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Impact of faba bean (*Vicia faba* L.) cultivation on soil microbiological activity

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Abstract. Faba bean (*Vicia faba* L.) is widely grown not only as an important protein source for food and feed, but as a component in different cropping systems to improve soil quality. Beans are grown using different soil management practices, moreover, legume seeds often are inoculated before sowing. Microorganisms, introduced in the soil as an inoculum, affect not only inoculated plants, but these microorganisms can remain in the soil for the next growing season and can also affect the subsequent crops. Seed inoculation can stimulate production of root exudates as well as change microbial diversity and structure. The aim of the present study was to estimate the soil microbiological activity in soils where faba beans were cultivated with different rhizobia inoculants obtained from collection of Latvia University of Life Sciences and Technologies. Another trial was established where faba beans were included in different crop rotations under two tillage systems. During both trials, soil microbiological activity was analysed. Soil respiration intensity was measured by changes of carbon dioxide. Soil enzymatic activity was assessed by dehydrogenase activity and fluorescein diacetate (FDA) hydrolysis intensity. The total number of bacteria, fungi and rhizobia was expressed as colony forming units (CFU) g^{-1} dry soil. Soil microbiological activity depended on the cultivated crop and the crop rotation. Faba bean inoculation method had less impact on the ratio between analysed microorganism groups than on the activity of soil enzymes.

Key words: tillage, crop rotation, Rhizobium, soil enzyme.

INTRODUCTION

Intensification of soil use in agriculture has caused the fear that soil quality and sustainability is decreasing. Soil management practices influence micro- and macro-organisms, so more environmentally friendly farming practices are being introduced in agriculture. That means thinking about improving soil cultivation, crop rotation systems and cultivation techniques. Attention is being paid to the impact of these processes on the quantity and composition of soil organic matter and availability of plant nutrients (Mikanova et al., 2009).

Legumes including faba beans (*Vicia faba* L.) are widely grown not only as an important protein source, but as a component in different cropping systems to improve soil quality. Currently, the use of legumes is expanding and developing also in the areas of processing, to improve the potential for legume production. Faba bean is increasingly investigated from the environmental point of view. Growing legumes not only reduces the required mineral nitrogen consumption, thereby reduces N_2O emissions from soil caused by microbiological processes and improves the structure of soil (Jensen et al., 2010; Jensen et al., 2012; Schwenke et al., 2015). In addition, faba bean is a good pre-crop for cereals (Mikanova et al., 2009; Köpke & Nemecek, 2010). Diversity of crop species, cropping intensity, and crop rotation affect microbial activity and microorganisms diversity. Legumes effect the following crop by influencing the amount of available mineral elements, and by changing the microbiological processes. Different crops create specific environment in the rhizosphere (Thomas & Kevan, 1993). The biological processes in the rhizosphere are influenced by both the change of nutritional composition and root exudates. Most of the fixed N in legumes is harvested, but experimental data suggest that legumes can deposit significant amounts of N in the soil (Lupwayi & Kennedy, 2007), affecting the soil P dynamics and rhizosphere properties during growth (Yadav & Verma, 2014; Maltais-Landry, 2015). The root exudates can influence the structure and function of soil microorganisms' community and activity. The metabolic characteristics and the diversity of soil microbial communities are known to be sensitive to soil management and may provide information on the status and activity of the microbial community as well as the resilience of the community to stress. Additionally, the size and diversity of specific functional microbial groups, such as rhizobia, nitrifying bacteria or arbuscular mycorrhizal fungi communities also have potential to characterise the effects of management on the sustainability of soil (Bending et al., 2000; Bending et al., 2004).

Often faba bean is grown using seed inoculation with rhizobia that can affect not only symbiotic nitrogen assimilation, but also the rhizosphere processes by altering the composition of the inoculated plant exudates. Seed inoculation stimulates production of phytohormones, siderophores, and release of phenolic compounds and enzymes in the soil (Siczek & Lipiec, 2016). Soil enzyme activity is a significant indicator of soil microorganisms' diversity if they reflect changes in enzymes activity. Activity of fluorescein diacetate (FDA) hydrolase, dehydrogenase, glycosaminidase, and phosphatases characterises microbial activity in soils. These enzymes are involved with decomposition of complex organic compounds and with nitrogen mineralization, and are correlated with fungal and microbial biomass (Kandeler, 2007).

Conventional tillage may negatively affect soil microbial and biochemical properties through a reduction of soil organic matter content that provides a substrate source for soil microorganisms. A decline in water-stable macroaggregates that provide a favourable microhabitat for soil microorganisms and changes in environmental conditions such as moisture and temperature (Balota et al., 2003; Kabiri et al., 2016). Soil biological and biochemical processes may also affect the formation of symbiotic associations and their effectivity. Legume cultivation using symbiotic microorganisms instead of mineral nitrogen fertilisers is of great importance.

Faba beans as an important leguminous crop are studied mostly as feed and food source. But the interaction of faba bean with soil microorganisms is investigated mostly from the symbiotic nitrogen fixation point of view. However, little information is available about the effect of faba bean on microbiological activity in soil under different agricultural management practices. The aim of this study is to compare the number of bacteria and fungi as well as soil enzymatic activity under different soil cultivation methods for faba bean.

MATERIALS AND METHODS

The description of experimental site. A stationary field experiment was carried out at the Study and research farm 'Peterlauki' of the Latvia University of Life Sciences and Technologies. The data were obtained during the period 2014–2016. Soil microbiological activity was determined in two sets of experiments: 1) Tillage experiment, where microbiological activity was compared in faba bean trials with different tillage methods, and 2) Rhizobia experiment, where microbiological activity was compared in faba bean trials with seed inoculation. Soil agrochemical properties are characterized in Table 1.

1) *Tillage experiment*. Soil microbiological activity in faba bean sown with conventional (CT) and reduced (RT) tillage was analysed. Conventional tillage consisted of ploughing to a depth of 22–23 cm with a mouldboard plough, in contrast to minimum shallow tillage (RT) to a depth of 10–12 cm with a disc harrow. The experiment had two replications. Each plot size was 24×100 m. The soil according to WRB 2015 was a Cambic Calcisol (Aric, Bathyraptic, Episiltic, Protostagnic). An additional stationary field experiment tested different crop rotation systems. The dataset described in this paper contains only the plots with faba beans sown after grains, and compared with winter wheat plots cultivated without crop rotation.

2) *Rhizobia experiment*. Soil microbiological activity was analysed in the experimental plots where faba bean was grown with and without rhizobia inoculation. The trials were established on a field where the pre-crop was cereals and where trials with rhizobia bacteria have not been carried out for more than 10 years. The soil according to WRB 2015 was an Endocalcaric Endoabruptic Luvisol (Aric, Endoclayic, Cutanic, Hypereutric, Ochric, Endoraptic, Anosiltic, Protostagnic, Epiprotovertic). Nitrogen fertilizer was applied only to the faba bean plots without rhizobia inoculation. The experiment included variants without seed inoculation (K), without seed inoculation but with additional mineral nitrogen fertilizer (KN), and different inoculation variants a) with individual rhizobia strains (RP023 and RV407) or a mixture (R), b) with mycorrhizae inoculum (M) and mycorrhizae with additional mineral nitrogen fertilizer (MN), and c) double inoculation with rhizobia strain and mycorrhizae (RP023M, RV407M or RM).

Table 1. Soil agrochemical properties at the depth of 0–20 cm

Experiment field	pH_{KCl}	Carbonate, %	C_{org}, %	P_2O_5, mg kg^{-1}	K_2O, mg kg^{-1}
Tillage experiment	6.8	0.46	1.06	134.5	245.3
Rhizobia experiment	7.2	1.37	1.39	247.4	328.3

Soil sampling. For the *tillage experiment*, the presented results were obtained in the spring (April) and at the end of the crop vegetation period (November). Soil samples were collected after the faba beans were harvested and the next crop (winter wheat) was sown. Soil samples from the *Rhizobia experiment* were collected after the yield was harvested.

For both experimental sets, soil samples were prepared similarly – soil samples were taken from 0–20 cm of soil layer using an auger with a 2-cm diameter. A composite sample of 10–15 drillings was taken from each plot. For the analysis of soil biological activity, field-moist samples were stored in plastic bags at 4 °C.

Biological activity was determined by soil respiration intensity. Soil basal respiration was determined by placing 50 g of field-moist soil and a beaker containing 5 mL of 0.1 M KOH solution into a 500 mL glass jar; the jar was sealed and placed in the dark at 30 °C for 24 hours. Afterwards, the KOH solution was removed and titrated with 0.1 M HCl to determine the amount of CO_2 evolved with the soil microbial respiration (Pell et al., 2005). Results were compared with average soil respiration intensity from all measurements obtained from 2010 until 2014, when the soil intensity assessment was initiated.

Soil moisture was determined by drying the sample for 24 hours at 105 °C.

Soil enzymatic activity. The dehydrogenase activity (DHA) was determined as described by Kaimi et al. (2007) using iodonitrotetrazolium chloride (INT). INT is reduced by the enzyme reactions to a red formazan (INTF), the concentration of which was determined spectrophotometrically at 460 nm.

Soil fluorescein diacetate hydrolysis activity was determined according to Schnürer & Rosswall (1982). Fluorescein diacetate was added to the soil sample and incubated at 24 °C. After incubation, fluoresceine, the product of enzymatic conversion of FDA, was determined spectrophotometrically at 490 nm. All measurements of soil enzymatic activity were done in four replicates.

Soil microbiological analysis was performed in 2016 after the crop was harvested. Total number of cultivable bacteria (Nutrient agar, *Scharlau* Chemie, S.A. Spain), fungi (Czapec agar, *Scharlau* Chemie, S.A.Spain) and number of *Rhizobia* on mannitol media were determined[1].

The experimental data were analysed by two-factor analysis of variance using software *ANOVA*. The parameters were considered as significant at $P < 0.05$. In the *Rhizobia experiment*, correlation coefficients between number of microorganisms and soil microbiological activity were determined.

RESULTS AND DISCUSSION

Legumes, including faba bean, are grown not only as an important source of protein but also as a component of crop rotation. Therefore, the microbiological activity of the soil in different bean sowings was estimated. In reduced tillage, there was consistently less microbial respiration under continuous cereal than under faba bean (Fig. 1). In conventional tillage, the same trend was apparent except in April 2015 when there was no significant difference between the crops. The values under winter wheat were always below average. In both years, microbiological activity was higher in the autumn, except in the case of faba bean with reduced tillage in 2016.

1 http://www.lf.llu.lv/sites/lf/files/2017-01/Eurolegume%20D3%201%20-%20Handbook%20of%20protocols.pdf

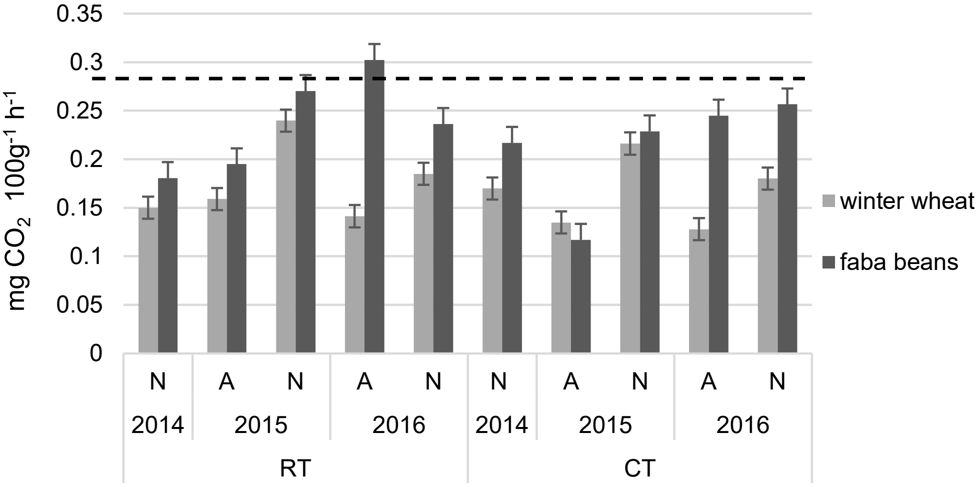

Figure 1. Soil respiration intensity in winter wheat and faba bean trials with reduced and conventional tillage: A – April; N – November; RT – reduced tillage; CT – conventional tillage; – – – – – average soil respiration intensity.

According to Copec et al. (2015), the soil treatment affects the proportion of small soil particles in the soil, which can affects soil water content, aeration, and temperature. In 2016, from the second decade of July, precipitation was 18 to 33 percent more than in 2015, which could have affected soil properties and microbial activity.

According to Kainiemi et al. (2015), there are no consistent results in the literature about tillage effect on soil microbiological activity. Some authors achieved higher fixed soil CO_2 emission in conventional tillage (La Scala et al., 2006), but Kainiemi et al. (2013) obtained higher soil respiration intensity after reduced tillage. In contrast to the results of Kainiemi et al. (2015), our results show that higher respiration intensity was in the soil collected in November (both tillages). Furthermore, soil respiration intensity is influenced by the crop rotation. Such effect was detected in conventionally tilled plots in 2016. Differences between microorganisms' activity in monoculture and crop rotation has been noticed previously. Lower soil respiration intensity in winter wheat monoculture has been related with decrease in microorganisms' biomass and diversity (Gajda & Martyniuk, 2005). In addition, Köpke & Nemecek (2010) emphasized the positive effect of faba beans on soil microbial diversity.

Activity of dehydrogenases were unstable (Fig. 2). Enzyme activity after faba bean cultivation was similar in the CT and RT soil, but in winter wheat plots significantly higher dehydrogenase activity was observed in conventionally tilled plot in 2016. In two experiment years from three raised dehydrogenase activity in conventionally tilled faba bean fields was observed. Tamm et al. (2016) noticed that more substantial dehydrogenase activity changes between soil layers were found in reduced tillage, but in conventionally tilled soil enzyme activity fluctuated less.

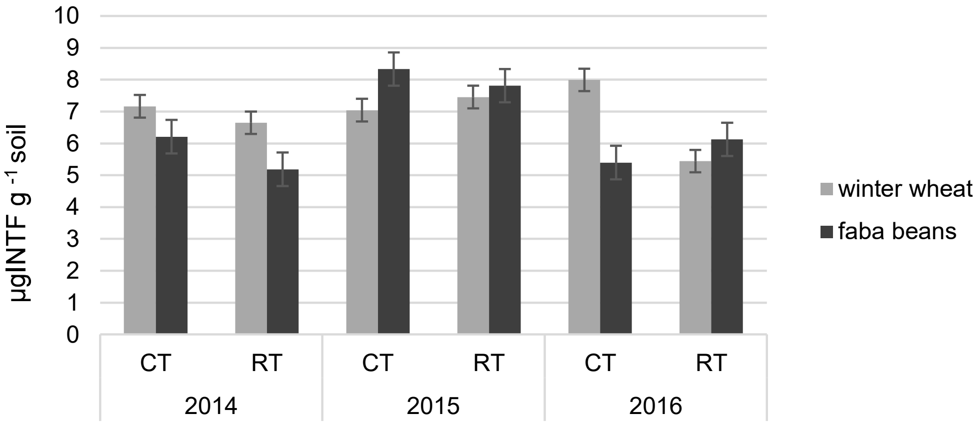

Figure 2. Dehydrogenase activity in winter wheat and faba bean trials at the end of the vegetation period: CT – convention tillage; RT – reduced tillage.

In contrast with dehydrogenase, in most cases activity of hydrolytic enzymes were higher in reduced tillage plot soils. Two thirds of the results indicated that the inclusion of faba beans in crop rotation after grains reduced the activity of hydrolytic enzymes at the end of the vegetation period. (Fig. 3).

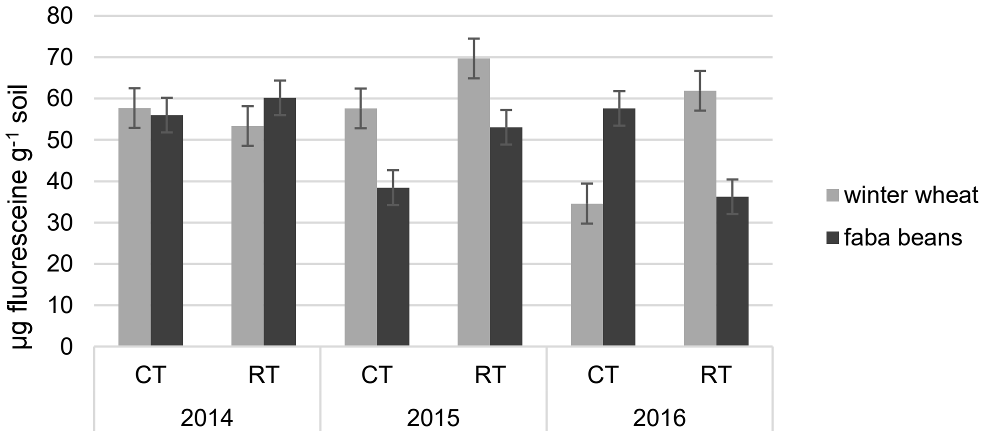

Figure 3. FDA hydrolysis intensity in winter wheat and faba bean trials at the end of vegetation period: CT – convention tillage; RT – reduced tillage.

Microbiological activity in the faba beans root zone was significantly higher at the end of vegetation period (Fig. 4). During flowering, faba bean is intensively fixing symbiotic nitrogen, and energetic processes are occurring more intensively in nodules (Hirsch, 1992; Kiers & Denison, 2008). At the end of the vegetation period, the progressive death of roots increases the carbon source for microorganisms, and a considerable amount of *Rhizobium* bacteria leave senescing nodules. The combination of these processes can also lead to higher soil respiration during this period.

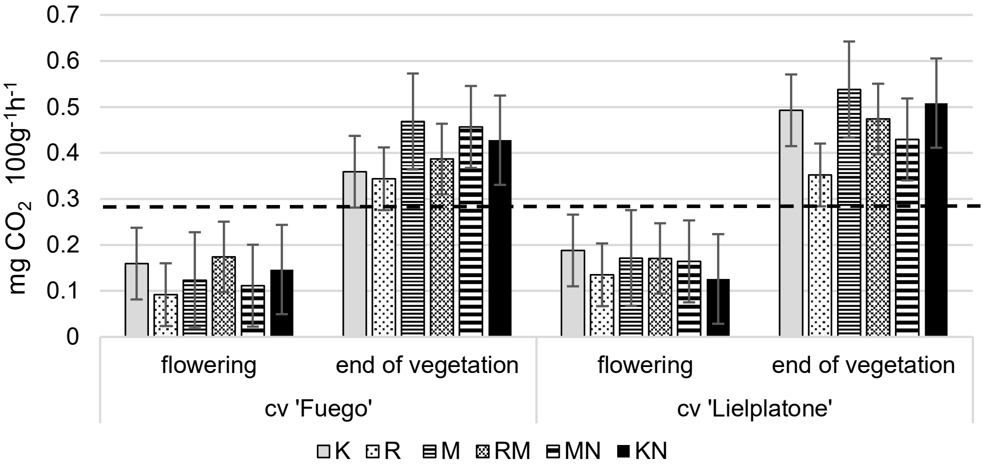

Figure 4. Soil respiration intensity in faba bean trials with different seed inoculation variants: K – control without seeds inoculation; R – inoculation with mixture of rhizobia strains RV407 and RP023; M – inoculation with mycorrhizae fungi; RM – inoculation with rhizobia bacteria and mycorhizae fungi; MN – inoculation with mycorrhizae fungi and with additional nineral nirogen fertilizer; KN - control without seeds inoculation, but with additional mineral nitrogen fertilizer; – – – – · average soil respiration intensity.

Dehydrogenase activity at the end of vegetation period varied between seed inoculation variants, but not significantly (Fig. 5). Values were lower in cv ‘Fuego’ than in cv ‘Lielplatone’. Highest dehydrogenase activity in cv ’Fuego’ was obtained with rhizobia strain RV407 and with mycorrhizae fungi, whereas the highest value in cv ’Lielplatone’ was observed with rhizobia strain RP023 followed by the double inoculation RP023M.

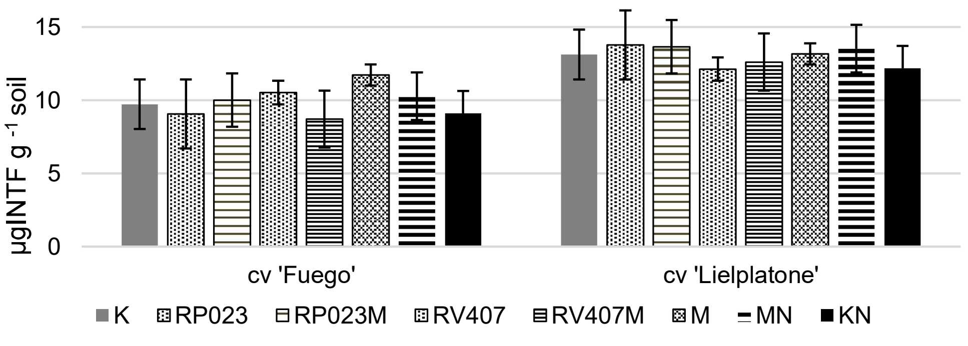

Figure 5. Comparision of DH activity in faba bean trials with different seed inoculation variants: K – control without seeds inoculation; RP023 – inoculation with rhizobia strains RP023; RV407 – inoculation with rhizobia strains RV407; M – inoculation with mycorrhizae fungi; RP023M – inoculation with rhizobia strains RP023 and mycorhizae fungi; RV407M – inoculation with mixture of rhizobia strains RV407 and mycorhizae fungi; MN – inoculation with mycorrhizae fungi and with additional nineral nirogen fertilizer; KN – control without seeds inoculation, but with additional mineral nitrogen fertilizer.

Fluorescein diacetate (FDA) results differed between inoculation variants and between cultivars (Fig. 6), with the lowest values in both cultivars from the RV407 rhizobial inoculant. For cv ‘Fuego’, the highest FDA hydrolysis intensity was observed in the treatment with both mycorrhiza and nitrogen fertilizer, but for cv ‘Lielplatone’ it was in the treatment with rhizobia strain RP023.

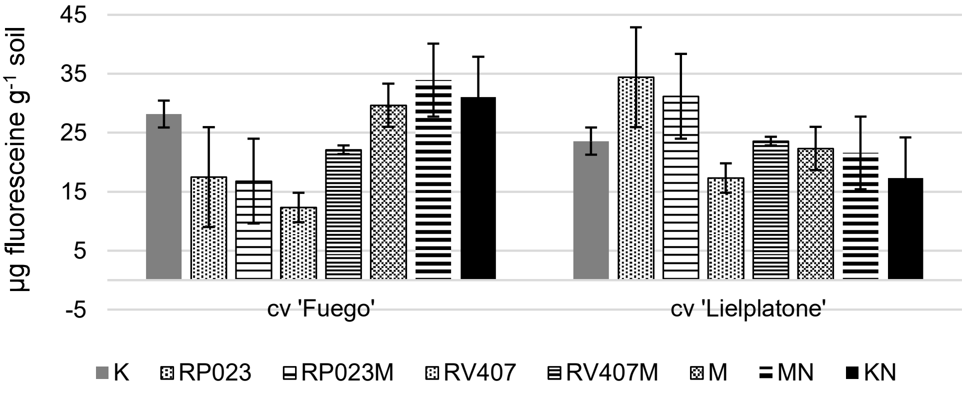

Figure 6. FDA hydrolysis intensity in faba bean trials with different seed inoculation variants Abbrevations are clarified in Fig. 5

Soil microbiological analysis showed that the number of microorganisms in the root zone was influenced more by the crop rotation and legume seed inoculation than it was by soil tillage method. Number of bacteria and fungi in the root zone of RT faba beans were about 71% and 55% higher respectively than in root zone of winter wheat, but in the CT plots 38% and 40% respectively. Differences between the number of microorganisms in RT and CT root zone were 21% and 30%, respectively.

In the rhizobia experiment, soil microbiological analysis at the end of the vegetation period showed differences between inoculation treatments. During the vegetation period, the number of rhizobia bacteria in the root zone increased by 19–32% for both faba bean cultivars. The highest increase was observed for treatments with double inoculation – for cv ’Fuego’ 32% increase, but cv ’Lielplatone’ 28%. A 2% decrease in rhizobia population was observed in mycorrhizae variant with additional nitrogen fertilizer for cv ’Lielplatone’. The total number of bacteria did not increase significantly. The highest increase was observed in variants with mycorrhizae: in the root zone of cv ‘Fuego’ by 16%, and in cv ‘Lielplatone’ by 49%. In the rhizobia experiment a significant correlation ($r = 0.43$) between total number of bacteria and dehydrogenase activity was detected.

CONCLUSIONS

Including faba bean in a crop rotation increased the number of soil microorganisms, regardless of tillage treatment.

Inoculation of bean seed influenced microorganism activity and interaction near plant roots.

Additional nitrogen fertilizer decreased the number of fungal colony forming units in the variant with mycorrhiza fungi inoculant.

Seed inoculation effectively increased rhizobia numbers.

The soil enzymatic activities were not similarly affected by different *Rhizobium* inoculation variants.

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