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Biostimulation of nutrient additions on indigenous microbial community at the stage of nitrogen limitations during composting

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A B S T R A C T

Microorganisms can play a crucial role in the efficiency for composting, which are essential for converting the organic wastes into a well-stabilized, value added product. However, the activity of most of the key functional microorganisms were inhibited due to the limited special nutrient substances or other physiochemical factors during composting, which further affected the quality of compost. The study was conducted to investigate the effects of enriched ammonium (NH_4^+-N) and organic nitrogen (Org-N) on indigenous microbial community and whether nitrogen (N) nutrient additions could modify the special species during composting. The results showed that the abundance and structure of bacterial community had distinctly diverse responses to different N nutritional treatments (no nutrient addition, NH_4^+-N addition, and Org-N addition). The addition of N sources enhanced the abundance of corresponding uncultured indigenous species negatively related to the factor of NH_4^+ and Org-N in redundancy analysis (RDA) during composting but the effect of NH_4^+ was more significant than Org-N. Nonmetric multidimensional scaling ordination (NMDS) demonstrated that both the two N additions changed bacterial community but had different duration for affecting bacterial composition. Conclusively, an optimized method for regulating the key stains with special biological capacity is proposed by controlling the single limiting-nutrient factor sharply decreasing at one of composting stages and negatively related to the key species in RDA.

1. Introduction

Organic wastes must be treated by appropriate disposal approaches, or they would lead to a serious threat to the environment and cause the loss of useful nutrients (Tilman et al., 2001). Composting is a controlled aerobic process and a sustainable method for managing organic solid wastes through effective biodegradation of microbes (Lim et al., 2016). In addition, it could convert the wastes into a well-stabilized, value-added product as a source of nutrients for plants and a conditioner for the soil, which could play double roles of microbial fertilizer and organic fertilizer.

Microorganisms play a crucial role in the efficiency of composting, and the maturity of the composts can be improved through effectively emending the activity of microorganisms (Brown et al., 2013). Therefore, the kinds of the functional microbial strains during composting will directly affect the quality of the final bioorganic fertilizer. Up till now, it has been widely known that inoculation of functional microbes could improve efficiency and quality of composts, but there are still many difficulties in describing the relevant mechanisms of inoculum to decompose polymeric compounds (Hachicha et al., 2012; Lei and VanderGheynst, 2000). It is prerequisite to isolate a number of species that could be cultured for preparing inoculum. In addition, there is a need to investigate whether the inoculated species were effective during composting. Considering the possible competitive relationship between indigenous microbes and inoculants and the low compatibility to environment, the results that the inoculated bacteria had no effect on the degradation of organic matter might be inevitable (Xi et al., 2016).

Biostimulation relying on the special ability of natural microorganisms was proposed to encourage indigenous bacteria by providing appropriate enrichment (Riser-Roberts, 1998). To date, available related studies have mainly focused on profiles of microbial inoculum and inoculation effect, yet rarely on stimulating the indigenous microorganisms. Given the fluctuations of temperature and moisture as well as sharp nutrient trends during composting, it was difficult to evaluate the effect of biostimulation on the composting process.

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Microbial population in composts have different enzymatic activities during composting, and most of the activities were related to polymeric carbon degradation, such as cellulolysis and ligninolysis (Vikman et al., 2002). Besides, most microorganisms showed the activities including amylolysis, proteolysis and xylanolysis, etc. at the bio-oxidative phase and they became more prominent as composting progressed. The study by Xue et al. (2010) suggested that the nitrifying bacteria increased NH_3 removal efficiency during composting. A number of key bacteria which influence the dynamic of precursors formed by the degradation of organic matter or synthetized by animalcule may control humic substances formation throughout composting (Wu et al., 2017). However, as the consumption of nutrient substances during composting, the decreased bioavailability of nutrients limited the activity of most of the functional microorganisms. Considering that microbial metabolic versatility, especially bacteria, is influential in all the events related to the biotransformation of organic substrates (Lopez-Gonzalez et al., 2015), it is necessary to enrich functional bacteria by providing appropriate enrichment to improve the quality of compost. Based on the relationship between microbial community compositions and physicochemical parameters during composting, a strategy has been proposed by Wang et al. (2015) to enhance the abundance of bacteria by adjusting physicochemical parameters, namely, the diversity of the microbial community may be controlled by physicochemical conditions, and it is almost universally accepted that bacteria could be better adapted to the physicochemical parameters which were positively related to these species. On the other hand, the concept of biostimulation was further explained by Smith et al. (1998), who suggested that biological diversity and function were likely to be influenced by growth-limiting resources, which always had a negative correlation with species. Therefore, it may be a practical way to stimulate the microorganisms by adjusting the limiting-nutrient factor during composting but it is unknown how to choose the most suitable environmental factors as the limiting-nutrient factor for biostimulation. Although a number of studies explored statistical relationship between microbial communities and nutrient factors (Lee et al., 2016; Xi et al., 2016; Zhao et al., 2016a), there has been a lack of studies to examine the effect of enrichment treatments of limiting-nutrient resources on the indigenous microbial population since the cooling phase of composting, especially the stages in which the content of nutritional source was insufficient to sustain the metabolism of most of microbial community. Based on the above considerations, two nitrogen (N) nutrients were added to relieve N stress and regulate the strains that may be affected by N insufficiency in this study.

In this study, we investigated physicochemical parameters and detected the dynamic changes of bacterial community using PCR-DGGE. Nutrient enrichment experiments were conducted to add inorganic-N (NH_4^+) and organic-N (amino acid) during composting, respectively. The aims of this study were to (1) investigate the limiting-nutrient factors for microbial community during composting, (2) compare the abundance and structure of indigenous bacterial community before and after NH_4^+ and Org-N addition, and (3) determine whether the stimulation method by improving limiting-nutrient factors was feasible during composting. This study could provide primary information to achieve biostimulation with enrichment cultures and further insight into the control of indigenous microbial communities during composting.

2. Materials and methods

2.1. Materials for composting

Composting materials were composed of chicken manure and wood chips. Chicken manure was collected from the College of Animal Science, Northeast Agricultural University, air-dried and smashed before composting. Sawdust (2 mm mesh size) originated from the timber mill (Harbin, China) were added to adjust the initial C/N ratio. Manure and sawdust were mixed at a ratio of 3:2 (dry weight ratio) to prepare a compost mixture, and approximately 15 kg mixtures were foisted loosely into a plastic cylinder with the diameter of 28 cm and height of 52 cm. The whole composting process was performed with four replicates for 60 days in the laboratory compost reactors referenced to Zhao et al. (2016b). The air (0.5 L/min) was supplied continuously by an external air compressor during composting. The piles were turned two times a week during the first month of the experiment, and thereafter every 5 days until the end of composting. During composting, distilled water was added to maintain the moisture content of each piles at around 60%. Samples were collected from different composting bins and then mixed in equal amount on day 0, 1, 2, 3, 5, 7, 9, 12, 15, 18, 21, 25, 30, 35, 40, 45, 50, 55 and 60. Each sample randomly divided into two subsamples. One part was dried and used for physicochemical analysis and the other was stored at −20 °C for the DNA analysis and further enrichment experiments of N source. The basic physicochemical characteristics of the chicken manure and the wood chips were shown in Table 1.

2.2. Enrichment experiments

Aerobic composting with no addition was conducted as a control (CK). To better understand how N additions influence the indigenous microbial community composition and diversity, two N-enrichment treatments were conducted as the design of small-sized systems to better explore the potential change in microbial communities (Gómez et al. (2016); Gat et al., 2016; Yang et al., 2016). A small amount of composting samples (200 g) of CK was collected as the initial material and cultivated in the Petri dish with the diameter of 150 mm under aerobic conditions for 20 days in the dark in the N-enrichment environment in which the other environmental conditions were controlled in accordance with the conditions of the first actual composting. For example, moisture content was kept at around 60% by watering when needed and temperature was controlled by using constant temperature incubator according to a trend of that in CK. Considering that ammonium (NH_4^+-N) and organic nitrogen (Org-N) were significantly reduced before day 40 and day 30 and gradually tended to be stable since day 40 and day 30 for CK, samples of CK in these two periods were chosen and treated with the addition of $(NH_4)_2SO_4$ (NH_4^+ treatment groups) and amino acid (Org-N treatment groups). NH_4^+ enrichment experiments were conducted on the samples-CK of day 40 by supplementing $(NH_4)_2SO_4$ as an inorganic

Table 1
Physical-chemical characteristics of the resource materials.

Resource materials	TOC (g kg^{-1})	TKN (g kg^{-1})	C/N ratio	Moisture content (%)
Chicken manure	385.6 ± 15	32.67 ± 1.31	11.8 ± 0.78	76.3 ± 0.94
Sawdust	513.6 ± 19	6.29 ± 0.05	81.65 ± 1.24	3.2 ± 0.02
Compost materials	442.7 ± 20	22.35 ± 0.85	19.81 ± 0.63	61.7 ± 1.04