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The impact of suspended oyster farming on nitrogen cycling and nitrous oxide production in a sub-tropical Australian estuary

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ABSTRACT

In this study we quantified nitrate (NO_3^-) reduction (denitrification, anammox and DNRA) and N_2O production in sediments and epibiont communities associated with Sydney Rock Oyster (*Saccostrea glomerata*) farming. In sediments beneath an active suspended oyster farm, DNRA accounted for 98% of NO_3^- reduction with rates of up to 169 ± 45 μmol N m^{-2} h^{-1}. Much of this DNRA was fuelled by NO_3^- derived from nitrification. Reference sediments had significantly lower DNRA rates of 83.8 ± 28.2 μmol N m^{-2} h^{-1}, however this constituted 96% of the sites total NO_3^- reduction. Fatty acid analysis showed that sediment organic matter was more labile in the oyster impacted sediments, facilitating subtle shifts in sediment oxygen demand which increased the Fe^{2+} availability with respect to the reference sediments. The difference in DNRA rate between the sites was attributed to autotrophic oxidation of soluble Fe^{2+} in sediments underlying the oyster cultures. DNRA was absent in the oyster shell epibiont communities and rates of anammox and denitrification were lower than in the sediments. Production of NH_4^+ from the oysters and their associated epibionts was larger than DNRA and reached a rate of 206.2 μmol N m^{-2} h^{-1}. Nitrous oxide production rates were generally low compared to other aquaculture systems and the net flux of N_2O for the combined oyster cultivation system (i.e. sediments plus epibionts) was negative, i.e. there was N_2O consumption in the sediments beneath the oysters. Overall, subtropical suspended oyster farming systems favour inorganic N retention over N loss.

1. Introduction

Mollusc aquaculture is a significant contributor to the world's total aquaculture value and volume (11.5% and 22.8% respectively) (FAO, 2014) and bivalve aquaculture accounts for some 70% of global mariculture. In Australia, farming of the Sydney Rock Oyster (*Saccostrea glomerata*) generated ~3000 tonnes of product worth $33 M in 2012 (DPI, 2014). While bivalve aquaculture is clearly a major contributor to global fisheries supply, there is uncertainty over the impact that it has on estuarine nitrogen cycling.

On the one hand, some studies conclude that filter feeding bivalves can contribute to the net removal of nitrogen (N) from coastal environments through the assimilation of suspended nutrients and enhanced denitrification in underlying sediments (Edebo et al., 2000; Carlsson et al., 2012; Smyth et al., 2013). Indeed suspended bivalve cultivation has for many decades been regarded as a mechanism for the 'bio-remediation' of eutrophic estuaries (Petersen et al., 2014; Nielsen et al., 2016). Conversely however, there is also strong evidence to suggest that bivalve cultivation performs an ecosystem disservice by 1) promoting sulphidic conditions and nutrient export from the underlying sediments through the local deposition of faeces and pseudofaeces, and 2) enhancing nutrient recycling in the water column due to the constant excretion of inorganic nutrients by the cultivated organisms (Christensen et al., 2003; Nizzoli et al., 2006, 2011; Higgins et al., 2013; Murphy et al., 2016).

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Whilst these conflicting conclusions may be the result of local factors such as the type, extent and intensity of bivalve cultivation and/or the hydrodynamics, eutrophication status etc. of the study site, part of the uncertainty arises as few studies have comprehensively assessed the overall impacts of bivalve aquaculture on nutrient dynamics. Many studies have focussed exclusively on the exchange of dissolved and particulate nutrients between the water column and cultivated organisms or the impacts of biodeposition on the nutrient fluxes from the underlying sediments (Christensen et al., 2000; Higgins et al., 2013; Lavoie et al., 2016). Furthermore, there is ambiguity surrounding the magnitude and direction of N losses in bivalve impacted systems due to the presence and measurement of different nitrate reduction pathways including denitrification, anammox and DNRA. While the first two are mechanisms of permanent N loss, DNRA acts to retain N within the environment (Tiedje et al., 1982). Hence the balance between DNRA and N_2 production is crucial for understanding the net role of suspended aquaculture operations on local nutrient budgets. To date only a handful of studies have measured the individual sediment nitrate reduction pathways within aquaculture production systems (Gilbert et al., 1997; Christensen et al., 2000; Nizzoli et al., 2006; Castine et al., 2012; Murphy et al., 2016); no aquaculture related studies have measured all three nitrate reduction rates simultaneously and there are no published rates of NO_3^- reduction beneath suspended Sydney Rock Oyster farms.

DNRA is proposed to be favoured over denitrification as the labile organic carbon to NO_3^- ratio (OC: NO_3^-) increases, and also in the presence of sulphide and/or Fe^{2+} (Tiedje et al., 1982; Straub et al., 1996; An and Gardner, 2002; Giblin et al., 2013; Hardison et al., 2015); typical of the conditions usually found in organic rich sediments. Consequently DNRA would be expected to be stimulated in sediments receiving aquaculture biodeposits. In the few aquaculture related studies where sediment DNRA has been measured, its rate was between 4 and 25 fold higher than N_2 production (Gilbert et al., 1997; Christensen et al., 2000; Nizzoli et al., 2006; Murphy et al., 2016). The paucity of information regarding the contribution of DNRA to nitrate reduction in sub-tropical suspended bivalve dominated estuaries means that a reliable assessment of their ecological impact cannot be made. This is an important knowledge gap given that oyster farming occurs in 17% of the estuaries along the New South Wales coast (DPI, 2014).

Another reason for the uncertainty in our understanding of bivalve aquaculture on local estuarine N budgets is the fact that very few studies have included estimates of the contribution of biofilms associated with bivalve aquaculture on N cycling (Holmer et al., 2015; Welsh et al., 2015). Bivalve shells are colonised by microbial biofilms and/or a thick layer of epiphytes that may contribute significantly to N cycling, however much of the emphasis of past work has been on the sediments (Welsh and Castadelli, 2004; Heisterkamp et al., 2013; Caffrey et al., 2016). Of the two studies that have looked at the contribution of bivalve biofilms to biogeochemical cycling, both found that the biofilms made a similar, and at times greater, contribution to inorganic N fluxes, oxygen demand and denitrification as did the sediments (Holmer et al., 2015; Welsh et al., 2015).

Also lacking in most aquaculture N cycling related studies is an assessment of how production influences N_2O dynamics. If suspended bivalve aquaculture enhances NO_3^-reduction, particularly denitrification in the underlying sediments, then one could reasonably expect a concomitant increase in N_2O production. Increased organic matter mineralisation and NH_4^+ production may also promote nitrification and N_2O production via nitrifier denitrification, especially under low oxygen conditions (Goreau et al., 1980). Bivalves themselves release N_2O produced by denitrifying organisms within surface biofilms and their digestive systems (Stief et al., 2009; Heisterkamp et al., 2013). Gut denitrification in particular can be an important source of N_2O, as differential expression of the genes for denitrification during the transition from oxic to anoxic conditions that occurs following ingestion can result in incomplete denitrification and high N_2O yields (Stief et al., 2009; Heisterkamp et al., 2016). Given the variable oxygen supply to sediments, the availability of organic matter, and the capacity of the animals themselves to release N_2O, one would assume that sediments associated with bivalve aquaculture would be hotspots of N_2O generation. However in high C loaded systems such as mangrove lined estuaries, N_2O can also be consumed (Erler et al., 2015b). While there are some measurements of N_2O production from individual bivalves (Heisterkamp et al., 2010), to date there are scant measurements of N_2O production from bivalve aquaculture systems (Welsh et al., 2015), and indeed from aquaculture operations in general (Hu et al., 2012). As yet there are no N_2O production rates available for Sydney Rock Oysters or Rock Oyster farms in Australia.

The overall objective of this study was to make a comprehensive evaluation of the impacts of oyster farming on the nitrogen dynamics of a sub-tropical estuary. More specifically we aimed to 1) determine the contribution of DNRA to NO_3^- reduction in sediments impacted by suspended oyster cultivation, 2) determine the relative contributions of the oysters, their shell epibiont communities, and the underlying sediments to inorganic N cycling, and 3) determine whether suspended oyster cultivation is a source or sink for N_2O.

2. Methods

2.1. Study site

Wallis Lake is a sub-tropical estuary system located on the south east coast of Australia. Geographical and geological details of the Wallis Lake estuary are given in Eyre and Maher (2010) and Maher and Eyre (2010). Two sampling sites were selected within the major oyster growing area of the estuary, one directly within an active oyster lease (in its 6th year of operation) (32.1835°S, 152.4884°E), and a second 'reference' site without active oyster cultivation (32.1816°S, 152.4829°E) (see Fig. 1 and KML file associated with this submission). Due to the patchy distribution of oyster leases in the studied estuary, a true un-impacted control site could not be studied, as it would have to be so far removed from the oyster cultivation area that it would not be comparable in terms of background biogeochemistry or hydrodynamics. Therefore we chose a 'reference' site as the comparison rather than a control. Both sites were within the tidal reach of the estuary.

2.2. Core collection and maintenance

Five undisturbed sediment cores (~25 cm) were manually collected in plexiglass core tubes (50 cm length x 9 cm internal diameter), from both sites for flux and process rate measurements during low tide on the 3rd Nov 2015 (water depth ~ 50 cm). Spare cores were also collected to gauge O_2 consumption. An additional set of 5 cores (~15 cm) were also collected from each site with PVC core tubes (20 cm length x 10 cm diameter) for later deployment of diffusive gradient and diffusive equilibration in thin films samplers (DGT and DET respectively). At each site, sediment samples (x3) were collected for C:N, loss on ignition (LOI), total Fe analysis, and fatty acid composition (polyunsaturated fatty acids (PUFA) and saturated fatty acid (SAFA). This involved colleting 10 cm of surface sediment using cut-off 50 ml syringes. Site water (100 L) was collected from a nearby boat ramp during the incoming flood tide.

All cores were transported to the field laboratory within 1 h and