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# Effects of nitrogen addition and litter properties on litter decomposition and enzyme activities of individual fungi

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ABSTRACT

Litter decomposition is an important process of C and N cycling in the soil. Variation in the response of litter decomposition to nitrogen (N) addition (positive, negative or neutral) has been observed in many field studies. However, mechanism about variability in individual fungal species response to N addition has not yet been well demonstrated in the literature. Therefore, the objective of this study was to investigate the effects of N addition and litter chemistry properties on litter decomposition and enzyme activities of individual fungi. Three fungal species (*Penicillium*, *Aspergillus*, and *Trichoderma*) were isolated from a subtropical mixed forest soil. An incubation experiment was conducted using the individual fungi with two types of litter (leaf of *Pinus massoniana* and needle of *Cryptocarya chinensis*) and different N addition levels (0, 50 and 100 for N-deficient treatments, and 500 and 1000 μg N for N-excessive treatments). Cumulative $CO_2$-C, enzyme activities, and lignin and cellulose loss were measured during the incubation period of 60 days. Litter decomposition and enzyme activities significantly varied with the fungal species, while the N addition and litter types greatly affected fungal enzyme activities. The N treatments significantly increased lignin-rich needle decomposition by lignocellulose decomposers (*Penicillium* and *Aspergillus*) but did not affect their leaf decomposition. On the contrary, The N treatments stimulated leaf decomposition by cellulolytic species (*Trichoderma*) but did not affect its needle decomposition. Correlation analysis showed that lignin in the litter was the key component to affect litter decomposition. Activities of N-acetyl-β-glucosaminidase and phenol oxidase were both positively correlated to litter decomposition. The fungi (*Penicillium* and *Aspergillus*) with higher production of N-acetyl-β-glucosaminidase showed higher litter decomposition ability. The low N addition levels stimulated *Penicillium* and *Aspergillus* litter decomposition, but they still required more N source (e.g., litter N source) to support decomposition. Depressed fungal litter N uptake (lower N-acetyl-β-glucosaminidase activities) only occurred at the highest N addition level. Litter decomposition of *Trichoderma* depended more on external N and its litter decomposition capability was the lowest among the three species.



## 1. Introduction

Litter decomposition is an important process of C and N cycling in the soil. Fungi are considered as the major litter decomposers because their extracellular enzymes can degrade complex compounds (Kjøller and Struwe, 2002; Berg and McClaugherty, 2003).

The effects of N addition on litter decomposition can be positive, neutral, or negative among different studies (Knorr et al., 2005). These contradictory results may be attributable to the vast variety of soil fungal species, which favor different substrates. For example, some Basidiomycete fungi can degrade lignin and humic materials (Blanchette, 1991); while some other fungi may target cellulose and chitin (Lynd et al., 2002; Lindahl and Finlay, 2006). Fungal litter decomposition is highly related to soil enzyme activities (e.g., β-glucosidase and phenol oxidase). Many studies focused on the change of total enzyme activities under N treatments (Micks et al., 2004; Waldrop and Zak, 2006). However, changes of individual fungal enzyme activities under N addition may be different to the total enzyme activity change. Several studies have shown that N treatments suppressed the phenol oxidase activity and decrease lignin degradation (Frey et al., 2004; Waldrop and Zak, 2006); nevertheless, Allison et al. (2009) reported that N addition stimulated phenol oxidase production by *Agrocybe praecox*, suggesting that N addition would promote lignin decomposition in soil dominated by

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*A. praecox*. Allison et al. (2008) reported that direct N fertilization had no effect on soil respiration in boreal ecosystems; however, N is commonly considered as a limiting nutrient for microbial growth in boreal forest soil and N addition should promote microbial C decomposition. Various responses to N addition might be attributable to the different response of individual fungi under the N treatments. Thus, study of litter decomposition by individual fungi under N treatments should provide better understanding of how C cycling response to N treatments in the field.

Chemical properties of litter (i.e., cellulose or lignin content) can affect the soil enzyme production and litter decomposition process (Berg, 2000; Berg et al., 2010). Different soil fungi favor to decompose specific compounds. Allison et al. (2009) showed that *Pholiota* and *Polyporus* were highly effective decomposers of lignin degradation in boreal forest soil. Osono and Takeda (2006) found that *Ascomycetes* (e.g., *Geniculosporium* and *Discosia artocreas*) preferentially decomposed carbohydrates. Commonly, litter with higher lignin content is usually more difficult for fungi to decompose; however, Voříšková et al. (2011) showed that the lignin content did not affect litter decomposition by a saprotrophic basidiomycete, *Hypholoma fasciculare*. Therefore, the effects of litter chemistry properties on litter decomposition in the field may vary with individual fungal, which needs further study.

In this study, individual fungal species were isolated from soil in a mixed pine and broadleaf forest of the Dinghushan Nature Reserve (DNR), where long-term N treatment experiments have been carried out for many years. Studies in the sampling site have shown positive effects of N treatments on litter decomposition rates (Mo et al., 2006, 2007). However, little information is available about the effect of N treatments on litter decomposition by individual fungal. Therefore, the objective of this study was to investigate the effect of N treatments and litter chemical composition (lignin and cellulose content) on litter decomposition by individual fungal species.

## 2. Materials and methods

### 2.1. Litter and soil sampling

In September of 2010, samples of litter and O-horizon soil were collected from the mixed pine and broadleaf forest of the DNR, in the mid-part of Guangdong Province in south China (112°30′39″–112°33′41″E, 23°09′32″–23°11′30″N). The climate in this region is subtropical monsoon with a distinctive hot-humid season (from April to September) and a cool-dry season (from October to March). The average annual rainfall in this region is 1680 mm, about 80% of which occurs in the hot-humid season. The mean annual temperature is 22.3 °C and relative humidity is 80%. Atmospheric N deposition rate was 38 kg $ha^{-1}$ $y^{-1}$ in 2001 (Mo et al., 2006). The forest has not been disturbed by artificial activities for more than 60 years. *Pinus massoniana* and *Cryptocarya chinensis* are the dominant tree species in this forest. Senesced litter samples were collected from *P. massoniana* (pine needle) and *C. chinensis* (broadleaf leaf) in the mixed forest. The litter samples were washed using deionized water, oven-dried at 65 °C, autoclaved at 121 °C for 15 min (Boberg et al., 2011), and then cut into pieces of 1 cm × 1 cm (for leaf) or 1 cm length (for needle). After sterilization, the litter samples were used to determine chemical properties. As shown in Table 1, the litter chemical properties between the two tree species are quite different. The soil was classified as lateritic red earth (oxisol), loamy with texture of loam (41% sand, 37.2% slit, and 21.8% clay) and pH of 4.0.

### 2.2. Fungal isolation and selection

The soil samples were stored in −20 °C after collection. Within one week after the soil collection, 10 g subsample of the frozen soil and several pieces of litters were mixed together and incubated in incubation bottles at 25 °C for one month. Soil field moisture was kept using sterile water. Then the pieces of decomposed litters were picked out and washed in sterile water. The suspension was diluted into $10^{-1}$ to $10^{-3}$. Inoculums (500 μL) were taken from each of the diluents and spread on agar plates. The agar plates contained (per liter) 1 g $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 5.0 g peptone, 18 g agar, 10.0 g glucose, and 3.3 mL 1% rose Bengal solution. To each 100 mL culture medium, 0.3 mL 1% streptomycin solution was added to avoid bacterial growth. For each dilution, at least five plates were used for isolation. The plates with inoculums were cultivated at 28 °C. For purification, hyphal tips of each fungal species were cut with a sterile Pasteur pipette and transferred to a new agar plate. The individual purified fungal species were stored at 4 °C. Fungal identifications were made to at least genus level based on morphological and ecological characteristics. Totally, 24 fungal species were found in the soil, majorly including *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Geotrichum* spp., and *Mortierella* spp. According to the traditional plate count method, biomass of *Penicillium*, *Aspergillus*, and *Trichoderma* were the first, second, and thirdly most in the soil, respectively. Nevertheless, fungal spores accounted in the biomass are not with the litter decomposition capability. Therefore, the biomass cannot be simply related to litter decomposition capability of each fungal species. The following preliminary incubation experiment was used to examine the decomposition capability.

**Table 1**
Chemical properties of the litters used for the incubation experiment (mean ± SE, $n = 3$).

| | *Pinus massoniana* litter (needle) | *Cryptocarya chinensis* litter (leaf) |
|---|---|---|
| C (%) | 55 ± 0.4 | 47 ± 1.6 |
| N (%) | 1.6 ± 0.01 | 1.6 ± 0.1 |
| C:N | 35 ± 0.1 | 29 ± 0.5 |
| Cellulose (%) | 11 ± 0.5 | 15 ± 0.5 |
| Lignin (%) | 33 ± 0.4 | 21 ± 1.1 |

For a two-week preliminary incubation experiment, 10 g sterile silica sand, 500 mg litter substrate, and 2 mL individual fungal inoculum were added into a sterile incubation pot. The silica sand was used as the basic reaction medium. The pot was sealed by a lid with air inlet and outlet port tubes. Each fungal species was incubated with each of the two types of litter and three replicates were set up for each treatment. To make the fungal inoculum, three 5 $mm^3$ cubes of mycelium-rich agar blocks were cut out from each fungal species purified plate using a sterile knife. Then the agar blocks were mashed, combined, and suspended in 75 mL sterile growth medium, and 2 mL individual fungal inoculums was taken from the 75 mL mixed sterile growth medium. The growth medium of inoculum contained (per liter) 500 mg $KH_2PO_4$, 150 mg $MgSO_4 \cdot 7H_2O$, 50 mg $CaCl_2 \cdot 2H_2O$, 20 mg ferric EDTA, and 0.1 mg thiamine HCl. Each incubation pot was weighted before incubation. Pots were incubated at 25 °C for two weeks. Accumulated $CO_2$ in the incubation pots was measured to represent the litter decomposition capability of the fungal species. For every three days, $CO_2$ concentrations within the headspace of incubation pot were determined using $CO_2$ analyzer (LI-COR Inc., Lincoln, NE, USA) with 0.1 ppm detection limit. Air circulated between the headspace and $CO_2$ analyzer at a flow rate of 0.5 L $min^{-1}$, which was controlled by a vacuum pump. In each measurement, $CO_2$ concentrations were recorded every 12 s for a 5 min period. The cumulative $CO_2$ was calculated using the method of Tang et al. (2011) and expressed as μg $CO_2$-C. All $CO_2$ measurements were conducted in the horizontal laminar flow table (ZHJH-C1109B). The $CO_2$ analyzer was also placed in the horizontal laminar flow table prior to experiment. Before the $CO_2$ measurements of each fungal species, the air inlet and outlet port tubes were wiped with 75% (v/v) ethanol, and the UV lamp in laminar flow table was turned on for at least