ARTICLE IN PRESS

Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Short communication

Green manure amendment enhances microbial activity and diversity in antibiotic-contaminated soil

Jiff Randy Caban[a], Saranya Kuppusamy[b], Jang Hwan Kim[a], Young-Eun Yoon[a], Song Yeob Kim[b], Yong Bok Lee[a,b,*]

[a] Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 52828, Republic of Korea
[b] Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea

ARTICLE INFO

Keywords:
Sulfathiazole (STZ)
Enzyme activities
Microbial richness and diversity
NPK fertilizers
Hairy vetch
Compost

ABSTRACT

The impacts of antibiotics on soil microbial activity and community structure are poorly understood. It is also important to identify strategies to reduce the problems that antibiotics inflict on soil biology. Hence, for the first time, this study investigated the effect of sulfathiazole (STZ) on soil microbial activity (measured in terms of dehydrogenase activity) and bacterial community structure (revealed by pyrosequencing analysis) in antibiotics contaminated soils (10 and 100 mg STZ kg^{-1} soil), and treated with (in)organic amendments (NPK, compost and hairy vetch). Dehydrogenase activity was significantly higher in the hairy vetch amended soils (with and without STZ). Pyrosequencing analysis revealed a concentration dependent impact of antibiotics on soil microbial richness and diversity. *Proteobacteria* and *Actinobacteria* were present in greater proportions even in soils with a high antibiotic concentration of 100 mg kg^{-1}. With 10 mg kg^{-1} STZ, the effect on microbial community richness and diversity was minimal. Meanwhile, with 100 mg kg^{-1} STZ, the disappearance of *Verrucomicrobia, Bacteroidetes and Planctomycetes* was observed. Even with a high antibiotic spiking concentration, a tremendous increase in the relative abundance of *Firmicutes* was seen in the hairy vetch amended soils which led to a considerably higher Gram-positive proportion than the compost and NPK treatments. We conclude that utilization of green manure (cover crop – hairy vetch) can be considered a viable strategy for safer crop production in antibiotic-contaminated soils. This is because it enhances soil microbial/enzyme activity, bacterial richness and diversity, notably the population of beneficial Gram-positive bacteria.

1. Introduction

Antibiotic consumption in agriculture is typically 4 to 5 times greater than that in human use. In fact, in 2010 alone, it was estimated that a total of 63,200 tons of antibiotics was utilized for the livestock industry worldwide and that amount is projected to increase to 105,600 tons by 2030 (Van Boekel et al., 2015; Kuppusamy et al., 2018). It should be noted, however, that antibiotics are poorly adsorbed in the mammalian gut and are designed to act in low doses, hence they are excreted from the body via manure or faeces in the shortest time possible. Different classes of antibiotics have different excretion rates. Sulfonamides for example, are characterized by having a 90% excretion rate, whereas other classes like tetracyclines, macrolides and beta-lactams have 65%, 50–100% and 85% excretion rates, respectively (Kim et al., 2016, 2017). Application of manure as fertilizer to agricultural land is a common practice around the world (Kumar et al., 2005), and this is one way how antibiotics are unintentionally introduced to the agricultural soils which may have potential impacts on soil health and crops (Kim et al., 2011). Sulfonamides are broad-spectrum antibiotics that are one of the most commonly used antibiotics in the livestock industry. In South Korea alone, sulfonamides rank as the 3rd most widely consumed antibiotics in agriculture with 98,845 kg consumed in 2015. Within this class, Sulfathiazole was the most heavily used, with 32,269 kg consumed in 2015 followed by Sulfamethoxazole and Sulfamethazine at 27,677 kg and 13,068 kg, respectively. In soil for example, sulfonamides' concentrations ranged from 200 ug kg^{-1} (Baran et al., 2011) to as high as 0.5 mg kg^{-1} (Michelini et al., 2012). On the other hand, studies on the potential impacts of sulfathiazole on soil health and crops are scarce.

Few studies regarding the effects of antibiotics on soil microbial activity (measured in terms of dehydrogenase enzyme activity) and microbial community structure exist, and furthermore the results are somewhat inconsistent. Consequently, more research is required in this context (Insam et al., 2015). In one study, Thiele-Bruhn and Beck

* Corresponding author at: Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 52828, Republic of Korea.
E-mail address: yblee@gnu.ac.kr (Y.B. Lee).

https://doi.org/10.1016/j.apsoil.2018.04.013
Received 21 February 2018; Received in revised form 6 April 2018; Accepted 30 April 2018
0929-1393/ © 2018 Elsevier B.V. All rights reserved.

(2005) found that the presence of sulpyrimidine and oxytetracycline (antibiotic spiking concentration = 1000 mg kg^{-1}) did not exhibit any significant effect on soil dehydrogenase activity. In contrast, the experiment by Boleas et al. (2005) showed that oxytetracycline (1 and 100 mg kg^{-1}) wielded a dose-dependent negative impact on soil dehydrogenase activity. Interestingly, some studies also suggested that antibiotic (e.g., sulfonamide) effects on soil microbial activity is most evident when microbial growth was stimulated by the addition of supplementary nutrient sources like glucose, manure, etc. (Hammesfahr et al., 2008; Gutierrez et al., 2010). In terms of microbial community structure, antibiotics are capable of inducing changes in community structure by affecting Gram-positive to Gram-negative bacterial ratio, bacteria to fungi ratio as well as encouraging/inhibiting growth of certain classes of bacteria (Thiele-Bruhn and Beck, 2005; Gutierrez et al., 2010; Reichel et al., 2014; Cui et al., 2014; Pinna et al., 2012; Liao et al., 2016). To the best of our knowledge, there are currently no studies available that explain the effects of sulfathiazole on soil microbial activity and community structure with simultaneous addition of nutrient sources.

The use of organic matter such as green manure/cover crop is especially environmentally friendly compared to chemical nutrient sources, and for this reason is recommended for sustainable agriculture. Because the availability of carbon substrates largely controls microbial growth in soil (Elfstrand et al., 2007), green manure amendments stimulate soil microbial growth and activity (Tejada et al., 2008). Moreover, incorporation of cover crops in a crop production system may impact on both soil biological and chemical processes, affecting the subsequent fate of agrochemicals (Zablotowicz et al., 1998). Previous studies have reported the effect of green manure on weed control, soil chemical/biological properties, crop growth and yield (Tejada et al., 2008; Muchanga et al., 2016). However, green manure's impact on soil microbial activity and community structure especially for bacteria in the antibiotic-contaminated soil is not available. In South Korea, legume-based green manure, notably hairy vetch (*Vicia villosa*) which is an important N source is widely used in sustainable agriculture (Caban et al., 2018). In the above context, for the first time, this study aims to get a clearer picture of the amendment effect of hairy vetch on soil microbial activity (dehydrogenase activity), bacterial richness and diversity (pyrosequencing analysis) in sulfathiazole contaminated soils. Compost which is one of the other commonly used organic amendments in agriculture including mineral fertilization (NPK) was used for treatment comparisons. The current study hypothesis is that green manure amendment enhances the microbial activity and diversity in antibiotic-contaminated soil.

2. Materials and methods

2.1. Soil, amendments and test compound

Soil that was previously free from antibiotics was collected in 2016 from the Gyeongsang National University Crop Research Farm, Jinju, South Korea. To ensure homogeneity, following collection, the soil was air-dried and passed through a 2 mm screen and mixed prior to use. Soil physicochemical properties were measured according to standard analytical procedures (Daquiado et al., 2016; Kuppusamy et al., 2017). The soil contained pH 6.7 ± 0.2, organic matter 14.6 ± 0.9 g kg^{-1}, total nitrogen 1.8 ± 0.4 g kg^{-1} and available phosphorous 79 ± 2.9 mg kg^{-1}. Exchangeable cations (cmol$^+$ kg^{-1}) like Ca^{2+}, Mg^{2+} and K^+ of the soil amounted to 9.2 ± 0.4, 1.2 ± 0.02 and 1.2 ± 0.03, respectively. Two organic amendments – compost and green manure and an inorganic amendment (NPK fertilizer – control) – were tested in this study. Urea, superphosphate and potassium chloride were the sources of inorganic N, P and K, respectively. Compost used was a livestock manure compost that contained 31.1% C, 2.2% N, 0.5% S and 14.4 C:N ratio. Field-grown dry and homogenized hairy vetch (*Vicia villosa*) with 44.1% C, 4.3% N, 0.3% S and 10.4 C:N ratio served as the source of green manure. In the case of inorganic fertilizers input, the amendment rates were 35, 30 and 18 kg ha^{-1} N, P and K, respectively. Compost treatment received 10 t ha^{-1} compost, while 6 t ha^{-1} hairy vetch was incorporated into the green manure treatment as per the amendment rates recommended in South Korean agricultural system. The test compound was STZ (analytical grade) with 99% purity purchased from Sigma-Aldrich (St. Louis, US). Stock solution (500 mg L^{-1} STZ) was prepared with methanol in amber color bottles (to prevent degradation of STZ) and stored at 4 °C.

2.2. Pot experiment

Homogenized soil samples were placed in 20 L polyvinyl chloride pots (4 kg of soil was filled pot^{-1}) to mimic the planting condition. The treatments were employed as follows: (1) NPK amended control soil without STZ; (2) 10 mg kg^{-1} STZ contaminated NPK amended soil; (3) 100 mg kg^{-1} STZ contaminated NPK amended soil; (4) compost amended control soil without STZ; (5) 10 mg kg^{-1} STZ contaminated compost amended soil; (6) 100 mg kg^{-1} STZ contaminated compost amended soil; (7) hairy vetch amended control soil without STZ; (8) 10 mg kg^{-1} STZ contaminated hairy vetch amended soil; and (9) 100 mg kg^{-1} STZ contaminated hairy vetch amended soil. STZ stock solution was added to 4 kg of each air-dried soil to generate nominal substance concentrations of 10 and 100 mg kg^{-1} of dry weight. Respective fertilizers were added to the top of each pot and the soil was mixed thoroughly and incubated in a greenhouse located at the Gyeongsang National University, Jinju, South Korea. Each pot was maintained at 50% water-holding capacity. Triplicate pots were prepared for each treatment and were randomized in the greenhouse side-by-side. Subsamples of soil (25–50 g) were collected on the 0th day and 30 days after incubation. Soil samples were then freeze-dried and stored in sealed plastic bags at −20 °C until required for analysis.

2.3. Analysis of soil dehydrogenase activity

Briefly, portions (3 g) of soil were taken in 50 mL centrifuge tubes, and 0.5 mL of 3% aqueous solution of TTC (2,3,5-triphenyl tetrazolium chloride), 30 mg $CaCO_3$ and 1.25 mL distilled water added. After incubating the samples for 24 h at 37 °C, TPF (2,3,5-triphenyl formazan) formed from TTC was extracted with methanol and analyzed at 485 nm (Wolinska et al., 2015) with a Varian Cary® 50 UV–Vis Spectrophotometer (Agilent Technologies, US). Dehydrogenase (DH) activity was expressed as mg TPF g^{-1} soil h^{-1}.

2.4. Pyrosequencing analysis

Soil genomic DNA for the PCR amplification was extracted from 0.5 g of triplicate soil samples (collected only at 30 days after incubation) using the FastDNA SPIN kit (MP Biomedicals, US) following the manufacturer's instructions. The extracted DNA was quantified using the Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, US) and stored at −20 °C until further use. PCR was carried according to Daquiado et al. (2016). Briefly, bacterial 16S rRNA genes were amplified using primers V1-9F (50-X-AC-GAGTTTGATCMTGGCTCAG-30) and V3-541R (50-X-AC-WTTACCGCGGCTGCTT-30), where X denotes a 7–11 nucleotide long barcode followed by a common linker AC. Each PCR mixture (50 mL) contained 1 × PCR buffer, 0.2 mM of dNTPs, 400 mM of each primer, 1 mg mL^{-1} of bovine serum albumin (Sigma-Aldrich, US), 1.25 U of taq polymerase (Roche, Germany) and 1 mL of 1/10 diluted DNA template. The PCR reactions were executed using the following program: initial denaturation of 94 °C for 5 min; 10 cycles at 94 °C for 30 s, 60 °C for 45 s and 72 °C for 90 s (the annealing temperature was reduced by 0.5 °C per cycle from the preceding cycle), and 20 cycles at 94 °C for 30 s, 55 °C for 45 s and 72 °C for 90 s. The PCR products were purified with QIAquick Gel Extraction kit (QIAGEN, Germany).

Download English Version:

https://daneshyari.com/en/article/8846605

Download Persian Version:

https://daneshyari.com/article/8846605

Daneshyari.com