Quantifying Potential N Turnover Rates in Hypersaline Microbial Mats by Using $^{15}$N Tracer Techniques

Oksana Coban,a,b Olivia Rasigraf,c,d,e Anniek E. E. de Jong,c,d Oliver Spott,f Brad M. Bebouta

aExobiology Branch, NASA Ames Research Center, Moffett Field, California, USA
bHydrology and Quantitative Water Management Group, Wageningen University and Research, Wageningen, The Netherlands
cDepartment of Microbiology, Radboud University Nijmegen, Nijmegen, The Netherlands
dNetherlands Earth System Science Centre (NESSC), Utrecht, The Netherlands
eGerman Research Centre for Geosciences (GFZ), Section 3.7 Geomicrobiology, Potsdam, Germany
fAgricultural Application Research, SKW Piesteritz GmbH, Cunnersdorf, Germany

ABSTRACT Microbial mats, due to stratification of the redox zones, have the potential to include a complete N cycle; however, an attempt to evaluate a complete N cycle in these ecosystems has not been yet made. In this study, the occurrence and rates of major N cycle processes were evaluated in intact microbial mats from Elkhorn Slough, Monterey Bay, CA, USA, and Baja California Sur, Mexico, under oxic and anoxic conditions using $^{15}$N-labeling techniques. All the major N transformation pathways, with the exception of anammox, were detected in both microbial mats. Nitrification rates were found to be low at both sites for both seasons investigated. The highest rates of ammonium assimilation were measured in Elkhorn Slough mats in April and corresponded to high in situ ammonium concentrations in the overlying water. Baja mats featured higher ammonification than ammonium assimilation rates, and this, along with their higher affinity for nitrate compared to ammonium and low dissimilatory nitrate reduction to ammonium rates, characterized their differences from Elkhorn Slough mats. Nitrogen fixation rates in Elkhorn Slough microbial mats were found to be low, implying that other processes, such as recycling and assimilation from water, are the main sources of N for these mats at the times sampled. Denitrification in all the mats was incomplete, with nitrous oxide as the end product and not dinitrogen. Our findings highlight N cycling features not previously quantified in microbial mats and indicate a need for further investigations of these microbial ecosystems.

IMPORTANCE Nitrogen is essential for life. The nitrogen cycle on Earth is mediated by microbial activity and has had a profound impact on both the atmosphere and the biosphere throughout geologic time. Microbial mats, present in many modern environments, have been regarded as living records of the organisms, genes, and phylogenies of microbes, as they are one of the most ancient ecosystems on Earth. While rates of major nitrogen metabolic pathways have been evaluated in a number of ecosystems, they remain elusive in microbial mats. In particular, it is unclear what factors affect nitrogen cycling in these ecosystems and how morphological differences between mats impact nitrogen transformations. In this study, we investigate nitrogen cycling in two microbial mats having morphological differences. Our findings provide insight for further understanding of biogeochemistry and microbial ecology of microbial mats.

KEYWORDS microbial mats, nitrogen, stable isotope labeling, incomplete denitrification, nitrous oxide

Microbial mats are multilayered sheets of Eukarya, Bacteria, and Archaea, which under moist conditions are usually held together by extracellular polymeric
substances secreted by these microorganisms (1). Microbial mats have been found in extreme environments where competition from other microbial groups and predation by grazing organisms are limited, such as the benthic-planktonic interface of hot springs, deep-sea vents, hypersaline lakes, and marine estuaries (2), as well as in dry environments like deserts (3). Most, if not all, biogeochemical processes that exist in aquatic ecosystems are considered to occur in microbial mats, due to the presence of highly diverse physiological groups of microorganisms therein (4, 5). Microbial mats have layered redox stratification during the day, when photosynthesis is occurring, and are completely anoxic during the night (6). This makes them ideal communities for the study of selective pressures acting separately on anaerobic and aerobic metabolism of the inhabiting microbes.

Microorganisms have an important role in the global nitrogen (N) cycle. The N cycle has had a profound impact on both the atmosphere and the biosphere throughout geologic time. There is significant evidence that N availability limits production of biomass on Earth today and has over geologic time (7–11). Briefly, the major microbial transformations of N are as follows: N-fixing microbes combine gaseous N with hydrogen to produce ammonia, present in these environmental conditions in its protonated form, ammonium (NH₄⁺), which is assimilated by microorganisms and further incorporated into organic compounds. Through ammonification, organic N can be converted back into NH₄⁺. This NH₄⁺ is oxidized to nitrite (NO₂⁻) and nitrate (NO₃⁻) with oxygen (O₂) as the terminal electron acceptor in the process known as nitrification. The produced NO₃⁻ can further undergo denitrification, mostly under anaerobic conditions, which is the stepwise reduction of NO₃⁻ via NO₂⁻ to nitrous oxide (N₂O) and dinitrogen (N₂) using organic carbon, hydrogen, or reduced inorganic species (e.g., Fe and S) as electron donors. Nitrate can also be reduced back to NH₄⁺ in the process known as dissimilatory nitrate reduction to ammonium (DNRA). Along with denitrification, anaerobic ammonium oxidation (anammox) is a N cycle transformation that contributes to net loss of fixed N in the environment. Here, NH₄⁺ and NO₂⁻ are combined to form N₂ with a side production of NO₃⁻ (12).

While extensive research has been performed on N cycling processes in soils and marine ecosystems, N cycling in microbial mats has received limited study. Most studies of N cycling in microbial mats have focused on the process of N₂ fixation (e.g., studies by Omoregie et al. in 2004, Moisander et al. in 2006, and Woebken et al. in 2015 [13–15]). This is likely due to both its importance as a source of reactive N in otherwise low-N environments and the relative ease with which it may be measured using acetylene reduction (16) and ¹⁵N₂ incorporation (17) techniques. Some studies have been focused on nitrification and denitrification in mat communities (3, 18–20). While nitrification rates have been quantified and a nitrifying community investigated in a few microbial mats (19, 21), the interactions between nitrification and other NH₄⁺-consuming processes, such as NH₄⁺ assimilation in microbial mats, have not been evaluated. It has been shown that assimilation of NH₄⁺ from the water column and ammonification from deeper layers can be the main sources of N for some microbial mats (22). Many other N transformations, like anammox and DNRA, have been overlooked in microbial mats. In the only study measuring anammox in hypersaline photosynthetic mats, this process was undetected (3). DNRA has been shown to occur in marine microbial mats (19) and along with ammonification and N₂ fixation can contribute to the pool of NH₄⁺ in mats.

Our knowledge of N cycling in microbial mats is at a rudimentary state, and there is a need for comprehensive studies evaluating a complete N budget for different types of microbial mats. In this study, we address the following objectives: (i) to detect and quantify the major N pathways in two photosynthetic microbial mats, (ii) to assess if there are substantial differences in N transformations between two different types of photosynthetic mats, and (iii) to explore the effect of environmental conditions on N cycling in studied microbial mats. The mats studied here were chosen as they grow in very different environments. One of the two mats studied (from Baja California,
Mexico) is constantly submerged, whereas the other mat (from Elkhorn Slough [ES], Monterey Bay, CA, USA) grows in an intertidal environment (Fig. 1). Both mats experience turbulence from storm events, but the disturbance caused by these events is likely far greater at the Elkhorn Slough field site. Physical disturbances resulting from a combination of wind-driven water movement, desiccation impacts on the attachment of the mats to sediment, and even disturbance by fauna result in far greater disruption of physical structure and integrity in the ES mats than the Baja mats. Differences in some nitrogen cycling processes, such as assimilation and ammonification, between the Baja mats and a mat very similar to the ES mat have previously been reported (22). Here, we present a more comprehensive study of nitrogen cycling processes in mats located in very different physical environments, which we hypothesized have a profound effect on nitrogen cycling. We determined the occurrence and rates of major N cycling processes in order to assess a complete N budget for two different types of microbial mats and for two seasons in one of them. For consistency, all rates were measured using 15N-labeling techniques.

RESULTS

Nitrification and nitrate assimilation/aerobic denitrification. The nitrification rate was determined as NO$_3^-$ production in microbial mat incubations amended with 15NO$_3^-$ under light. In the same incubations, measured NO$_3^-$ consumption reflected a NO$_3^-$ assimilation/aerobic denitrification rate. In general, while the NO$_3^-$ concentration decreased significantly over experimental time in all incubations, the 15N dilutions of the NO$_3^-$ pool were low for both types of microbial mats, ranging from 0.1 to 0.2 atom% for ES mats in October to 0.4 atom% for Baja mats in February (Fig. 2). These correspond to low NO$_3^-$ production rates, which were calculated to be 3.1 ± 1.7 mmol N m$^{-2}$ day$^{-1}$ in ES October mats, 5.6 ± 3.2 mmol N m$^{-2}$ day$^{-1}$ in ES April mats, and 6.5 ± 5.1 mmol N m$^{-2}$ day$^{-1}$ in Baja February mats. Gross NO$_3^-$ consumption rates were higher than NO$_3^-$ production rates: 28.1 ± 5.1 mmol N m$^{-2}$ day$^{-1}$ in ES October mats, 23.9 ± 16.2 mmol N m$^{-2}$ day$^{-1}$ in ES April mats, and 47.8 ± 23.5 mmol N m$^{-2}$ day$^{-1}$ in Baja February mats. Net NO$_3^-$ consumption rates were slightly lower than gross NO$_3^-$ consumption rates and were 25.0 ± 5.2 mmol N m$^{-2}$ day$^{-1}$ in the ES October mats, 18.3 ± 13.1 mmol N m$^{-2}$ day$^{-1}$ in the ES April mats, and the highest in Baja mats, at 41.3 ± 20.3 mmol N m$^{-2}$ day$^{-1}$. Means of all the estimated rates were somewhat higher in Baja mats than in ES mats, although the differences were not statistically significant (P > 0.05).

Ammonification and ammonia assimilation. Ammonification and ammonia assimilation rates were evaluated as NH$_4^+$ production and consumption, respectively, in microbial mat incubations amended with 15NH$_4^+$ and nitrapyrin for nitrification inhibition under light (Fig. 3). The results reveal strong gross NH$_4^+$ consumption by ES microbial mats in October (14.9 mmol N m$^{-2}$ day$^{-1}$), but weak NH$_4^+$ production (2.4 mmol N m$^{-2}$ day$^{-1}$), as indicated by a significant decrease in the NH$_4^+$ concentration and a weak decrease of 15N signal. Data for ES April mats show a significant decrease of both the 15N signal and NH$_4^+$ concentration, which reflects strong NH$_4^+$ production (22.3 ± 23.0 mmol N m$^{-2}$ day$^{-1}$) and gross NH$_4^+$ consumption (85.1 ± 66.5 mmol N m$^{-2}$ day$^{-1}$). For Baja February mats, a strong decrease in 15N signal is observed, but no substantial decrease in NH$_4^+$ concentration, which means NH$_4^+$ production and gross NH$_4^+$ consumption are almost equal, resulting in a rather balanced N turnover (24.0 ± 2.9 and 17.8 ± 3.7 mmol N m$^{-2}$ day$^{-1}$, respectively). In the case of the results for Baja February mats, a higher NH$_4^+$ production rate than a gross NH$_4^+$ consumption rate resulted in the mat being a net producer of NH$_4^+$ (−6.3 ± 6.6 mmol N m$^{-2}$ day$^{-1}$). The means of both net and gross consumption rates were somewhat higher in ES mats in April than in ES mats in October and Baja mats in February, although the differences were not statistically significant (P > 0.05).

Anammox and denitrification. For anammox rate evaluation, the nonrandom distribution approach for 15N in N$_2$ was used based on the analyses of 28N$_2$ and 15N$_2$ after amendment of the microbial mat incubations in the dark with 15NO$_3^-$ and NH$_4^+$. A 15N enrichment in N$_2$ was, however, not detectable in any of the incubations, and the 28N$_2$
level was not higher than that of laboratory air (data not shown); therefore, anammox activity was not detected.

Denitrification was assessed in microbial mats as production of \(^{15}\text{N}\)-labeled \(\text{N}_2\) and \(\text{N}_2\text{O}\) gases after amendment of the microbial mat incubations in the dark with \(^{15}\text{NO}_3^-\).

Interestingly, \(^{15}\text{N}\) enrichment in \(\text{N}_2\) was lacking in all investigated mats and seasons. \(^{15}\text{N}\) enrichment in \(\text{N}_2\text{O}\), however, was found in all incubations, although each data set

**Fig 1** View of the Baja (A) and Elkhorn Slough (ES [C]) field sites and samples of microbial mat (inset) from each of the field sites. Cross-sectional views of the Baja (B) and ES (E) mats are shown with representative oxygen microelectrode profiles under lighted (yellow symbols) and dark (black symbols) conditions. The oxygen microelectrode profiles of the Baja mat (C) and ES mat (F) are plotted at the same spatial scale as the mat cross sections. Error bars in the microelectrode profiles represent the standard deviation of 3 profiles taken in the same location at steady state. The view of the ES field site in panel D shows a very heterogeneous distribution of mats, mats of apparently different thicknesses, and desiccation features resulting in mats wetted to different degrees. In contrast, the mats on the floor of the saltern where the Baja mats were collected (A) can be almost completely flat across tens of meters of horizontal distance. Photo credits: Skylar J. Laham (A), Leslie Bebout (B), Natalie Jones (E).

**Fig 2** Nitrate turnover for ES mats in October and April and Baja mats in February.
represents different patterns. In the of Baja February mats, weak $^{15}$N$_2$O enrichments were detected; however, all the N$_2$O concentrations were below the limit of quantification (<0.3 ppmv). This corresponds to a maximum N$_2$O production rate below $1.0 \pm 0.4 \mu$mol N$_2$O-N m$^{-2}$day$^{-1}$. A peak of enrichment of about 0.4 atom% was observed after a 1-h incubation time (Fig. 4).

ES mats in October produced a strong $^{15}$N enrichment of N$_2$O at time step zero (less than 5 s of incubation) (Fig. 5a). $^{15}$N enrichment of N$_2$O abundance increased from $75.2 \pm 10.4$ atom% at time zero to $86.9 \pm 1.3$ atom% at incubation time 3 h and subsequently decreased to $74.0 \pm 9.5$ atom% at the end of the experiment. As for N$_2$O production rates, N$_2$O production was found to be the highest at incubation time 1 h ($4.0 \pm 0.8$ mmol N$_2$O-N m$^{-2}$day$^{-1}$), and the average rate was $2.3 \pm 1.7$ mmol N$_2$O-N m$^{-2}$day$^{-1}$.

In contrast to ES October mat data set, the $^{15}$N data for N$_2$O in ES April mats (Fig. 5b) indicated a consistent increase in $^{15}$N abundance over time that reached $2.9 \pm 1.0$ atom% after 6 h of the experiment. N$_2$O production also increased over incubation time, with an average of $5.2 \pm 4.1 \mu$mol N$_2$O-N m$^{-2}$day$^{-1}$.

**DNRA.** DNRA rates were calculated from the isotopic enrichment of the NH$_4^+$ pool after addition of $^{15}$N-labeled NO$_3^-$ to microbial mats in the dark in the same incubations that were used for the denitrification rate measurement. ES microbial mats in April exhibited a higher DNRA rate than ES mats in October ($1.8 \pm 0.8$ and $0.4 \pm 0.2$ mmol N m$^{-2}$day$^{-1}$, respectively), and generally, ES mats exhibited higher DNRA rates than Baja mats in February ($0.2$ mmol N m$^{-2}$day$^{-1}$) ($P = 0.022$) (Fig. 6).

---

**FIG 3** Ammonium turnover (without nitrification) for ES mats in October and April and Baja mats in February. (Due to loss of replicates, only one value is shown for ES October.)

**FIG 4** $^{15}$N abundance of nitrous oxide in Baja mats in February.
Nitrogen fixation. The nitrogen fixation rate was evaluated by two techniques: $^{15}$N isotope labeling and acetylene reduction assay (ARA). Due to experimental failure with $N_2$ fixation rate measurement for Baja February and ES October mats, only data for ES April mats will be shown here. ES April microbial mats were preincubated under light prior to being placed in the dark for $N_2$ fixation rate determination during the night. The average rate of $N_2$ fixation as measured by the $^{15}$N-labeling technique was found to be $8.1 \pm 3.1 \mu$mol N m$^{-2}$ day$^{-1}$, and the average rate of ethylene production as measured by ARA was $555.4 \pm 257.5 \mu$mol C$_2$H$_4$ m$^{-2}$ day$^{-1}$ (Fig. 7). The highest rates for ARA were observed after 1 h of incubation time and the lowest after 0.5 h ($P < 0.001$). As for $N_2$ fixation rates measured by the $^{15}$N-labeling technique, the highest rate was found to be also after 1 h of incubation ($P = 0.003$); however, the rate at 0.5 h of incubation was not taken into account for the significant difference calculation due to very high variation between replicates.

DISCUSSION

Detection and quantification of the major N pathways in the ES and Baja microbial mats. In this study, N turnover rates were evaluated for ES microbial mats in October and April and for Baja microbial mats in February. Nitrate turnover was estimated under light (oxic) conditions such that NO$_3^-$ consumption would reflect NO$_3^-$ assimilation/aerobic denitrification and that NO$_3^-$ production would be assigned to ni-
A key finding was that nitrification in both microbial mats occurred only at very low rates. All investigated samples of microbial mats were characterized by much higher NO$_3^-$ consumption than NO$_3^-$ production rates. This is evidenced by significant NO$_3^-$ concentration decreases reflecting high NO$_3^-$ consumption rates and weak $^{15}$N dilution that corresponds to low NO$_3^-$ production rates. However, the calculated gross NO$_3^-$ consumption is also dependent on the expression of the pool dilution effect and therefore could be underestimated, considering that $^{15}$N dilution was low, especially in ES October mat incubations. Nevertheless, considering the near equality of the calculated net and gross consumption rates and that net consumption rate calculation is independent of $^{15}$N data, then estimated NO$_3^-$ turnover is accurate. In other words, net turnover rates that are close to gross ones are another confirmation that nitrification rates are low at both sites for investigated seasons. This is additionally supported by the concentration ratio of NH$_4^+$ to NO$_3^-$ at all sampling sites, which is always $>1$ (Table 1).

There are several possible explanations for the low nitrification rates. Likely, there is competition for NH$_4^+$ between ammonia oxidizers and cyanobacteria and diatoms, which are the main structural components of the photosynthetic microbial mats (23). As activity of ammonia-oxidizing bacteria is also inhibited by high salinity, this could be another possible explanation of low nitrification rates in studied hypersaline microbial mats (24). Additionally, photoinhibition of nitrifying microorganisms has been previously shown (25, 26) and could be partially responsible for quantified low nitrification rates.

High rates of NO$_3^-$ consumption were observed in all investigated mats and seasons. Since it is not possible to differentiate between NO$_3^-$ assimilation and aerobic denitrification (denitrification occurring in the mat under lighted conditions) in these experiments, the NO$_3^-$ consumption reflects a total of these two processes. Some denitrification activity under anoxic conditions in the form of $^{15}$N$_2$O production was indicated in all the investigated mat samples (see below), and therefore aerobic denitrification might occur as well in studied microbial mats. However, as we do not know about the extent of denitrification in the presence of O$_2$ in microbial mats, and whether or not denitrification is confined to layers below the upper oxic layers, it is not feasible to compare these two data sets. High rates of denitrification (e.g., 2 to 22 mmol N m$^{-3}$ sediment h$^{-1}$) under oxic conditions (“aerobic denitrification”) have been previously shown (27–29), and this could be a sink for NO$_3^-$, along with microbial assimilation in the investigated mats under light. In microbial mats, deeper layers (below 3 mm) are usually anoxic, and denitrification could occur there even under lighted conditions.

Ammonium is likely present in higher concentrations than other nitrogenous nutrients in these mats (22). Multiple pathways of NH$_4^+$ consumption are possible: i.e., microbial assimilation, nitrification, and anammox. However, anammox was found to
As nitrapyrin was added to inhibit nitrification in our incubations, all the NH$_4^+$ consumption should be assigned to microbial assimilation. It has been shown that photosynthetic microbial mats, in particular from the Baja location, are able to fulfill their N requirements from inorganic N present in the water column and via pore water through diffusive flux (22). In this study, while ES mats showed comparable rates of NH$_4^+$ and NO$_3^-$ consumption, Baja mats had very low NH$_4^+$ assimilation rates relative to both NO$_3^-$ consumption and NH$_4^+$ assimilation in ES mats. Such differences can be possibly explained by very high in situ water NH$_4^+$ concentrations in ES in April when also NH$_4^+$ assimilation was found to be the highest. Elevated NH$_4^+$ concentrations in ES may stimulate NH$_4^+$ assimilatory gene transcription rates necessary for acquiring these nutrients; that may not be the case for Baja mats when NH$_4^+$ concentrations remain low all the time. Still, as NH$_4^+$ and NO$_3^-$ water concentrations are within the same range in Baja mats (Table 1), it remains unclear why rates of NO$_3^-$ consumption were found to be higher in these mats and needs to be further investigated. Given that NH$_4^+$ is generally preferred over NO$_3^-$ as a substrate due to the lower energy cost for NH$_4^+$ assimilation (30), high rates of NO$_3^-$ consumption in Baja microbial mats are probably related to aerobic denitrification. As for NH$_4^+$ production, the possible pathways are ammonification and DNRA. Since the NH$_4^+$ turnover experiments were conducted under light and the resultant oxygen production via photosynthesis (6) and given that DNRA occurs optimally under anoxic conditions (31), we assume here that most of the NH$_4^+$ production was via ammonification. As the NH$_4^+$ production in the Baja mats was higher than its consumption, ammonification could fulfill the N requirements for these mats. Recycling via ammonification (along with assimilation from the water column and N$_2$ fixation) has previously been shown to be an important source of N in Baja microbial mats (22).

DNRA rates, measured in the dark, when microbial mats are anoxic (6), were the highest in ES microbial mats in April, when the NH$_4^+$ concentration and NH$_4^+$ assimilation rates were also highest. Baja mats exhibited a very low DNRA rate relative to those measured in ES mats. Factors causing the difference in DNRA rates between these mats should be investigated further.

Anammox was not observed in either of the mats studied. Anammox has been found to be absent in other microbial mats (21, 32), and to the best of our knowledge, no study has documented the occurrence of this process in these ecosystems. This might imply the absence of zones featuring favorable conditions for anammox in microbial mats. Although mats are exposed to anoxic environments below 2 mm when photosynthesis occurs, other factors, and not O$_2$ absence, may limit anammox in these zones. Risgaard-Petersen et al. (33) suggested that anammox is of very limited significance in environments that periodically experience N limitation, and this could be the case for microbial mats. Other studies have, however, measured high anammox

---

### TABLE 1 Nutrient concentrations and environmental parameters in overlaying water at the time of microbial mat collections

<table>
<thead>
<tr>
<th>Field site</th>
<th>Sample description</th>
<th>Concentration, µM</th>
<th>Salinity, %</th>
<th>Surface water depth, m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration Area 5, Exportadora de Sal S. A. de C.V., Guerrero Negro, Baja California Sur, Mexico (27° 41' 15.22&quot; N, 113° 55' 17.92&quot; W)</td>
<td>Baja February</td>
<td>Ammonium: 1.6 ± 0.6, Nitrate: 0.8 ± 0.3</td>
<td>98</td>
<td>1</td>
</tr>
<tr>
<td>Elkhorn Slough, Monterey Bay, CA, USA (36°48'46.61&quot; N, 121°47'4.89&quot; W)</td>
<td>ES October</td>
<td>Ammonium: 1.4 ± 1.1, Nitrate: 0.9 ± 0.9</td>
<td>55</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>ES April</td>
<td>Ammonium: 21.2 ± 1.6, Nitrate: 1.2 ± 0.5</td>
<td>55</td>
<td>0.1</td>
</tr>
</tbody>
</table>
activities in marine suboxic zones with very low inorganic N concentrations (34, 35). Our finding of very high rates of NH₄⁺ assimilation may imply that anammox bacteria in microbial mats are outcompeted by other microorganisms for NH₄⁺. Also, large amounts of organic matter in microbial mats (with C/N ratios in these mats of between 5.8 and 7.2 [data not shown]) would result in heterotrophic denitrification outcompeting anammox bacteria, which are slower growing. Fluctuating conditions are also known to be less beneficial for anammox microbes (36).

Amendment with ¹⁵NO₃⁻ under anoxic (dark) conditions did not result in ¹⁵N₂ enrichment in any investigated microbial mat samples. However, these incubations resulted in very different patterns of ¹⁵N₂O enrichment. Baja microbial mats in February revealed slight ¹⁵N enrichment in N₂O; however, all the N₂O concentrations were below the limit of quantification. This could be evidence of weak denitrifier activity in these mats. In the case of the ES October data set, a strong ¹⁵N₂O labeling occurs at time step zero (only a few seconds of incubation), which would not be expected in case of a progressive microbial N₂O production by denitrification. The calculated evolution of labeled N₂O might indicate increasing N₂O production at the beginning (ca. for 1 h), followed by a decreasing contribution of labeled N₂O. However, over the experimental duration of 6 h, no changes in ¹⁵N-N₂O or total N₂O concentration occurred (with the exception of the immediate labeling of N₂O at time zero). While this might invoke the presence of an abiotic mechanism of N₂O production using the added labeled source at the beginning of the experiment (37), consideration of the entire time course of the incubation provides no clear evidence of microbial N₂O production by denitrification (as would be expected from the N₂O concentration increasing over time). In these incubations, ZnCl₂, which is routinely used as a preservative in ¹⁵N tracer studies, was added to incubation bottles prior to sampling to stop microbial activity. Considering that these studies do not directly measure N₂O production via denitrification, it is unclear if the high concentrations and ¹⁵N abundance of N₂O are an artifact of abiotic N₂O production in our study or if it is rather the case that that ZnCl₂ does not inhibit activity of denitrifying enzymes, except for N₂O reductase. In either of these cases, precautions should be taken when using ZnCl₂ as a preservative in similar studies.

In the case of ES microbial mat incubations in April, where headspace was transferred directly without prior addition of ZnCl₂, the ¹⁵N composition of N₂O indicated increasing ¹⁵N abundance over time. However, the analyzed N₂O concentrations were very low (most of them below <0.3 ppmv), and hence, while there is an indication for microbial N₂O production, the reliability of this data set is questionable. The differences between both data sets (ES October and ES April) are obvious, however, and clearly indicate that they behave differently with respect to the observed N turnover.

N₂O can be produced via several N transformation processes such as co-denitrification, heterotrophic nitrification, nitrifier denitrification, and incomplete denitrification to N₂O (38, 39). In the present work, N₂O production was determined based on the ¹⁵N abundance of N₂O and the ¹⁵N abundance of NO₃⁻ calculated via the mole fraction of ⁴⁵N₂O and ⁴⁶N₂O, assuming denitrification as the only relevant source of microbial N₂O production. In fact, according to the ¹⁵N approach of Spott and Stange (39), no hints could be found that a hybrid N₂O production by, e.g., co-denitrification took place. However, it should be taken into account that NO₃⁻ reduction to NO₂⁻ and further reduction of NO₂⁻ to N₂O can be separated. Many denitrifying microorganisms can reduce NO₃⁻ to NO₂⁻ and excrete NO₂⁻ into the environment, which can be further used by another functional group, such as nitrifiers. However, under the experimental conditions used here (anoxic), nitrifier denitrification could not have occurred.

Most of the denitrification rates in microbial mats reported by other studies were measured either using an acetylene inhibition technique or by isotope pairing technique evaluation that only quantifies ¹⁵N₂ and not N₂O production (18–21, 40). Using these techniques, it is impossible to evaluate whether incomplete denitrification to N₂O took place in the microbial mats. A direct comparison of incomplete denitrification
rates found in our study and other studies cannot, therefore, be made. However, there are reports of incomplete denitrification for similar ecosystems. For instance, Abed et al. (32) reported high rates of N$_2$O emissions from cyanobacterial soil crusts that were assigned to incomplete denitrification and reported to make up to 54 to 66% of the total produced gases during denitrification. In contrast, Molina et al. (41) have reported that microbial mats in evaporitic ponds in high-altitude wetlands in Chile may be sinks of N$_2$O. Further work is needed to understand the fluxes of N$_2$O between microbial mats and the atmosphere.

It is not clear why the microbial mats used in these studies exhibited incomplete (to N$_2$O, rather than to N$_2$) denitrification. Incomplete denitrification has been shown to occur under low O$_2$ concentrations as N$_2$O reductase is more sensitive to O$_2$ than the other denitrifying enzymes (42). However, considering that under natural conditions microbial mats are completely anoxic in the dark and that our experiments were carried out in dark and after flushing with N$_2$, O$_2$ was not present during the incubations and would therefore not inhibit N$_2$O reductase. Incomplete denitrification has been shown to occur in agricultural lands and soils under carbon-limited conditions (i.e., a C/N ratio of <2) (43, 44). The microbial mats used for these studies exhibited C/N ratios of between 5.8 and 7.2 (data not shown) and so are outside the range of C/N ratios that are known to trigger incomplete denitrification. N$_2$O reduction might be inhibited by increased salinity (45) as N$_2$O reductase is a periplasmatic enzyme that is more sensitive to environmental stress than NO$_3^-$ reductase, which is membrane bound (46).

Another possible explanation for denitrification with N$_2$O as the end product instead of N$_2$ is copper limitation. Copper is a cofactor of the N$_2$O reductase, and its shortage has shown to limit the activity of this enzyme, causing N$_2$O accumulation in cultures of denitrifying bacteria (47). Sulfide has also been shown to inhibit N$_2$O reduction, resulting in production of N$_2$O and not N$_2$ as the major product of denitrification (48). Further research is needed on possible causes of incomplete denitrification in the studied microbial mats.

Nitrogen fixation was evaluated using both stable isotope and acetylene reduction assay techniques. It has been reported numerous times that a conversion factor from C$_2$H$_4$ production to N fixation should be evaluated for an individual system (49). In our study, the C$_2$H$_4$/$^{15}$N$_2$ assimilation ratio was found to be 69 and so is on the higher end of reported values by other studies (50–52). Low solubility of N$_2$ gas when introduced as a gas bubble is one of reasons for underestimating N$_2$ fixation rates when using the $^{15}$N$_2$ tracer method (49, 53). Nevertheless, the obtained N$_2$ fixation rate for the ES April data set is 1 to 2 orders of magnitude lower than other estimated N turnover rates (i.e., NH$_4^+$/NO$_3^-$ assimilation), and it is evidence that N fixation is probably an insignificant source of N in these mats in comparison with recycling/assimilation from a water column. Still, while assimilation of N from ammonification in deeper mat layers can supply the N demand of anabolism in the surface layers, N$_2$ fixation likely remains an important source for “new” (external to the mat) N necessary for the production of new mat biomass. Low N$_2$ fixation rates can be explained from a bioenergetic perspective, because energetically expensive N$_2$ fixation generally does not dominate in the presence NH$_4^+$ or NO$_3^-$.

Bioenergetic considerations may be of greater relative importance in hypersaline environments, where more energy is required to overcome salt stress (54). A similar conclusion about N cycling in other hypersaline microbial mats was recently reported by investigators using completely different methods (55). Specifically, these investigators also saw evidence for an inhibition of nitrification, as well as suppression of denitrification and annamox, and observed the N isotopic signature of efficient NH$_4^+$ recycling.

**Overview of N cycles of the ES and Baja microbial mats and differences between them.** Analysis of the N transformations in the ES microbial mats revealed low rates of nitrification and high rates of NO$_3^-$ consumption that may indicate both NO$_3^-$ assimilation and aerobic denitrification (Fig. 8). There was a significant seasonal difference in NH$_4^+$ assimilation rates in these mats that may be explained by seasonal fluctuations of in situ water NH$_4^+$ concentrations (higher in spring compared to fall) and/or the availability of organic matter on top of, or below, the surface of the
photosynthetic layers of the mats (see comments about the physical structure the ES mats below). Anammox was undetectable, DNRA rates were low in both measured seasons, and the N\textsubscript{2} fixation rate was found to be low in spring (not measured in fall). Incomplete denitrification to N\textsubscript{2}O was observed; however, its rate was very low. We also detected low rates of N\textsubscript{2} fixation in ES mats in April (not measured in October).

Overall, the N cycle in these mats was characterized by high rates of recycling of nutrients from the water column, with seasonal variations depending on the nutrient concentrations.

Baja microbial mats were also characterized by low rates of nitrification (Fig. 8). However, these mats were found to have a higher affinity for NO\textsubscript{3}\textsuperscript{-} compared to the ES mats. Also, rates of NH\textsubscript{4}\textsuperscript{+} assimilation from the water were found to be lower than rates of NO\textsubscript{3}\textsuperscript{-} consumption in these mats. This might indicate the presence of an additional sink for NO\textsubscript{3}\textsuperscript{-} in the Baja microbial mats consistent with what would be expected from aerobic denitrification. Baja mats also had rates of ammonification far higher than those in the ES mats in October. Baja mats were similar to ES mats in having low DNRA rates and an apparent absence of the anammox process. Evidence for incomplete denitrification to N\textsubscript{2}O was also found in these mats.

**Macroscale constraints on microscale processes in microbial mats.** While both mats exhibited a great capacity for consumption of both NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} from the water column, somewhat higher mean rates of ammonification and indications of aerobic denitrification distinguish the Baja mats from the ES mats. We hypothesize that these differences in N cycling processes likely result from differences in morphological characteristics of the mats, which are, in turn, related to the environments in which they grow; Baja mats are constantly submerged, whereas ES mats grow in a highly dynamic intertidal environment. The degree to which the physical integrity of the mats is disrupted by turbulence and storm events likely changes the availability of regenerated nitrogen from lower layers to the active photosynthetic zone. While the upper 3 to 4 mm of each mat have very similar redox-related depth stratification (Fig. 1), the delivery of the substrates necessary for these transformations to the respective redox zones conducive to them is likely impacted over larger spatial scales.

Physical disturbances resulting from a combination of wind-driven water movement, desiccation impacts on the attachment of the mats to sediment, and even disturbance by fauna result in far greater disruption of physical structure and integrity in the ES mats than the Baja mats (Fig. 1). ES mats are commonly observed to be forming on the surface of older layers of mat and on top of deposits of filamentous green algae, which grow in abundance during some seasons of the year. The profound physical disturbance of the ES mat likely results in a highly variable flux of NH\textsubscript{4}\textsuperscript{+} from lower layers of mat, as well as the possibility of the lower layers of the mat coming into contact with oxygen from the water and even air during low tides. In contrast, the Baja mat is far more structurally cohesive, and the upper layers of the mat likely receive an uninterrupted and much higher flux of NH\textsubscript{4}\textsuperscript{+} diffusing from lower layers. These differences
in physical structure would explain the observed differences in measured rates of aerobic denitrification and ammonification and likely result in profound differences in depth-related microbial community compositions, even in the upper layers of the mat. The depth-dependent microbial community composition and nitrogen cycling functional genes in these mats are presently being investigated using iTag and omics techniques.

**Conclusions.** Respiratory N transforming pathways were dominated by incomplete denitrification to N\textsubscript{2}O, a finding that revealed photosynthetic mats as being net sources of this important greenhouse gas. Low rates of N fixation in Elkhorn Slough microbial mats hint at a rather closed N transformation cycle, based on recycling and assimilation as the main sources of N in the studied mats at the sampling times investigated here. Differences in N consumption may in large part be explained by structural differences in the mats imparted by physical factors in their environments and by NH\textsubscript{4}+ concentrations in surrounding water. The underlying biological processes driving the observed activities should be investigated with improved depth resolution in future studies using omics techniques in order to reveal the responsible organisms and pathways.

**MATERIALS AND METHODS**

**Study areas and microbial mat sampling.** This study evaluated microbial mats collected from two distinct locations. The first study area is the salt works managed by Exportadora de Sal SA, and located near Guerrero Negro, Baja California Sur, Mexico (further referred to as “Baja February” (GPS coordinates in Table 1). Microbial mats at this location are permanently covered with 0.5 to 1 m of water at 80 to 100% salinity and can grow up to 10 cm thick (6) (Fig. 1A to C). Sections (20 by 25 cm) of microbial mats were collected from the seawater concentration Area 4, on the dike separating Area 4 from Area 5 (Area near 5), on 25 February 2016 and transported to NASA Ames Research Center (Moffett Field, CA) within 3 days. During the transportation, microbial mats were kept moist with the brine from the sampling site.

The second study area is located within the Elkhorn Slough (ES) estuary at Monterey Bay, CA, USA (GPS coordinates in Table 1). This mat (further referred to as “ES mat”) is up to 1 cm thick, and its areal extent varies with seasonal changes in water flow, tides, and nutrient inputs (56) (Fig. 1D to F). The average salinity of the field site water is 55%. The mats were collected at this experimental site twice, on 19 October 2015 (further referred to as “ES October”) and on 5 April 2017 (further referred to as “ES April”). After collection, the samples were kept moist with ambient seawater and brought to the laboratory at NASA Ames Research Center (Moffett Field, CA) within 2 h.

Nutrient concentrations and environmental parameters of water from sampling sites at the time of microbial mat collections are given in Table 1. Upon arrival, all samples were immediately prepared for incubations for biogeochemical rate measurements.

**Incubation setup.** Incubations were prepared by cutting small subcores (11-mm diameter, 10-mm depth) from whole sections of intact microbial mat with plastic core tubes, and the cores, still contained within the plastic core tubes, were placed on the bottom of 10-ml serum vials for the N\textsubscript{2} fixation measurements and in 38-ml serum vials for the other experiments. A 1% agar solution made using water from respective sampling site was added to each bottle up to the level of the top of the core tube. The agar served to physically fix the plastic core into position in the bottom of the bottle and to ensure that the only contact between the cores and the overlying water was across the top of the mat (across the mat-water interface). Fifteen milliliters of filtered (0.2-μm pore size) seawater from a boat ramp neighboring the sampling location (for ES mats) and ambient filtered water (for Baja mats) was then added to each 38-ml bottle, and bottles were subsequently sealed with a rubber stopper. Two milliliters of water was added to the 10-ml serum vials that were used for N\textsubscript{2} fixation measurement. Three replicates were set for each sampling time and treatment. Prior to the injection of a labeled compound, all samples were preincubated for 16 h under light for day incubations and for 24 h in the dark for dark incubations. Samples for N\textsubscript{2} fixation rate measurements were preincubated under light for 24 h, although the experiment was performed in the dark. Day incubations were performed under irradiance provided by white LED lamps (≈1,200 μE m\textsuperscript{-2} s\textsuperscript{-1}), and night incubations were performed under dark conditions (covered by aluminum foil). Night incubations were purged with N\textsubscript{2} for 15 min prior to the start of the experiment. All the incubations were performed on a shaker table with a rotation speed of ≈30 rpm and constant temperature of +16°C. One milliliter of water sample for nutrient analysis was taken at times zero, 0.5, 1, and 6 h, filtered with a 0.2-μm syringe filter, and stored at 20°C. Ammonium analysis was performed using a colorimetric method as described by Parsons et al. (57), and NO\textsubscript{3}\textsuperscript{-} was analyzed using a microplate reader-based colorimetric method adapted from the protocol of Ringquet et al. (58). Samples for 15N analysis were taken and preserved as described in the next section.

**Biogeochemical rate measurements.** The rate of each specific N transformation process (nitrification, NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}+ assimilation, ammonification, anammox, denitrification, DNRA, and N\textsubscript{2} fixation) was measured for two seasons (fall and spring) in ES mats and one season (winter) in Baja mats. All the rates were measured as potential rates (maximum rates under optimal conditions i.e., anoxic in dark/oxic under light) without limitation by substrate availability and with diffusive solute transport aided through

April 2021 Volume 87 Issue 8 e03118-20 aem.asm.org 12
Nitrogen Turnover in Microbial Mats

In acknowledgment of the possibility of circadian rhythms, all incubations under light were done during daylight hours (between 9:00 a.m. and 3:00 p.m.), and all incubations in the dark were done close to and after sunset (6:30 p.m. to 6:30 a.m.). Rates are presented as amounts of N (mol) per surface area of microbial mat (m²) per time (day).

Nitrification and nitrate assimilation/aerobic denitrification rate measurement. Nitrate production (nitrification) and consumption (NO₃⁻ assimilation and denitrification under aerobic conditions) were monitored using an isotope dilution approach. One hundred microliters of 10 atom% Na¹⁵NO₃ was injected into the vials, such that the final concentration of Na¹⁵NO₃ was 100 μM. Incubations were performed under light. As NH₄⁺ was present in ambient water at each sampling site (Table 1), it was not added to the incubation vials. Water samples (14 ml) were taken destructively at times zero, 0.5, 1, 3, and 6 h, filtered with a 0.2-μm syringe filter, and stored at 20°C until further ¹⁵NO₃ analysis. Samples for ¹⁵N in NO₃⁻ analysis were further prepared by bacterial denitrification assay (59) and analyzed on a Thermo Finnigan GasBench + PreCon trace gas concentration system interfaced to a Thermo Fisher Scientific (Bremen, Germany) DELTA V Plus isotope ratio mass spectrometer (IRMS) at the Stable Isotope Facility of UC Davis, Davis, CA, USA. The gross nitrication and NO₃⁻ consumption rates were calculated as previously described (27), and the net nitrification rates were calculated as NO₃⁻ concentration change per day as the difference between the gross nitrication and consumption rates.

Ammonification and ammonia assimilation rate measurement. Ammonium production (ammonification) and consumption (NH₄⁺ assimilation) were monitored using an isotope dilution approach. In order to distinguish NH₄⁺ assimilation from nitrification, nitrapyrin (a nitrification inhibitor) was added to all vials at a final concentration of 0.5 mg ml⁻¹ (60). Nitrapyrin was shown to effectively inhibit nitrification in liquid cultures at concentrations of 0.5 to 1.0 mg ml⁻¹ for up to several days (61, 62). One hundred microliters of 10 atom% ¹⁵NH₄Cl was injected into the vials, such that the final concentration of ¹⁵NH₄Cl was 100 μM. Incubations were run under light conditions. Incubations were stopped by removing all the water from incubation vials at times zero, 0.5, 1, 3, and 6 h. The water samples were then filtered with a 0.2-μm syringe filter and stored at 20°C until further ¹⁵NH₄ analysis. Samples for ¹⁵N in NH₄⁺ analysis were prepared by hypobromite-azide method as per Zhang and Altabet (63). The ¹⁵N of the resultant N₂O was measured on a Thermo Fisher Scientific (Bremen, Germany) Finnigan DELTAplus XP IRMS at the University of Utrecht, Utrecht, Netherlands. The gross ammonification and NH₄⁺ assimilation rates were calculated as previously described (27), and the net ammonification rates were calculated as NH₄⁺ concentration change per hour as the difference between the gross ammonification and NH₄⁺ assimilation rates.

Anammox rate measurement. To estimate the potential rate of anammox in studied microbial mats, the experimental setup related to the isotope pairing technique (IPT) was used. Fifty microliters of 99 atom% ¹⁵NH₄Cl and 50 μl of NaNO₂ were injected into the vials, such that the final concentration of ¹⁵NH₄Cl was 50 μM and the final concentration of NaNO₂ was 50 μM. Incubations were run in the dark. For the ES October samples, incubations were stopped at 0, 0.5, 1, 3, and 6 h by adding 200 μl of a 7 M ZnCl₂ solution. Afterwards, the samples were stored at +4°C upside down for 3 months before the headspace was transferred to evacuated 12-ml vials (Exetainer; Labco, Ltd., United Kingdom). For the Baja February and ES April samples, headspace was directly transferred at 0, 0.5, 1, 3, and 6 h to evacuated 12-ml Exetainer vials. The ¹⁵N in N₂ was analyzed using a Thermo Fisher Scientific GasBench + Precon gas concentration system interfaced to a Thermo Fisher Scientific DELTA V Plus IRMS at the Stable Isotope Facility of UC Davis, Davis, CA, USA. The rates of anammox were calculated from the mole fraction of ²⁹N₂ and ³⁰N₂ and of ⁴⁴N₂O and ⁴⁵N₂O, respectively, in the sampled headspace of samples using the equation for hybrid N gas formation of Spott and Stange (39).

Denitrification and DNRA rate measurement. In the ES October samples, denitrification rates were measured using the experimental setup related to the IPT after injection of 100 μl of 99 atom% Na¹⁵NO₃ into the vials such that the final concentration of Na¹⁵NO₃ was 100 μM. Addition of 99% labeled Na¹⁵NO₃ led to very high ¹⁵N content in N₂ and therefore for further mat incubations with the Baja February and ES April samples, 10 atom% Na¹⁵NO₃ was used instead. Incubations were run in the dark. For the ES October samples, incubations were stopped at 0, 0.5, 1, 3, and 6 h by injecting 200 μl of a 7 M ZnCl₂ solution. Afterwards, the samples were stored at +4°C upside down for 3 months before the headspace was transferred to evacuated 12-ml incubation vials. For the Baja February and ES April samples, incubations were stopped at 0, 0.5, 1, 3, and 6 h by direct transfer of headspace to evacuated 12-ml Exetainer vials. The ¹⁵N in N₂ and NO₃⁻ was analyzed using a Thermo Fisher Scientific GasBench + Precon gas concentration system interfaced to a Thermo Fisher Scientific DELTA V Plus IRMS at the Stable Isotope Facility of UC Davis, Davis, CA, USA. The rates of denitrification and complete denitrification to N₂O were calculated from the mole fractions of ²⁹N₂ and ³⁰N₂ and of ⁴⁴N₂O and ⁴⁵N₂O, respectively, in the headspace atmosphere of samples using equations 1 and 2 of Spott and Stange (39).

DNRA rates were quantified in the same vials used for denitrification incubations. For ES October samples, additional incubations were set with 10 atom% Na¹⁵NO₃ additions identical to ES April and Baja February denitrification incubations. After headspace was sampled for the denitrification rate estimation, 14 ml of water from the vial was filtered with a 0.2-μm syringe filter and stored at 20°C until further ¹⁵N analysis. Samples for ¹⁵N in NH₄⁺ analysis were prepared by the hypobromite-azide method as described by Zhang and Altabet (63). The ¹⁵N of the resultant N₂O was measured on a Thermo Fisher Scientific (Bremen, Germany) Finnigan DELTAplus XP IRMS at the University of Utrecht, Utrecht, Netherlands. The rates of DNRA were calculated using the isotope mixing approach (64).

Nitrogen fixation rate measurement. Unlike other light incubations, all the samples for N₂ fixation rate measurements were preincubated under light for 16 to 24 h before the beginning of the experiment that was run in the dark as the light period has been shown to be required for energy and
redundant generation in other photosynthetic microbial mats (65). One milliliter of headspace was replaced by 99 atom% 15N2, resulting in the isotopic composition of 15N2 of 18.3 atom%. Microbial mat biomass was transferred to scintillation vials at times zero, 0.5, 1, 3, and 6 h and frozen at −20°C. Afterwards, the biomass was dried, homogenized, weighed into tin capsules, and analyzed for 15N content with IRMS using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Ltd., Cheshire, United Kingdom) at the Stable Isotope Facility of UC Davis, Davis, CA, USA. The rates of N2 fixation were calculated as the relative contribution of the 15N2 pool to the microbial mat biomass using the isotope mixing approach (64). The N2 fixation rate as nitrogenase activity was also estimated with the acetylene reduction assay (ARA) as previously described (65). Five hundred microliters of water was exchanged for acetylene, which was injected through the stopper into the aqueous phase to start the incubation. One milliliter of headspace was transferred at 0, 0.5, 1, 3, and 6 h to the Exetainer vials containing a saturated solution of sodium chloride (NaCl) and inverted for storage (the stored gas thereby being trapped by the NaCl solution below it and glass above it). Ethylene concentrations were quantified using a Shimadzu GC-14A gas chromatograph with a flame ionization detector (FID) and a 2-m Porapak N column held at 80°C.

### Statistical analysis
Statistical analyses for differences in biogeochemical process rates were performed using SigmaPlot software (v.13.0; Systat Software, Inc.). Normality of the data was checked using the Shapiro-Wilk test. A one-way analysis of variance (ANOVA) was performed to check for differences between the two types of microbial mats and seasons. When significant differences were found, treatment means were separated with a pairwise multiple comparison test (Tukey’s, Dunn’s, or Holm-Sidak) at a P value of 0.05. The rates presented are means of three replicates. Error bars represent ±1 standard deviation. As replicates represented biological samples, standard deviations of the measured rates were found to be sometimes very high due to well-known (e.g., Dillon et al. [66]) heterogeneity of microbial mat samples.

### ACKNOWLEDGMENTS
Funding was provided by the U.S. Department of Energy (DOE) Genomic Science Program and NASA, both through the Exobiology and ISFM programs. Oksana Coban was supported by an appointment to the NASA Postdoctoral Program at the NASA Ames Research Center, administered by the Universities Space Research Association under contract with NASA. Olivia Rasigraf and Anniek E. E. de Jong were supported by the Netherlands Organization for Scientific Research (NESSC 024.001.001).

We thank Mike S. M. Jetten for providing laboratory facilities for 15NH4+ sample preparation and Carina van der Veen and Thomas Roeckmann for assistance with 15NH4+ measurements. We are also grateful to Angela Detweiler for help with the experiments. An anonymous review substantially improved an earlier version of the manuscript. We are appreciative of access to the Guerro Negro, Baja California field site and logistical support provided by Exportadora de Sal, S.A. de C.V. We thank Jeff Cann, Associate Wildlife Biologist, Central Region, California Department of Fish and Game, for coordinating our access to the Moss Landing Wildlife Area to collect Elkhorn Slough mats.

No competing financial interests exist.

### REFERENCES


