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# Composition variability of spent mushroom substrates during continuous cultivation, composting process and their effects on mineral nitrogen transformation in soil

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ABSTRACT

Spent mushroom substrates (SMSs) are a typical by-product of mushroom production, which is rich in nutrient like nitrogen. The reuse of SMSs as soil amendments has become the focus of attention. Due to its nutrient content, SMSs could contribute to reduce the use of non-renewable resources, such as peat. Recently, a SMSs recycling strategy was adopted in an edible mushroom industry Ltd., China. The waste materials experienced a continuous cultivation, composting process which sequentially includes the cultivation of *Pleurotus eryngii*, the cultivation of *Pleurotus ostreatus*, the composting process, and were finally used as soil amendments. The main objective of this study was to investigate the composition variability of SMSs during continuous cultivation, composting process and their effects on mineral nitrogen transformation in amended soil. The components analysis suggested the relative moisture and polysaccharide content of SMSs decreased by 33.6% and 17.1%, respectively, during the continuous cultivation, while protein increased by 29.5%. Moreover, relative humic acid content of SMSs increased by 18.6% during composting process while biodegradable matter (BDM), polysaccharide and protein decreased by 38.6%, 79.4% and 50.0%, respectively. The results of a 42-day incubation experiment suggested that addition of spent mushroom composts (SMCs) can significantly enhance the mineral nitrogen contained in soil. With the amendment of SMCs, 39.4% of input nitrogen was converted into mineral nitrogen within day 42. Combined application of SMCs/urea was considered to be a better strategy, since it provided soil with more mineral nitrogen than SMCs alone throughout the whole incubation.

## 1. Introduction

China is one of the most important mushroom-producing countries contributing over 80% of the global total output of mushrooms and yearly production > 20 million tons (Li, 2012; Phan and Sabaratnam, 2012).As a by-product of mushroom industry, spent mushroom substrates (SMSs) have also shown a dramatic increase over the years (Kapu et al., 2012). About 5 kg of SMSs are produced from each kilogram of mushrooms (Paredes et al., 2009) and the quantity of SMSs generated by mushroom production in China was 13.2 million tons per year in 2010 (Gao et al., 2015). Traditionally, incineration has been applied for the final disposal of abandoned SMSs, which could cause a series of environmental problems including air pollution. Thus, it is necessary to adopt new techniques for the beneficial use of SMSs in value-added applications.

SMSs have potential uses in many fields, such as soil-less growing medium (Medina et al., 2009; Ribas et al., 2009), soil and water bioremediation (García-Delgado et al., 2013; Jordan et al., 2008; Lau et al., 2003; Li et al., 2012), energy feedstocks (Finney et al., 2009), animal feeds (Li et al., 2001) and organic amendments (Courtney and Mullen, 2008; Paula et al., 2017). However, most of these applications are not viable, and are unable to completely solve the disposal problem of SMSs; only agricultural use is an economically and ecologically acceptable way (Paredes et al., 2016). Considering the nutrient-rich residues contained in SMSs, mushroom production companies in China have already established a continuous cultivation which means to reuse the original spent substrates as the medium of another mushroom. Moreover, the residual wastes after harvesting can be composted and used as soil amendments. So far, the whole recycling of SMSs has been realized.

Qingyuan, a county located in the southwestern part of Zhejiang Province, is one of the main cultivation areas of edible mushroom in China. Being considered as one of the largest species of edible mushroom, *Pleurotus eryngii* (also known as king trumpet mushroom) is widely cultivated in Qingyuan. In order to recycle SMSs, local edible mushroom industries tend to reuse the SMSs of *Pleurotus eryngii* (SMSs-

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PE) as cultivation medium of another common edible mushroom, *Pleurotus ostreatus*. Subsequently, the collected residues (SMSs-PO) of mature *Pleurotus ostreatus* are composted to make spent mushroom composts (SMCs), which can be used as soil amendments. The recycling process of SMSs has been defined as a continuous cultivation, composting process. As reported, the use of compost for soil amendment is a promising agricultural practice environmentally and economically viable (Cicatelli et al., 2014). However, the nutrients contained in SMSs are not available for plants immediately (Curtis and Suess, 2006). This is particularly the case for nitrogen (Hackett, 2015). Maher et al. (2000) has reported that only 10.8% of the total N in SMCs was present in the plant available forms of nitrate and ammonium while Bercher and Pakula (2014) reported that mineral N contained < 10% of total N in SMSs. All the literatures mentioned above suggest that nitrogen content contained in SMCs is slowly released to soil through mineralization (Stewart et al., 2000). Although some studies have discussed the nitrogen fractions in SMSs or properties of SMSs/SMCs-amended soil, few researchers have focused on the release process of mineral nitrogen from SMSs/SMCs to soil. In other words, the mineralization of nitrogen in SMSs/SMCs needs to be thoroughly evaluated since it is the main process regulating nitrogen availability.

Hence, in this study, we first analyzed the physico-chemical properties of SMSs at different stages of the continuous cultivation, composting process (including the prepared cultivation mediums as PCMs, the reused cultivation medium as SMSs-PE, the raw composting material as SMSs-PO, and the product of compost as SMCs). Moreover, an indoor incubation experiment was conducted under field conditions to evaluate variations of mineral N transformation in soil amended with SMSs at different stages. Soils amended with traditional nitrogen fertilizers including urea and ammonium sulfate were set up as control groups in this work.

## 2. Materials and methods

### 2.1. Materials

The prepared cultivation medium (PCMs), SMSs of *Pleurotus eryngii* (SMSs-PE), SMSs of *Pleurotus ostreatus* (SMSs-PO) and SMCs, used in the present study were generously provided by an edible mushroom industry Ltd., located in Qingyuan county (Zhejiang Province, China). The detailed information about the PCMs was recorded in a Chinese patent (patent number: CN201310312985.7, Wu et al., 2013). The formulation of PCMs contains sweet potatoes (5–10%), sawdusts (30–40%), wheat brans (10–20%), bagasses (5–15%), straws (10–30%), calcium carbonates (5–8%) and etc. After harvesting fruit bodies of *Pleurotus eryngii*, residual mediums were collected as SMSs-PE. Afterwards, SMSs-PE (93%) was combined with sawdust (5%), $CaSO_4$ (2%), mineral salt (0.5%) to produce mediums for *Pleurotus ostreatu* growing. Similarly, the residual mediums of *Pleurotus ostreatu* were collected as SMSs-PO. Finally, the SMSs-PO was supplemented with $CaSO_4$ (2%), mineral salt (0.5%), small amounts of lime water (to neutralized the organic acid during composting) and conducted with a combined static, heap-turning composting process to obtain the mature compost, which was collected as SMCs. As shown in Fig. S1(c) and (d), mixed materials were built into static heaps. Static piles were periodic turning to ensure adequate aeration using turning machines. Additionally, forced ventilation was not used in this case. The composting process was completed in three-months.

Considering land-use and soil properties, the loamy soil samples used in the incubation experiment were collected from Hangzhou, Zhejiang Province, China (30°18′20″N, 120°4′21″E), which served as a typical subtropical cropland. The main background values of soils were presented in Table 1. The soil samples were collected from the top layer in soil profile, as described by other researchers (Zhao et al., 2014; Tong and Xu, 2012) (0–20 cm). According to published works, all the fresh materials and soil were air dried and pulverized through a 2-mm sieve prior to experiments (Tong and Xu, 2012; Zhao et al., 2014).

**Table 1**
Physicochemical characteristics of selected soils.

| Soils | | |
|---|---|---|
| Sampling site | | Hangzhou, Zhejiang Province of South China (30°18′20″N, 120°4′21″E) |
| pH (1:2.5, w/v) | | 6.37 |
| SOM (%) | | 3.5 |
| CEC (cmol/kg) | | 12.8 |
| Texture (%) | Sand | 24.2 |
| | Silt | 60.4 |
| | Clay | 15.4 |

### 2.2. Component analysis of SMSs/SMCs

Generally, several parameters related to nutritional ingredients of SMSs/SMCs were measured to evaluate the changes that occurred during the continuous cultivation, composting process. Moisture content was determined through an oven-drying method. The pH values were measured by extracting SMSs/SMCs in distilled water with a ratio of 1:5 (w/v), using a Mettler Toledo pH meter. Electrical conductivity (EC) and total dissolved solids (TDS) were analyzed through a water extract (in the ratio of 1:5, w/v) using conductivity meter (DDBJ-350, LeiCi, China). Prior to chemical analysis, all the materials were pulverized to pass through a 0.15-mm sieve. Total carbon, hydrogen and nitrogen contents were assessed using a CHN elemental analyzer (Flash EA 1112, ThermoFinnigan), while the C/N ratio was calculated subsequently. Protein component was assessed using Coomassie Brilliant Blue method (Sedmak and Grossberg, 1977). Polysaccharide component was obtained by using phenol-sulfuric acid method, as described elsewhere (Masuko et al., 2005). Ash content was measured based on China National Standards (GB/T 12532-2008 determination of ash content in edible mushroom). Cellulose, hemicellulose and lignin contents were analyzed according to the Van-Soest method recorded in Agricultural Handbook (No. 379). After NDF (Neutral detergent fiber), ADF (acid detergent fiber), and ADL (acid detergent lignin) were all determined; the cellulose content was estimated as ADF-ADL, the hemicellulose was calculated as NDF-ADF, while the lignin content was equal to the value of ADL. Contents of total humic acid and BDM (biodegradable matter) were detected by the methods described by Bao (2000) and Zhao et al. (2002), respectively.

### 2.3. Characterization of substrate samples

FTIR (Prestige-21 Japan) analyses were performed to characterize the changes of chemical functional groups with a wavelength range from 4000 to 400 $cm^{-1}$. In order to describe the morphological changes in the samples, a scanning electron microscopy (SEM) (FEI-quanta 200F, Netherland) was performed under acceleration voltage of 30 kv with a largest magnification of 10 K.

### 2.4. Incubation experiment

To evaluate the mineral N transformation in soil, incubation experiments were designed under simulated field conditions. A series of 250 mL beakers were prepared for incubation experiments. Each beaker was filled with different treatments, which contains 600 g of soil and corresponding amount of nitrogen source (Table 2). A constant concentration value of 400 mg N/kg was added into treatments with different nitrogen nutrient sources, according to the application rates of nitrogen fertilizer in actual field (Ju et al., 2009). As typical forms of N fertilizer, urea and ammonium sulfate were both chosen for chemical fertilizers. Based on the total content of nitrogen measured in Table 3, detailed dosages of SMSs-PE, SMSs-PO and SMCs were adjusted and