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Characterization of organic carbon in decomposing litter exposed to nitrogen and sulfur additions: Links to microbial community composition and activity

Yehong Xu [a,b], Jianling Fan [a], Weixin Ding [a,*], Anna Gunina [c,d], Zengming Chen [a,b], Roland Bol [e], Jiafa Luo [f], Nanthi Bolan [g]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Department of Agricultural Soil Science, Georg-August-University of Göttingen, 37077 Göttingen, Germany
[d] Department of Soil Biology and Biochemistry, Dokuchaev Soil Science Institute, Moscow, Russian Federation
[e] Institute of Bio- and Geosciences, IBG-3: Agrosphere, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany
[f] Land and Environment, AgResearch, Hamilton 3240, New Zealand
[g] Global Centre for Environmental Remediation, University of Newcastle, NSW 2308, Australia

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ABSTRACT

Understanding the links between litter chemical transformations and functional microbial communities is key to elucidating the mechanisms of litter decomposition processes under nitrogen (N) and sulfur (S) deposition. Carbon (C)-13-labelled *Pinus massoniana* needles were incubated in a subtropical plantation forest soil exposed to: no amendment (Control), N amendments of 81 (N1) and 270 (N2) mg kg^{-1}, S amendments of 121 (S1) and 405 (S2) mg kg^{-1} and combined N and S amendments. Litter decomposition was measured as litter-derived carbon dioxide (CO_2) emissions and the litter C pools were partitioned using a two-pool model. Relationships between litter residue chemistry (assessed by ^{13}C nuclear magnetic resonance spectroscopy analysis) and microbial community composition (probed by phospholipid fatty acid analysis, PLFA) and activity (the metabolic quotient, qCO_2) were investigated. Over the 420 days incubation period, N and S additions (except N and S addition alone at low rate) significantly increased litter decomposition by 7.2–18.9% compared to the Control. Decomposition was stimulated by 10.2–61.9% during the initial 56 days (stage 1) and in contrast, 8.3–42.1% inhibition was measured during 57–420 days (stage 2) across the addition treatments. Stimulation on litter-derived CO_2 emissions under the N and S additions was largely dependent on the loss of O-alkyl C, a dominant component of the litter active C pool. During the initial 7 days, N and S additions increased the ratio of fungal to bacterial PLFAs compared to the Control, which was accompanied by the increases in methoxyl C. The activity of microbes, particularly gram-negative bacteria, was also increased by N and S additions at stage 1, which was related to di-O-alkyl C. In contrast, fungal activity decreased under N and S additions at stage 2, accompanied by lowered C availability and increased methoxyl C. Alkyl C and aromatic C in the litter had positive relationships with the half-life of the slow C pool. Accordingly, the residue recalcitrance was increased under N and S additions compared with Control at stage 2, and was largely responsible for the inhibition of litter decomposition. Thus, N and S deposition is likely to increase the persistence of litter-derived recalcitrant C in subtropical forest soils in the long term.

1. Introduction

The decomposition of plant litter represents an important process for converting photosynthetic products into soil organic matter (Austin et al., 2016; De Deyn et al., 2008). There is growing recognition of the value of plantation forests in enhancing carbon (C) sequestration in soil (Kelty, 2006). With suitable temperature and adequate rainfall, southern China accounts for 63% of the national plantation areas (SFA, 2007). However, this area has also been highlighted as one of the regions that are most severely affected by nitrogen (N) and sulfur (S) deposition in the world (Nowlan et al., 2014). It has been reported that N additions accelerate the decomposition of loblolly pine litter by nearly 100% (Sanchez, 2005). Nonetheless, many studies have demonstrated a considerable inhibition effect of N on lignin degradation (Hagedorn et al., 2012; Hobbie et al., 2012), resulting in an increase of the relative content of aromatic compounds (Baumann et al., 2009). This occurs due to the inhibition of some ligninolytic microorganisms (Waldrop et al., 2004), suppressed microbial production of oxidative enzymes

* Corresponding author.
E-mail address: wxding@issas.ac.cn (W. Ding).

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(Craine et al., 2007), or formation of more recalcitrant substances through linking N with lignin-like compounds (Davidson et al., 2003). However, N additions were found to increase the decomposition of the slowly decomposable parts in pastures and crop residues when total soil N was ≤0.1% (Finn et al., 2015). Compared with N deposition, less attention has been paid to S deposition. Wang et al. (2010) observed that S deposition suppressed litter decomposition in a broadleaf forest and a coniferous forest due to the inhibition of enzymatic activities under low-pH conditions. In contrast, additions of S increased the decomposition of hazel (*Corylus avellana* L.) litter by stimulating the growth of saprotrophic fungi (Newsham et al., 1992). A lack of clear responses of litter decomposition to N and S deposition raises the question of whether plantation forests still make a large contribution to C sequestration in soil under N and S deposition.

Microorganisms are the primary decomposers of plant litter (Swift et al., 1979). Feng and Simpson (2009) reported that the microbial metabolic activity transiently increases when labile C substrates are abundant, but is not influenced in the long term. It is suggested that bacteria can grow rapidly on labile C substrates and dominate in the initial stage of litter decomposition (Paterson et al., 2008). However, Valmaseda et al. (1991) found that decomposition of wheat straw by ligninolytic fungi consisted of an initial colonization stage of consumption of free sugars, and a subsequent degradation stage of breaking down the polysaccharides and lignin. In a review, Geisseler and Scow (2014) concluded that microbial activity is affected by numerous factors, such as physiological stress and shifts from a bacteria- to fungi-dominating microbial community. To date, few studies have linked the functional microbial community with the detailed changes in residue chemistry during decomposition (Baumann et al., 2009, 2011).

The various forms of C present in the litter have different chemical nature, and play a critical role in meditating the rate of litter decomposition (Baldock et al., 2004). Since last decades, solid-state ^{13}C crosspolarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy has been proposed as a valuable tool to characterize organic matter at a molecular level (Bonanomi et al., 2013, 2016). Cozzolino et al. (2015) reported that easily decomposable compounds, such as O-alkyl C, progressively decreased during the composting of municipal organic wastes. However, an increase in O-alkyl C and a decrease in alkyl C were observed during the later decomposition stage of cover plants in the Savanna region of Central Brazil, presumably due to enhanced microbial degradation of polyphenols and some long-chain aliphatics into smaller molecules (Carvalho et al., 2009). Phenolic C and methoxyl C compounds are relatively decomposable in the initial litter decomposition stage, and these C functional groups reflect stable lignin when decomposition rates decrease sharply in the late stage (De Marco et al., 2012). In an experiment with 64 litter types selected from Mediterranean and temperate environments, litter decay rates were highly correlated with the ratio of carbohydrate to methoxyl C (Bonanomi et al., 2013). In contrast, C mineralization rates of seven typical Australian plant materials were positively related to carbonyl C in litter over the first three days and thereafter negatively to aromatic C and phenolic C (Wang et al., 2004). With inconsistent changes and unclear roles of residue chemistry during litter decomposition, it remains a challenging issue to be fully explained.

In this study, we investigated the effects of N and S deposition on the litter decomposition processes in the subtropical forest soil. ^{13}C-labelled needles of *Pinus massoniana* were incubated in an Ultisols soil exposing to different levels of N and S additions. The extent of litter decomposition was assessed by litter-derived microbial respiration during 420 days. Changes in residue chemistry were characterized by solid-state ^{13}C CPMAS NMR spectroscopy, whose sensitivity can be largely enhanced for isotopically enriched materials (Kelleher et al., 2006). The functional microbial community compositions were examined by phospholipid fatty acid (PLFA) analysis in conjunction with compound-specific isotope analysis. We hypothesized that N and S deposition change the chemical composition of litter through effects on microbial decomposition processes, and the transformed residue chemistry would in turn shift the composition and activity of microbial community. Therefore, the objectives of this study were to: (1) understand the relationships between litter decomposition and residue chemistry under N and/or S additions; and (2) link the microbial community composition and activity with changes in residue chemistry.

2. Material and methods

2.1. Soil and ^{13}C-labelled litter sampling

Soil was sampled from a plantation forest dominated by *Pinus massoniana*. The plantation was located at the Yingtan Agro-ecological Experimental Station, Chinese Academy of Sciences, Yingtan City, Jiangxi Province, China (28°15′N, 116°55′E). The region is characterized by a humid, mid-subtropical monsoon climate, with a mean annual temperature of 17.6 °C and a mean annual precipitation of 1795 mm. Surface soil (0–20 cm) was collected randomly in a 10 m × 10 m grid in May 2012. Totally, 50 kg soils were sampled and composited to reduce the heterogeneity. The soil is derived from quaternary red clay and characterized as a Typic Plinthudult (Ultisols) according to the US Department of Agriculture soil taxonomy. It is made up of 11% sand, 31% silt and 58% clay. The soil pH is 4.69 and bulk density is 1.48 g cm^{-3}. The soil has 3.08 g kg^{-1} organic C with a $\delta^{13}C$ value of −23.52‰, 0.50 g kg^{-1} total N and 0.21 g kg^{-1} total S. The NO_3^--N concentration is 6.17 mg kg^{-1} and the NH_4^+-N concentration is 6.18 mg kg^{-1}.

Two-year old *Pinus massoniana* seedlings were selected from a local nursery based on the similar heights and stem diameters. They were labelled with ^{13}C-enriched carbon dioxide (CO_2, 99.9 atom% ^{13}C) in a labelling cabinet within a climate-controlled growth room for two months (Tavi et al., 2013). After harvest, the pine needles were rinsed, dried, cut into lengths of 2 cm and used for the incubation experiment. The litter had 444.0 g kg^{-1} organic C with a $\delta^{13}C$ value of 270‰, 15.7 g kg^{-1} total N and 1.42 g kg^{-1} total S.

2.2. Incubation experiment

The fresh soil was mixed thoroughly, sieved (2 mm) and stored at 4 °C before incubation. Subsamples of 100 g (on the oven-dried basis) were weighed into 500 mL glass jars, mixed with 0.5 g (on the oven-dried basis) of pine needle litter to create a homogeneous mixture and packed to the same bulk density as in the field (1.48 g cm^{-3}). Then NH_4NO_3 and/or Na_2SO_4 solution was added to simulate future deposition conditions (Dentener et al., 2006). There were three replicates of nine treatments: no N or S amendment (Control), N amendments at rates of 81 mg N kg^{-1} (N1) and 270 mg N kg^{-1} (N2), S amendments at rates of 121 mg S kg^{-1} (S1) and 405 mg S kg^{-1} (S2), and four combined N and S amendments (N1S1, N1S2, N2S1 and N2S2). The treatments with higher S additions than those of N were used to simulate the in situ deposition conditions (Fan et al., 2009; Xu et al., 2004). After adjusting the soil moisture to 60% water holding capacity, the glass jars were covered with perforated cling film and incubated in the dark at 25 °C. Water was replenished gravimetrically every three days during the incubation period.

A group of glass jars (27 jars in total) were used to determine the CO_2 fluxes on days 1–9, 16, 25, 38, 56, 66, 112, 127, 179, 224, 297, 365 and 420. A separate set of jars were used for sampling soil and litter samples on days 1, 3, 7, 56, 112, 224 and 420 (189 jars in total). The litter residues in these destructive samples (termed as residues hereafter) were retrieved from the soil by tweezers. The litter residues were freeze-dried to easily separate the attached soil particles, and then washed, rinsed, freeze-dried again and ground (Bertrand et al., 2006). Residues harvested on days 7, 56, 224 and 420 were used for NMR analysis. Part of the soil was stored at 4 °C before chemical analysis, and the other part was freeze-dried and conserved at −20 °C prior to the PLFAs extraction.