

Microbial associations with macrobiota in coastal ecosystems: patterns and implications for nitrogen cycling

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In addition to their important effects on nitrogen (N) cycling via excretion and assimilation (by macrofauna and macroflora, respectively), many macrobiota also host or facilitate microbial taxa responsible for N transformations. Interest in this topic is expanding, especially as it applies to coastal marine systems where N is a limiting nutrient. Our understanding of the diversity of microbes associated with coastal marine macrofauna (invertebrate and vertebrate animals) and macrophytes (seaweeds and marine plants) is improving, and recent studies indicate that the collection of microbes living in direct association with macrobiota (the microbiome) may directly contribute to N cycling. Here, we review the roles that macrobiota play in coastal N cycling, review current knowledge of macrobial–microbial associations in terms of N processing, and suggest implications for coastal ecosystem function as animals are harvested and as foundational habitat is lost or degraded. Given the biodiversity of microbial associates of macrobiota, we advocate for more research into the functional consequences of these associations for the coastal N cycle.

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Nitrogen (N) is often a limiting nutrient in coastal marine systems, but human activities have doubled the availability of this nutrient over the past century, particularly via fertilizer production to sustain increases in agriculture (Galloway *et al.* 1995). In coastal ecosystems receiving anthropogenic N, excess N can fuel harmful algal blooms, and the decomposition of organic (dead

algal) material via bacterial respiration depletes dissolved oxygen (DO), potentially leading to hypoxia (Howarth *et al.* 2011). However, coastal ecosystems (estuaries, marshes, reefs, and the nearshore pelagic ocean) are also well-known sites of N removal via microbial activity. This removal attenuates the land–sea flux of N and can ameliorate the effects of N pollution in coastal regions. At the same time, newly recognized forms of microbial N metabolism, such as anammox, conversion of nitrite (NO_2^-) and ammonium (NH_4^+) directly into gaseous nitrogen (N_2) (Table 1), and the identification of new microbial contributors (eg archaea; Könneke *et al.* 2005) have increased our appreciation of the complexity of coastal N cycling. In open waters, light and DO gradients in the water column govern microbial transformations of the marine N cycle, and “new” N is primarily supplied by upwelling and biological N fixation (Figure 1; Table 1). Coastal systems, however, also host a diversity of macrobiota (multicellular flora and fauna) that contribute to N cycling through N production, retention, and removal (eg Nelson *et al.* 2013). Macrobiota can also locally change DO concentrations via metabolic activities, thereby promoting a range of N metabolisms over a scale of only micrometers to millimeters (Figures 2 and 3; Table 1). Macrofauna and macroflora clearly host microbial communities that are capable of a diversity of N metabolisms (Figures 2 and 3), though our understanding is still limited. As the distribution and abundance of marine macrobiota change rapidly due to harvest pressure, invasions, habitat fragmentation, pollution, other local disturbances, and global climate change, the effects

In a nutshell:

- Genomic discovery methods reveal a diversity of microbial taxa associated with coastal marine species
- The metabolic activities of marine flora and fauna are an important component of nitrogen (N) cycling in coastal marine environments
- Microbial associates of marine macrobiota facilitate N processing in coastal ecosystems, including ameliorating negative environmental consequences, such as eutrophication associated with animals' nitrogenous waste
- To aid management and to better predict coastal ecosystem function, we argue for an improved understanding of the microbial associates of marine macrobiota and their quantitative contributions to coastal N cycling

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Table 1. Key microbial nitrogen metabolisms in the coastal ocean

Nitrogen metabolism	Definition
Anammox (anaerobic ammonium oxidation)	Chemoautotrophic organisms from the bacterial phylum Planctomycetes convert nitrite (NO_2^-) and ammonium (NH_4^+) directly into gaseous nitrogen (N_2).
Denitrification	Heterotrophic facultative anaerobic bacteria reduce nitrates (NO_3^-) and NO_2^- to ultimately produce N_2 via intermediate gaseous nitrogen oxide intermediates (NO and N_2O).
Dissimilatory nitrate reduction to ammonium (DNRA)	Reduction of NO_3^- to NH_4^+ via NO_2^- intermediate by microbial organisms.
Nitrification	A two-step transformation by bacteria and archaea where NH_4^+ is oxidized to NO_2^- , which is further oxidized to NO_3^- .
Nitrogen fixation	Atmospheric elemental nitrogen (N_2) is converted into biologically available forms (eg NH_4^+).

of these changes on associated microbial diversity and N cycling functions are unknowns in coastal biogeochemistry and ecology. Here, we evaluate the current understanding of the role of microbial associations with macrobiota in coastal N processing, highlighting where these associations could be important at an ecosystem level.

Although N is a key element in amino acids and therefore critical to all life on Earth, the N cycle includes multiple transformations (eg N fixation, nitrification, denitrification, dissimilatory nitrate reduction to ammonium [DNRA], anammox; Table 1) carried out primarily or exclusively by microorganisms. Increasingly, scientists are discovering bacterial and archaeal roles in N transformations, and it has recently become apparent that associations with macrobiota enhance these transformations. Indeed, review of the macrobiota-associated N transformations indicates that every link in the N cycle has identifiable microbe–macrobiota associations in the natural environment that can alter the rate of N cycling (Figures 2 and 3; Table 2). We highlight this rapidly emerging area of interaction between microbes, animals, and macrophytes (seaweeds and marine plants).

Macrobiota can locally transform the environment in ways that favor particular microbial activities. First, macrobiota can serve as a predictable and comparatively resource-rich surface for microbial populations, especially for those environments where water motion is constant and solid substrate is limited. Thus, the surfaces of macrobiota can be a renewing physical resource for microbial colonization. This contrasts with soft sediment environments where the mud–sand matrix is persistent, providing a stable spatial structure for a microbial population (Laverock *et al.* 2011).

A second key role specific to macrofauna is as a source of regenerated N as urea or NH_4^+ , which is quantitatively important to productivity in a range of marine systems (Gilbert *et al.* 1982; Bracken 2004; Roman and McCarthy 2010). A critical role for animal excretion (production of N-containing compounds [NH_4^+ , urea] as by-products of metabolic processes) is demonstrated across diverse taxa, from zooplankton (Dugdale and Goering 1967; Saba *et al.* 2011) to whales (Roman and McCarthy 2010). Although this N is ultimately derived from N uptake lower in the

food chain, its availability as NH_4^+ , a comparatively more accessible form of dissolved inorganic nitrogen (DIN) for microbes, means that macrobiota and their aggregations contribute to biogeochemical hotspots in aquatic systems and ameliorate nutrient limitation. This is especially the case for biogenic habitats (formed by or produced by living organisms) such as reefs and kelp beds (Allgeier *et al.* 2013). Further, filter feeders can locally concentrate N from filtering particulate organic nitrogen (PON) from a large volume of water (Pather *et al.* 2014). Animal aggregations can deliver 5- to 177-fold increases in N loading over anthropogenic N delivery, even in areas highly disturbed by human activities (Allgeier *et al.* 2013). Similarly, large marine mammals transport and concentrate oceanic N near the sea surface through release of large fecal plumes at feeding areas. The contribution of marine mammals (estimated to be 2.3×10^4 metric tons N yr^{-1}) exceeds the combined inputs from all rivers in the Gulf of Maine system (Roman and McCarthy 2010). Mussel aggregations in coastal areas have been shown to augment NH_4^+ concentrations (Aquilino *et al.* 2009) and are associated with increased N processing (Pfister *et al.* 2014a). In saltmarshes within the southeastern US, intertidal pools with small nekton (free-swimming organisms) experienced a 17–109% increase in N concentration as compared with pools without nekton (Galloway *et al.* 1995). The relatively low energetic cost to macrophytes of using NH_4^+ as a dissolved inorganic N source may increase the importance of animal contributions. In sum, in N-limited coastal systems, animals can have a major positive effect on coastal productivity (Figure 2).

■ Macrobiota as microbial N-cycling hotspots

Diversity of marine microbe–macrobiota associations

The number of described microbial associations with macrobiota in the coastal oceans has expanded rapidly in recent years and now covers a diversity of marine taxa, including invertebrates (WebTable 1), macrophytes (WebTable 2), and vertebrates (WebTable 3). These studies indicate broad phylogenetic diversity of

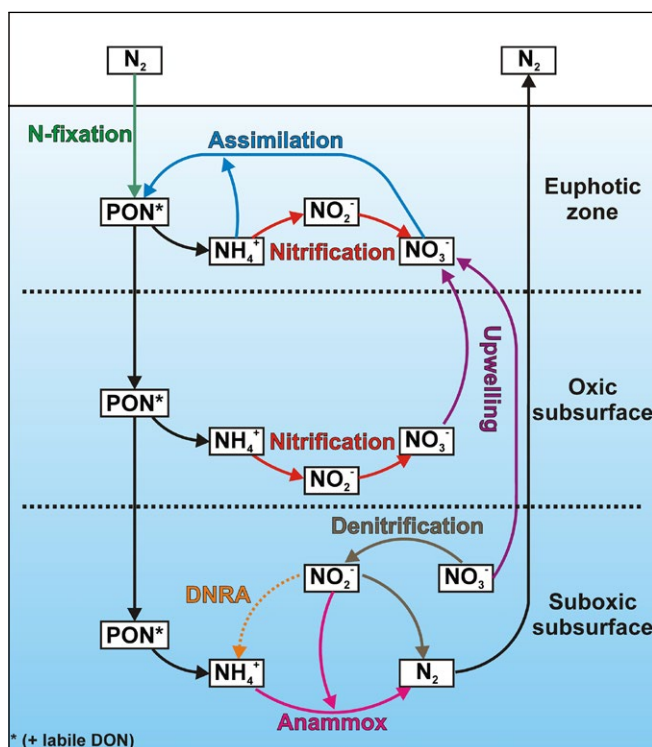


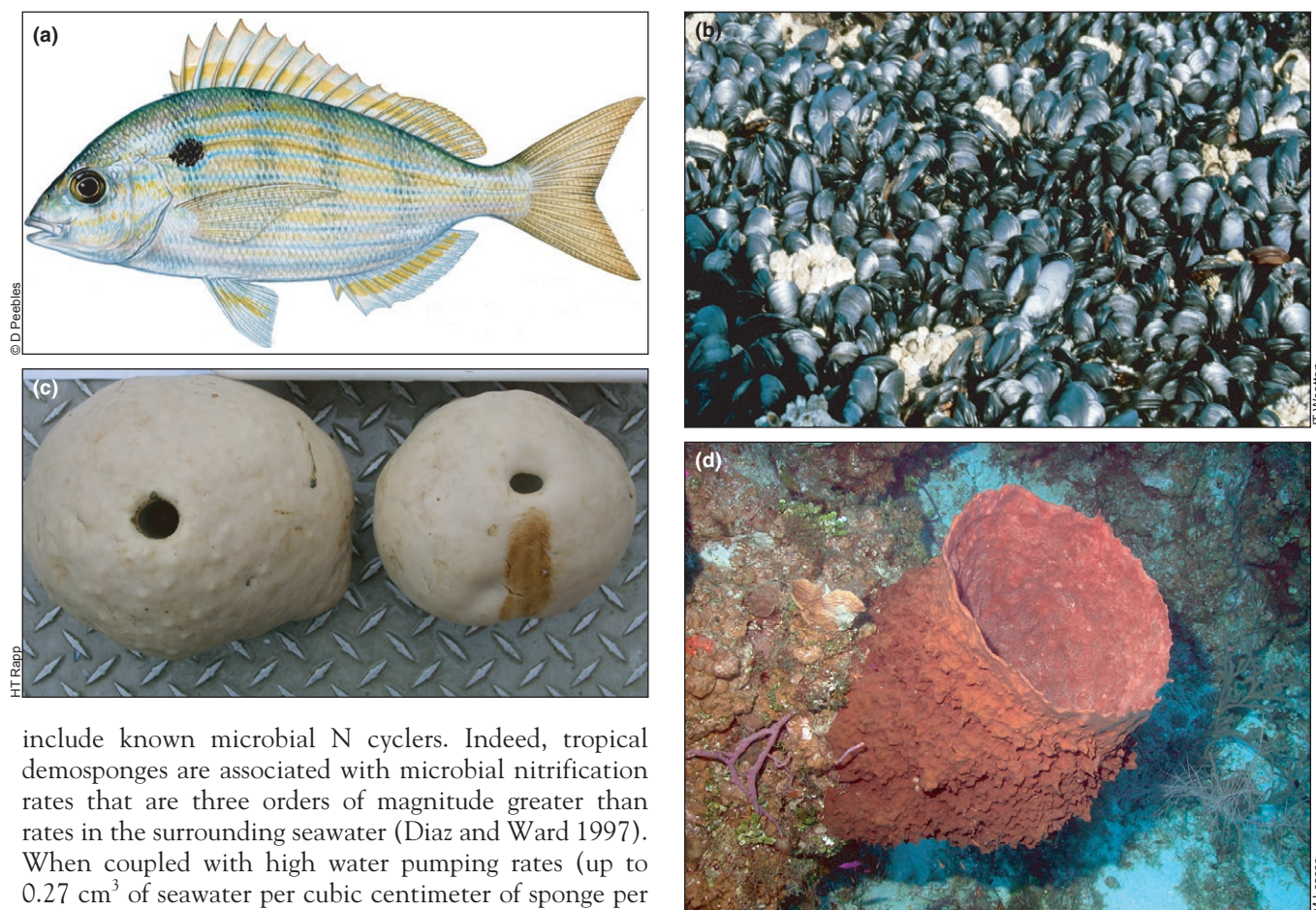
Figure 1. A schematic diagram of three different environments in the ocean. In the euphotic zone, aerobic processes and assimilation dominate, and nitrogen (N) inputs come from mainly upwelling and mixing of nitrate (NO_3^-) from deeper waters. Where light is limiting but the environment remains oxic, aerobic N transformations occur. Where suboxic conditions exist, anaerobic N transformations dominate. Dissolved oxygen (DO) levels can depend on the metabolic activities of animals and thus may influence the dominant N metabolisms. PON: particulate organic nitrogen, DON: dissolved organic nitrogen. For additional detail, see Capone *et al.* (2008).

microbial taxa hosted on and within macrobiota, and there is emerging evidence of selection for or promotion of particular microorganisms by macrobiota (Sunagawa *et al.* 2010; Apprill *et al.* 2014) when compared to surrounding seawater. For example, marine macrophytes enhance local microbial diversity relative to surrounding unvegetated sediments (Delille *et al.* 1996), and new microbial taxa have been discovered in association with seagrass (Lucas-Elío *et al.* 2011). Distinct microbial communities are found on the surface of seagrass leaves (Törnblom and Søndergaard 1999), roots and rhizomes (Nielsen *et al.* 2001), and in the rhizosphere (Shieh *et al.* 1989). Selection for particular microbes by macrobiota is demonstrated by some macrobiotic species hosting a shared “core” microbiome (collection of microorganisms in a shared location) among individuals; for instance, despite their geographic isolation, humpback whales (*Megaptera novaeangliae*) from different ocean basins possess similar skin-surface-associated bacterial communities (Apprill *et al.* 2014).

Understudied functions of marine microbe–macrobiota associations

Within this overall microbial diversity, a small but growing number of studies have shown unique microbe–macrobiota associations involving known microbial N-cycling taxa (Table 2). In some cases, it is possible to identify these organisms by 16S ribosomal RNA (rRNA) gene sequencing, because certain families have defined and specific N-cycling roles. This is true for N-fixing and anammox bacteria, as well as nitrifying archaea and bacteria (Francis *et al.* 2007; Zehr and Kudela 2011). In contrast, some N-cycling processes are sustained by a wide diversity of microbial groups and are considered “broad” processes: denitrification, for instance, is performed by microorganisms from all three domains of life (Archaea, Bacteria, Eukaryota) (Zumft 1997). For these processes, metagenomics – in which a mixture of primarily microbial genetic material is recovered directly from environmental samples (rather than isolates) and sequenced – has provided insight into host-associated microbial functional diversity. This is particularly powerful when paired with N-cycling rate measurements and can be used to determine linkages between a species of macrobiota, their hosted microbes, and N cycling. For example, Ribes *et al.* (2012) showed that, among three sponge species, two host diverse microbial communities with high nitrification and NH_4^+ uptake rates, whereas the third species hosts a low microbial diversity community with low measured N uptake rate. Though the two sponge species hosting a high diversity of microbes both demonstrated rapid N metabolism, the microbial communities associated with each sponge species were distinct (Ribes *et al.* 2012). The potential “functional convergence” of these microbial communities was supported by metagenomic analyses, which revealed that genes for different enzymes in the denitrification pathways were present in both microbial communities, although the taxonomic composition differed (Fan *et al.* 2012).

Microbial associates of macrobiota (Figures 2 and 3; Table 2) may result in either N loss or N fixation. Nitrogen loss occurs via denitrification (Heisterkamp *et al.* 2013), while animal excretion of NH_4^+ may accelerate N loss through anammox (Bianchi *et al.* 2014). Because they also host known N fixers (Fiore *et al.* 2010), animals may act as direct conduits of N flux to and from coastal ecosystems. To date, tropical corals and sponges are the best-understood macrobiota (eg Knowlton and Rohwer 2003), and corals are viewed as “holobionts” consisting of the coral animal, its photosynthetic symbionts, and coral-associated bacteria and archaea (Rohwer *et al.* 2002) that interact through linked carbon (C) and N cycling. Sponges appear to harbor specific bacteria (of the phylum Poribacteria) that



include known microbial N cyclers. Indeed, tropical demosponges are associated with microbial nitrification rates that are three orders of magnitude greater than rates in the surrounding seawater (Diaz and Ward 1997). When coupled with high water pumping rates (up to 0.27 cm^3 of seawater per cubic centimeter of sponge per second [Reiswig 1974]), these sponges are substantial contributors to N processing in local seawater nutrient profiles, where concentrations of nitrate (NO_3^-) are typically at the nanomolar level (Southwell *et al.* 2008). Sponges are similarly important for C cycling on reefs (de Goeij *et al.* 2013), showing dissolved organic carbon (DOC) cycling rates that equal those of plankton in the water column, and demonstrating how the C and N cycles can be linked by animal activity. Marine animals such as these filter-feeding sponges are effective integrators, concentrators, and processors of relatively large volumes of seawater and its chemical and biological constituents.

A key way in which sessile animals promote a diverse array of N metabolisms is by generating strong DO gradients through their respiration. Due to photosynthetic symbionts at the interface between the animal and surrounding seawater, sponge and coral surfaces are highly zoned with respect to DO, as are the nitrifying microbes hosted at the oxygenated surface and denitrifying taxa found in the deeper, anoxic areas (Fiore *et al.* 2010). Shelled marine invertebrates also have sharp DO gradients, with values varying from 0 to $1200 \mu\text{M}$ over a scale of less than 3 mm from gut to shell (Heisterkamp *et al.* 2013). In a review of animal effects on nitrification and denitrification in soft sediment communities, the presence of animals increased nitrification by a factor of 3.0 and denitrification by a factor of 2.4. Further, we expect

Figure 2. Several groups of marine macrofauna (invertebrates and vertebrates) have demonstrated the ability to locally alter the surrounding seawater DO levels and promote N metabolisms. (a) Pinfish (*Lagodon rhomboides*), by moving from nearshore seagrass habitat to offshore waters, export remineralized N with excretion rates greater than the sum of local river and submarine groundwater discharge (Nelson *et al.* 2013). (b) High rates of nitrification have been measured in association with sessile marine invertebrates such as *Mytilus galloprovincialis* (Welsh and Castadelli 2004) (closely related *Mytilus trossulus* pictured). (c) Anammox has been measured in association with sponge tissue, as evidenced by depletion of NH_4^+ and NO_3^- paired with production of gaseous N_2 in airtight vials containing the sponge *Geodia barretti* (Hoffmann *et al.* 2009). (d) Sponges are net sinks of NO_3^- , providing evidence that denitrification likely occurs in association with these organisms in cases where the host sponge slows or stops pumping and tissue becomes hypoxic/anoxic (Fiore *et al.* 2010).

that N fixation by microbial populations (via the nitrogenase enzyme) will be favored under low DO conditions, while near-anoxic conditions can drive anaerobic N loss processes (denitrification and anammox), assuming that NO_3^- is available. The removal of biologically available N could result in the production of nitrous oxide (an intermediate for denitrification and an ozone-

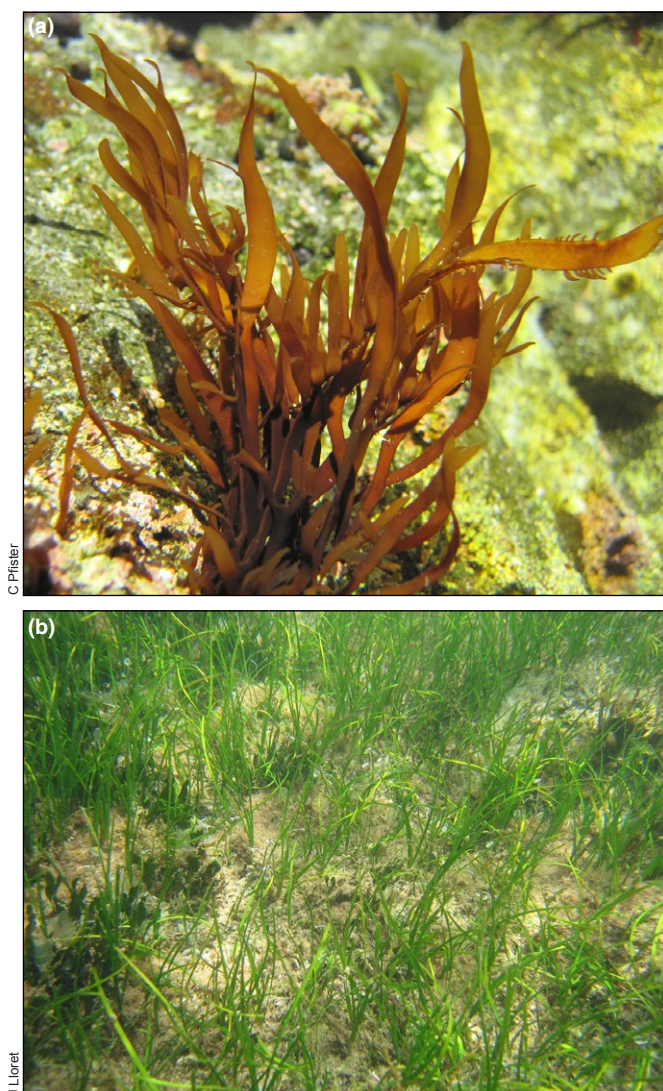


Figure 3. As with macrofauna (Figure 2), marine macroflora (seaweeds and plants) differentially promote microbial N-metabolizing associates and processes via alteration of local seawater DO levels. (a) The Pacific Northwest intertidal seaweed *Prionitis sternbergii* assimilates invertebrate-excreted NH_4^+ from local seawater (Pather *et al.* 2014). (b) Nitrogen fixation occurs at high rates in rhizospheric sediments of seagrasses as a function of plant–bacteria interactions (Welsh 2000) (*Cymodocea nodosa* pictured).

destroying greenhouse gas), as demonstrated within the anoxic guts of diverse animal taxa (Stief *et al.* 2009). Production of nitrous oxide was highest in filter- and deposit-feeders – groups that could increase in abundance with coastal eutrophication (nutrient enrichment) (Murray *et al.* 2015). Macrophytes also affect DO gradients, through photosynthesis and respiration. Although microbial associations with marine macroflora are only just beginning to be described (eg Miranda *et al.* 2013), there is great potential for macrophyte–microbial interactions via shared resources such as C and N.

Positive ecological roles of macrobiota as hosts for microbial function

Although species diversity in marine systems can affect ecosystem function (Worm *et al.* 2006), these systems are also home to species that play particularly crucial foundational, dominant, or keystone roles (Paine 1966; Power *et al.* 1996; Estes *et al.* 2011). Across multiple ecosystems, keystone species – including seastars and sea otters – are those with effects on a community trait (eg species diversity) that are disproportionate to their abundance. Abundant species with structural importance are termed foundational species and are represented in marine systems by seagrasses, algae, and mussels – these have key, community-level effects, often via habitat provision. Ecologically dominant species are those with higher abundance than that of competitors within an ecosystem, with or without relatively higher impact on ecosystem dynamics. Due to their documented importance in terms of abundance or ecosystem-level influence, we expect foundational species, keystone species, and ecological dominants to be particularly important with regard to the N cycle. Shallow coastal marine foundational habitats such as coral reefs, marsh grasses, seagrasses, mangroves, and mussel beds facilitate the existence of other species and provide ecosystem services (eg Bracken 2004), as well as host microbial nutrient processing (Welsh 2000; Rosenberg *et al.* 2007). Although information is limited on the effects of foundational species loss on microbial communities in marine habitats, work in terrestrial ecosystems shows major negative consequences for ecosystem structure (Ellison *et al.* 2005), and thus related microbial community function. Worldwide, foundational species, as well as ecological dominants, are experiencing rapid declines as a result of overharvesting, pollution, and global environmental change (Orth *et al.* 2006), but are also the target of restoration efforts. Because foundational species likely serve as critical hosts for microbial contributors to N cycling, it is necessary to understand whether the microbiome shapes the functional outcome of restoration.

If a particular animal or macrophyte species is capable of enhancing N metabolisms, then do any of these macrobiota have disproportionate effects? Are there foundational species, keystone species, or ecological dominants with respect to the metabolism of N in coastal marine ecosystems? Certainly, foundational species could have a correspondingly greater impact in N transformations due to the surface area they provide via thalli (vegetative tissue) in the case of macrophytes, or the shells, skin, and carapaces of animals. The shell area of animals has been shown to harbor from 50% to 94% of the observed N function (eg bivalves [Welsh and Castadelli 2004; Heisterkamp *et al.* 2013]), suggesting that abundance and body/thallus size scales directly with contribution to microbial activity.

Table 2. Nitrogen-cycling processes demonstrated to occur in association with macrobiota, and their ecological importance

Nitrogen cycling process	Macrobiota association(s)	Insight from association	Quantifying the effect	Reference(s)
Nitrification	Corals, sponges, bivalves, benthic crustaceans, benthic polychaetes	Microbes can compete with zooxanthellae for N	Benthic macrobiota-associated nitrification rates are threefold higher than background rates	Corredor <i>et al.</i> (1988); Diaz and Ward (1997); Fiore <i>et al.</i> (2010); Hentschel <i>et al.</i> (2002); Wafar <i>et al.</i> (1990)
Denitrification/anammox	Corals, sponges, bivalves, benthic crustaceans, benthic polychaetes	Animals generate low DO microsites that allow anaerobic N cycling to occur	On average, benthic macrobiota-associated denitrification rates are 2.4-fold higher than background	Fiore <i>et al.</i> (2010); Heisterkamp <i>et al.</i> (2013); Pfister <i>et al.</i> (2010); Hoffmann <i>et al.</i> (2009)
Nitrous oxide production	Bivalves and other mollusks	Animals generate low DO microsites that allow anaerobic N cycling to occur	Animals may increase N ₂ O emissions by 32–103%; extremely high N ₂ O yields relative to overall N cycling (ca 50%)	Stief <i>et al.</i> (2009); Heisterkamp <i>et al.</i> (2013)
Nitrogen regeneration and transport	Zooplankton	Animals provide ammonium for anammox	Association enhances oceanic N loss via anammox by 27–40%	Bianchi <i>et al.</i> (2014)
Nitrogen fixation	Corals, sea urchins, ship-worms, sponges	Animals generate low DO microsites that facilitate this process; may be of particular importance in symbioses	N-fixation is elevated relative to background rates in sediments and water	Fiore <i>et al.</i> (2010); Lesser <i>et al.</i> (2004); Cardini <i>et al.</i> (2014)

Microbe–macrobiota associations as novel avenues for species interaction

In addition to the need to understand direct fitness linkages between host macrobiota and microbial communities, it is important to consider how interactions could indirectly affect other species. Indirect effects have been a focus in ecological research for more than two decades (Wootton 1994), and the new appreciation for microbial diversity and function explores novel ways in which species interact. For instance, NH₄⁺ excreted by polychaete worms (*Hediste diversicolor*) is utilized by surrounding algae but also supplies nitrifying bacteria that provide NO₃⁻ to these primary producers (Heisterkamp *et al.* 2012). Benthic-regenerated N likely enters the water column, as up to one-half of the nutrients used by phytoplankton in the coastal ocean are produced in coastal sediments (Jørgensen 1983). Similarly, the metabolic activities of schooling fish concentrate N through remineralization and enhance N locally for other species, including primary producers (Durbin and Durbin 1998). In the case of seagrasses, which often serve as foundational species, alterations to the forms of N available are expected throughout the community. For example, N fixation is enhanced in the seagrass rhizosphere (Welsh *et al.* 1996), representing concentrated “new” N for the seagrass, as well as for other species (Duarte *et al.* 2005). Not only does animal-based nutrient remineralization enhance microbial metabolisms (Welsh and Castadelli 2004), it also increases eukaryotic access to N (Bracken 2004). Whether eukaryotes compete with bacterial and

archaeal microbes for this animal-regenerated N remains to be determined.

■ Hypotheses to further our understanding of N-based microbial function in the coastal ocean

Concerns about how marine ecosystems will respond to continued animal harvest and loss of habitat-forming foundational species (eg seagrasses and corals), while anthropogenic N inputs increase, require that we extend current efforts to understand the functional role of microbe–macrobiota associations. We offer four hypotheses regarding these associations in coastal N cycling that we suggest are worthy of future study. While our examples concentrate on the N-limited coastal ocean, the hypotheses listed below are broadly applicable to nutrient cycling by macrobiota in other ecosystems.

Hypothesis 1: macrobiota select for a microbiome composed of N-cycling microbes

The specificity of microbial communities with their animal and macrophyte hosts remains underexplored. If microbial interactions with animal or macrophyte hosts are highly specialized, then continued loss of macrobiota will affect microbial diversity and the range of microbial functions. For those macrobiota whose microbiome taxa have been genetically sequenced in tandem with microbiota from the surrounding seawater, unique taxa occur in association with the host. In a study of tide pool mussels, anemones, and a seaweed,

multiple microbial taxa collected from within macrobiotic tissues were absent from the water column (Pfister *et al.* 2014b). Similarly, sponges host distinct microbial taxa as compared with microbial samples taken from the surrounding seawater (Hentschel *et al.* 2002). However, an improved understanding of whether microbial taxa have obligate relationships with host macrobiota is required. Targeted manipulative experiments and biogeographic studies will reveal host–microbe specificity.

If macrobiota represent an inert physical surface for biofilms, then associated microbes are expected to have a cosmopolitan distribution among different hosts. If, on the other hand, hosts exert different selection pressures via resource availability, then high beta-diversity (or diversity differences among hosts) is expected, including the evolution of distinct microbial assemblages among hosts. Little is known about whether similar animal or macrophyte taxa host similar microbial assemblages. A study of several macrobiota substrates in rocky intertidal environments showed that each hosted unique microbial taxa, verifying association specificity (Pfister *et al.* 2014b), and strong differentiation of microbial communities between co-occurring species of coral again suggests specificity in microbe–macrobiota relationships (Sunagawa *et al.* 2010).

Hypothesis 2: N-cycling microbial associates provide benefits to macrobiota

The microbial diversity hosted by macrophytes and animals (WebTables 1–3) motivates investigation into whether microbes affect host fitness, or if hosts are indifferent to these colonists. Of the diversity of microbial taxa associated with macrobiota, it is still unclear how quantitatively important N cyclers are, and whether macrobiota benefit as well. Mussel studies suggest that microbial N uptake may ameliorate NH_4^+ accumulation to levels that would otherwise be toxic to animals. Although California mussel (*Mytilus californianus*) excretion rates and densities should theoretically result in millimole per liter local NH_4^+ concentrations (Pfister *et al.* 2010), only $1.26 \mu\text{mol L}^{-1}$ concentrations have been measured above mussel beds (Aquilino *et al.* 2009), suggesting that a combination of advection and uptake decrease NH_4^+ concentrations from millimolar to micromolar levels. In mussel aquaculture, animals have been observed to neither contribute nor deplete N locally, despite locally dense animal populations (Asmus and Asmus 1991), suggesting that uptake by microbes and photosynthetic organisms is quantitatively important.

Hypothesis 3: microbe–macrobiota associations broaden microbial N-cycling function

Cases of microbial taxa in association with macrobiota are accumulating rapidly (WebTables 1–3), and there is strong evidence that microbial metabolisms are

enhanced via microbial–animal associations (Table 2). Because we expect greater diversity and rates of N metabolisms in association with animals and macrophytes, it is probable that macrobiota numerically concentrate existing N functions, enhance the range of energetically possible functions, or both. The strong DO gradients along the surface of animal tissues or the thallus of an alga, and within tissues and digestive tracts, suggest that the metabolic activities of macrobiota create a range of oxidative states, and may therefore promote multiple N metabolisms in proximity to macrobiota (Figures 2 and 3). Whether direct microbial associations, or an alteration of the resources available to microbes in the immediate vicinity of the animal, are responsible for these functional consequences remains to be determined.

Other aspects of macrobiotic life histories could also determine the importance of microbe–macrobiota relationships and the traits of the associated microbes that colonize macrobiota. Motile and migratory organisms translocate microbes and N as they move about. Long-lived species with persistent structural components (eg shells or stipes [stems]) could select for stable, persistent microbial assemblages, while non-equilibrium microbial communities might be associated with fast-growing, rapidly senescing macrobiota.

Hypothesis 4: novel ecological links among macrobiota are facilitated by microbial N-cycling function

Little is known about whether microbial associations can determine the outcome of interactions with their host macrobiota. Symbionts vary in the fitness benefits they impart to hosts (Lema *et al.* 2014), suggesting that microbial communities may have the ability to mediate interactions among macrobiota (both individuals and species). Microbial presence can alter the attractiveness and palatability of prey and therefore the attack rate upon macrofaunal species, altering consumer fitness (Burkepile *et al.* 2006). The above relationships encourage a more complete understanding of how and when microbes affect the interactions of macrobiota and impart a fitness advantage to one species at the expense of the other.

Microbe–macrobiota relationships could also generate positive interactions through N cycling. For example, a microbiome that locally reduces otherwise toxic levels of NH_4^+ provides benefits for the host species as well as any surrounding species. Because animals and macrophytes change local DO levels through respiration and photosynthesis, host macrobiota could favor particular microbial taxa and metabolisms, to the benefit or detriment of neighboring species. The photosynthetic enhancement of DO by macroalgae and seagrasses, for instance, could favor the oxidative process of nitrification, in turn benefiting host animals by local microbial use of animal-excreted NH_4^+ . Conversely, concentration of N and C by

microbiota creates microbial hotspots of production, respiration, and N cycling that could subsequently affect the fitness of other microbiota.

■ Implications for coastal ecosystem function

Natural N inputs and cycling are likely changing as macrobiotic diversity and biomass decline in many aquatic ecosystems (Worm *et al.* 2006) – part of a larger pattern of global “defaunation” (Dirzo *et al.* 2014) and habitat loss. Human activities have greatly reduced large consumer biomass (Worm *et al.* 2006), yet scientists know little about how their loss has affected nutrient cycling and retention, although the loss of excretion products likely has a substantial effect (Croll *et al.* 2005). If macrobiota host microbial communities with important roles in N cycling, then animal and macrophyte loss will be accompanied by the alteration of N processing in marine ecosystems. This wide-scale depletion of macrobiota-enhanced N processing will likely coincide with increased anthropogenic N loading to coastal ecosystems. The many associations between microbes and macrobiota have been identified as a “new imperative for the life sciences” (McFall-Ngai *et al.* 2013); it is therefore timely to investigate to what degree microbes in association with macrobiota are important components of the coastal marine N cycle and what their role may be in ameliorating eutrophication. Given the range of human activities that affect the distribution and abundance of macrobiota and inputs of N to coastal ecosystems, these functional relationships deserve greater scrutiny in order to advance mechanistic understanding of their contribution to ecosystem processes and the implications for coastal ecosystem function and restoration.

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