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Rhizosphere isoflavones (daidzein and genistein) levels and their relation to the microbial community structure of mono-cropped soybean soil in field and controlled conditions

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A B S T R A C T

Despite an increase in the understanding of the soybean isoflavones involved in root-colonizing symbioses, relatively little is known about their levels in the rhizosphere and their interactions with the soil microbial community. Based on a 13-year experiment of continuous soybean monocultures, in the present study we quantified isoflavones in the soybean rhizosphere and analyzed the soil microbial community structure by examining its phospholipid fatty acid (PLFA) profile. Two isoflavones, daidzein (7, 4′-dihydroxyisoflavone) and genistein (5,7,4′- trihydroxyisoflavone), were detected in the rhizosphere soil of soybean plants, with the concentrations in the field varying with duration of mono-cropping. Genistein concentrations ranged from 0.4 to 1.2 μg g^{-1} dry soil over different years, while daidzein concentrations rarely exceeded 0.6 μg g^{-1} dry soil. PLFA profiling showed that the signature lipid biomarkers of bacteria and fungi varied throughout the years of the study, particularly in mono-cropping year 2, and mono-cropping years 6–8. Principal component analysis clearly identified differences in the composition of PLFA during different years under mono-cropping. There was a positive correlation between the daidzein concentrations and soil fungi, whereas the genistein concentration showed a correlation with the total PLFA, fungi, bacteria, Gram (+) bacteria and aerobic bacteria in the soil microbial community. Both isoflavones were easily degraded in soil, resulting in short half-lives. Concentrations as small as 1 μg g^{-1} dry soil were sufficient to elicit changes in microbial community structure. A discriminant analysis of PLFA patterns showed that changes in microbial community structures were induced by both the addition of daidzein or genistein and incubation time. We conclude that daidzein and genistein released into the soybean rhizosphere may act as allelochemicals in the interactions between root and soil microbial community in a long-term mono-cropped soybean field.

1. Introduction

Soybean (*Glycine max*) is one of the most economically important crops in the world. The climatic conditions in Northeast China are particularly suitable for soybean cultivation and since the early 1980s the area planted with soybean has increased dramatically. Nowadays, soybean production in Northeast China is characterized by extensive monocultures. In some areas, soybean is the only crop grown, and a normal crop rotation cannot be achieved because the soil in early spring is often too wet or waterlogged for the early seeding of alternative crops, such as spring wheat (Liu and Herbert, 2002). However, continuous monoculture of soybean results in yield decline and quality deterioration. Despite the implementation of recommended field crop management practices, including the planting of new resistant cultivars, nutrient management and pest management, depressed production remains a problem.

Productivity decline in a long-term mono-cropped soybean field can be caused by numerous biotic and abiotic factors, all of which are interrelated, most notably in the build-up of soil pathogens and allelochemicals (Liu and Herbert, 2002; Qu and Wang, 2008). Allelochemicals are substances produced by members of one species that influence the behavior or growth of members of another species (Whittaker and Feeny, 1971). A few studies have indicated that several phenolic acids, such as 2, 4-di-tert-butylphenol and vanillic acid, are allelochemicals responsible for the shift in soil microbial communities in continuously cultivated soybeans (Colpas et al., 2003; Herrig et al., 2002; Li et al., 2010; Qu and Wang, 2008). Although these phenolic acids were detected in

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soybean tissues and root exudates, as well as their amended soil (Cesco et al., 2010; Zhang et al., 2000), the experiments were designed to study their effects on soil microorganisms involved phenolic acids at arbitrary concentrations rather than at concentrations similar to what is normally detected in the soil of mono-cropped soybean fields. At more biologically relevant concentrations, the phenolic acids acted as carbon sources, but had little effect on the soil fungi and microbial communities (Qu and Wang, 2008). Such phenolic-acid-based allelochemicals are unlikely to explain the shift in soil microbial communities in mono-cropped soybean fields, as fungal growth was stimulated more than bacterial growth (Liu and Herbert, 2002).

Soybean bio-synthesizes a wide variety of secondary metabolites in addition to phenolic acids. Isoflavones, with a backbone of 3-phenyl-1-benopyran-4-one, are one such compound and are of particular importance in interactions between roots and microorganisms, such as the establishment of rhizobium-legume symbiosis (Antunes et al., 2006; Cooper, 2007). In particular, isoflavones may induce rhizobial resistance to infections of the root by microbial pathogens (Werner, 2001, 2007). Three isoflavones daidzein (7, 4′-dihydroxyisoflavone), genistein (5, 7, 4′- trihydroxyisoflavone) and glycitein (4′, 7-dihydroxy-6-methoxyisoflavone) are found in soybean tissues and root exudates of different cultivars and during different growth stages (Graham, 1991; Pueppke et al., 1998; Shi et al., 2010). Daidzein is a ubiquitous compound identified in all cultivars, while genistein and glycitein are strongly cultivar specific (Cesco et al., 2010; Pueppke et al., 1998). Although much effort has gone into identifying isoflavones in soybean tissues and root exudates, there have been few follow-up studies on the quantification of isoflavones in the soybean rhizosphere (Cesco et al., 2010). In the past, it has been very difficult to unambiguously quantify rhizosphere isoflavones due to methodological limitations. However, the application of modern techniques, such as ultra-performance liquid chromatography (UPLC) (Eljarrat and Barcelo, 2001), allow greater effort to be directed toward quantifying isoflavones in the soybean rhizosphere.

Research on root exudates has focused on the contribution of root-derived carbon to soil microorganisms (Farrar et al., 2003; Lu et al., 2002). Less attention has been paid to the release of bio-active compounds, such as allelochemicals, from roots, and to the effect of such compounds on soil microbial communities. Root-derived allelochemicals play an essential role in interactions among soil organisms (Bais et al., 2006; Kong et al., 2008). A few studies have clearly shown that daidzein and genistein, together with other isoflavones released from soybean roots, can affect soil microbial growth and diversity, particularly fungi populations (Colpas et al., 2003; Werner, 2001).

There is a wealth of information on the role of soybean isoflavones in root-colonizing symbioses, including rhizobia, arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria (Cooper, 2007; Larose et al., 2002; Ramos-Solano et al., 2010; Sugiyama et al., 2007). However, because there is apparently less information known about isoflavone levels in the soybean rhizosphere and isoflavone interactions with the soil microbial community, further studies are justified. A few soybean varieties produce considerable amounts of isoflavones in their living roots and root exudates (Antunes et al., 2006; Graham, 1991; Pueppke et al., 1998). Therefore, the concentration of isoflavones exuded from soybean roots could be much higher locally in the rhizosphere. Under this scenario, it is likely that isoflavones would affect the soil microbial community structure. However, it needs to be clarified if microbial community structure is affected by exudation or addition of soybean isoflavones. Accordingly, a 13-year study of continuous soybean monocultures was conducted for the purpose of quantifying isoflavones in the rhizosphere by UPLC. In addition, the phospholipid fatty acid (PLFA) profiles (Zelles, 1999) of the soybean monocultures were analyzed to better understand their accompanying soil microbial communities. Furthermore, the changes in the soil microbial community structure in an incubation experiment involving the addition of two isoflavones, daidzein and genistein, at typical soybean rhizosphere concentrations were evaluated. Thus, we aimed at further enhancing the understanding of the role of rhizosphere isoflavones in the interactions between long-term mono-cropped soybeans and the corresponding soil microbial communities.

2. Materials and methods

2.1. Soybean cultivar, chemicals and field experimental site

One soybean cultivar, Hefeng-25, was used in this study. This cultivar was selected based on its commercial importance in the local soybean industry. Daidzein and genistein were obtained from Sigma–Aldrich Co. Other organic solvents and chemicals were purchased from local suppliers.

The field experimental site was located at the Experimental Station of Heilongjiang Academy of Agricultural Sciences (Harbin, Heilongjiang province, China, 47°26′ N, 126°38′ E). The experimental region represents the typical soil and climate types in Northeast China. The soil is a Luvic Phaeozem (FAO classification) with a pH of 5.6 ± 0.06, organic matter content of 23.99 ± 0.74 g kg^{-1}, total N 1.14 ± 0.05 g kg^{-1}, exchangeable K (extracted with ammonium acetate) 140.27 ± 8.22 mg kg^{-1} and Oslen-P 22.02 ± 0.40 mg kg^{-1}. As in a continental monsoon climate zone, the region is dry and cold in winter and warm and humid in summer. The experimental region has a mean annual air temperature of 4.8 °C and annual precipitation of 550 mm, of which 80% falls in July and August. Average air temperatures in January and July are −18 °C and +23 °C, respectively, and the frost-free period is between 120 and 160 days in length, with an early frost in September and late frost in May. Soybean and maize (*Zea mays*) are major crop plants in this region.

2.2. Greenhouse experiments

A series of greenhouse experiments were conducted to examine the occurrence and level of isoflavones in both soybean tissue and soil at various growth stages, and the results were used to identify an optimum growth stage for field samplings late. A randomized, complete-block design was used with 12 testing blocks and 6 replicates. Two pre-germinated soybean seeds (Hefeng-25) were planted in plastic pots (12 cm × 17 cm) containing 3200 g soils collected from the same origin as in the experimental station described above. Pots without soybean plants served as the controls. All pots were placed in a greenhouse maintained at 20 or 30 °C night and day time temperatures respectively and 65–90% relatively humidity. Pots were randomized and irrigated with tap water once a day. All pots were regularly hand-weeded during the experiments. Soybean plants were harvested separately at the seedling, pod and seed maturity stages. The tissue of the harvested plants and their rhizosphere soil were sampled as described below.

2.3. Field trials

A 13-year experiment of continuous soybean monocultures was conducted at the experimental station described above. Before the start of the continuous soybean mono-cropping experiment in 1996, the field used had been previously planted with maize for several seasons. The field was divided into numerous plots (7 m × 10 m) in a completely randomized design. Each plot was