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REVIEW

Nitrogen Fate and Adaptation of the Microbial Community Responsible for Ammonia Removal in a Biofilter Treating Waste Gas from Livestock Manure Composting

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Abstract

Treating NH_3-loaded gases is necessary for improved livestock management. Nitrification, the sequential oxidation of NH_3 to NO_3^- via NO_2^-, is an important microbial process for effective long-term NH_3 removal. Denitrification, a microbial respiration process that reduces NO_3^- and NO_2^- to N_2 under anaerobic conditions, can also contribute to nitrogen conversion in biofiltration systems. Understanding these microbial processes is imperative to control NH_3 removal better and achieve nitrogen balance in biofiltration. In this review, we discuss the functions and compositions of the microbial community responsible for nitrification and denitrification in a biofiltration system, along with the relationship between these processes and the nitrogen mass balance. Our results indicate that both nitrification and denitrification could be achieved by a consortium of microbes well adapted to the ecosystem in a full-scale biofilter. Moreover, the microbial community was controlled by substrate availability. Nitrogen removal potential was up to 39% in a laboratory-scale biofilter with intermittent water recirculation, and the unknown nitrogen loss was considered mainly denitrified. Under gradual accumulation of nitrogenous compounds, the gamma proteobacterial group contributes to NH_3 oxidization. These findings will improve our understanding of microbial fluctuations and the complex behavior of nitrifiers and denitrifiers within an NH_3-loaded biofiltration system.

Discipline: Agricultural Environment
Additional key words: denitrification, microbial community composition, nitrification, nitrogen fate

Introduction

A large amount of ammonia (NH_3) is emitted during the composting of livestock manure as a byproduct of the aerobic decomposition of organic matter. Based on the emission rates of NH_3 of various composting processes (Hojito et al. 2003), a survey of livestock manure management by Japanese farmers (Ministry of Agriculture, Forestry and Fisheries 2011), and inventories of nitrogen excretion (LEIO 1998, Hojito et al. 2003, Ogino et al. 2017), the estimated emission rates of NH_3 from Japanese livestock farms are approximately 6%, 13%, 8%, and 29%-45% of the total nitrogen contained in the manure of milking cows, beef cattle, pigs, and poultry, respectively. NH_3 causes environmental degradation, including eutrophication and acidification (Steinfeld et al. 2006, Sutton et al. 2008), in addition to an odor problem (Kuroda et al. 1996, Parker et al. 2012). Its concentration in the air is regulated as per the Offensive Odor Control Law of Japan (Ministry of the Environment 1995). Thus, the treatment of NH_3-loaded gases is necessary for better management of livestock farming.

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Compared with other physical and chemical deodorization technologies, biological techniques are often used to treat NH_3 because of their relatively low cost and high removal efficiency (Wani et al. 1997, van Groenestijn & Kraakman 2005). Biofiltration is a promising odor and gas treatment technology (Chen & Hoff 2009). Although the treatment of high concentrations of NH_3 (i.e., > 100 ppm) from the process of composting livestock manure is challenging, it can be achieved by biofiltration with longer contact time between the gas and packing material (Fukumori & Doshu 1984).

The basic design and operating standard of biofiltration systems have been well established. However, improvements in engineering and biofilter modeling could depend on additional studies of the microbiology and chemical reactions involved (Williams & Miller 1993, Devinny & Ramesh 2005). Improved understanding of the microbial aspects is especially important for better control of the removal of NH_3 and nitrogen balance in the biofiltration process. In this review, we examined the compositions and functions of the microbial community responsible for nitrification and denitrification in full-scale and laboratory-scale rockwool biofilters.

NH_3 removal mechanisms and nitrogen fate in a biofiltration system

In a biofiltration system, NH_3 removal is achieved by absorption and nitrification. Theoretically, to achieve an ion balance, half of the incoming NH_3 is converted to nitrite (NO_2^-) or nitrate (NO_3^-) by nitrifying microorganisms, and the other half is absorbed into water as ammonium (NH_4^+), resulting in the accumulation of NO_2^- or NO_3^- and residual NH_4^+ in the system (Joshi et al. 2000, Baquerizo et al. 2009, Ottosen et al. 2011). This nitrogen accumulation leads to the failure of NH_3 removal (Smet et al. 2000) or requires the additional treatment of the percolating effluent water. Practically, however, nitrogen loss in biofiltration systems has been reported (i.e., 22%-35% of the inlet nitrogen; Malhautier et al. 2003). Furthermore, when a biofilter is operated with water circulation to minimize wastewater volume, the concentration of inorganic nitrogen gradually increases. However, removal of NH_3 can be achieved under nitrogen-accumulating conditions in a full-scale rockwool biofilter despite an unknown nitrogen fate (Sato 2000). Denitrification, a microbial respiration process that reduces NO_3^- and NO_2^- to dinitrogen (N_2) via nitric oxide (NO) and nitrous oxide (N_2O) under anaerobic conditions, can contribute to nitrogen removal in the biofiltration system.

Characterization of the microbial community associated with NH_3 oxidation in a full-scale rockwool biofilter for compost waste-gas treatment

Rockwool is a manufactured mineral fiber produced from slag or igneous rocks. A biofilter packed with a mixture of rockwool, urethane, zeolite, dried chicken feces, and rice husks has been developed as a replacement for soil biofilter (Fukumori et al. 1990). Yasuda et al. (2009) studied the performance of a rockwool biofilter treating compost waste gas by measuring the gases and potential nitrification and denitrification activities; a diagram of the rockwool biofilter used for analysis is shown in Figure 1. The inlet NH_3 concentration peaked after compost turning and then slowly declined, but the biofilter effectively removed the NH_3 gas. The inlet concentrations differed among sampling points, with the peak NH_3 concentration at inlets 2 and 3 being double of that of inlet 1 or higher. The kinetics of oxidation of NH_4^+ in the packing materials were studied by Yasuda et al. (2010). The estimated values for the Michaelis–Menten constant K_m (mM) were similar among depths at the same sampling site in the biofilter and tended to increase in the order of site a < b < c (Table 1). Site a was exposed to gas emitted from the initial stages of the composting process, and site c was exposed to gas from the later stages (Fig. 1).

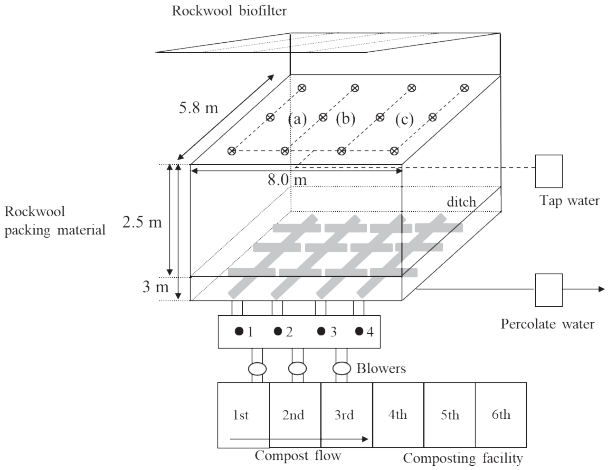

Fig. 1. Diagram of biofilter and composting facility used for analysis

Black circles, sampling points of inlet air (1-4); letters, sampling points of outlet air and packing materials (a-c); circles with a cross, water nozzles (reprinted from *Bioresource Technology*, vol. 100, Yasuda et al., Evaluation of full-scale biofilter with rockwool mixture treating ammonia gas from livestock manure composting, 1568-1572, Copyright [2009], with permission from Elsevier).

The inlet NH_3 concentration could have affected the microbial substrate affinity. Furthermore, the 140-cm depth from the midpoint site b, where the concentration of NH_3 was assumed to be high, had the highest NO_2^- + NO_3^- production rate V_{max} (μmol [g dry sample]$^{-1}$ hr^{-1}) (Table 1).

Chemolithotrophic NH_3-oxidizing bacteria and NO_2^--oxidizing bacteria traditionally have been considered responsible for nitrification. As for NH_3-oxidizing bacteria, *Nitrosomonas* spp. that require a high concentration of ammonium sulfate have been isolated from deodorization biofilters (Hatayama et al. 1999). Metagenomic studies, however, have recently identified a novel gene that resembles NH_3 monooxygenase, the central enzyme of NH_3 oxidation in bacterial nitrification, possessed by mesophilic Crenarchaeota (Venter et al. 2004, Treusch et al. 2005). Although an autotrophic NH_3-oxidizing archaeon has been isolated from marine and terrestrial environments (Könneke et al. 2005, Tourna et al. 2011), the existence of NH_3-oxidizing archaea in biofilters has not been previously reported.

Table 1. Estimated values of kinetic parameters K_m and V_{max}

Sample origin (site-depth)	K_m (mM, NH_4^+)	V_{max} (μmol [g dry sample]$^{-1}$ h^{-1})
a- 50 cm	0.05 (0.01)	0.29 (0.01)
a-140 cm	0.09 (0.03)	0.25 (0.01)
b- 50 cm	0.32 (0.07)	0.19 (0.01)
b-140 cm	0.24 (0.16)	0.36 (0.03)
c- 50 cm	0.68 (1.24)	0.22 (0.09)
c-140 cm	1.05 (0.88)	0.24 (0.05)

Values in parentheses represent standard errors (reprinted from *Microbes and Environments*, Vol. 25 [2010] No. 2, 111-119, Yasuda et al., with permission).

In a previous study (Yasuda et al. 2010), we examined the community structures of NH_3-oxidizing bacteria and archaea by PCR-denaturing gradient gel electrophoresis (DGGE) targeting *amoA* genes (Fig. 2). The overall patterns for NH_3-oxidizing bacteria varied, without common bands even within the same reactor. In contrast, the archaeal *amoA* banding patterns appeared to be more similar among samples. A correspondence analysis showed the DGGE profile of *amoA* of beta-proteobacterial NH_3-oxidizing bacteria of the deeper midpoint sample (b-140 cm) to be distinguishable from the others. The sequence of the specific band contributing to the pattern of sample b-140 cm (band B6) was related to *Nitrosospira multiformis* ATCC25196 (similarity: 93%) (Fig. 3). In contrast, the intense bands in sample a- and b-50 cm (bands B7-B9) were clustered together with *N. briensis* within *Nitrosospira* cluster 3. Although both *N. multiformis* and *N. briensis* are grouped within *Nitrosospira* cluster 3, the cell activity is higher for *N. multiformis* than for *N. briensis* (Belser 1979). These results suggest an association between the composition of the NH_3-oxidizing bacterial community and NH_3-oxidation kinetics of the samples. The presence of the crenarchaeal *amoA* gene sequences was confirmed, and their sequences were related to those belonging to soil and sediment groups. However, no clear association between NH_4^+-oxidation kinetics and this group was observed.

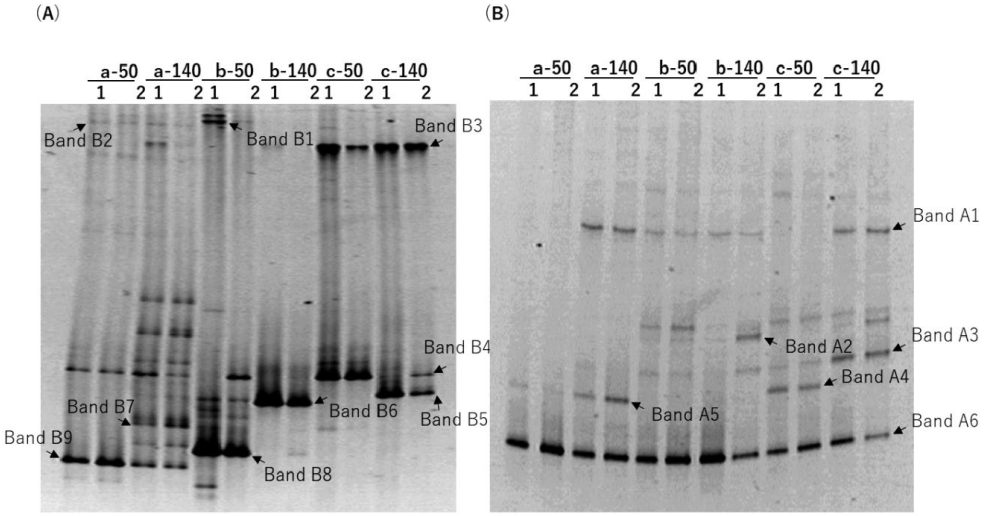

Fig. 2. Duplicate denaturing gradient gel electrophoresis profiles of ammonia-oxidizing bacterial (A) and archaeal (B) *amoA* fragments of the biofilter packing materials
Site a: 50 cm, 140 cm depth; site b: 50 cm, 140 cm depth; site c: 50 cm, 140 cm depth as shown in Figure 1. Bands indicated by black arrows were excised and sequenced (reprinted from *Microbes and Environments*, Vol. 25 [2010] No. 2, 111-119, Yasuda et al., with permission).

Characterization of the denitrifying bacterial community in a full-scale rockwool biofilter for compost waste-gas treatment

Few studies have examined the denitrifying bacterial community and the relationships between these microbes and nitrogen loss in gas purification systems, probably because a biofilter is generally considered to be a strongly aerobic ecosystem. We examined the physiological characteristics of the denitrifying bacterial

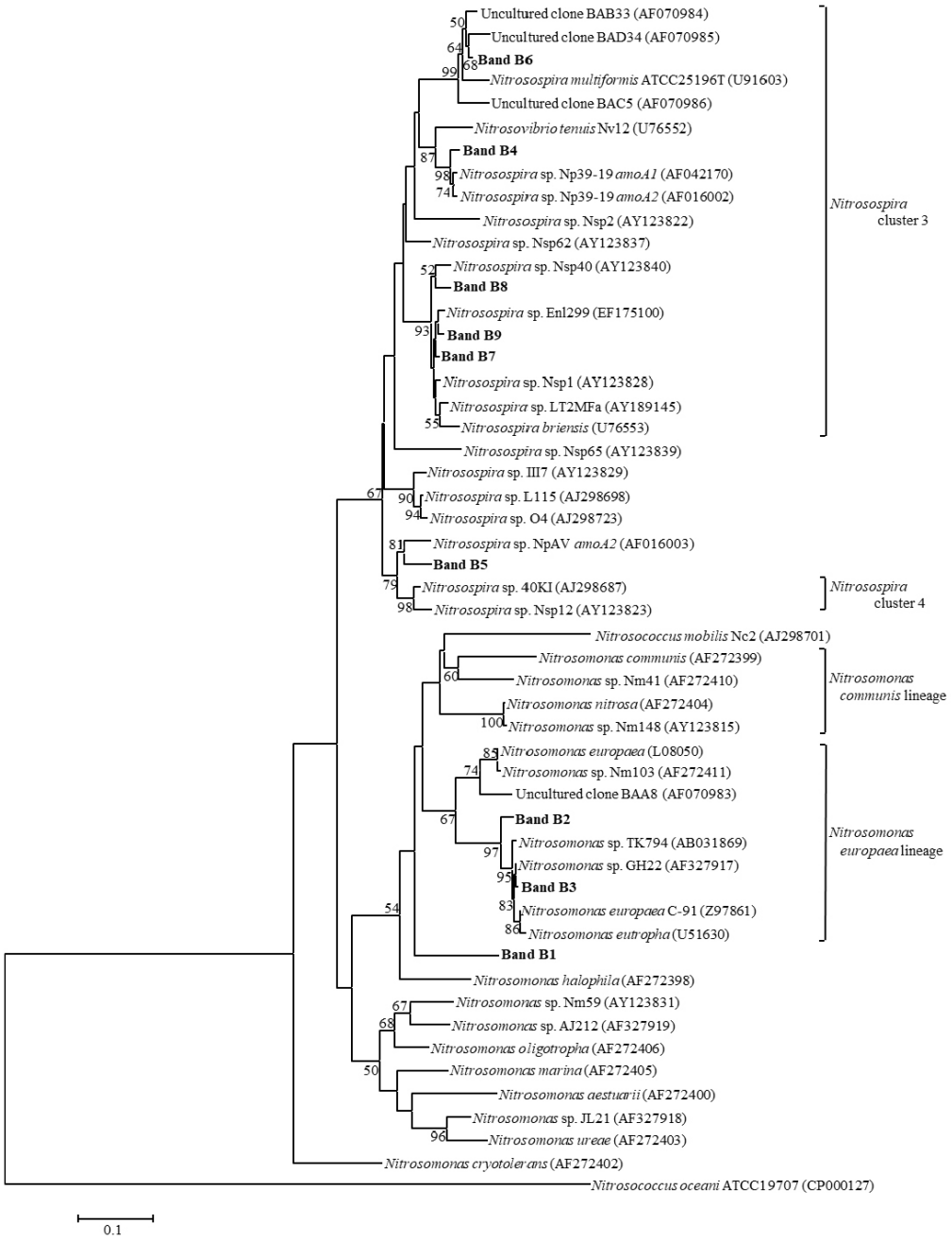

Fig. 3. Neighbor-joining tree based on ammonia-oxidizing bacterial *amoA* fragments (296 bp)
Bold letters represent sequences retrieved from denaturing gradient gel electrophoresis bands of rockwool packing materials. Accession numbers of sequences are indicated in parentheses. Scale bar indicates five replacements per 100 nucleotides. Bootstrap values (%) obtained with 1,000 resamplings are shown at branch points (when > 50%). *Nitrosococcus oceani* was used as an outgroup (reprinted from *Microbes and Environments*, Vol. 25 [2010] No. 2, 111-119, Yasuda et al., with permission).

community in response to different electron donors and the denitrifying bacterial community structure in a full-scale rockwool biofilter (Yasuda et al. 2017a). We conducted anoxic incubation with ^{15}N-labeled NO_3^- in the presence of different electron donors (compost extract, NH_4^+, hydrogen sulfide, propionate, and acetate). The responses of the rockwool samples to the different electron donors were compared with those of activated sludge (Fig. 4). Denitrification activity after 1 day of incubation was well-induced by the addition of compost extract with the rockwool samples, indicating that the dust originating from the compost materials in the waste-gas stream is an important source of organic matter for the denitrifying bacteria. With activated sludge, however, the addition of acetate induced the greatest increase in the potential denitrification among the electron donors.

The denitrifying bacterial community was analyzed using PCR-DGGE that targets the following denitrifying genes: *nirK* (encoding a copper-containing NO_2^- reductase), *nirS* (encoding a cytochrome cd_1-containing NO_2^- reductase), and *nosZ* (encoding N_2O reductase). The DGGE profile indicated differences in the distribution patterns of the denitrifying genes. The distribution of *nirK* was spread in a vertical direction, and the distribution of *nosZ* was horizontally spread within the biofilter (Fig. 5). Water is supplied from the top of the biofilter and is not circulated, whereas gas is supplied from the bottom. Therefore, NO_3^- concentrations gradually change in the vertical direction. The horizontal distribution of *nosZ* could represent the presence of composting gases from different composting stages. The horizontal and vertical stratification of the denitrifying bacterial community might be attributed to the operational conditions of the composting and biofiltration facilities. The sequences of the denitrifying genes were primarily related to those of Bradyrhizobiaceae, Alcaligenaceae, and Phyllobacteriaceae and to the environmental clones from the activated sludge, freshwater environments, guts

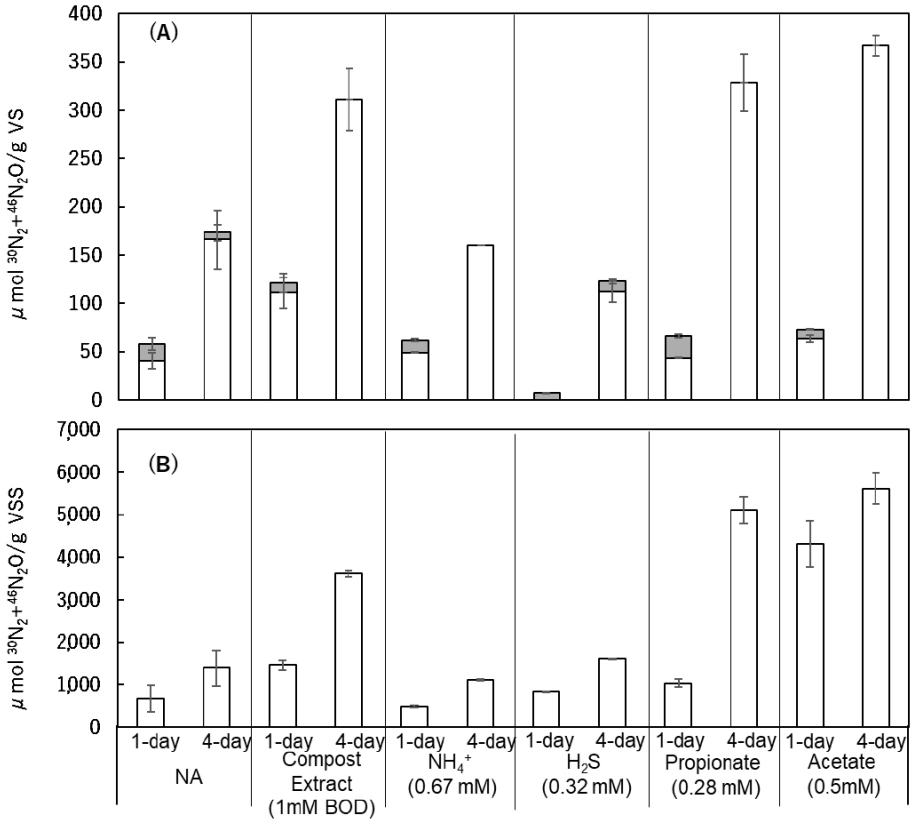

Fig. 4. $^{30}N_2$ and $^{46}N_2O$ production of (A) rockwool samples and (B) activated sludge after 1 and 4 days of batch incubation with the addition of different electron donors
NA: without addition of an electron donor. Error bars indicate the range (n = 2) except for NA, which is the SD (n = 4). White bars represent the portions of $^{30}N_2$, and gray bars represent those of $^{46}N_2O$. VS: volatile solids, VSS: volatile suspended solids (reprinted by permission from Springer Nature, *Applied Microbiology and Biotechnology*, Characterization of the denitrifying bacterial community in a full-scale rockwool biofilter for compost waste-gas treatment, Yasuda et al., Copyright [2017]).

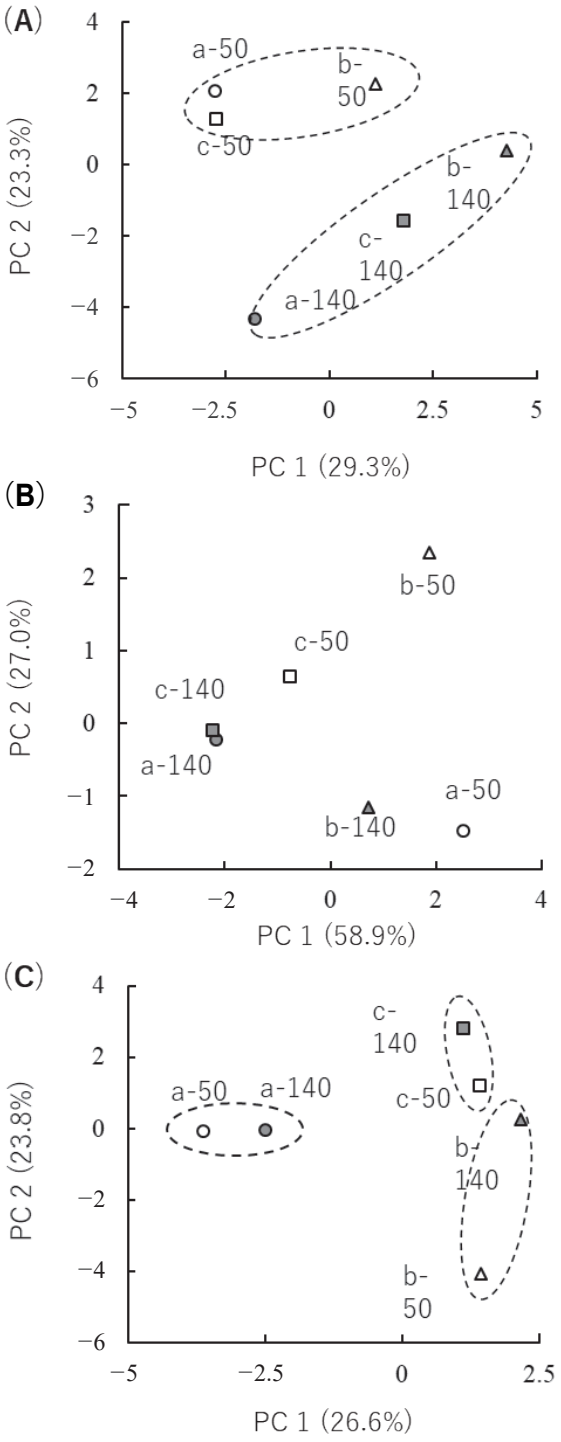

Fig. 5. Plots from principal component analyses of denaturing gradient gel electrophoresis bands of (A) *nirK*, (B), *nirS*, and (C) *nosZ* gene fragments

Letters and numbers within the plots represent the sampling positions (site and depth [cm]) in the biofilter shown in Figure 1 (reprinted by permission from Springer Nature, *Applied Microbiology and Biotechnology*, Characterization of the denitrifying bacterial community in a full-scale rockwool biofilter for compost waste-gas treatment, Yasuda et al., Copyright [2017]).

of earthworms or other invertebrates, and agricultural soil. Some *nirK* fragments were related to those from microaerobic environments. The microbial consortia in the biofiltration system may be affected by the changes in the types of composting facilities.

Responses of the community structure of total bacteria and NH_3-oxidizing and denitrifying microbes during nitrogen accumulation in an NH_3-loaded biofilter

A laboratory-scale biofilter with rockwool mixtures was operated to estimate the nitrogen balance and investigate the relationships between the structure of microbial community and nitrogen loss (Yasuda et al. 2013, 2017b). To mimic the types of biofilters often used in livestock farms, the NH_3-loading rate was 49-63 g m^{-3} day^{-1}, and the system was operated with water circulation for 436 days. Large proportions of nitrogen accumulated in the packing materials as NH_4^+ and NO_3^-. The percentage of nitrogen accumulated in the circulation water was 5%-10% (Fig. 6). Approximately 30%-39% of the inlet nitrogen was still not recovered after day 150. In addition to denitrification, an anaerobic NH_4^+ oxidation (anammox) reaction may contribute to nitrogen removal (Waki et al. 2010). However, based on a batchwise ^{15}N tracer experiment examining the presence of anammox and denitrification activity in the packing material, the unknown nitrogen was considered to be mainly denitrified (Yasuda et al. 2013).

During the operation of the laboratory-scale biofilter, the changes in the abundance as well as compositions of the community of the NH_3-oxidizing archaea and bacteria occurred in a different manner between archaeal and bacterial *amoA*. However, our results indicated that an increase in free NH_3 concentrations in circulation water could affect both microbial community structures. The increase in free NH_3 also caused a temporal decline in reactor performance. Dominant *amoA* sequences after this transition were related to Thaumarchaeota Group I.1b for archaea and to *Nitrosomonas europaea* lineages and one subcluster within *Nitrosospira* sp. cluster 3 for bacteria (Yasuda et al. 2013). The proportions of genera for which at least one denitrifying strain or species possessing *nosZ* had been characterized were assessed by metabarcoding sequencing analysis. The changes in the proportions agreed with the observed nitrogen loss. In addition, the relative abundance of the genus *Nitrosococcus* (gammaproteobacteria) increased when electron conductivity gradually increased from 24.4 to 84.7 mS cm^{-1} (Yasuda et al. 2017b). A shift from betaproteobacterial to gammaproteobacterial NH_3-oxidizing bacteria was noted. This latter bacterial group

could also contribute to oxidization of NH_3 during the later period of reactor operation (Fig. 6). The results of this study also highlight the adaptation of microbial communities to environmental changes, especially the gradual accumulation of nitrogenous compounds.

Future perspective

From the analysis of the full-scale rockwool biofilter, the potential nitrification activity was estimated to be sufficient to oxidize at least the half the incoming NH_3 during the sampling period, and more NH_3 was also removed via absorption into the water. These results suggest that denitrification can occur within the biofilter under appropriate conditions, and water circulation could enhance nitrogen removal in the system. Denitrification needs to be taken into account when the nitrogen mass balance in the biofilter is modeled. In addition, substrate availability might be a key factor in controlling the microbial community responsible for nitrification and denitrification in the biofilter.

High-NH_3-load operation could be achieved by improving the air-to-water contact performance, but this would raise the treatment cost of the percolating effluent water unless it could be used as a fertilizer or for other purposes. Optimizing the process regarding electron donor dosage (Yasuda et al. 2020) and the balance between the biomasses of nitrifying and denitrifying microbes remains to be clarified in future research.

Conclusion

In this paper, we reviewed our studies of the functions and compositions of the microbial community responsible for nitrification and denitrification in a biofiltration system as well as their relationships with the nitrogen mass balance. Both nitrification and denitrification can be achieved by a consortium well adapted to the ecosystem, and the microbial community was controlled by substrate availability in the full-scale biofilter. The nitrogen removal potential is up to 39% in the laboratory-scale biofilter with intermittent water recirculation. The nitrogen not accounted for was considered to be mainly denitrified. Moreover, we observed that the gammaproteobacterial group could contribute to oxidization of NH_3 under the gradual accumulation of nitrogenous compounds. This shift of bacterial community from betaproteobacteria

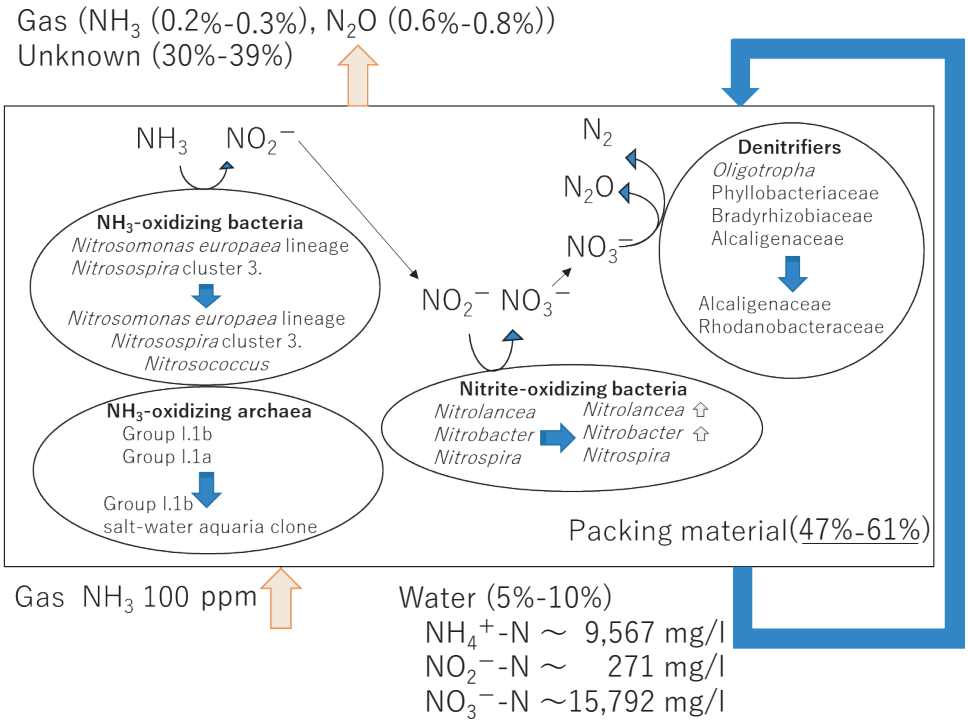

Fig. 6. Changes in the microbial community associated with nitrification and denitrification during nitrogen accumulation in a laboratory-scale rockwool biofilter with water circulation

Nitrogen allocations after day 150 of the operation (day 150, 317, and 436) as described by Yasuda et al. (2013) are shown as percentages of inlet nitrogen (sum of inflow NH_3-N and the initial amounts of the total nitrogen at day 0) in parentheses.

demonstrates functional redundancy by the adaptation of the nitrifiers to the reactor conditions. These findings improve our understanding of the microbial fluctuations and complex behavior of nitrifiers and denitrifiers within NH_3-loaded biofiltration systems.

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