Spatiotemporal evolution and assembly processes of ammonia-oxidising prokaryotic communities in 1000 years coastal reclaimed soils

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Abstract

Coastal marshes are transitional areas between terrestrial and aquatic ecosystems and vulnerable to climate change and anthropogenic activities. In recent decades the reclamation of coastal marshes remarkably increased and their effects on microbial communities present in coastal marshes have been studied with great interest. However, most of these studies focused on microbial community composition and diversity. The processes underlying functional community assembly and spatiotemporal effect often ignored. Therefore, community structure and assembly mechanisms of ammonia-oxidising prokaryotes in long-term reclaimed coastal marshes have not been studied. Here using qPCR and IonS5TMXL sequencing platform, we investigated spatiotemporal dynamics, assembly processes and diversity patterns in ammonia-oxidising prokaryotes in over 1000 years reclaimed coastal salt marsh soils. The taxonomic & phylogenetic diversity and composition of the ammonia-oxidizers showed apparent spatiotemporal variations along reclamation of soil. The phylogenetic null modelling-based analysis showed across all sites, the archaeal ammonia-oxidising community assembled by deterministic process (84.71%). The ammonia-oxidising bacterial community was formed more by a stochastic process in coastal marshes and at stage 60 years ($|\beta NTI|<2$), despite its relatively dominant deterministic process (55.2%). The deterministic assembly process and nitrification activity in reclaimed soils was positively correlated. Archaeal amoA gene abundance were also positively correlated with the nitrification rate. Our study revealed that during the 1000 years of reclamation coastal marshes both ammonia-oxidising communities responded differently to diversity change and assembly processes and nitrification activity. These findings provide a better understanding of how long-term reclamation affect soil N cycling and assembly dynamics of ammonia-oxidising communities.

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ABSTRACT

Coastal marshes are transitional areas between terrestrial and aquatic ecosystems and vulnerable to climate change and anthropogenic activities. In recent decades the reclamation of coastal marshes remarkably increased and their effects on microbial communities present in coastal marshes have been studied with great interest. However, most of these studies focused on microbial community composition and diversity. The processes underlying functional community assembly and spatiotemporal effect often ignored. Therefore, community structure and assembly mechanisms of ammonia-oxidising prokaryotes in long-term reclaimed coastal marshes have not been studied. Here using qPCR and IonS5^{TM}XL sequencing platform, we investigated spatiotemporal dynamics, assembly processes and diversity patterns in ammonia-oxidising prokaryotes in over 1000 years reclaimed coastal salt marsh soils. The taxonomic & phylogenetic diversity and composition of the ammonia-oxidizers showed apparent spatiotemporal variations along reclamation of soil. The phylogenetic null modelling-based analysis showed across all sites, the archaeal ammonia-oxidising community assembled by deterministic process (84.71%). The ammonia-oxidising bacterial community was formed more by a stochastic process in coastal marshes and at stage 60 years ($|\beta NTI|<2$), despite its relatively dominant deterministic process (55.2%). The deterministic assembly process and nitrification activity in reclaimed soils was positively correlated. Archaeal *amoA* gene abundance were also positively correlated with the nitrification rate. Our study revealed that during the 1000 years of reclamation coastal marshes both ammonia-oxidising communities responded differently to diversity change and assembly processes and nitrification activity. These findings provide a better understanding of how long-term reclamation affect soil N cycling and assembly dynamics of ammonia-oxidising communities.

Keywords: Coastal Salt Marshes; Deterministic; Stochastic; βNTI; Community Assembly; Ammonia Oxidiser

Introduction

Salt marshes are the most productive and valuable habitats and play a crucial role in the protection of coastal regions, while being vulnerable to climate change and anthropogenic destruction (Bowen*et al.* , 2013, Deegan *et al.* , 2012, Sousa *et al.* , 2008). Reclamation of coastal wetlands has turned out to be a popular activity around the world to provide more space for anthropogenic activities. Therefore, wide areas of coastal marshes are now being used for agriculture, transportation infrastructure, and industry. However, this large-scale reclamation activity has caused unprecedented damage and interference to coastal marshlands. Reclamation creates significant environmental threats, therefore becoming a global issue. Nitrification and denitrification of nitrogen in salt marshes has been incorporated by either plant biomass or the local microbiota (Verhoeven *et al.* , 2006). The primary and rate-limiting stage of nitrification is known to be ammonia oxidation driven by ammonia-oxidising archaea (AOA) and bacteria (AOB), which play a significant role in maintaining the nitrogen cycle (Rotthauwe *et al.* , 1997, Zhang *et al.* , 2017). Recent studies have investigated spatiotemporal variations in microbial communities, but debate exists over the drivers and how microbially driven mechanisms are ultimately affected (Brankatschk *et al.* , 2011, Nemergut *et al.* , 2007, Sigler & Zeyer, 2002).

The microbial community composition is associated with the surrounding environment, and consequently environmental conditions can alter the microbial community structure (Gutknecht *et al.* , 2012). Numerous studies have indicated that the trajectories, such as the disentangled relationship between ecological mechanisms and edaphic factors exhibited by microbial communities are helpful to structure microbial populations in disturbed and natural soil (Dini-Andreote *et al.* , 2015a, Ferrenberg *et al.* , 2013). Several anthropogenic and natural activities are responsible for controlling the association between environmental and microbial assembly dynamics and it would also affect biogeochemical cycling and ecosystem functioning (Hutchins & Fu, 2017). Both assembly dynamic stochastic and/or deterministic processes control microbial biogeography and differ in their relative contribution to microbiota assembly over different tempo-spatial scales (Zhou & Ning, 2017b). However, spatial scales can be a deterministic factor when correlated with functional traits (niche), population size, and environmental factors, affecting the dispersion of different taxa (Hanson *et al.* , 2012). The ancient theory of a niche shows that biodiversity regulation is based on biotic (inter taxa interaction) and abiotic (environmental heterogeneity) factors. A central theme in ecology is the interpretation of variables that regulate spatiotemporal differences in the composition of microbial communities, along with

environmental factors (Tilman, 1988, Van der Maarel & Franklin, 2012).

Limited studies have investigated the successional pattern of microbial communities based on agricultural land, post-mining regions, and glacial foreland over chronosequence (Hüttl & Weber, 2001, Kuramae *et al.* , 2010, Wu *et al.* , 2012). A chronosequence of soil formation may depend on spatiotemporal fluctuations in ammonia oxidiser populations and the dynamics of ecosystem development over a time scale (Walker *et al.* , 2010). In recent years, extensive work in molecular biology has aided the investigation of AOA and AOB ecology via*amoA* gene surveys. The null model-based β-diversity (βNTI) is useful inferring the stochastic and deterministic processes of community assembly for soil microbial communities. In this study, we analysed community structure, composition and assembly dynamics of ammonia oxidisers using effective statistical methods, including the Mean Pairwise Distance standardised-effect size (ses.MPD), Nearest Related Index (NRI), Mean Nearest Taxon Distance standardised-effect size (ses.MNTD), Nearest Taxon Index (NTI), beta Mean Nearest Taxon Distance (βMNTD), and beta Nearest Taxon Index (βNTI). In this context we hypothesised that (1) The reclamation of soil is a human concept that captures a wide range of natural and anthropogenic processes that modify the environment and due to modification in environment the soil biogeochemistry will be different enough to impact on ammonia oxidizers community composition and assembly process. (2) the functional species abundance is also an important determinant of ecosystem functions and the identity of those species. Therefore, the assembly process can necessarily influence soil microbial abundance, diversity and composition, with downstream impacts on the ecosystem functions.

MATERIALS AND METHODS

2.1 Study area description and field soil sampling

The present study was conducted on the south coast of Hangzhou Bay (121°05'-121deg35''E, 30deg0'-30deg25'N) in Cixi County, Zhejiang Province, China. The study area is a typical northern subtropical monsoon climate zone, with a mean annual precipitation of 1273 mm and temperature of 16.0degC. Salt marshes formed from the Qiantang Estuary saltwater sediments and diked lands were exposed to tidal effects at an elevation range from 2.6 to 5.7 m (Guo *et al.* , 2000). The tidal salty marsh was eventually reclaimed for crop growth, beginning 1000 years ago with the formation of dikes at different historical stages. Based on the construction times of dikes, the years of soil reclamation were calculated, and a detailed description is available online in Chinese at (www.cixi.gov.cn). In this study, around 1000 years of ancient spanning of soil chronosequence was identified, including undisturbed coastal salty marsh (T0) as control and soil reclaimed 5-years (T5), 20-years (T20), 50-years (T50), 60-years (T60), 120-years (T120), 200-years (T200), 220-years (T220), 280-years (T280), 500-years (T500), and 1000-years (T1000) ago.

Distances between these soils were no more han 30 km in a similar topography. Soil samples were collected and mixed from six random locations, and then six mixed soils sampled from the same reclaimed year were considered replicates. A stainless-steel auger (3-cm diameter) was used for soil collection. Briefly, soil samples were taken from the plough layer (0–20 cm), and a total of 66 samples were obtained representing specific reclamation years (**Fig. S1**). The taxonomic class of soil samples was fluvisol as per Food and Agriculture Organization/United Nations Educational, Scientific and Cultural Organization (FAO/UNESCO), which was formed from fluvial deposits around the Qiantang River, and the soil texture was silty loam (Cheng *et al.* , 2009, Roth *et al.* , 2011). In December 2015, reclamation site samples (T5-T1000) were collected from a traditional soybean-broccoli rotation field during active broccoli growth from crop free sites to avoid the possible impact of plant roots. Compound fertilisers were applied @ 900 kg*ha^{-1}, of which nitrogen (N):phosphorus (P_2O_5):potassium (K_2O) was estimated to be 1:1:1. The salt marsh soils (T0) were sampled in the high-tide area under *Phragmites australis* . Samples were immediately taken to the laboratory on ice packs and then sieved through a 2 mm mesh to remove roots and another residue. After that, sieved soils were divided into three parts; two parts were kept at -80degC and 4degC for total DNA extraction, mineralised N content, and nitrification analysis. The third part was air-dried for the determination of physicochemical parameters of the soil.

2.2 Measurements of soil physicochemical properties

Soil physicochemical properties were described in our previous studies (Wang*et al.* , 2015). Briefly soil electrical conductivity (EC) was measured using a platinum electrode in water to soil (5:1) suspension. Soil pH was measured using a glass electrode at a soil to water ratio of (1:2.5). Soil organic matter (SOM) was determined using wet digestion by the potassium dichromate method. The soil total nitrogen (TN) was measured by the micro-Kjeldahl method using digestion in H_2SO_4 followed by steam distillation. Soil available phosphorus (AP) was extracted with sodium bicarbonate and then determined using the molybdenum-blue method. Soil available potassium (AK) was extracted with ammonium acetate and determined by flame photometry. Ammonium (NH_4-N) and nitrate nitrogen (NO_3-N) were determined by Auto Analyzer 3 (Bran+ Luebbe GmbH, Germany) (Kempers & Luft, 1988, Searle, 1984).

2.3 Measurements of potential nitrification rate

Potential nitrification rate was quantified by method as described (Taylor *et al.* , 2013). In brief explanation; soil sampled were incubated at three different NH_4^+concentrations level (3.25, 25 and 100mg NH_4^+ per kg soil). The soil samples incubated for 96h at 25 degC, subsequent $NO_2^- + NO_3^-$concentrations were determined by FIA-6000 multi-channel automatic flow injection analyser (Beijing Jitian Instrument Co., Ltd.).

2.4 Total DNA extraction

The soil total genomic DNA was extracted using the Soil DNA kit (Omega Bio-Tek, Inc., Norcross, GA, USA) according to the manufacturer's instructions from 0.5 g of soil. The purity and quality of DNA were measured by 1% agarose gel electrophoresis and a spectrophotometer, respectively (Nanodrop, PeqLab, Germany).

2.5 Amplification and sequencing of the *amoA* gene for AOA and AOB

The *amoA* gene of archaea and bacteria was amplified by polymerase chain reaction (PCR) using Arch-*amoA* F (5'- STAATGGTCTGGCTTAGACG-3'), Arch-*amoA* R (5'-GCGGCCA TCCATCTGTATGT-3') and *amoA* -1F (5'-GGGGTTTCTACTGGTGGT -3') and *amoA* -2R (5'- CCCCTCKGSAAAGCCTTCTTC -3') respectively. PCR was performed with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM/μL forward and reverse primers, and 10 ng/μL DNA template. The following cycling parameter were used: incubation at 95 for 5 min, followed by 30 cycles of 95 for 30 s, 56°C and 58°C for 60 s, 72°C for 60 s and 35 s (archaea and bacteria, respectively), and a final 10 min extension at 72. The PCR product was recovered and purified by 2% agarose gel electrophoresis and GeneJET Gel Extraction kit (Thermo Fisher Scientific USA). According to the manufacturer's instructions, sequencing libraries were constructed using the Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific). The library quality was estimated with a Qubit@ 2.0 Fluorometer (Thermo Scientific), and the library was sequenced on an Ion S5TM XL platform.

2.6 Quantification of the *amoA* gene in AOA and AOB

To estimate the *amoA* functional gene for AOA and AOB, quantitative PCR (qPCR) was performed. The ammonia-oxidising archaeal*amoA* gene was quantified by fluorescence qPCR using the primers shown above (section 2.3). Absolute quantification was carried out on the Applied Biosystems QuantStudio^{TM} 6 Flex Real-Time PCR System (Life Technologies Corporation, Carls-bad, CA, USA) amplifier using Hieff$^{(r)}$ qPCR SYBE$^{(r)}$ Green Master Mix (YEASEN). The fluorescence qPCR reaction volume was 20 μl, containing 10 μl 2 × SYBR Green Mix, 0.8 μl upstream and downstream primers (10 pmol / μl), 1 μl diluted total DNA template, and 7.4 μl double distilled water. The protocol for the PCR reaction was as follows: predenaturation at 95°C for 5 min; 40 cycles at 95°C for 30 s, annealing at 56°C and 58°C for 60 s, and extension at 72°C for 60 s and 35 s (archaea and bacteria, respectively); the melting curve temperature range was 65–95°C.

2.7 Sequencing data processing and analysis

Single-end reads were assigned to samples based on their unique barcode, and Cutadapt (V1.9.1) was used for quality filtering to obtain high-quality clean reads (Martin, 2011). Reads were analysed by the Silva

database using the UCHIME algorithm to classify and delete chimera sequences (Edgar *et al.* , 2011, Haas *et al.* , 2011). The Uparse program grouped the purified sequences (Uparse v7.0.1001). The same operational taxonomic units (OTUs) were assigned sequences with 97% identity (Edgar, 2013). The Mothur algorithm (Yang *et al.* , 2014) was used to perform annotated OTU analysis to obtain representative species and count the number of OTUs per sample in taxonomic information (Kingdom, Phylum, Class, Order, Family, Genus, and Species level). Archaeal and bacterial OTUs were identified and assigned in the archaeal and bacterial Silva Database (Haas *et al.* , 2011). For multiple sequence alignment, MUSCLE software (Version 3.8.31) was used to examine the phylogenetic relationship between different OTUs and the classification of the dominant species in different samples (Edgar, 2004).

2.8 Phylogenetic diversity matrices analysis

The Mean Phylogenetic Distance (MPD) determined by Net Relatedness Index (NRI) for all co-occurring populations revealed 'basal dispersal' within the population, while the Net Taxon Index (NTI) measures the Mean Nearest Taxon Distance (MNTD) between populations, thus calculating the population 'terminal' phylogenetic dispersion (Webb, 2000). The MPD and MNTD standardised effect size was measured using the ses.MPD and ses.MNTD commands in the 'Picante' package of R (Kembel, 2010). The distribution of 999 null values, computed by shuffling the tip labels in the tree, was used to account for temporal changes of the species pool. ses.MPD. observed ses.MNTD. The observed values were multiplied by -1 to be equivalent to the NRI and NTI (Kembel, 2010).

NRI= -(MPDobserved–MPDrandomised)/(sdMPDrandomised) (Webb, 2000)

NTI = -(MNTDObserved - MNTDRandomised)/ (sdMNTDRandomised) (Kembel, 2010)

The beta Means Nearest Taxon Distance (βMNTD) was computed as previously described (Stegen *et al.* , 2012). The beta Nearest Taxon Index (βNTI) and standard deviation of βMNTD were calculated in R with the package MicEco by using command ses.comdistnt (abundance.weighted= True) (Zhang *et al.* , 2019). The significance was calculated using 9999 permutations with the 'ade4' package in R. Overall environmental and geographical differences were calculated as standardised Euclidean distances between samples.

2.9 Statistical analyses

Soil physicochemical properties and comparison of gene copy numbers between samples were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test by SPSS 25.0 for Windows. Discrepancies were considered statistically significant at $P < 0.05$. Soil ecosystem function (potential nitrification) correlation with environmental parameters, *amoA* gene abundance diversity and assembly processes were calculated by Spearman's correlation analysis. Alpha diversity analysis namely observed-species, Chao1, Shannon, Simpson, ACE, and good-coverage, were calculated with QIIME (Version1.7.0). Beta diversity analysis was performed on QIIME software (Version1.7.0) to evaluate the differences in community complexity among samples and to calculate the Unique Fraction (UniFrac) distance. The Unweighted pair group method with arithmetic mean (UPGMA) sample clustering was conducted in R software (Version 2.15.3). NMDS (non-metric multi-dimensional scaling) analysis and non-parametric Analysis of Similarity (ANOSIM) based on the Bray-Curtis distance for analysing compositional differences between ammonia oxidiser prokaryotic communities were performed in R software (Version 2.15.3).

Temporal variations in phylogenetic beta diversity were analysed by Permutational Multivariate Analysis of Variation (PERMANOVA) using R function ADONIS based on the Bray-Curtis distance. The effect of physical and chemical factors on ammonia-oxidising microbial communities was analysed by canonical correspondence analysis (CCA). The cca-envfit function was used to test the impact of each environmental factor on the distribution of species. Spearman rank correlation coefficient analysis was used to correlate the relationship of environmental factors and reclaimed soil stages with ammonia oxidiser microbial abundance (alpha diversity) and species distribution. We conducted correlation analyses to assess the relationship of environmental variables with NTI and NRI to examine the change in community structure by Spearman's correlation. The mechanisms of ammonia oxidiser community assembly were determined by calculating

βNTI. Briefly, if βNTI > 2 or βNTI < -2, deterministic processes may be important in shaping the community composition across all sites, whereas stochastic processes may play a significant role in community assembly processes when the values of βNTI are between -2 and 2. The βNTI > 2 revealed significantly more phylogenetic turnover than predicted, which is often interpreted by chance as variable selections, while βNTI < 2 referred to less phylogenetic turnover than expected, i.e., homogeneous selection (Stegen et al. 2013). If the measured βMNTD values did not bring clarity to significant differences from the null distribution of βMNTD, e.g. |βNTI|<2, the observed phylogenetic variability was not the consequence of selection (Hardy, 2008). To overcome this problem, the Bray-Curtis-based Raup-Crick metric (RC_{bray}) was further determined as described by (Stegen *et al.* , 2013). Briefly, the values of (RC_{bray}) ranged between -1 and 1, and we compared |βNTI|<2 and (RC_{bray}) values. The relative contribution of dispersal limitation was estimated as the percentage of pairwise comparison between |βNTI| < 2 and (RC_{bray}) values > 0.95, whereas |βNTI| < 2 and (RC_{bray}) values > -0.95 indicated homogenous dispersal. The undominated process was calculated as |βNTI| < 2 and (RC_{bray}) values > 0.95. The undominated concept described a state wherein the primary cause of variations between population compositions was neither dispersion nor selection, namely ecological drift (population sizes fluctuating due to stochastic birth and death events) (Stegen *et al.* , 2015). We applied ordinary least-squares regression analysis to determine the slope of the association among phylogenetic relatedness with environmental factors. The correlation between nitrification activity, alpha diversity and assembly processes (stochastic/deterministic processes related to beta diversity were calculated by spearman correlation. Using partial Mantel tests with Pearson's correlation coefficient and 999 permutations, the relationships between βNTI, geographical distance, and Euclidean distances in environmental parameters were analysed.

RESULTS

3.1 Soil physicochemical variables

The statistical results of physical and chemical properties of soils with different reclamation years showed in (**Table S1**). The soil pH decreased with the increase of reclamation age which showed that continuous reclamation would help to improve soil pH. Electron conductivity and AK gradually declined from 4653.83 to 131.16 μs·cm-1 and 327.66 to 108.33 mg/kg respectively after 1000 years' reclamation. Concentrations of AN and TN significantly decreased with reclamation stages. Soil organic matter proportionally increased with reclamation age and sharply increased after long term (1000 years) reclamation period.

3.2 AOA and AOB community composition

A total of 840 and 714 archaeal and bacterial OTUs, respectively, were obtained, and community' annotations were performed on the OTU sequences by comparison to the Silva132 database. The archaeal community shared OTUs (14.4%) in all soil samples (**Fig. S2A**), and coastal salt marsh soil exhibited the highest number of unique OTUs (55.8%) compared with reclaimed soils. The archaeal community had 457 annotated OTUs (54.40%). The overall proportion of annotated OTUs at the phylum level was 24.17%. The cumulative archaeal community composition is shown in (**Fig. S2B)** . The relative abundance of AOA phylum*Crenarchaeota* in coastal salt marsh soil was 19.1%, and it increased to 48.9% after five years of reclamation and then decreased (from 12.3 to 6.6% in the soil 20 and 50 years after reclamation, respectively), showing an asymmetrical distribution along with chronosequence. A linear increase in relative abundance was found with reclamation time ranging from 60 to 280 years (from 39.1 to 56.6%, respectively) and reached the highest abundance in soil reclaimed 500 years ago (63.2%). Abundance decreased in the 1000-year-old reclaimed soil (10.3%). The relative abundance of the phylum *Thaumarchaeota* decreased linearly with reclaimed years, with 27.0% in the marsh soil and 0.1% in the 1000-year reclaimed soil. The proportion of OTUs shared by ammonia-oxidising bacteria in the soil during different reclamation years was 16%, with the lowest unique OTUs in T500 (0.5%) and the highest in T0 (17%) (**Fig. S2C**). The abundance of Proteobacteria, the most predominant bacterial phylum across all soil samples (19.1%), was detected, and it was the highest (61%) in the salt marsh and decreased with the number of reclaimed years (from 42% in 5-year reclaimed soil to 3% in 1000-year reclaimed soil). The relative abundance of *Nitrosospira* and*Nitrosomonas* was 5 and 3%, respectively, in 5-year reclaimed soil and decreased at later reclamation stages **(Fig. S2D)** .

3.3 The quantification archaeal and bacterial amoA gene abundance

To quantify the number of*amoA* gene copies in archaeal and bacterial communities across all reclaimed soils, quantitative PCR was used (**Fig. 1A**). Reactions were performed in triplicate for all soils, with an efficiency of 92% and 96% for archaeal and bacterial *amoA* genes, respectively (r^2 = 0.99). Archaeal *amoA* gene abundance increased progressively with reclaimed time (from marsh to 1000-year reclaimed soil). Bacterial *amoA* gene abundance showed a similar trend along reclamation time. In the reclaimed soils, both archaeal and bacterial *aomA* gene abundance was greater than that in the marsh and youngest reclaimed soil. In addition, Pearson's correlation analysis showed that the abundance of the archaeal *amoA* gene was negatively correlated with pH, electron conductivity (EC), and available potassium (AK) and positively correlated with soil organic matter (SOM), available nitrogen (AN), total nitrogen (TN), and reclamation years (**Table S2**). Bacterial *aomA* gene abundance was positively associated with AP and negatively associated with EC.

3.4 Taxonomic and phylogenetic alpha diversity analysis

The archaeal and bacterial diversity and richness indices showed different trends among reclaimed soils. Alpha diversity (OTUs, Chao1, Shannon, Simpson, Ace, and Goods coverage) of archaeal and bacterial*amoA* genes is listed in **(Table 1)** . Considerable variation in AOB diversity and richness was found among all reclaimed soils. From the Shannon index, the highest diversity was found in reclaimed soils at 20–280 years ($P < 0.05$). The lowest AOB diversity was found in T0, T5, and T1000. From the ACE index, AOB richness was significantly higher in T120, T200, and T220, which decreased sharply in T1000. The archaeal diversity indices of Shannon and Simpson decreased from the coastal salt marsh to early reclaimed soil (5-50 years), while another climax was observed 220 years after reclamation, followed by a decrease in long-term reclamation. The richness indices decreased in reclaimed soils compared with the salt marsh soil.

Faith's phylogenetic diversity (PD) values of ammonia oxidiser communities generally decreased along with soil chronosequence. To determine whether the AOA and AOB community structure assembled via stochastic or deterministic processes among reclaimed soil, the NRI and NTI were calculated. Based on the NRI and NTI values, we found that AOA and AOB NRI and NTI values were above 2 in all reclaimed soils, except for the AOA community (NTI < 2) 50 years after soil reclamation and the AOB community (NTI > -2) in marsh soil (**Fig. S3**). Most of the NRI and NTI values showed that populations of co-occurring ammonia oxidisers were more phylogenetically related than predicted by chance. In addition, in both communities, we correlated NTI and NRI values with environmental variables, including AN and TN, which were not significant. AOA NTI and NRI values were significantly and positively associated with pH ($P < 0.01$) and were not correlated negatively with NO_3^--N ($P < 0.01$) EC and AK (**Fig. S4**). AOB NTI and NRI values were negatively associated with EC and AK, whereas other environmental factors were not significantly associated.

3.5 Taxonomic and phylogenetic beta diversity analysis

Distribution of the AOA and AOB community were distinct in later reclamation stages based on NMDS analysis (**Fig. 2**). The composition of ammonia oxidisers in the coastal salt marsh was significantly different from all reclaimed soils. Similarly, AOA and AOB communities 5 and 1000 years after soil reclamation also separated from other reclaimed soils. ANOSIM analysis revealed significant differences in AOA and AOB community composition (**Table S3**). The AOA community showed non-significant variations between T120 and T200 (R = 0.183, P = 0.077), T20 and T50 (R = -0.0260, P = 0.571), T60 and T120 (R = 0.067, P = 0.206), and T280 and T500 (R= -0.022, P = 0.546), indicating that temporal variations in the AOA community were noted with a long reclamation time. The community structures of AOB were similar after 5–120 years of soil reclamation. The community structure of AOB was not significant between T20 and T60 (R = 0.098, P = 0.111), T120 and T200 (R = 0.107, P = 0.188), T60 and T120 (R= 0.007, P = 0.444), and T20 and T50 (R= 0.119, P = 0.157). The distance-based community dissimilarity (Weighted UniFrac distance) and βMNTD were used to measure the dissimilarity between different reclaimed soils. The weighted UniFrac values were greater than βMNTD, and both analyses showed that dissimilarity increased with the number of reclamation years (**Fig. S5**).

3.6 Quantitative analysis of the ammonia-oxidising community assembly process

The ammonia oxidiser community assembly process was calculated by the βNTI and RC_{bray} to reveal whether community assemblage mechanisms could explain the assembly process of ammonia oxidisers. By counting the deviations of phylogenetic turnover, we found that AOA and AOB community assembly mechanisms showed that deterministic processes were dominant (84.71 and 55.2%, respectively) with βNTI were greater than 2 or less than -2. The stochastic process was secondary (15.29 and 44.80%, respectively) with βNTI values between 2 and -2 (**Fig. 3**). Variable selection contributed a larger fraction to the ammonia-oxidising community, followed by dispersal limitations. Furthermore, at each reclamation time, the AOB community in marsh soil and 60 years of reclaimed soil were assembled by stochastic processes (79.50 and 50.50%, respectively), which were influenced by dispersal limitations **(Table 2)** . Dispersal limitations and ecological drift (undominated processes) were secondary variables in the AOA community assembly process.

3.7 Spatiotemporal variations in phylogenetic beta diversity and community composition

The spatiotemporal variations in ammonia oxidiser community composition and phylogenetic beta diversity were analysed by weighted UniFrac dissimilarity, βMNTD, βNTI, and PERMANOVA based on ADONIS the Bray-Curtis distance. The PERMANOVA results showed that there were significant variations in archaeal and bacterial communities between salt marsh and reclaimed soils ($P < 0.05$) (**Table S4**). Between undisturbed salt marsh and reclaimed soils, the most considerable variations in community structure were noted ($F = 26.8$, $R^2 = 0.73$, $P < 0.001$). Significant differences among reclaimed soils were found in AOA and AOB communities, while the maximum variation was noted in soils 50–500 years after reclamation ($F = 73.5$, $R^2 = 0.88$ and $F = 14.0$, $R^2 = 0.58$, respectively). AOA and AOB communities showed non-significant variations in reclaimed soils between adjacent close reclamation years ($P > 0.05$). Temporal variations were detected 0–1000 years after soil reclamation, indicating significant changes in the relative OTU composition. The weighted UPGMA cluster analysis indicated significant phylogenetic turnover in ammonia-oxidising prokaryotic community composition (**Fig. S6A, B**). Phylogenetic turnover at the phyla level in the AOA community from the coastal salt marsh to long-term reclaimed soil was associated with shifts in beta diversity composition, and AOB community composition was not correlated with phylogenetic turnover. Based on βMNTD, we found that the dissimilarity of AOA and AOB communities increased with geographic distance and environmental variables (**Fig. 4**). To validate whether different geographic distance-controlled community assembly, we calculated the correlation between βNTI values and spatial distance (**Table S5**). The results indicated that the deterministic process of geographic distance was greater than that of an environmental variable ($r = 0.172$, $P = 0.001$ and $r = 0.119$, $P = 0.005$) in the process of AOA community assembly. In contrast, the AOB community was partially negatively correlated with environmental variables and geographic distance.

3.8 Quantifying the roles of environmental variables and geographical distance in an ammonia oxidiser community

To reveal the relationships between environmental variables and AOA/AOB groups, canonical correspondence analysis (CCA) was conducted. The environmental variables (TN, SOM, AN, NO_3^--N, available phosphorus (AP,) EC, pH, AK, and NH_4^+-N) were selected based on their variance inflation factors (VIFs). They explained 57.4% and 58.8% of the variance in AOA and AOB, respectively (**Fig. 5A, B**). Variance partition analysis (VPA) revealed that the relative influence of environmental and spatial parameters on the composition of AOA and AOB communities was 55.43% and 42.55% included geographic distance (7.25% and 7.07% respectively). The community composition of AOA influenced by NH_4^+-N and AOB influenced by EC (3.66% and 6.77% respectively) (**Fig. 5C, D**). Spearman correlation coefficient analysis was used to correlate AOA and AOB phyla with environmental variables. Archaeal phylum Thaumarchaeota positively associated with pH and EC and negatively correlated with AP and NO_3^--N. Furthermore, phylum Crenarchaeota was significantly and positively associated with TN and negatively associated with NO_3^--N and AK (**Table S6**). The bacterial phylum Proteobacteria showed a positive correlation with NH_4^+-N, pH, EC, and AK ($P < 0.001$) and a negative association with SOM, TN, and AN ($P < 0.001$). The βNTI values were also correlated with environmental variables, indicating that AOA βNTI was positively correlated with

EC, AK, AP, NH_4^+-N, and NO_3^--N and negatively correlated pH. The AOB βNTI values were negatively associated with EC and AK and positively correlated with pH, AN, AP, and NH_4^+-N (**Fig. S7**).

3.9 Potential Nitrification rate and ammonia oxidiser community assembly processes

To assess how reclamation of coastal marshes affect the potential nitrification rate, the nitrification rate was calculated. Nitrification activities among reclaimed cultivated soils shown in (**Fig. S8**) indicated temporal effects over potential nitrification rate which increased from costal salt marsh soil to early cultivation at stage 5 (from 14% to 20%). Potential nitrification rate increased progressively along reclamation stages and maximum nitrification rate noted in reclamation stage 220 years. The nitrification rate was positively correlated with archaeal *aomA* gene abundance ($P < 0.01$). Furthermore, bacterial *amoA* gene abundance was not associated with nitrification rate (**Fig. 6A,B**).

Further we performed Pearson correlation analysis to assess the role of quantified soil physiochemical parameters on potential nitrification (**Table S7**). The nitrification rate was significantly positively correlated with reclamation age, SOM, AN and TN. Potential nitrification rate was negatively correlated with pH, EC and AK concentrations. To further investigate the impact of ammonia oxidisers diversity and assembly deterministic/stochastic assemblages on potential nitrification rate, we calculated the correlation between alpha diversity Shannon index, |βNTI| values and potential nitrification rate. AOB Shannon diversity were positively correlated with potential nitrification rate while negatively correlated with |βNTI| values. In contrast the AOA Shannon diversity negatively and |βNTI| values were positively correlated with potential nitrification rate**(Fig. 6)** . Therefore, AOA and AOB differently response to diversity change, assembly processes and nitrification activity.

DISCUSSION

4.1 Succession of an ammonia-oxidising prokaryotic community along a coastal soil chronosequence

Communities change in an orderly manner with time in a particular assumed environment, which is defined as succession (Begon *et al.* , 1996). Macro-ecologists have shifted their attention from standard vegetation description to the study of community dynamics, while micro-ecologists are still unable to develop a well-established framework to address microbes in successional environments (Fierer*et al.* , 2010). Several studies have shown that the composition and distribution of microbial communities (fungal, archaeal, and bacterial) changes with temporal variations (Anderson *et al.* , 2008, Dimitriu *et al.* , 2010, Li *et al.* , 2014). Salt marshes are particularly active ecosystems, and apart from their environmental significance, few studies have discussed the primary succession of microbial communities in these habitats to investigate the trends and processes at the phylogenetic level that drive archaeal, bacterial, and fungal assembly dynamics (Bowen *et al.* , 2009b, Keith-Roach *et al.* , 2002, Liu *et al.* , 2020, Salles*et al.* , 2017). Although much of our understanding of the assembly and dynamics of the microbial community depends on taxonomy-based evaluations (i.e., based on the 16S rRNA gene), less focus has been devoted to the distribution in natural systems of functional genes. In the present study, we investigated the successional patterns AOA and AOB communities in an undisturbed salt marsh and reclaimed cultivated soil spanning 1000 years of ecosystem development, providing a unique and dynamic landscape to study the pattern of functional microbial communities. Some studies indicated that the archaeal *amoA* gene is more abundant than that of bacteria in marine and terrestrial environments (Leininger *et al.* , 2006b, Mincer *et al.* , 2007, Shen *et al.* , 2008). Conversely, mounting evidence from various oceans and coasts has shown that the abundance of bacterial*amoA* genes in some regions is higher than that of archaeal*amoA* (Caffrey *et al.* , 2007, Mosier & Francis, 2008, Santoro *et al.* , 2008). We showed that the archaeal *amoA*gene was more abundant in an undisturbed coastal salt marsh than the bacterial *amoA* . Along with reclamation years, archaeal*amoA* gene abundance significantly increased, while bacterial*amoA* gene abundance was asymmetrical and comparatively lower than that of archaea (**Fig. 2**). These findings suggested that the AOA*amoA* gene dominated not only costal marshes but also in reclaimed soil.

Marine N cycling by AOA and AOB can be affected by several factors, including physiochemical variables and

external nutrient availability (Bowen *et al.* , 2009a). Here, we also found that abiotic parameters, including EC, pH, NH^+_4-N, NO^-_3-N, and SOM, had significant effects on the composition and diversity of ammonia oxidiser prokaryotic communities in reclaimed soils, which is an agreement with previous publications. For example, the availability of oxygen (Gleeson *et al.* , 2010), phosphorus (Herfort *et al.* , 2007), pH (Li *et al.* , 2015), soil type (Huang *et al.* , 2012), NH^+_4-N, and NO^-_3-N (Li *et al.* , 2012) have been identified as essential parameters influencing the distribution and variety of ammonia-oxidising organisms in various habitats (Behrendt*et al.* , 2017). Previous studies indicated that AOA richness dominated in acidic soil, and AOB dominated in neutral or alkaline soil (Nicol *et al.* , 2008). The current study revealed that soil pH and ammonia-oxidising prokaryotic community (AOA and AOB) richness continuously decreased with the succession of the soil, indicating that soil pH plays a vital role in ammonia-oxidising microbial composition. The changes in SOM, TN, and AN concentration in the soil environment were closely associated with dissimilarities in AOB community composition (Yang *et al.* , 2020). It was previously shown that AOA was more abundant in soil with lower concentrations of AN, while AOB increased correspondingly (Jia & Conrad, 2009). Our study showed that AN and SOM was significantly correlated with the AOB community in reclaimed soils. Moreover, the relative abundance of archaeal phylum Thaumarchaeota was positively correlated with EC and pH, while Crenarchaeota was significantly and positively correlated with TN. The bacterial *amoA* gene harboured in phylum Proteobacteria was positively correlated with pH, EC, AK, and NH_4^+-N and negatively correlated with TN, AN, and SOM concentration. Interestingly, both ammonia oxidiser communities responded differently to the number of reclamation years; therefore, both communities occupied separate ecological niches (Brankatschk *et al.* , 2011).

Our results suggest that differences in phylogenetic beta diversity of ammonia oxidiser communities could be described by temporal variability in nutrient availability (NH_4^+-N, SOM, AP, and AN), as well as immigration of microbial input (uncultured bacterial species and uncultured prokaryotic accumulation), and the amplitude of variation in environmental factors (EC and pH) from coastal salt marshes to reclaimed cultivated soils. Soil buffering capacity and crops became more dominant as succession proceeded, which possibly reduced the amplitude of variations, resulting in the reduction of phylogenetic turnover in AOB (**Fig. 5**). These temporal effects were further correlated with edaphic factors from coastal salt marshes to long-term reclaimed soils, indicating that the AOA community was dominantly driven by environmental variables compared to the AOB community (**Fig. S4**). Archaeal and bacterial richness continuously decreased from coastal salt marshes to long-term reclaimed cultivated soils, while the accumulation of uncultured bacteria species was observed at later reclamation stages. Phylogenetic diverse ammonia oxidiser taxa were observed at later stages than at primary reclamation stages; therefore, our study supports 'the theorem of diversity begets diversity' (Whittaker, 1972), which assumes community evolution towards species complexity.

4.2 Deterministic processes determine the assembly of ammonia oxidiser communities

Assembly processes that form the community's structure have recently been of considerable interest (Chase, 2010, Zhou & Ning, 2017a). It has previously been stated that stochasticity has been reduced across successive phases (Dini-Andreote *et al.* , 2015b, Tripathi *et al.* , 2018), but there was no improvement in the assembly mechanism during succession based on the time scale. According to niche-based theory, deterministic factors, including species traits, interspecies interactions, and environmental conditions, govern community structure (Chesson, 2000). Our study showed that AOA and AOB communities were assembled by deterministic process (84.71 and 55.2%, respectively), as well as reclamation of coastal marshes. Our results are contradictory with previously reported studies; the pattern of increasing deterministic process might be due to long-term reclamation of coastal salt marshes over 1000 years, while previous studies demonstrated that short successional stages (more than one century) (Dini-Andreote*et al.* , 2015b, Liang *et al.* , 2020), artificial soil disturbance, and cropping system also influence the deterministic process (Jiang & Patel, 2008). A study reported that spatial distance had a significant role in the soil bacterial community (Jiang & Patel, 2008), and our study also revealed that spatial distance (geographic and environmental) was significantly correlated with AOA and AOB community structure. In our study, the AOA community was positively correlated with geographic and environmental distance, while the AOB community was negatively associated with environmental distance and partially correlated with geographic distance. The

unmeasured environmental variables also affected phylogenetic community assembly. Previous studies have shown that unmeasured environmental factors have an intensifying impact on deterministic processes that suppress stochastic processes (Wang *et al.* , 2013). Our AOB community results showed that phylogenetic turnover is associated with geographical distance instead of environmental variables; therefore, deterministic processes are dominant compared to stochastic processes, which supports the argument given by (Stegen & Hurlbert, 2011). Other studies have shown that as geographic distance increases, if phylogenetic turnover also increases, it will significantly influence deterministic processes (Wang*et al.* , 2013). In this study, the heterogeneous community selection of AOA was strongly associated with geographical distance. βNTI correlations with environmental variables indicated that EC and AK were negatively related to the AOB community, while AOA community was partially negatively correlated with pH.

4.3 Spatiotemporal variations in nitrification rate and its linkage with environmental variables and diversity changes

Nitrification rate is fundamental indicators for soil to supply nitrogen for plant growth and nitrogen transformation in the environment (Neill*et al.* , 1995). Previous studies indicated that ammonia oxidation was higher in acidic soils with AOA, while bacterial ammonia oxidation was higher in neutral or alkaline soils (Erguder *et al.* , 2009, Lehtovirta *et al.* , 2009, Leininger *et al.* , 2006a). We found that pH decreased from alkaline to neutral with the reclamation of coastal salt marshes, and the relative abundance of the *amoA* gene and nitrification rate was higher in archaea compared to the bacterial community. In addition to soil pH, the availability of nutritional gradients SOM and TN played important roles in the nitrification rate (Dai *et al.* , 2018, Yao *et al.* , 2011, Zhou *et al.* , 2015). The cultivation of crops and utilisation of fertiliser increased soil organic matter and total nitrogen, and a similar trend was observed in our study. These results suggesting that land management practices, i.e., reclamation of salt marshes to cultivated soil increased soil organic matter and total nitrogen. Another study indicated that the ammonium concentration is the key factor determining the relative contribution of AOB and AOA in nitrification in agricultural soils (Ouyang *et al.* , 2017). Our study presented contradictory findings that ammonium concentration was not correlated with nitrification rate (**Table S1**). Interestingly, ammonium concentration was also not associated with AOA community distribution and *amoA* gene abundance (**Table S1 & S5**), which might be due to the potential nitrification rate that is also not correlated to ammonium concentration. The gene abundance can reflect functional ability while the functional gene abundance is a significant predictor of functional redundancy (Xun *et al.* , 2019). Previous studies indicated that loss in soil microbial diversity significantly affect specialised functional capacity such as potential nitrification and denitrification activity (Philippot *et al.* , 2013). Our findings also suggesting that AOA gene abundance increased significantly along with reclamation stages and was positively correlated with nitrification rate**(Fig.2)** . The relationship between reductions in functional gene diversity and the destruction of ecosystem structure and service has been extensively studied using α-diversity but particularly lacking on β -diversity (Mori *et al.* , 2018). We observed significant negative correlation between AOA taxonomic α-diversity and nitrification rate while contrast correlation with AOB βNTI values taxonomic α-diversity. The phylogenetic βNTI values of AOA and AOB were correlated positively and negatively respectively with nitrification rate. The Our α-diversity and phylogenetic β -diversity contradictory findings with nitrification rate support previous studies that taxonomic diversity has relatively little impact on ecosystem functioning than phylogenetic or functional diversity(Hooper *et al.* , 2005, Krause *et al.* , 2014, Nielsen *et al.* , 2011).

CONCLUSION

We investigated temporal and spatial variations in an ammonia oxidiser community from a coastal salt marsh (0 years) and long-term (1000 years) reclaimed soils and observed the heterogenicity of diversity, composition, and phylogenetic structure of AOA and AOB communities. During the 1000 years of reclamation, there was a clear evolution of the*amoA* gene harboured in AOA and AOB communities. The soil ecosystem was significantly destroyed by the reclamation of coastal wetlands, which also altered soil physicochemical parameters. The major soil physicochemical parameter changes included a decrease in pH, EC, and NH_4^+-N and increase of SOM, AN, and TN. A decrease in substrate concentration and pH subsequently led

to a decrease in ammonia oxidiser prokaryotic communities. We investigated the relative importance of stochastic and deterministic processes in nitrification rate and shaping AOA and AOB communities. In the assembly processes of ammonia oxidiser groups, deterministic processes were dominant over stochastic processes. Our results indicated that reclamation stages affect assembly processes and significantly alter diversity pattern of ammonia oxidisers. These findings suggesting that reclamation from coastal salt marshes to cultivated soil and deterministic assembly processes increased the soil nitrification activity. These findings provide a better understanding of how soil N cycling, reclamation of coastal salt marshes, long-term land management, and cultivation of crops affect ammonia-oxidising communities, including assembly dynamics, *amoA* gene abundance, and community distribution patterns. More research into biodiversity will strengthen our knowledge of collective assembly mechanisms and the effects of community functions, which will be critical to ensuring ecosystem sustainability.

List of Abbreviations

AOA: Ammonia Oxidising Archaea

AOB: Ammonia Oxidising Bacteria

FAO/UNESCO: Food and Agriculture Organization/United Nations Educational, Scientific and Cultural Organization

N: Nitrogen

PCR: Polymerase Chain Reaction

qPCR: quantitative Polymerase Chain Reaction

OTUs: Operational Taxonomic Unit

EC: Electron Conductivity

AK: Available Potassium

SOM: Soil Organic Matter

AN: Available Nitrogen

TN: Total Nitrogen

AP: Available Phosphorous

PD: Faith Phylogene1c Diversity

VIFs: Variance Inflation Factors

VPA: Variance Partition Analysis

MPD: Mean Phylogenetic Distance

NRI: Net Relatedness Index

MNTD: Mean Nearest Taxon Distance

NTI: Net Taxon Index

βMNTD: beta Mean Nearest Taxon Distance

βNTI: beta Net Taxon Index

ANOVA: Analysis Of Variance

NMDS: Non-metric Multi-Dimensional Scaling

PERMANOVA: Permutational Multivariate Analysis Of Variation

CCA: Canonical Correspondence Analysis

RCbray: Bray-Curtis-based Raup-Crick metric

ANOSIM: Analysis of Similarity

UPGMA: Unweighted pair group method with arithmetic mean

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data supporting the findings of this study are available on request from the corresponding authors (Hui Cao, Feng Wang).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

S.H. and Y.Y. were major contributors in manuscript writing, analysed sequence data and prepared figures and/or tables. S.L. contributed in reagents/material/analysis. S.Y. analysed the physiochemical parameters. D.C. contributed in sample collection and performed qPCR. C.H. and W.F. conceived and developed the original framework, reviewed drafts of the paper, approved the final draft. All authors read and approved the final manuscript.

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Table Titles

Table. 1. The alpha diversity of AOA and AOB community along soil chronosequence.

Table. 2. The relative importance (%) of each assembly process that governs the turnover of ammonia oxidising community.

Figure Legends

Figure 1. Histogram of quantitative archaeal and bacterial*aomA* gene abundance per gram dry soil. Bars labelled with different superscript letters indicate a significant difference (Duncan's test, $P < 0.05$) among reclamation stages.

Figure 2. Bray-Curtis distance-based non-metric multi-dimensional scaling plot at the OTU level in reclaimed soils.**(A)** AOA community, **(B)** AOB community.

Figure 3. Boxplot of βNTI values across reclamation years. Solid lines inside the box represent median values. Scatter plot distribution based on Mantel test. Spearman's correlation r shows the relationship of βNTI values of AOA and AOB communities with geographic distance and environmental variables (Euclid-based distance).

Figure 4. Boxplot indicating phylogenetic dissimilarity based on βMNTD at temporal scales. Different letters above boxes indicate significant differences (Duncan's test, $P < 0.05$). Scatter plots indicate the correlation between βMNTD values and spatial parameters (geographical distance and environmental parameters). Spearman correlation (r) and probability values are provided.

Figure 5. Relationship between environmental variables and microbial community structures. The length of the arrow represents the degree of correlation between environmental variables and community structures: **(A)** archaeal community, **(B)** bacterial community. The quantitative contribution of environmental and spatial parameters based on variance partition analysis indicated the percentage of individual parameter variance on community structures of ammonia oxidizers: **(C)** archaeal community, **(D)** bacterial community.

Figure 6. The relationship between taxonomic and phylogenetic diversity and nitrification rate. **A** correlation analysis of archaeal *amoA* gene abundance with nitrification rate, **B**correlation between bacterial *amoA* gene abundance and nitrification rate. **C** ammonia oxidiser archaeal correlation between taxonomic alpha diversity and nitrification rate. **D** AOB correlation with taxonomic alpha diversity and nitrification

rate bacterial community. **E** |βNTI| values of AOA correlation with Nitrification rate. **F** AOB |βNTI| values with nitrification rate. The Spearman correlation denoted by (r), and probability values (P) are provided.

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