The community structure of soil Sarcodina in Baiyun Mountain, Guangzhou, China

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ABSTRACT

Community structures of soil Sarcodina in 7 different habitats within Baiyun Mountain in Guangzhou, China were investigated with qualitative and quantitative analyses. The abundance, dominance, species diversity and community similarity index of soil sarcodina with different physicochemical parameters were comparatively analyzed. A total of 67 species of sarcodina belonging to 4 Super-groups, 6 First ranks and 14 Second ranks were identified. The first dominant group was Tubulinea, followed by Flabellinea, with dominance of 59.7% and 13.4%, respectively. The highest abundance of sarcodina appeared in autumn of Site 5, reaching $1.20 \times 10^3$ ind g$^{-1}$; the lowest in spring of Site 2 with $1.73 \times 10^1$ ind g$^{-1}$. Margalef’s biodiversity index ranged from 1.26 (winter of Site 6) to 2.51 (summer of Site 1). Statistical analyses showed the sarcodina abundance was positively correlated with organic matter, soil moisture, soil pH, ammonia nitrogen and total nitrogen, but the correlation coefficient of total potassium was negative. Total phosphorus, nitrate nitrogen and sulphate showed no significant effect on sarcodina abundance in the present study.

1. Introduction

Sarcodina, especially testate amoebae, occur in many types of terrestrial habitats and are a dominant group of soil protozoa for their large biomass and production reaching that of earthworms [18,34]. In this research, we use the term sarcodina in the classical sense to include all amoeboid-like organisms including naked and testate amoebae, although we recognize that this is no longer accepted as a valid group in modern taxonomic nomenclature [1]. As a significant predator of bacteria and epiphytic microorganisms, soil sarcodina play a major role in nutrient mineralization, energy flow, regulation of microflora populations, and maintain or increase soil fertility and productivity by releasing soluble nutrients in their phagocytic activity [2,5,9,20,24,37].

There is further well-established evidence that soil sarcodina are valuable bioindicators in a variety of terrestrial habitats because their tiny dimensions, short generation time and high abundance in such habitats [3,14,16]. On the other hand, their biodiversity has been considered similar as a biological index reflecting the stability of natural and disturbed soil systems [10,14]. However, sarcodina in soil environments are less well understood compared to ciliates and flagellates and other organisms [2,3]. Two main reasons may contribute to this. Firstly, amoeboid organisms are notoriously difficult to identify to the level of species [6] and it is accepted that they are a relatively under-studied group because of nomenclatural and taxonomical problems [17]. Secondly, the heterogeneous structure of terrestrial habitats [5] results in the occurrence of numerous and diverse microhabitats harboring a large variety of species, many of which require special efforts [32].

As, historically, soil sarcodina have been under-sampled on a global scale, and additional sampling has, in the past, revealed the presence of endemic species in geographically distant locations [6]. Therefore, much further work studying a wide range of local faunas (and accurately identifying the species detected) is required to study their community characteristics for a better understanding of soil sarcodina species distribution on a global scale. Although terrestrial sarcodina have been studied to some extent in other countries [2,5,6,34,36], little information is available about soil sarcodina community, and there is no recent work on the relationship between soil sarcodina ecological characteristics and physicochemical properties in China, especially in the south subtropical hilly area. The present study specifically aims at analyzing the species abundance, dominance, biodiversity and community similarity index of soil sarcodina at 7 different habitats within Baiyun Mountain, as well as effects of soil physicochemical parameters on sarcodina abundance.
2. Materials and methods

2.1. Description of the Baiyun Mountain

Baiyun Mountain (23°09’–23°13’ N, 113°16’–113°19’ E), a branch range of Jiulian Mountain of Guangdong, is made up of more than 30 hills and covers an area of 32.27 km² with its highest peak, Moxing Summit, measuring 382 m a.s.l. The regional soil of Baiyun Mountain is latosolic red soil and the content of soil organic matter is medium; nutrient elements like nitrogen (N) and phosphorus (P) are scarce and soil fertility is poor. Mean annual temperature is 21.4 °C, mean annual precipitation is 1,689.3–1,876.5 mm, belonging to the southern subtropical monsoon climate. Covered by natural broad-leaved secondary forest, artificial Pinus massoniana forest and mixed coniferous broad-leaved forest, Baiyun Mountain possesses a large variety of natural resources and rich vegetation types. With the forest coverage of more than 95%, Baiyun Mountain absorbs 2800 tons of carbon dioxide and emits 2100 tons of oxygen every day, adequate for nearly 3 million people to breathe freely. So it is called the “Lung of Guangzhou”, having particular important ecosystem function [35].

2.2. Sampling and sample treatment

All samples were collected quarterly during September 2005 and June 2006 in the Baiyun Mountain. In total, 7 habitats, 4 on the southern slope and 3 on the northern, were selected as sampling sites according to the elevation and the main vegetation characteristics (Fig. 1).

Sampling was conducted according to the “parallel leaping method” described by Foissner [14]. Briefly, ten replicated surface soil samples (0–5 cm soil depth), including litter, surface soil and fine plant roots, were collected randomly from an area of up to 100 m² in each site, and immediately mixed together. Samples were then sealed in sterile plastic bags and then brought back to the laboratory for the following analytic determination.

2.3. Analytical methods

2.3.1. Analysis of physicochemical parameters of soil samples

The physicochemical parameters of each soil sample were determined following standard procedures [23]. Each pooled sample was weighed, then dried at 60 °C for 72 h, and re-weighed to determine soil moisture (SM) content. The pH values were obtained by using a 1:5 soil/water solution with electrical conductivity (automatic, temperature-compensated conductivity meter). The content of organic matter (OM), total nitrogen (TN), total phosphorus (TP), total potassium (kalium, TK) and sulphate (SO₄²⁻) were analyzed by the acid-dichromate oxidation method, the semi-micro Kjeldahl method, the ammonium molybdate spectrophotometry method, the flame photometer method and barium sulphate turbidity, respectively. Determination of ammonia nitrogen (AN) was processed by the indophenol blue colorimetric method, nitrate nitrogen (NN) by the copperized cadmium reduction method. Soil mechanical composition of each dried sample was analyzed by a hydrometer method.

2.3.2. Qualitative determination of soil sarcodina

All samples were processed with the non-flooded method described by Foissner [14,15] after air-dried for at least one month, but using a larger amount of soil to increase the number of species found. Live specimens were observed by bright-field and differential interference contrast microscope (magnifications, ×100–1000; Nikon, YS2-H or E800). Taxa of sarcodina species were determined according to Adl et al. [1] Lee et al. [21] and Shen et al. [31].

2.3.3. Quantitative determination of soil sarcodina

The quantitative analysis of soil sarcodina was based on the modified “most probable number” (MPN) method [11,22] after the soil samples were air-dried for one month. According to the preliminary experiment, dilution degree of 10³–10⁵ was adopted.

2.4. Data processing and statistical analyses

The Gleaso-Margalef formula was used to calculate sarcodina community diversity index:

\[ d = (S - 1)/\ln N \]

where \( S \) is species number; \( N \) is 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 [22]. The Jaccard formula [25] was used to calculate community similarity index of the sarcodina that were from different soil samples:

\[ J = c/(a + b - c) \]

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

The relationships between sarcodina abundance and single-physicochemical-factors were examined bivariate correlation. The stepwise regression analysis was conducted to reveal the integrated effect of multi-factors on the sarcodina abundance and the correlation degree between these factors and sarcodina abundance. The hierarchical cluster was conducted to analyze the similarity of physicochemical characters of 7 sampling sites. All statistical analyses were performed using SPSS 11.5 software.

Fig. 1. Location and distribution contour map (unit: m above sea level) of soil sampling sites (1–7). Elevation and mean temperature of Sites 1–7 were 88 m and 25 °C, 163 m and 25.4 °C, 269 m and 28.2 °C, 343 m and 26.8 °C, 269 m and 26 °C, 166 m and 23.1 °C, 82 m and 22 °C, respectively.
The main physicochemical parameters at the 7 investigated sites of the Baiyun Mountain are listed in Table 1. Most of physicochemical factors, such as SM, OM, TN, AN, are much different at different sampling sites. Others like pH, TK, and SO4 showed no significant differences.

The relations between sarcodina abundance and soil physicochemical parameters were assayed by bivariate correlation and multiple stepwise regressions. In bivariate correlation, OM (P = 0.000), SM (P = 0.007), AN (P = 0.010), TN (P = 0.024) and TK (P = 0.032) affected sarcodina abundance significantly, whereas other factors had no significant effects (P > 0.05). However, in multiple regression, the impact of soil pH became significant. According to a multiple stepwise regression analysis, sarcodina abundance was negatively correlated with TK (P = 0.032); whereas a positive correlation was found with the contents of OM (P = 0.000), SM (P = 0.002), pH (P = 0.036), AN (P = 0.010) and TN (P = 0.024). Among these factors, the most significant one was OM (R² = 0.920), followed by SM (R² = 0.908), pH (R² = 0.908), AN (R² = 0.723) and TN (R² = 0.608), while the effect of TK (R² = 0.560) was the least. On the other hand, TP, NN and SO4 showed no significant effect (P > 0.05) on sarcodina abundance in the present study.

### 3. Results

#### 3.1. Physicochemical parameters

The main physicochemical parameters at the 7 investigated sites of the Baiyun Mountain are listed in Table 1. Most of physicochemical factors, such as SM, OM, TN, AN, are much different at different sampling sites. Others like pH, TP, TK and SO4 are slightly different. The hierarchical cluster dendrogram revealed that soil physical and chemical properties at sites 2 and 7 are most similar, and that of sites 4 and 5 are disparate from other sites (Fig. 2).

#### 3.2. Community structure of soil sarcodina

A total of 67 species of sarcodina, belonging to 4 Super-groups, 6 First ranks and 14 Second ranks were identified in all soil samples in our study. Among these 67 species, 28 species were found at half or more of the sampling points and the most prevalent species occurring at all 7 sites were Centropyxis hirsuta, Cyclopyxis eury-stoma, Lamtopyx callistoma, Plagiopyxis minuta, Propagpyxis nuda, Trinema enchelys and Vahlkampfia vahlkampfia. The percentages recorded for each major taxonomic group, Super-group and First rank, are as follows: Amoebazoa, Acanthamoebidae (45); Acanthamoebidae, Flabellinea (13.4); Amoebazoa, Tubulinea (59.7); Chromalveolata, Stramenopiles (1.5); Excayata, Heterolobosea (9.0) and Rhizaria, Cercozoa (11.9). The first dominant group was Tubulinea and it contributed about 60% of the total species identified.

#### 3.3. Sarcodina abundance and diversity

The highest number of sarcodina was found in autumn at Site 5, reaching 1.20 × 10^5 ind. g^-1, where the highest annual mean abundance of sarcodina was found. On the contrary, Site 2 in the spring had the lowest abundance, only 1.73 × 10^3 ind. g^-1 and the lowest annual mean abundance. Margalef’s biodiversity index of all samples ranged from 1.26 (winter of Site 6) to 2.51 (summer of Site 1) and results of the ecological analysis indicated that Site 2, 6 and 7 had a much lower biodiversity than other sites (LSD-test, P < 0.05).

### 3.4. Sarcodina community similarity indices

Jaccard coefficients among 7 habitats/sites ranged from 0.351 (Site 5 and 6) to 0.567 (Site 2 and 7), and the details of each pair are listed in Table 2. Results showed that the sarcodina community similarity indices among the 7 sites were moderately dissimilar (0.25 < J < 0.50) to moderately similar (0.50 < J < 0.75).

### 3.5. Relationship between sarcodina abundance and soil physicochemical parameters

The relations between sarcodina abundance and soil physicochemical parameters were assayed by bivariate correlation and multiple stepwise regressions. In bivariate correlation, OM (P = 0.000), SM (P = 0.007), AN (P = 0.010), TN (P = 0.024) and TK (P = 0.032) affected sarcodina abundance significantly, whereas other factors had no significant effects (P > 0.05). However, in multiple regression, the impact of soil pH became significant. According to a multiple stepwise regression analysis, sarcodina abundance was negatively correlated with TK (P = 0.032); whereas a positive correlation was found with the contents of OM (P = 0.000), SM (P = 0.002), pH (P = 0.036), AN (P = 0.010) and TN (P = 0.024). Among these factors, the most significant one was OM (R² = 0.920), followed by SM (R² = 0.908), pH (R² = 0.908), AN (R² = 0.723) and TN (R² = 0.608), while the effect of TK (R² = 0.560) was the least. On the other hand, TP, NN and SO4 showed no significant effect (P > 0.05) on sarcodina abundance in the present study.

### 4. Discussion

#### 4.1. Sarcodina community composition

Although both the total species number and abundance are different, the dominant groups in the 7 habitats are the same, all with Tubulinea of which the species composition were mainly testate amoebae, as the first dominant group. This accords with other terrestrial regions of the world [43, 22, 28], though their dominant species were different. Ekelund and Rønne [12] considered that testate amoebae are K-selected; whereas, most other Protzoa (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. On the other hand, Foissner [14] emphasized the important function that the shell serves in enabling testate amoebae to conserve their body water, and that is why Tubulinea was the first dominant group in most terrestrial habitats.

4.2. Sarcodina community similarity indices of different habitats

Protozoa inhabit environments which are heterogeneous in time and space and their distributions in field situations are patchy [5]. The soil sarcodina communities between the 7 habitats ranged from moderately dissimilar to moderately similar. However, the sarcodina communities between Baiyun Mountain and the terrestrial habitats of other regions were even much lower, and appeared extremely dissimilar to moderately dissimilar (Table 3). This not only validated soil protozoa diversity in microhabitats, but reflected the pronounced effect of vegetation and environmental factors on soil protozoa communities [33].

4.3. Sarcodina abundance and biodiversity

Sarcodina have high abundance that usually reaches $10^5$ ind. g$^{-1}$, second only to flagellates in soils, globally. In many zones such as the middle tropical zone, northern tropical zone, warm temperate zone and temperate zone, the abundance of soil sarcodina is even higher than that of flagellates [22,28]. However, relatively low abundances of soil sarcodina were reported here, only $10^3$–$10^5$ ind. g$^{-1}$, which may be related to the regional soil characteristics of Baiyun Mountain.

Ning and Shen [28] 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. Vegetation and soil physicochemical parameters affect the characteristics of the sarcodina fauna, therefore, the community characteristics of soil sarcodina can indicate the environmental status in which they reside. The lower biodiversities at sites 2, 6 and 7 might imply that these 3 sites/habitats are more unfavorable for soil sarcodina or have suffered the frequent human interference.

4.4. Relationship between sarcodina abundance and soil physicochemical parameters

Each soil habitat has its own distinctive "biological space" with regards to the level of microbial biomass [30]. The abundance and species of soil organisms depend on the type and physicochemical characteristics of the soil [29]. On the other hand, soil habitats are complicated micro-ecological environments and their characteristics are determined by the interaction of the main physicochemical factors. Between them, the architecture of the habitable soil pore network which is determined by soil texture and structure, and the moisture in the soil have considered as the two most important factors in the regulation of soil protozoan populations. Their interaction establishes the basic environmental surrounding for soil sarcodina [12]. However, little research has been reported about the effect of multi-physicochemical-factors on sarcodina abundance, though the effects of single-physicochemical-factors of soil parameters such as soil moisture, pH, OM and nutrition salt (TN, TP and TK) on sarcodina abundance have been reported to some extent [20,24,26].

Soil habitat is a complex system where so many inorganic, organic, and biological factors coexist. In the present study, the results of an integrated effect of multi-factors on soil sarcodina abundance performed by the stepwise regression analysis reveals a relationship between abundance and soil pH that was not revealed by bivariate correlation. This might due to the coexistence and interactions between many soil physicochemical factors. The integrated effect of multi-factors on the soil sarcodina of Baiyun Mountain showed that the correlations between sarcodina abundance and most physicochemical factors were similar to that in other terrestrial habitats [22,28]. Liao et al. [22] reported that sarcodina abundance was negatively correlated with soil pH, but this was quite the reverse in our present study. This difference could be related to the more salty condition in mangrove forest habitats. 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.

There is little information about the relationship between sarcodina abundance and soil AN and NN content, though TN has been reported to possibly effect soil protozoa abundance [22,28]. We observed that AN showed more significant effect than TN and NN showed no significant effect on sarcodina abundance, indicating the content of AN could actually have more effect on the sarcodina abundance than that of NN when considering the effect of the TN. The regional soil of Baiyun Mountain is latosolic red soil with shortage of certain nutrient elements, which may account in part for the little correlation between sarcodina abundance and TP and SO$_4^{2-}$ reported here; though Liao et al. [22] reported that sarcodina abundance correlated significantly with TP in air-dried soil samples of Dongzhaihang Mangrove forest.

4.5. Notes on the most probable number (MPN) method

We recognize that the most probable number (MPN) method might under-estimate total protozoan numbers if the organisms were killed during the setting up of the cultures, or if they were unable to grow on the food offered [8,19]. It is also possible that this method cannot reactivate all cysts and the species present when they are less numerous and provides no information on the relative abundances of different genera or species, also, this method cannot determine the standing stock at the time of sampling. To obtain a relative most probable abundance, we think it is sufficient since the MPN method is commonly used for enumerating soil protozoa. And it really has no problem for correlation and comparative analysis of relative most probable abundances if all the dates are obtained from the same quantitative method in the present study.
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References


