Salt marsh nitrogen cycling: Where land meets sea

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Abstract

Salt marshes sit at the terrestrial-aquatic interface of oceans around the world. Unique features of salt marshes that differentiate them from their upland or offshore counterparts include high rates of primary production from vascular plants and saturated saline soils that lead to sharp redox gradients and a diversity of electron acceptors and donors. Moreover, the dynamic nature of root oxygen loss and tidal forcing leads to unique biogeochemical conditions that promote nitrogen cycling. Here, we highlight recent advances in our understanding of key nitrogen cycling processes in salt marshes and discuss areas where additional research is needed to better predict how salt marsh N cycling will respond to future environmental change.

Salt marshes are hot spots for nitrogen removal

Salt marshes occupy a unique ecological niche between the land and sea. However, their precarious position in the terrestrial to marine continuum leaves them vulnerable to anthropogenic disturbances from both directions (Fig. 1). From the land, salt marshes intercept terrestrial runoff, often laden with nitrogen (N) from anthropogenic sources including sewage waste disposal, excess fertilizer, and atmospheric deposition from fossil fuel combustion [1]. From the sea, upwelled nutrients can be advected into estuaries to support salt marsh production [2]. At the same time, rising sea levels can waterlog soils, leading to increased ponding [3] and decreased productivity that inhibits the natural ecogeomorphic feedbacks that have allowed these ecosystems to survive thousands of years of global change [4]. Extensive historic salt marsh loss, combined with unprecedented rates of global change, have dramatically shrunk the spatial extent of marshes, with an estimated loss rate of 0.28% y^{-1} between 2000 and 2019, causing billions of dollars of economic damages [5].

Loss of salt marsh habitat also threatens adjacent waters, in part because microbes that reside in salt marsh soils can remove N before the negative consequences of that N can be realized. Although our atmosphere is made predominantly of di-nitrogen (N_2) gas, many ecosystems, including salt marshes, are limited by the amount of biologically available N. While N can limit growth, too much N from land or sea is also negative and can lead to a series of deleterious effects including excess algae growth, low oxygen conditions, and decreases in biodiversity [6]. However, the complex cycling and removal of N by salt marshes can mitigate downstream eutrophication. Micro-organisms cycle this N by converting it to different forms that exploit energetic gradients and promote cell growth. Several unique features of salt marshes differentiate them from terrestrial and offshore habitats and make them hot spots for N removal,

because they lead to widely fluctuating redox conditions that favor microbial N transformations (Box 1). Below, we review recent literature on microbial N cycling in salt marshes and how oscillating redox conditions facilitate N removal and support critical ecosystem services.

Mineralization

The process of N mineralization (Box 2) converts organic N to inorganic ammonium (NH_4^+). The fraction of mineralized N not immobilized as microbial biomass can be assimilated by primary producers or other microbes or it can be oxidized. Mineralization rates span a wide range, from 3-122 g N m^{-2} y^{-1} [7], are broadly related to climatic factors (e.g., temperature) and ecosystem properties (e.g., soil composition, microbial biomass, C:N ratios) and decrease with depth in the soil [8,9]. Since mineralization of organic N is the principle means by which reactive N is recycled back to primary producers, this process plays a key role in determining overall ecosystem productivity, particularly when external inputs are low.

Salt marshes are strongly N limited and, although N loading rates from surrounding watersheds and coastal upwelling can be high, mineralization supports 50-200% of plant N demand [7]. Excess NH_4^+ production is generally either immobilized in microbes or enters into other N cycling pathways, rather than being exported from the system via lateral fluxes [7]. Ammonium production can occur through cleavage of N-rich compounds by extracellular enzymatic degradation of proteins and polypeptides; this is a multi-step process whereby proteases and peptidases, respectively, cleave amino acids and amides that are further degraded by amidases, releasing NH_3. Microbial uptake of substrates is size restricted so particulate and dissolved forms of organic N must first be degraded into low molecular weight components. The dissolved organic N (DON) pool is smaller in coastal soils, compared to terrestrial soils and the ocean, and it remains poorly characterized [10]. Moreover, there is a limited understanding of

how DON composition varies with salt marsh plant species, tidal inundation, and soil redox [11,12].

Salt marsh plants affect mineralization through litter production and by altering soil redox conditions and carbon availability [12,13]. These effects can be species specific, but marsh plant-microbe interactions are often poorly characterized. Indirect evidence suggests that salt marsh plant rhizodeposition differs between rapid growth and flowering life stages [14] but environmental conditions, nutrient availability, and the interactions among them, likely affect rates and composition as well [15-18].

Unlike well-drained terrestrial soils and much of the ocean, salt marsh soils are saturated and anoxic within millimeters of the surface. Tidal wetting and drying affect N mineralization by altering redox conditions and organic matter vulnerability to decomposition [19]. Oscillating redox conditions created by tides, bioturbation, and root translocation affect mineral-organic matter associations, depolymerization of macromolecules, and whether microbial decomposition proceeds through aerobic or anaerobic pathways [13,20,21]. Ultimately, the likelihood that low molecular weight products of depolymerization and fermentation will be mineralized depends on kinetic and thermodynamic constraints on microbes.

Recent methodological advances have allowed better estimation of net and gross mineralization rates. The former is often based on accumulation of NH_4^+ and nitrate (NO_3^-) over time and captures the net outcome of productive and consumptive processes. Highly sensitive ^{15}N tracer techniques estimate gross mineralization as the dilution of added $^{15}NH_4^+$ and/or $^{15}NO_3^-$ by the same compounds produced at natural abundance. Lastly, there have been efforts to measure hydrolytic enzymes involved in mineralization across salt marsh habitats [22]. Emerging frameworks that consider both the free energy yield of the electron acceptor and the

nominal oxidation state of organic carbon [23] offer a promising way forward in predicting substrate availability to microbes and the efficiency of mineralization [8].

N_2 fixation

Nitrogen fixation (N_2 fixation) is a critical process in the N cycle that connects largely unreactive atmospheric N_2 to the biologically active pools of N (Box 2). It can help ease N limitation and increase primary production. Photoautotrophs, chemolithotrophs, and heterotrophs, can perform N fixation (collectively referred to as diazotrophs), where N_2 is transformed into ammonia (NH_3) and rapidly protonated to NH_4^+. This NH_4^+ is then incorporated into microbial biomass, or it may leak out of the cell into the environment. N_2 fixation is energetically expensive, requiring 16 ATP for every mole of N_2 fixed.

One of the first N budgets for a salt marsh demonstrated that ~10% of the total N inputs to the marsh came from N_2 fixation and most of this was due to rhizosphere bacteria [24]. Since then, a variety of studies demonstrated N_2 fixation rates ranging from 0-60 mg N m^{-2} d^{-1} with a median rate of 12.6 mg N m^{-2} d^{-1} and a total annual N addition to salt marshes each year of 1.7 Tg N y^{-1} [25]. Overall, salt marsh N_2 fixation contributes only modestly to the global N budget. Most of these rates are from the acetylene reduction assay, which is logistically simpler and more cost-effective than using a $^{30}N_2$ label. However, this method has numerous artifacts, including causing shifts in the microbial community, particularly among sulfate reducing bacteria, which are important N_2 fixers in salt marshes [26].

On an individual salt marsh basis, N_2 fixation may be an important N source, albeit one that is challenging to accurately measure. This is especially true for younger salt marshes that have low nutrient stocks and N limited primary production. In fact, higher rates of N_2 fixation were observed in younger, artificially created salt marshes [27]. As salt marshes age, a variety of

changes take place that could limit N_2 fixation, such as decreases in light availability and increases in concentrations of sulfide and NH_4^+. For example, Tyler et al. [28], used a natural salt marsh chronosequence to show that summer N_2 fixation rates were 2-3 fold higher in a young marsh compared to older marsh. They also demonstrated that N_2 fixation exceeded N demand in the young salt marshes.

Other controls on N_2 fixation include organic matter availability [29], which is important for heterotrophic N_2 fixers, and oxygen concentrations, which inhibit nitrogenase. The role of dissolved inorganic N in controlling N_2 fixation is an active mystery. Some studies clearly demonstrate that N_2 fixation is down regulated in the presence of NH_4^+ and NO_3^- [30], while others show no change [31]. This lack of consistent inhibition in the presence of dissolved inorganic N might hint at the type of diazotroph present, as well as their reason for fixing N [31]. For example, some N_2 fixation may occur as a mechanism to maintain intracellular redox balance [31], rather than to fill an N deficit.

The application of next generation sequencing approaches reinforces the ubiquity and high diversity of salt marsh diazotrophic communities. Predicted metabolic potential for soil N_2 fixation was widespread across a salt marsh-to-sea transition zone [32]. Nitrogen fixers associated with roots of the grass *Elytrigia atherica* were more abundant at higher compared to lower elevations [33]. Bulseco et al. [34] demonstrated an increase in N fixation genetic signatures with depth in a New England salt marsh. And recent *Spartina alterniflora* root microbiome studies demonstrate the importance of N fixing sulfur cycling prokaryotes that simultaneously increase N availability and detoxify sulfide from the root zone [22,35]. The microbial community associated with *S. alterniflora* is a function of plant phenotypic characteristics, with N_2 fixing sulfate reducers predominating in the areas associated with the tall

143 form, and N_2 fixing methanogens more typically associated with short form [36]. Understanding
144 how N_2 fixation rates will change under future climate conditions is imperative for predicting
145 future salt marsh productivity and thus their ability to keep pace with sea level rise and sequester
146 carbon.

147 **Nitrification**

148 Nitrification, the oxidation of NH_4^+ to NO_3^-, is carried out by bacteria and archaea known
149 as ammonia-oxidizers, or more colloquially, as nitrifiers (Box 2). The canonical ammonia-
150 oxidizing bacteria (AOB) have been joined by ammonia-oxidizing archaea (AOA) and, most
151 recently, complete ammonia oxidizers known as comammox bacteria. AOA and AOB oxidize
152 NH_4^+ to nitrite (NO_2^-), while comammox carry the process all the way to NO_3^-. Nitrification rates
153 and abundances reported in salt marshes vary over orders of magnitude, with rates ranging from
154 0-1 $x10^3$ μmol N g^{-1} day^{-1} and nitrifier abundances show similar variability [37]. Factors that
155 drive differences in abundance, diversity, and activity of nitrifiers include salinity, oxygen,
156 temperature, sulfide, and pH [38]. However, a primary characteristic of salt marshes that
157 distinguishes them from other coastal or marine systems is the dominance of plants. Typically,
158 higher nitrification rates and nitrifier abundances are associated with vegetated marsh soils [39].
159 However, in a recent study of the *S. alterniflora* microbiome [22], AOB were enriched in the
160 root/rhizome samples, while AOA and comammox were higher in soils separated from plant
161 roots. Higher absolute abundances of AOA and AOB were also found in bulk versus vegetated
162 soils along a salt marsh chronosequence [40]. Conflicting results may simply reflect complex
163 dynamics in salt marsh soils, but they may also be due to different plant species compositions.

164 Soils dominated by different salt marsh plant species have different nitrifier communities,
165 activities (e.g., [41,42]), and abundances that can vary over several orders of magnitude.

166 However, it is not clear whether plants are impacting the nitrifiers by species-specific root
167 exudates [43] or differential O_2 loss from roots [44]. Two studies of comammox conducted in the
168 same region with similar plant species, reported conflicting results. In one study, pH and salinity
169 were the most important factors driving comammox distribution [45], while a second reported
170 plant species as the major driver [46]. One confounding factor affecting studies on the
171 interactions between plants and nitrifiers in salt marshes is that both the patterns of plant
172 zonation and controls on nitrifier communities are dictated by soil chemistry, which is driven by
173 the dynamics of groundwater flow and tidal flooding [47]. Thus, disentangling plant effects from
174 soil chemistry effects can be difficult, and may contribute to conflicting results.

175 The effect of plants on nitrifiers, although still not fully resolved, explains why salt
176 marshes are often categorized as more terrestrial than marine. However, the presence of saltwater
177 in marshes makes them more similar in some ways to marine systems. Salinity is a critical factor
178 regulating abundance, activity, and community structure of nitrifiers [38,48]. However, the
179 nitrifier communities in salt marshes differ significantly from those in marine systems. In marine
180 systems, AOA are consistently more abundant than AOB [49], but in salt marshes, AOB
181 sometimes outnumber AOA [40,42], suggesting more variation in salt marshes relative to marine
182 environments.

183 AOA community composition also differs between salt marshes and oceans [50], with
184 *Nitrososphaera* increasing in abundance as the environment transitions from offshore to the
185 coastal zone (Fig. 2). Studies of AOB communities suggest a transition from freshwater to
186 marine waters, with *Nitrosomonas* being more abundant at the freshwater end, and *Nitrosospira*
187 and uncultivated AOB being more abundant in brackish and marine systems (reviewed in
188 Bernhard and Bollman [38]). *Nitrosomonas* is also associated with more eutrophic environments,

189 compared to *Nitrosospira* [51,52]. Unfortunately, a global quantitative analysis of AOB
190 distribution has not been done.

191 Comammox have not yet been detected in ocean systems, but their abundance rivals and
192 even outnumbers AOB in some salt marshes [37,45], suggesting an important role for these
193 organisms. The apparent absence of comammox in oceans has led the hypothesis that
194 comammox does not grow well in high salinity and a recent study demonstrated low saline
195 tolerance of the cultured comammox, *Nitrospira inopinata* [53]. However, their abundance in
196 salt marshes and other estuarine systems suggests more ecophysiological diversity among these
197 nitrifiers, and our current inventory of comammox diversity is far from complete. Regardless of
198 whether NH_4^+ is oxidized via AOA, AOB, or comammox bacteria, the process requires a supply
199 of oxygen to generate oxidized N. Therefore, in salt marshes where redox conditions oscillate,
200 nitrification forms a critical link between the mineralization of NH_4^+ and the production of
201 oxidized N needed for subsequent dissimilatory NO_3^- transformation and loss. Thus, nitrifiers
202 play an outsized role in the N removal benefits provided by salt marshes, even though they do
203 not directly remove N themselves.

204 **Dissimilatory NO_3^- loss and transformation**

205 While nitrification requires the presence of oxygen, there are three important
206 dissimilatory NO_3^- loss and transformation processes that require the absence of oxygen —
207 denitrification and anammox (anaerobic ammonium oxidation), which generate N_2 that is lost
208 from the system, and dissimilatory NO_3^- reduction to ammonium (DNRA), which transforms
209 NO_3^- into its most reduced form (Box 2). Unlike the open ocean, where oxidized N is typically
210 upwelled from deep NO_3^- rich seawater, in marshes this NO_3^- is provided either by anthropogenic
211 sources, coastal upwelling, or via nitrification of mineralized NH_4^+. Thus, the unique oscillating

redox gradients in salt marshes allow close coupling between the oxidation of NH_4^+ and the reduction of NO_3^-, which facilitates N loss from the system.

Denitrification — Salt marshes are hot spots for denitrification, which is the multistep conversion of NO_3^- through several intermediates to N_2 (Box 2). Many microbial taxa have the genetic capacity for denitrification [54,55] but estimates are difficult due, in part, to the complex dynamics of tidal flooding and oxidation within the rhizosphere. However, one analysis of published studies suggests that the median salt marsh denitrification rates (14-28 mg N m^{-2} d^{-1}) are higher than estuarine and coastal shelf sediments [25] and terrestrial forests [56] and on par with agricultural soils [57].

Denitrifying microbes require a supply of NO_3^-, which, in many salt marshes is primarily provided via *in situ* nitrification [58] even under elevated supplies of anthropogenic NO_3^- [59]. Since denitrification also requires low oxygen conditions, the varying redox profiles of salt marshes provide both the supply of oxygen needed for NO_3^- production from nitrification and the anoxic conditions needed to facilitate NO_3^- respiration by denitrifiers. These factors are strongly driven by plant traits, such that plant species diversity can further differentiate rates of denitrification and the structure of the microbes responsible [60,61]. Although denitrification is typically associated with heterotrophic metabolisms that require a supply of organic matter as an electron donor, recent work also indicates that autotrophic denitrification can occur in salt marsh soils [34,62], though its contribution to total denitrification is unclear.

Each step in the denitrification pathway (Box 2) is facilitated by different enzymes and the process is modular [63,64], as not all microbes contain all the genes essential for complete denitrification. Critically, a key intermediate in the denitrification pathway is the conversion of nitrogen oxide (NO) to nitrous oxide (N_2O), a potent greenhouse gas, which, in canonical

denitrification is converted in the final step to N_2. However, many microbial taxa lack the gene that facilitates the final step in the denitrification pathway, leading to N_2O production [63]. Nitrous oxide can also be produced biotically and abiotically during nitrification, making it challenging to determine which pathway is the proximate source of N_2O [65]. In salt marshes with low supply of ambient NO_3^-, nitrification appears to dominates N_2O production however, under high NO_3^-, denitrifiers appear to dominate N_2O production and consumption [66]. Lastly, recent work examining N_2O production in marsh fungi also suggests a role for fungal denitrification in N_2O production [67], though its quantitative importance remains uncertain.

Anammox — anaerobic ammonium oxidation — is an autotrophic microbial pathway where NH_4^+ is oxidized to N_2 under anoxic conditions using NO_2^- as the electron acceptor [68]. Anammox is an important pathway for N loss in oceans, where it is a major, and often the dominant N_2 producing process in deeper marine sediments [69] and oxygen minimum zones [70]. This can be contrasted with terrestrial systems, where little was known about anammox. However, a recent review of 1212 observations found that while anammox rates were extremely low in natural forests and grasslands, they were fairly high in wetlands (averaging 2.17 nmol N g^{-1} hr^{-1}) and in crop lands (averaging 1.6 nmol N g^{-1} hr^{-1}) [71].

In salt marshes anammox appears to be responsible for 1-12% of NO_3^- reduction to N_2. Rates of anammox fall within the lower end of the range of those typically reported for marine systems [72,73]. However, the low importance of anammox is less due to lower rates of anammox, and more due to the high rates of denitrification and DNRA, thereby reducing the relative importance of anammox. High organic matter availability is an important factor affecting the partitioning of NO_3^- reduction away from anammox [74] but higher salinity [72] and hifh sulfide concentrations [75] may also reduce the importance of anammox.

Dissimilatory Nitrate Reduction to Ammonium (DNRA) — DNRA is the microbial reduction of NO_3^- to NH_4^+. This process is important because unlike denitrification or anammox, it retains fixed N in the ecosystem. There are two very different pathways for DNRA, one is heterotrophic fermentative DNRA and the other is carried out by autotrophic bacteria using sulfide or other reduced compounds as the electron donor [76]. Several studies have suggested that fermentative DNRA is favored over denitrification under conditions when organic carbon availability is high and nitrate concentrations are low [74,77,78].

The advent of new molecular techniques and wider use of ^{15}N tracers has revealed that DNRA is common in many habitats. It was first reported to be an important NO_3^- reduction pathway in coastal marine sediments as well as some freshwater environments where rates can be quite high [76]. DNRA tends to be a less important NO_3^- reducing process in deeper marine sediments. For example, in Arctic continental shelves DNRA made up less than 10% of total NO_3^- reduction [79]. Because DNRA was believed to require sustained anaerobic conditions it was thought to only occur in saturated soils and sediments. However, this view began to change when Silver et al. [80] reported that up to 75% of NO_3^- reduction in tropical soils was due to DNRA. Since then, a variety of studies in terrestrial soils have reported the occurrence of DNRA. A review of 85 studies found a mean global rate of DNRA of 0.31 $\mu g\ N\ g^{-1}\ d^{-1}$ [81] with the highest rates in paddy soils and the lowest in unfertilized crop lands.

DNRA is an important process in many salt marsh and mangroves systems and while the number of studies is small, typical rates fall within a range of 0.7 to 8 $mg\ N\ m^{-2}\ d^{-1}$. In a compilation of 14 studies Giblin et al. [82] found that DNRA accounted for anywhere between 3 to 99% of the total salt marsh soil NO_3^- reduction, and in 30% of studies DNRA was the

dominant NO_3^- reduction pathway. Although rates are on average lower than those of denitrification, they can be a relatively high percentage of the total NO_3^- reduction.

The controls on DNRA are not well understood and while the relative availability of organic matter to NO_3^- is predictive in some cases, it does not hold all the time. In terrestrial systems, Cheng et al. [81] found precipitation was the main factor stimulating DNRA, however, others found that rates can be influenced by plant type [83] and soil N availability [83,84]. In aquatic soils, DNRA tends to be favored under condition of high organic matter, and sulfide concentrations were the most accurate predictor of the DNRA [75] in estuarine sediments, consistent with laboratory studies [85]. Other studies found salinity to be an important control, with DNRA being less important than denitrification at low salinities [86,87]. Nutrients also play a role. Peng et al. [65] found that while NO_3^- reduction in a natural temperate salt marsh was dominated by DNRA, large N additions shifted the balance to favor denitrification. Other studies found that N additions increase both processes, although denitrification showed greater stimulation than DNRA [59]. Discrepancies between these studies may reflect differences in the amount and types of N added [88]. Plant species may also play a role. Studies in China where *S. alterniflora* is invading mangrove wetlands suggested that this grass enhanced denitrification by almost three-fold while decreasing DNRA by nearly 90%.

Concluding remarks and future directions

While we understand many of the individual controls on microbial N cycling, more questions remain regarding how these processes will respond in aggregate to changing environmental conditions (see Outstanding Questions Box). Ultimately, N from natural and anthropogenic sources and N fixation and mineralization determine the extent of primary productivity in salt marshes, with ecosystem-wide consequences. New discoveries, such as the

identification of abundant comammox microbes in salt marshes, further demonstrate that we have an incomplete understanding of the taxonomic breadth of organisms underlying these pathways. While we are beginning to document the genetic capacity of these processes through metagenomics, we have very little information on what genes are being expressed, what proteins are being produced, and how microbial interactions with each other and with marsh vegetation affect N cycling. Coupling manipulative field and lab experiments with measurements of rates and metatranscriptomics and metaproteomics will provide new insights into controls on these critical steps in the marsh N cycle.

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526 **Figure legends**

527

528 Figure 1: Aerial view of the Great Marsh located Northeastern Massachusetts. The Great Marsh
529 forms part of the study areas of the NSF funded Plum Island Long-Term Ecological Research
530 Site (PIE-LTER). The photograph captures the essence of marshes being influenced by both
531 terrestrial and marine forces, with the terrestrial uplands behind and to the left of the camera
532 view that trends toward fully marine systems in the distant downstream toward the top of the
533 photo. This image was taken using a helium blimp and kite from heights of 300-500 feet in
534 August, 2009 by James S. Aber, Susan W. Aber and Vinton Valentine.

535

536 Figure 2. Relative distribution of archaeal *amo*A sequences in different habitats, based on data
537 from Alves et al. [50]. The top figure shows the distribution of sequences from all habitats; the
538 bottom figure shows the distribution of sequences from marine and estuarine/coastal habitats,
539 broken down into sub-habitats. NT = *Nitrosotalea*, NS = *Nitrososphaera*, NP = *Nitrosopumilus*.

540

541 Fig. I: Contrasts on the timescales and frequency of oscillations that affect ecosystems processes
542 along the terrestrial to ocean continuum, highlighting the divergence of salt marshes from both
543 terrestrial and deep ocean habitats.

544

545 Figure II: Summary of key nitrogen cycle processes and the oxidation states associated with
546 those transitions. Although organic matter (OM) has variable oxidation states, the N that is
547 mineralized as reduced N becomes part of the N pool that feeds into microbial N cycling in salt
548 marshes. Colored boxes and arrows indicate specific processes (blue - anammox, red -

549 denitrification, purple - DNRA, green -N_2 fixation, orange - nitrification, and brown -
550 mineralization).

551 **Box 1: Marshes in the middle.**

552 Aside from navigating the combined effect of anthropogenic disturbance on land and
553 rising waters from the sea, the location of salt marshes between terrestrial upland and oceanic
554 habitats creates other unique features with important implications for our understanding of mash
555 nitrogen cycling (Fig. I). Along a terrestrial to ocean continuum, vegetated terrestrial soils (left
556 panel) experience diel fluctuations in photosynthesis and, depending on location, seasonal
557 variation in temperature and episodic patterns of precipitation and drought. Deep ocean
558 sediments (right panel), by contrast, have exceedingly stable temperature, light, pH, salinity, and
559 oxygen levels and experience seasonal and episodic deposition of organic material from the
560 surface ocean. Salt marshes (center panel) by contrast, have all the same oscillations observed in
561 terrestrial systems, coupled with tidal forcing that induces patterns of inundation and drying on a
562 12-hour 25-minute cycle and on the lunar cycle of spring and neap tides. Further, they have
563 variation in salinity driven by the extent of precipitation and drought, which further influences
564 the timing, delivery, and relative importance of terrigenous vs oceanic inputs. The combined
565 effect of these multiple oscillating processes happening in marshes, results in highly dynamic
566 redox environments that enhances the cycling and removal of N from natural and anthropogenic
567 sources.

568

Box 2: Key processes in the marsh nitrogen cycle.

The nitrogen (N) cycle in salt marshes contains several key N transformations (Fig. II, Key Figure) that are often mediated by diverse groups of microbes that facilitate different steps. Below we briefly describe the key steps in the N cycle, and the genes that are diagnostic for each step.

Mineralization is the conversion of high molecular weight organic material into ammonium (NH_4^+) facilitated through hydrolysis of complex organic material into simpler compounds. In salt marshes, the molecular analysis of mineralization lags behind the rest of the N cycle, although there have been efforts to quantify extracellular enzymes and the Carbohydrate-Active enZYmes Database (CAZY), will allow future exploration of the genetic underpinnings of mineralization.

Nitrogen fixation is a critical process carried out by micro-organisms that convert N_2 gas to NH_4^+. N_2 Fixation can be carried out by a wide array of diazotrophs, is catalyzed by the enzyme nitrogenase, and can be detected using the diagnostic gene *nifH*, recent work has emphasized the importance of also considering *nifD* or *nifK* [89].

Nitrification is the two-step process that converts NH_4^+ first to NO_2^- and then to NO_3^-. Typically thought to result from two different microbes, the recent discovery of complete ammonia oxidizers (comammox) revised our understanding [90]. The first step of nitrification is considered rate limiting and the *amoA* gene that encodes ammonia monooxygenase is considered diagnostic.

Denitrification is the conversion of NO_3^- through multiple steps into N_2 gas. Incomplete denitrification occurs when the full process is truncated and leads to release of the greenhouse gas N_2O. Different genes encode different steps in the pathway but the most commonly used

592 diagnostic genes are *nirS/nirK* and *nosZ* that encode nitrite and nitrous oxide reductases,
593 respectively.

594 Anammox is an autotrophic process where the oxidation of ammonium to dinitrogen gas
595 is carried out using nitrite as an electron acceptor. It is anaerobic and strongly inhibited by
596 oxygen [68]. The most common gene used to study anammox is hydrazine synthase, encoded by
597 *hzsCBA*.

598 Dissimilatory nitrate reduction to ammonium (DNRA) is the microbial conversion of
599 NO_3^- to NH_4^+. DNRA is carried out by both heterotrophic and autotrophic organisms and is
600 important because it retains fixed nitrogen in marshes where it can be used to support primary
601 production. The *nrfA* gene is most commonly used to study DNRA, although DNRA may not be
602 restricted to bacteria with the *nrfA* gene.
603
604