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Significant seasonal variations of microbial community in an acid mine drainage lake in Anhui Province, China☆

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ABSTRACT

Acid mine drainage (AMD),characterized by strong acidity and high metal concentrations, generates from the oxidative dissolution of metal sulfides, and acidophiles can accelerate the process significantly. Despite extensive research in microbial diversity and community composition, little is known about seasonal variations of microbial community structure (especially micro eukaryotes) in response to environmental conditions in AMD ecosystem. To this end, AMD samples were collected from Nanshan AMD lake, Anhui Province, China, over a full seasonal cycle from 2013 to 2014, and water chemistry and microbial composition were studied. pH of lake water was stable (~3.0) across the sampling period, while the concentrations of ions varied dramatically. The highest metal concentrations in the lake were found for Mg and Al, not commonly found Fe. Unexpectedly, ultrahigh concentration of chlorophyll *a* was measured in the extremely acidic lake, reaching 226.43–280.95 μg/L in winter, even higher than those in most eutrophic freshwater lakes. Both prokaryotic and eukaryotic communities showed a strong seasonal variation. Among the prokaryotes, *"Ferrovum"*, a chemolithotrophic iron-oxidizing bacterium was predominant in most sampling seasons, although it was a minor member prior to September, 2012. Fe^{2+} was the initial geochemical factor that drove the variation of the prokaryotic community. The eukaryotic community was simple but varied more drastically than the prokaryotic community. Photoautotrophic algae (primary producers) formed a food web with protozoa or flagellate (top consumers) across all four seasons, and temperature appeared to be responsible for the observed seasonal variation. *Ochromonas* and *Chlamydomonas* (responsible for high algal bloom in winter) occurred in autumn/summer and winter/spring seasons, respectively, because of their distinct growth temperatures. The closest phylogenetic relationship between *Chlamydomonas* species in the lake and those in Arctic and Alpine suggested that the native *Chlamydomonas* species may have been both acidophilic and psychrophilic after a long acclimation time in this extreme environment.

1. Introduction

Metalliferous mining activity has produced large quantities of sulfidic mineral wastes (such as pyrite or arsenopyrite) all over the world. These mine wastes are continuing sources of environmental contamination that may persist for decades and even centuries after mining activity ceased. One of the most serious ecological problems caused by mine wastes is the occurrence of acid mine drainage (AMD), which is usually characterized by low pH (below 3.5) and high levels of iron, toxic metals such as aluminum, manganese, lead, cadmium, and zinc, as well as metalloids, of which arsenic is generally of the greatest concern (Bruneel et al., 2011).

AMD generates when metal sulfide minerals, particularly pyrite (FeS_2), come in contact with oxygen and water. The overall reaction can be written as follows:

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

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$Fe^{3+}$is the predominant oxidant at low pH and limits the overall reaction rate. The abiotic oxidation rate of Fe^{2+} to $Fe^{3+}$is slow below pH 3. Acidophilic organisms, however, can generate energy by converting Fe^{2+} to Fe^{3+} in acidic environment. Thus, the rates of pyrite oxidation and AMD acidification are greatly increased in the presence of iron-oxidizing species such as *Acidithiobacillus ferrooxidans* (Johnson, 1998). Many researchers have reported microbial community structures in various acidic environments (Méndez-García et al., 2015). These studies provide comprehensive insights into the role of acidophilic microorganisms in generating AMD and mediating elemental cycling in acidic ecosystems.

Despite these recent advances, seasonal shifts of acidophilic community in AMD environment are poorly understood (Tan et al., 2009; Edwards et al., 1999; Volant et al., 2012), because most studies are usually carried out with only a few samples from one season. Furthermore, prokaryotic microorganisms are usually the focus and reports on acidophilic eukaryotes are scarce. However, microbial eukaryotes are usually present in some AMD environments, and the diversity and abundance of eukaryotes could be even higher than those of prokaryotes. In these cases, acidophilic eukaryotes could play a critical role in acidic ecosystems (Zettler et al., 2002).

The objective of this study was to examine seasonal variations of both prokaryotic and eukaryotic acidophiles in an AMD lake in Anhui Province, China. We hypothesize that these two communities may jointly contribute to acid generation and elemental cycling in such extreme environment. Our results expand the current knowledge of acidophilic microbial community structure and function in response to geochemical conditions in AMD environment.

2. Materials and methods

2.1. Study site and sample collection

The Nanshan pyrite mine is located in eastern Anhui Province, China (Fig. 1). The region has a warm and humid subtropical monsoon climate, with an annual precipitation around 1100 mm and an annual mean temperature of 16 °C. The average daily temperature is highest in July (~28 °C) and lowest in January (~3 °C).

Open cast pyrite mining activities in the Nanshan mine started in 1910s and waste products were disposed of in a nearby refuse dump. Pyrite in exposed waste ores is oxidized by acidophiles and large amounts of metal ions and H_2SO_4 are released, resulting in the formation of an AMD lake since 1970s. The AMD lake is approximately 540 m long, 240 m wide, and 15 m deep, with an area of about 1.0×10^5 m^2 at present.

To investigate the seasonal variations of planktonic acidophilic community in the lake, AMD samples were collected on Sep 17th, 2013 (autumn), Dec 23rd, 2013 (winter), May 12th, 2014 (spring), and Aug 10th, 2014 (summer). We recognized that the physical hydrology of the Nanshan lake was important to the study of the horizontal and vertical variations of geochemical parameters and microbial community. Unfortunately the Nanshan mine was still in production at the time of sampling and waste products were being disposed of into a refuse dump. Because of safety issues (extremely acidic lake water) and possible interference to mining activity, it was not possible to collect vertical samples to study seasonal turnover or thermal stratification. Thus, in each season, four surficial water samples, named as AMD-1, AMD-2, AMD-3, and AMD-4, from four locations around the lake (Fig. 1), were collected into 2.5-

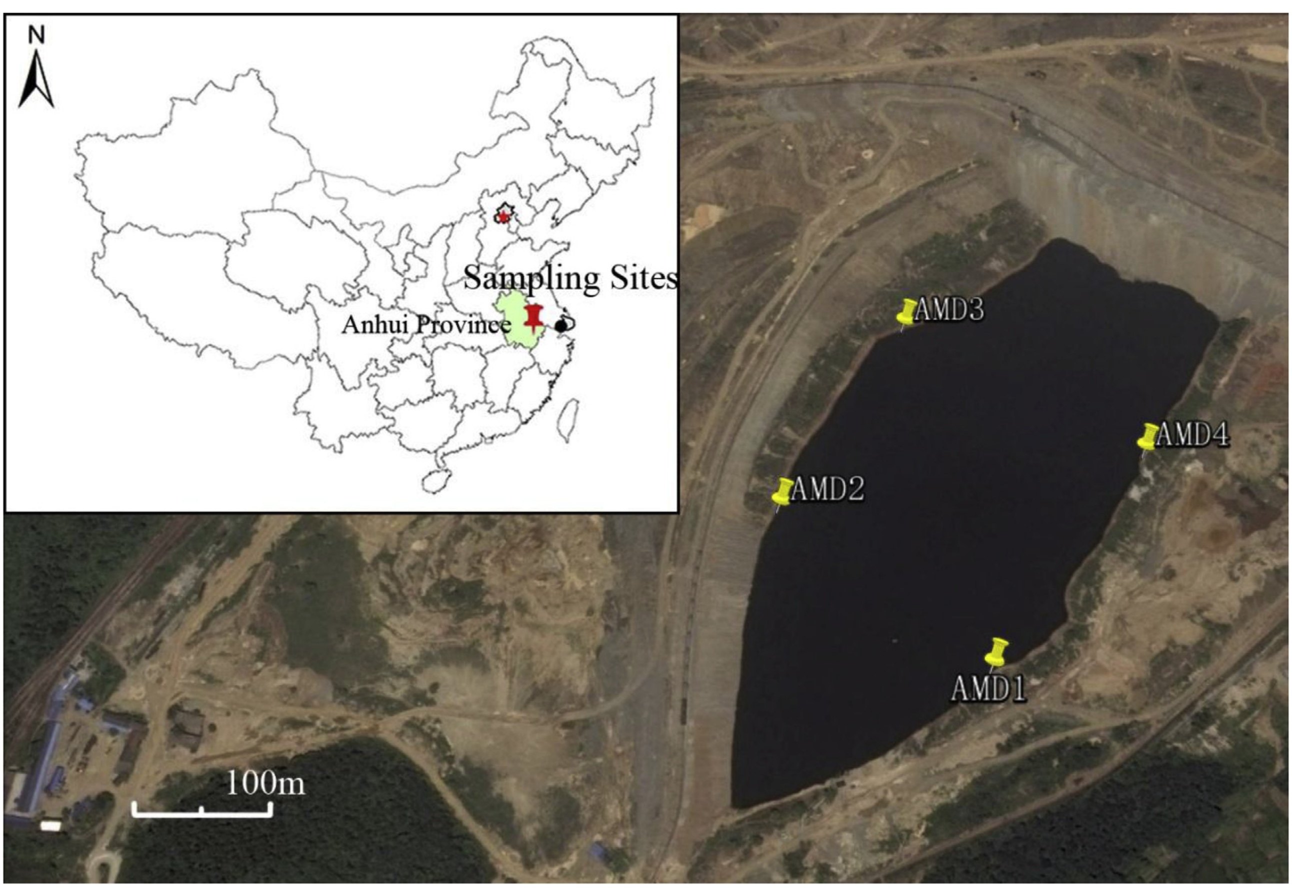

Fig. 1. Locations of four AMD sampling sites around the Nanshan AMD lake in Anhui Province (green area), China. The red star on the national map of China represents Beijing, the capital of China. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)