Effects of gaps in the forest canopy on soil microbial communities and enzyme activity in a Chinese pine forest

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ABSTRACT

Gaps in the forest canopy play an important role in forest ecology, affecting biological dynamics, nutrient cycling, and plant succession. The influence of gap size on soil microbial communities and soil enzyme activity is poorly understood. Chinese pine (*Pinus tabulaeformis*) is one of the main afforestation species in northern China, and the creation of artificial gaps in Chinese pine forests is a common silvicultural practice to reduce forest density. We compared the microbial communities and the activity levels of several key enzymes in a Chinese forest among sites with closed canopies and canopies with small gaps ($40–50\,m^2$) and large gaps ($100–120\,m^2$) in October 2015, one year after the gaps were created. Soil samples from a depth of 0–10 cm were collected, and soil microbial community composition was evaluated using phospholipid fatty acid analysis. The activity levels of soil enzymes were also analyzed. Overall, the amounts of phospholipid fatty acids and the activity level of soil enzymes were highest in the sites with small gaps, followed by sites with closed canopies and sites with large gaps. Gram-positive bacteria were mainly responsible for the difference between microbial communities in sites with small gaps and sites with closed canopies. The activity levels of soil β-glucosidase and L-leucineaminopeptidase were also highest in sites with small gaps and lowest in those with large gaps. Soil acid phosphatase activity was similar in sites with small gaps and closed canopies and was lowest in sites with large gaps. Redundancy analysis indicated that dissolved organic carbon was the common driving factor affecting the variations in microbial communities and enzyme activity. Our results suggest that small gaps are beneficial for microbial communities. Further study of gap treatments is needed to determine the proper gap size for forest management.

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1. Introduction

Gaps in the forest canopy provide space for new trees to grow, creating environmental heterogeneity. Therefore, they play an important role in changing the structure of tree stands and renewing the forest (Franklin et al., 2002; Gray et al., 2012; Whitmore, 1989). The harvesting of trees to create gaps is considered a sustainable practice (Li et al., 2012). The size of a gap reflects the magnitude of the disturbance and the degree of environmental heterogeneity (Muscolo et al., 2014). Environmental factors such as net radiation, temperature, and moisture are affected by the size of the gap (Brown, 1993; De Freitas and Enright, 1995; Gray et al., 2002). Furthermore, changes in microclimate induced by gaps dramatically influence local biogeochemical cycling (Scharenbroch and Bockheim, 2008a).

Forest soil, an essential part of the ecosystem in which nutrients are recycled and many organisms live, is also influenced by forest canopy gaps. Previous studies have focused on carbon cycling and nutrient release from the surface soil at sites with different-sized gaps. Some studies reported lower litter input and fine root biomass at sites with gaps than at sites with closed canopies, as well as varying rates of litter decomposition at sites with different-sized gaps (Lin et al., 2015; Ritter, 2005; Saner et al., 2009; Zhang and Liang, 1995). Relative to undisturbed closed forest, lower amounts of labile carbon and lower rates of soil respiration were detected as gap size increased (Scharenbroch and Bockheim, 2008a). Soil nitrogen and phosphorus fractions also appear to be affected by gaps (Hu et al., 2016; Scharenbroch and Bockheim, 2008b).

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(Allison, 2012; Veres et al., 2015; Waldrop and Firestone, 2004) and react quickly to changes in the environment (Barreiro et al., 2015; Burns et al., 2013). The influence of gaps on soil microbial communities is relatively complex. The creation of a gap may result in improved understory vegetation and changed soil microclimates that are beneficial for microbial communities (Bolat, 2014; Muscolo et al., 2007a); however, reduced litter input and increased radiation may harm microorganisms (Schliemann and Bockheim, 2014; Xu et al., 2016). Several studies have investigated the effect of gap size on soil microbial biomass and soil enzyme activity. One study suggested that small gaps enhance soil organic matter content, microbial biomass, and enzyme activity and concluded that creating small gaps was an appropriate method of forest management (Muscolo et al., 2007a). As gap size increased, microbial biomass and soil respiration decreased (Schliemann and Bockheim, 2014; Xu et al., 2016). In addition, different microbial communities react differently to changes in gap size. According to Arunachalam and Arunachalam (2000), the bacterial population was positively correlated with gap size, whereas the fungal population showed an insignificant negative correlation.

Chinese pine (*Pinus tabulaeformis*), one of the main species used for afforestation, is primarily distributed in the arid and semiarid zones of China. Trees were planted too densely in the 1950s and were not tended afterward, creating many low-efficiency forests, the management of which is of interest to the Chinese government and to scholars (Cheng et al., 2014). Previous studies reported that gaps in Chinese pine forests influence the physical and chemical properties of soil, as well as the growth of seedlings and shrubs (Han et al., 2012, 2013; Li et al., 2012). It is widely accepted that gap creation is beneficial for the management of Chinese pine forests (Han et al., 2013). However, few studies have addressed the effects of gaps on the soil microbial community structure in Chinese pine forests.

The specific objective of the present study was to investigate how soil microbial communities respond in the short term to the creation of different-sized gaps. We hypothesized that (1) compared with closed-canopy sites, sites with gaps would contain a lower concentration of phospholipid fatty acids (PLFAs) due to reduced litter input and that as the gap size increased, the microbial communities would decline; and that (2) the shifts in microbial communities would be closely correlated with changes in the chemical properties of soil at sites with different-sized gaps.

2. Material and methods

2.1. Study region

This study was conducted in Badaling Forest Park (40°17′N, 115°55′E) in Beijing, northeastern China. This region is a part of Yanshan Mountain and the topography is fragmented, with altitudes ranging from 550 m to 1238 m. It is in a warm-temperate zone and has a semi-humid continental monsoon climate. The mean annual temperature is 12.2 °C and the mean annual precipitation is 598 mm. The zonal vegetation is warm-temperate deciduous broad-leaved forest, and the main species are *Quercus liaotungensis*, *Quercus mongolica*, *Populus davidiana*, and *Betula* spp. However, the area is almost devoid of its original vegetation, which remains only sparsely distributed in the mid-altitude regions. The forests in low-altitude regions are pure stands resulting primarily from artificial afforestation in the 1950s and include the main silvicultural species Chinese pine (*P. tabulaeformis*), Oriental arborvitae (*Platycladus orientalis*), and locust (*Robinia pseudoacacia*). The ground vegetation is dominated by herbs, including *Girardinia cuspidata*, *Carex lanceolata*, and *Oplismenus undulatifolius*. Bushes such as *Vitex negundo* var. *heterophylla*, *Deutzia grandiflora*, and *Spiraea trilobata* are sporadically distributed.

Chinese pine mainly grows on shaded and partially shaded slopes, at densities of 1400 stems ha^{-1}. Sporadic patches of diseased or dead trees are present due to the high density of planting (Geng et al., 2007). To improve the health of the forest, diseased trees are often felled and removed. The soil is formed from a granite parent material, and the soil type is Hapli-Ustic Cambosol, according to Chinese Soil Taxonomy (Gong, 1999). The general soil organic matter content at 0–20 cm is 40–60 g kg^{-1} and is medium grade according to Geng et al. (2010).

2.2. Experimental design and soil sampling

A previous study reported that small gaps are the predominant type of canopy gap in Beijing forests (Tan et al., 2000). In autumn 2014, gaps were created by felling unhealthy trees in a monoculture Chinese pine forest at altitudes of 600–700 m. A year later, we selected 15 randomized sites with similar aspect, slope topography, and original soil condition and divided them into categories (Suppl. Fig. 1). These were small gap (1–3 trees felled, 40–50 m²), large gap (5–8 trees felled, 100–120 m²), and closed canopy (195–210 m²). The distances between the centers of adjacent sites were at least 50 m. The gap size was calculated by marking the canopy drip line (Brokaw, 1982). Generally, undergrowth was more abundant at sites with gaps than at those with closed canopies.

Soil samples were collected in October 2015. There was no rainfall for three days before sampling, and no rain fell during sampling. At each site, a cross transect was placed across the center. Along each of the two lines of the cross, avoiding trunks and site edges, forest litter was carefully removed and six randomly distributed subsamples of soil (0–10 cm depth) were collected using a specific cylindrical corer device (100 cm³ in volume). The 12 subsamples from each site were combined to form a composite sample. The soil was sealed in plastic bags and transported to the lab in a portable container maintained at 4 °C.

After visible roots, residues, and rocks were removed from each sample, the fresh soil in the sample was separated into three subsamples. One was air-dried at room temperature and used to determine the chemical properties of the soil, another was stored at −80 °C for microbial analysis, and the third was stored at 4 °C and analyzed for soil enzyme activity. Enzyme analyses should be performed as soon as possible after sample collection to prevent alteration and degradation of enzyme activity (Wallenius et al., 2010); however, in many cases, sample storage is unavoidable. Sample storage is acceptable if the ability to compare soils is unaffected by the storage (Peoples and Koide, 2012). Dick (2011) recommended storing samples for enzyme analysis at 4 °C. In several previous studies, samples were stored no longer than one week for enzyme analyses (Brouns et al., 2016; Domínguez et al., 2016; Duan et al., 2015), but other studies have used longer storage time (Tischer et al., 2015; Zhang et al., 2015). In the present study, the activity level of each enzyme was determined on the same day, with randomized order of samples, to ensure that results between different gap sizes would be comparable. The whole process of enzyme analysis was completed within 10 days after collection. Enzymes were analyzed in the following order: acid phosphatase, N-acetyl-glucosaminidase, β-glucosidase, L-leucineaminopeptidase.

2.3. Laboratory analysis

2.3.1. Soil chemical properties

Soil pH was determined using an FE20K pH meter (Mettler Toledo, Zurich, Switzerland), with a ratio of soil to water of 1:5 (m/v). Soil organic carbon was determined using the potassium dichromate capacity method/dilution heat method. Soil total nitrogen was measured using the Kjeldahl method. Soil dissolved