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Foraging association between myxomycetes and fungal communities on coarse woody debris

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ABSTRACT

Myxomycetes are one of the major microbial predator groups found in detrital food webs within terrestrial ecosystems. They are typical inhabitants of coarse woody debris (CWD). However, the association between fungal communities and the foraging habits of myxomycetes has not been explored sufficiently in the field. Our study aimed to find community relationships between myxomycetes and fungi, a potential prey, on CWD, and the trophic status of saproxylic myxomycetes using stable isotope analysis of their sporocarps. Records of sporocarps present on 184 *Pinus densiflora* logs during a 3-year period listed 37 species of myxomycetes and 45 species of fungi. Ordination analysis using occurrence data of 34 dominant species (17 myxomycetes and 17 fungi) revealed their dynamic succession during log decay. Fungal dominants were clearly divided into two groups—earlier and later— and the majority of myxomycetes occurred during the middle stages of log decay between the first and second groups of fungal dominants. Species level associations between fungal and myxomycetes communities were rare. Isotopic nitrogen ($\delta^{15}N$) values of myxomycetes were significantly higher than those of wood-decay fungi, but few myxomycetes showed $\delta^{15}N$ values higher than those of ectomycorrhizal fungi. Isotopic carbon ($\delta^{13}C$) values of myxomycetes were not significantly different from those of fungi. $\delta^{15}N$ values of myxomycetes and fungi and $\delta^{13}C$ of myxomycetes significantly increased with an increase in wood decay. However, these positive correlations between stable isotope profiles and wood decay disappeared after the values were calibrated by subtracting the $\delta^{15}N$ and $\delta^{13}C$ values of sapwood from those of myxomycetes and fungi. While saproxylic myxomycetes have long been assumed to be bacterivous, the results of this study suggest that wood-decay fungi could be an important part of their diet, and their foraging associations might be non-species specific.

1. Introduction

Among dead plant tissues, coarse woody debris (CWD) is a quantitatively and qualitatively important food source in the detrital food web within forest ecosystems for the following reasons. They store more than 73 billion tonnes of carbon across the world (Pan et al., 2011), they constitute nearly one-half the volume of plant tissues input annually to the forest floor (Vogt et al., 1986), and they host a variety of saproxylic communities (Stokland et al., 2012). Dead plant tissues are consumed by decomposers, such as fungi and bacteria, which are, in turn, foraged by microbial feeders and detritus feeders (Schmitz, 2010). Foraging behaviour of microbial feeders is important for nutrient release from dead plant tissues (de Vries et al., 2013). The microbes immobilize nutrients within their bodies, making it unavailable for uptake by plants, but then these nutrients are mineralized and become plant nutrients when the microbes are consumed. Microbial feeders also affect the decay rate of dead plant tissues by controlling the decay activity of microbes (Crowther et al., 2012). Thus, the knowledge of interspecific foraging linkages in detrital food webs has great ecological significance.

The myxomycetes are a group of protists that belong to the supergroup Amoebozoa (Schnittler et al., 2017) and typically inhabit CWD (Stokland et al., 2012). The life cycle of myxomycetes consists of two very different trophic stages—the uninucleate amoebae and the plasmodium, a distinctive multinucleate structure (Stephenson et al., 2011). Myxomycetes act as predators of other microorganisms, such as bacteria, fungi, cyanobacteria and algae (Stephenson et al., 2011). Their amoebae can reach high densities in detritus (Feest and Madelin,

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1985; Urich et al., 2008; Geisen et al., 2015; Taylor et al., 2015, 2017). The foraging activity of their plasmodia within dead wood has the ability to mineralize nutrients by consuming microorganisms and excrete wastes (Fukasawa et al., 2017a). Previous studies have demonstrated that several species can complete the spore-to-spore life cycle using living or dead cells of *Escherichia coli* as prey, particularly in their amoebae stage under culture conditions (Madelin, 1984; Ishibashi et al., 2001), suggesting that bacteria is an important food source for myxoamoebae (Stephenson et al., 2011). However, the foraging habits of the majority of myxomycete species have not been explored sufficiently in the field.

There are several field observations reporting that the plasmodia of myxomycetes feed on fungal fruit bodies. For example, plasmodium of the Physarales species *Badhamia utricularis* is known to feed on fungal sporocarps in the field (Elliott, 1914; Harada, 1977) and has lytic enzymes that work on fungal fruit bodies (Miyairi et al., 1994). Similarly, the Physarales species *Physarum compressum* and Stemonitales species *Stemonitis herbatica* are known to overgrow basidiomycetes *Pleurotus* mushrooms and fructify on them (Desrumaux et al., 2003). Studies under controlled laboratory conditions confirmed that the plasmodia of some species caused lysis on the fungal hyphae cultured on artificial media and that the preference for specific fungal species as a food source is different among myxomycete species (Elliott and Elliott, 1920; Howard and Currie, 1932). These results suggest that fungivory might also be common in myxomycetes, particularly in their plasmodial stage.

The ratio of carbon (C)- and nitrogen (N)-stable isotopes (δ^{13}C and δ^{15}N) are widely used to reveal the trophic interactions in detrital food webs (Hyodo et al., 2010). Because δ^{13}C values of consumers are similar (< 1‰ difference) to that in their diet and δ^{15}N values are ~3‰ higher than that in their diet, we can estimate the C source of consumers by their δ^{13}C values, and their trophic positions by their δ^{15}N values (DeNiro and Epstein, 1978; Minagawa and Wada, 1984). Recently, based on the assumption that myxomycetes are bacterivore, Tiunov et al. (2015) applied stable isotopic measurements to myxomycetes collected from leaf and woody litter on the forest floor to estimate the δ^{13}C and δ^{15}N values of soil bacteria, those of which are practically difficult to measure the isotopic signature directly. However, it is unknown whether the myxomycete communities would utilize the fungal communities as food resources on CWD, or whether there are species-level associations between the two soil organisms.

Here, we aim to identify the trophic status of saproxylic myxomycetes by analysing the stable isotopes of their sporocarps and those of fungi that are fruiting on CWD. Because their communities change dramatically during CWD decomposition, we conducted a community analysis of myxomycetes and fungi using the presence/absence dataset of their sporocarps on CWD in a variety of decay stages to determine the possible species-level associations between the two organisms (Fukasawa et al., 2015).

2. Materials and methods

2.1. Study site

This study was conducted in a secondary forest dominated by oak (*Quercus serrata*) and pine (*Pinus densiflora*) in the northern part of the central island of Japan (Mt. Chitose; 38°14′N, 140°21′E; altitude 245 m). Canopy tree composition in this site was described in Fukasawa et al. (2017b). The aspect is a gentle northwestern slope with a mean total annual precipitation of 1238 mm, mean annual temperature of 12.0 °C (period 2001–2010, Japan Meteorological Agency) and maximum snow depth of approximately 2 m at the nearest weather station (Yamagata; 38°15′N, 140°21′E; altitude 153 m). The area of interest was a shrine forest (approximately 100 ha) managed by the Forestry Agency of Japan. Pine wilt disease caused by the North American native pinewood nematode *Bursaphelenchus xylophilus* was first observed in 1982 in this area and has caused severe dieback of *P. densiflora* during recent decades. The area had undergone CWD management (felling of infected trees and fumigation with pesticides, such as methylcarbamodithioic acid ammonium) to prevent the spread of pine wilt disease.

2.2. Myxomycetes–fungal community on CWD

The presence of myxomycetes and fungal sporocarps was recorded on 184 *P. densiflora* logs (diameter, 10–74 cm; mean = 33 cm) within an area of approximately 1 ha in the study site 18 times from May to October in 2013, 2014 and 2015 (once each month). The logs were classified into the five decay classes (DC) according to the criterion described by Fukasawa (2012) ranging from recently dead hard logs (DC I) to strongly decayed fragile logs (DC V) (27, 35, 26, 54 and 42 logs for DCs I, II, III, IV and V, respectively). To eliminate within-stem variation in DC (Pyle and Brown, 1999), we selected a stem section in which DC was uniform for each CWD (ca. 2 m along the stem).

Fungi and myxomycetes were identified in the field or were collected for later identification by morphological observation using microscopes. In the case we were not confident of morphological identification, we used molecular techniques for several fungal specimens with using internal transcribed spacer 1 and 2 regions of rDNA (amplified by the primers ITS1F/ITS4) with the National Center for Biotechnology Information (NCBI) database as a reference (sequence similarity > 97%). As a consequence, 7 fungal species, *Callistosporium luteo-olivaceum*, *Femsjonia uniseptata*, *Gymnopilus liquiritiae*, *Gloeoporus dichrous*, *Hygrocybe* sp., *Leucogyrophana arizonica*, *Pholiota lubrica*, were identified from their DNA. Among them, the two fungal species (*Femsjonia uniseptata* and *Hygrocybe* sp.) were new to science and the first one was described as a new species using a specimen obtained from this site (Shirouzu et al., 2017). Myxomycetes were identified to species or variety level according to their morphology. Thus, the list of recorded species included different taxonomic levels, but here, for simplicity, we use the term “species” for all identified organisms. All occurrences were recorded as binary data regardless of the number and size of the sporocarps or the area they occupied on CWD. The frequencies of each species on logs were calculated as the number of records of the species/the total number of CWD × 100. Dried specimens of collected fungi and myxomycetes are archived in National Museum of Nature and Science in Japan (*F. uniseptata*, #TNS-F-54018), herbaria of Fungus/Mushroom Resource and Research Center of Tottori University (*Hygrocybe* sp., #TUMH 62171–62183) and the private collection of Y. Fukasawa at Tohoku University (the remaining specimens).

2.3. Stable isotope analysis

Mature fruiting bodies of myxomycetes and fungi and sapwood samples were taken from the logs during the study period. Overall, 112 myxomycete (29 species including 6 varieties), 86 fungi (29 species), and 33 sapwood samples were collected and analysed.

All samples collected were oven dried at 60 °C for at least 48 h and stored in capped glass vials at room temperature. Before analysis, the samples were ground to powder form with a MM400 ball mill (Retsch GmbH, Haan, Germany) and put into tin capsules. Their isotopic compositions were determined using an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific, USA), coupled with the Flash 2000 elemental analyser (Thermo Fisher Scientific, Waltham, MA, USA) at Okayama University, Okayama, Japan. The isotopic compositions of N and C were expressed in the δ-notation relative to international standards (atmospheric N or Vienna Pee Dee Belemnite): $\delta X = [(R_{sample}/R_{standard}) - 1]$, where R is the ratio of the heavier isotope to the lighter isotope. The analytical precision was < 0.2‰ for δ^{13}C and δ^{15}N values. Along with isotope analyses, N and C contents (as mass %) were determined in all samples.

Since the bulk stable isotope profile of individual organism is strongly affected by their dietary substrates, δ^{13}C and δ^{15}N values in fungal and myxomycete sampes were calibrated by subtracting δ^{13}C