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An ecological analysis of soil sarcodina at Dongzhaigang mangrove in Hainan Island, China

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ABSTRACT

The community structure of soil sarcodina in three different habitats within a typical mangrove forest in Dongzhaigang, Hainan, China was investigated with qualitative and quantitative analyses. The three habitats were Site A (bare land without vegetation), Site B (artificially planted mangroves) and Site C (natural mangroves). The abundance, species diversity, dominance and community similarity index of soil sarcodina in fresh and air-dried soils with different physical/chemical properties were comparatively analyzed. Statistical analyses showed that the sarcodina abundance was positively correlated with moisture, salinity, organic matter (OM), total nitrogen (TN), total phosphorus (TP) and sulfate (SO_4^{2-}) of the mangrove soil, but the correlation coefficients with pH and total potassium (kalium, TK) were negative. The abundance and diversity index of sarcodina followed the order of Site A < Site B < Site C in both fresh and air-dried soils; Site B showed the highest community similarity with Site C; whereas, Sites A and C had the smallest community similarity in both fresh and dried samples from these three different habitats.

1. Introduction

Mangrove ecosystems are typical wetland systems in coastal deposits of mud and silt throughout the tropic latitudes, mainly distributed in the Southeast of Asia, and Central and South America. They are the transition areas between terrestrial ecosystems and marine ecosystems, possessing typical ecological characteristics of intertidal belts. These areas play an important role in maintaining and improving biological environments, purifying air, reversing wastewater pollution, mitigating natural disasters in bays or estuarine areas and providing biodiversity protection in coastal marshes. They are treated as "maritime forests" for their special ecological, economical, tourism and recreational value [17]. The importance of mangroves has attracted increasing attention in recent years, especially after the heavy tsunami in the Indian Ocean [4]. Since then, increasing research has been conducted on mangrove ecosystems in Asia.

Although some mangrove animals have been studied, the focus has largely been on macrobenthic fauna [9,11,18] and soil microorganism [29,35,36]. However, due to their small body size (ranging upward from several to dozens of μm) and difficulty in identification [6], only limited information is available on soil protozoa in the mangrove forest habitats [1,2,12,19,24,25].

As a main consumer group of bacteria and epiphytic microorganisms, sarcodina play a bridge role in nutrient cycling and energy flow, and are significant members of aquatic food chains. In this research, we use the term sarcodina in the classical sense to include all naked and shelled amoeboid protists, although we recognize that that this is no longer accepted as a valid group in modern taxonomic nomenclature. Sarcodina have high abundance that usually reaches 10^8 individuals g^{-1} [6], second only to flagellates in soils, globally. In many zones such as the middle tropical zone, north tropical zone, warm temperate zone and temperate zone, the abundance of soil sarcodina is even higher than that of flagellates [22]. In the typical zones of China, the most abundant protozoa are sarcodina and flagellates; whereas, ciliates are extremely limited quantitatively. All of these observations highlight that sarcodina and flagellates, especially the sarcodina, occupy an important place among soil protozoa [22].

In addition, because of their small sizes soil sarcodina can adapt easily to extremely small pore spaces. Some larger species can

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penetrate through soil granules with diameters of only 1 μm; and thus their active and predatory spaces are extended greatly [23]. These advantages enable sarcodina to play an even more important role in food chain and energy transmission. There is further well-established evidence that sarcodina are important in promoting decomposition, transformation of sediment, soil formation and development; and accelerating mineralization of carbon and nitrogen, etc. [6]. However, there are also some free-living, pathogenic sarcodina species, such as *Naegleria fowleri* and some *Acanthamoeba* species. *N. fowleri* can invade the central nervous system by penetrating through the human nasal mucosal membrane, migrating through olfactory nerves to the brain, and thus induce meningitis. Some *Acanthamoeba* species can also invade the human central nervous system and eyes [26].

Soil sarcodina are of theoretical and practical importance, such as the recognition that they help support the structure and function of mangrove forests, and more broadly have led to policies promoting protection and rational utilization of mangrove forest and soil animal resources. Hence, substantial research has been devoted to the Dongzhaigang mangrove forest, especially clarifying the community structure of mangrove soil sarcodina, analyzing the existence of harmful species and exploring the relationships between soil sarcodina and their habitats. In this research report, our goals were specifically to analyze the species abundance, biodiversity and community similarity index of soil sarcodina in three different habitats; i.e., bare land, man-made mangroves, and natural mangroves. Through research such as this, we have substantially enriched our knowledge of typical mangrove forests in Dongzhaigang, Hainan, China. Based on the present study, some suggestions and references for bare land reestablishment, as well as species selection and planting, are provided in the Discussion.

1.1. Natural status of the research area

Dongzhaigang National Mangrove Nature Reserve occupies 44.51% of the mangrove forest and is the largest mangrove everglade of Hainan Island [20]. This nature reserve, authorized and founded by the People's Government of Guangdong Province in 1980, was designated as a national nature reserve in 1986. It was included in the List of Wetlands of International Importance in 1992, mainly used to protect the mangrove forest ecosystem.

Dongzhaigang (110°30′–110°37′E, 19°51′–20°01′N) is a shoalwater bay formed by continental sink during the Great Qiongzhou Earthquake of 1605. The total everglade area is 5400 m^2, with 2065 m^2 of mangrove forest, and 3335 m^2 of mudflat and shoalwater area. Dongzhaigang has a typical subtropical monsoon marine climate. Annual average air temperature ranges from 23.3 °C to 23.8 °C; Annual average rainfall reaches 1676.4 mm, and average sunlight is 2200 h [15].

2. Materials and methods

2.1. Soil sampling

All soil samples were collected from three different habitats in April of 2006. Site A (110°34′25″E, 19°54′53″N) was bare land without any vegetation; Site B (110°34′21″E, 19°54′52″N) was a *Sonneratia apetala* artificially planted forest; and Site C (110°34′23″E, 19°54′58″N) was a natural mangrove habitat dominated by *Bruguiera gymnorrhiza* with a few individuals of *Ceriops tagal* (Fig. 1). Ten replicated surface soil samples (0–5 cm) with fine plant roots in each sample site were collected using the "parallel leaping method". Usually, about 10 small parallel sub-samples were taken from one site of about 100 m^2, then mixed thoroughly to form a composite sample (about 3 kg) and immediately placed inside a sterilized plastic bag, sealed and transferred back to the laboratory for the following analyses.

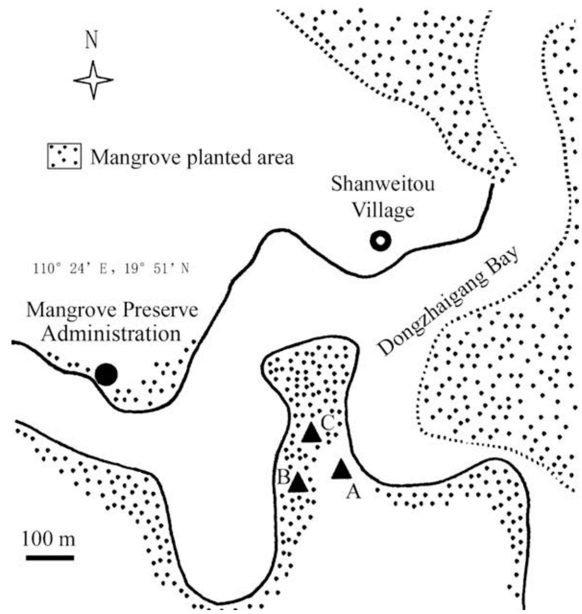

Fig. 1. Map of the location of the sampling sites (A–C).

2.2. Analytical methods

2.2.1. Qualitative investigations of soil sarcodina

All samples were qualitatively analyzed following the "non-flooded Petri dish method" described by Foissner [7]. Live specimens were observed by bright-field and differential interference contrast microscopy (magnifications of ×40–1000; using a Nikon, YS2-H or E800 microscope). The identification of sarcodina species was done mainly according to Shen et al. [28].

2.2.2. Quantitative investigations of soil sarcodina

Fresh soil samples were analyzed using a direct counting method [7]. Air-dried soil samples were based on the modified "most probable number" method (MPN) [27]. According to our preliminary experiment, dilution factors of 10^4–10^6 were adopted.

2.2.3. Determination of physical/chemical parameters of soil samples

The physical/chemical parameters of each soil sample were determined according to standard procedures [30]. Each pooled sample was weighed, dried at 60 °C for 72 h, and re-weighed to determine the moisture content. The pH and salinity values were obtained using a 1:5 soil/water solution by electrical conductivity (automatic, temperature-compensated conductivity meter). Analyses of organic matter were made using the acid-dichromate oxidation method, total nitrogen (TN) using the semi-micro-Kjeldahl method, total phosphate (TP) using the Ammonium molybdate spectrophotometry method, total potassium (TK) using the flame photometer method and SO_4^{2-} using Barium sulfate turbidity. Soil mechanical composition of each dried sample was analyzed by a hydrometer method.

2.3. Data processing and statistical analysis

The Gleason–Margalef formula was used to calculate the sarcodina community diversity index:

$$d = (S - 1)/\ln N$$

where S is the species number; N is the total number of individuals of all species; d is the diversity index.

The dominance was the ratio of the dominant group number and the total species number [21]. The Jaccard formula [34] was used to calculate community similarity analysis of the sarcodina that were obtained from different soil samples:

$$J = c/(a + b - c)$$

where J is the similarity index; a and b are the total number of species in sites 1 and 2, respectively; and c is the number of species common in both sites 1 and 2.

Statistical analyses were done using SPSS 13.0 software.

3. Results

3.1. Community structure of soil sarcodina in the mangrove forest

3 Classes, 5 Orders, 28 Genera, and 40 species of sarcodina were found all total in the soil samples (Table 1). The first dominant group was Amoebida (19 species) with dominance of 0.48, followed by Arcellinida (12 species) with dominance of 0.30 and Heliozoida (five species) with dominance of 0.13. These three Orders together accounted for 90% of the total species identified in the present study. Meanwhile, the rare order was Monothalamia with only one species detected.

3.2. Sarcodina abundance and biodiversity

The abundance of soil sarcodina in three habitats decreased in the order of Site A < Site B < Site C (Table 1), with the highest number in the dried sample from Site C, reaching 1.20×10^6 ind./g; while the lowest one was found in the fresh sample from Site A, with only 9.42×10^4 ind./g. Results also indicated that the Margalef's biodiversity index of sarcodina in both fresh and dried samples varied among the three habitats and followed the order of Site A < Site B < Site C.

3.3. Sarcodina community similarity indexes

The sarcodina community similarity indexes ranged from 0.31 to 0.55 fresh soil (FS) and 0.53 to 0.69 dried soil (DS) between different sites in the mangrove forest, and the index of dried samples was larger than that of fresh ones. The order of similarity index was distributed as follows: Site A and Site C (FS 0.31, DS 0.53) < Site A and Site B (FS 0.50, DS 0.58) < Site B and Site C (FS 0.55, DS 0.69) in both fresh and dried samples.

The sarcodina community similarity indexes between fresh and dried soils from the same sample site ranged from 0.25 to 0.5 in Site A and Site B, and 0.5 to 0.75 in Site C. The order of similarity index was as follows: Site A (0.44) < Site B (0.48) < Site C (0.54).

3.4. Physical/chemical parameters of soil samples in the mangrove forest

The main physical and chemical factors at the three habitats of the mangrove forest are listed in Table 2.

3.5. Relationship between sarcodina abundance and physical/chemical properties

3.5.1. Correlation in fresh samples

3.5.1.1. Relationship between sarcodina abundance and soil moisture, salinity, and pH. According to a multiple stepwise regression analysis, the sarcodina abundance (ind./g) was negatively correlated with pH but positively with soil moisture and salinity (Table 3). Among these three factors, the most significant one for the sarcodina abundance was pH, followed by soil moisture, and the last was salinity.

3.5.1.2. Relationship between sarcodina abundance and soil OM, TN, TP, TK and SO_4^{2-}. Based on a multiple stepwise regression analysis, the sarcodina abundance was positively correlated with OM, TN, TP and SO_4^{2-} ($P < 0.01$), but negatively correlated with TK ($P < 0.05$) (Table 3). The effect of TN on the sarcodina abundance was the highest, followed by TP, OM and SO_4^{2-}, while TK showed the least effect.

3.5.2. Correlation in dried samples

3.5.2.1. Relationship between sarcodina abundance and soil moisture, salinity, and pH. There was a significant negative correlation between sarcodina abundance and pH; whereas, a positive correlation was found with moisture and salinity in dried samples (Table 3). Also, the most significant factor for the sarcodina abundance was pH, followed by soil moisture, while salinity was the least.

Table 1
Soil sarcodina species found from three different habitats in the mangrove forest.

Sarcodina species	Bare land		Man-made mangrove		Natural mangrove	
	FS	DS	FS	DS	FS	DS
Acanthamoeba castellanii					+	
Acanthamoeba polyphaga					+	+
Acanthocystis myriospina	+	+	+		+	
Actinophaerium eichhorni		+		+		+
Actinophrys sol				+	+	+
Amoeba gorgonia		+		+		
Amoeba limicola		+	+	+	+	+
Amoeba sol						+
Arcella discoides	+	+	+	+		+
Arcella gibbosa			+	+	+	+
Arcella vulgaris		+	+	+	+	+
Cashia angelica	+		+		+	
Cashia limacoides			+	+	+	+
Centropyxis constricta		+		+		+
Corythion dubium	+	+				+
Cyclopyxis arcelloides		+		+		+
Cyphoderia trochus				+		+
Difflugia acuminata			+			
Difflugia avellana	+	+	+	+	+	+
Difflugia corona	+	+	+	+	+	+
Difflugia globulosa			+	+	+	+
Difflugia gramen		+		+	+	+
Filamoeba nolandi					+	+
Glaeseria mira	+		+	+		+
Lesquereusia epitominum			+	+	+	
Lieberkühnia wagneri	+	+	+			+
Mayorella cypressa				+	+	+
Naegleria gruberi		+		+		+
Nebela dentistoma						+
Platyamoeba placida		+	+	+	+	+
Polymyxa palustris	+	+	+	+	+	
Raphidiophrys elegans	+	+	+	+	+	+
Raphidiophrys viridis		+	+	+	+	+
Rosculus ithacus		+		+	+	+
Saccamoeba lucens		+				
Trichamoeba villosa	+	+	+	+	+	+
Trinema enchelys				+	+	+
Vahlkampfia vahikampfia	+	+	+	+	+	+
Vannella miroides				+		+
Vannella platypodia	+	+		+	+	+
Total species (S)	13	23	20	29	25	32
Abundance ($\times 10^3$ ind./g)	94.2	117	227	387	695	1204
Margalef's diversity	1.05	1.89	1.54	2.18	1.78	2.21

+: sarcodina species appeared in the samples; FS: means fresh soil samples; DS: means dried soil sample.

Table 2
Main physical/chemical factors at the three habitats of the mangrove forest.

Environmental factors	Bare land	Man-made mangrove	Natural mangrove
pH	6.51	6.16	4.77
Water percentage (%)	24.2	35.6	45.4
Salinity	19.243	25.695	24.288
OM (g/kg)	31.267	42.087	150.276
TN (g/kg)	1.345	0.831	3.962
TP (g/kg)	0.511	0.568	0.626
TK (g/kg)	13.094	11.953	11.652
SO_4^{2-} (g/kg)	2.001	2.895	5.503

3.5.2.2. Relationship between sarcodina abundance and soil OM, TN, TP, TK and SO_4^{2-}. The relationships between sarcodina abundance and soil chemical properties, namely OM, TN, TP, SO_4^{2-} and TK, were all positive except TK, and followed the same trend as that in fresh samples (Table 3). Among these five factors, TN was most significant, followed by OM, TN, SO_4^{2-}, while the effect of TK was the least.

As stated above, the sarcodina abundance was significantly negatively correlated with pH, but the relationships with soil moisture and salinity were positively correlated. The most significant of these three factors for sarcodina abundance was pH, followed by soil moisture, and the last was salinity. Among the variables OM, TN, TP, SO_4^{2-} and TK, of different soil chemical properties, sarcodina abundance positively correlated significantly with TN, TP, OM and SO_4^{2-}, but negatively correlated with TK. Among these five factors, TN was the most significant, followed by OM and TN, SO_4^{2-}, while the effect of TK was the least.

4. Discussion

4.1. Sarcodina community composition

4.1.1. Analysis of sarcodina communities in different faunal habitats

Compared with other published studies on the sarcodina community, soil sarcodina community composition in Dongzhaigang, Hainan, China was similar to that of the typical zones in China, with both Amoebina and Arcellinida as dominance groups, though their dominant species were different (Table 4). Foissner emphasized the important function that the shell serves in enabling testate amoebae to conserve their body water [7]. That is why Arcellinida was the first dominant group in typical zones of China [22]. However, this absolute predominance group in terrestrial habitats was far less than Amoebina, one group of the naked amoeba in the typical mangrove forest in Dongzhaigang. This observation may be related to the typical characteristic of the ecological environment in mangrove forests as compared to other locales in China.

Table 3
Relation comparisons between soil sarcodina abundances and environmental factors in mangrove soil and terrestrial soils.

Environmental factors	Correlation degree between sarcodina's abundance and environmental factors		
	Habitat in mangrove forest		Habitat in typical zones of China
	FS	DS	
pH (p)	−[a] 1[b]	− 1	+ 2[c]
Water percentage (W)	+ 2	+ 2	+ 1
Salinity (S)	+ 3	+ 3	−[d]
OM (OM)	+ 2	+ 2	+ 1
TN (TN)	+ 1	+ 1	+ 2
TP (TP)	+ 3	+ 3	+ 3
SO_4^{2-} (SO)	+ 4	+ 4	−
TK (TK)	− 5	− 5	− 4

[a] +: positive correlation, −: negative correlation.
[b] Correlation degree (1 means the largest effect of this factor on sarcodina abundance, followed to a lesser degree by 2, 3, in sequence).
[c] Number 2 is temperature.
[d] −: no data.

Table 4
Comparison of soil sarcodina dominant groups and rare groups between Dongzhaigang mangrove and typical zones of China.

Groups	Dongzhaigang mangrove	Soils in typical zones of China
The 1st dominant groups	Amoebina (0.48)	Arcellinida (0.46)
The 2nd dominant groups	Arcellinida (0.30)	Amoebina (0.30)
The 3rd dominant groups	Heliozoa (0.13)	Gromiida (0.15)
The rare groups	Monothalamia	Heliozoa and Leptomyxida

Number in bracket means degree of dominance.

A major difference between the mangrove forest soil and the other terrestrial soils is that the drought in the general terrestrial soils may be permanent. The mangrove forest is substantially affected by seawater tidal influences, especially the forest's soil moisture content that depends on flood tides, which can help to maintain the soil's wetness. Therefore, the testate amoebae have no obvious advantages, through their water conservation as shell-bearing species, in the mangrove environment; though elsewhere their water conservation ability can significantly enhance their survival during drought conditions. By contrast, the naked amoebae were found to be the first dominant group in the mangrove soil. This may be attributed to their capacity for rapid encystment and excystment, short reproductive cycle, and hence relative facile adaptive capacity to environmental changes [6]. With respect to testate amoebae, Volz [33] discovered that excystment appeared several days after rewetting the dry soil samples and that the excystment time increased gradually with drying time. This phenomenon is called postponed excystment [21], which can be explained as an adaptive mechanism to withstand severe and extended drought conditions when available moisture is insufficient to sustain growth and where too rapid excystment might lead to fatal dehydration. This is a defense mechanism originating from long-term evolution and adaptation of the protozoa. Ekelund and Rønn [6] considered that testate amoebae are K-selected; whereas, most other protozoa (smaller naked amoebae and flagellates for example) are *r*-selected, especially the groups that feed on bacteria. This K-selection may enable testate amoebae to adapt to the permanent drought climate of general terrestrial soils and helps to explain why testate amoebae are the first dominant group of the sarcodina in our analyses.

4.1.2. Analysis on the differences of soil sarcodina composition among three mangrove habitats

Although both the total species number and the abundance are quite different, the dominant and rare groups in the three habitats are almost the same, all within the first dominate group of Amoebida. It further illustrates that the difference of sarcodina dominant groups mainly depended on the difference between mangrove forest soil and terrestrial soils.

The physical/chemical parameters, such as soil texture, moisture and the litter stock differed greatly because of different vegetation, although the three habitats were separated by only a small distance (100 m), and there were no differences in hypsography. The topsoil of natural mangrove forest soil was quite loose and could provide habitats for more sarcodina; whereas, prior research [5] has shown that in the bare land, the soil is more compact with much smaller pore size and the sarcodina distribution is more restricted.

Secondly, soil moisture content in the natural mangrove forest was significantly higher than that of man-made mangroves and in bare land. That is why the mangroves have the highest species diversity. Ning and Shen [22], using multiple regression stepwise analyses, analyzed the relationships between soil protozoa abundance and soil moisture, temperature and pH of typical zones in China and found that the most significant factor was the soil moisture. Thirdly, abundant deposits of residues and litter were found in bottom mud in the natural mangrove. The litter not only provided enough organic matter, humus and other nutrients for soil sarcodina, but also greatly increased available area for sarcodina movement (especially for naked amoebae). Lastly, the synergic effect of several factors such as soil texture, moisture and litter content, together with soil pH and salinity, can enhance the differences in soil sarcodina distribution among different mangrove habitats.

4.2. Relationship between soil sarcodina encystment/excystment and mangrove environmental changes

Encystment and excystment are the common characteristics of protozoa living in various kinds of ecosystems, especially for the soil protozoa [21]. Encystment is an effective strategy for soil protozoa to resist environmental pressure (starvation, desiccation, nutritional deficiency, extreme environment, etc.) or in some cases is a stage of the reproduction process [6]. The reproductive cysts of certain amoebae are resistant to digestion in the animal alimentary canal and are released in the feces, thus enhancing the dispersal of their offspring in soil, air and water [3]. Sarcodina cysts have extremely high biological stress tolerance; Goodey [10] found that they could remain viable after 47 years air dried; and they are widespread in soil, water and even in the dry air. Kingston and Warhurst [13] reported that the density of sarcodina cysts was 9.1/m^3 air, and Lawande [14] isolated many kinds of sarcodina from a sand storm in Nigeria.

As for soil sarcodina, starvation and shortage of certain growth factors (vitamins, for example) are the main factors favoring encystment among a wide range of other environmental variables. Moreover, encystment can also occur when soil protozoa incur density dependent stress such as congestion, even though they have enough food [6]. Abiotic factors, including desiccation, high salinity, extreme pH, and anaerobic conditions, are important factors promoting encystment [31]. Excystment occurs rapidly when the environment is recovering, such as adequate available moisture and oxygen, enough food and sufficient soil organic matter [31]. Consequently, soil sarcodina encystment and excystment are closely linked with mangrove environmental changes.

4.3. Variation analysis of sarcodina abundance

4.3.1. Variation analysis of soil sarcodina abundance in fresh and dried samples

Soil sarcodina abundance in the three habitats of the present study declined in the order of Site A < Site B < Site C in both the fresh and the dried samples. The abundance in dried samples was much higher than that in fresh samples. This variation was related to the specific microhabitat [32], as well as possible characteristics of the direct counting and culture method.

Fresh samples contained more moisture and, therefore, most of their sarcodina were likely in a trophic stage. Thus, the direct counting method was suitable to quantify these active protozoa. This method avoided counting protozoa that are in a resting stage. However, the direct counting method could under-estimate total sarcodina numbers because some of them are very small and cannot be readily separated from the soil particles [27]. On the other hand, most sarcodina in a resting stage were capable of reviving, and these individuals were reactivated when the dried sediment was wetted and the culture method was used. Hence, sarcodina abundance obtained by our two methods accounted for the sarcodina abundance in fresh samples as well as the individuals in a resting stage. This is the most likely reason why the sarcodina abundance in dried samples was much greater than that in fresh samples – it included the excysted as well as active stages.

4.3.2. Relationship between sarcodina abundance and soil physical/chemical factors

In order to analyze the extent of influence of soil physical/chemical factors on sarcodina abundance, we compared the relationships between sarcodina abundance and soil physical/chemical factors of typical zones in China (Table 3). Results showed that the sarcodina abundance was distinctly correlated with pH, moisture, OM, TN, TP and TK, and the degree of correlation of each pair was different. Sarcodina abundance was negatively correlated with pH in the mangrove forest, but was quite the reverse in terrestrial habitats.

The optimal pH value for nutrient absorption by plants is from 4.5 to 5.5. Therefore, under the salty condition in mangrove forest habitats, the mildly acidic pH can favor enhanced plant nutrition and hence release of organic nutrients supporting protozoan growth as well as creating a more favorable osmotic balance for the protozoa. Furthermore, weak acidic conditions favor sarcodina growth, which can be restrained by higher or lower pH conditions. Owing to the influence of acid rain, terrestrial soils are acidified and they restrain the growth and reproduction of sarcodina greatly, which may explain why the sarcodina abundance was positively correlated with the pH of terrestrial soil, but negatively correlated with the milder mangrove soil pH.

Little research has been reported about the effect of multi-physical/chemical factors on sarcodina abundance, though numerous have been published about single-physical/chemical factors. The optimal pH value range of soil testate amoebae is narrow [7], and likely equally so for non-testate amoebae. Take the non-shelled amoebae, *Vampyrellids,* for example. The highest abundance appears at pH 7.0, and their vital movements are restrained at pH 4.0 or 8.5. In addition, high OM content, high levels of dissolved organic carbon (DOC) in mangroves and samples with fine roots were usually accompanied with large protozoan abundance [1,8,19], which may account in part for the relatively high abundances of sarcodina reported here.

4.4. The sarcodina community similarity indexes of different mangrove habitats

The soil sarcodina similarity indexes between habitats, ranging from moderately dissimilar to moderately similar, validated soil protozoa diversity in microhabitats and reflected the pronounced effect of vegetation and environmental factors on soil protozoa [32]. Community distribution of soil sarcodina in the man-made mangrove fell in between the bare land and the natural mangrove. Moreover, the man-made mangrove was a transition zone from bare land to natural mangrove. Accordingly, developmental stages of this sample area, viz. the degree of vegetation restoration, can be concluded from the soil sarcodina similarity index of these habitats. Ning and Shen [22] explained the important significance of community similarity indexes and assessment of biodiversities as reflecting the complexity and stability of communities themselves as well as the ecological or environmental quality. The results of the present study also validated this point.

5. Research prospect

Dongzhaigang national nature reserve is presently the largest and best mangrove nature reserve for natural environment conservation in China. As a fast growing tree species, *S. apetala* was a relatively recent introduction from Bengal. Owing to its superior cold resistance and rapid growth compared to other species, the *S. apetala* forest has been artificially planted extensively in Dongzhaigang [16]. However, this *S. apetala* is nonviable in most locations with bare land (about 1/3 of the total everglade). This may be explained by differences of the soil physical/chemical properties and reflected as well in the distribution variation of sarcodina community. Biodiversities of different communities not only reflect the complexity and stability of communities themselves, but the ecological or environmental quality of their habitats as well [22]. As the largest, or second largest group of soil protozoa, the sarcodina have immense influence on plant growth [22].

Based on a comparative analysis of all of our results, we conclude that the planting of mangrove flora, and its consequent soil enrichment in part through support of rich and diverse protozoan communities, is a practicable method for bare land reestablishment. That is, to rebuild a "soil protozoa–bacteria–*S. apetala* forest" system by applying humus from natural mangrove forests as biofertilizer, and thus to create a favored environment for both flora and microorganism communities to flourish.

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