Microbial composition and diversity of the long term application of organic material in upland soil

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Key words: Microbial diversity, Colony forming units (CFUs), Biolog, Denaturing gradient gel electrophoresis (DGGE)

Abstract

Organic and chemical fertilizer amendments are an important agricultural practice for increasing crop yields. In order to maintain the soil sustainability, it is important to monitor the effects of fertilizer applications on the shift of soil microorganism, which control the cycling of many nutrients in the soils. Here, culture-dependent and culture-independent approaches were used to analyze the soil microorganism and community structure under six fertilization treatments, including green manure, rice straw compost, rapeseed cake, pig mature compost, NPK +pig mature compost, NPK and control. Both organic and chemical fertilizers caused a shift of the cultural microorganism CFUs after treatments. Bacterial CFUs of the organic fertilization treatments were significantly higher than that of chemical fertilization treatments. The DGGE profiles of the bacterial communities of the samples showed that the green manure treatment was a distinct difference in bacterial community, with a greater complexity of the band pattern than other treatments. Cluster analyses based on the DGGE profile showed that rice straw compost and pig mature compost had a similar banding pattern and clustered together firstly. Rapeseed cake, NPK, NPK + pig manure compost and control clustered together in other sub-cluster and clearly distinguished from green manure.

Introduction

Organic farming has often been shown to improve soil fertility by increasing soil organic matter and supporting the living organism in soil. Soil microbial diversity is a key indicator of soil microbial function and can be affected by management practices. Traditionally, soil microbial communities are investigated using methods based on isolating and culturing the microorganism. However, cultivation method is that only a small fraction of microorganism is cultivatable. The drawbacks of the cultivation method can be overcome by using phospholipid fatty acid (PLFA), Biolog and molecular techniques such restriction fragment length polymorphism (RFLP),

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denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP). The objective of this study was to examine the soil microbial composition and diversity of long term organic and conventional fertilizer in upland soil by using Biolog and PCR-DGGE methods

Materials and methods

Soil samples were collected from a long-term experiment field at Suwon, Gyeonggi Province of Korea. The soil samples (0-20 cm) from seven individual plots per treatment were collected. The total numbers of culturable bacteria and fungi were determined as colony forming units (CFUs) on agar plates by dilution plate methods. Soil bacterial community function was determined by using Biolog Eco MicroPlate (Biolog Inc., CA, USA). A soil suspension was prepared by adding 4 g soil to 36 mL sterile saline solution. This solution was vigorously shaken for 30 min to dislodge bacteria before diluting to 10^{-3}. A 150 μL aliquot of the bacterial suspension was inoculated into each well of a Biolog EcoPlate (Biolog Inc., Hayward, Ca). The plates were incubated at 25°C and read every 24 h with microplate reader at wavelengths of 590 nm. Soil DNAs were extracted using the FastDNA® SPIN kit for soil (Bio 101 inc, USA) according to the manufacturer's instructions. For soil bacteria, polymerase chain reaction (PCR) conditions for primer GC-341f and 518r were performed. DGGE was performed by using 8% acryamide gel with a 40% to 60% denaturant gradient. The electrophoresis was run in a Dcode Universial Detection System Instrument (Bio-Rad Laboratories, USA) at a constant temperature of 60°C for 12.5 h at 60 V. After running, the gels were stained with EtBr and then photographed with UV transillumination.

Results and Discussion

Both organic and chemical fertilizers caused a shift of the cultural microorganism CFUs after treatments (Table 1). Bacterial CFUs of the organic fertilization treatments were significantly higher than that of chemical fertilization treatment and control. There was no significant difference in the actionomyces and fungi CFUs among the treatments.

Table 1 Microorganism colony formation units (CFUs) of the soil fertilization treatments by the plate counting method (log CFUs g^{-1}soil)

Treatment	Bacteria	Actinomyces	Fungi
Green manure	7.1a[a]	6.3a	4.4a
Rice straw compost	6.9ab	5.9a	4.2a
Rapeseed cake	6.8ab	5.9a	4.2a
Pig mature compost	6.6ab	6.1a	4.7a
NPK+ Pig mature compost	6.4b	6.3a	5a
NPK	6.3b	6a	4.6a
Control	6.1b	6.3a	4.6a

[a] Means followed by the same letter within each column are not significantly different at $P < 0.05$.

The average well color development (AWCD), richness (R) and Shannon- Weaver index (H) varied for different soil sample (Table 2). The AWCD value was the highest in green manure, the lowest in control. The values represent the metabolic activity of soil bacterial community in using the carbon sources, suggesting that the effect of green manure on soil bacterial community metabolic function is opposite. It is clearly indicated that the richness as evaluated by the number of carbon sources used and the metabolic diversity as calculated by Shannon-Weaver index were remarkably the highest in Green manure. It is infers that catabolic diversity of the soil bacterial community could be increased by applying green manure.

Table 2. Effect of different treatments on catabolic diversity of the soil bacterial community as evaluated by average well color development (AWCD), Richness (R) and Shannon- Weaver index

Treatment	AWCD	R	H
Green manure	1.47a[a]	27.75a	3.20a
Rice straw compost	0.90b	21.22ab	2.88ab
Rapeseed cake	0.63b	17.0ab	2.61abc
Pig mature compost	0.91b	19.11ab	2.68abc
NPK+ Pig mature compost	0.72b	17.67ab	2.58abc
NPK	0.58b	14.33b	2.39bc
Control	0.42b	11.44b	2.12c

[a] Means followed by the same letter within each column are not significantly different at $P < 0.05$.

The DGGE profiles of the bacterial communities of the samples are shown in Fig. 1. The green manure treatment showed a distinct difference in bacterial community, with a greater complexity of the band pattern than other treatments. Cluster analyses based on the DGGE profile showed that rice straw compost and pig mature compost had a similar banding pattern and clustered together firstly. The rapeseed cake expeller, NPK, NPK + pig mature compost and control clustered together in other sub-cluster and clearly distinguished from green manure treatment.

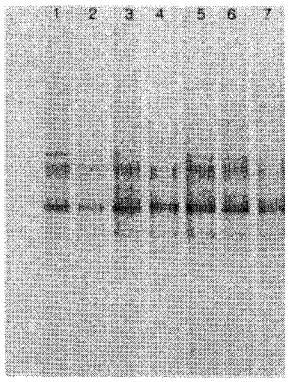

Fig. 1 DEEG profiles of bacterial 16S rRNA gene fragments of soil samples under different treatments. Lane: 1 green manure, 2 Rice straw compost, 3 Rapeseed cake, 4 Pig mature compost, 5 NPK + pig mature compost, 6 NPK, 7 Control.

Conclusions

These results show that long term fertilization had effects on soil microbial communities and the use of green manure mainly increased the catabolic diversities of bacterial communities.

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