Journal of Advances in Science and Technology
Vol. 17, Issue No. 1, March-2020, ISSN 2230-9659

An analysis on Soil Ammonia Oxidisers under different Nitrogen use Efficiency Strategies

Sudarshan Medagani[1]*, Dr. Neelu Jain[2]

[1] Research Scholar

E-Mail: sudarshanmedagani@gmail.com

[2] Associate Professor, SSSUTMS

E-Mail: info@sssutms.co.in

Abstract - The initial and rate-limiting step in nitrification is the oxidation of ammonia, which is carried out by both ammonia-oxidizing archaea (AOA) and bacteria (AOB). Environmental factors that influence the quantity, composition, and activity of AOA and AOB communities in soil nitrification are not fully defined, and the relative importance of these two groups in nitrification is still up for debate. AOB populations were found to be rising, as predicted, but there was no discernible influence of JX soil on any other taxa studied. And moreover, stable-isotope DNA probing demonstrated that during active nitrification, AOA outcompeted bacteria by factors of 37.0-, 10.5-, and 1.91-fold in the ZY, JD, and LZ soils, respectively, whereas in the JX soil, AOB but not AOA were tagged. Nitrogen-oxidizing bacteria (NOB) were discovered in greater numbers than nitrogen-fixing bacteria (AOA and AOB), and acetylene completely inhibited 13CO2 uptake by nitrifying populations. Molecular phylogenetics suggests that AOA associated with soil fosmid 29i4 catalysed archaeal ammonia oxidation within the soil group 1.1b branch. In the ZY, LZ, and JX soils, 13C-AOB belonging to the Nitrosomona communis lineage did the bulk of the ammonia oxidation, but in the JD soil, AOB similar to Nitrosospira cluster 3 did most of the work. The 13C-NOB was predominated by Nitrospira rather than Nitrobacter. Under microaerophilic conditions, the relative activity of AOA and AOB indicates that AOA is more advantageous than AOB. According to these results, soil physiochemical properties have a crucial role in determining ammonia oxidizer and nitrite oxidizer activities.

Keywords - Ammonia oxidizers, Soil, Nitrogen, Efficiency strategies

----------X----------

INTRODUCTION

In both nitrification and denitrification, nitrous oxide (N2O) is created as a byproduct and is formed as an intermediary. When it comes to global warming and the depletion of the stratospheric ozone layer, N2O production and emission are of critical relevance. Roughly 70% of the total N2O in the atmosphere comes from soil microbial activities. As more and more nitrogen (N) is added to agricultural soils via fertilisation, N2O emissions from soils have skyrocketed. Nitrification's contribution to total N2O generation has been shown to rise following ammonium fertilisation in both laboratory and field investigations, suggesting an uptick in nitrifier activity. Since community shifts were only detected for nitrifiers when wastewater was used in place of mineral fertiliser, it was unclear if fertilising also resulted in a shift in the community structure. Despite abundant evidence from field studies showing that ammonia oxidizer communities may vary among soil types (see below), there have been relatively few experimental research addressing the in situ dynamics of nitrifier populations.

The 16S rRNA genes have been the primary focus of research on the aerobic ammonia-oxidizing nitrifiers in soils. Two monophyletic groupings may be made out of the known ammonia oxidizers that are only found in pure culture. Two species, Nitrosococus oceani and Nitrosococus halophilus, are the only representatives of the subclass of the class Proteobacteria. The other group consists of two separate genera under the family Proteobacteria: Nitrosomonas and Nitrosospira. In

order to better understand the diversity of ammonia-oxidizing bacteria and clones within the -Proteobacteria, 16S rRNA gene sequences were used to classify these organisms into at least seven distinct groups. Nitrosospira species from clusters 2, 3, and 4 predominated in soils. Soil acidity was shown to affect the community structures of ammonia oxidizer 16S rRNA clones, with Nitrosospira cluster 2 being established at low soil pH. Soil ammonium concentration was another selection factor, with Nitrosospira cluster 3 predominating in early successional soils with high ammonium concentrations and Nitrosospira clusters 2 and 4 predominating in old successional soils with low ammonium concentrations. Large populations of Nitrosomonas species were also found in fields to which manure or compost had been applied. Ammonia oxidizer populations were similarly impacted by grassland management, with more diversity seen in unimproved soil compared to that of improved soil. Cluster 3 of Nitrosospira and cluster 7 of Nitrosomonas predominated in N-fertilized soil.

Ammonia monooxygenase is the central enzyme for all aerobic ammonia oxidizers. Due to its accurate reflection of the phylogeny of the ammonia oxidizers, the gene coding for a component of this enzyme (amoA) is a good target for environmental investigations. Soil ammonia oxidizers have mostly been studied using the 16S rRNA gene, despite the fact that the amoA gene is unique and universal to all ammonia oxidizers.

Targeting the nirK genes and the 16S rRNA genes, respectively, allowed us to compare the denitrifier population's composition to that of the whole bacterial community. The nitrite reductase enzyme (nirK) is encoded by this gene and requires copper. Denitrification relies on two important enzymes, NirK and the cytochrome cd1-containing nitrite reductase (NirS), which are structurally distinct but functionally comparable. Since this physiological category is common across widely divergent taxa, nitrite reductase genes serve as functional flag genes of denitrifying bacteria. Denitrifiers may be identified in environmental materials by using either gene. Recent research using polymerase chain reaction (PCR) tests indicated that nirK was more often found in soils, whereas nirS was more commonly found in marine sediments. Cloning of both genes and terminal restriction fragment length polymorphism (T-RFLP) analysis of nirS genes from environmental samples were used to investigate denitrifier community structures. The present research presents the first use of T-RFLP for community analysis using nirK genes.

REVIEW OF LITERATURE

The application of litter or dung on a continuous basis creates a reservoir of nutrients in the soil that releases those nutrients slowly over the course of many years, increasing the amount of primary and secondary nutrients (Ginting et al., 2003). However, soil bacteria do not make 100% of the N and other nutrients accessible for plant absorption in the first year after application. In the first year, plants may take use of around 55% of the nitrogen (N) in PL, and in subsequent years, only about 45% is usable (Eghball et al., 2004). Sharpley et al. (1993) demonstrated that long-term PL administration led to a greater rise in NO# $-N and total N in treated plots than in untreated ones (2.5 and 1.2 fold, respectively). The environmental dangers of using organic wastes in agricultural production have sparked a renewed interest in quantifying N mineralization in soils (Sharpley et al., 1998). However, there is a dearth of research on the mineralization of land-applied PL in comparison to other types of waste. While (Tyson and Cabrera, 1993) observed organic N mineralization rates of 0.4- 5.8% in composted and non-composted PL, respectively, after 8 weeks of incubation at 25°C, Sanchez and Mylavarapu (2011) demonstrated that higher rates of PL application during 60 days of incubation resulted in greater N mineralization rates.

When PL is applied directly to the soil surface in pastures or no-till fields, the rapid rate of mineralization of organic N results in significant ammonia losses (Cabrera et al., 1993). When the quantity of litter generated exceeds the area of land accessible and transportation to other places is not economically viable, a useful management method is to apply composted PL to pastures (slower rate of N mineralization) (Brinson et al., 1994). These and other management strategies to reduce N loss from land-applied PL have been shown to be beneficial, but it is unclear what influence they have on the soil microbes that mediate crucial processes in the waste's transformation.

The southern states provide the most to America's yearly production of 8.6 billion broilers and 13 million Mg of PL (National Agricultural Statistics Service, 2012). Excreta, bedding, feathers, and waste feed are all part of the PL that is spread on fields to fertilise various crops (Kelleher et al., 2002; Nahm, 2003). As an organic amendment, PL is widely employed in agricultural soils in the United States due to its high nutritional content (especially nitrogen, phosphorus, potassium, calcium, and other plant nutrients) (Edwards and Daniel, 1992; Sims and Wolf, 1994). Soil organic matter, oxygen diffusion rate, water retention capacity, and aggregate stability are all enhanced after PL application; however, these changes are more significant in agricultural soils than pasture soils (Adeli et al., 2009). However, disposal of PL is often restricted to surrounding places because to the significant transportation expense involved. Over application in the local fields causes an accumulation of excess nutrients that are bad for the crops and the environment, as a consequence of

this surplus PL creation (He et al., 2009). By increasing total soil carbon and microbial biomass, PL treatment enhances soil quality (Watts et al., 2010). Excessive PL application has been linked to elevated surface soil salinity and nitrate and phosphorus buildup.

MATERIALS AND METHODS

Physicochemical properties of the soil

Some of the soil studies conducted are summarised in Table 1; further information about these and other analyses may be found in the Appendices.

Soil OXC value represents the soil's propensity to accept electrons since all oxidising agents are included in, but electron suppliers (such as soil organic matter) are not.

Microcosms were developed to study the influence of floods on the redox status of paddy soil. Each 1500 ml polyethylene container (height: 30 cm; diameter: 8.0 cm) was filled with 1.5 kg of soil and 2-3 cm of standing water and kept at 25 1C for 60 days. We were able to measure the oxygen content of the air at several altitudes thanks to a microelectrode sensor for oxygen (Unisense OX 50; Science Park, Aarhus, Denmark). At 100 mm intervals down to a depth of 5 mm, the tip of a microsensor was inserted into the soil to measure the dissolved oxygen content. Subsequently, a spatially resolved extended oxygen profile of the soil was produced, spanning a depth of 20 cm.

DNA-SIP microcosm and gradient fractionation

The construction of DNA-SIP microcosms is shown in Figure 1. The treatments were conducted in an incubator at 28.1C and 60% of the soil's maximum water-holding capacity, with triplicate microcosms for each treatment. Following incubation, duplicate soil samples were collected for each treatment and kept in a freezer at 80 °C until molecular analysis could be performed. Following the removal of the gradient medium in the ultracentrifuge tube, sterile water was injected into the tube at a controlled rate of 0.38 ml min—1 using a syringe pump to create the DNA fractions (New Era Pump Systems Inc., Farmingdale, NY, USA).

Real-time PCR quantification of the amoA genes

Using real-time quantitative PCR of the amoA genes, we determined the relative abundances of AOA and AOB in the total DNA after incubating the soil microcosms for 56 days. As previously reported, real-time polymerase chain reaction was utilised to assess the efficiency of 13C incorporation into the genomes of AOA and AOB in the fractionated DNA across all buoyant density gradients from the DNA-SIP microcosms (Lu and Jia, 2013).

Pyrosequencing, cloning and phylogenetic analysis

Sample-specific barcode sequences of 6 nucleotides were fused to the 3' end of the forward primer, whereas adapter B was fused to the 3' end of the reverse primer. Table S1 contains the annealing and extension temperatures utilised for polymerase chain reaction primers. PicoGreen Kits (Invitrogen, Shanghai, China) were used for gel purification and quantitative analysis of the PCR products, and the resultant sequences were pyrosequenced on a GS FLX Titanium sequencer (Roche Diagnostics Corporation, Branford, CT, USA). The raw pyrosequencing data were cleaned of sequence chimaeras using PyroNoise, a technique provided in the mothur software package (Schloss et al., 2009). Denoised sequence reads were matched to their proper samples using sample-specific barcode sequences, and only 4,300 bp sequences with an average quality score of 430 and no dubious base calls were included in the subsequent analyses. The major lineages of AOA, AOB, and NOB were assigned taxonomically by binding the sequences of the 16S rRNA gene and the amoA gene into operational taxonomic unit (OTU)s at 97% similarity levels. In order to acquire a representative sequence of the 16S rRNA or amoA gene for each OTU, Mothur was utilised. All OTUs were assigned to a taxonomic category using neighbor-joining phylogenetic trees based on sequencing data from the amoA and 16S rRNA genes. Taxonomically labelled reference sequences from GenBank were compared using the Kimura 2-parameter distance (determined in MEGA 4.0), and bootstrap values were determined with a total of 1000 replicates (Tamura et al., 2007).

Statistical analysis

Canonical correspondence analysis (CCA) and the biological environmental network (BIO-ENV) were used to identify the abiotic factors likely to affect the microbial population of AOA and AOB in the four paddy soils under in situ conditions (Supplementary Table S5). Following construction of the soil physico-chemical property matrix with the environmental factors as covariates, a variation partitioning analysis was performed in R using the vegan package. Spearman's correlation analysis was used to examine the potential relationship between soil properties and 13C-DNA AOA/AOB ratios (Supplementary Table S6). After doing a one-way analysis of variance, multiple comparisons were undertaken using Tukey's post hoc tests. An independent t-test was run to find out whether there were statistically significant differences between the groups. All analyses were performed using the statistical programme SPSS Inc. (Cary, NC, USA) version 13.0 for Windows, and

statistical significance was determined using a P value of 0.05.

RESULTS

Accession numbers of nucleotide sequences

Nucleotide sequences derived from the 13C-archaeal and bacterial amoA genes, respectively, have been submitted in GenBank with the accession codes KF999673-KF999679 and KJ949142-KJ949150. The amoA and 16S rRNA pyrosequencing readings have been submitted to the DNA Data Bank of Japan with the accession numbers DRA002271 and DRA001268.

Soil nitrification activity

Soil nitrification activity was measured by measuring the concentration of nitrates in microcosms that had been incubated with or without acet- ylene, a suicide inhibitor of autotrophic ammonia oxidation (Figure 2a). We found no significant difference in the amounts of inorganic nitrogen between the 13CO2-labeled and 12CO2 control microcosms at day 56 after urea fertilisation led to the stepwise formation of $NO3^{-1}$ in the absence of C2H2 (Supplementary Figure S2). Net nitrification activity was calculated as the rate at which soil nitrate concentration increased, and this value decreased from ZY (9.46) to JD (3.03) to LZ (1.43) to JX (1.43) each day, assuming linear kinetics.

There was also a rising trend in the soil ammonium concentrations, which came to B119, 243, 600, and 706, mg NH4 -N g^{-1} d.w.s. for the ZY, JD, LZ, and JX soils, respectively (Supplementary Figure S2). After 56 days of incubation, the formation of nitrate in any of the soils examined was totally halted by the addition of C2H2. Nitrate was created in all soils examined throughout the 56-day incubation period, and pairwise comparisons of soil microcosms amended with and without C2H2 showed that the consumption of soil ammonium from urea hydrolysis was restored in approximately stoichiometric amounts (Supplementary Figure S2).

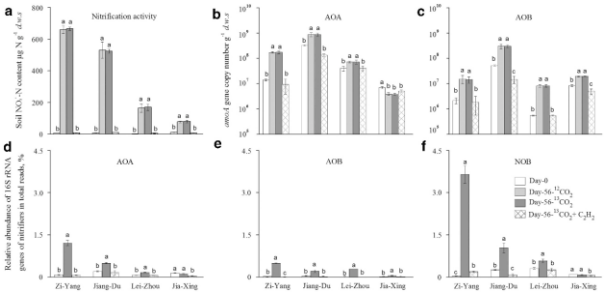

Figure 1: Ammonia and nitrite oxidizer communities and nitrification activity in soil microcosms throughout a 56-day incubation period. Total DNA extracted from microcosms contains three different types of ammonia-oxidizing organisms: ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB). Soil nitrate production (a) was measured to determine nitrification activity in soil microcosms treated with 12CO2, 13CO2 or 13CO2 C2H2 for 56 days. The amoA gene copy counts of AOA (b) and AOB (c) were assessed using real-time quantitative PCR. Pyrosequencing was performed at the total microbial community level in SIP microcosms using the universal primer pair 515F–907R, and the relative abundance of AOA (d), AOB (e) and NOB (f) is expressed as the ratio of the targeted 16S rRNA gene reads to the total microbial 16S rRNA gene reads in each microcosm. Each soil microcosm got 100 mg urea- Ng^{-1} d.w.s. on a weekly basis. Day 56 denotes a soil microcosm that has been incubated for 56 days. Microcosms of soil were incubated with 13CO2 in the presence of the nitrification inhibitor acetylene, which is represented by 13CO2 C2H2 (C2H2). Sites for paddy field soil samples are indicated by numbers adjacent to the x axis in Figure 1. Microcosms were used for all treatments, and there were three of them. Standard deviations of the average of the triplicate microcosms are shown by the error bars.

Population size and composition of the soil-nitrifying communities

Quantitative polymerase chain reaction of the amoA genes in microcosms was used to estimate the number of AOA and AOB present on days 0 and 56. (Figures 2b and c). When urea was added to the ZY, JD, and LZ soil microcosms on day 0, the copy counts of the archaeal amoA genes rose by 12.4, 2.67, and 1.83-fold by day 56, respectively (from 1.4 107 to 1.7 108, 3.3 108 to 8.8 108, and 4.0 107 to 7.3 107 g— 1 d.w.s.). This was true even in the absence of C2H2 (Figure 2b). However, in the JX soil, the copy counts dropped from 7.0 106 to 5.1 106. From day 0 to day 56 in the ZY, JD, LZ, and JX soils, the number of bacterial amoA gene copies grew by 7.27-, 5.66-, 14.8-, and 2.38-folds, respectively, from 2.2 x 106, 5.3 x 107, 5.5 x 105, and 8.4 x 106 g— 1 d.w.s (Figure2c). C2H2 totally eliminated the upsurge in amoA gene abundance in all four soils' AOA and AOB populations.

The whole microbial population in the SIP microcosms of the four soils was sequenced using pyrosequencing of the 16S rRNA genes after 0 and 56 days of incubation. In all soils examined on day 0, the relative abundance of AOA was higher than that of AOB, but after 56 days of incubation, the AOB population seemed to have been stimulated to a larger degree than the AOA population. Day 0 AOA relative abundance was 0.88%, Day 16 AOA relative abundance was 1.60%, and Day 56 AOA relative abundance was 1.64% in the ZY soil, the JD soil, and the LZ soil, respectively. These numbers

imply a 14.0, 3.15, and 2.38-fold increase, respectively (Figure 2d). After 56 days of incubation, the microcosm's AOB relative abundance had risen by 22.5-fold (ZY), 4.92-fold (JD), and 138-fold (LZ) (Figure 2e). Additionally, the NOB population was boosted by 83.1, 4.01, and 1.83% in the ZY, JD, and LZ soils, respectively (Figure 2f). There were small decreases in AOA and NOB in the JX soil, while AOB increased by a factor of 2.26 after 56 days of incubation.

Total DNA was extracted from 13C-labeled microcosms on days 0 and 56, and pyrosequencing of the amoA genes was conducted. High-quality amoA read counts for AOA and AOB were B280 000 and 182 000, respectively (Supplementary Table S2). Both the AOA and AOB amoA gene phylogeny (Supplementary Figure S3) and the 16S rRNA gene phylogeny (Supplementary Figure S3) were very similar (Supplementary Figure S4). Nine different operational taxonomic units (OTUs) were found within four clusters, all of which were assigned to the soil group 1.1b lineage. These clusters were named the soil fosmid 29i4 cluster, the soil fosmid 29i4-associated cluster, the soil fosmid 54d9 cluster, and the Nitrososphaera viennensis cluster (Supple- mentary Figure S3a). Thermophilic AOA-like sequences were also discovered by phylogenetic analysis of archaeal 16S rRNA genes, but no readings were able to be attributed to the soil fosmid 54d9 cluster (Supplementary Figure S3b). Analysis of the amoA and 16S rRNA genes revealed that AOB has a close genetic relationship with the Nitrosomonas communis cluster and the Nitrosospira clusters 0 and 3. (Supplementary Figure S4). In agreement with the 16S rRNA gene analysis, the bacterial amoA genes grouped into seven OTUs (Supplementary Figure S4a) (Supplementary Figure S4b). All NOB 16S rRNA gene sequences were found to belong only to the genera Nitros- pira and Nitrobacter (Supplementary Figure S5). Nitrite oxidizer communities were dominated by Nitrospira moscoviensis-like OTU-1. Three distinct OTUs made up the Nitrospira defluvii cluster, whereas two belonged to the Nitrospira marina cluster. Within the grouping of Nitrobacter hamburgensis, only a single OTU-7 was found (Supplementary Figure S5).

SIP of active nitrifying communities

After incubating the four paddy soils for 56 days, complete DNA was extracted using isopycnic centrifugation to separate the 13C-labeled DNA from the 12C native DNA (Figure 1). According to real-time quantitative PCR of amoA genes in the fractionated DNA, AOA populations were strongly labelled in the ZY and JD soils, whereas AOB populations were strongly labelled in all four soils evaluated (Figure 3). In the SIP control microcosms (12CO2 and 13CO2 C2H2), the largest amoA copy counts of both AOA and AOB were observed in the 'light' fractions expected for the unlabeled DNA, with a buoyant density of B1.720 g ml^{-1}. There was a slight increase in the number of copies of the archaeal amoA gene in the 'heavy' portion of the LZ soil in the 13CO2-labeled microcosms (Figure 3e), but there was no obvious peak of archaeal amoA genes in the JX soil (Figure 3g). Contrarily, in the 'heavy' DNA fractions of all four soils studied, the majority of bacterial amoA genes were recoverable. Peaks in bacterial amoA gene copy number were seen in the heavier portions of the LZ and JX soils, with buoyant densities of 1.740 g ml^{-1}, whereas the ZY and JD soils had peaks in the lighter fractions, with densities of 1.730-1.735 g ml^{-1}. (Figures 3f and h). AmoA gene labelling was not seen in SIP microcosms supplemented with 13CO2 C2H2, indicating that ammonia oxidation was required for 13CO2 uptake by AOA and AOB (Figure 3). Moreover, the pyrosequencing of 16S rRNA genes at the level of the whole microbial community indicated substantial enrichments of AOA, AOB, and NOB, which accounted for up to 30.0%, 36.0%, and 58.1% of the total microbial communities in the 'heavy' DNA fractions, respectively (Supplementary Table S3). The SIP control microcosms lacked this (Supplementary Table S3), indicating that the active nitrifiers studied led chemo- lithoautotrophic lifestyles (Supplementary Results).

Genes encoding for 13C-aminoacyl-CoA synthetase were used to build clone libraries for phylogenetic study (Figure 4a). Twenty percent of the annotated archaeal amoA genes in the JD soil were found to be affiliated with the 29i4 cluster. Furthermore, in the ZY soil, 20% of the tagged archaeal amoA genes belonged to the N. viennensis cluster, whereas in the JD soil, 20% did, and in the LZ soil, 20% did as well (Table 2). The 13C-labeled 16S rRNA genes yielded the same findings (Figure 4b). Members of the 29i4 cluster dominated the archaeal communities found in the ZY, JD, and LZ soils, with 94.3%, 71.6%, and 64.0% of the 13C-labeled 16S rRNA genes, respectively. Furthermore, in the JD and LZ soils, respectively, 21.8% and 36.0% of the tagged 16S rRNA genes had strong sequence similarity with AOA within the N. viennensis cluster (Table 2). Supplemental Figure S6a shows that in the ZY, JD, LZ, and JX soils, respectively, 95%, 50%, 100%, and 50% of the 13C-labeled bacterial amoA genes were phylogenetically close to Nitrosospira cluster 3. (Table 2). Half of the 13C-labeled amoA genes in the JD soil were part of an AOB similar to that of Nitrosomonas communis, but in the JX soil, all of the amoA genes were associated with the Nitrosospira cluster 0. The 13C-labeled 16S rRNA genes showed similar results (Supplementary Figure S6b). For example, in the ZY, JD, LZ, and JX soils, B91.4%, 38.2%, 100%, and 55.4% of the 13C-16S rRNA genes, respectively, could be attributed to the Nitrosos-pira cluster 3, respectively (Table 2). Also, in the JD soil, 61.8% of the 13C-labeled AOB were found in the Nitrosomo- nas communis cluster, but in the JX soil, only 43.6% of the active AOB were found in the Nitrosospira cluster 0 group. Known NOB were associated with the 13C-labeled 16S rRNA genes of nitrite oxidizers (Supplementary Figure S7), with

Nitrospira-like NOB being more common than Nitrobacter-like NOB (Table 2).

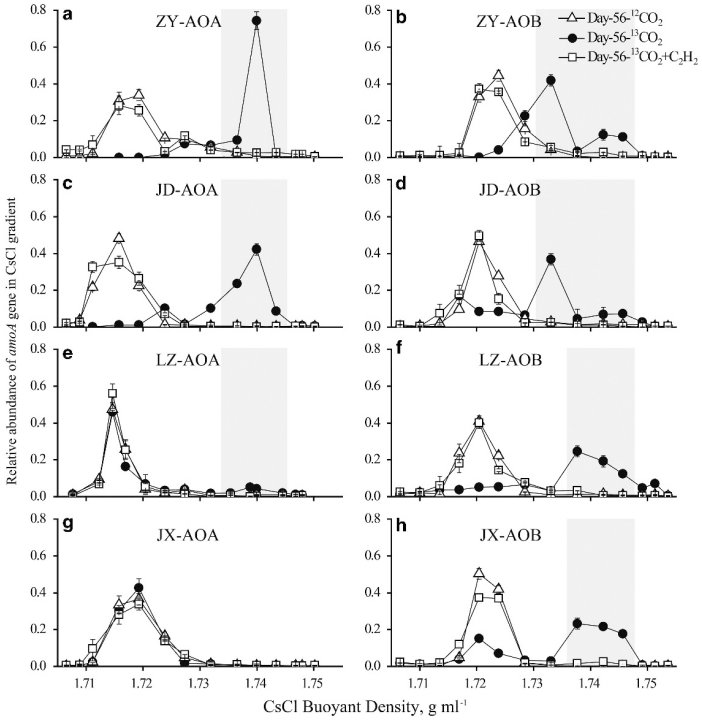

Figure 2: Soil microcosms were cultured with either 12CO2, 13CO2, or 13CO2 C2H2 þ for 56 days, and the amoA genes of archaea and bacteria were counted over the full buoyant density gradient of the DNA fractions. The ratios of gene copies in each DNA fraction to the total number of amoA genes over the whole DNA fraction gradient for each treatment constitute the normalised data. The shaded region denotes the active fractions (13C indicated) from the labelled microcosms. Soils from ZY, JD, and LZ all had an active fraction for 13C-AOA with a buoyant density between 1.735 and 1.745 mg l— 1. We found 13C-AOB in soil active fractions with DNA buoyant densities ranging from 1.73 to 1.74 mg l— 1 in ZY and JD soils and from 1.73 to 1.74 mg l— 1 in LZ and JX soils. Standard errors from three independent technical replicates of the microcosm are shown as error bars. The rest of the labelling follows the pattern established in Figures 1 and 2.

CONCLUSION

The results of this study might largely reflect the functional process of nitrification under field conditions, although the incubation of SIP microcosms could not entirely reproduce the physiochemical and biological characteristics of the paddy soils in situ.

REFERENCES

1. Abalos D, De Deyn GB, Kuyper TW et al. Plant species identity surpasses species richness as a key driver of N2O emissions from grassland. Glob Change Biol 2014;20:265–75.
2. Bardgett RD, Mommer L, De Vries FT. Going underground: root traits as drivers of ecosystem processes. Trends Ecol Evol 2014;29:692–9.
3. De Vries FT, Bloem J, Quirk H et al. Extensive management promotes plant and microbial nitrogen retention in temperate grassland. PLoS One 2012;7:e51201.
4. De Vries FT, Jørgensen HB, Hedlund K et al. Disentangling plant and soil microbial controls on carbon and nitrogen loss in grassland mesocosms. J Ecol 2015;103:629–40.
5. Hadfield J. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R Package. J Stat Softw 2010;33:1–22.
6. Le Roux X, Schmid B, Poly F et al. Soil environmental conditions and microbial build-up mediate the effect of plant diversity on soil nitrifying and denitrifying enzyme activities in temperate grasslands. PLoS One 2013;8:e61069.
7. Strauss SL, Reardon CL, Mazzola M. The response of ammoniaoxidizer activity and community structure to fertilizer amendment of orchard soils. Soil Biol Biochem 2014;68: 410–8.
8. Subbarao GV, Rondon M, Ito O et al. Biological nitrification inhibition (BNI): is it a widespread phenomenon? Plant Soil 2007;294:5–18.
9. Wedin DA, Tilman D. Species effects on nitrogen cycling: a test with perennial grasses. Oecologia 1990;84: 433–41.
10. Wei B, Yu X, Zhang S et al. Comparison of the community structures of ammonia-oxidizing bacteria and archaea in rhizoplanes of floating aquatic macrophytes. Microbiol Res 2011;166:468–74
11. Yao H, Campbell CD, Chapman SJ et al. Multi-factorial drivers of ammonia oxidizer communities: evidence from a national soil survey. Environ Microbiol 2013;15:2545–56.

Corresponding Author

Sudarshan Medagani*

Research Scholar

E-Mail –sudarshanmedagani@gmail.com