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Ammonium enrichment reduces the diversity and changes the composition of ammonia-oxidizing microbial communities in agricultural soil media

SHERLY ASNATH SAUKOLY, VINCENTIA IRENE MEITINIARTI, RULLY ADI NUGROHO, AGNA SULIS KRAVE♥

Faculty of Biology, Universitas Kristen Satya Wacana. Jl. Diponegoro No. 52-60, Salatiga 50711, Central Java, Indonesia.
Tel./fax.:+62-298-321212. ♥email: agnakrave@gmail.com

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Abstract. *Saukoly SA, Meitiniarti VI, Nugroho RA, Krave AS. 2024. Ammonium enrichment reduces the diversity and changes the composition of ammonia-oxidizing microbial communities in agricultural soil media. Biodiversitas 25: 916-923.* Nitrification is the process of ammonium oxidation to form nitrites and nitrates. Ammonium availability in the soil is one of the factors influencing the activity, abundance, and diversity of ammonia-oxidizing microorganisms (AOM). This study aimed to determine the effect of enriching a soil media with different ammonium concentrations on the abundance and diversity of AOM as well as the potential for nitrification. Soil as the media was prepared from an agricultural land receiving cow dung sewage. The media was then placed in the microcosms, and was added to a nitrification medium containing ammonium at three levels; 0 (control soil), 200 (low ammonium-enriched soil), and 500 mg L^{-1} (high ammonium-enriched soil). The microcosms were further incubated for 42 days at room temperature. The nitrification potential determination was based on the formation of nitrite from ammonium oxidation. The abundance of AOM and the potential nitrification were measured every 14-day interval, including day 0 as the initial condition. The AOM composition was analyzed based on the similarity level of the *amoA* gene sequence to the NCBI BLAST GenBank database. However, this analysis was only conducted for the control and high ammonium-enriched soil. This study indicated that ammonium enrichment increased the nitrification activity and the abundance of AOM, but it decreased the diversity of AOM communities. There were positive correlations between nitrification and nitrate potential, as well as between ammonium content and AOM abundance. Negative correlations appeared between pH and nitrification potential, nitrate concentrations, ammonium concentrations, and MPN. The diversity of ammonium-oxidizing archaea (AOA) in the control soil was higher than in the high ammonium-enriched soil. The enrichment with ammonium also changed the AOA composition. The *Candidatus Nitrosocosmicus oleophilus* strain of the MY3 chromosome was the most dominant archaea in the control and high ammonium-enriched soil. This implies that the high diversity of AOA in this soil may be beneficial for further use of the soil as a source for inocula in the development of nitrifiers-based biofertilizers.

Keywords: Ammonium enrichment, *amoA* gene, AOM diversity, *Nitrosocosmicus oleophilus*, potential nitrification

INTRODUCTION

Nitrogen is essential for plant growth and development (Fathi and Zeidali 2021). Even though plants require a large amount of macronutrients, such as nitrogen, this element is often present in small quantities or in a form that the plant cannot use (Grzyb 2021; Muratore et al. 2021). In general, nitrogen is absorbed by plants in the form of ammonium (NH_4^+) and nitrate (NO_3^-) (Beeckman et al. 2018). On agricultural land, it is more difficult for plants to absorb ammonium because it binds strongly to the soil, while nitrates and nitrites are more soluble in water, so plants easily absorb them (Amir et al. 2012; Nguyen et al. 2017). Nitrates become available in soil through ammonium oxidation, called the nitrification process (Musiani et al. 2020). Nitrification is essential in providing more available nitrogen nutrients for plants (Ayiti and Babalola 2022).

The nitrification process is carried out by two groups of microorganisms with the *amoA* gene: ammonia-oxidizing archaea and ammonia-oxidizing bacteria (Baskaran et al. 2020). Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) are nitrifiers that are easily found in various environments, including agricultural soil (Azziz et al. 2016). Bernhard (2010) and Wright et al. (2023) stated that AOA and AOB obtain their metabolic energy from the oxidation of ammonium to hydroxylamine by ammonia monooxygenase (AMO). The former hydroxylamine is then converted to nitrite by hydroxylamine oxidoreductase (Kessel et al. 2015; White and Nicolai 2016). This process produces little energy, resulting in very slow nitrifier growth (Bernhard 2010). Further, the resulting nitrites are oxidized to nitrates (Koch et al. 2015). This oxidation is catalyzed by nitrite oxidoreductase (Chicano et al. 2021).

In most soil ecosystems, AOA is known to be more dominant than AOB (Gao et al. 2018). The abundance of AOB in soil is 10^4-10^6 g^{-1}, whereas the abundance of AOA is higher than 10^7 g^{-1} in soil (Okano et al. 2004; Leininger et al. 2006). So far, information on the abundance, diversity, composition, and activity of ammonia-oxidizing microorganisms (AOM) in soil in Indonesia, especially

agricultural soil, is still limited. There is limited information available on the influence of various environmental factors in the tropics on the abundance and activity of AOM. Environmental factors such as soil type, pH, moisture, and the availability of substrates in the soil can affect the abundance, diversity, composition, and nitrification activity of AOA and AOB (Zhang et al. 2014a; Tago et al. 2015; Siliakus et al. 2017). Shen et al. (2012) reported that the abundance and composition of the AOB community in most rice-growing soil in China were significantly influenced by fertilization. Changes in pH due to fertilization also affect AOA, which is known to be more responsive to fertilization at pH below 5.09 (Shen et al. 2012; Wang et al. 2015). In a study by Lourenço et al. (2022), the fertilization of organic and inorganic materials did not change the community structure of AOB and denitrifying fungi in sugarcane plantation soil. *Nitrosospira* is the dominant AOB in soil enriched with mixed organic and inorganic fertilizers. Verhamme et al. (2011) reported that in the Craibstone area, Scotland, with sandy loam soil (pH of 7.5), AOA and AOB could grow in ammonium with 200 µg NH_4^+-N g^{-1} soil concentrations. However, it has not been identified whether the low abundance of AOM in tropical soil can be increased by enriching the soil with ammonium. This study aims to determine the effect of ammonium enrichment on the abundance and diversity of AOM in soil media taken from agricultural land receiving effluent of cow manure from the adjacent upstream cowshed. The soil showed a greater abundance of nitrifiers as previously revealed by molecular analysis of *the amoA* gene. This study strives to demonstrate that ammonium enrichment can decrease the diversity and change the composition of AOM.

MATERIALS AND METHODS

Soil sampling and analysis

Soil samples were collected from agricultural land in Bumiharjo Hamlet, Sumogawe Village, Getasan Sub-district, Semarang District, Central Java, Indonesia (7°22'46.9"S110°26'42.0"E). The preliminary molecular detection indicated that the soil at this site contained a diverse group of nitrogen-fixing microorganisms and ammonia-oxidizing archaea. The soil materials were taken using a ring sampler with dimensions of height*diameter = 10 x 4 cm at a depth of 0-10 cm from the soil surface. Approximately, a total of 10 kg of composited soil material of the top 10 cm was collected from the study site. All the collected soil materials were mixed and sieved using a 2 mm mesh to separate the soil material from the rocks, plant roots, and organisms. Furthermore, a 50 g sample of the sieved soil was used for the analysis of physical and chemical properties, while the remaining part was used for soil media preparation. Soil moisture content was determined by weight loss after drying (100°C, 24 h). Soil pH_{H_2O} was measured with a pH electrode by the slurry method of 1:5, w:v; sample: distilled deionized water (Kusuma et al. 2014). The carbon content (C) was measured using the potassium dichromate oxidation method (Walkley and Black 1934), while the determination of total organic nitrogen (N) content used the Kjeldahl method (Kjeldahl 1883). The C/N ratio was calculated from the comparison of the C and N content of the soil.

Preparation of soil media and nitrification medium

Soil media was prepared by enriching the sieved soil material with a nitrification medium to stimulate nitrification activity and the growth nitrifier community. A liter of nitrification medium contained 0, 200, or 500 mg $(NH_4)_2SO_4$; 725 mg KH_2PO_4; 1136 mg Na_2HPO_4; 50 mg $MgSO_4 \cdot 7H_2O$; 20 mg $CaCl_2 \cdot 2H_2O$; 1 mg Fe.EDTA; and 1 mL trace elements (Agustiyani et al. 2004). The trace elements were made by dissolving 10 mg $Na_2MoO_4 \cdot 2H_2O$, 20 mg $MnCl_2 \cdot 4H_2O$, 10 mg $ZnSO_4 \cdot 7H_2O$, 0.2 mg $CoCl_2 \cdot 6H_2O$, and 2 mg $CuSO_4 \cdot 5H_2O$ in 1000 mL aquabides (Agustiyani et al. 2004). All of the nitrification medium ingredients were dissolved in 1000 mL of distilled water and were sterilized at 121°C for 20 minutes.

Experimental design and sample collection

The experiment to examine the effects of ammonium enrichment on the nitrification potential, abundance, and composition of AOM was carried out in 36 microcosms, made of PVC with dimensions of height*diameter = 11.5 x 7 cm. Each microcosm was filled with 161 g of soil media containing 60 mL nitrification medium to produce 40% soil moisture content. Three different treatments of soil media were applied, these controlled soil media (0 mg L^{-1} $(NH_4)_2SO_4$), soil media with a low amount of ammonium enrichment (200 mg L^{-1} $(NH_4)_2SO_4$), and soil media with a high amount of ammonium enrichment (500 mg L^{-1} $(NH_4)_2SO_4$).

All 36 microcosms were randomly located in an incubation chamber under room temperature. The experiment lasted 42 days and sampling was carried out on every 14-day interval, including day 0 as the initial condition. Hence, each soil media treatment and incubation time treatment was conducted in 3 replicate microcosms. The soil media in the microcosms was kept moist by adding a nitrification medium once a week to restore the soil media from weight loss due to evaporation. This experiment used a discarded sample method to determine the nitrification potential, physio-chemical analysis of the soil media, and analysis of the abundance and composition of AOM.

Determination of nitrification potential

Nitrification potential was determined by the method of Berg and Rosswal (1985). Total 5 g samples of soil media that were withdrawn from microcosms were put into a 100 mL Erlenmeyer, added with 0.2 mL sodium chlorate 1.5 M and 40 mL ammonium sulfate 1 mM, and then it was covered with a rubber cap. The suspension of the soil media sample was divided into 2 parts; one part was incubated for 5 hours at room temperature and the other part was incubated at -20°C as a control. After the incubation, 10 mL potassium chloride 2 M was added to both parts and then centrifuged at 50 rpm for 10 minutes. Five mL of the supernatant was reacted with 3 mL

phosphate buffer of pH 8 and 2 mL nitrite reagent. The density of the formed purplish-pink color was measured at a wavelength of 520 nm with a spectrophotometer (Shimadzu UVmini-1240 UV-Vis spectrophotometer).

Ammonium and nitrate determination

Before determination, the available ammonium and nitrate in the soil media sample were extracted according to Mawaddah et al. (2016). Fifteen gram soil media samples were dissolved in 100 mL aquabides, shaken for 1 h^{-1}, and centrifuged at 50 rpm for 10 minutes. The resulting supernatants were used as soil extracts for the determination of ammonium and nitrate concentrations. The determination of ammonium concentration used the phenate method (Weatherburn 1967). The ammonium present in the sample was reacted with phenol to form indophenol. In this determination, 0.1 mL soil extract was reacted with 2 mL phenol solution, 5 mL oxidizing solution (12.5 mL sodium hypochlorite 5% and 50 mL alkali citrate), and lastly, 2 mL sodium nitroprusside 0.5% was added as a catalyst. The absorbance of the blue color due to indophenol development was measured at a wavelength of 660 nm.

The nitrate concentrations were measured using the Forster's method (1995). Nitrosalicylic acid was formed by the reaction of nitrate and salicylic acid under highly acidic conditions. The complex is yellow under basic (pH >12) conditions with maximal absorption at 410 nm. In this determination, 0.5 mL of a sample extract was reacted with 0.5 mL salicylic acid 5% and 5 mL NaOH 4 M in a test tube. After incubation for 1 hour, the absorbance of the yellow color was measured by a spectrophotometer at a wavelength of 410 nm. The absorbance was calibrated to a standard curve of N-nitrate (0-100 mg $N\text{-}NO_3^-$ L^{-1}) and was directly proportional to nitrate content in the samples.

MPN determination of AOM

The Most Probable Number (MPN) method was used to determine the abundance of ammonia-oxidizing microorganisms (AOM) in the soil media samples. Suspensions of soil media samples from a dilution level of 10^1-10^5 were grown in a sterile ammonium Ca-carbonate medium (Subba-Rao 1999) for 6 weeks at room temperature. The MPN determination for each sample and dilution level were carried out in 3 replications. At the end of the incubation period, one drop indicator solution (0.1 g of diphenylamine in 50 mL of concentrated H_2SO_4) was added to each test to detect the formation of nitrite. A positive test was indicated by the formation of a blue color (Rowe et al. 1977). The number of positive tests from the 3 selected dilution concentrations was used to determine the AOM abundance based on the MPN statistics table (McBride et al. 2003).

Analysis of AOM

The AOM composition in the soil media samples was analyzed based on the sequence of *the amoA* gene. The analysis began with DNA extraction of 0.4 g soil samples using the Power Soil DNA Isolation Kit that was processed according to the protocols of MoBio Laboratories Inc., San Diego, CA, USA. The quality of the isolated DNA was examined on 1% agarose gel electrophoresis. DNA extracts of good quality were stored at -20°C for further analyses. The *amoA* gene in the DNA extracts was amplified using primers Arch-amoA-for/Arch-amoA-rev for AOA communities (Wuchter et al. 2006), and primers amoA-1F/amoA-2R for AOB communities (Rotthauwe et al. 1997). Amplification conditions of the *amoA* target sequence with primers Arch-amoA-for/Arch-amoA-rev were as follows: 95°C for 5 min; 40 cycles of 95°C for 30 s, 53°C for 45 s, 72°C for 45 s; final extension of 72°C for 10 min (Wuchter et al. 2006), while amplification conditions for priers amoA-1F/amoA-2R were as follows: 94°C for 5 min; 36 cycles of 60°C for 90 s; 72°C for 90 s; 94°C for 60 s; final extension 72°C for 10 min (Rotthauwe et al. 1997). Furthermore, the resulting PCR products were sequenced using the Illumina MiSeq platform. A paired-end sequencing on the Illumina MiSeq platform was prepared using the Nextera XT kit according to the protocols (Illumina, San Diego, CA, USA). The resulting nucleotide sequences of all amplicons were compared to the GenBank NCBI BLAST database (https:/www.ncbi.nlm.nih.gov/) to determine the sequence similarity between the *amoA* in the samples being studied and the registered *amoA* sequences in the database.

Statistical analysis

A one-way nonparametric Kruskal-Wallis test was used to analyze the effect of ammonium treatment. The nonparametric Kruskal-Wallis test was used because the data did not fulfill the assumptions of ANOVA. Furthermore, a Mann-Whitney test was conducted to find out the effects of the incubation time on the pH, nitrification potential, availability of ammonium, nitrate, and AOM abundance. The relationship between nitrification potential and pH, availability of ammonium, nitrate, and AOM abundance was analyzed using the Spearman correlation test. All the statistical analyses were performed using the IBM SPSS Statistics 22 for Windows.

RESULTS AND DISCUSSION

Soil characteristics

The soil material used in this study contained AOM with an MPN value of $1.4\times10^5\pm89.48$ cell g^{-1} and had a nitrification potential of 6.36 ± 0.012 mg NO_2^--N g^{-1} soil h^{-1}. The soil had a humidity of 37.9%±0.45, a pH of 7.38±0,08, an ammonium content of 0.09±0.49 mg g^{-1} soil dry weight, a nitrate content of 8.48±0.14 mg g^{-1} soil dry weight, total nitrogen of 1.73%±0.02, total organic C of 5.4%±0.05, and a C/N ratio of 3.12±0.28.

Effect of NH_4^+ enrichment on pH, nitrification potential, nitrate, and ammonium

The pH in the soil media decreased incubation, which lasted on day 42 (Figure 1.A). The pH decrease in the high ammonium-enriched soil was significant ($p \leq 0.05$), much higher than in the low ammonium-enriched soil. In the high ammonium-enriched soil, the pH decreased from 7.47 on day 0 to 6.74 on day 42. In contrast, in the low ammonium-enriched soil, the pH decrease was relatively low, from

7.46 at the start of the incubation period to 7.31 at the end. The pH in the control soil tended to be stable from the initial to the end of the incubation period (Figure 1.A). The nitrification potential and nitrate concentration in the high ammonium-enriched soil continued to increase until the day 28th and remained relatively stable until the 42nd day. In contrast, the ammonium content in the soil media continued to decrease until the end of the incubation period (Figure 1.B). The low ammonium-enriched soil displayed an increase in nitrification potential and nitrate concentrations until the 14th day. Then, the nitrification potential and nitrate concentrations were relatively stable until the end of the 42-day incubation period. The increasing nitrification potential and nitrate concentrations and the decreasing ammonium concentrations in the control soil were detected at lower concentrations than those in the high and low ammonium-enriched soils.

Correlation between nitrification potential, nitrate, ammonium, soil pH, and AOM abundance

The correlation test showed a positive relationship between nitrification potential and nitrate concentrations in the soil medium and between ammonium concentrations and MPN values (Table 1). Negative correlations were observed between nitrification potential and soil pH, nitrate concentrations and soil pH, ammonium concentrations and soil pH, and MPN and soil pH (Table 1).

Effect of ammonium enrichment on the abundance and diversity of soil AOM

The abundance of AOM in the soil media increased after being enriched with ammonium. The abundance of AOM in the low and high ammonium-enriched soils was significantly higher than in the control soil (Table 2).

Table 2. Effects of ammonium enrichment on the abundance of AOM in soil media

Incubation time (days)	Abundance (cell g^{-1})		
	Control	Low	High
0	1.4×10^5	1.3×10^5	1.3×10^5
14	1.1×10^5	2.5×10^5	2.5×10^5
28	9.5×10^4	1.46×10^5	2.5×10^5
42	9.5×10^4	1.1×10^5	2.5×10^5

Table 1. Spearman correlation coefficients among the soil properties

	Nitrification potential	NO_3^--N	NH_4^+-N	Soil pH H_2O	MPN
Nitrification potential					
NO_3^--N	0.814**				
NH_4^+-N					
Soil pH H_2O	-0.750**	-0.743**	-0.710**		
MPN			0.565**	-0.356*	

Note: only significant correlations are shown at *P ≤ 0.05, and **P ≤ 0.01

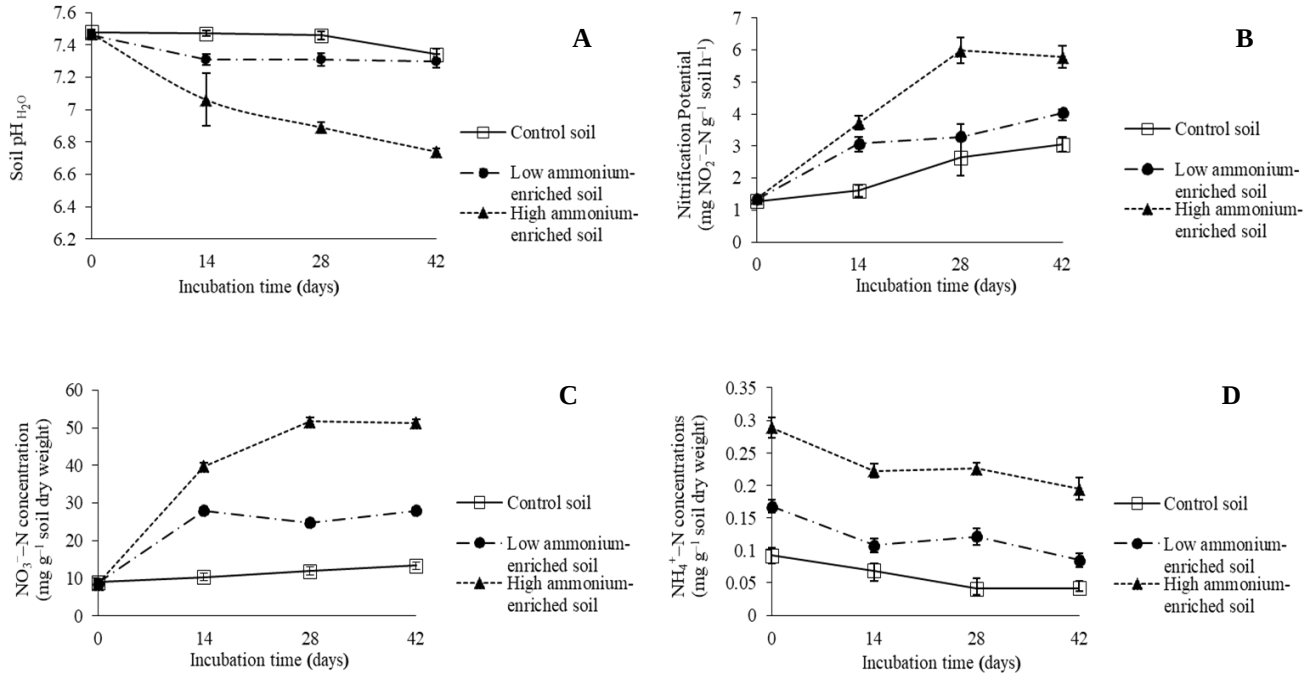

Figure 1. Effects of ammonium enrichment on: A. Soil pH H_2O, B. Nitrification potential, C. Nitrate concentration, and D. Ammonium concentration in the soil media

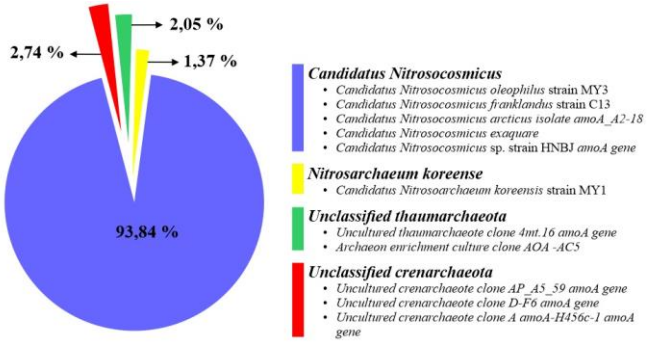

Figure 2. Composition (%) of the AOA communities in the control soil

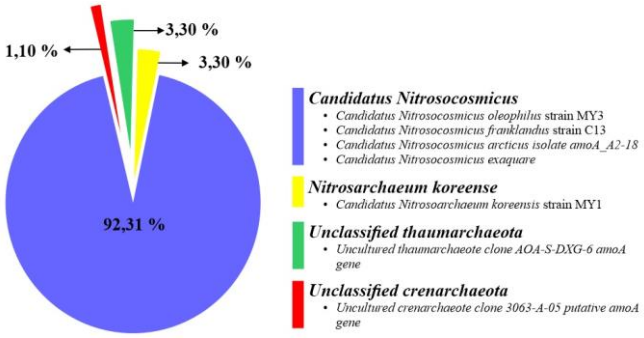

Figure 3. Composition (%) of the AOA communities in the high ammonium-enriched soil

Table 3. The Composition of AOA in the control and high ammonium-enriched soils.

Archaea	Soil		Similarity (%)	Accession number
	Control	High		
Candidatus Nitrosocosmicus oleophilus strain MY3	✓	✓	97.03-99.16	CP012850.1
Candidatus Nitrosocosmicus franklandus strain C13	✓	✓	90.50-99.47	KU290366.1
Candidatus Nitrosocosmicus arcticus isolate amoA_A2-18 *amoA gene*	✓	✓	90.73-96.19	MK978763.1
Candidatus Nitrosocosmicus exaquere	✓	✓	94.07	CP017922.1
Candidatus Nitrosoarchaeum koreensis MY1	✓	✓	100.00	HQ331117.1
Candidatus Nitrosocosmicus sp. strain HNBJ *amoA gene*	✓	-	96.48-100.00	MK396102.1
Uncultured Thaumarchaeota clone AOA-S-DXG-6 amoA gene	-	✓	94.48	KC735250.1
Uncultured Thaumarchaeota clone 4mt.16 amoA gene	✓	-	98.84	JX026227.1
Archaeon enrichment culture clone AOA -AC5	✓	-	95.34	JX026227.1
Uncultured Crenarchaeote clone AP_A5_59 amoA gene	✓	-	97.47	HM589803.1
Uncultured Crenarchaeote clone 3063-A-05 putative amoA gene	-	✓	97.37	GU129385.1
Uncultured Crenarchaeote clone D-F6 amoA gene	✓	-	92.11	JF935733.1
Uncultured Crenarchaeote clone A amoA-H456c-1 amoA gene	✓	-	99.11	GU208181.1

AOM-extracted DNA from the control and high ammonium-enriched soils showed band quality that met the requirements for sequencing. In addition, the nitrification activity in the control and high ammonium-enriched soils showed significant differences when it was compared to the low ammonium-enriched soil, so that only the control and high ammonium-enriched soils were selected for analysis of AOM diversity.

The sequences of the *amoA* gene obtained from both soils were then identified to determine the degree of similarity of the sequences to the NCBI BLAST database. The control soil resulted in 1131 sequences, but only 400 of the NCBI BLAST identified them, while the other 731 sequences were not identified. Of the 400 identified sequences, 146 were from AOA, and 254 were derived from non-AOB bacteria. PCR-DNA sequencing of high ammonium-enriched soil resulted in a total of 644 sequences. Of these sequences, 293 were identified in the NCBI BLAST database, and 351 were not placed. Of the 293 identified sequences, 91 were identified as AOA, and 202 were confirmed as non-AOB.

Based on the revealed *amoA* gene sequences, the exposed AOA group was a member of two phyla, namely Thaumarchaeota phylum (genus *Candidatus Nitrosocosmicus*, *Nitrosorchaeum koreensis*, unclassified Thaumarchaeota) and Crenarchaeote phylum (unclassified Crenarchaeote). Some sequences were closely related to *amoA* gene from *Candidatus Nitrosocosmicus oleophilus* strain MY3 with a degree of similarity of 97.03 to 99.16% (Table 3). *Candidatus Nitrosocosmicus* was the most dominant AOA genus in the control and high ammonium-enriched soils that composed 93.84 and 92.31%, respectively (Figures 2 and 3).

Discussion

Effect of ammonium enrichment on nitrification activity

The abundance of AOM and the pH were controlling factors for the nitrification process in the soil. This study showed a pH decrease in the soil media with the progress of incubation time. The pH decrease in soil media occurred after the addition of ammonium. According to Corley and Tinker (2016); Buthelezi and Buthelezi-Dube (2022), the addition of ammonium sulfate significantly decreases soil pH. This occurred through the oxidation of ammonium to nitrate in the soil media was followed by the release of H^+ ions, resulting in soil acidification (Msimbira and Smith

2020). Ammonium that underwent nitrification increased nitrate availability, followed by a soil pH decrease. This can be seen in the continuous reduction of pH from the 14th day of incubation (Figure 1.A).

The potential for nitrification indicates the nitrifying activity of AOM in the environment (Hazard et al. 2021). Nitrification potential is commonly determined by measuring short-term nitrite or nitrate production in soil. Stopnisek et al. (2010) revealed that soil nitrification potential was not affected by the enrichment of a medium with different ammonium concentrations. This study showed, however, the opposite response. Ammonium enrichment increased the nitrification potential and nitrate concentrations in the soil media (Figure 2). This increase in nitrification potential and nitrate concentrations co-occurred with a decrease in ammonium concentrations from the 14th to the 42nd day incubation period. This shows that the AOM communities in the soil media can oxidize ammonium to nitrite and nitrate.

Furthermore, the AOM communities may assimilate the inorganic ammonium for growth (Hink et al. 2018) by utilizing exergonic energy from the oxidation of ammonium to nitrite (Maia and Jose 2014). This study also showed a negative correlation between nitrification potential, nitrate and ammonium content, and soil pH. Increased ammonium concentrations in the soil media due to the ammonium enrichment treatment can lower the pH. Decreased soil pH supports nitrification and increases nitrate concentrations (Chen et al. 2021). A negative correlation also occurred between the soil pH and MPN. This shows that activity and the rise of AOM abundance will be followed by a decrease in pH. The AOM, especially the archaea group, has a wide pH range, but their preference for acidic soil is higher (Li et al. 2018).

Ammonium is a substrate that influences the abundance of AOM communities. He et al. (2018) also reported a positive correlation between ammonium concentrations and the MPN of AOM. In addition, there were positive correlations between ammonium concentrations with the nitrification potential and the nitrate concentrations in the soil media. Ammonium level in the soil affects the nitrification rate, affecting the concentrations of nitrate produced. Thus, these findings are consistent with the study of He et al. (2018).

Effects of ammonium enrichment on AOM abundance

The MPN test showed that the AOM abundance increased in response to the ammonium enrichment in the soil media. This effect does not agree with the studies of Stopnisek et al. (2010); Verhamme et al. (2011) and Tzanakakis et al. (2018). They reported that ammonium concentrations did not affect the growth and abundance of AOA, possibly because the AOA found in their study was oligotrophic. According to Cavagnaro et al. (2008), adding ammonium to organic farming soil also does not affect the AOB abundance. According to Berg et al. (2015) and Sauder et al. (2017), AOA has a high affinity for ammonia and can grow in conditions of low ammonia availability. Meanwhile, AOB grows better when the ammonium concentration is higher. This occurs in field studies using a microcosm (Okano et al. 2004). According to Guo et al. (2021), adding urea as a source of ammonia in the soil significantly increased the abundance of AOM in the soil. Lu et al. (2012) also reported that adding urea in tea garden soil pointedly increased the relative proportion of total AOA 16S rRNA genes from 0.28 to 7.86% with an incubation period of 8 weeks.

Diversity of AOM

The study showed that the AOA community was relatively more diverse in the control soil than in the high ammonium-enriched soil. Thaumarchaeota was the most dominant AOA member in the control and high ammonium-enriched soils. According to Tsiknia et al. (2015) and Azziz et al. (2016), ammonia-oxidizing Thaumarchaeota are the predominant AOA communities in most soil and, those are one of the main nitrification drivers in terrestrial environments. At the species level, *Candidatus Nitrosocosmicus* was the most dominant species found in the control and high ammonium-enriched soils (Figures 2 and 3). According to Song et al. (2016), Duan et al. (2018), and Xu et al. (2022), *Candidatus Nitrosocosmicus* is dominant and widely distributed in the soil. This study also revealed that the *Candidatus Nitrosocosmicus oleophilus* MY3 strain is the most prevalent species in the control and high ammonium-enriched soils followed by *Candidatus Nitrosocosmicus franklandus.*

In this study, *Candidatus Nitrosocosmicus oleophilus*, *Candidatus Nitrosocosmicus franklandus*, *Candidatus Nitrosocosmicus arcticus*, and *Candidatus Nitrosocosmicus exaquare* were AOA species which were found to grow well in the control and high ammonium-enriched soils. This finding suggests that the AOA strain of *Candidatus nitrosocosmicus* has a higher ammonia tolerance than another member of AOA, so they can adapt well to soil with low or high ammonium (Jung et al. 2016; Lehtovirta et al. 2016; Kits et al. 2017). However, in agricultural settings, high ammonium concentrations can limit AOA growth (Sterngren et al. 2015). Besides *Candidatus Nitrosocosmicus*, this study also revealed the presence of the genus Nitrosarchaeum koreense in the control and high ammonium-enriched soils. *Candidatus Nitrosarchaeum koreense* and *Candidatus Nitrosocosmicus exaquere* are cosmopolitan AOA in terrestrial environments that have a relatively high affinity for ammonia and oxygen (Jung et al. 2011; Sauder et al. 2017). Other detected archaea were the *Unclassified thaumarcheota* and *Unclassified Crenarchaeota*. In this study, no *amoA* gene was detected from members of AOB, even though a specific *amoA* primer for AOB was used. It seems that this matter was not related to the pH factor. The AOB was found in soil with a neutral pH of 7.4-8 (Claros et al. 2013; Zhang et al. 2014b), corresponding to the control soil with a pH of 7.38. AOB grew in soil with high concentrations of ammonium, but low pH due to the addition of ammonium as a limiting factor of AOB growth in soil (Ouyang et al. 2016; Hayatsua et al. 2021). The abundance of AOB in the soil material used in this study was likely below the detection level of the method employed. According to Yao et al.

(2013), the population of AOB in tropical soil is indeed low, so it is difficult to detect. It can be inferred that the AOA communities played a more significant role in the nitrification process in the agricultural soil being examined in this study.

Thus, it can be concluded that ammonium enrichment can reduce the diversity of AOM and change its community composition in the soil. The treatment also increases AOM abundance, nitrification potential, and nitrate concentrations in the soil media. Nevertheless, members of the AOA group remain the dominant AOM communities following the enrichment of soil media with ammonium. *Candidatus Nitrosocosmicus oleophilus* strain of MY3 is the most dominant.

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