THE VARIATIONS OF FUNGAL COMMUNITY STRUCTURE AND COMPOSITION DURING HAZE EVENTS IN BEIJING, CHINA

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Abstract - Haze events have become a severe air pollution problem in Beijing, since 2013. Airborne microbes are important components of particulate matter, which may threaten human healthy. However, the variations of fungi in haze events remain unclear. In this study, we characterized the airborne fungal community structure and composition in response to nine haze events that occurred between October 1, 2015, and January 5, 2016. We also explored the key environmental factors that significantly affect airborne fungal community structure. Our results indicated that although airborne fungal alpha diversity and community structure were stable during haze events, particulate matter, gaseous pollutants and relative humidity still had obvious effect on airborne fungal community structure. Meanwhile, we also found that Cladosporium, Aspergillus, Cordyceps and Torulawereenriched on haze days. Our study extends the knowledge about the airborne fungal characteristics in haze events.

Keyword - Fungal Community Structure and Composition, Correlation, Haze

I. INTRODUCTION

Severe haze events frequently occur in Beijing during fall to winter [1]. Although the variations of chemical compositions of particulate matter in haze events have been widely researched [2], the understanding of airborne microbial community structure and composition in haze events still limited, especially the knowledge on airborne fungal variations in haze events is very rare.

Airborne fungi are important composition of airborne microbe, and may threaten human heath because of they include allergens and pathogens [3]. Previous studies have shown that Ascomycota, Basidiomycota and Zygomycota were predominant fungal phyla in atmosphere [4]-[7]. However, the results about variations of airborne fungal community structure and composition in haze events are divergent in different reports. In terms of alpha diversity of airborne fungal community, Yan et al.reported that Shannon index in heavy haze samples were higher than in non-haze and light haze samples, but it did not show obvious distinguish between non-haze and light haze samples [8]. Du et al.pointed out that ACE index decreased during haze events occurring in summer and winter [9]. Besides, some studies found that there were no significant correlations between alpha diversity of fungal community and haze events [10, 11]. In the aspect of community structure, only one study about the effect of haze events on fungal community structure had been reported up to now, which indicated fungal community structure had significantly different between haze and non-haze days [8]. As similar to the fungal community composition in atmosphere, the fungal phyla in particulate matter of haze events occurred in Beijing were also dominated by Ascomycota, Basidiomycota and Zygomycota[8]-[11]. At the genus level, Aspergillus was identified as the predominant genus in haze by cultured method[12]. Moreover, other genera, such as Cladosporium and Penicillium,were found as main airborne fungi by high-throughput sequencing [8], [9], [11], [13]. In addition, the relative abundance of Aspergillus and Penicillium were increased, whereas Cladosporium was decreased in haze events [8]. The significant effects of temperature, PM_{10}, NO_2, SO_2 and CO on airborne fungi community structure in haze events were also observed [8]. Besides, Li et al indicated that season had greater effects on airborne bacterial community than haze events [14]. However,researches on airborne fungal community in haze events are not only rare but did not take into account the effects of seasonal changes.Therefore, the information of airborne fungal community structure and composition was limited, and the key environmental factors that significantly affect airborne community structure were also unclear.

To explore the variations of airborne fungal community structure and composition in haze events in Beijing, and identify the key environmental factors that caused these variations, samples were collected during haze events that occurred during October 1, 2015 to January 5, 2016 and were divided into three seasonal stages referring the results of Li et al [14]. The airborne fungal alpha diversity, community structure and composition in samples were researched based on data from ITS genes high-throughput sequencing. Meanwhile, the correlations between environmental factors ($PM_{2.5}$, PM_{10}, SO_2, NO_2, O_3, CO, temperature, relative humidity, air pressure and wind speed) and fungal community structure were also calculated. We hope these can extend the knowledge of airborne fungi in haze events and provide data support for the prevention hazy hazards.

II. METHODS

2.1 Sample Collection

Collected airborne samples contained nine haze processed during October 1, 2015 to January 5, 2016.

The sample site locates on the roof of the Center of Life Sciences (CLS) Building (40°01'28''N, 116°16'41''E, ~20 m above ground) at the China Agricultural University, Beijing. There are no pollution sources nearby sample site. Samples were collected by a high-volume air sampler (flow rate: 1.03 m^3/min, Model KB-1000, Qingdao Genstar Electronic Technology Co., Ltd., Qingdao, China) equipped with a fiberglass filter. Sample time usually was eight hours, and may be adjusted according to the duration of the haze events.

In addition, according to the result of Li et al [14], nine haze events were divided into three stages (stage I, II and III represent bioaerosol samples collected from fall, early winter and mid-winter) for subsequent analysis [14].

2.2 Pollution levels identify

The concentrations of pollutants (i.e., $PM_{2.5}$, PM_{10}, SO_2, NO_2, CO and O_3) were recorded from the Beijing Municipal Environmental Monitoring Center (http://www.bjmemc.com.cn/). Meteorological parameters (i.e., temperature, relative humidity and wind speed) were recorded from the China Weather Network (http://www.weather.com.cn/) and air pressure was obtained from the National Meteorological Center (http://www.nmc.cn/).

In order to explore the characteristics of airborne fungal community structure and composition at different pollution levels, all samples were divided into five groups ("clean", "slight polluted (SP)", "yellow", "orange" and "red") from non-haze to severe haze events according to the research of[15].

2.3 High-throughput sequencing and Data analysis

Metagenomic DNA was extracted by using a MO BIO PowerSoil® (MO BIO Laboratories, Carlsbad, CA, United States) DNA isolation kit and a GenMag Spin® (GenMag Biotechnology, Beijing, China) Viral DNA Kit. ITS1 region of the ITS genes was sequenced on an Illumina (San Diego, CA, United States) HiSeq platform at Novogene (Beijing, China) to determine airborne fungal community structure and composition. During high-throughput sequencing, primers 1737F (GGAAGTAAAAGTCGTAACAAGG)/2043R (GCTGCGTTCTTCATCGATGC) were applied for amplifying of ITS1 region.

After cutting off the barcode and primer sequence, quality controlling and removing the chimera sequences, the effective tags were clustered at 97% similarity and annotated using the Unit Database. Alpha diversity of fungal community was calculated using QIIME software. Redundancy analysis and ANOSIM test were performedusing the Vegan package (v.2.2-1) in the R computing environment. Pearson correlation analysis was conducted using SPSS version 18.0.

III. RESULTS AND DISCUSSION

3.1 The variations of airborne fungal community structure in haze events.

After quality control, a total of 1,956,816 effective tags were obtained from 35 samples. Alpha diversity including observed species, Chao1 richness and Shannon index were shown in Fig.1. This result indicated that all alpha index did not show significant different between haze and non-haze days among three stages, except for Chao1 richness significant decrease in haze events at stage I. Meanwhile, the Pearson correlations of alpha index and environmental factors indicated that only Shannon show significant positive correlation with SO_2 and wind speed waspositively correlated with observed species and Chao1 (Table 1). ANOSIM test was performed to assess the difference of fungal community structure between different groups. As shown in Table 2, airborne fungal community structure did not have significant difference between haze and non-haze days at each stage, whereas there are obvious distinctions among different stages. These results indicated that the effect of haze events on airborne fungal community structure was not detected. Although the difference between fungal community structures in haze and non-haze day was observed in previous study, the difference was small (ANOSIM, R=0.076, p=0.006) [8]. Meanwhile, other studies also indicated that airborne fungal community structure was stable under haze processes [10], [16].

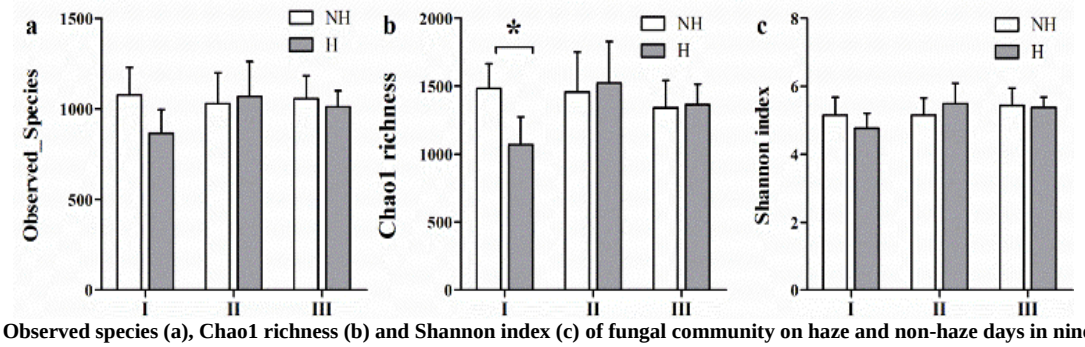


Fig. 1 Observed species (a), Chao1 richness (b) and Shannon index (c) of fungal community on haze and non-haze days in ninehaze events during October 1, 2015, to January 5, 2016. I, II and III represent three stages, respectively. "NH" and "H"represent samples from non-haze andhaze days, respectively. Error bars represent SD of samples from non-haze or haze days in each haze event, respectively. T-test, ∗p< 0.05.

	Observed species	Chao1	Shannon
$PM_{2.5}$	-0.043	-0.024	0.16
PM_{10}	-0.053	-0.049	0.122
SO_2	0.16	0.189	**0.402***
NO_2	-0.197	-0.219	0.214
O_3	0.133	0.097	-0.284
CO	-0.005	0.000	0.291
AP	0.109	0.046	0.238
T	-0.097	-0.092	-0.283
RH	-0.027	0.02	0.143
WS	**0.385***	**0.363***	0.133

AP, T, RH and WS represent air pressure, temperature, relative humidity and wind speed, respectively.* *P*< 0.05 (2-tailed)

Table1: Pearson's correlation coefficients between alpha diversity index and pollutants, meteorological parameters.

		R value	*P* value
Stages	I vs II	0.1709	0.007
	I vs III	0.3717	0.001
	II vs III	0.2760	0.002
Haze vs Non-haze	Stage I	-0.0357	0.572
	Stage II	0.1905	0.149
	Stage III	0.0394	0.283

Table2: ANOSIM tests on the fungal community between each stages and between haze & non-haze samples.

3.2 The key environmental factors affecting airborne fungal community structure in haze events.

Redundancy analysis was conducted to analysis the relations between airborne fungal community structure and environmental factors. The result was shown in Fig.2, airborne fungal community structure on non-haze days were positively correlated with temperature, air pressure and wind speed, and the airborne fungal community structure on haze days at stage II and stage III were significantly affected by PM_{10}, SO_2, NO_2, CO and relative humidity. Yan et al. also found that PM_{10}, NO_2, SO_2, CO and relative humidity were important factors affecting airborne fungal community structure during haze events in Beijing [8]. These results implied that although haze events did not significantly change airborne fungal community structure, particulate matter, gaseous pollutants and relative humidity still had obvious effect on airborne fungal community structure.

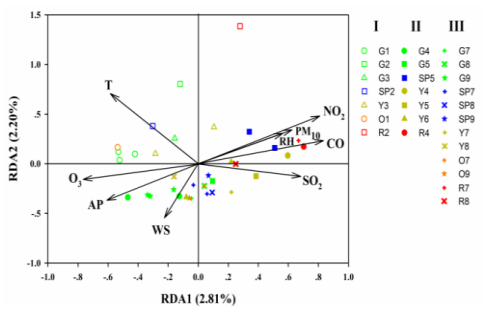


Fig.2 Redundancy analysis (RDA) of biological with environmental parameters. "G", "SP", "Y", "O", and "R", respectively, represent haze pollution levels green, slightly polluted, yellow, orange and red. Nos. 1 to 9 represent the nine haze events during October 1, 2015, to January 5, 2016. Only significant environmental variables are shown in this figure.

3.3 The enrichment of fungi in haze events.

In this study, 7 phyla covering 31 classes, 105 orders, 248 families, and 623 genera were detected across all samples. The relative abundances of fungal phyla are shown in Fig.3, Ascomycota was the most predominant phyla in air atmosphere (expect for the No.3 haze samples). At the genus level, the relative abundances of Cladosporium, Aspergillus, Cordyceps and Torula were increased on haze days, and showed significant positive correlation with $PM_{2.5}$ (Fig.4). The relative abundances of Davidiella, Selenophoma were decreased on haze days and negatively correlated with $PM_{2.5}$. Cladosporium, Aspergillus, Davidiella and Selenophoma were common fungi in environment. Same as this study, Yan et al.found Aspergillus enriched on haze days [8], whereas [10] found that the relative abundance of Selenophoma was decrease on haze days [10]. However, Yan et al. also found the relative abundance of Cladosporium was decrease on haze days during June to November [8]. The reason for this distinction may be different sampling time and it is worth further research.

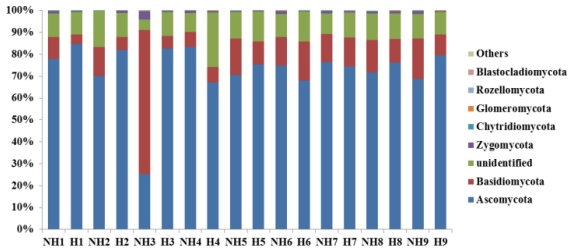


Fig.3 Fungal community compositions at the phyla level during Nos. 1 to 9 haze events. "H" represents haze pollution samples, while "NH" represents the non-haze samples.

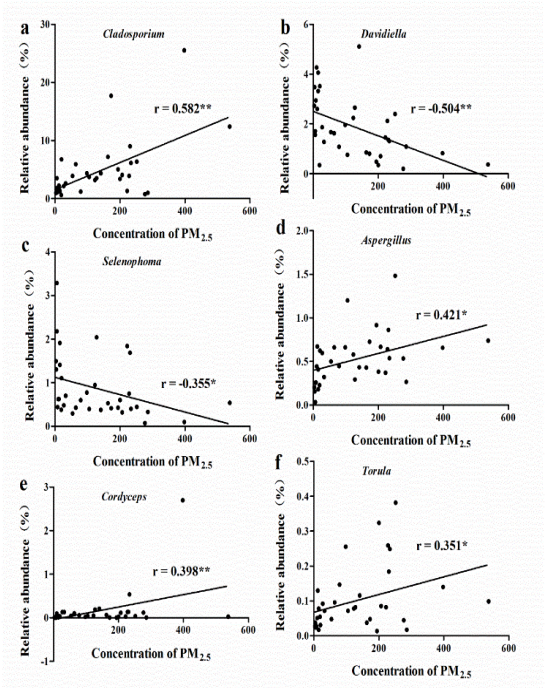


Fig.4 Pearson's correlations analysis between fungi and $PM_{2.5}$. Only the genus that the relative abundance exceeding 0.1% were analyzed. ** p<0.01 (2-tailed), * P< 0.05 (2-tailed).

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