, which suddenly and rapidly becomes a nitrogen source for the system when the MA mat collapses in summer, causing the so called "dystrophic crises" (Viaroli et al., 1996, 2008).

The main hypotheses of this study are that in Mediterranean eutrophic lagoons, where MA are present all year round, macroalgae drive a major fraction of DIN fluxes, suppress N loss via denitrification and have a stronger effect on the benthic metabolism compared to MPB. To test these hypotheses, we assessed primary production and nitrogen uptake by MPB and MA in the Sacca di Goro (Po River Delta, Northern Italy) and we compared N retention by MPB and MA with N losses via denitrification.

2. Material and methods

2.1. Study sites

The study was performed at two field sites within the eutrophic Sacca di Goro lagoon (Viaroli et al., 2006). Station Giralda (44° 49′ N 12° 17′ E) is located in the western part of the lagoon, close to the inlet of turbid, nutrient-rich freshwater from the Po di Volano canal; water depth averages 70 cm (Fig. 1). Sediments are generally bioturbated by surface (i.e. *Corophium insidiosum*) and deep (i.e. *Neanthes succinea*) burrowers and consist of a soft mud colonised by benthic diatoms. Station Gorino (44° 48′ N 12° 19′ E) has a mean depth of 60 cm and muddy-sand sediments. It is located in the eastern area of the Sacca di Goro, where drifting mats of the macroalga *Ulva* sp. develop. Long term monitoring of this site suggests a strong control of water hydrochemistry by MA, with inorganic nitrogen drops and oxygen peaks during the spring, when macroalgae exhibit maximum growth rates (Viaroli et al., 2006). Station Giralda and station Gorino are micro-tidal, with average daily depth variations of ±30 cm.

2.2. Sampling programme

Measurements were performed in the framework of a European project (NICE, Nitrogen Cycling in Estuaries, contract MAS3-CT96-0048), according to an experimental approach defined during an intercalibration workshop and detailed in a protocol method handbook (Dalsgaard et al., 2000). Undisturbed sediment samples were collected from station Giralda on 03.26.97, 05.10.97, 06.18.97, 07.29.97, 09.5.97, 11.11.97, 12.10.97, 01.13.98 and 02.17.98. Sediment samples were collected with cores of 2 different dimensions (diameter×length), each for a distinct purpose: sediment characterisation (5×30 cm, n=3) and flux measurements, (8×40 cm, n=6 to 10). At Gorino sediments were collected in patches with floating mats of *Ulva* sp. and in areas devoid of macroalgae on 04.03.97, 06.10.97, 08.21.97, 09.18.97, 10.16.97, 11.26.97, 01.27.98 and 03.10.98. Sediment characterisation and flux measurements in sediment without MA was performed as detailed for station Giralda (same cores, same replicates) while fluxes in sediments covered with MA were determined in squared Plexiglas chambers (n=6) (see below). Sediment in the cores and chambers used for flux measurements were levelled to a thickness of about 10 cm; the water column overlying sediments had a depth of about 30 cm. Water volume in cores with MA-free sediments was about 1.5 l while that in chambers with MA was about 12 l.

At both sampling sites about 100 l of water were collected on every sampling campaign for core maintenance, preincubation and incubation procedures.

2.3. Sediment features

Benthic microalgal biomass was measured in triplicate as chlorophyll-*a* (Chl-*a*) concentration in the top 0.5 cm of sediment and determined spectrophotometrically after extraction with 90% acetone (Lorenzen, 1967). Bulk density was determined as the ratio between wet weight and volume (typically 5 ml) of sediment. Organic matter content (OM) was measured as percentage of weight loss by ignition (450 °C, 2 h) from dried sediment.

Biomass of macroalgae was estimated by random positioning of a plastic frame over the sediment surface (30×30 cm, n=5), harvesting of enclosed vegetal matter, drying at 60 °C after removal of ephiphytes and other organisms and weighing.

2.4. Flux measurements

Fluxes of DIN and O_2 across the sediment–water interface and denitrification rates were measured during light and dark incubations of intact sediment cores and flux chambers. Sediments with MPB from stations Giralda and Gorino were collected by a hand corer and maintained in cylindrical Plexiglas liners; water stirring was ensured by a 4 cm long Teflon-coated magnetic stirring bar, suspended 6 cm above the sediment surface and driven by an external rotating magnet at 40 rpm. Three to five cores were incubated in the light and three to five cores were incubated in the dark. Sediment sampling in areas covered with MA was done with a hand held box corer made of 1 mm thick steel plate, fitting precisely Plexiglas flux chambers (20×20×40 cm, w×d×h, n=6). The corer was pushed 10 to 15 cm into the sediment, dug out with a shovel, transferred underwater in the flux chamber and then carefully removed. When removing the corer a gap of 2 to 3 mm was left between the sediment and flux chamber walls, however, the sediment block expanded horizontally,