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Soil microbial activity of faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) rhizosphere during growing season

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ABSTRACT

Plant–microorganism relationships in the rhizosphere soil are crucial for nutrient cycling, plant productivity, and health. The study was conducted to assess the effects of faba bean and wheat on enzyme activities and metabolic potential in the soil rhizosphere as well as their changes during the vegetative season. The field experiment was performed on a Haplic Luvisol. Faba bean (*Vicia faba* L.) cv. Granit (F) and spring wheat (*Triticum aestivum* L.) cv. Kandela (W) rhizosphere soils were taken for analysis three times during the vegetation. The activities of dehydrogenase, protease, urease, and acid phosphomonoesterase as well as the metabolic diversity of the microbial community were assessed. Although soil enzyme activity was related to the sampling terms, the activities of dehydrogenase, protease, and urease were higher under F than W at the later growth phases. Similarly, metabolic diversity indices were significantly greater under F than W at the flowering stage of faba bean and head emergence of wheat. Significant differences between plants and sampling terms were observed in respect to utilization of different groups of carbon substrates, especially carboxylic acids, amides, and amines. This result demonstrates higher soil microbial activity and functionality in the rhizospheres of faba bean than wheat. The plant effect on soil microbial activity increased with time. This study expands our knowledge of the ecological effects of plant species on interactions between the plant rhizosphere and soil microorganisms.

1. Introduction

During plant growth and development, diverse processes occurring in the soil adjacent to the plant roots affect the properties of the soil and, as a result, rhizosphere activity and microbiota. Among different factors (plant species and relatedness, soil chemical properties, and spatial localization) affecting the community composition in the plant rhizosphere, plant species has been regarded as the best predictor of the composition of fungal and bacterial communities (Burns et al., 2015). Plants influence soil microorganisms surrounding their roots by root morphology, excretion of exudates and other substances that can be both repellent and chemoattractant signals, and the presence of dead root cells (Badri and Vivanco, 2009; Bais et al., 2006; Bakker et al., 2012; Marschner et al., 2004; Weisskopf et al., 2006). The composition of microbial communities in the rhizosphere is affected mainly by nutrients provided by plant roots and by modification of redox gradients and pH (Schmidt et al., 2011).

Root exudates with signaling, nutritional, and antibiotic capabilities is the main factor controlling activity and biodiversity of the rhizosphere microorganisms, which subsequently stimulate or inhibit plant development and productivity. It was shown that selected root exudates enhanced to a different degree both hydrolase activities and microbial growth (Falchini et al., 2003). Study by Eisenhauer et al. (2017) reveal that plant diversity by increasing root-derived organic inputs significantly increased fungal and bacterial biomass. However, as reported recently by Steinauer et al., (2016) root exudate diversity determines soil microbial communities and function and thus is a crucial link between plant diversity and soil microorganisms.

In the plant rhizosphere, soil microorganisms play an essential role by transformation and mineralization of nutrients (Marschner et al., 2011). They affect the availability of nutrients through modification of the surrounding conditions by secretion of different molecules with chelating, solubilizing, reducing, and oxidizing activities.

Previous studies have shown that, at the same field sites, plant species or even cultivars can influence the composition of bacterial communities in the soil rhizosphere (Smalla et al., 2001). Based on the PCR-DGGE of 16S rDNA, i.e. a molecular technique that allows studying uncultivable microorganisms, Marschner et al. (2004) showed that the rhizospheres of canola, chickpea, and Sudan grass differ in terms of bacterial community structure. These differences were affected by the

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type of soil; in clay soil, the community structures of canola and Sudan grass were similar and differed from those of chickpea. PCR-DGGE analysis of the oat and common vetch rhizosphere showed differences between plants in relation to richness index, PCR-DGGE profiles and soil bacterial community structures (Qiao et al., 2012). With respect to faba bean and wheat it was shown that both, microbial biomass carbon and phosphorus, and mycorrhizal colonization of roots differ between rhizosphere of these plants (Tang et al., 2016). Plant species were found to influence significantly denitrification and nitrification activity as well as free-living bacteria with the N_2-fixation ability in grassland ecosystems (Patra et al., 2006). The study by Appuhn and Joergensen (2006) showed strong differences between plant species in terms of microbial colonization of roots. It revealed significantly higher muramic acid content of the *Fabaceae* in relation to the *Brassicaceae* and the *Poaceae* roots. Fungal carbon and bacterial carbon in rhizosphere soil were greater under *Fabaceae* than wheat (Appuhn and Joergensen, 2006).

Soil enzymes are known to play an essential role in nutrient cycling by mediating biochemical processes; they enable plant residue decomposition and release of plant available nutrients. Enzymes maintain soil health; they are vulnerable sensors of environmental changes and are used to assess the effects of soil management practices and quality (Heidari et al., 2016). Soil enzymes were reported to change with time and space and showed greater activity in plant rhizosphere in comparison with that in bulk soil (Ge et al., 2017).

Cultivation of cereals (a major source for human nutrition) requires the development of a sustainable farming system that relies more on fixed nitrogen from legumes. The cultivation area of legumes in the EU, including Poland, is low (3% and 1.5%, respectively) in comparison with the USA (15–25%). Cultivation of legumes supplies material for the manufacture of feed and food products and is associated with economic and ecological benefits for agriculture and the environment. Faba bean is an important legume crop; it occupies the third position among legumes cultivated in the EU, after pea and soybean. There is a need to increase our knowledge of the faba bean effects on soil quality in comparison with cereals. Due to the great consequence of plant–-microorganism relations in the rhizosphere soil for nutrient cycling and plant growth and health, it is essential to recognize factors affecting microbial parameters in the soil environment. However, our knowledge of the relations between plants, their developmental stages, and soil microbial activity is limited. We took this study to assess faba bean and wheat effects on enzyme activities in rhizosphere soil and metabolic potential under field conditions as well as their changes during the vegetative period. The aim of this experiment was to investigate the effect of *Fabaceae* (faba bean) on microbial communities in the rhizosphere in comparison to cereals (wheat). One model cultivar (recommended for cultivation in the study site) was selected from both species.

2. Material and methods

2.1. Site description and soil sampling

The field study was performed in Lublin, Poland (51°15′N, 22°35′E). The soil is characterized as a Haplic Luvisol (FAO 1998) derived from loess. Clay, silt, and sand contents in the 0–20 cm soil layer were 70 g kg^{-1}, 290 g kg^{-1}, and 640 g kg^{-1}, respectively, pH was 6.1 (H_2O), and organic carbon was 8.97 g kg^{-1}. According to the Kjeldahl method, total N was 0.75 g kg^{-1} and available K (determined by flame emission spectrometry (FAES) after wet sulphuric acid digestion of samples), P (determined by spectrophotometric method, measuring the intensity of the blue color of phosphate-molybdenum blue), and Mg (determined by flame atomic absorption spectrometry (FAAS)) contents were 153, 114, and 39 mg kg^{-1}, respectively, determined at the time of establishment of experiment. The soil of the experimental site was under long-term (30 years) conventional tillage. Crop rotation in the study area included cereals (wheat and barley), legumes, and root crops (sugar beets). The investigation was conducted in 2016. The average yearly air temperature and rainfall in 2016 were 9.1 °C and 726.7 mm, respectively, as measured by the meteorological station close to the experimental field. The amount of rainfall during the faba bean growing season (April–August) was 314 mm, and it was similar to the long-term average (316 mm) (Siczek et al., 2017). Faba bean (*Vicia faba* L.) cv. Granit (F) and spring wheat (*Triticum aestivum* L.) cv. Kandela (W) were used as the test crops. The plots (2 m × 3 m) were designed in three repetitions and were randomly organized.

Faba bean and wheat rhizosphere soil from the 0 to 15 cm soil layer was taken for microbial analysis. Rhizosphere soil i.e. the soil adhering tightly to the roots was collected by first shaking off the loosely adhering soil. Soil was collected three times during the plant vegetation, at the same day for both plants, at T1 corresponding to the 5–6 leaf stage of faba bean (BBCH 15-16) and stem elongation of wheat (BBCH 31), T2 – the flowering stage of faba bean (BBCH 65) and head emergence of wheat (BBCH 56), and T3 - the pod formation stage of faba bean (BBCH 80) and ripening of wheat (BBCH 89). Water content in rhizosphere soil used for analysis was 7.1, 5.0 and 10.5% (w/w) at T1, T2, and T3 under faba bean and under wheat these values were 9.2, 3.7 and 11.1%, respectively, whereas pH (in H_2O) values were respectively 6.27, 6.20 and 6.21 under F and 6.29, 6.27, 6.29 under W. Directly after sampling, the soil was sieved through a 0.2-cm mesh and the samples were used immediately for measurements or stored at 4 °C for a short period of time.

2.2. Microbial parameters

The activity of soil dehydrogenase was measured according to the Thalmann (1968) method, modified by Alef (1995), and urease activity was assayed as described by Zantua and Bremner (1977). The activity of protease was measured with the Ladd and Butler (1972) method modified by Alef and Nannipieri (1995) and the Tabatabai and Bremner (1969) method was used for acid phosphomonoesterase activity measurement. Each analysis was performed in three repetitions. The results were converted into oven-dry (105 °C) weight of soil.

The Biolog EcoPlate™ (Biolog Inc., Hayward, CA, USA) system was used for evaluation of the metabolic diversity of the microbial community (Insam, 1997). The wells on the plate were inoculated with 120 µL of the soil suspension prepared as described by Frąc et al. (2014) and were incubated at 27 °C. The absorbance values at 590 nm were noted every 12 h, and readings obtained at 48 h were used to calculate the following indices: average well color development (AWCD), Richness (R), and Shannon-Weaver index (H), following Garland and Mills (1991).

2.3. Statistical analysis

Statistical analyses were carried out with Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA, 2011) using GLM Univariate Analysis and the two effects (time and plant) were tested as independent variables. Cluster analysis was performed to group the features and treatments based on the standardized data from the average absorbance values. In order to eliminate the effects of inoculum density, the data were standardized according to AWCD (Garland, 1997).

3. Results

3.1. Enzyme activities

The results of the present study clearly indicated that the effect of plant species on soil enzymes was related to the sampling time. At T1, the protease activity was higher under W than F ($P < 0.05$) by 27%, and the other analyzed enzymes (dehydrogenase, urease and acid phosphomonoesterase) did not differ statistically between the