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Dynamics of ^{13}C natural abundance in wood decomposing fungi and their ecophysiological implications

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Abstract

Factors that affect the $\delta^{13}C$ values of fungi need to be analyzed for the progress of isotope-based studies of food-chain or organic matter dynamics in soils. To analyze the factors that control $\delta^{13}C$ values of the fungal body, basidiomycete and ascomycete species were grown on a beechwood substrate (six species) and in glucose medium (nine species), and the $\delta^{13}C$ value of produced fungal body was compared to that of the carbon source. The ^{13}C enrichment ($\Delta\delta^{13}C$) in the fungal aggregates compared to the decomposed wood varied from 1.2 to 6.3‰ among six species. In the glucose substrate experiment, the degree of ^{13}C enrichment in the hyphal mat was relatively small and varied from -0.1 to 2.8‰ among nine basidiomycetes species depending on their growth stage. Calculated $\delta^{13}C$ values of the respired CO_2 were lower than those of the hyphal mat, organic metabolites and the glucose used. The degree of ^{13}C enrichment was affected by fungal species, substrate and growth stage. Fungal internal metabolic processes are the plausible mechanism for the observed isotopic discrimination between fungal bodies and substrates. Especially, dark fixation of ambient CO_2 and kinetic isotope fractionation during assimilation and dissimilation reactions could well explain $\Delta\delta^{13}C$ dynamics in our experiments. Through the analysis of field $\Delta\delta^{13}C$, we could know undisturbed fungal status about starvation, aeration and type of decomposition.

Keywords: Carbon isotopes; Detritus food chain; Fungi; Invertebrates; Isotope fractionation; Beech wood; White rot; Brown rot

1. Introduction

Fungi inhabit primarily in the aerobic terrestrial soil and play two kinds of important ecological roles which cannot be replaced by any other class of organisms. One is the decomposition of plant-derived lignin-rich polymers and humus by the saprophytic fungi (Rayner and Boddy, 1988). The other is the symbiotic nutritional relationships with vascular plants by the mycorrhizal fungi and endophytes (Smith and Read, 1997). Carbon, nitrogen and phosphorus cyclings in forest cannot be maintained without these saprophytic and symbiotic fungi. In many terrestrial ecosystems, biomass of fungal hyphae exceeds that of bacteria, and fungal respiration largely contributes to the soil respiration (West et al., 1987; Alphei et al., 1995). Hyphal biomass in the soil is one of the primary food sources for diverse soil invertebrates (Beare et al., 1992). However, dynamics of fungal biomass in the field and the relative importance of hyphae for the food source of the invertebrates have been unclear.

In the grazing food chains of aquatic communities, carbon isotope ratios ($^{13}C/^{12}C$; $\delta^{13}C$) in animal tissues are known to reflect the $\delta^{13}C$ of their diet, although small ^{13}C enrichments (≤ 1.0‰) between dietary and body sometimes occurred (DeNiro and Epstein, 1978; Fry and Sherr, 1984; Wada et al., 1998). So, $\delta^{13}C$ of animal bodies has been used to infer their primary food sources. $\delta^{13}C$ indices were applied also to the soil invertebrates in the detritus food

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chains to infer their energy and carbon sources (Neilson et al., 1998; Ponsard and Arditi, 2000; Santrucková et al., 2000; McNabb et al., 2001). Ponsard and Arditi (2000) showed that soil detritivores were enriched in ^{13}C relative to litter by 2.0–5.0‰ on monthly average. The large ^{13}C enrichments of soil detritivores relative to litter suggested the possibilities of the selective feeding of the ^{13}C enriched microbial fraction on the litter. Santrucková et al. (2000) showed that litter-feeding soil invertebrates respire CO_2 that is enriched in ^{13}C relatively to average litter carbon, and suggested that they selectively absorb some ^{13}C-enriched organic fraction out of the whole ingested litter.

What is the ^{13}C enriched fraction of litter utilized by soil invertebrates? The most plausible answer to this question would be fungal hyphae and/or bacterial cells colonized on and inside the litter. The information about the $\delta^{13}C$ values of the fungal sporocarps in the field and of the incubated hyphal aggregates and yeast cells has been accumulated. Basidiocarps of wood decomposing and litter decomposing fungi are known to be enriched in ^{13}C relative to wood and litter. Hobbie et al. (1999) and Kohzu et al. (1999) showed that the degree of ^{13}C enrichment of these saprophytic fungal sporocarps relative to their substrates ($\Delta\delta^{13}C$:$\delta^{13}C_{fungi} - \delta^{13}C_{substrate}$) were larger than +3.0‰ on average. This $\Delta\delta^{13}C$ is large enough to explain the $\delta^{13}C$ difference between soil invertebrate and litter by the selective feeding on fungal hyphae. In fact, Tayasu (1998) reported the significant ^{13}C enrichment in the termite relative to the litter with which they cultivates fungus comb enriched in ^{13}C. However, the degree of the carbon isotope discrimination during fungal growth can be highly variable in natural environment, and knowledge about the patterns of variation in $\Delta\delta^{13}C$ values and the mechanisms responsible for the variation is lacked at present. Therefore, in order to effectively apply the $\delta^{13}C$ indices to the soil detrital food webs, we must know what controls the $\delta^{13}C$ values of the fungal body in natural environments.

The variations in $\Delta\delta^{13}C$ values depend primarily upon the carbon isotope effects accompanied with fungal metabolic reactions. Some possible factors that affect the $\Delta\delta^{13}C$ variation are substrate quality (C:N ratio, lignin content, etc.) (Fernandez and Cadisch, 2003), saprophytic fungal species (e.g. brown rot vs. white rot fungi) (Henn et al., 2002; Abraham and Hesse, 2003), and the growth conditions of fungi (Henn et al., 2002), because these factors control the flux distribution among the fungal internal metabolic pathways. However, such factors have not been thoroughly considered in these previous studies of fungal $\delta^{13}C$ dynamics. In the present study, nine basidiomycetes and one ascomycetes species were used in the experiments to elucidate the interspecies $\delta^{13}C$ variation. Two carbon sources (glucose and beech-wood) were used as substrates to examine the effect of the quality of carbon source on fungal body $\delta^{13}C$. In the glucose substrate experiment, $\delta^{13}C$ values of the fungal body were compared between growth and autolysis period to know the effect of growth condition.

2. Materials and methods

2.1. Fungal strains

We used the following 10 different wood-decomposing fungi in the wood decomposing experiments and in the glucose substrate experiments.

White rot fungi: *Daedaleopsis tricolor* (Bull.: Fr.) Bond. et Sing. (IFO 6269); *Ganoderma applanatum* (Pers.) Pat. (IFO 8346); *Lenzites betulina* (L.: Fr.) Fr. (FFPRI L5b); *Panus rudis* Fr. (IFO 8994); *Polyporus anceps* Peck (*Dichomitus squalens*) (CBS432.34); *Poria subvermispora* Pilát (*Ceriporiopsis subvermispora*) (FP90031); *Trametes versicolor* (L.: Fr.) Quél. (FFPRI 1030).

Brown rot fungi: *Laetiporus sulphureus* (Fr.) *Murrill var. miniatus* (Jungh.) Imaz. (IFO 6497) *Tyromyces palustris* (Berk.: Curt.) Murr. (FFPRI 0507).

Soft rot fungi: *Chaetomium globosum* Kunze ex Fr. (IFO6347)

Three species of white rot fungi (*L. betulina*, *P. rudis*, *T. versicolor*), two species of brown rot fungi (*L. sulphureus*, *T. palustris*) and one soft rot fungus (*C. globosum*) were used in the wood-decomposing experiment. In the glucose substrate experiment, all species except *L. sulphureus* were used.

2.2. Wood degradation experiment

Beech (*Fagus crenata* Blume) wood was collected at Ashiu (35°22′N, 135°55′E), Japan, and cut into blocks (20×20×5 mm, 1.2–1.5 g dry weight). The wood blocks were sterilized with ethylene oxide gas (ES-15, Hirayama Manuf. Co., Kasukabe, Japan) and placed on a layer of quartz sand saturated with modified Trion's synthetic medium (85 ml per incubation bottle): $KH_2PO_4 \cdot 613$ mg, $MgSO_4 \cdot 7H_2O$ 246 mg, K_2HPO_4 114 mg, $CaCl_2$ 55.5 mg, $FeSO_4$ 20 mg, $ZnSO_4 \cdot 7H_2O$ 3.52 mg, $CuSO_4 \cdot 5H_2O$ 0.38 mg, $MnSO_4 \cdot H_2O$ 0.031 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.025 mg and thiamine hydrochloride 5 mg in 1000 ml distilled water (pH 6.0). Organic carbon content of this medium, consisting of thiamine, was negligible compared with that of wood (more than 500 mg C). Fungal pellets for inoculation were prepared from submerged culture using JIS9501 medium: glucose 40 g, peptone (Mikuni Co., Tokyo, Japan) 3 g and malt extract (Nacalai Co., Kyoto, Japan) 15 g in 1000 ml distilled water. The amount of fungal pellets inoculated on each wood block was less than 2.0 mg dry weight. Each wood block on the medium-saturated quartz sand was aseptically placed in a sterilized wide-mouthed bottle (900 ml) and incubated at 26 °C in the dark. Inside the bottle, aseptic conditions were maintained, although fresh air could pass through the loosely shut cap.