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High soil microbial activity in the winter season enhances nitrogen cycling in a cool-temperate deciduous forest

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ABSTRACT

The soil microbial community plays essential roles in bioavailable nitrogen (N) cycling, and microbial dynamics during the plant dormant season could affect N cycling in the subsequent growing season. Despite frequent observations of seasonal shifts in microbial community composition in forests, the biogeochemical consequences for bioavailable N cycling have not been well characterized. Here we examine the relationship between microbial community dynamics and bioavailable N dynamics throughout a year in a cool-temperate deciduous forest, with a focus on the dormant season. Specifically, we analyzed temporal changes in abundances of N-cycling microbial populations and N concentrations and transformations in soils. We also assessed temporal changes in bacterial community composition by deep sequencing of 16S rRNA genes. Even though the bacterial community composition was stable throughout the year, we found a drastic increase in abundances of total bacterial and fungal populations in the mid-winter, followed by a subsequent decrease during the snowmelt period in the early spring. We also found a temporary increase in abundances of NH_3-oxidizing bacterial population in the mid-winter and of denitrifying bacterial and fungal populations during the snowmelt period. Correspondingly, soil NH_4^+, NO_3^-, and dissolved organic N concentrations sequentially peaked in the winter and early spring. These results suggest that successive growth of litter degraders, ammonifiers, nitrifiers, and denitrifiers in the dormant season drives the subsequent bioavailable N transformations. High microbial N metabolic activities during the dormant season could also support plant growth during the growing season and increase the efficiency of annual N cycling in the forest via the seasonal partitioning of N between plants and microbial communities.

1. Introduction

Soil microbial communities play fundamental roles in ecosystem N cycling through the production and consumption of bioavailable N. The supply of bioavailable N to plants provided by microbial communities likely limits the productivity of forests in temperate and boreal climatic regions (Vitousek and Howarth, 1991; Canfield et al., 2010). Since microbial biomass N, in addition to plant biomass N, is the main component of organic N in forest soils, microbial communities function as a reservoir and source of bioavailable N in forests. Microbial cells have even a much higher protein content than plant litter (Neidhardt et al., 1990), and a large amount of labile N is stored in their cells as they grow, which is later released when they die. Microbial communities also express diverse N metabolisms and convert various complex compounds of organic N in litter and root exudates of plants into smaller molecules that can be subsequently assimilated by both microbes and plants. Microbes and plants may also compete for major N nutrients, such as NH_4^+ and NO_3^- (Kuzyakov and Xu, 2013). Thus, the ecological behavior of soil microbial communities is an essential factor influencing bioavailable N cycling and therefore primary production of forests.

Temperate and boreal forests are characterized by the seasonal photosynthetic activity of plants (Voříšková et al., 2014). Deciduous plants shed leaves in autumn and become dormant in winter and early

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spring. Microbial activity tends to decrease as the temperature drops (Price and Sowers, 2004), and therefore is generally considered to be low in winter. However, microbial growth and metabolism can continue to occur even at subzero temperatures (Bakermans and Skidmore, 2011). Indeed, plant litter decomposition (Mullen et al., 1998; Jaeger et al., 1999; Schmidt et al., 2007) as well as high enzymatic activity related to litter decomposition (Lipson et al., 2002; Schweiger et al., 2010; Voříšková et al., 2014) and high soil mineralization rates (Hishi et al., 2014; Zhang et al., 2014) have been observed in the snow covered forests. The large supply of fresh litter from deciduous trees in autumn, as well as microbial cryophilic ability, could enable these microbial activities in winter, together with the lack of competition for N with plants.

In many cool-temperate and boreal forests, soil freeze–thaw event occurs repeatedly at the onset of snowfall in winter and when snow melts in early spring. This event could accelerate the microbial activities through the physically fragmentation of plant litter, fine roots, and necromass (Hosokawa et al., 2017). In contrast, repeated soil freeze–thaw events could also damage microbial cells, causing a release of microbial biomass N into soils (Shibata et al., 2013; Shibata, 2016). That microbe-derived N could be lost through denitrification or leaching. Relatively large N_2O emissions through denitrification have been frequently observed in the snowmelt period due to increased anaerobiosis and substrate availability (Wagner-Riddle et al., 2017; Congreves et al., 2018). Nitrate leaching could also occur in this period (Ohte et al., 2004). However, a considerable portion of the microbe-derived N could be taken up and mineralized by microbes and then further utilized by plants to meet their strong N demand in the following growing season (Mullen et al., 1998; Jaeger et al., 1999). Thus, soil microbial activity in winter may play an important role in bioavailable N cycling in the subsequent growing season.

Microbial community analyses also suggest that there could be cryophilic or cryotolerant microbes that maintain N metabolism and growth in low temperatures and even in partially frozen forest soils (Bardgett et al., 2005; Schmidt et al., 2007). A study in the Arctic tundra showed that distinctly different microbial taxa are potentially active in winter and summer in the active layer overlaying permafrost, whereas microbial abundance was similar between the seasons (Schostag et al., 2015). Studies in the alpine tundra in the United States showed that bacterial and fungal community compositions changed seasonally, that microbial taxa potentially involved in litter degradation likely dominated the soil community during the snow cover period, and that soil microbial biomass peaked during the snow cover period (Schadt et al., 2003; Lipson and Schmidt, 2004). Moreover, recent studies in temperate and boreal forests have shown that bacterial and fungal community compositions change seasonally (Schweiger et al., 2010; Voříšková et al., 2014; Zhang et al., 2014; Žifčáková et al., 2016). A study using metatranscriptome analysis in a conifer forest in the Czech Republic showed that major microbial transcription in winter and summer was originated from different taxa and that bacterial contribution to the microbial transcription increased from 67% in summer to 84% in winter (Žifčáková et al., 2016).

Despite these observations, the relationship between seasonal microbial community dynamics and N dynamics has not been well characterized in temperate and boreal forests. In particular, biogeochemical consequences of microbial community dynamics during the plant dormant season on N cycling have not been thoroughly examined. Because many climatic events that potentially affect the microbial community composition and functioning occur during relatively short time periods in the winter season, such as snowfall, snowmelt, and repeated soil freeze–thaw cycles, frequent field investigations are needed for the precise examination. However, such investigations are practically more laborious in the winter season than in the other seasons. In fact, none of the microbial ecological studies described above involved more than four temporal data points during any given year or more than one or two points during the dormant season.

The purpose of this study is to illustrate the relationship between seasonal microbial community dynamics and bioavailable N dynamics throughout a year in a cool-temperate, deciduous forest. We specifically focus on how climatic events during the plant dormant season (winter to early spring), such as snowfall, snowmelt, and soil freeze–thaw cycles, affect microbial population abundance, community composition, and ecological functions, and relevant bioavailable N dynamics. We examined the growth and death of total bacterial and fungal populations by examining temporal change in abundances of their 16S rRNA and 18S rRNA genes, as described in Blazewicz et al. (2014). We also analyzed the growth and death of NH_3-oxidizing bacterial and achaeal populations (nitrifiers that control the rate-limiting step of nitrification) (Isobe et al., 2011a), and of nitrite-reducing bacterial and fungal populations (denitrifiers that are responsible for a key step of denitrification) (Wei et al., 2015a). We also characterized seasonal changes in bacterial community composition and their genetic potential for litter degradation and ammonification by sequencing their 16S rRNA gene followed by through a computational prediction approach.

We here hypothesized based on the previous ecological observations that there is seasonal N partitioning between plants and microbes and within microbial community in the forest. In particular, we hypothesized that some microbial taxa or functional groups actively degrade the litter or necromass and take up the mineralized N during the plant dormant season, that they release their biomass N into soil through the cell breakage in snowmelt period that could be utilized by plants in the following growing season, and that the different sensitivity to substrate availability and soil temperature among the microbial taxa or functional groups shape the different community composition across season.

2. Materials and methods

2.1. Study site

The study site was established in a natural, deciduous, broad-leaved forest in the Shibecha branch of the Hokkaido Forest Research Station, Field Science Education and Research Center, Kyoto University (located in the northern part of Japan). The forest is located at the boundary of the boreal and temperate climatic regions. The mean annual precipitation and air temperature (1981–2010) were 1169.7 mm and 5.8 °C, respectively. The mean annual maximum snow depth (1987–2010) was 68.7 cm. The soil is classified as Andosols. Soil pH and total C content of the soils around this study site are 5.4 and 11.2%, respectively. Other soil properties around the site were reported in Christopher et al. (2008) and Urakawa et al. (2015b).

We established twelve 5 × 12 m study plots on the near lower end of a gentle slope, Six plots were located in a row on the west- and east-facing slopes in the forest. The distance between each adjacent plot was 3.5–11.1 m depending on the micro topography. The lower end of each plot was 4.3–8.4 m from the bottom of the slope. The forest is dominated by Japanese oak, *Quercus crispula*, and each plot had two or three individuals of Q. *crispula*, whose average diameter at breast height was about 30 cm. The understory layer was densely covered by dwarf bamboo, *Sasa nipponica*, with maximum heights of about 80–100 cm.

The study was conducted from October 2013 to September 2014. Snowpack depth was measured at three points in each plot using a meter stick at the sampling dates described below. Soil temperature at 0- and 5-cm depth was measured in each plot at 30-min intervals using thermal sensors. The beginning of the growing season was monitored by sap-flow measurements of Q. *crispula* using Granier sensors.

2.2. Soil sampling

Mineral soil samples at a depth of 0–10 cm (A horizon) were collected using a hard, sharp shovel throughout the study period in each plot after removal of the organic layer (O horizon). Soil sampling was conducted at about 1-week intervals from mid-March to late-May (nine