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Responses of soil microbial communities to a short-term application of seaweed fertilizer revealed by deep amplicon sequencing

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ABSTRACT

Numerous studies have reported soil damage from chemical fertilizer application and an obvious promotional effect of seaweed fertilizer fermented with *Sargassum horneri* on the growth of tomato roots and seedlings due to its alginate oligosaccharide. However, few studies have assessed the effects of the fermented seaweed fertilizer on ecological environment and microorganisms in soil. Herein, our objective is to uncover microbial and soil environmental responses to *Sargassum horneri*-fermented seaweed fertilizer. After treated tomato-planting plots with *Sargassum horneri* fermented seaweed fertilizer, soil bacterial community compositions based on 16S rRNA gene amplicon sequencing, enzyme activities in soil and crop yield were analyzed. The bacterial α-diversity was strongly influenced by seaweed fertilizer amendment after 60 days. Non-metric multidimensional scaling (NMDS) analysis showed that a difference in bacterial community compositions between day 0 and day 60 was obvious for soil treated with seaweed fertilizer. The community variation could be caused by invertase activity and dehydrogenase activity in canonical correlation analysis (CCA). Protease activity, polyphenol oxidase activity and urease activity showed an obvious correlation with community variation in the Mantel test. The fertilization increased tomato yield by 1.48–1.83 times, Vc content by 1.24–4.55 times and lycopene content by 1.20–2.33 times. In the present study, a possible reason for bacterial community variation was discovered, which will provide an economical dilution rate of seaweed fertilizer for optimal crop yield and quality. Meanwhile, our study will be beneficial for developing a possible substitute for chemical fertilizer and an improved understanding of soil microbial functions and soil sustainability.

1. Introduction

Since the first Green Revolution, chemical fertilizers have been extensively applied to sustain global agricultural production (Leita et al., 1999; Tilman, 1998). Currently, excessive application of chemical fertilizers, pesticides, and herbicides is common in modern high-intensity agricultural ecosystems all over the world and is considered a main cause for a decrease in soil quality and fertility, reduction of soil microbial diversity, and contamination of groundwater resources in agricultural ecosystems (Chaudhry et al., 2009; Kaur et al., 2008). On the other hand, agriculture sector is the largest contributor to these non-CO_2 global greenhouse gas (N_2O, CH_4 and fluorinated gases) emissions (Simpson et al., 2014), for instance, about 82% of non-CO_2 emissions in Central and South America were attributed to the agriculture sector in 2005. Synthetic fertilizer and pesticides use in the recent past caused tremendous increase of global greenhouse gas emissions by agricultural sector (Davidson and Kanter, 2014).

High yields in agricultural production and a clean environment without pollution are both anticipated goals pursued by human beings. A substitute for chemical fertilizer is urgently needed.

As a natural resource, seaweed is not only harmless to the environment but also rich in various nutrients that are beneficial to plants. In addition to organic compounds such as proteins, amino acids, lipids, cellulose, vitamins and phenols, seaweed is also rich in alginate, fucoidan, laminarin and other polysaccharides that are not present in terrestrial plants (Khan et al., 2009; Ugarte et al., 2006). Greater concentrations of minerals and plant hormones have also been found in marine seaweed (Nabti et al., 2017). As a biofertilizer, seaweed is a sustainable alternative to chemical fertilizers. Furthermore, advanced production technology enhances the nutrient content of seaweed

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extract (Kadam et al., 2015; Wang et al., 2016a). Previous studies have reported the beneficial effects of seaweed extract application in the agriculture domain, such as early seed germination and seedling establishment, improved crop yield and quality, increased resistance to environmental stresses, and regulation of the soil micro-ecosystem (Bai et al., 2013; El Kaoaua et al., 2013; Mohamed and El-Sehrawy, 2013).

Understanding soil microbial properties in response to seaweed fertilization helps us know about soil microbial functions through optimized fertilization practice. Numerous studies have revealed that the soil microbial community is an important component of the soil ecosystem and is known to be the main driver of soil health and quality (Xue et al., 2013). Biological processes are essential and responsible for many critical ecosystem functions in soil, such as nutrient cycling, toxin removal, the decomposition of organic matter and the formation of soil aggregates (Stark et al., 2008). In addition, microorganisms in soil play an important role in promoting plant growth. Although the concealed links between soil microbial communities and the soil ecosystem are still poorly understood, interaction effects have been observed between fertilization practices, the diversity and abundance of soil microbial communities, and plant quality (van der Heijden and Wagg, 2013). Some studies have reported that soils in organic farming regimes had higher microbial activity and diversity than those in conventional farming systems (Mader et al., 2002). Recently, an increasing number of studies focus on the effects of fertilization and soil management practices such as organic farming, crop rotation, straw amendments and biostimulant application on soil microbial communities (Chen et al., 2014; Garbeva et al., 2004; Hartmann et al., 2015; Tejada et al., 2011; Venter et al., 2016). However, few studies have reported on the effects of seaweed fertilizer application on the soil microbial community.

To establish seaweed as an effective biofertilizer, more in-depth studies are needed to determine the relationship between seaweed fertilizer use and the soil microbial community. In a previous study, we used the identified strain *Bacillus litoralis* to ferment *Sargassum horneri* and found that the fermented seaweed fertilizer with alginate oligosaccharides promoted root and seedling growth of tomato (Wang et al., 2016a, 2017).

In the current study, we determined basal soil biochemical properties including total C, total N, and enzyme activity and explored the effects of seaweed fertilizer application on soil microbial communities in a field trial of tomato. Soil was augmented with seaweed root-irrigation and sampled after 60 days of application. Soil bacterial diversity and community composition were analyzed by using a deep sequencing approach. Due to microbial ecological functions being closely related to soil biochemical properties, We hope to investigate (i) the short-term effects of amendment with seaweed fertilizer on the soil bacterial diversity and community composition, (ii) the relationship between bacterial community composition and soil biochemical properties after short-term seaweed fertilizer application, and (iii) whether soil and crop qualities were improved.

2. Materials and methods

2.1. Site description

Soil samples were collected from a long-term fertility experiment site located at the Yantai Agri-Expo Garden, Yantai City, Shandong Province, China (37.494°N, 121.286°E). The region has a temperate monsoon climate, with mean annual precipitation of 672.5 mm and a mean annual temperature of 12.6 °C. The soil is brown soil with loamy particle size-classes and good structure (Udic Luvisols, FAO) (Li et al., 2014).

2.2. Experimental design

In June 2015, 5 experimental plots were used to plant tomato (*Lycopersicon esculentum* Mill) for approximately 60 days. This field was used to plant tomato for 5 consecutive years prior to this experiment. As a base fertilizer, commercial chemical manure (N-P-K 16-16-16) was regularly applied for tomato plantation at the content of 1500 kg ha^{-1} every time. Each plot consisted of 14 plants in double rows (1.0 m wide, 4.0 m long) with row interval of 0.2 m. The treatments with fermented seaweed fertilizer at three concentrations of W6, W9 and W12 were conducted; and commercial seaweed fertiliser Leili (CKL) were used as the positive control and the water only (CK0) were used as the negative control.

During the experimental period of 60 days, one experimental plot was subjected to root-irrigation with 10 L of water for 5 times. One experimental plot was subjected to treatment with Leili seaweed fertilizer (Leili 2000, Beijing) at the concentration of 6 ml L^{-1} for 5 times. Fermented seaweed fertilizers (Wang et al., 2016a) with 60 ml, 90 ml, and 120 ml of fermented seaweed extract were added in 10 L of water, and applied to soil at three concentrations for 5 times, respectively. The nutritional compositions of commercial Leili seaweed fertilizer were composed of 18% solid content, 2% alginate acid, 0.1% amino acid, 8% N, 2% P_2O_5, and 4% K_2O. Fermented seaweed fertilizer contained 4.5% solid content, 2% alginate acid, and 0.2% alginate oligosaccharide.

2.3. Soil sampling

In each plot, three single pore soil samples were randomly collected as three replicates. Totally 30 soil samples were collected from 5 plots (CK0, W6, W9, W12 and CKL) and 2 time points (tomato seeding time as day 0 and tomato harvesting time as day 60). Each replicate was collected from top layer of soil with the depth of 0–20 cm after different treatments on day 0 in June and on day 60 in August. The soil samples were preserved in a zip lock bag. Each replicate was divided into two portions. One portion was homogenized and air-dried to pass through a 0.15 mm mesh sieve for evaluating soil properties. Another portion was stored at −80 °C for future high-throughput sequencing.

2.4. Determination of physico-chemical characteristics

Total organic carbon (TOC) and total nitrogen (TN) were determined using an elemental analyzer (Vario MACRO cube, Elementar, Germany). C/N ratios were estimated by the ratio of TOC to TN. Soil moisture was determined by the percentage between soil samples before and after drying at 105 °C. Soil enzyme activities were determined according to methods described (Guan, 1986). The method of ninhydrin colorimetry is used for determination of soil protease activity. In brief, 2 g of air-dried soil were mixed with 10 ml 1% pH 7.4 phosphate buffer solution and 0.5 ml of toluene. The mixed liquids were incubated at 30 °C for 24 h and then filtered. Proteins were precipitated with sulfuric acid and sodium sulfate. Ninhydrin solution was added and the mixture was boiled for 10 min. Absorbance was measured at 560 nm.

For polyphenol oxidase activity analysis, 1 g dry soil sample mixed with 10 ml 1% pyrogallol solution was incubated at 30 °C for 2 h. Then, the mixture was extracted with citric acid-phosphate buffer and ether for 30 min. The colored ether phase was colorimetrically assessed at 430 nm.

Indophenol blue colorimetry was used for determination of urease activity. In brief, 1 ml toluene was added to 2 g air-dried soil. After 15 min, 10 ml urea solution (10%) and 20 ml of citrate buffer (pH 6.7) were added and mixed by shaking. The samples were incubated at 37 °C for 24 h. After incubation, sodium phenol and sodium hypochlorite were added and after 20 min absorbance was determined at 578 nm.

To measure invertase activity, the toluene, sucrose solution and phosphate buffer were added in to 5 g soil, cultured at 37 °C for 24 h and quickly filtered. Then, 1 ml filtrate was removed, and 3 ml 3,5-dinitrosalicylic acid was added. The mixture was then incubated in a boiling water bath heat 5 min. The volumetric flask was then moved to tap water for cooling for 3 min. Absorbance was determined at 508 nm.

The dehydrogenase activity in soil was determined using 2,3,5-