Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Similar positive effects of beneficial bacteria, nematodes and earthworms on soil quality and productivity

Xianping Li[a,b,1], Chunliang Liu[a,b,1], He Zhao[a], Fei Gao[a], Guanning Ji[a], Feng Hu[a], Huixin Li[a,c,*]

[a] College of Resources and Environmental Science, Nanjing Agricultural University, Nanjing 210095, China
[b] National Engineering Research and Technology Center for Red Soil Improvement, Ecological Experiment Station of Red Soil, Chinese Academy of Sciences, Yingtan, Jiangxi 335211, China
[c] Jiangsu Collaborative Innovation Center for Solid Organic Waste Utilization, Nanjing 210095, China

ARTICLE INFO

Keywords:
Bio-fertilizer
Enzyme activity
Functional diversity
Soil productivity
Free-living nematodes
Phosphate-solubilizing bacteria

ABSTRACT

Bio-fertilizers are thought to be overwhelmingly superior to chemical fertilizers for the improvement of soil quality and productivity. However, the comprehensive effects of bio-fertilizer on the soil ecosystem and the possibility of using multiple soil beneficial biota are still not well understood. A two-year field study was conducted to examine how crop yield, soil biochemical properties, enzyme activities, and functional diversity responded to different bio-fertilizers in the sweet potato phase of a double-cropping system (sweet potato and rapeseed). Six fertilizer treatments, including one chemical treatment (CF), two organic treatments (OM, organic manure; MC, organic manure plus chemical fertilizer) and three bio-fertilizer treatments (MCN containing nematodes, MCE containing earthworms, and MCP containing phosphate-solubilizing bacteria) were compared. The soils under CF had the lowest values for all soil parameters in comparison with the other treatments. Among the organic and bio-fertilizer treatments, OM performed better in improving soil biological properties such as soil respiration and microbial biomass, while soils under all these treatments had similar soil enzyme activities and functionality. Crop yield was positively correlated with soil nutrient levels, microbial biomass and enzyme activity, and most of the biochemical variables were highly intercorrelated. Our results indicated that organic fertilizers and bio-fertilizers were superior to chemical fertilizers and generally improved soil quality and productivity to similar levels in the field.

1. Introduction

Conventional agriculture, which greatly depends on chemical fertilizers and pesticides, has caused serious environmental problems worldwide, such as soil degeneration, ground water pollution, and crop quality decline (Ju et al., 2009; Zhang et al., 2013). Bio-fertilizer usually contains living microorganisms, which are extremely advantageous in enriching soil fertility and promoting plant growth. It is also more environmentally friendly and appears to be a promising practical application for modern sustainable agriculture (Malusá et al., 2012; Singh et al., 2016; Singh et al., 2011).

The beneficial effects of multiple soil microorganisms are well documented. Such effects include the nitrogen fixation of *Rhizobium* (Graham and Vance, 2000), the phosphate mobilization of *Aspergillus* (Pradhan and Sukla, 2006), and the plant growth promotion of *Pseudomonas* (Ahmad et al., 2008). These microorganisms enhance plant nutrient acquisition through a wide range of processes, such as transformation of insoluble soil nutrients, suppression of plant pathogens and stimulation of plant growth (Alori et al., 2017; Singh et al., 2016, 2011). Similar functions are also performed by other soil fauna, such as soil nematodes and earthworms. Soil nematodes are highly abundant and widely distributed in almost all terrestrial ecosystems (Bongers and Ferris, 1999). Free-living nematodes dominate in soil, representing 60% to 80% of the soil nematode community, and perform ecosystem functions such as regulating organic matter decomposition, maintaining nutrient cycling, and suppressing plant disease (Freckman, 1988; Neher, 2010; Yeates and Wardle, 1996). In addition, as the largest component of animal biomass in soil, earthworms play significant roles in supporting ecosystem services through soil formation, water regulation, nutrient cycling, primary production, climate regulation and pollution remediation (Blouin et al., 2013; Lavelle et al., 2006). Although the important ecological roles of soil fauna such as nematodes and earthworms are widely acknowledged, few studies have explored the possibility of using these organisms as "fertilizers" to improve soil

* Corresponding author at: College of Resources and Environmental Science, Nanjing Agricultural University, Nanjing 210095, China.
E-mail address: huixinli@njau.edu.cn (H. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.apsoil.2018.06.016
Received 8 June 2018; Accepted 25 June 2018

fertility and crop productivity in the field.

Soil microorganisms are widely applied as integrative bioindicators for quantifying the effects of fertilization not only because of their critical roles in maintaining soil health and productivity but also because they respond quickly to any modification of soil properties (Kennedy and Smith, 1995; Schloter et al., 2003). Application of bio-fertilizer (containing beneficial microorganisms) is expected to enhance soil quality and productivity through stimulating soil microbial activities (Shen et al., 2015, 2011). Compared to bio-fertilizer with specific beneficial microorganisms, nematodes and earthworms also strongly interact with a wide range of soil microbial groups. Previous studies have shown that free-living nematodes promote the growth of crop plants through altering the soil microbial community (Djigal et al., 2004; Mao et al., 2006). It was also demonstrated that adding earthworms to soil would improve soil microbial biomass, enzyme activities and plant productivity (Paz-Ferreiro et al., 2014; Tao et al., 2009) (but see Caravaca and Roldán, 2003). However, the influence of different bio-fertilizers on the soil microbial community and their potential to sustain soil productivity are still not well understood and characterized in the field.

Here, we integrated three kinds of organisms, phosphate-solubilizing bacteria, native free-living nematodes, and epigeic earthworms, with composted manure to explore their potential as bio-fertilizers. We examined how crop yield, soil biochemical properties, enzyme activities, and functional diversity responded to bio-fertilizer in the sweet potato phase of a double-cropping system (sweet potato and rapeseed) for two years. Soil enzyme activities and functional diversity are widely adopted to evaluate the effects of different fertilizers on soil fertility and quality (Islam et al., 2011; Tao et al., 2015) because soil enzyme activities could be more easily related to changes in the soil microbial community structure (Waldrop et al., 2000; Zhang et al., 2015), whereas soil functional parameters, such as carbon utilization profile, provide comprehensive information on multiple soil microbial functions (Zak et al., 1994). The aims of our study were to 1) compare the effects of chemical fertilizer, organic fertilizer, and bio-fertilizer on crop yield, and 2) investigate the effects of different soil beneficial organisms on soil microbial community activity and functioning.

2. Materials and methods

2.1. Site and experimental design

The study was conducted in Jiangxi Institute of Red Soil (116°55′N, 28°13′E), Jiangxi Province, China. The region is sited in a typical subtropical monsoon landscape zone, with a mean annual precipitation of 1 788 mm and a mean annual temperature of 17.6 °C. The soil is sandy loam Ultisol (U.S. soil taxonomy), with very low soil organic matter (total C, 5.73 g/kg; total N, 0.41 g/kg; total P, 0.31 g/kg). A double-cropping system with local varieties of sweet potato (*Ipomoea batatas* L.) and rapeseed (*Brassica napus* L.) was set up in an agricultural field in 2014.

The fertilizer treatments included one chemical, two organic and three bio-fertilizer treatments, as follows: 1) CF (chemical fertilizer), 2) OM (organic manure), 3) MC (organic manure plus chemical fertilizer), 4) MCN (organic manure plus chemical fertilizer plus nematodes), 5) MCE (organic manure plus chemical fertilizer plus earthworms), and 6) MCP (organic manure plus chemical fertilizer plus phosphate-solubilizing bacteria). For CF, the fertilizer N was applied in 152.72 kg N per ha, P in 116.20 kg P_2O_5 per ha, and K in 166.32 kg K_2O per ha according to the typical local fertilizer application amount, and this treatment was used as the control. Organic manure was applied with composted pig manure (total N, 13.6 g/kg; total P, 2.67 g/kg; total K, 1.51 g/kg; available P, 0.61 g/kg and available K, 0.15 g/kg) in 11 290 kg/ha, which had an amount of N equal to that of the chemical fertilizer treatment. In terms of MC treatment, 40% of the amount of the chemical fertilizer and 60% of the composted manure were mixed to maintain an equal amount of N as CF and OM.

Based on our years of experience in both the laboratory and in the field, another three treatments (MCN, MCE and MCP) were constructed by adding nematodes, earthworms, and phosphate-solubilizing bacteria separately to the organic–inorganic compound fertilizer. The mixed population of resident nematodes, mainly constituted by bacterial-feeding species, were extracted from the local soil using the method described by Mao et al. (2006), with some modifications for boosting the number of nematodes effectively in the field (Mao et al., 2006). Briefly, for each plot under MCN treatment, 3 kg of soil mixed with 30 kg of pig manure were incubated at 20 °C in the dark for 4 weeks; at this point, the density of nematodes was estimated to have reached 50 individuals per gram of dry soil, and the mixed soil was added back to the plot. In addition, a reduced amount of composted pig manure was supplied to maintain an equal amount of N as was present in the CF treatment. For MCE, we chose a compost earthworm, *Eisenia fetida*, for several reasons. First, this species can exert similar functions as other soil earthworms, such as improving soil structure and promoting nutrient cycling. Second, this species also grows rapidly and adapts well to many environments. It has been widely raised in factories, which will make it easy to obtain at a low price for farmers if this treatment is promoted in the future. Moreover, the interactions between the earthworms and composted manure were expected to improve soil quality more significantly in the field. In this study, nearly 1 300 individuals of *E. fetida* (with a mean mass of 0.3 g) were added to the plot, resulting in an abundance of 20 g/m^2, which was 2 times higher than average abundances (approximately 10 g/m^2) of local soil earthworms observed in the nearby undisturbed grassland (Liu et al., 2004).

Since P is one of the essential plant growth-limiting nutrients, and many soils are P deficient, a phosphate-solubilizing microorganism was used in the MCP treatment. Under MCP treatment, a bacterial solution (1×10^{12} cfu/ml) of *Bacillus thuringiensis* B1 (a promising phosphate-solubilizing bacterium isolated from garden soil in our lab, see Wang et al., 2014 for details) was prepared. For each plot, a mixture of the bacterium and the MC fertilizer described above was added together (5×10^{12} cfu/m^2). These treatments were arranged in a completely randomized design, and three replicates were used for each treatment. Each plot was 20 m^2 (5 m × 4 m) and was separated from its neighbors by concrete barriers (60 cm deep and 10 cm aboveground). All the chemical fertilizers, pig manure composts and bio-fertilizers listed above were applied as basal fertilization before planting sweet potato each year, and additional mineral fertilizers (152.72 kg N per ha, 116.20 kg P_2O_5 per ha, and 166.32 kg K_2O per ha) were appended to all plots before planting rapeseed. The fresh tubers of sweet potato were gathered and weighed in September after the harvest.

2.2. Soil sampling and biochemical properties analysis

Soil samples were collected at a depth of 0–20 cm after the sweet potato was harvested. Five soil cores with a diameter of 4 cm were randomly sampled and mixed for each plot. The soil samples were stored at 4 °C until soil biochemical properties were measured. Soil total organic carbon (TC) and total nitrogen (TN) were analyzed using an automated elemental analyzer (Vario MAX CN, Elementar Co., Germany). Total phosphorus (TP) was measured by NaOH fusion and colorimetric procedures (Olsen and Somers, 1982). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were estimated by the fumigation-extraction method described by Vance et al. (1987) (Vance et al., 1987). Briefly, two fresh soil samples (10 g dry weight equivalent) were prepared and inoculated in 250-ml centrifuge tubes. Then, one of the soil samples was fumigated with 0.5 ml ethanol-free chloroform for 24 h. Both fumigated and non-fumigated soils were extracted with a 40 ml 0.5 M K_2SO_4 solution. The organic carbon and nitrogen in extracts were determined by an automated C/N analyzer, and they were converted to MBC and MBN using conversion factors of 0.38 and 0.45, respectively (Vance et al., 1987). Soil respiration (SR)