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Plant-specific effects of sunn hemp (*Crotalaria juncea*) and sudex (*Sorghum bicolor* × *Sorghum bicolor* var. sudanense) on the abundance and composition of soil microbial community

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ABSTRACT

We investigated the effect of sunn hemp (leguminous) and sudex (non-leguminous) on the abundance and composition of microbial community in soil with a low nutrient content for two years. Abundance of microbial groups was determined by phospholipid fatty acid (PLFA) analysis. Pyrosequencing analysis was used to compare the composition of bacterial communities in the rhizosphere with that in the bulk soil of the unplanted control. The concentration of microbial PLFA increased during cultivation period and decreased after incorporation of plant residues, which implies that the responses depended on living plants. Overall, the bacterial PLFA concentration was lower with sunn hemp than sudex, despite the above-ground biomass was about five times greater with the former than with the latter crop. This indicates that the increase in bacterial PLFA with sudex is plant-specific. Pyrosequencing analysis revealed that the abundance of *Arthrobacter*,*Bacillus*, and *Sphingomonas* were the highest in the bulk soil and decreased in the rhizospheres of the cover plants. The proportions of *Gaiella* and the nitrogen fixing bacteria *Mesorhizobium* increased with sunn hemp, while those of cyanobacteria, including *Calothrix*, *Leptolyngbya*, and *Nostoc*, increased greatly with sudex. Our results indicated that the high level of bacterial PLFA with sudex could be primarily explained by the colonization of cyanobacteria. We show that the impacts of cover plants on the abundance and composition of the microbial communities depend more on the plant species than on the aboveground plant biomass, both during growth and incorporation stages. Our results also suggest that non-leguminous as well as leguminous plants harbor beneficial microbes such as nitrogen-fixing bacteria to cope with nutrient-poor environments.

1. Introduction

Improving soil fertility is an important goal for a sustainable agricultural system. Cover crops are widely used to improve soil properties and reduce weed infestation and soil erosion. Both leguminous and non-leguminous plants can be used as cover crops. Sunn hemp (*Crotalaria juncea* L.) is a legume from subtropical East Asia and is also cultivated in temperate regions. The main role of leguminous plants is nitrogen input in a symbiotic relationship with nitrogen-fixing bacteria. Sudex (*Sorghum bicolor* × *S. bicolor* var. sudanense) is a non-leguminous plant widely used in crop rotation systems. Both non-leguminous and leguminous cover crops improve the chemical and physical properties of the soil such as the organic matter content and water-holding capacity (Ramos et al., 2010).

Cover crops affect the activity, biomass and composition of the soil microbial community by supplying root exudates and debris during their growth period (Elfstrand et al., 2007; Liu et al., 2010; White and Weil 2010; Liu et al., 2014). The effect varies depending on the plant species (Li et al., 2012; Lorenzo et al., 2013; Xavier et al., 2013). There are also some differences between the microbial properties of the rhizosphere and bulk soil (Mouhamadou et al., 2013). Highly specific relationships are formed between the plants and soil microbes such as symbiotic nitrogen-fixing bacteria and pathogens.

Soil microorganisms have beneficial effects on the crops. Nitrogen-fixing bacteria supply nitrogen through symbiotic and non-symbiotic interactions. This is particularly important in N-deficient soils (Knelman et al., 2012). Moreover, some rhizosphere bacteria facilitate the uptake of plant nutrients through phosphate solubilisation and siderophore production (Ahmad et al., 2008).

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Decomposers mineralise the plant debris and rhizodeposits, releasing the nutrients (Elfstrand et al., 2007). The chemical composition and carbon-nitrogen (C/N) ratio of incorporated plant matter can affect the microbial decomposition (Marschner et al., 2003; Tejada et al., 2008).

Various biochemical and molecular techniques have been used to monitor the changes in the abundance and composition of soil microbial community. For example phospholipid fatty acid (PLFA) analysis can be used to determine the changes in the biomass of microbial groups (Kourtev et al., 2002; Wu et al., 2013). Pyrosequencing provides a deeper characterisation of microbial diversity than PLFA but it is an expensive technique (Torres-Cortes et al., 2012; Bakker et al., 2013).

The aim of this study was to determine the influence of two cover crops, sunn hemp and sudex on the abundance and composition of soil microbial community before and after their incorporation. The following hypotheses were tested: (a) leguminous cover crops are more effective in accumulating biomass and have a greater impact on the abundance of microflora than non-leguminous plants and (b) plants harbor specific bacteria that improving nutrient supply in nutrient-poor soils.

2. Materials and methods

2.1. Study field

The experiment was conducted in a field located in Eumseong, Chungbuk Province, Republic of Korea (36.56N, 127.45E). The field was prepared for ginseng cultivation by dumping soil up to a depth of approximately 5 m in 2009. This sandy loam soil with the low nutrient content had never been fertilized. The plots were constructed using a randomised block design with four replicates (each 8×10 m) per treatment. The cover crops were seeded (100 kg ha^{-1}) on 30 June 2010 and 8 June 2011. Field incorporation by rotary tillage was performed on the 14 October of each year. Control plots were left fallow, and all plots were hand-weeded from July to August each year.

2.2. Soil and plant samplings

The surface soil between the plants was sampled at a depth of 0–20 cm with an auger for analysis of microbial PLFA and soil chemical properties. The soil samples collected from 4–5 locations were homogenized by hand mixing, and plant debris and roots were removed. Sampling was conducted during the growing season (on 17 September 2010, and 15 September 2011) and twice after the incorporation of plant residues (on 16 May 2011, and 4 May in 2012). For pyrosequencing analysis, soil samples were taken from the rhizosphere of cover plants and the bulk soil of control plots on 5 September 2011. Five plants per plot were uprooted and the soil attached to the roots was homogenized and considered rhizosphere soil. Soil samples were freeze-dried prior to PLFA and pyrosequencing analysis. Aboveground plant biomass, including leaves and stems, was determined in three quadrats (each 1×0.5 m) per plot on 28 September 2010 and 15 September 2011.

2.3. Analyses of chemical properties of the soil and plant nutrient composition

Soil moisture was measured after drying the soil at 105 °C for 48 h. The nitrate concentration in the soil was determined using an auto-analyser (AutoAnalyser 3, Bran + Luebbe, Germany). The available phosphate was analyzed using the Lancaster method, and the soil organic content was determined according to the Tyurin method (RDA, 2002). The carbon-to-nitrogen (C/N) ratio was determined by a C/N analyzer (Vario Max CN, Elementar, Germany). The exchangeable cations in the soil and the plant nutrients were extracted with 1 N NH_4OAc (pH 7.0) and measured using the inductively coupled plasma analysis (Integra XMP, GBC, Australia).

2.4. PLFA analysis

Analysis of PLFAs was performed using the method of Li et al. (2006). Lipids were extracted from 5 g of soil with a solvent (chloroform:methanol:citrate buffer, 1:2:0.8 v/v/v) and separated into neutral lipids, glycolipids, and phospholipids on a silica column. The concentration of Gram-negative bacteria was determined by summing the fatty acids 18:1 ω7c, cy19:0 ω8c, and 17:1 ω8c. For Gram-positive bacteria, it was taken as a total of i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0. Actinomycetes were determined by summing 10Me16:0, 10Me17:0, and TBSA10Me18:0. The fatty acid 18:2 ω6, 9c was chosen as a bioindicator of fungi (Frostegard and Baath, 1996). Aerobes and anaerobes were represented by the 16:1 ω7c and cy19:0, respectively. The ratio of monounsaturated fatty acids (16:1 ω5c, 17:1 ω8c, and 18:1 ω7c) to saturated fatty acids (14:00, 15:00, 16:00, 17:00, 18:00, and 20:00) was used as an indicator of microbial stress (Kaur et al., 2005). The concentration of each PLFA was calculated using 19:0 peak as an internal standard. All analyses were performed using the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA).

2.5. Pyrosequencing and sequence analysis

We conducted pyrosequencing analysis to compare the bacterial community composition of the sunn hemp and sudex rhizosphere soil with the composition of bulk soil in the control plot. Pyrosequencing was performed according to Chun et al. (2010), and the samples from only three replicated plots were analyzed. Genomic DNA was extracted from 0.5 g of freeze-dried soil using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, USA). An aliquot (0.5 μg) of DNA obtained in the polymerase chain reaction was used for pyrosequencing. In brief, the V1–3 region of the bacterial 16S rRNA gene was amplified. The forward primer was V1–9F (5′-CCTATCCCCTGTGTGCCTTGG CAGTC-TCAG-AC-AGTTT-GATCMTGGCTCAG-3′) and the reverse primer was V3-541R (5′-CCATCTCATCCCTGCGTGTCTCCGAC-TCAG-AGAGCTG-AC-WTTACCGCGGCTGCTGG-3′). The sequences were determined using a 454 GS FLX Titanium Sequencing system (Roche, Branford, CT, USA). Sequencing reads shorter than 300 bp or containing two or more unresolved nucleotides were removed. The extended EzTaxon database was used to compare the sequencing reads. The taxonomic classification was conducted using a criterion of ≥94% identity for genus and ≥75% identity for phylum.

2.6. Data analysis

Soil chemical properties and ratios of PLFA indicators were subjected to repeated measures analysis of variance followed by Fisher's least significant difference (LSD) test. The aboveground biomass and nutrient content of the two cover plants were compared using the *t*-test. The analyses of pyrosequencing data were conducted with a cut-off value of 97% similarity for assigning phylotypes. Relationships between communities were determined using the Fast UniFrac online tool for principal coordinate analysis of UniFrac distances. The percentage and number of operational taxonomic units (OTU) of the genera analyzed by pyrosequencing were compared for the three treatments using LSD test. All statistical analyses were conducted using SAS v9.1 (SAS Institute, Cary, NC). Rarefaction curves were generated with an OTU defined