



Reclamation negatively influences arbuscular mycorrhizal fungal community structure and diversity in coastal saline-alkaline land in Eastern China as revealed by Illumina sequencing



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ABSTRACT

Arbuscular mycorrhizal (AM) fungi play a vital role in the succession of the plant community, as well as the enhancement of nutrient uptake and stress tolerance in their host plants. To obtain a better understanding of changes in the AM fungal community composition and diversity in response to reclamation with vegetation successions, Illumina sequencing was performed with samples that were collected from the coastal saline-alkaline fields of Eastern China. This study consisted of 3 reclamation stages consisting of 2 years (since 2011), 6 years (since 2007), and 34 years (since 1979) in combination with 4 vegetation statuses, namely bare land, seepweed (*Suaeda salsa*), couch grass (*Imperata cylindrical*), and corn (*Zea mays*). A total of 742 OTUs (Operational Taxonomic Units) from 20,474 sequences affiliated with 4 Glomeromycota families were obtained, with Glomeraceae and *Glomus* being the most abundant family and genus, respectively. Gigasporaceae, the second-most dominant family, primarily inhabited bare land at the early reclamation stage (2–6 years). With vegetation successions, there were decreasing trends for both Acaulosporaceae and Gigasporaceae and an increasing trend in Glomeraceae, along with a decreasing trend in soil electrical conductivity (EC) and an increasing trend in soil NO₃⁻-N content. The diversity indices of Shannon (*H'*), abundance (*Chao1*) and dominance (*D*) for AM fungi also decreased with decreasing soil EC or increasing soil NO₃⁻-N content, and the Alatalo evenness index (*E_a*) of AM fungi increased, and it was inversely correlated with soil pH. Our results clearly showed changes in community structure and decreases in diversity indices for soil AM fungi in coastal saline-alkaline lands during the past 30 years of reclamations.

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1. Introduction

Coastal saline-alkaline land is the buffer zone that contacts land and sea, and it is characterized by its low fertility, high salinity, intermittent drought, changeable temperatures, and an unstable sandy substrate (Yamato et al., 2012). Its high salinity has several effects, such as physiological drought in plants through osmotic potential (Feng et al., 2002), cell toxicity by accumulating sodium (Na⁺) and chloride (Cl⁻) ions (Juniper and Abbott, 1993; Munns, 2002), and nutrient imbalance from the reduced uptake of nutrients from soils (Adiku et al., 2001). Therefore, it is difficult to engage in the direct exploitation and utilization of coastal lands, and more attentions should be paid to the reclamation processes

performed on coastal lands. For instance, soil properties, especially the salinity level, could change in coastal lands as a result of reclamation (Shi et al., 2005; He et al., 2015), and this change would bring about vegetation succession (Robertson et al., 1993; El-Ghani and Amer, 2003; González-Alcaraz et al., 2014). However, soil microbes play important roles in soil formation (Rillig and Mummey, 2006) and nutrient cycling (van der Heijden et al., 2008), influencing the reclamation process in coastal land, and correspondingly shifting their community compositions (Steenwerth et al., 2002; Fu et al., 2012). However, knowledge about the concomitant changes in functional soil microbes in coastal land as a consequence of reclamation with vegetation succession is still inadequate.

Among soil microbes, arbuscular mycorrhizal (AM) fungi are known for their vital role in the primary and secondary succession of the plant community as well as for increasing nutrient uptake

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and the tolerance of their host plants to salinity and other abiotic stresses (Beena et al., 2000; Mohammad et al., 2003). It is well known that AM fungi are wide spread in both natural and disturbed ecosystems, such as farmland (Vestberg et al., 2011), forests (Coughlan et al., 2000), grasslands (van der Heijden, 2004), deserts (He et al., 2002) and coastal lands (Kowalchuk et al., 2002; Bohrer et al., 2004; Shi et al., 2005). A number of studies have reported changes in the spore community structure and the diversity of AM fungi in terms of morphology in saline-alkaline lands (Beena et al., 2000; Oliveira et al., 2005), such as in Iran (Aliasgharzadeh et al., 2001) and China (Wang et al., 2004). In comparison with the poor representatives of spore analyses, modern molecular methods have become more informative approaches for the ecological studies of AM fungi (Santos et al., 2006; Higo et al., 2015). For example, Kowalchuk et al. (2002) surveyed AM fungi in Dutch coastal lands by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), Yamato et al. (2012) investigated AM fungi in Japanese coastal lands by cloning and sequencing, and Krishnamoorthy et al. (2014) studied AM fungi in Korean coastal reclaimed lands by terminal restriction fragment length polymorphism (T-RFLP). However, these technologies are still thought to be insufficient for exploring AM fungal diversity (Sogin et al., 2006). Recently, next generation sequencing (NGS) methods, such as Illumina sequencing (Cox et al., 2010), have been revolutionizing microbial ecology studies (Metzker, 2010) by comprehensively revealing the microbial community in terms of its diversity and biogeography, which are inaccessible by either genetic fingerprinting, clone libraries, or culture-reliant methods (Rousk et al., 2010). These methods are helpful for acquiring a better understanding of the soil AM fungal community composition and diversity.

In the coastal area of Jiangsu Province, Eastern China, reclaimed lands have increased by more than 230,000 ha over the last 50 years (Chung et al., 2004). Along with the reclamation processes for coastal saline-alkaline lands, the vegetation has also shifted from halophytes to non-halophytes (Chen et al., 1999; Rozema and Flowers, 2008). For example, only halophytes, such as seepweed (*Suaeda salsa*), can survive at the early stage. Non-halophytes, e.g., couch grass (*Imperata cylindrical*), then become dominant at the middle stage. During the late period, crops such as corn (*Zea mays*) can grow well. Because soil AM fungi can be influenced by their host plants, their composition and diversity may shift during the reclamation period. Hence, the objectives of this study were to examine the changes in AM fungal community structure and diversity in response to reclamation with vegetation successions through Illumina sequencing and to explore the impacts and the mechanisms of environmental variables in the AM fungal community parameters.

2. Materials and methods

2.1. Description of sites and sampling

Soil samples were collected from the reclaimed lands (32°44'42"–32°46'53"N, 120°49'45"–120°55'43"E) along the coastal levees at Jianggang Town, Dongtai City of Jiangsu Province, Eastern China in July 2013. Eight types of sites were selected (Fig. 1), including the following 3 reclamation stages: (1) 2 years, 2011BL and 2011SS, bare land (BL) and seepweed (SS)-grown land within the levee reclaimed in 2011; (2) 6 years, 2007BL, 2007SS and 2007IC, BL, and SS- and couch grass (IC)-grown lands within the levee reclaimed in 2007; and (3) 34 years, 1979BL, 1979IC and 1979ZM, BL, and IC- and corn (ZM)-grown lands within the levee reclaimed in 1979. There was no vegetation for the 2011BL, 2007BL and 1979BL sites (≥ 3 m in diameter), and the plant community consisted only of seepweed at the 2011SS (>10 m in diameter) and

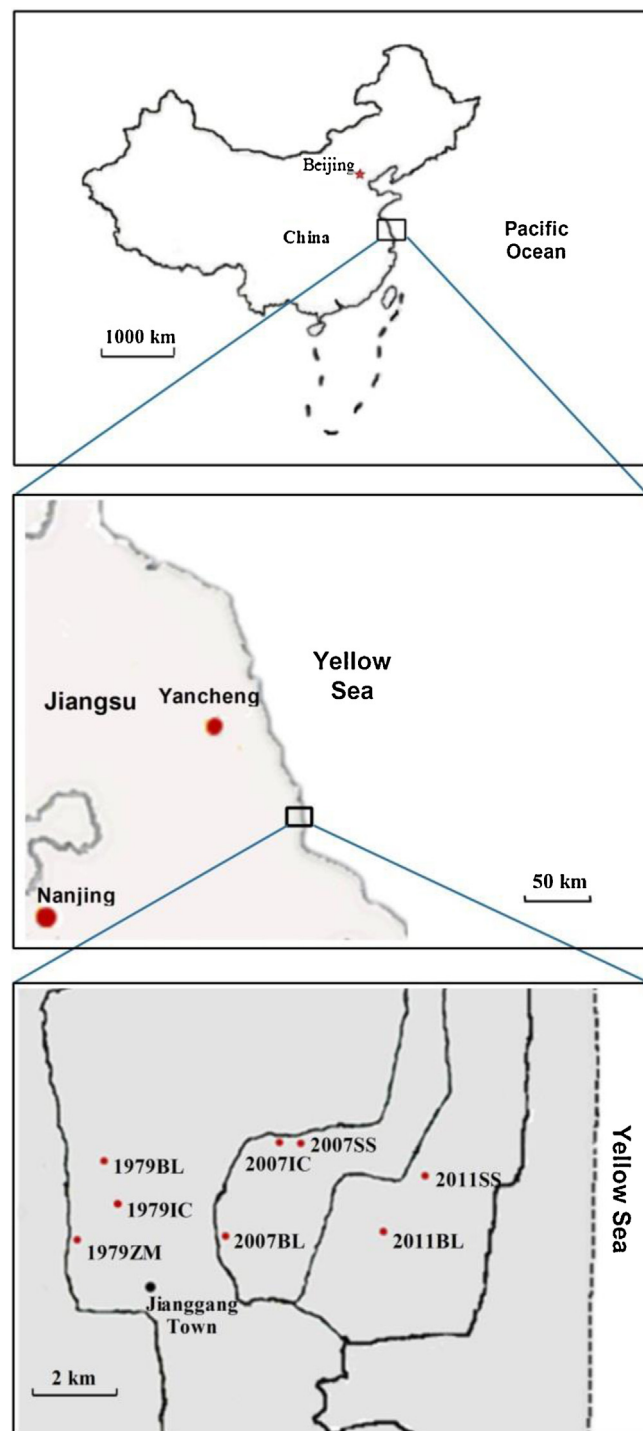


Fig. 1. The location of sample sites in Jiangsu Province of China. Samples were taken in 2011, 2007, and 1979, from soil along the levee that was reclaimed in 2011, 2007, and 1979, respectively; BL, bare land; SS, seepweed (*Suaeda salsa*); IC, couch grass (*Imperata cylindrical*); and ZM, corn (*Zea mays*).

2007SS sites (4–8 m in diameter), only couch grass at sites 2007IC (4–8 m in diameter) and 1979IC (3–5 m in diameter), and only corn at site 1979ZM (>5 m in diameter). Each type of site included 3 replicate samples, and the distances among replicates were greater than 10 m. Each sample was collected from 5 points at a 0–10 cm depth with each point separated from the others by 30–50 cm, and then the samples were mixed and divided into two parts, with one for soil chemical property analysis and another for the molecular analysis of soil AM fungi.

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