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Variation in benthic metabolism and nitrogen cycling across clam aquaculture sites

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ABSTRACT

As bivalve aquaculture expands globally, an understanding of how it alters nitrogen is important to minimize impacts. This study investigated nitrogen cycling associated with clam aquaculture in the Sacca di Goro, Italy (*Ruditapes philipinarum*) and the Eastern Shore, USA (*Mercenaria mercenaria*). Ammonium and dissolved oxygen fluxes were positively correlated with clam biomass; *R. philippinarum* consumed ~6 times more oxygen and excreted ~5 times more NH_4^+ than *M. mercenaria*. There was no direct effect of clams on denitrification or dissimilatory nitrate reduction to ammonium (DNRA); rather, nitrate availability controlled the competition between these microbial pathways. Highest denitrification rates were measured at sites where both water column nitrate and nitrification were elevated due to high densities of a burrowing amphipod (*Corophium* sp.). DNRA exceeded denitrification where water column nitrate was low and nitrification was suppressed in highly reduced sediment, potentially due to low hydrologic flow and high clam densities.

1. Introduction

The presence of bivalve aquaculture in coastal ecosystems has large implications for coastal nitrogen (N) dynamics. As nutrient pollution continues to be problematic in coastal waters worldwide concurrent with the rapid expansion of the bivalve industry (FAO, 2014), the influence of bivalve aquaculture on N removal pathways is of increasing interest. Implementing bivalve aquaculture as a means to promote N removal and mitigate coastal eutrophication is a current topic of debate (e.g. Stadmark and Conley, 2011; Rose et al., 2012). Effective resource management requires an understanding of the net effect of bivalve cultivation on N cycling, both recycling and removal pathways, and particularly how this changes with different environmental conditions. This study investigates the mechanistic drivers that influence the effects of clam cultivation on benthic N cycling pathways by sampling two clam species that are farmed across a range of environmental conditions.

As infaunal organisms, cultivated clams both directly and indirectly affect sediment N cycling rates and benthic metabolism through bioturbation, biodeposition, excretion, and respiration, which subsequently influence microbial metabolic pathways (reviewed in Laverock et al., 2011). Clam bioturbation transports particles and water, including solutes (e.g. O_2, NO_3^-), through sediments. Through feeding and biodeposition, clams actively deliver organic carbon to the sediments from the water column, fueling microbial decomposition pathways, enhancing microbial respiration and oxygen demand, and thereby substantially changing redox gradients (Aller, 1982; Kristensen et al., 1985) and impacting redox sensitive microbial processes such as nitrification and denitrification (Stief, 2013). Benthic infauna, including cultivated clams, also excrete dissolved inorganic and organic N, directly increasing benthic N fluxes to the water column and potentially providing substrate (e.g. NH_4^+) for microbial processes such as nitrification and ANAMMOX (Welsh et al., 2015). Bivalves can thus influence both microbial N removal and recycling pathways in coastal sediments.

Bivalves may enhance N removal by promoting denitrification, the microbially mediated pathway that reduces nitrate (NO_3^-) to inert N_2 gas. This bivalve-facilitated, denitrification enhancement results both from biodeposition of organic matter to sediment microbial communities (Newell et al., 2002; Kellogg et al., 2013; Smyth et al., 2013) and

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by provision of habitats for denitrifying microorganisms (Heisterkamp et al., 2010; Welsh et al., 2015). However, some studies have reported no significant effect of bivalves on denitrification rates (Higgins et al., 2013; Erler et al., 2017). Additionally, often overlooked is the effect bivalves have on inorganic N regeneration pathways. High densities of bivalves, found in cultivation settings, may accelerate N recycling processes through bivalve excretion and stimulation of microbial ammonification including dissimilatory nitrate reduction to ammonium (NH_4^+) (DNRA) (Dame, 2011; Murphy et al., 2016; Erler et al., 2017), which retain bioavailable N in the aquatic ecosystem.

The question of whether bivalves promote N removal or retention remains equivocal. The discrepancy among previous studies on how bivalves influence benthic N cycling pathways is in part due to differences in the bivalve species studied (e.g. epifaunal oysters or mussels versus infaunal clams), but also likely due differences in the environmental conditions under which bivalves are farmed. Bivalve aquaculture can occupy expansive regions across estuarine environmental gradients. Few studies that investigate N cycling associated with bivalve aquaculture, and specifically clam aquaculture, have captured the natural environmental variability across which the practice exists. Moreover, few studies have investigated the partitioning of NO_3^- reduction between denitrification and DNRA, which is ecologically important as DNRA retains bioavailable N in the system as NH_4^+ whereas denitrification removes it. Those studies that do provide simulatenouse measurements of denitrification and DNRA are restricted to single study systems. Therefore, we were interested in directly comparing different study systems, which are heavily exploited for infaunal clam cultivation and where previous studies found contrasting results regarding denitrification and DNRA at clam cultivation sites. We chose to sample clam aquaculture in the Sacca di Goro, Italy, where denitrification was reportedly higher than DNRA (Nizzoli et al., 2006) and in coastal Virginia, US, where DNRA generally dominated NO_3^- respiration (Murphy et al., 2016).

The objective of this study was to investigate how sediment N cycling associated with clam aquaculture varies across different environmental conditions and between two commonly cultivated infaunal clam species: *Ruditapes philipinarum* (Italy) and *Mercenaria mercenaria* (US). Across the natural environmental gradients in which clam aquaculture exists, we were specifically interested in (1) comparing N cycling and metabolic rates between the two cultivated clam species and determining the direct contribution of these clams to benthic rates and (2) determining the factors that influence the competition between microbial denitrification and DNRA at clam aquaculture sites. By studying two clam species exposed to different environmental conditions and farming practices, we sought to highlight the challenge in generalizing ecological responses across all bivalve aquaculture and, more specifically, across all clam cultivation practices. We hypothesized that both clam species would significantly increase benthic oxygen demand and inorganic N fluxes; however, the contribution of clams to these benthic processes would differ across sites depending on site-specific factors and clam species physiology. We expected that the degree to which N is removed through denitrification relative to N recycled through DNRA would change depending on the availability of labile organic carbon and NO_3^- (Tiedje, 1988), factors that would vary broadly across estuarine gradients and clam aquaculture sites.

2. Methods

2.1. Study sites

The Sacca di Goro is a lagoonal system of the Po River Delta, Italy. Approximately 26 km^2 with an average depth of 1.5 m, the lagoon hosts a substantial clam aquaculture industry, with about 1/3 of the area occupied by clam cultivation. The system is generally divided into three areas based on hydrologic characteristics: the eastern portion is shallow with low energy and slow water exchange; the central region is tidally influenced, and the western portion is riverine dominated with freshwater flow from the Po di Volano (Fig. 1A). The lagoon, particularly the eastern region, typically experiences periodic dystrophic events in the early summer when macroalgae bloom. Drastic changes to the hydrodynamics of the system were made over the past 20 years to improve water flow and alleviate dystrophic events, including channel construction along the southern sand spit and dredging of internal canals to increase flow to the Adriatic Sea (Viaroli et al., 2006). The manila clam, *R. philippinarum*, is farmed in privately leased portions of the lagoon at densities ranging from 100 to > 2000 individuals m^{-2}. Growers collect juvenile clams at the mouth and directly outside the lagoon, transport them to individual leases within the lagoon; after approximately 9 months market-sized clams are hydraulically harvested.

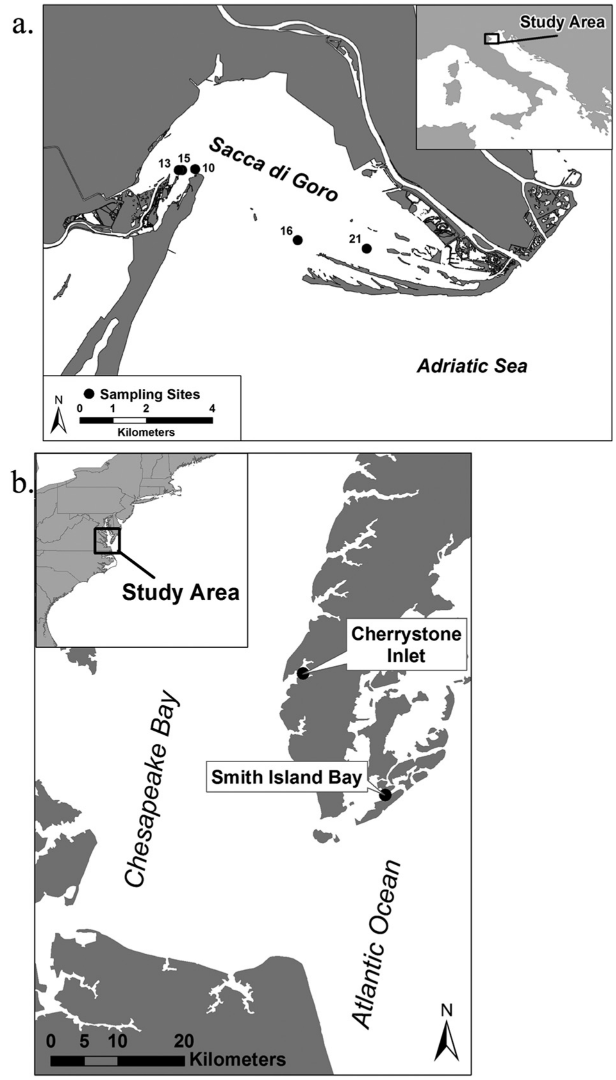

Fig. 1. Study sites in the Sacca di Goro, Italy (a) and the Eastern Shore, VA, USA (b).

Clam aquaculture occupies large subtidal areas on both the Chesapeake Bay-side and Atlantic-side of the Eastern Shore peninsula of Virginia (Emery, 2015). Cherrystone Inlet (ES-23), located on the Chesapeake Bay-side of the Eastern Shore, is a small shallow embayment (~6 km^2, mean water depth of 1.1 m) that receives little freshwater discharge. Smith Island Bay (ES-33) is the southern-most lagoon, located on the eastern side of the Eastern Shore and is protected from the Atlantic Ocean by a barrier island (Fig. 1B). In both locations, the hard clam, *M. mercenaria*, is cultivated in privately owned leases in subtidal regions. Clams are sourced from land-based hatcheries and nurseries and planted in the sediments at ~8–15 mm in shell length.