Increasing Inundation Frequencies Enhance the Stochastic Process and Network Complexity of the Soil Archaeal Community in Coastal Wetlands

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ABSTRACT Coastal wetlands are experiencing frequent flooding because of global climate changes, such as the rising sea level. Despite the key role of archaea in soil biogeochemical cycles, the assembly processes and co-occurrence patterns of archaeal communities in coastal wetlands in response to increasing inundation frequencies remain elusive. In this study, we established an *in situ* mesocosm with an inundation frequency gradient to investigate the response of soil archaeal community toward increasing inundation frequencies in monocultures of *Spartina alterniflora* and a mangrove species, *Kandelia obovata*. Both neutral community model and null model analyses suggested that stochastic processes are dominant in governing the archaeal community assembly and that the stochastic processes are enhanced with increasing inundation frequencies. Increasing inundation frequencies significantly increased the community niche width. Moreover, archaeal community in *S. alterniflora* soil displayed lower niche overlap and higher stochasticity than in *K. obovata* soil. Co-occurrence network analysis revealed that the network complexity increases with increase in the inundation frequencies. Soil water content is the most decisive factor influencing the archaeal communities. Overall, we found that increasing inundation frequencies enhance the stochastic processes and network complexity of the soil archaeal community in coastal wetlands. This study could enhance our understanding on the response of soil archaeal communities in coastal wetlands toward global change.

IMPORTANCE Coastal wetlands, subjected to regular disturbances by periodic tides, are highly productive and important in the regulation of climate change. However, the assembly mechanisms and co-occurrence patterns of soil archaeal communities in coastal areas remain poorly known, especially for their responses to increasing inundation frequencies. In this study, we aimed at unraveling these uncertainties by studying typical estuarine ecosystems in southern China. We show that increasing inundation frequencies enhance the stochastic processes and network complexity of the soil archaeal community. This study offers a new path for an improved understanding of archaeal community assembly and species coexistence in coastal environments, with a special focus on the role of inundation frequency.

KEYWORDS archaea, assembly processes, co-occurrence network, coastal wetlands, inundation frequency gradients

Archaea play an important role in mediating soil biogeochemical cycles (1–3). Thus, investigating the role of soil archaeal community is of great ecological importance and has received considerable attention (4–6). Disentangling the assembly processes and species coexistence patterns in diverse microbial communities is a central goal in microbial ecology (7–9). The proposed species coexistence theories are divided into 2

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categories, the niche-based theory and neutral theory; the former emphasizes the differences in the ecological strategy of co-occurring species, and the latter is based on the dispersal and stochastic processes (10–13). Deciphering the contributions of deterministic and stochastic processes to the microbial community assembly could elucidate the ecological strategies of co-occurring species (13, 14). Knowledge of these processes could advance our understanding on the fundamental mechanisms of establishment and maintenance of microbial diversity and allow us to decipher the responses of microbial communities to environmental changes (8, 9, 15–17). Assembly processes (18–20) and co-occurrence patterns (21–23) of soil archaeal communities across various ecosystems have been extensively studied. For instance, Juottonen et al. found that the archaeal communities in boreal Swedish lakes are dominated by deterministic processes (20). Conversely, the soil archaeal communities in tropical rainforest in Brunei Darussalam were shown to be mainly governed by stochastic processes (18). These controversial findings indicate that archaeal community assembly processes could vary across different ecosystems. To date, our understanding on the assembly processes and co-occurrence patterns of the archaeal community in coastal wetlands is inadequate. We speculate that the assembly processes and co-occurrence patterns of archaeal community in coastal wetlands are distinct from those in other terrestrial ecosystems or water bodies because coastal areas are subjected to long-term anaerobic-aerobic fluctuations and high-salinity environments.

The ongoing climate change is expected to continue accelerating the rising rate of the global mean sea level in the near future (24, 25). Due to the continuing circumstance, coastal wetlands are experiencing more frequent flooding. Growing evidence suggests that prolonged inundation frequencies significantly impact the soil microbial communities in coastal wetlands (26–30). For example, Chambers et al. found that the microbial community structure of brackish soil in Florida Bay is more strongly influenced by increases in inundation rather than by salinity (26). In addition, Franklin et al. revealed that both the diversity and composition of bacterial community in Phillips Creek salt marsh in Virginia vary with increasing inundation frequencies (29). However, despite the important role of archaea in driving ecological functions, we still know less about the response of the archaeal community in coastal wetlands to increasing inundation frequencies. This knowledge gap impedes our ability to accurately assess the change in coastal wetlands under rising sea level scenarios. In a previous study, we found that increasing inundation frequencies enhance the deterministic processes that dominant the bacterial community assembly and reduce the network complexity of bacteria in a *Spartina alterniflora* salt marsh (30). Considering the large differences between bacteria and archaea in terms of environmental adaptations, cellular structure, and cellular metabolisms (31–33), we question whether the responses of soil archaeal and bacterial communities to increasing inundation frequencies in coastal wetlands are consistent.

The mangrove ecosystem, located in the intertidal zone of tropical and subtropical areas, is an important component of coastal wetlands and plays a key role in climate regulation (34). *S. alterniflora*, which is native to North America, was first introduced to China for ecological engineering in 1979 (35). Since then, *S. alterniflora* has spread vastly along most of the coastlines in China (35–37). Currently, the native mangroves and *S. alterniflora* can be commonly found coexisting in single habitats in southern China (36). The target site of our study, Zhangjiang River Estuary, is the largest concentrated area and the northernmost region of natural mangrove forests in China, with the distribution area of 167 ha (38). Over the span of a decade, the coverage of *S. alterniflora* has enlarged from 57.94 ha to 116.11 ha in this region (38). Extensive studies have revealed that *S. alterniflora* invasion into mangroves in China has strong impacts on the soil microbial communities and their functions (39–41). Although we have deciphered the response of the soil bacterial community to increasing inundation frequencies in an *S. alterniflora* ecosystem (30), we still know less about differences in the response of the soil archaeal community to increasing inundation frequencies between mangroves and *S. alterniflora* salt marshes. This understanding would help us to better

FIG 1 (A) Principal-coordinate analysis (PCoA) depicts the weighted UniFrac distance of archaeal communities among soils along inundation frequency gradients; (B) variance partitioning analysis (VPA) shows the independent and shared contributions of plant species and soil physiochemical properties to the archaeal community structure; (C) aggregated boosted tree (ABT) analysis shows the relative influences of each soil physiochemical property on the archaeal community structure. TN, total nitrogen; TC, total carbon; KO, *Kandelia obovata*; SA, *Spartina alterniflora*; n, number of samples.

assess the ecological consequences of *S. alterniflora* invasion in the context of rising sea levels.

To address the aforementioned uncertainties, we established an *in situ* mesocosm with inundation frequency gradients and planted with monocultures of *S. alterniflora* and a mangrove species, *Kandelia obovata*, in Zhangjiang River Estuary. Subsequently, we validated the main findings of the mesocosm experiment by assessing *S. alterniflora* soils distributed in natural habitats experiencing different inundation frequencies. We aimed to answer the following questions. (i) How do the assembly processes and co-occurrence patterns of archaeal community change in response to increasing inundation frequencies? (ii) What are the differences in the archaeal community assembly and co-occurrence network between *K. obovata* and *S. alterniflora* soils in response to increasing inundation frequencies? Overall, this study could advance our understanding on the response of archaeal communities in coastal wetlands to increasing inundation frequencies.

RESULTS

Increasing inundation frequencies strongly influenced the soil archaeal community.

K. obovata grew best under an inundation frequency between 7.73% and 13.90%, and *S. alterniflora* performed the best under an inundation frequency between 21.40% and 29.20% (see Fig. S3 in the supplemental material). The results of pairwise permutational multivariate analysis of variance (PERMANOVA) indicated significant ($P < 0.05$) differences in archaeal communities between different elevations of the mesocosm for both *K. obovata* and *S. alterniflora* soils (Tables S3 and S4). Soils at different elevations displayed high numbers of unique amplicon sequence variants (ASVs) (Fig. S4). To identify the main factors influencing archaeal communities, we measured 17 soil physicochemical properties, including, e.g., soil water content, salinity, and pH (Tables S5 and S6 and Fig. S5 and S6). Principal-coordinate analysis (PCoA) showed a clear differentiation of archaeal communities along the inundation frequency gradients in axis 1 (43.38% explanation) and axis 2 (33.36% explanation) (Fig. 1A). Variation partitioning analysis (VPA) showed that archaeal communities are primarily influenced by soil physicochemical properties rather than plant species (Fig. 1B). Aggregated boosted tree (ABT) analysis revealed a nonzero influence of all the 17 predictors on archaeal communities, and

FIG 2 (A) Relative abundances of phyla for *K. obovata* and *S. alterniflora* soils; (B) changes in the relative abundances of the phyla along with inundation frequencies for *K. obovata* and *S. alterniflora* soils. The relationships were examined by both linear and quadratic models and were selected based on the lower value of Akaike's information criterion (AIC). Solid and dashed lines indicate significant and insignificant correlations at a P value of <0.05 .

soil water content was found to be the most decisive factor (Fig. 1C). The crucial role of soil water content in influencing the archaeal communities of *K. obovata* ($r=0.48$; $P<0.001$) and *S. alterniflora* ($r=0.53$; $P<0.001$) soil was further confirmed by Mantel tests (Table S7).

We found significant distance-decay relationships of archaeal communities for both *K. obovata* and *S. alterniflora* soils, with no significant difference between them ($P=0.15$) (Fig. S7A and B). With increasing inundation frequencies, the within-group dissimilarities of *S. alterniflora* soil significantly decreased ($R^2_{\text{adj}}=0.05$; $P<0.01$), but this trend was not found in *K. obovata* soil (Fig. S8). Beta diversity partitioning revealed that Repl is dominant in archaeal beta diversity in both *K. obovata* (72.91%) and *S. alterniflora* (73.66%) soils (Fig. S7C). In addition, the contribution of Repl to beta diversity increased with increase in the degree of change in inundation frequency (Fig. S7C). Next, we performed 50-fold cross-validation to identify archaeal biomarkers that are strongly associated with changes in inundation frequency, which included communities such as *Woesearchaeia*, *Halobacteria*, *Thermoplasmata*, Marine Benthic Group A, *Micrarchaeia*, and others (Fig. S9). *S. alterniflora* soil had significantly higher relative abundances of *Nitrososphaeria* and *Heimdallarchaeia* ($P<0.05$) than those of *K. obovata* soil, whereas it displayed significantly lower relative abundances of *Bathyarchaeia*, *Lokiarchaeia*, and *Nanohaloarchaeia* ($P<0.05$) than those of *K. obovata* soil (Fig. S10).

The topmost 5 abundant phyla were *Thaumarchaeota* (46.66%), *Crenarchaeota* (33.06%), *Euryarchaeota* (10.44%), *Asgardaeota* (8.43%), and *Diapherotrites* (0.30%) (Fig. 2A). We used both the linear and quadratic models to assess the relationship between inundation frequency and the relative abundances of the dominant phyla. Results showed an inconsistent response of different archaeal populations to changes in the inundation frequencies, with responses being hump shaped in some (e.g., *Diapherotrites*), linear in some (e.g., *Thaumarchaeota*), and nonsignificant ($P>0.05$) in some taxa (e.g., *Asgardaeota*) (Fig. 2B).

Two-way analysis of variance indicated that inundation frequency significantly influenced both the observed operational taxonomic units (OTUs) and Faith's phylogenetic diversity (PD) ($P<0.001$), whereas plant species mainly affected the observed OTUs ($P<0.01$) (Table S8). Spearman correlations showed that both the observed OTUs and Faith's PD were significantly correlated with multiple soil physicochemical properties in both *K. obovata* and *S. alterniflora* soils ($P<0.05$) (Table S9). These results are also supported by the classification random forest analyses, with soil water content demonstrated as the most important factor affecting alpha diversity (Fig. S11). Observed OTUs in *K. obovata* soil were relatively higher than those of *S. alterniflora* soil ($P=0.05$) (Fig. S12A). We also found significant positive correlations between inundation frequency

FIG 3 The assembly processes of soil archaeal community. (A and B) The neutral community model (NCM) quantified the contributions of neutral processes in community assembly for *K. obovata* (A) and *S. alterniflora* (B) soils. m , estimated migration rate; N , number of sequences per community; R^2 , fit of the parameter based on nonlinear least-squares fitting. (C) Relative contributions of different ecological processes in governing community assembly based on the null model. (D and E) Relationships between inundation frequency and the relative contribution of stochastic process based on the NCM (D) and null model (E), respectively. Solid and dashed lines indicate significant and insignificant correlations at a P value of <0.05 , respectively. *, $P < 0.05$; ***, $P < 0.001$.

and alpha diversity ($P < 0.001$) (Fig. S12B). The alpha diversity for both *K. obovata* and *S. alterniflora* soils exhibited a hump-shaped response to inundation frequency (Fig. S12B).

Increasing inundation frequencies enhanced stochastic processes that dominate the archaeal community assembly. In this study, both the neutral community model (NCM) and null model were applied to determine the community assembly processes. Results of all the analyses indicated that the stochastic processes dominate the archaeal community assembly (Fig. 3A to C). Specifically, archaeal communities of *K. obovata* ($R^2 = 0.61$; $P < 0.001$) and *S. alterniflora* ($R^2 = 0.66$; $P < 0.001$) soils well fitted the NCM (Fig. 3A and B). We further used the null model to delineate the relative contributions of different ecological processes toward the community assembly. Results showed that stochastic processes, mainly dispersal limitation and undominated processes, dominated the community assembly, with higher stochasticity demonstrated in *S. alterniflora* soil than in *K. obovata* soil (Fig. 3C). Mantel tests showed that the community assembly is significantly influenced by several soil physicochemical properties, of which the soil water content is the most significant factor (Table S10). The relative contribution of stochastic processes was shown to be significantly and positively correlated with inundation frequency ($P < 0.05$); however, the results obtained from the null model of *K. obovata* soil demonstrated no such correlation (Fig. 3D and E). In addition, nearest taxon index (NTI) values did not change significantly with increasing inundation frequency in either *K. obovata* ($P = 0.89$) or *S. alterniflora* ($P = 0.32$) soils (Fig. S13A). The value of betaNTI increased significantly ($P < 0.001$) with increase in the degree of change in inundation frequency (Fig. S13B).

To better understand the mechanisms of archaeal community assembly, we analyzed the niche width and niche overlap of the archaeal community (Fig. 4). The results indicate that archaeal communities in *K. obovata* (mean = 2.13) and *S. alterniflora* (mean = 1.95) soils have comparable niche widths ($P = 0.057$) (Fig. 4A). Nevertheless, the niche overlap of archaeal communities in *K. obovata* soil was significantly higher than that in *S. alterniflora* soil (0.06 versus 0.04; $P < 0.001$) (Fig. 4C). In addition, the niche width of archaeal communities increased significantly with increasing inundation

FIG 4 The differences of niche width (A) and niche overlap (C) between *K. obovata* and *S. alterniflora* soil. Significant difference was examined by Wilcoxon rank sum test. (B and D) Relationships between inundation frequency and niche width (B) and niche overlap (D). ***, $P < 0.001$.

frequency in both *K. obovata* and *S. alterniflora* soils ($P < 0.001$) (Fig. 4B). Niche overlap of *K. obovata* soil was significantly and positively correlated with inundation frequency ($P < 0.001$), while this trend was not found for *S. alterniflora* soil (Fig. 4D).

Increasing inundation frequencies enhanced the complexity of the co-occurrence network. All the 120 soil samples collected from the mesocosm were used to construct the co-occurrence network. We obtained a total of 343 nodes and 1,406 edges (Fig. S14A). The distribution of the number of degrees for individual nodes obeyed a power-law distribution ($R^2 = 0.86$; $P < 0.001$), which indicated a scale-free network and non-random distribution pattern (Fig. S14B). Within this network, we further identified key-stone species and found significant relationships between the relative abundance of keystone species and inundation frequency ($P < 0.001$), with distinct response patterns between *K. obovata* and *S. alterniflora* soils (Fig. S15 and Table S11).

Subsequently, we constructed the subnetwork for each elevation of *K. obovata* and *S. alterniflora* soils (Fig. 5A). We found that both the numbers of nodes and edges were significantly correlated with inundation frequency ($P < 0.001$) (Fig. 5B and C). We used the average degree (avgK) to characterize the network complexity. The results showed that network complexity was significantly and positively correlated with inundation frequency in both *K. obovata* ($R^2_{\text{adj}} = 0.13$; $P < 0.01$) and *S. alterniflora* ($R^2_{\text{adj}} = 0.36$; $P < 0.001$) soils (Fig. 5D). Moreover, the proportion of mutual exclusion (negative correlations) within the subnetworks was significantly and negatively correlated with inundation frequency ($P < 0.05$) (Fig. 5E). Additionally, we observed significant relationships between the relative contribution of stochastic processes and avgK ($P < 0.01$); however, the results from the null model of *K. obovata* soil demonstrated no significant relationships between the 2 parameters (Fig. S16).

Archaeal community assembly and co-occurrence pattern: evidence from natural *S. alterniflora* soil. To validate the results obtained from the mesocosm experiment, we collected *S. alterniflora* soils experiencing different inundation frequencies and used them to determine the community assembly processes and co-occurrence networks. The soil water content of natural *S. alterniflora* soils ranged from 40.30% to 59.14% (Table S1). Similar to the results obtained from the mesocosm experiment, the archaeal communities of these soils were found to exhibit a significant distance-decay relationship ($r = 0.12$; $P < 0.05$) (Fig. S17). Both NCM and null model analyses suggested that stochastic processes were dominant in the archaeal community assembly (Fig. 6A and B and Fig. S18). Moreover, the number of nodes ($R^2_{\text{adj}} = 0.08$; $P < 0.05$), number of edges ($R^2_{\text{adj}} = 0.15$; $P < 0.01$), and avgK ($R^2_{\text{adj}} = 0.34$; $P < 0.001$) were significantly correlated with soil water content (Fig. 6C to E).

FIG 5 Co-occurrence pattern of archaeal community. (A) Co-occurrence networks in each elevation for *K. obovata* and *S. alterniflora* soils. (B to E) Relationships between inundation frequency and number of nodes (B), number of edges (C), average degree (avgK) (D), and proportion of mutual exclusion (E) within the network. The relationships were examined by both linear and quadratic models and were selected based on the lower value of AIC. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

DISCUSSION

Increasing inundation frequencies enhance stochastic processes governing the archaeal community assembly. Generally, the deterministic and stochastic processes are recognized to simultaneously govern the community assemblies (7, 8). Extensive studies have been conducted to determine the balance between stochastic and deterministic processes governing archaeal community assembly across various ecosystems, namely, freshwater lake (20), forest (18, 42, 43), coastal zone (44), desert (45), and agricultural (46) ecosystems. In this study, the results of both the NCM and null model analyses suggested that unlike the deterministic process-dominated bacterial community assembly (30), soil archaeal communities are dominated by stochastic processes in

FIG 6 Assembly processes of the archaeal community in soils distributed in natural habitats. (A) The NCM quantified the contributions of neutral processes in community assembly. (B) Relative contributions of different ecological processes in governing community assembly based on the null model. (C to E) Relationships between soil water content and number of nodes (C), number of edges (D), and avgK (E). The relationships were examined by both linear and quadratic models and were selected based on the lower value of AIC. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

coastal wetlands (Fig. 3 and 6). This might be due to differences in environmental adaptations, cellular structure, and cellular metabolisms between bacteria and archaea (31–33). Our findings are consistent with those of other studies that have reported the dominant role of stochastic processes in structuring the soil archaeal communities in forests (18, 43) and farmland (47). However, other studies have also revealed only a minor role of stochastic processes in governing the archaeal community assemblies in the Namib Desert (45) and in agricultural fields across eastern China (46). One of the possible reasons for our contradictory findings might be the superior adaptability of archaea in response to high salinity (48, 49) and anoxic conditions (50). Thus, the changes in inundation frequency in this study might exert less selective pressure on archaea than other environmental forces. Another possible reason might be that the balance between stochastic and deterministic processes in community assembly are scale dependent and probably variable across different biomes (7, 51, 52).

Our results suggest that the relative contribution of stochastic processes increases significantly with increase in inundation frequency ($P < 0.05$) (Fig. 3D and E). The deterministic process includes both the abiotic and biotic factors that influence the presence or absence and the relative abundances of species (53). However, the stochastic process comprises probabilistic dispersal, unpredictable disturbance, and random shifts in relative abundances of species (54). Soil water content was the most decisive factor influencing archaeal community assembly processes (Table S10). A large amount of nutrients is available in seawater that could serve as constant resources for soil microorganisms. Indeed, we found strong and positive correlations between the soil water content and substrate concentrations (e.g., total carbon [TC], carbon-to-nitrogen ratio [C/N], and salinity) (Fig. S5 and S6). Consequently, with increasing inundation frequency, the community niche width increased significantly ($P < 0.001$) (Fig. 4). These consequences could be responsible for fewer biotic competitions among species. In this case, the community assembly is likely to be dominated by stochastic birth-death and dispersal events rather than by environmental factors (7). Moreover, dispersal limitation plays an important role in archaeal community assembly (Fig. 3C), and increases in inundation frequency could facilitate the species movements. Nevertheless, the experimental verification of archaea in response to environmental changes is essential because statistical analyses of alterations in the community niche width and niche overlap might not fully reflect the reality.

Studies have revealed an important role of plants in structuring soil microbial communities (55–57). In this study, we also found differences in the response of archaeal communities to increasing inundation frequencies between *K. obovata* and *S. alterniflora* soils (Fig. 2 to 4 and Fig. S9 and S15). The archaeal community in *S. alterniflora* soil displayed significantly lower niche overlap than that in *K. obovata* soil ($P < 0.001$) (Fig. 4C). High niche overlap between species increases the competitions among them for resources (58). This might be one of the reasons for the observation of more stochasticity in *S. alterniflora* soil than in *K. obovata* soil (Fig. 3). In addition, higher fine root biomass of *S. alterniflora* relative to *K. obovata* could lower the soil bulk density (41) and contribute to the dispersal of species. Overall, our results indicate that *S. alterniflora* colonization in the subtropical mangroves could modify the soil archaeal communities and their responses to increasing inundation frequencies. This study has advanced our knowledge of the responses of archaeal community assembly to environmental changes, particularly inundation frequency. However, we investigated the response of soil archaeal communities only by using amplicon sequencing in the present study. Determining the response of microbial functions to increasing inundation frequencies will be another interesting topic to explore in future studies. From a combination of mechanistic and functional aspects, we can better understand the evolution of ecosystem functions in the context of global change.

Increased inundation frequency increases the network complexity. The co-occurrence network provides novel insights into the potential interactions among microbial members and has been broadly applied to study the interactions in numerous

ecosystems (6, 9, 21, 59). In this study, a power-law distribution pattern was observed in the network (Fig. S14), which is similar to that observed in the agricultural ecosystems of the North China Plain (6), in the wetlands of the Qinghai-Tibetan Plateau (52), and in *S. alterniflora* wetlands of the Zhangjiang River Estuary (30). The observed non-random distribution pattern indicates that this network is a typically scale-free network, which means that most nodes have few links, while a few nodes have a large number of links.

We found that increasing inundation frequency significantly increased the avgK ($P < 0.01$), which indicates that the complexity of co-occurrence networks is enhanced with increasing inundation frequency (Fig. 5D). Our findings are well supported by those of other similar studies that have reported more bacterial interactions in high-moisture soils than in low-moisture soils in the urban environments of Chicago (60) and the southern Loess Plateau (61). However, our previous findings indicating that increasing inundation frequencies strongly reduce the bacterial network complexity in *S. alterniflora* soil in the Zhangjiang River Estuary (30), which contradicts the current results. Our previous findings (30) are consistent with those of a study by Zhang et al., according to which the soils with the highest water content exhibit the lowest network connectivity of methanotrophic communities in the Qinghai-Tibetan Plateau (52). The discrepancy in the aforementioned results might be due to differences in the ecological niches for each microbial population (62). In this study, the archaeal community niche width strongly increased with increase in inundation frequency (Fig. 4B). The wider the ecological niche of a species, the more it is likely to be a generalized species; otherwise, it tends to be a specialized species (63, 64). This suggests that increase in inundation frequency releases the resource limitation of archaea. Additionally, increased inundation frequency slightly influenced the niche overlap (Fig. 4D), which indicates that archaeal taxa might strive for different substrates, thus allowing more species to coexist in a given habitat (64). Indeed, a high archaeal alpha diversity with a low proportion of mutual exclusion was observed with increasing inundation frequency (Fig. 5E and Fig. S12). Overall, our study provides evidence of the archaeal coexistence patterns in coastal wetlands and, more importantly, their responses to increasing inundation frequencies. This is important for understanding the evolution of soil functions in coastal wetlands under global change scenarios (9). However, we considered only archaea in the construction of co-occurrence networks in the present study. Future studies considering broader microbial communities (such as bacteria, fungi, and viruses) are essential to comprehensively assess the responses of soil microbial communities to environmental changes.

How species coexist within a community is one of the key questions in microbial ecology (65). We found that the network complexity tends to be high when soil archaeal communities are largely governed by stochastic processes (Fig. S16). Our findings are supported by a study which demonstrated that microbial associations are high when communities are primarily driven by stochastic processes (e.g., dispersal limitation) in agricultural soils across eastern China (66). Interestingly, our previous study revealed that bacterial network complexity tends to be low when communities are largely governed by deterministic processes (30). Unlike stochastic-process-dominated assembly, deterministic-process-dominated community assembly may specifically favor certain microorganisms, thereby causing a reduction in the network complexity. Microbes with small body sizes and high dispersal capability could exhibit complex interactions with each other within an ecological niche (67, 68). A community dominated by stochastic processes tends to be less constrained by resources, and the concomitant decrease in the community niche overlap will be more conducive to stable coexistence of species (65). Species coexistence theories have been widely used in macroecological research on plants and large animals (10–13). Nevertheless, the application of macroecological laws in microbial ecology is greatly hampered by the fact that microorganisms are difficult to observe directly due to their huge quantity and small size (69). Therefore, high-throughput isolation of microorganisms is crucial for validating the interspecies interactions. In the present study, the main results obtained from the *in situ* mesocosm were well supported by those

from the natural habitats, which greatly eliminates the uncertainty caused by simulated experimental conditions, making the results more reliable. The current study was conducted at the local spatial scale. However, as sea levels continue to rise, most coastal wetlands will predictably experience longer periods of inundation (24, 25). Therefore, the study of soil microbial communities in coastal wetlands at a larger spatial scale (e.g., regional) in response to environmental changes is particularly important.

Conclusions. Going back to the scientific questions we asked in the beginning, based on the results obtained from the mesocosm and natural habitat experiments, we affirm that archaeal communities in coastal wetlands are primarily governed by stochastic processes. Furthermore, increasing inundation frequency strongly enhances the stochastic processes and increases the network complexity. Network associations tend to be high when stochastic processes are enhanced in the community assembly. Moreover, compared with the effect of changes in inundation frequency, the effect of plant species is lesser on the archaeal community in coastal wetlands; however, the effects of *S. alterniflora* invasion on mangrove soil microbial community cannot be ignored under the scenario of global climate change.

MATERIALS AND METHODS

Experimental design. The experiment design in this study was divided into 2 parts, an *in situ* mesocosm system and natural *S. alterniflora* soil environment. The *in situ* mesocosm was designed and established in the Zhangjiang River Estuary (23°55'N, 117°26'E) in Fujian Province, China, in accordance with the method described in similar studies (70–72). Briefly, a subplatform (4 m long and 2 m high for each) was established, with continuous elevation gradients. Two subplatforms that were located along the coast-to-sea direction were combined as one platform to obtain extensive elevations (Fig. S1A). After the combination, each platform contained 11 elevations at a 25-cm vertical interval (Fig. S1B). To meet the requirement of the number of replicates, we totally built 6 subplatforms which induced 3 platforms in the intertidal zone (Fig. S1A). A pressure transducer (HOBO U20L-04 water level) was installed for monitoring the inundation frequencies at the highest and lowest elevations. The inundation frequency varied between 0.02% to 53.60%, which decreased gradually with increasing elevation (Fig. S1C). After establishment of the mesocosm, 6 polypropylene buckets (with 25-cm inner diameter and 33-cm height) were placed on each elevation of every platform. Three holes (1-cm diameter) were poked at the bottom of each bucket and covered with a nylon screen (1-mm mesh) to facilitate the vertical exchange of seawater. Intact soil cores from a depth of approximately 20 cm were collected from nearby bare mudflats and placed into the buckets. Next, the 6 buckets on each elevation were divided into 2 groups, with 3 buckets each for *K. obovata* and *S. alterniflora* planting. In March 2017, we planted 2 *K. obovata* propagules and 1 *S. alterniflora* seedling in the buckets (Fig. S1D). Finally, this arrangement produced 9 replicates per elevation for each plant species. This mesocosm allowed us to achieve continuous inundation frequencies within a relatively small spatial scale and eliminate the effects of other environmental changes. In September 2018, plants were harvested and biomass was determined (73).

Subsequently, to verify the results obtained from the mesocosm experiment, we collected *S. alterniflora* soils, which were distributed along different elevations and had been experiencing different inundation frequencies, from natural habitats (Fig. S2 and Table S1). Natural *S. alterniflora* habitats were chosen because the elevation range of their distributions in the intertidal zone is closer to the mesocosm setting than that to the mangroves. The elevation value for each natural *S. alterniflora* soil was measured using real-time kinematics (RTK; GNSS G970 RTK-GPS; UniStrong Science & Technology, Inc., Beijing, China). As described in Table S1, the lowest and highest elevation values were approximately 10.39 m and 11.97 m, respectively, which roughly corresponded to elevation 3 and elevation 8 of the mesocosm, respectively.

Collection of soil samples. Soil samples were collected in September 2018 during neap tide. Although plants in some buckets in the mesocosm were dead or flushed away before soil sampling, at least 5 replicates remained per elevation for each plant species (Table S2). Three intact soil cores (from a depth of 20 cm) surrounding the plants were randomly collected from each bucket and then completely homogenized as one sample. At this stage, a total of 120 soil samples were collected from the mesocosm (Table S2). Similarly, 42 soil samples were collected simultaneously from the natural *S. alterniflora* soils (Table S1). All 162 soil samples were stored in Ziploc bags and sealed with gummed tapes after removal of the fine roots and other debris. Soil samples were stored on ice during the transportation to laboratory.

Measurements of soil physicochemical properties. We determined a total of 17 soil physicochemical properties of the 120 soil samples collected from the mesocosm. In brief, soil water content was measured gravimetrically (74); soil salinity and pH were determined using an FE20 digital meter (Mettler Toledo, Shanghai, China); NO_3^- and NH_4^+ concentrations were measured after extraction of soils with 2 mol/liter of KCl by using a San⁺⁺ continuous-flow analyzer (Skalar, Breda, Netherlands); soil total carbon (TC) and total nitrogen were determined using a 2400 II CHN elemental analyzer (PerkinElmer, Boston, MA), and then the carbon-to-nitrogen ratio (C/N) was calculated. Other soil elements (including P, K, Ca, Mg, Mn, Al, Fe, and Cu) were digested with 1:2:1 (vol/vol/vol) nitric acid (HNO_3), hydrofluoric

acid (HF), and perchloric acid (HClO₄) in a hot plate, and the content of each element was determined using an ICP Optima 8000 (PerkinElmer, Waltham, MA). For the 42 soil samples collected from natural *S. alterniflora* habitats, soil water content was determined gravimetrically.

Extraction of soil total DNA and high-throughput sequencing. Soil DNA was extracted using a FastDNA SPIN kit (MP Biomedicals, Santa Ana, CA) according to the manufacturer's instructions. Archaeal 16S rRNA genes were amplified using the primer pair 524F-10-ext (5'-TGYCAGCCGCCGCGTAA-3') and Arch958-modR (5'-YCCGGCGCTTGAATCAATT-3') (75). High-throughput sequencing was performed on the MiSeq PE300 platform (Illumina, Inc., San Diego, CA).

Bioinformatic analyses. Raw sequences were processed using the amplicon sequence variant (ASV) method in the Quantitative Insight into Microbial Ecology 2 (QIIME2) pipeline (76). The raw sequences with average quality scores of <20 or read lengths of <50 bp were filtered using Trimmomatic (77) and merged using FLASH software (78). The filtered sequences were denoised using the DADA2 algorithm (79). The taxonomy of each sequence was analyzed using the Ribosomal Database Project Classifier algorithm (<http://rdp.cme.msu.edu/>) against the SILVA database (<https://www.arb-silva.de/>). Finally, a total of 2,695 and 1,207 ASVs were obtained from the 120 and 42 soil samples collected from the mesocosm and natural settings, respectively. For downstream analyses, soil samples were rarefied to the minimum number of sequences per sample; 120 and 42 soil samples were rarefied to 9,829 and 16,258 sequences, respectively.

Statistical analyses. Principal-coordinate analysis (PCoA) was performed based on weighted UniFrac dissimilarities. A pairwise permutational multivariate analysis of variance (PERMANOVA) was conducted to determine the significant differences in archaeal communities across elevations. We performed variation partitioning analysis (VPA) to analyze the archaeal community structure and to evaluate the independent and shared contributions of plant species and soil physicochemical properties. Furthermore, we used Mantel tests to examine the significance of relationships between archaeal community structure and each soil physicochemical property. Then, we performed aggregated boosted tree (ABT) analysis by using the *gbmplus* package in R software (version 3.6.0) to evaluate the relative influence of each soil biogeochemical property on archaeal community structure (80). A classification random forest analysis was conducted using the *rffPermute* package in R to identify the most important predictors influencing archaeal alpha diversity. Both linear and quadratic models were used to assess the relationships between soil physicochemical properties and archaeal communities (e.g., the relative abundance of dominant phyla), and the most suitable model was selected based on a low Akaike's information criterion value. The distance-decay relationship was assessed for both *K. obovata* and *S. alterniflora* soils, and the significant difference between 2 slopes was analyzed and compared through standardized major axis regression by using the *SMATR* package in R (81). To further understand the diversity patterns and the underlying mechanisms of archaeal communities, compositional dissimilarities between soils were subdivided into replacement and richness difference components (Podani family and Sørensen dissimilarities) by using the *adespatial* package in R. The random forest model was applied using the *randomForest* package in R (importance = TRUE, proximity = TRUE, and ntree = 1,000) to identify the archaeal taxa on the class level that accurately predicted inundation frequencies (82). Cross-validation was performed 50 times by using the *rfcv* function to select the appropriate classes. Levin's niche width and niche overlap indices were calculated using the *spaa* package in R (83). Niche width was calculated based on the diversity of the species in the community, and each individual point represents the niche width of a species. Niche overlap was computed between each pair of species, and each point represents the overlap between any two species.

The neutral community model (NCM) was applied to estimate the relative contribution of the stochastic process toward archaeal community assembly (84). After that, the relative contributions of different ecological processes (e.g., dispersal limitation) were evaluated using the null model approach (8). The nearest taxon index (NTI) and beta nearest taxon index (betaNTI) were calculated using the *ses.mntd* function (85) and null model (8) in the *picante* package in R, respectively. NTI values were used to evaluate the phylogenetic community assembly on a within-community scale, in which the positive and negative values indicated clustering and overdispersion of taxa, respectively, across the overall phylogeny (86). A combination of betaNTI values and Bray-Curtis-based Raup-Crick index (RC_{bray}) was applied to estimate the relative contributions of ecological processes (homogeneous selection, variable selection, dispersal limitation, homogenizing dispersal, and undominated processes) toward the community assembly (87, 88). Pairwise betaNTI values of less than -2 and greater than +2 indicated homogeneous selection and variable selection, respectively. RC_{bray} of less than -0.95 or greater than +0.95 indicated significant deviations from the null model expectation. In case of |betaNTI| of <2, with RC_{bray} less than -0.95 or greater than +0.95, the contribution of homogenizing dispersal or dispersal limitation, respectively, was suggested. Otherwise, when |betaNTI| was <2, with |RC_{bray}| of <0.95, shifts in the community composition were considered undominated. The significance of the relationship between betaNTI and soil physicochemical properties was examined using Mantel tests.

A co-occurrence network was constructed using CoNet in Cytoscape software (version 3.7.2) (89). ASVs that were detected in fewer than 5 soil samples were discarded. The relationship between ASVs was examined simultaneously by Pearson correlation, Spearman correlation, Bray-Curtis dissimilarity, and Kullback-Leibler dissimilarity. Values of *p* were computed with 1,000 permutations and adjusted using Benjamini Hochberg's correction (89). Subnetworks were generated from the network by preserving the presented nodes and edges. The topological parameters of each network were computed using the NetworkAnalyzer plugin (90). The universal role of each node was analyzed using the *netcarto* package in R. In this study, the connector hub and connector were defined as keystone species. The relative

abundances of keystone species in the network were calculated by normalization based on the standardized relative abundance (z-score) (91).

Data availability. All raw sequences were deposited to the Genome Sequence Archive at the National Genomics Data Center, Beijing Institute of Genomics (China National Center for Bioinformation), and Chinese Academy of Sciences, under accession number CRA003181, which is publicly accessible at <https://bigd.big.ac.cn/gsa>.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 11.8 MB.

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