Anaerobic Ammonium Oxidation in Acidic Red Soils

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Anaerobic ammonium oxidation (anammox) has been proven to be an important nitrogen removal process in terrestrial ecosystems, particularly paddy soils. However, the contribution of anammox in acidic red soils to nitrogen loss has not been well-documented to date. Here, we investigated the activity, abundance, and distribution of anammox bacteria in red soils collected from nine provinces of Southern China. High-throughput sequencing analysis showed that *Candidatus Brocadia* dominates the anammox bacterial community (93.03% of sequence reads). Quantification of the hydrazine synthase gene (*hzsB*) and anammox 16S rRNA gene indicated that the abundance of anammox bacteria ranged from $6.20 \times 10^6$ to $1.81 \times 10^9$ and $4.81 \times 10^6$ to $4.54 \times 10^8$ copies per gram of dry weight, respectively. Contributions to nitrogen removal by anammox were measured by a $^{15}$N isotope-pairing assay. Anammox rates in red soil ranged from 0.01 to 0.59 nmol N g$^{-1}$ h$^{-1}$, contributing 16.67–53.27% to $N_2$ production in the studied area, and the total amount of removed nitrogen by anammox was estimated at 2.33 Tg N per year in the natural red soils of southern China. Pearson correlation analyses revealed that the distribution of anammox bacteria significantly correlated with the concentration of nitrate and pH, whereas the abundance and activity of anammox bacteria were significantly influenced by the nitrate and total nitrogen concentrations. Our findings demonstrate that *Candidatus Brocadia* dominates anammox bacterial communities in acidic red soils and plays an important role in nitrogen loss of the red soil in Southern China.

Keywords: red soils, anaerobic ammonium oxidation, high-throughput sequencing, *Candidatus Brocadia*, nitrogen removal

INTRODUCTION

Anaerobic ammonium oxidation (anammox) is a principal microbe-driven process that removes excess N from an ecosystem and oxidizes $NH_4^+$ with $NO_2^-$ or $NO_3^-$ to form $N_2$ gas under anaerobic autotrophic condition (Mulder et al., 1995). The discovery of anammox challenged the established concept attributing atmospheric dinitrogen gas ($N_2$) from fixed nitrogen in the environment to heterotrophic denitrification. The anammox process is mediated by bacteria belonging to the monophyletic order of *Candidate Brocadiales*, of the phylum *Planctomycetes* (Jetten et al., 2010). Presently, there are five different genera of anammox bacteria that have been described, including *Candidatus Brocadia* (Strous et al., 1999; Kartal et al., 2008), *Candidatus Jettenia* (Quan et al., 2008),...
Anammox bacteria are broadly distributed in many kinds of natural environments, including aquatic and terrestrial ecosystems. Candidatus Scalindua was almost the only genus found in marine sediments, such as South China Sea (Hong et al., 2011a), Bohai Sea (Dang et al., 2013), and Arabian Sea (Woebcken et al., 2008). In contrast to marine environments, all of these genera of anammox bacteria have been detected in soil ecosystems (Wang et al., 2015; Yang et al., 2017). Zhu et al. (2011) found that the 16S rRNA gene sequence of fertilized paddy soil were related to four different genera of anammox bacteria. Anammox bacteria related to Candidatus Brocadia, Kuenenia, and two novel unidentified clusters were found to dominate in the 12 typical paddy soils collected in southern China (Yang et al., 2015). Shen et al. (2017) detected three genera of anammox bacteria by Illumina-based 16S rRNA gene sequencing in a vegetable field, including Candidatus Kuenenia, Brocadia, and Jettenia. Nonetheless, another study showed that anammox bacteria in rice paddy soils were consisted of mainly Candidatus Scalindua (Wang and Gu, 2013), which was regarded as the dominant genus in marine environments. In addition, anammox have been reported to be an important nitrogen removal pathway in soil ecosystems, which accounts for 0.4–37% of the total N₂ production in soil ecosystems (Zhu et al., 2011; Yang et al., 2015; Shan et al., 2016, 2018; Xi et al., 2016). Yang et al. (2015) suggested that ~10% of applied N-based fertilizers was lost via the anammox process. Until now, however, there has been limited evidence for the existence and role of anammox bacteria in acidic and natural red soils.

Red soil is widely distributed and covers an area of nearly 2.04 × 10^6 km² in southern China, accounting for 6.5% of the total farmland area, and is one of China's most important agricultural soils (Xu et al., 2003). On the other hand, red soils are acidic (pH: 4.2–5.9), nutrient deficient (total N ranges from 1.4 to 2.0 g kg⁻¹, Total P: 0.6–0.9 g kg⁻¹), poor in organic matter (ranging from 10 to 100 g kg⁻¹) and have low water-holding and supplying capacity (Wilson et al., 2004). Fe (total iron: 11.7–148.0 g kg⁻¹) and Al oxides are often the dominant clay minerals in red soils. Increasing evidence shows that soil characteristics (e.g., soil moisture, pH, and nutrient conditions) may significantly affect the activity and community of soil ammonia oxidation bacteria and archaea (Nicol et al., 2008; Erguder et al., 2009; Gleeson et al., 2010; Gubryrangin et al., 2011; Liu et al., 2017). We hypothesized that the activity, abundance, and diversity of anammox bacteria in red soil would be lower than in other natural habitats. Besides, the anammox population in red soil is expected to be related to a limited number of genera that can be adapted to acidic and nutrient-deficient conditions.

In this study, 10 representative red soil samples from nine provinces of southern China were collected to (i) analyze the diversity and structure of anammox bacteria by Illumina-based 16S rRNA gene sequencing; (ii) determine the abundance of anammox bacteria by quantitative PCR (qPCR) analysis of the hydrazine synthase gene (hzsB) and 16S rRNA gene; (iii) evaluate the potential rates and contribution of anammox and denitrification to N₂ production using the ¹⁵N-labeling approach; (iv) analyze the relations between the physicochemical characteristics of red soil and activity, abundance, and community structure of anammox bacteria. This study provides a new understanding of the community composition and N loss contribution of anammox bacteria in non-fertilized acidic red soils.

### MATERIALS AND METHODS

#### Samples Collection of Red Soil

A total of 10 red soil samples from nine provinces were collected from the natural fields in southern China (Figure 1 and Table 1) during July 2017. These fields were not affected by a substantial amount of nitrogen fertilizer. Red soil samples (depth: 0–20 cm, Supplementary Figure 1) of four replicates (ca. 500 g each subsample) were collected from 10 natural red soils. Soil samples were immediately placed in sterile plastic bags, sealed, and transported to the laboratory on ice. The collected samples were subsequently divided into three parts. The first part was incubated to determine anammox and denitrification activities immediately, the second part was stored at 4°C for subsequent analysis of physicochemical properties, and the third part was stored at −80°C for DNA extraction and molecular analyses.

#### Analyses of Red Soil Physicochemical Properties

pH of red soil was measured at a soil/Milli-Q water ratio of 1:2.5 with a pH analyzer (Mettler Toledo S220, Switzerland). Dissolved inorganic nitrogen (NH₄⁺, NO₂⁻, and NO₃⁻) was extracted from the soil using 2 mol/L KCl at a liquid to solid ratio of 100. The extraction process was as follows. Freeze-dried and sieved 100 mg of a sediment sample was placed in a polyethylene centrifuge tube, and a 2 mol/L KCl solution was added to it. It was agitated on a rotary shaker for 8 h. After that, the sediment was centrifuged for 10 min at 3500 rpm, and the supernatant was used to measure the dissolved inorganic nitrogen concentration. Concentrations of NH₄⁺, NO₂⁻, and NO₃⁻ in the supernatants were determined by spectrometric detection methods described by Wu et al. (2016) and Guan et al. (2017). Total nitrogen (TN), organic carbon (%), and the C:N ratio in each sample were analyzed on an elemental analyzer (IsoPrime 100, Elemental, Germany) after leaching with 0.1 M HCl to remove sedimentary carbonate.

#### Measurement of Potential Anammox and Denitrification Rates

The potential rates of anammox and denitrification in the red soils were measured as described by Risgaard-Petersen et al. (2003) and Hou et al. (2012) with a slight modification, and their relative contributions to N₂ production were calculated based on these rates. Briefly, 30 g of field moist soils was transferred to a 250 mL glass bottle with helium-purged water.
at a volume ratio of 1:5. The mixture was purged with helium for 30 min. The resulting soil slurries were transferred into gas-tight 12.5 mL helium-flushed glass vials (Labco Exetainters, United Kingdom) under helium. Next, these slurries were incubated for approximately 24 h to remove residual NO$_3^-$ and dissolved oxygen at in situ sampling temperature. The $^{15}$N atom% (Fn, represents the fraction of $^{15}$N in total NO$_3^-$) was calculated by taking into account $^{15}$N atom% of stock solutions and any residual ambient $^{14}$NO$_3^-$ as determined by difference, ranging from 0.80 to 0.98. After pre-incubation, these vials with slurries were divided into three groups, which were spiked through the septum with helium-purged stock solutions of (1) $^{15}$NH$_4^+$ ($^{15}$N at 99.6%), (2) $^{15}$NH$_4^+$+$^{14}$NO$_3^-$, and (3) $^{15}$NO$_3^-$ ($^{15}$N at 99%). The final concentration of $^{15}$N in each vial was ~100 µM. Samples for dissolved gas analysis were preserved with 200 µL of a 50% ZnCl$_2$ solution and analyzed within 8 h. Membrane inlet mass spectrometry (MIMS, Hiden) was employed to measure the concentrations of $^{29}$N$_2$ and $^{30}$N$_2$ produced during the incubation period. Finally, the developed methods were used to calculate the rates of both anammox and denitrification and their potential contribution to N$_2$ production.
DNA Extraction and PCR Amplification

Approximately 0.3 g of red soil was used for genomic DNA extraction using the PowerSoil DNA Isolation Kit (Mobo, United States). DNA quality and quantity were checked on a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, United States). Anammox bacteria were targeted by the barcoding primers A438f and A684r. The forward primer of A438f was attached to a unique 8 bp barcode sequence. More details of the primers and PCR amplification program are given in Supplementary Table 1. The amplified products were verified by electrophoresis in a 1.0% agarose gel and then purified using a MiniBEST agarose gel DNA extraction kit (TaKaRa, Beijing, China) before high-throughput sequencing.

High-Throughput Sequencing and Analysis

Data analysis of high-throughput raw sequences was conducted in the Mothur software v.1.35.1 following the standard protocol1 (Schloss et al., 2009). The obtained reads were processed by removing tags and primers before the sequence read numbers per sample were grouped together. The quality-trimmed sequences were aligned to the newly developed anammox 16S rRNA gene databases. The chimeric sequences were identified and removed by the Chimera-uchime. High-quality anammox bacterial sequences were used to generate a distance matrix and cluster with the average neighbor algorithm. Representative sequences for each operational taxonomic unit (OTU) as defined by 97% sequence identity were obtained for further diversity analyses (Zhao et al., 2013). Phylogenetic analysis of the representative anammox bacterial 16S rRNA gene sequences from each OTU was conducted with the MEGA 7.0 software. A heat map was constructed based on the abundance of top 30 OTUs. Principal coordinates analysis (PCoA) was conducted by Normalized weighted Unifrac (Lozupone et al., 2006). Canonical correspondence analysis (CCA) was performed using the CANOCO 5.0 software. The plots in this study were created in SigmaPlot (version 12.5).

qPCR Analysis of hzsB and 16S rRNA Genes of Anammox Bacteria

The abundance of the hzsB gene and 16S rRNA gene of anammox bacteria was determined in triplicate on a Bio-Rad iQ5 thermal cycler (Bio-Rad Laboratories) with primer sets HSBeta396F-HSbeta742R and A438f-A684r, respectively. More details of qPCR assays are given in Supplementary Table 1. Plasmids carrying the targeted gene fragments were extracted from Escherichia coli DH5α hosts using a Plasmid Mini Preparation Kit (TaKaRa, Beijing, China). Standard curves were built using 10-fold serial dilution of the plasmid with target anammox bacterial genes: hzsB and 16S rRNA gene. Specificity of the amplified products was checked by examination of a single melting peak and the presence of a unique band of the expected size in a 2% agarose gel stained with ethidium bromide. The results with efficiency and correlation coefficient above 90% and 0.97 were employed in this study.

Statistical Analysis

The relations among the activity, abundance of anammox bacteria, and different environmental factors were examined by Pearson correlation analyses using Statistical Analysis System (SAS 9.4).

Nucleotide Sequence Accession Numbers

The raw Illumina reads of 16S rRNA gene sequences of anammox bacteria were deposited in the NCBI short-read archive under the Accession No. SRP140525.

RESULTS

Physicochemical Properties of Red Soils

The concentrations of NH\textsubscript{4}⁺, NO\textsubscript{3}⁻, NO\textsubscript{2}⁻, TN, and C (%) and the ratio C:N of red soils from nine provinces of Southern China are listed in Table 1. The pH values of all collected red soil samples were relatively low, ranging from 4.40 to 6.02. High concentrations of NH\textsubscript{4}⁺ were characteristic of all red soils, ranging from 27.25 to 69.12 mg kg\textsuperscript{-1}. NO\textsubscript{2}⁻ concentrations (ranging from 3.29 to 300.61 mg kg\textsuperscript{-1}) were lower than those of NH\textsubscript{4}⁺.
but peaked in sample CZ (300.61 mg kg$^{-1}$) in Anhui Province. The concentrations of NO$_2^-$ were relatively low and ranged from 2.25 to 5.40 mg kg$^{-1}$. The soil TN and C% contents were lower than those in other agricultural soils, which varied from 70 to 570 mg kg$^{-1}$ and 0.056 to 0.433%, respectively. Such differences may be the result of less application of fertilization in natural red soils. Most of the samples had a low C:N ratio (ranging from 5.33 to 10.05). Nonetheless, samples LS and YL showed relatively higher C:N ratios (13.69 and 14.00). The physicochemical properties of red soils in this study were in the same range as other reported values for red soils (Wilson et al., 2004).

The Potential Rates of Anammox and Contribution to N$_2$ Production

The potential anammox and denitrification rates were determined in red soils based on the incubation of $^{15}$NO$_3^-$. The potential anammox rates ranged from 0.01 ± 0.00 to 0.59 ± 0.07 nmol N g$^{-1}$ dry red soil h$^{-1}$, while denitrification rates ranged from 0.01 ± 0.01 to 1.63 ± 0.19 nmol N g$^{-1}$ dry red soil h$^{-1}$ (Figure 2 and Supplementary Table 2). Denitrification rates were higher than anammox rates, suggesting that denitrification dominated the total nitrogen loss in red soils. The highest anammox and denitrification rates were both observed in CZ in Hunan province. The red soil collected in SC, YL, MZ, and GZ showed relatively lower potential anammox rates. The relative contribution of anammox to N$_2$ production varied from 16.67 to 53.27%.

Abundance of Anammox Bacteria

The $hzb$ gene and anammox 16S rRNA gene were used to estimate the abundance of anammox bacteria in red soils by the qPCR method. The abundance of anammox bacteria ranged from $6.20 \times 10^5$ to $1.81 \times 10^9$ ($hzb$ gene) and $4.81 \times 10^6$ to $4.54 \times 10^8$ (anammox 16S rRNA gene) copies per gram of dry weight (Figure 3). The highest abundance of anammox bacteria was observed in sample CZ, while other samples had values between $10^5$ and $10^8$ copies/g. $hzb$ was slightly more abundant than the 16S rRNA gene of anammox bacteria (the $hzb$/anammox 16S rRNA gene ratio was 2.18) in red soils. The presence of anammox bacteria was ascertained in the current study by the positive correlation between $hzb$ and ANA 16S rRNA gene ($r = 0.818, P < 0.001, n = 10$).

Community Composition and Phylogenetic Analysis of Anammox Bacteria

A total of 0.14 million of anammox raw sequences (15000 sequence reads per sample except NP, which contained only 7222 sequences reads) were subjected to denoising and trimming of sequences. After quality controls, ~12000 reads per sample was filtered as high-quality reads for further analysis (Table 2).

Based on the 0.03 distance level, 138 OTUs (77 ± 7 OTUs per sample, $n = 10$) of the anammox 16S RNA gene were obtained (Table 2). The highest number of OTUs was seen in sample GZ (84 OTUs). The Chao1 and ACE richness estimators of anammox bacteria ranged from 89.169 to 107.521 and 107.027 to 164.916, respectively. Higher richness of anammox bacteria was found in sample BS. The Shannon index showed that the highest diversity of anammox bacteria was detected in sample NP (0.624), whereas sample MZ (0.263) had the lowest diversity among red soils. The evenness of anammox bacteria was low in red soil and ranged from 0.060 to 0.150. Good’s coverage values varied from 0.995 to 0.996, suggesting that the libraries were adequately
large. Rarefaction curves (Supplementary Figure 2) and a rank-abundance curve (Supplementary Figure 3) for the anammox bacterial 16S rRNA gene at 97% similarity showed that the high-throughput sequencing could supply enough bioinformation to investigate the community composition and diversity of anammox bacteria in the current study.

The community structure of anammox bacteria in red soil at the genus level is depicted in Figure 4. Gene sequences of anammox bacteria mainly belonged to Candidatus Brocadia (93.03%), Candidatus Scalindua (0.09%), Candidatus Anammoxoglobus (0.01%), Candidatus Kuenenia (0.04%) and unclassified (6.83%). Candidatus Brocadia (93.03%) was the dominant genus in acidic and natural red soils, whereas Candidatus Scalindua was more abundant in sample GZ. A heatmap (Figure 5A) and phylogenetic tree (Figure 5B and Supplementary Figure 4) of dominant anammox OTUs (top 30 OTUs, 99% sequences were obtained) indicated that the top 30 OTUs were affiliated with only one cluster, which was closely related to Candidatus Brocadia fulgida. OTU 1 was the most abundant sequence in all the samples and had average relative abundance of 27.39.

**Correlation Analysis of Anammox Potential Rates, Gene Abundances, and Red Soil Properties**

Pearson correlation analysis was used to illustrate the correlations among anammox potential rates, gene abundances, and red soil properties (Figure 6 and Supplementary Table 3). The results indicated that anammox rates positively correlated with NO$_3^-$ (coefficient = 0.889, $P < 0.001$). hzsB abundances positively correlated with TN (coefficient = 0.637, $P < 0.05$) and anammox 16S rRNA abundances (coefficient = 0.942, $P < 0.001$). Anammox 16S rRNA abundance also positively correlated with TN (coefficient = 0.652, $P < 0.05$). In addition, C% positively correlated with NH$_4^+$ (coefficient = 0.779, $P < 0.001$) and TN (coefficient = 0.993, $P < 0.001$) in red soils. As revealed by CCA, pH and NO$_3^-$ were found to be the main factors affecting the distribution of anammox bacteria in the red soils (Supplementary Figure 5).

**DISCUSSION**

Recently, increasing evidences showed that anammox bacteria play a significant role in nitrogen loss in agricultural soils, particularly paddy soils (Zhu et al., 2011; Wang and Gu, 2013; Wang et al., 2014; Nie et al., 2015; Yang et al., 2015). In the present study, we present the evidence of anammox process for the first time in 10 different acidic red soils of Southern China by combining the $^{15}$N-labeling approach, qPCR analysis, and high-throughput sequencing. The potential anammox rates, ranging from 0.01 ± 0.00 to 0.59 ± 0.07 nmol N g$^{-1}$ dry red soil h$^{-1}$ (Figure 2 and Supplementary Table 2), were examined in all the selected red soils, revealing the same range as that found in agricultural soils (Long et al., 2013; Nie et al., 2015) and forest soils (Xi et al., 2016), but lower than other values reported for fertilized paddy soils and agricultural fields (Zhu et al., 2011; Yang et al., 2015; Shen et al., 2016, 2017). Red soils in China were found to be characterized by low pH, TN, and organic carbon, in contrast to other fertilized agricultural soils (Table 1). The physicochemical properties of red soils were found to be the most important factors influencing the potential anammox and denitrification rates, especially soil organic carbon and TN. Soil organic carbon can enhance microbial activities and improve physical and chemical conditions of the soil (Pullman et al., 2000; Zhang et al., 2009). These results indicated that the characteristics of red soil may influence the activity of anammox bacteria. Other studies also showed that flooding and fertilization conditions made the paddy soils a suitable habitat for anammox bacteria (Shen et al., 2017). Zhu et al. (2011) found many microniches were existed in a paddy soil; thus, it could support the various ecophysiologies of the different anammox genera and facilitate the anammox activity. In addition, it should be noticed that slurry incubations may underestimate the in situ anammox activity due to the following reasons. Firstly, the incubation conditions at higher moisture may affect the microbial community, which results in underestimation of the denitrification and anammox rate. Finally, the incubation time of 24 h may not be enough to eliminate the initial $^{15}$NO$_3^-$ in sample CZ (Fn: 0.80) and LS (Fn: 0.87), which may underestimate the potential rates. Different potential rates of

**TABLE 2 | Estimates of sedimentary 16S rRNA gene sequence richness and diversity in different red soils.**

| Samples | High-quality reads | OTUs$^a$ | Chao1$^a$ | ACE$^a$ | Shannon$^a$ | Evenness$^b$ | Coverage |
|---------|--------------------|----------|-----------|--------|-------------|------------|----------|
| BS      | 12450              | 74       | 89.169    | 107.027| 0.334       | 0.077      | 0.996    |
| CZ      | 11823              | 77       | 89.366    | 130.129| 0.542       | 0.125      | 0.995    |
| FZ      | 12425              | 78       | 93.469    | 135.885| 0.444       | 0.102      | 0.995    |
| GZ      | 12280              | 84       | 105.999   | 164.916| 0.530       | 0.120      | 0.995    |
| LS      | 12501              | 82       | 96.499    | 140.765| 0.365       | 0.083      | 0.995    |
| MZ      | 12461              | 80       | 95.796    | 138.615| 0.263       | 0.060      | 0.995    |
| NP      | 5824               | 64       | 97.833    | 132.182| 0.624       | 0.150      | 0.995    |
| SC      | 12500              | 81       | 99.562    | 148.988| 0.320       | 0.073      | 0.995    |
| SY      | 12349              | 75       | 91.721    | 126.715| 0.361       | 0.084      | 0.996    |
| YL      | 12404              | 82       | 107.521   | 137.841| 0.493       | 0.112      | 0.995    |

$^a$Richness and diversity were determined based on 0.03 distance.
$^b$Evenness was calculated by dividing Shannon index by Ln (OTUs).
Anammox were observed in 10 samples from nine provinces of Southern China (Figure 2). Cultivation history, land use patterns, and a cropping system are the important factors influencing the physicochemical properties of red soil in different provinces (Shen et al., 2013). Anammox contributed 16.67 to 53.27% to red soil N\textsubscript{2} production (Figure 2 and Supplementary Table 2),...
suggesting that anammox should be recognized as a significant N sink in red soil. On the base of the soil density, the average potential anammox rate, and red soil area in southern China, N loss attributed to anammox was estimated to reach \( \sim 2.33 \) Tg N per year. This result was comparable to previously reported values (2.50 Tg N per year) in paddy soil in Southern China (Yang et al., 2015), demonstrating the importance of anammox in red soil ecosystems. Pearson correlation analyses showed that the potential anammox rates positively correlated with NO\(^-\textsubscript{3}\) (coefficient = 0.889, \( P < 0.001 \)) (Figure 6 and Supplementary Table 3). Previous study on freshwater sediments also suggested that NO\(^-\textsubscript{3}\) is a factor controlling anammox rates (Yoshinaga et al., 2011). Anammox bacteria are favored in environments where NO\(^-\textsubscript{3}\) is available (Trimmer et al., 2003; Engström et al., 2005; Yang et al., 2015).

The abundance of anammox bacteria ranged from \( 6.20 \times 10^5 \) to \( 1.81 \times 10^9 \) \( hzsB \) copies/g and \( 4.81 \times 10^6 \) to \( 4.54 \times 10^8 \) (anammox bacterial 16S rRNA gene), which fall within the ranges reported in Pearl River Estuary (1.4–20 \( hzsB \) copies/g; Wang et al., 2012), Yangtze Estuary (3.67–822 \( 10^5 \) 16S rRNA copies/g; Zheng et al., 2016), and paddy soils (0.7–1.4 \( 10^7 \) \( hzs \) copies/g; Nie et al., 2015). Anammox bacterial abundances in red soils were confirmed by means of the anammox \( hzsB \) gene and 16S rRNA gene (Figure 3), and abundances of these two target genes correlated (\( r = 0.818, P < 0.001, n = 10 \)). In this study, TN concentration was identified as an important factor influencing the anammox bacterial abundance in selected red soils (Figure 6 and Supplementary Table 3). It can be speculated that higher TN concentration may provide an environment favorable for the distribution and growth of anammox bacteria (Shen et al., 2016). In line with other studies (Metz et al., 2003; Etchebehere and Tiedje, 2005; Yang et al., 2017), a positive correlation between anammox activity and abundance (\( hzsB \) or 16S rRNA gene) was not observed. These results indicated that anammox rates are limited not by the presence of the population capable of anammox but by the availability of NO\(^-\textsubscript{3}\) or NH\(^+\textsubscript{4}\) as substrate. If anammox bacterial abundance correlated with their activities, it suggested that anammox bacterial abundance has a potential to predict anammox activity (Dale et al., 2009). Concentrations of NO\(^-\textsubscript{3}\) and/or NH\(^+\textsubscript{4}\) have been reported to significantly affect the anammox rate (Shen et al., 2013). Lisa et al. (2015) found that physiological nature of an organism and the environmental conditions of the system are the two important factors influencing whether the genetic potential may or may not predict the potential for that process. Furthermore, the presence of anammox bacteria detected by gene amplification is not an indication of their level of activity as would be indicated by active community (cDNA) qualification.

Some studies have reported that Candidatus Scalindua is the exclusive anammox genus in marine ecosystems (Schmid et al., 2007; Hong et al., 2011a,b). In this study, Candidatus Brocadia dominated the anammox community in acidic red soils. In total, 93.03% sequence reads of anammox bacteria were related to Candidatus Brocadia (Figure 4) and the dominant
OTUs (TOP 30) were all affiliated with Candidatus Brocadia fulgida (Figure 5). Candidatus Brocadia was reported to be the most common anammox genus in terrestrial ecosystems, including paddy soils (50%, Yang et al., 2015) and Chinese agricultural soils (75%, Shen et al., 2013). Some studies showed that Candidatus Brocadia possesses diverse metabolic pathways (Gori et al., 2011) and better adaptability to terrestrial soils than do other anammox genera (Shen et al., 2013). Kartal et al. (2016) also found that Candidatus Brocadia fulgida can use short-chain organic acids as alternative electron donors to reduce NO\(^-\) to N\(_2\). Candidatus Brocadia belongs to the r-strategists, making it suitable for the higher substrate concentration, and it will outcompete other anammox genera (Strous, 1999; Gao et al., 2018). CCA suggests that pH and NO\(^-\) content had a significant contribution to anammox bacterial community structure, which may be attributed to an increased supply of NO\(^-\) via reduction of NO\(_3^-\). Similar results have also been observed in the sediments of the Dongjiang River (Sun et al., 2013) and Mai Po Nature Reserve (Li et al., 2011).

Thus, both the presence and activity of anammox bacteria were for the first time shown in 10 different acidic red soils from nine provinces in Southern China. These data expand the knowledge of the distribution and N loss contribution of anammox bacteria in acidic red soils. Our high-throughput sequencing analysis indicates that Candidatus Brocadia dominates the anammox bacterial community. Anammox contributed 16.67 to 53.27% to red soil N\(_2\) production, which was the vital N sink (2.33 Tg N per year) in red soils of Southern China. These results demonstrate the lower diversity but higher contribution of anammox bacteria to the removal of fixed N from acidic red soils.

**AUTHOR CONTRIBUTIONS**

JW, YH, XH, LJ, and XW performed the research. JW, YH, SC, GC, YL, TH, YHH, and XL analyzed the data. JW and YH wrote the paper. All co-authors substantially contributed to commenting and revising it and read and approved the final manuscript.

**FUNDING**

This work was financially supported by National Natural Science Foundation of China (Grant Nos. 41576123 and 31870100), National Key Basic Research Program of China (Grant No. 2015CB452902), Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA11020202), Natural Science Foundation of Guangdong (Grant No. S201302012823), Scientific Research Project of Guangzhou (Grant No. 201504010005), and Research Foundation for Talented Scholars of Guangzhou University (Grant No. GU2017001).

**ACKNOWLEDGMENTS**

We would like to thank Chunyu Zhao, Jiaqi Ye, Tongxin Li, and Yuwei Li for their kind help; Jinxing Wu, Mingshan Chen, and Lihao Chen are gratefully acknowledged for their detailed and patient favor in red soil sampling.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.02142/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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