



## Temporal variations of soil microbial community under compost addition in black soil of Northeast China



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### ARTICLE INFO

#### Keywords:

Compost addition  
Temporal variation  
Black soil  
Soybean  
Biolog EcoPlates™  
Soil microbial community

### ABSTRACT

Soil microorganisms play crucial roles in regulating dynamics of organic matter in agroecosystem, and are conspicuously influenced by agricultural practices. To reveal the effect of compost addition on soil microorganisms, we examined the soil properties, soil microbial biomass, activity, diversity and communities at soybean seedling, flowering and mature stage in a 1-year compost addition experiment system in Northeast, China. Soil microbial communities were determined using Biolog EcoPlates™. Structural equation model (SEM) was applied to disentangle the direct and indirect effects of compost on soil microbial properties. The results showed that compost addition significantly enhanced soil organic matter, electrical conductivity, total nitrogen, total phosphorus (P), available P, available N and kalium. Soil microbial biomass, activity and diversity showed significant temporal variations during the cropping season, but unaffected by compost addition. Moreover, highest soil microbial biomass was found in the seedling stage, while soil microbial diversity and activity were lowest at the same time. SEM showed that temporal change exhibited a stronger effect on the soil microbial community composition than compost addition. Although the soil microbial community composition was unaffected by compost addition in the seedling and flowering stage, it was significantly affected in the mature stage. Our findings highlight the significant effect of compost addition on soil nutrient availabilities, and emphasize temporal change to be a stronger determinant than 1-year compost addition in shaping microbial communities in black soil of Northeast China.

### 1. Introduction

In countries like China, with high input of chemical fertilizer and frequent soil tillage, soil degradation has been recognized as a widespread problem (Cao et al., 2009; Liu and Diamond, 2005). Soil quality and fertility, as well as crop yield, have declined due to soil degradation (Cao et al., 2009; Kirschenmann, 2010; Liu and Diamond, 2005). On the other hand, with the intensive agricultural and animal husbandry practices, more and more organic solid wastes (e.g. animal dung and straw) were produced and has become one of the major pollution sources in China (Zhen et al., 2014).

Composting of organic solid wastes is an effective strategy for organic waste recycling (Santos et al., 2011) and beneficial practice for soil restoration (Scotti et al., 2016). Previous studies have demonstrated that compost addition to soil not only provides an important source of nutrients (Duong et al., 2013; Evanylo et al., 2008; Gil et al., 2008), but also increased soil carbon stocks (Evanylo et al., 2008; Hemmat et al., 2010), improved soil structure (Celik et al., 2004) and water-holding capacity (Caravaca et al., 2002), enhanced crop yield and suppressed

soil-borne pathogens (Pane et al., 2013; Zaccardelli et al., 2013). Compost amendments, therefore, maintain and enhance the fertility and productivity of agricultural soils (Pérez-Piqueres et al., 2006).

Soil microorganisms play key roles in agroecosystem by regulating dynamics of organic matter and plant nutrient availability (Kibblewhite et al., 2008; O'Donnell et al., 2005), and it was proposed that soil microbial parameters could be used as valuable indicators to assess soil quality (Morra et al., 2010). Previous researches mainly focused on the influence of compost on soil physical and chemical properties (Duong et al., 2013; Guo et al., 2016; Tejada et al., 2009). While, Araujo et al. (2015) and Zhen et al. (2014), observed that soil microorganisms were also sensitive to compost addition significantly. For instance, soil microbial biomass was increased by 3.2–242% after compost application (García-Gil et al., 2000; Martínez-Blanco et al., 2013). Other studies showed that compost addition may have positive (Bernard et al., 2012; Zhen et al., 2014), neutral (Nair and Ngouajio, 2012) or even negative (Martínez-Blanco et al., 2013) effect on soil microbial diversity and activity.

However, there remain much unstudied temporal variations of soil

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microbial community under compost addition (Saison et al., 2006; Zhen et al., 2014). For instance, Saison et al. (2006) reported that change in soil microbial community structure was detected 4 days after compost addition, while 6 months later the effect disappeared partially. Zhen et al. (2014) reported that soil microbial biomass, diversity and amounts of cultivable microorganisms were significantly enhanced by compost addition regardless of sampling time under controlled laboratory conditions. These varying observations suggest that temporal variations of soil microorganism under compost addition conditions still need further investigation.

Black soil is a vital soil resource for crop production distributed in Northeast China (Xu et al., 2010; Yin et al., 2015). However, serious soil erosion and fertility deterioration has occurred in this region over the past several decades (Liu et al., 2003; Yao et al., 2017). To study the responses of plants, soil properties and microorganisms to compost addition, a controlled gradient compost addition experimental system was therefore been established in an agroecosystem on the Songnen Plain. We tested the following hypotheses: H<sub>1</sub>, the soil physical and chemical properties, soil microbial biomass, activity, diversity and community will be enhanced by compost addition; H<sub>2</sub>, there will be temporal variations in the soil microbial community under compost addition condition; H<sub>3</sub>, there will be dose-dependent effect of compost addition on soil and microbial properties.

## 2. Materials and methods

### 2.1. Field experiment design

A field experiment was conducted in Northeast Agricultural University's experimental farm in eastern Songnen Plain, China (45°45'45"N, 126°54'46"E) in 2016. This region has a typical monsoon climate, with annual average temperature about 4–5.5 °C, and annual precipitation about 400–500 mm (70%–80% during summer, Ding et al., 2017). The temperature along the experimental period are shown in Fig. A.1. The soil is classified as Mollisol, with pH of 6.3, soil organic matter of 25.8 g/kg, total N of 1.1 g/kg, total P of 0.5 g/kg.

Soybean (*Glycinemax* (L.) Merrill) was planted on 6th May and harvested on 29th September. The compost used was obtained through 45 days on-farm composting of cow manure and plant residues. Chemical analyses of compost showed a pH of 8.0, 386.1 g/kg total organic carbon, 18.4 g/kg total N, 399.8 mg/kg NO<sub>3</sub>-N, 212.9 mg/kg NH<sub>4</sub>-N and C: N ratio of 21.0. Compost were applied as basal fertilizer before soybean planted, and four levels of compost addition were applied. In a complete randomized design, there were four treatments: (1) no compost addition (CK); (2) 11.3 Mg/ha compost addition (low level of compost addition, LC, equal to 200 kg N/km<sup>2</sup>); (3) 22.5 Mg/ha compost addition (moderate level of compost addition, MC); (4) 45 Mg/ha compost addition (high level of compost addition, HC). There were four replicates for each treatment, and a total of 16 plots (5 m × 4.5 m each and 2 m separation from each other) were in a randomized arrangement.

### 2.2. Sampling

Soil samples were collected on 4th June (seedling stage); 24th July (flowering stage) and 27th August 2016 (mature stage). In total, 48 soil samples were collected. Briefly, five soil cores (near plant roots, 20 cm deep, 5 cm diameter) from each plot were randomly collected and mixed with one composite sample. The soil samples were then packed in an ice box and transported to laboratory. Roots and debris were picked out manually from fresh soil. Subsamples for Biolog EcoPlates™ and microbial biomass analyses were sieved (1-mm sieve) and stored at 4 °C until analysis within two days. Subsamples for chemical and physical properties were air-dried and sieved (1-mm and 0.25-mm sieve, respectively), then stored at room temperature until analysis.

### 2.3. Soil physical and chemical analyses

Soil pH was determined in 1:2.5 (v/v) soil/water extracts using a combination glass electrode. Electrical conductivity (EC) was determined in 1:5 (v/v) soil/water extracts using electrical conductivity detector. Soil gravimetric moisture was determined by drying at 105 °C for 8 h. Soil bulk density was determined from oven dried undisturbed cores as mass per volume of oven dried soil. Soil organic matter (SOM) content was determined by the potassium dichromate oxidation-ferrous sulphate titrimetry (Walkley and Black, 1934). Total nitrogen (TN) was determined as ammonium-N by steam distillation after digestion with H<sub>2</sub>SO<sub>4</sub> (Bremner and Mulvaney, 1982). Available N (AN) was determined by the alkaline hydrolysis diffusion method. Total phosphorus (TP) was measured by digesting soil using the H<sub>2</sub>SO<sub>4</sub>-HClO<sub>4</sub> method and then measured using Mo-Sb colorimetry method (Murphy and Riley, 1962); available P (AP) was extracted using 0.5 mol/L NaHCO<sub>3</sub> solution and determined as described above. The available kalium (AK) was extracted with NH<sub>4</sub>OAc and determined by flame atomic absorption spectrometry (Schollenberger and Simon, 1945).

### 2.4. Soil microbial biomass carbon

Soil microbial biomass carbon (MBC) was measured using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). In brief, paired 10 g fresh soil samples were either fumigated or non-fumigated with CHCl<sub>3</sub> for 24 h. The samples were then extracted with 25 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> (soil/extractant ratio 1:2.5). Total organic C in the fumigated and non-fumigated samples was determined by the potassium dichromate oxidation-ferrous sulphate titrimetry. MBC was then calculated using a conversion factor of 2.64 (Vance et al., 1987).

### 2.5. Soil microbial community

Biolog EcoPlate™ (BIOLOG inc., Hayward, CA, USA) was used to characterize soil microbial community. Each Biolog EcoPlate™ consist of 96 wells containing 31 carbon sources and water as control, with each replicated three times. As the carbon source is utilized, the tetrazolium violet dye is reduced, developing a purple color (Bartelt-Ryser et al., 2005). 10 g fresh soil was added to 100 mL Tris buffer (pH = 7.5) and shaken for 10 min. After shaking, samples were centrifuged for another 10 min at 2,600g. The supernatant was extracted and diluted to achieve 10<sup>-3</sup> dilution, from which 150 uL liquid was inoculated in wells of the Biolog EcoPlate™. The inoculated plates were incubated at 25 °C in the dark for 10 days and color development of each well was determined by measuring absorbance at 590 nm using a BioTek plate reader (BioTek Inc., USA) every 24 h (Bartelt-Ryser et al., 2005).

### 2.6. Statistical analyses

Soil microbial activity was indicated by average well color developing (AWCD), which was calculated by averaging the absorbance values for 31 substrate wells (Garland, 1997). Shannon diversity index was calculated as follows:  $H' = -\sum_{i=1}^N P_i(\ln P_i)$ , where  $P_i$  is the ratio of the activity on a particular substrate to the sum of activities on all substrates (Staddon et al., 1997). Simpson diversity index was calculated as  $1/D$  ( $D = \sum(n_i(n_i - 1))/(N(N - 1))$ ), where  $N$  is the total carbon sources ( $N = 31$ ), and  $n_i$  is the relative absorbance of well (Staddon et al., 1997). Pielou evenness index was calculated as  $J = H'/\ln S$ , where  $S$  is the total number of utilized carbon sources (Pielou, 1969).

Two-way ANOVAs were used to examine the effects of compost addition, temporal change (sampling time) and their interaction on soil properties (bulk density, pH, soil moisture, EC, SOM, TN, TP, AP, AK and AN), soil MBC, AWCD, AWCD of six categories of substrates

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