Crab bioturbation significantly alters sediment microbial composition and function in an intertidal marsh

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ABSTRACT

Crabs are among the most important benthos in intertidal marshes. Numerous studies have shown that they can influence soil properties and soil-surface water exchange through bioturbation. However, little is still known about the effects of crab burrows on sediment microbes. In this study, we examined the effects of crab burrows on the community structure and interaction pattern of soil microbes in sediments in an intertidal marsh of the Yellow River Delta, north China using high-throughput sequencing and co-occurrence network analyses. Results showed that crab bioturbations altered soil microbial community composition both within the fungal and bacterial communities. Network analyses results indicated that the modularity of fungal networks decreased after crab bioturbation. Further, bacterial and fungal functional predictions indicated the relative abundances were significantly lower for fungal saprotrophs and saprotroph-pathotrophs in the soil mound sediment, while significantly higher for bacterial chemoheterotrophs and aerobic chemoheterotrophic in the burrow wall sediment. Our results demonstrate that crab bioturbation can modify the microbial community structure and function, and thus nutrient cycling through degradation of organic matter, a key ecosystem processes in intertidal marsh.

1. Introduction

Ecosystem engineers are that organisms can modulate the availability of resources for other species through physical structures, physiology, and behavioral activity (Jones, 1995). Studies about soil ecosystem engineers (e.g. earthworm, ant) in terrestrial ecosystems have revealed the impacts on soil structure, soil heterogeneity, soil nutrient availability, gas diffusion and maintaining species diversity by their burrowing and foraging (Wolters, 2000; Wu et al., 2013; Bertrand et al., 2015; Wu et al., 2015 a,b). Benthos can alter sediment structures by digging burrows (Kristensen et al., 2008). In addition, they can change the chemical properties of sediments by foraging and excretion (Wieltschnig et al., 2008). There has been growing evidence that benthic invertebrates in aquatic sediments can also increase microbial activity and alter nutrient cycling (Wang et al., 2010; Luo and Gu, 2018).

Benthic invertebrates affect the energy flow and nutrient cycle of estuary wetland ecosystems. They achieve functions through physiological activities, such as burrowing, excretion, and bioirrigation, which

strongly affect the biogeochemical cycle processes of wetlands, particularly carbon (C) and nitrogen (N) cycling in sediments (Lohrer et al., 2004; Eldridge et al., 2015). Crabs are one of the most abundant benthos in coastal wetlands. They can directly affect soil properties and ground water-surface water exchange through burrows (Stahl et al., 2014; Hu et al., 2015; Xiao et al., 2019). Studies have found that burrowing crabs are strongly associated with mangrove trees and the existence of their burrows counteracts the reduced redox potential in mangrove sediments (Robertson and Daniel, 1989; Gillis et al., 2019). Ferreira et al. (2007) revealed that lowest pyrite-Fe, degree of pyritization and acid volatile sulfide values in summer are related to increased crab activity such as foraging and reproduction. Most of these studies focus on the effects of bioturbation on nutrient cycling and plant growth (He and Silliman, 2016; Luo and Gu, 2018; Gutiérrez et al., 2018). However, little is known about the effects of crab bioturbation on microbial community composition and diversity. Bioturbation increases oxygen penetration, alters the physical and chemical conditions in sediments, controls the composition of microbial communities, alters microbial activity and

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Fig. 1. Soil microbial diversity in the soil mound sediment (SM), burrow wall sediment (WS), and no-bioturbated sediment (CT) with crabs. Mean $\pm$ SE. *: $P < 0.05$; **: $P < 0.01$.

benthic metabolism, and the burrowing activity can create habitats for other small organisms (Derksen et al., 2019).

Both bacteria and fungi are important components of soil microbial assemblages and provide important ecological functions, including nutrient cycling and interactions with vegetation (Falkowski et al., 2008). Soil carbon is the energy source on which microbes depend (Delgado-Baquerizo et al., 2016). It is generally believed that fungi and bacteria have different substrate preferences. Fungi tend to decompose recalcitrant carbon, whereas bacteria tend to assimilate soil labile C (Myers et al., 2001; Courty et al., 2010). Studies have shown that soil microbial community structures are affected by environmental factors such as temperature and precipitation (Guo et al., 2018; Ochoa-Hueso et al., 2018). Soil pH is an important environmental factor to predict the response of microbial α diversity to global changes due to physiological constraints on microorganisms (Rousk et al., 2010). Compared with abiotic factors, biotic factors have been less studied in regard to the influence of microbes.

In recent years, the development of high-throughput sequencing technology has enhanced soil microbial studies (Bates et al., 2013). Further, the application of biological network analysis is useful to study interactions of microbes. This study applied high-throughput sequencing technology to study soil microbes that are affected by crab bioturbation. The bacterial and fungal communities of the crab burrows were described, and their microbial networks were analyzed. The effects of crabs on the composition of, and interactions among soil microbial communities were evaluated. Our hypotheses were as follows: (1) the structure of bacterial and fungal communities change during bioturbation; and (2) crab burrows change the microbial community structure, and thereby the interaction pattern of the entire microbial network; and (3) the functions of bacteria and fungi in such ecosystems are affected by crab bioturbation.

2. Materials and methods

2.1. Study area and sample collection

The study site was the Yellow River Delta coastal wetlands (37°35'–38°12'N, 118°33'–119°20'E, 0–15m above the sea level). It is a warm temperate continental monsoon climate with an average annual

temperature of 11.7 °C–12.6 °C, and annual precipitation of 551.6 mm, 70% in summer. Rain and high temperatures typically occur during the same period. The main soil types in the study area are fluvo-aquic and saline soils. We randomly selected 6 active crab burrows (i.e. crabs were observed). We collected crab bioturbated soil samples from soil mound sediment (SM), burrow wall sediment (WS), and non-bioturbated sediment as the control (CT) ($n = 6$ for each treatment, total of 18 replicates). We collect soil mound and burrow wall sediments to 5 cm depths. Non crab bioturbated sediment sampling depths were also 5 cm. All sediment samples were stored on ice, and immediately transported to the laboratory.

2.2. Soil DNA extraction, quantification, and Illumina MiSeq sequencing

Total DNA was isolated from soil samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The final DNA concentrations and purification were determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). The DNA quality was checked by 1% agarose gel electrophoresis. The V3–V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (ACTCC-TACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT), and for the ITS rRNA gene were amplified with primers ITS1F (CTTGGTCATTAGAGGAAGTAA) and ITS2R (GCTGCGTCTTCATC-GATGC). Purified amplicons were pooled in equimolar concentrations, and then paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) in the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) according to its standard protocols.

2.3. Statistical and co-occurrence network analyses

Raw FASTQ files were quality-filtered by Trimmomatic and merged by FLASH (Magoc and Salzberg, 2011) using the following criteria: (1) if the mean quality score < 20 , reads were truncated over a 50 bp sliding window, and reads shorter than 50 bp were discarded after quality control; and (2) ambiguous characters were discarded. After chimeras and singletons were detected and removed, operational taxonomic units (OTUs) were clustered on the basis of 97% similarity with the USEARCH tool (v.7.0 <http://drive5.com/uparse/>) and classified using the SILVA

Fig. 2. Soil microbial composition in the soil mound sediment (SM), burrow wall sediment (WS), and no-bioturbated sediment (CT).

database (Quast et al., 2013) for bacteria and the UNITE database (Abarenkov et al., 2010) for fungi. The functions of soil bacteria and fungi were separately identified using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) (Louca et al., 2016) and FUNGuild (Nguyen et al., 2016).

The effects of crab bioturbation on the microbial community structure, diversity and function relative abundance were determined by contrasting the three soil treatments using one-way analysis of variance (ANOVA). Characterization of bacterial and fungal α -diversity was achieved by applying the Chao 1 and Shannon indices. The effects of crab burrows on the soil microbial community were visualized using non-metric multidimensional scaling (NMDS) and assessed through a permutation multivariate analysis of variance (PERMANOVA). The Psych package in the R program (V.3.6.1) was used for co-occurrence network analyses. Only significant differences ($P < 0.05$), absolute correlation values > 0.8 , and relative abundance $> 0.2\%$ OTUs were considered for construction of the network. The networks were visualized using the interactive Gephi platform and the Fruchterman-Reingold layout algorithm.

3. Result

3.1. Abundance and richness of microbial communities

Sequencing results were clustered into 7999 bacterial and 1156 fungal OTUs, with a 97% identity threshold. For bacterial OTUs, A total

of 112 (1%) OTUs contributed 50% of the total bacterial OTU abundances. While for fungal OTUs, 12 (1%) OTUs contributed 50% of the total fungal OTU abundances. Significant differences were noted in the bacterial α -diversity between SM and WS. The bacterial Chao 1 index was significantly higher in mound sediments (SM) than in wall sediments (WS) or control sediments (CT) ($P < 0.01$). The fungal Chao 1 index of the CT was significantly higher than that of the SM or WS ($P < 0.01$). In contrast, no significant differences were noted in the fungal Shannon index among the three different sample sources (Fig. 1).

3.2. Taxonomic composition

A total of 53 bacterial phyla and 13 fungal phyla were observed. The seven phyla of bacteria with higher relative abundance were Proteobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Actinobacteria, Gemmatimonadetes, and Acidobacteria. The dominant bacterial phyla were Proteobacteria and Bacteroidetes in crab bioturbated sediment (SM, WS), representing 32.3%–35.1% and 21.1%–34.8%, respectively (Fig. 2). The dominant bacterial phyla in non-bioturbated sediment were Proteobacteria and Firmicutes, representing 25.7% and 10.6%, respectively. Crab burrows also had a significant influence on specific taxa. The relative abundances were higher for Proteobacteria and Bacteroidetes while lower for Actinobacteria and Gemmatimonadetes in the two crab bioturbated sediment than in the non-bioturbated sediment. While the relative abundances of Actinobacteria and Gemmatimonadetes were lower in the bioturbated sediment than in the non-bioturbated sediment

Fig. 3. Non-metric multidimensional scaling (NMDS) plot of bacterial and fungal community structures in the soil mound sediment (SM), burrow wall sediment (WS), and non-bioturbated sediment (CT) with crabs.

Table 1

Permutation multivariate analysis of variance (PERMANOVA) on the effects of crab bioturbation on bacterial and fungal assemblages in sediment. R^2 shows the percentage of variation attributed to the treatment; **: $P < 0.01$. SM: soil mound sediment, WS: burrow wall sediment, and CT: no-bioturbated sediment.

Disturbance	Fungal		Bacterial	
	<i>P</i>	R^2	<i>P</i>	R^2
SM vs WS	0.01**	0.24	0.01**	0.31
SM vs CT	0.01**	0.26	0.01**	0.30
WS vs CT	0.01**	0.31	0.1	0.12

(Fig. 2).

Ascomycota and Basidiomycota were the dominant fungal phyla in all three type sediments, representing 70.3%–83.1% and 5.5%–17.3% of classified OTUs, respectively (Fig. 2). The dominant classes across all samples were Dothideomycetes and Sordariomycetes (phylum Ascomycota). Tremellomycetes were significantly higher in the crab bioturbated sediment than the non-bioturbated sediment. In contrast, the relative abundance of Agaricomycetes was significantly higher in the mound sediments than in the other sediments (Fig. 2).

Significant differences were noted in the bacterial β -diversity, as assessed by mass, among the three type samples (Fig. 3, $P < 0.01$). The fungal communities differed significantly between mound and wall or control sediments ($P < 0.01$, Table 1).

3.3. Co-occurrence network analysis

Network analyses based on Spearman's correlations revealed some effects of crab bioturbation on microbial interactions (Fig. 4). Networks were separately generated for bacteria and fungi in each sediment type, and topological properties were determined to characterize each network (Table 2). Results showed that crab bioturbation changed not only the community composition of fungi and bacteria, but also the relationships among microbes. The average degree of interaction was higher in the bacterial network than that in the fungal network. The modularity of bacterial and fungal networks, except the bacterial network in wall sediments, was greater than 0.4, indicating a modular structure. The modularity of soil fungal networks decreased after crab bioturbations. The clustering coefficient of networks was higher in crab bioturbated sediments (SM and WS) than in non-bioturbated sediments.

3.4. Responses of microbial functional structure to crab bioturbation

Bacterial functions were predicted according to the FAPROTAX database. The 30 functional groups with the highest relative abundances were considered. The main bacterial functional groups among the three type samples were chemoheterotrophy, aerobic chemoheterotrophy, fermentation, respiration of sulfur compounds, sulfur respiration, thio-sulfate respiration, hydrocarbon degradation, and nitrate reduction (Fig. 5). Among these 30 functional groups, chemoheterotrophy and bacterial aerobic chemoheterotrophy showed significant differences among the three types of soil samples, with the relative abundances of chemoheterotrophic and aerobic chemoheterotrophic bacteria being significantly higher in wall than control sediments.

The soil fungi were mainly divided into four functional types - saprotrophs, pathotrophs, symbiotrophs, and pathotroph-saprotrophs based on the fungal trophic mode (Fig. 6). The crab burrows had a significant effect on the pathotrophic and symbiotrophic fungi. The relative abundance of saprotrophs was significantly lower in mound than in wall or control sediments ($P < 0.05$), with a reduction by 71.6% and 57.6%, respectively. Pathotroph-saprotroph types were less abundant in mound than in control sediments ($P < 0.05$).

4. Discussion

4.1. Bacterial and fungal taxonomic shifts

Diversity among bacteria was highest in mound sediment while fungal diversity was highest in control sediments. Fungi tend to decompose more recalcitrant compounds and are regarded as *K*-selected, while bacteria decompose labile compounds and are regarded as *r*-selected (Paul, 2007). In this study, the mound soil was suitable for the growth of *r*-selected microbes, while the control soil was more suitable for the growth of *K*-selected. Compared with the control soils, the diversity of fungi in the bioturbated mound and burrow wall soils was reduced, consistent with the results of Booth et al. (2019). On the other hand, the reduced fungal diversity in crab bioturbated sediments might be due to the fact that crab activity destroys the hyphal networks in the burrows, and that the crabs may compete with fungi for food. Additionally, crabs may feed on fungi directly (Kristensen et al., 2010). Studies had shown that the diversity and structure of microbial communities are linked with ecosystem function (Graham et al., 2016). Microbial diversity enhances multiple ecosystem functions, and a loss in soil biodiversity threatens ecosystem functioning (Wagg et al., 2014, 2019). Therefore, a decrease of fungal diversity could have negative effects on ecological functions.

Fig. 4. Interactions of bacteria (a, b, c) and fungi (d, e, f) in the soil mound sediment (a, d); burrow wall sediment (b, e); and no-bioturbated sediment (c, f) with crabs. The line between each pair of nodes represents strong positive (red) or negative (green) interactions with adjusted $P < 0.05$ and absolute correlation values > 0.8 . Only relative abundances $> 0.2\%$ bacterial (a, b and c) or fungal (c, d and e) OTUs were included in the analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Topological properties of bacterial and fungal co-occurrence networks affected by crabs. SM: soil mound sediment, WS: burrow wall sediment, and CT: no-bioturbated sediment.

	Sample	Node	Edges	Average degree	Clustering coefficient	Modularity	Average diameter
Bacterial	CT	82	415	10.122	0.587	0.531	7
	WS	79	544	13.772	0.684	0.362	9
	SM	62	219	7.065	0.658	0.583	10
Fungal	CT	43	154	7.163	0.525	1.692	8
	WS	56	111	3.964	0.728	0.665	5
	SM	68	214	6.294	0.732	0.494	12

We showed that the relative abundances of Proteobacteria and Bacteroidetes were significantly increased, while Actinobacteria were significantly decreased after crab bioturbations. Actinobacteria exhibit slow growth rates even when resources are abundant, but they are able to degrade recalcitrant lignin and cellulose, to produce polyphenolic compounds (Pankratov et al., 2011; Eisenlord et al., 2013). Crab feeding behavior of physical scrapping and their digestion of organic matter provides labile substrates for copiotrophic groups, which is beneficial to their growth and metabolism (Kristensen and Pilgaard, 2001). The change in bacterial community structure indicated that the composition of the bacterial community had changed from an oligotrophic group to a copiotrophic group, and thus overall bacterial community degradation ability was decreased following crab bioturbation.

4.2. Crabs impact soil microbial networks

Complex ecological networks typically increase the stability of food webs and ecosystems (Neutel et al., 2007; Zelezniak et al., 2015). Our results showed that both the co-occurrence networks and degree of connectivity were altered by crab bioturbation. Different patterns in the co-occurrence networks occurred for bacteria and fungi, as also noted by Xiao et al. (2018).

Crab burrows create new microhabitats, thereby changing the physicochemical properties of organic matter; these different environmental conditions are likely the major drivers of microbial communities and co-occurrence network variations among soils (Smith et al., 2014). *Helice tientsinensis* crabs are omnivores that mainly live on coastal wetland plants, microalgae, and sediments (He and Silliman, 2016).

When crabs ingest sediments in the burrows, they also digest and absorb organic matter and microbes from the sediments (Skov and Hartnoll, 2002). This could directly reduce microbial biomass. On the other hand, both crabs and fungi contribute to the decomposition of plant litter, so there is likely food resource competition between the two organisms. Zelezniak et al. (2015) found that metabolic dependence is a major driver of species co-occurrence. As a result, co-occurrence groups can be mutually beneficial, as different species can more efficiently utilize limited resources and coexist across various niches. The habitat changes caused by crab bioturbation limit the survival of fungi; however, the utilization efficiency of resources can also be improved by a greater number of species connections (Morrien et al., 2017). The high modularity of their networks reflects that biological interactions are more frequent within the modules than outside the modules (Chagnon et al., 2012). Studies have shown that modularity of networks has a negative relationship with a community's ability to adapt to changes in biotic or abiotic factors (Eldridge et al., 2015). Because the modularity of fungal networks decreased after crab bioturbation, which may improve tolerance and resilience from environmental and resource changes.

Our results suggested that whether or not they are disturbed by crabs, bacteria tend to have more connections than fungi. This difference may result because bacterial diversity is higher than fungal diversity, and generally grow faster than fungi (Rousk and Bååth, 2011). Nevertheless, more connections don't necessarily mean that bacteria are more important than fungi. Other studies have demonstrated that the changes in fungi in intertidal sediments have synergistic effects on the development of both microbial communities and their function (Booth et al., 2019). Collectively, bacteria and fungi may play complementary roles in

Fig. 5. Main functional structure of soil bacteria in the soil mound sediment (SM), burrow wall sediment (WS), and no-bioturbated sediment (CT).

Fig. 6. Main functional structure of soil fungi in the soil mound sediment (SM), burrow wall sediment (WS), and no-bioturbated sediment (CT). Mean $\pm$ SE. *: $P < 0.05$.

various habitats.

4.3. Crabs and soil microbial functions

The most abundant microbial function in all three soil types was

chemoheterotrophy. This was not surprising, as most bacteria are chemoheterotrophs. Furthermore, the chemoheterotrophy and bacterial aerobic chemoheterotrophy in the burrow wall soils were significantly higher than those in the control soils. The increase in the relative abundance of chemoheterotrophy and aerobic chemoheterotrophy

induced by bioturbation is likely due to O₂ injection and the increased bioavailability of sedimentary organic matter. A recent investigation reported a CO₂ flux increment of 200% after bioturbation in a mudflat, and an increment of 8000% in a salt marsh (Fanjul et al., 2015). Thus, the increase in relative abundance of chemoheterotrophy and aerobic chemoheterotrophy may have increased levels of CO₂ flux.

Fungal communities are highly diverse, both taxonomically and functionally, and fungal communities likely control the rates of key ecosystem processes and directly affect the community structure of plants and animals, through either pathogenic or mutualistic interactions (Anderson and Cairney, 2004; Falkowski et al., 2008; Peay et al., 2008; Gessner et al., 2010). In our study, significant differences were noted in fungal functions between the control and mound soils (Fig. 6). After crab burrows were formed, the relative abundance of pathotrophic-saprotrophic and saprotrophic fungi in the mound soils was significantly decreased, as compared with the control soils (Fig. 6).

Saprotrophic fungi play an important role in the decomposition of organic matter and nutrient cycling in forest soils (Talbot et al., 2014). Saprotrophs can compete with ectomycorrhizal fungi for soil nitrogen (Fernandez and Kennedy, 2015). A reduction in the relative abundance of saprotrophic fungi may lead to reduced decomposition and increased sequestration of available carbon (Gadgil and Gadgil, 1971; Gadgil, 1975; Fernandez and Kennedy, 2015). Lenhart et al. (2012) reported that saprotrophic fungi are drivers of methane production. Therefore, a reduction in the relative abundance of saprotrophic fungi may reduce methane emissions. A reduction in the corresponding functional fungal abundance can thus have different effects on various plants. Pathological vegetative fungi in soil can have negative effects (Anthony et al., 2017). Therefore, compared with the non-bioturbated control soils, the reduced relative abundance of pathotrophic-saprotrophic fungi in bioturbated soils may have a positive effect on plants.

5. Conclusion

Our findings suggest that crab bioturbation likely alters both the bacterial and fungal communities in intertidal wetland soils, and affects the interaction patterns among microbial taxa. The composition of bacterial communities changed from an oligotrophic group to a copiotrophic group. The fungal community had increased connections between internal communities in response to crab bioturbation. Functional predictions of fungi and bacteria indicated that crab bioturbations decrease the relative abundance of pathotrophic-saprotrophic and saprotrophic fungi in the mound sediments, while increasing the dominance of chemoheterotrophy and aerobic chemoheterotrophy. Consequently, our results indicate that crab bioturbations change the roles of microbes in nutrient cycles, and should be considered when studying ecosystem functions of coastal wetlands.

CRediT authorship contribution statement

Chuanjing Wu: Writing - original draft, Formal analysis, Data curation. **Haitao Wu:** Supervision, Conceptualization, Methodology, Project administration. **Dandan Liu:** Data curation, Formal analysis. **Guangxuan Han:** Writing - review & editing, Resources. **Panpan Zhao:** Formal analysis, Visualization. **Yujuan Kang:** Data curation, Formal analysis, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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