JFES
Journal of Forest and Environmental Science

Effects of Experimental Drought on Soil Bacterial Community in a *Larix Kaempferi* Stand

Beomjeong Kim[1,+] and Byoungkoo Choi[2,*]

[1]*Graduate School of Forestry and Environmental Systems, Kangwon National University, Chuncheon, Korea*
[2]*Division of Forest Sciences, Kangwon National University, Chuncheon, Korea*

Abstract

Drought alters soil microorganisms; however, it is still not clear how soil microbes respond to severe drought conditions. In this study, the responses of soil bacterial community to experimental drought in a coniferous stand were examined. Six 6 m x 6 m plots with three replicates of control and drought treatments were delimited. PCR amplification and Illumina sequencing were conducted for cluster analysis of soil bacterial community and species richness and species diversity was analyzed. Along the 393 days of simulated drought from July 2016 to October 2017, soil bacterial species diversity slightly increased whereas species richness decreased in both control and roof plots. Moreover, soil bacterial species richness more decreased in roof plots than in controls. Combining these results, soil bacterial activity might have been altered by simulated drought.

Key Words: climate change, experimental drought, soil bacterial community, soil microorganism, throughfall exclusion

Introduction

More frequent and severe drought in multiple forested regions was forecasted by several climate models (IPCC 2014). Drought alters soil bacterial community through osmotic stress and resource competition (Chodak et al. 2015). The soil microbial community responds to drought depending on the physiological tolerance and metabolic flexibility of the constituent microbes (Fuchslueger et al. 2014). Soil bacterial vulnerability to drought depends on different groups of soil bacteria (Uhlirova et al. 2005; Schimel et al. 2007). Also, soil bacterial communities are considered to directly influence on a wide range of ecosystem processes (Langerheder et al. 2006). Thus, understanding reaction of soil bacteria to drought stress is important because soil bacteria play a critical role in the functioning of forest ecosystems (Bauhus and Khanna 1999; Nannipieri et al. 2003; Chodak et al. 2015). However, few studies have examined

in South Korea specifically under experimental drought condition. The objective of this study was to examine the soil bacterial community response to simulated drought.

Materials and Methods

Site description

The experimental drought was carried out from 15 July The experimental drought was carried out from 15 July 2016 to 20 October 2017 in *Larix kaempferi* stand located at the experiment forest area of Kangwon National University (37°47′30″N, 127°49′49″E) at an elevation of 569 m, in Gangwon province, Korea. 53-year-old Japanese larch (*Larix kaempferi*), 8-year-old Korean pine(*Pinus koraiensis*), *Zanthoxylum schinifolium*, *Aralia elata*, *Lindera obtusiloba*, and *Cornus alba* dominated the stand. The understory vegetation consisting mainly of *Dryopteris crassirhizoma* and *Rubus crataegifolius* covers about 80% of the soil surface

during growing season. Recent 30-year climate records indicated mean annual precipitation of 1,450 mm year^{-1} and mean annual temperature was 11.5°C, with a mean August maximum of 30°C and a mean January minimum of -9°C. The soil is classified as B2 (Korea Forest Service) with loam and its depth ranges between 30 and 60 cm.

Experimental design and measuring rainfall, soil moisture and soil temperature

TThree translucent roofs, each 6 x 6 m, were constructed 1.5 m above the forest floor in July 2016 in order to simulate severe drought. Control plots of the same size were also established adjacent to the three exclusion plots. All measurements were conducted in core zone with 1 m buffer zone to avoid edge effect.

Soil moisture and soil temperature were measured hourly using probes (1000A, IStech, Korea) installed at a soil depth of 10 cm and 30 cm in each plot, and data were collected using data logger (GL840, ALTHEN, Germany). Also, throughfall water was hourly detected by a tipping bucket rain gauge (IS-7857, Davis, USA) which was installed above 1.5 m above the ground adjacent control and treatment plots.

Soil sampling

One soil sample from control plots and two soil samples from throughfall exclusion plots for soil bacterial community analysis were collected at a soil depth of 10 cm on 11 August 2016 and on 8 September 2017. Soil samples were stored in 50 ml Falcon tubes and were kept in a -35°C refrigerator. After this conditioning, samples were sent to the commercial laboratory (ChunLab, Inc, Korea) in order to analyze soil bacteria community in September 2017.

PCR amplification and Illumina sequencing

PCR amplification was carried out using primers targeting from V3 to V4 regions of the 16S rRNA gene with extracted DNA. For bacterial amplification, primers of 341F and 805R. The amplifications were performed under the following conditions: initial denaturation at 95 °C for 3min, followed by 25 cycles of denaturation at 95 °C for 30 sec, primer annealing at 55 °C for 30 sec, and extension at 72 °C for 30 sec, with a final elongation at 72 °C for 5 min. Then, secondary amplification for attaching the Illumina

NexTera barcode was carried out with i5 forward primer and i7 reverse primer. The condition of secondary amplification is identical to previous one apart from the amplification cycle set to 8.

Using 2% agarose gel electrophoresis and visualized under a Gel Doc system (BioRad, Hercules, CA, USA), and the PCR product was confirmed. The amplified products were purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). Equal concentrations of purified products were pooled together and removed short fragments (non-target products) with Ampure beads kit (Agencourt Bioscience, MA, USA). The quality and product size were estimated on a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) using a DNA 7500 chip. Mixed amplicons were pooled, and the sequencing was performed at Chunlab, Inc. (Seoul, Korea), with Illumina MiSeq Sequencing system (Illumina, USA) according to the manufacturer's instructions.

Data analysis

Changes in soil bacterial communities between the treatment and control plots following 15 months throughfall exclusion were compared utilizing the Shannon-Weaver diversity index (H'). The Shannon-Weaver index accounted for measurements of richness with those of evenness of the species present. The Shannon-Weaver index (H') is defined (Shannon and Weaver 1949). Uniformity of Pielou Index was used in order to quantify how equal the soil bacterial community was numerical. The Pielou's evenness index (Pielou 1966). All statistical analyses were performed using R version 3.4.2, and in all cases, significance was accepted at p levels < 0.05.

Results and Discussion

The total number of members comprised of the classifiable sequences was 65 phyla. The endemic soil bacterial community of the experimental area was largely dominated by members of the *Proteobacteria* and *Acidobacteria*, with relatively lower abundances of *Chloroflexi*, *Actinobacteria*, and *Verrucomicrobia* (Fig. 1). The results of the species diversity, evenness, and richness over the exclusion period were shown in Table 1. Significant difference seemed not exist in the bacterial diversity and evenness

Table 1. Comparison of the diversity, evenness, and richness in control and roof plots over the simulated drought

	H'	e	ACE	Chao1	ACE (%)	Chao1(%)
BC	6.57	0.80	130,024	3,945		
BR	6.80	0.80	131,987	6,214		
AC	6.59	0.84	121,975	3,647	-6.19	-7.55
AR	6.84	0.81	117,396	5,743	-11.05	-7.58

H' is Shannon-Weaver Diversity Index and e is Pielou evenness Index.
Chao and abundance-based coverage estimator (ACE) are represented as raw values for single sampling time and percent change over 13 months through fall exclusion.
B = August 2016, A = September 2017; C = control, R = roof

over the experiment. In August 2016, Shannon-Weaver Diversity Index values (H') were 6.57 and 6.80 in control and roof plots, respectively. Subsequently, H' values slightly increased in both control and roof plots after 13 months exclusion (6.59 and 6.84, respectively), but the increments were trivial. In addition, there was no change in species evenness (e) over the experiment in both plots. However, estimated values of Chao and abundance-based coverage estimator (ACE) had a different trend with indices of species diversity. In August 2016, species richness was slightly higher in control plots (ACE = 130,024 and 131,987; Chao1 = 3,945 and 6,214 in control and roof plots, respectively).
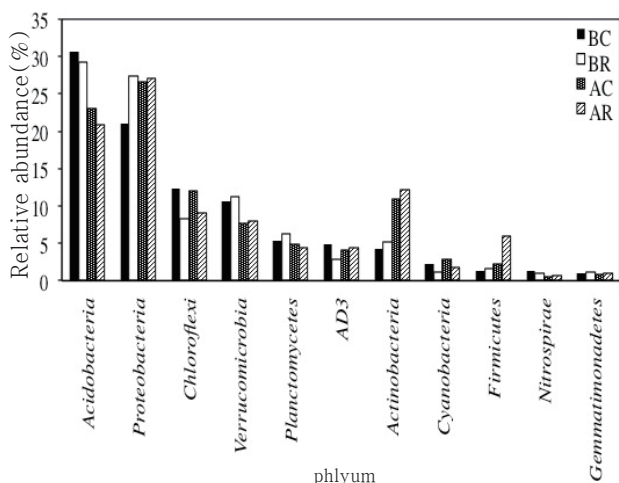

Fig. 1. Relative abundance of dominant bacterial phyla in the soil bacterial communities. Each bar which is coded with different pattern represents a single sampling time. B = August 2016, A = September 2017; C = control, R = roof.

Species richness decreased in both control and roof plots after 13 months throughfall exclusion, but there was higher decrement of richness in roof plots (ACE = 121,975 and Chao1 = 3,647 in control, ACE = 117,396 and Chao1 = 5,743 in exclusion plot). This result indicates the number of soil bacterial species observed increased while the equitability of species distribution decreased in roof plots compared to the control.

There were increases and decreases in soil bacterial abundances over the experimental period. After 13 months throughfall exclusion, the relative abundance of *Acidobacteria* increased the most (7.67% and 8.37% in control and roof plots, respectively), and with relatively less increment, the relative abundance of *Verrucomicrobia* increased (2.97% and 3.31% in control and roof plots). *Planctomycetes* abundance was increased more under the simulated drought (1.84%) compared to control (0.56%). There were slight increases in abundances of *Chloroflexi*, *AD3*, and *Chlamydiae* only in control plots (0.35%, 0.84%, and 0.17%, respectively), while *Proteobacteria* abundance marginally increased (0.35%) only in the roof plots. On the other hand, *Actinobacteria* abundance decreased most in both control and roof plots (6.68% and 6.95%, respectively), and only in control plots, *Proteobacteria* abundance 5.55% decreased. The decrement of Firmicutes abundance was five times higher (4.35%) in roof plots than control (0.87%). And, *Chloroflexi* and *AD3* abundances only decreased under the simulated drought (0.81% and 1.59%, respectively). Over the 13 months simulated drought, soil bacterial species richness more decreased in roof plots. These results agree with Sheik et al.(2011) that the diversity of soil microbes in warmed treatment combining were generally less abundant and community structure was significantly different when compared with the control. Moreover, they found microbial populations were declined in warmed plots with the lack of rainfall. Combining these results, it was obvious that soil microbial activity has been restrained by drought effects. More precise study is needed to determine major limiting factors which affect soil microbial community. Also, the research period of this study was not enough to ascertain the drought effects on soil microbial community. Thus, longer term experiment should be conducted for future research.

Acknowledgement

Reference

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215: 403-410.

Bauhus J, Khanna PK. 1999. The significance of microbial biomass in forest soils in: Going underground - ecological studies in forest soils (Rastin N, Bauhus J, eds). Research Signpost, Trivandrum, India, pp 77–110.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114-2120.

Chodak M, Gołębiewski M, Morawska-Płoskonka J, Kuduk K, Niklińska M. 2015. Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. Ann Microbiol 65: 1627.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27: 2194-2200.

Fu L, Niu B, Zhu Z, Wu S, Li W. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28: 3150-3152.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460-2461

Fuchslueger L, Bahn M, Fritz K, Hasibeder R, Richter A. 2014. Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. New Phytol 201: 916-927.

Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. 2012. PANDAseq: paired-end assembler for illumina sequences. BMC bioinformatics 13: 31.

Myers EW, Miller W. 1988. Optimal alignments in linear space. Comput Appl Biosci 4: 11-17.

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G. 2003. Microbial diversity and soil functions. Eur J Soil Sci 54: 655–670.

Patrick DS, Sarah LW, Thomas R, Justine RH, Martin H et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75: 7537-7541.

Schimel J, Balser TC, Wallenstein M. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88: 21386–21394.

Sheik CS, Beasley WH, Elshahed MS, Zhou X, Luo Y, Krumholz LR. 2011. Effect of warming and drought on grassland microbial communities. ISME J 5: 1692–1700.

Langenheder S1, Lindström ES, Tranvik LJ. 2006. Structure and function of bacterial communities emerging from different sources under identical conditions. Appl Environ Microbiol 72: 212-20.

Uhlirova E, Elhottova D, Třiska J, Šantrůčková H. 2005. Physiology and microbial community structure in soil at extreme water content. Folia Microbiol 50: 161–166.