



Transformation of organic nitrogen fractions with different molecular weights during different organic wastes composting

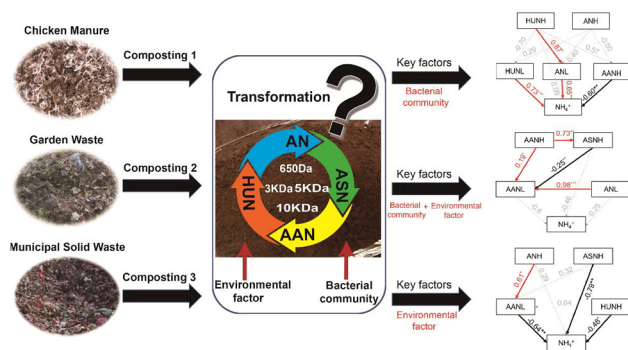
Haixuan Zhou^a, Yue Zhao^a, Hongyu Yang^a, Longji Zhu^a, Baiyan Cai^b, Sheng Luo^c, Jinxiang Cao^c, Zimin Wei^{a,*}

^a College of Life Science, Northeast Agricultural University, Harbin 150030, China

^b College of Life Science, Heilongjiang University, Harbin 150080, China

^c Yi'an County Agricultural Technology Promotion Center, Heilongjiang 161500, China

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed to investigate different transformation mechanisms of organic nitrogen (N) fractions during composting with different raw materials, and the contributions of bacterial communities and environmental factors to organic N fractions transformation. The results showed that high molecular weight organic N was first degraded to low molecular weight organic N and then turned into NH_4^+ during chicken manure composting. In comparison, organic N fractions were stored in composts rather than mineralization during garden waste and municipal solid waste composting. Meanwhile, bacterial communities, environmental factors and the combination of them were the main drivers of N transformation during chicken manure, municipal solid waste and garden waste composting, respectively. In conclusion, the proposed organic N transformation mechanisms in this study provided a theoretical basis for improving the quality of compost products.

1. Introduction

With the increase of economy and population, the amount of solid wastes (e.g., municipal solid wastes, agricultural wastes and garden wastes) has increased significantly in China (NBS, 2016). Landfill and

combustion are the commonest ways to treat solid wastes due to their low costs. However, because of poorly management for solid wastes, the water, farmland and air are becoming polluted. More and more countries have implemented policies to limit the landfill and combustion disposal of solid wastes and promote biological treatment (Hodge

* Corresponding author.
E-mail address: weizimin@neau.edu.cn (Z. Wei).

et al., 2016). Composting is a reliable and environmental friendly technology to treat solid wastes (Maeda et al., 2017), which can not only promote the recycle of N, P and K elements, but also effectively improve soil condition and farm product quality (He et al., 2014; Wang et al., 2015; Zhao et al., 2017). Therefore, composting is widely used to manage kinds of organic solid wastes recently.

Nitrogen (N) is one of the most important nutrients controlling net plant primary production in ecosystems (Jones et al., 2004). Organic N was the main form of N during composting, which could be found in various molecular forms such as proteins, peptides, amino acids, nucleic acids, and amino sugars etc. (Wang & Zeng, 2017). In the composting N cycle, the rate-limiting step of microbial decomposition of organic N is the depolymerization of proteins to oligopeptides and amino acids by extracellular proteases, rather than the subsequent mineralization of amino acids to ammonium (Mooshammer et al., 2012; Murphy et al., 2003; Schimel & Bennett, 2004). However, most studies on composting N cycle have focused on N mineralization and the fate of inorganic N (NH_4^+ , NO_3^- and NO_2^-), rather than on the transformation in organic N fractions. N transformation during composting is very complicated, because N fractions can be transformed continuously accompanying with N loss (Bao et al., 2008). Based on Keeney and Bremner's (1964) classification of organic N fractions, total hydrolyzable organic N (THN) can be divided into amino acid N (AAN), amino sugar N (ASN), amine N (AN) and hydrolyzable unknown N (HUN). Plants available N of composts is highly dependent on organic N fractions. Therefore, a detailed understanding of organic N fractions transformation will strongly improve our knowledge about the utilization of composts.

It is generally accepted that functional and ecological differences in organic N molecules are related to molecular weights (Warren, 2014). The molecular weights of organic N fractions may range from several hundred to several thousand Dalton (Da). The < 1 kDa fraction of organic N turns over rapidly (~hours), while the > 1 kDa fraction turns over much more slowly (~days–months) (Jones et al., 2004). Low molecular weight (LMW) dissolved organic nitrogen (DON) can be taken up directly by microorganisms and plants (Warren, 2014). In comparison, high molecular weight (HMW) DON generally requires extracellular enzyme mediated degradation to LMW DON. Ultrafiltration using a tangential flow filtration (TFF) system can separate organic N into different molecular weight fractions. Many previous studies have investigated organic N fractions in the environment based on the acid-hydrolysis method (Stevenson, 2008; Zhang et al., 2006), however, few studies focused on the transformation of organic N by separating hydrolyzable organic N into different molecular weight fractions.

In this study, three composting experiments were conducted with different raw materials. Concentrations of organic N fractions with different molecular weights and forms were measured throughout composting. The goals of this study were to (1) analyze the distribution and changes of organic N fractions during composting, (2) compare transformation relationships of organic N fractions during composting of different materials, (3) determine critical factors regulating the transformation of organic N fractions during composting of different materials. These may facilitate to understand the relationships between organic N fractions and compost materials, which contributes to apply composting products according different needs.

2. Materials and methods

2.1. Composting experiment design

The raw materials of chicken manure (CM), garden waste (GW) and municipal solid waste (MSW) were collected from Harbin, China. The physicochemical properties of these raw materials, including pH, moisture contents, organic matter and C/N are summarized in Table 1. Nine lab-scale composting experiments were carried out for 60 days in the special compost reactors as described by Zhao et al. (2016). Three replicates of each raw material were carried out and the average of the

Table 1
Physical and chemical properties of raw materials of composts.

Materials	pH	MC (%)	C/N	OM (%)
CM	7.28	71.4	21.6	63.2
GW	6.62	62.4	27.6	66.5
MSW	5.05	70.7	25.4	52.3

MC: moisture content; OM: organic matter.

three replicates were used for further statistical analysis. The temperature during composting was controlled according to the description given by Zhao et al. (2016). The samples were collected on days 0, 7, 14, 23, 37, 45, and 60 of the composting period for analyzing concentrations of organic N fractions. And samples of 0, 7, 14, 37 and 60 days were selected for analyzing microbial community composition. Each of the samples was divided into two parts, where one was air-dried and milled to 0.15 mm to analyze physicochemical properties, and the other was freeze-dried, ground to 1 mm, and stored at -80 °C for DNA extraction.

2.2. Extraction and determination of organic N fractions

The samples were digested by the Kjeldahl method to measure the total soil N (TN), which were further measured in the hydrolysate prepared by refluxing the compost samples with 6 mol L⁻¹ HCl (Keeney & Bremner, 1964). Portions of AAN, ASN, AN and HUN were identified after acid hydrolysis. The procedure is briefly described as follows: each compost sample (1 g), octanol (100 µL) and 6 mol L⁻¹ HCl (50 mL) were combined in a 150 mL flask. Afterwards, flasks were sealed and placed in an oven at 105 °C for 12 h. Then the hydrolysate was filtered and subsequently neutralized by NaOH. Afterwards, the tangential flow filtration (TFF) system equipped with membrane packages of 650 Da, 3 kDa, 5 kDa and 10 kDa (Pall Corporation) was used to separate hydrolyzable organic N fractions into MW < 650 Da, 650 Da < MW < 3 kDa, 3 kDa < MW < 5 kDa, 5 kDa < MW < 10 kDa and MW > 10 kDa. Different organic N fractions were determined by steam distillation with different additives (Thomas, 1982; Wang et al., 2017), where THN with NaOH after Kjeldahl digestion with H₂SO₄ and catalyst (K₂SO₄ and CuSO₄) mixture; AN with MgO; the sum of AN and ASN with phosphate-borate buffer; AAN with phosphate-borate buffer after treatment with 0.5 mol L⁻¹ NaOH at 100 °C and ninhydrin (pH 2.5, 100 °C) to convert AAN to NH₄⁺. The amount of other organic N fractions were calculated as follows:

$$\text{ASN} = (\text{AN} + \text{ASN}) - \text{AN}$$

$$\text{HUN} = \text{THN} - (\text{AAN} + \text{AN} + \text{ASN})$$

2.3. Physicochemical analysis

Composting temperature was monitored with a digital thermometer every day. The pH values were measured using a digital pH meter by aqueous suspension of compost samples (sample-water ratio 1:10 w/v). Moisture content was assessed based on the weight loss after drying samples at 105 °C for 24 h until a constant weight. The dry compost samples were ground into fine powder passing through a 100-mesh sieve to determine the contents of carbon and nitrogen. Total nitrogen (TN) was measured by the Kjeldahl method. Total organic carbon (TOC) was measured by the total organic carbon analyzer (TOC-VCPH, Shimadzu, Japan). Ammonia nitrogen (NH₄⁺-N) was measured according to NaRSH'S colorimetry after extracted by 2 mol L⁻¹ KCl (1:10 ratio) at 200 rpm for 1 h and filtered.

2.4. DNA extraction and PCR-DGGE

Total DNA of samples was extracted using soil DNA kit (Omega

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