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Diversity and co-occurrence network of soil fungi are more responsive than those of bacteria to shifts in precipitation seasonality in a subtropical forest

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ABSTRACT

Precipitation regime change presents serious ecological consequences in diverse ecosystems, particularly forests. However, little attention has been paid to soil microbial community sensitivity to precipitation changes despite the potential for significant feedbacks on the large carbon stocks in subtropical forests. Here we examined the responses of both fungal and bacterial communities in aspects of diversity and co-occurrence network to a manipulative alteration of precipitation seasonality (MAPS) by reducing dry-season rainfall, increasing wet-season rainfall, while keeping the total annual rainfall unchanged. Our results showed that MAPS significantly reduced the diversity of the fungal community but not that of the bacterial community. The richness and abundance of *Sordariomycetes* were reduced, while the abundance of ectomycorrhizal fungi was elevated by MAPS. The co-occurrence network of fungal OTUs (fungal network) was also changed more than the bacterial network. The fungal network under the MAPS treatment featured more links and clusters than in the control, implying the interactions among fungal OTUs could be intensified. Ectomycorrhizal fungi were suggested to affect fungal network structure under MAPS through their functions in phosphorous nutrition, as indicated by the significant correlation between fungal network structure and soil phosphorous content. The MAPS treatment also changed the correlation relationships of fungal network structure with soil enzymes, which were not found for bacterial networks. The activities of several lignocellulose degrading enzymes were positively correlated with overall fungal diversity. Our results indicate that the altered precipitation seasonality in a subtropical forest can more readily influence soil fungal than bacterial communities, with potential for serious consequences such as degradation of recalcitrant organics in soil.

1. Introduction

Water availability is one of the most important factors controlling soil biogeochemical processes (Parton et al., 1987). Projected alteration in precipitation regimes, resulting from global climate change, will likely be characterized by more variable rainfall patterns with greater frequency of extreme drought and floods (Huntington, 2006). Water constitutes 70–90% of the mass of microbes (Atlas and Bartha, 1998), and as a basal solvent, often co-limits microbial communities with other factors (Zhang et al., 2015). Sudden changes in soil moisture, such as those brought on by drought and subsequent rain events, can result in physiological stresses to microbes from rapid changes in water potential (Harris, 1981). The tolerance or sensitivity to water potential differs in different microbial groups, for example, Gram + bacteria and hyphae fungi can tolerate lower water potential than Gram-bacteria (Harris, 1981). Thus there can be differential responses of different microbial groups to alteration of precipitation regimes. Specifically, bacteria and fungi are the two main constitutes of soil microbes, with different, though sometimes overlapping, roles in

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soil biogeochemical processes. Due to differences in cell structure, physiological traits, community diversity and inter-species interactions (James et al., 1995; Koide et al., 2005; Kooijman et al., 2016), bacterial versus fungal communities likely experience different susceptibility to altered precipitation regimes.

The diversity or composition of soil fungal and bacterial communities could be sensitive to changes of rainfall patterns (Hawkes et al., 2011; Classen and Cregger, 2012; Sudderth et al., 2012), or show no significant changes (Bi et al., 2012; Mchugh and Schwartz, 2015; Paradis et al., 2015). In some studies, bacteria are more influenced by soil moisture (Zeglin et al., 2013), while other studies show that fungi showed higher sensitivity to soil moisture (Cregger et al., 2012; Kaisermann et al., 2015). This inconsistency could come from a lack of comparative precipitation change settings (Beier et al., 2012), different methods used in measuring microbial community and different ecosystem types. Soil properties, water-related stress history and between-taxa interactions can help explain the response patterns of microbial communities to precipitation changes (Fierer et al., 2003; Hawkes et al., 2011). Relative to (semi) arid or agricultural ecosystems, studies on microbial responses to precipitation changes in warm, wet ecosystems are scarcer. A recent study in a wet tropical forest indicated that reduced precipitation lowered fungal abundance (Waring and Hawkes, 2015). Bacterial diversity could also be reduced by reduced precipitation in tropical forests, but pre-exposure to drought increased the resistance of bacterial communities to the subsequent drought in another tropical forest study (Bouskill et al., 2013).

Soil microorganisms usually do not live in isolation, but instead form complex inter-species networks that, to a great extent, regulate the structure of an ecological community (Freilich et al., 2010) and consequently the functions of the ecosystem (Fuhrman, 2009). Network analysis has proven a powerful way to study the interactions between different entities in a system, and has recently been used to study complex ecological systems, such as the human microbiome (Greenblum et al., 2012), marine bacterioplankton (Gilbert et al., 2012), bacterial or fungal communities in soils (Barberán et al., 2012; Lu et al., 2013), and communities in soils under elevated CO_2 (Zhou et al., 2011b). Although network analysis has its own pitfalls (Faust and Raes, 2012), it has intrinsic power and usefulness in revealing information about community organizations and member interactions, keystone species and their responses to changed environment that cannot easily be understood by routine microbial community analyses (Lu et al., 2013). For example, novel interactions can be predicted by network analysis among members of under-characterized phyla, providing valuable information for the co-culturing of different species (Faust et al., 2012). The bacterial communities, *Actinobacteria* was found to be the keystone phylum to connect other bacterial members (Zhou et al., 2011b). *Sordariales* and *Hypocreales* played similar “connecting” roles in one study of fungal co-occurrence networks (Lu et al., 2013). However, there still lack studies to inspect and compare network structural changes between bacteria and fungi in response to altered precipitation regimes, which could provide new information about the impacts of climate change and microbial feedbacks in soils.

Tropical and subtropical forests are hot spots of global C cycling and highly sensitive to precipitation changes (Nepstad et al., 2002; da Costa et al., 2010). Here we conducted a study in a south subtropical forest in China, manipulating the precipitation seasonality to cause more intensified drier and wetter events while keeping total annual precipitation unchanged, mimicking the future rainfall patterns as predicted by Zhou et al. (2011a). We used amplicon-sequencing technology to inspect the diversity and composition of bacterial and fungal communities. Recently-developed FUNGuild and molecular ecological network analyses were also used to help delineate multi-aspect pictures of how soil microbes respond to the alteration of precipitation regimes. Since fungi usually has a lower abundance and diversity than bacteria in the whole community, we hypothesized that (i) fungal community diversity would be more sensitive to the alteration of precipitation regimes; and (ii) fungal community may harbor a network structure more susceptible to the alteration of precipitation regimes. The results of this study could provide valuable clues to infer the impacts of precipitation changes on biological and ecological processes in a subtropical forest.

2. Materials and methods

2.1. Site description and experimental design

This study was conducted at the Heshan National Field Research Station of Forest Ecosystem (112° 50′ E, 22° 34’ N) located in Guangdong province, southeastern China. The climate in this region is subtropical monsoon with a distinct wet season from April to September and a dry season from October to March. The mean annual precipitation is 1700 mm, and the mean annual air temperature is 21.7 °C (Wang et al., 2010; Zhao et al., 2017). The soil is classified as Ultisol according to the USDA soil taxonomy (Soil Survey Staff, 2010). The mean slope of the experimental area is 15°. Vegetation is dominated by two evergreen broad-leaved tree species, *Schima superba* and *Michelia macclurei* (Chen et al., 2017).

Eight 12 × 12-m^2 experimental plots were established in 2012. The total study area was about 1 ha. Four plots were assigned to manipulative alteration of precipitation (hereafter MAPS treatment), which had a drier dry season and wetter wet season (see below), and four plots were designed as controls (hereafter control treatment). The distance between plots was about 3 m. In each MAPS plot, a set of steel frames was set up on 16 standing steel pillars to support throughfall reduction shelters and water addition sprinklers (Fig. S1). The supporting frames were kept 1.5 m above the ground. Ten to twelve rainfall exclusion sheets, covering 67% of the total plot area, were used in each MAPS plot to exclude throughfall during the dry season. The exclusion sheets were made of polyethylene materials with 95% of light transparency to minimize potential shading effects; surface soil temperature (10 cm) was not significantly affected by the exclusion sheets (Student *t*-test, $P = 0.43$; supplementary material Fig. S2). The exclusion sheets were deployed, along with the slope of the plots, and connected to PVC troughs located at the lower slope end to allow the excluded throughfall to drain out of the plots (Fig. S1). The throughfall reduction rate (i.e., 67%) was adopted according to other throughfall reduction experiments for tropical forests (Brando et al., 2008; da Costa et al., 2010). Each MAPS plot had 25 automated sprinklers at the center of steel frames, with a spraying diameter of 2.5 m and showering 50 L of water per hour. The control plots were set up without throughfall reduction and water addition facilities, but the four sides of each plot were trenched using 1-m height PVC boards to a depth of 60–80 cm to prevent lateral water flow, as done in each MAPS plot. Automatic rain gauges (Davis Instrument, MD, USA) were installed under the canopy of the plot (area without shelters) to record the amount of throughfall.

The experiment lasted for two hydrological years: 2013 (1 October 2012 to 30 September 2013) and 2014 (1 October 2013 to 30 September 2014). The MAPS treatment was applied to four plots from October to March with throughfall reduction and from June to September with water addition. During the dry season, 67% of the total throughfall was excluded by opening the exclusion sheets. During the wet season, the sheets were folded and an equivalent amount of water to that excluded during the dry season was added