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Fungal succession and decomposition of composted aquatic plants applied to soil

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ABSTRACT

Few studies have documented the pattern of fungal colonization and decomposition of composted aquatic plants applied for amending soil. In the present study, we demonstrated the fungal succession and its relationship with chemical changes in decomposing composted aquatic plants applied to soil, by performing a 6-month litterbag experiment incubating three types of composted aquatic plants differing in the years of composting. A total of 189 operational taxonomic units (OTUs) of fungi were detected, including 115 and 34 OTUs in Ascomycota and Basidiomycota, respectively. The OTU richness generally increased with the progression of decomposition and was not significantly different among the three types of composted aquatic plants. Redundancy analysis indicated the relationships among the fungal assemblages, type of composted aquatic plants, duration of decomposition, and chemical changes in composted aquatic plants. Fungal succession was generally characterized by the cumulative increase of major OTUs and was affected by the contents of extractives, total carbohydrates, total carbon, and total nitrogen in decomposing composted aquatic plants.

1. Introduction

Fungi play a central role in the decomposition of plant residues on soil because they are capable of actively decomposing recalcitrant structural components in the plant cell wall, such as lignin and cellulose (Osono, 2007; Van der Wal et al., 2013). A diverse array of fungi takes part in plant litter decomposition, leading to chemical changes in litter and the concomitant colonization, replacement, and succession of fungal species (Hudson, 1968; Voříšková and Baldrian, 2013). Studying the relationship between fungal succession and changes in chemical composition of litter is crucial for understanding the functional roles of fungal diversity in decomposition processes.

Aquatic plants have often been used as organic amendments for soil, either immediately after being harvested from water or after being composted on land for several years, because they are a rich source of essential nutrients and organic compounds with the potential to improve plant yields and soil conditions (Gunnarsson and Petersen, 2007). The recent increase of overgrowing aquatic plants in water bodies worldwide (Chambers et al., 2008) has promoted interest in the effective utilization of composted aquatic plants (CAPs) as organic amendments and in the pattern of fungal succession and decomposition of CAPs on soil. Previous studies have examined the decomposition processes and fungal colonization of aquatic plants in aquatic environments (Howard-Williams and Davies, 1979; Webster and Benfield, 1986; Xie et al., 2004). However, data are lacking about the fungi and decomposition of CAPs in terrestrial environments. We hypothesize that fungal colonization and succession have critical relationships with chemical changes in decomposing CAPs on soil.

The purposes of the present study were to examine: (i) the taxonomic richness and composition of fungi associated with decomposing CAPs on soil, by metabarcoding the internal transcribed spacer (ITS) region of nuclear ribosomal DNA as the fungal genetic marker; (ii) the pattern of successional change of fungal assemblages in decomposing CAPs; and (iii) the relationship between fungal succession and chemical changes during the decomposition. Three types of CAPs were used: all three were

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derived from Lake Biwa, the largest lake in Japan, and they differed regarding the number of years of composting. A field experiment was performed using litterbags to incubate CAPs on soil of an experimental agricultural field, which is a proxy for the surface amendment of CAPs in farmlands.

2. Materials and methods

2.1. Materials

We used composted aquatic plants (CAPs) harvested from the south basin of Lake Biwa, Shiga Prefecture, Japan. The local government of Shiga Prefecture harvests aquatic plants (mostly submerged macrophytes) either superficially or thoroughly by uprooting. The harvested aquatic plants are transported to an open field on the southeast side of Lake Biwa, Ohmihachiman City, piled up in several rows, each measuring approximately 5 × 20 m and approximately 2 m in height, and left exposed generally for 1 to 2 y, for composting. The piled aquatic plants are mixed using an excavator once or twice per year during the first 2 y of composting to ensure aeration and maturation.

We used three types of CAP (CAP0, CAP1, and CAP2) differing in the years of harvest and the period of composting. CAP0 were non-composted aquatic plants harvested in June 2012 and kept in the composting field for one week. Hence, the materials denoted CAP0 had not been kept long enough for composting, but they were referred to using the terminology CAP for the sake of simplicity. CAP1 were harvested in June 2011 and composted for 1 y (i.e. one-year-old composted aquatic plants), and as a result became fragile but still remained fibrous. CAP2 were harvested in June 2010 and composted for 2 y (i.e. two-year-old composted aquatic plants), whereby they became more fragmented and clumped, with a few fibrous plant materials remaining. Note that CAP0, CAP1, and CAP2 differed not only in the years of composting but also in the original composition of aquatic plants because they were harvested in different years and in different parts of the lake. The composition of plant species in CAP0, CAP1, and CAP2 was not determined but consisted mainly of submerged macrophytes that predominated in the south basin of Lake Biwa, such as *Hydrilla verticillata*, *Potamogeton maackianus*, and *Ceratophyllum demersum* (Ohtsuka et al., 2004). We procured CAP0, CAP1, and CAP2 in June 2012, each from the upper part of a single pile to minimize soil contamination and the heterogeneity of plant material, and took them back to the laboratory.

2.2. Litterbag experiment

The composts were dried in an oven at 40 °C for 1 week to a constant mass and enclosed in litterbags to be used for the study of decomposition (Osono et al., 2006). Leaf litter (4.98–5.02 g, 5.00 g on average) was placed in each litterbag (24 × 18 cm) made of nylon with a mesh size of approximately 2 mm. A total of 108 litterbags were prepared, 36 for each of CAP0, CAP1, and CAP2. The decomposition experiment was performed for 6-months from June to December 2012. Six quadrats (1 × 1 m) were established in an area (measuring 5 × 5 m) of the experimental field of the Center for Ecological Research (established in 1998), Kyoto University, in Shiga, Japan. Mean annual temperature of the experimental agricultural field was 14.4 °C, and mean annual precipitation was 1665 mm for 10 y from 2007 to 2016. Mean monthly temperature during the experimental period in 2012 increased from 20.6 °C in June to reach 27.0 °C in August, and then decreased to 4.1 °C in December. Monthly precipitation was 92–302 mm from June to December 2012. In the research area, weeds were mowed every year but no experimental plants were cultivated for several years. Weed species include *Imperata cylindrica*, *Desmodium paniculatum*, *Artemisia indica* var. *maximowiczii*, *Equisetum arvense*, *Solidago altissima*, *Trifolium repens*, and *Cyperus microiria*.

Litterbags were placed on soil within the quadrats in June 2012, with 5 bags per CAP type in each quadrat. The litterbags were fixed to soil using vinyl-coated wires to prevent movement and ensure good contact between the bags and the litter layer. The remaining six litterbags of each CAP type were stored for analysis of the initial chemical properties. Sampling of the bags was performed 5 times: 0.5, 1, 2, 4, and 6 months after initiation of the experiment. On each sampling occasion, six litterbags per CAP type were retrieved from six quadrats. The collected litterbags were transported to the laboratory and used for mass determination, DNA analysis, and chemical analyses.

A portion of samples (0.5 g mean wet weight) in the litterbags was kept in a tube containing cetyltrimethylammonium bromide (CTAB) lysis buffer and stored at −20 °C until DNA extraction. The remaining samples were oven-dried to a constant mass at 40 °C, and mean values of remaining mass were calculated for each sampling and CAP type, taking the sample portions used for DNA analysis into account. The dried subsamples were ground in a laboratory mill so that they could pass through a 0.5-mm screen and used for chemical analyses as described below.

2.3. DNA extraction, PCR amplification, and pyrosequencing

The methods of DNA analysis followed those described in Matsuoka et al. (2016a). Whole DNA was extracted from a total of 90 samples (3 CAP types × 5 collections × 6 replicates) for DNA analysis using the modified CTAB method described by Gardes and Bruns (1993). For direct 454 sequencing of the fungal internal transcribed spacer 1 (ITS1), we used a semi-nested PCR protocol. First, the entire ITS region was amplified using the fungus-specific primers ITS1F (Gardes and Bruns, 1993) and LR3 (Vilgalys and Hester, 1990). PCR was performed in a 20 μl volume with the buffer system of KOD FX NEO (TOYOBO, Osaka, Japan), which contained 1.6 μl of template DNA, 0.3 μl of KOD FX NEO, 9.0 μl of 2 × buffer, 4.0 μl of dNTP, 0.5 μl each of the two primers (10 μM), and 4.1 μl of distilled water. The PCR conditions were as follows: an initial incubation for 5 min at 94 °C; followed by 20 cycles of 30 s at 95 °C, 30 s at 58 °C for annealing, and 90 s at 72 °C; and a final extension of 10 min at 72 °C. The PCR products were purified using ExoSAP-IT (GE Healthcare, Little Chalfont, Buckinghamshire, U.K.) and diluted by adding 225 μl of sterilized water. A second PCR was then conducted targeting the ITS1 region using ITS1F fused with the eight base pair DNA tag (Hamady et al., 2008) for post-sequencing sample identification, and the reverse universal primer ITS2 (White et al., 1990). PCR was performed in a 20 μl volume with the buffer system of KOD Plus NEO (TOYOBO), which contained 1.0 μl of template DNA, 0.4 μl of KOD Plus NEO, 2.0 μl of 10 × buffer, 2.0 μl of dNTP, 1.2 μl of $MgSO_4$ (25 mM), 0.8 μl each of the two primers (5 μM), and 11.8 μl of distilled water. The PCR conditions were as follows: an initial incubation for 5 min at 94 °C; followed by 25 cycles of 30 s at 95 °C, 30 s at 60 °C, and 90 s at 72 °C; and a final extension of 10 min at 72 °C. PCR products were purified with ExoSAP-IT and quantified with NanoDrop. Amplicons were equimolarly pooled and purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The pooled product was sequenced with a GS Junior sequencer (454 Life Science, Branford, USA) at Roche Diagnostics, Tokyo, Japan.

2.4. Bioinformatics

The procedures used for bioinformatics analyses followed those described in Matsuoka et al. (2016b). In the pyrosequencing, 141,115 reads were obtained. These reads were trimmed with sequence