IMPACT OF MIXED-CULTURE CULTIVATION ON MICROBIAL ACTIVITIES IN RHIZOSPHERE SOIL

Kintl A., Elbl J., Záhora J., Hynšt J.

Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic

E-mail: antonin.kintl@mendelu.cz

ABSTRACT

The aim of this study was to describe the effect of mix culture on the microbial activity in the roots zone. Under the term of mixed culture we understand the cultivation of two different crops at same field simultaneously, in particular mixture of leguminous and non-leguminous crops. The need to cultivate these crops for improving soil fertility and sustainability is often neglected, but positive influence of legumes in crop rotation is widely recognized. This work deals with impact of mixed-culture cultivation on microbial activities in rhizosphere, because they have direct influence on leaching of nutrients from soil. Substrate Induced Respiration (SIR) and content of ammonia nitrogen in microbial biomass (INDEX of nitrogen availability) were chosen as a main indicator of microbial activities in soil. SIR and INDEX were determined in soil sample, which were removed from rhizosphere of Winter Pea (WP), Winter Wheat (Control). Moreover, soil sampling was performed from rhizosphere of Wheat and Winter Pea (W+WP), which both plants species grew together. Significantly the highest SIR was found in variant W+WP (13.6 μg CO_2-C $g^{-1}\cdot h^{-1}$) in comparison with the control. The highest content of ammonia nitrogen was found in variant W+WP (369.5 mg·kg^{-1}). Based on the results from this experiment, authors conclude that the cultivation of mixed-culture has a positive effect on microbial activity and decrease in leaching of mineral nitrogen.

Key words: winter wheat, winter pea, mineral nitrogen, microbial activity

Acknowledgments: This work was supported by the project NAZV No.: QJ1220007 and the project CZ.1.07/2.3.00/20.005.

INTRODUCTION

Intercropping can be broadly defined as a system where two or more crop species are grown in the same field at the same time during a growing season (Ofori & Stern, 1987). Moreover authors state: intercrops generally reduce the yields of the individual components from their expected yields in a monoculture; however the combined seed yield is oft en higher than the mono-crops. Leaching of nitrate nitrogen (NO_3^--N) from intensive agro-systems is a main environmental problem in many countries (Di & Cameron, 2002). The use of legumes grown in rotations or intercropping is now regarded as an alternative and sustainable way of introducing N into lower input agrosystems (Fustec et al., 2010). Mixing species in cropping systems may lead to a range of benefits that are expressed on various space and time scales, from a short-term increase in crop yield and quality, to longer-term agro-ecosystem sustainability, up to societal and ecological benefits (Malezieuxet et al., 2009). The success of intercrop farming systems depends initially on effective nitrogen fixation and more importantly, on subsequent transfer of nitrogen to the non-legume (Stern, 1993).The distance between the cereal and legume root systems is important because N is transferred through the intermingling of root systems (Fujita et al., 1992). Fustec et al. (2010) describe this transfer from legumes to the release of N compounds by legume roots, a process named rhizodeposition, then the uptake by the companion crop. Legumes are able to accumulate substantial quantities of nitrogen, and the soil's population of microbes has an enormous capacity to cycle this N in the right conditions (Jarvis et al., 1996).

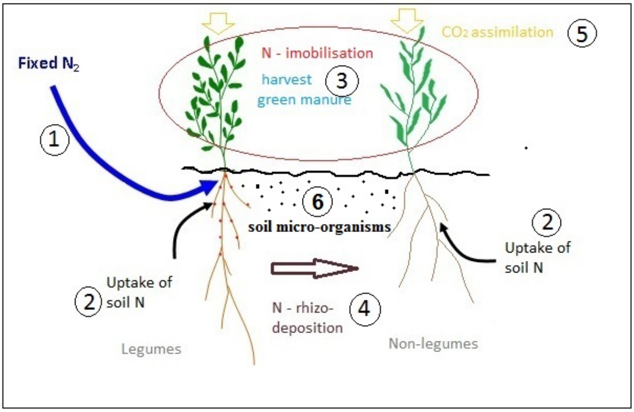

Fig. 1. Flows of nitrogen during the growing mixed culture. 1. Biological fixation of N_2. 2. Uptake from the soil Nmin. 3. Immobilization of nitrogen in plant biomass. 4. Nitrogen rhizodepozition. 5. CO2 assimilation.6. Soil micro-organisms (Schmidtke, 2008).

MATERIAL AND METHODS

Field experiment

Area of our interest is the agricultural region, which is located 8 km north of the city Prostějov. Experimental site is situated near the protection zone of underground drinking water source "Kvartér řeky Moravy". This site is located according Quitt (1975) in the climatic region T2, where annual climatic averages are of 350-400 mm in growing season and 200-300 mm in winter precipitation and 8-9 °C mean of annual air temperature. The experiment was based on the black earth, moderate, loess without skeleton (BPEJ 30100).

Two replicate 2x10 m plots per treatment were arrayed in blocked design. These variants were prepared: *Winter wheat* - 140 kg N ha^{-1} yr^{-1}. Mix culture *Winter Wheat* and *Winter Peas* without fertilization. Soil for laboratory analysis were taken in accordance with ČSN ISO 10 381-6.

Substrate Induced Respiration

Substrate Induced Respiration (SIR) was determined by measuring the CO_2 production from soils incubated in serum bottles for 4 h after the addition of glucose. Field-moist soil (5 g) was added to three replicate serum bottles as described for the determination of BR in the previous paragraph, and 2ml of a glucose solution was added to each bottle (4 mg C g^{-1} of dry soil). Bottles were sealed with butyl rubber stoppers, and soils were incubated at 25 °C. After 2 and 4 h, a 0.5 ml sample of the internal atmosphere was analyzed by gas chromatography (see previous paragraph). SIR was calculated from the CO_2 increase during the 4 h incubation period (4–2 h). The bottles were further processed as described for BR measurement. The amount of glucose amendment necessary for maximal respiratory response and linearity of CO_2 evolution during first 4 h were both checked in pilot experiments (data not shown) (Šimek 2011).

Index of nitrogen availability

Elbl et al. (2013) describe it as method for measuring the content of available nitrogen in soil. Available soil nitrogen is estimated from NH_4^+-N production during 7 day waterlogged incubation. The whole method is divided into two procedures. The first procedure is used to determine the content of NH_4^+-N before incubation. The second procedure is used to determine the content of NH_4^+-N, which is released from the microbial biomass. The contents of NH_4^+-N were performed by extraction with 2 M KCl. Extraction was realized in sealed glass containers. From each replication (control, winter wheat with field pea etc.) was collected 20 g of soil. This sample was inserted in glass containers and shook for 60 min with 2 M KCl. After shaking, the determinations of NH_4^+-N were made by distillation-titration method according Peoples et al. (1986). Elbl et al. (2013) further state: The amount of NH_4^+-N was measured after 7 day incubation in an incubator at 40 °C. Samples were prepared for incubation as follows: 50 ml of distilled water and 20 g soil sample (from each replication) were placed into of 125 ml incubation bottle. After 7 day incubation, 50 ml of 4 M KCl was added and this solution was shaked for 60 min. Subsequently, the suspension was filtered and NH_4^+-N was determined in the filtrate as above. The results were expressed in mg of in mg of NH_4^+-N kg^{-1} of soil. The index of nitrogen availability was first described by Bundy & Meisinger (1994).

Statistical analysis

Potential differences in values of respiration and index of nitrogen availability were analyzed by one-way analysis of variance (ANOVA) in combination with the Tukey´s test. All analyses were performed using Statistica 10 software.

RESULT AND DISCUSSION

The results of Substrate Induced Respiration and Index of nitrogen availability were determined during the first year of the present experiment.

Substrate Induced Respiration (SIR)

Soil samples for determination of SIR and INDEX of nitrogen availability were removed from rhizosphere of Winter Wheat (Control - C), Winter Pea (WP), Winter Wheat and Winter Pea (both plants species grew together - W+WP). Significantly the highest SIR (ANOVA, $P < 0.05$) was found in variant W+WP (13.6 μg CO_2-C $g^{-1}\cdot h^{-1}$) in comparison with the control variants.

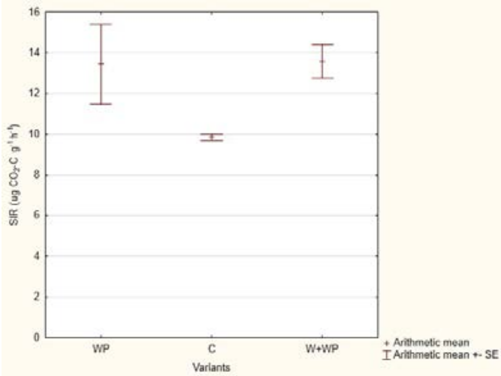

Fig. 2 Production of carbon dioxide ($\overline{x}$ from each variant; n = 3; ± σ) - SIR

SIR is proportional to active microbial biomass and its decreasing value may be attributed to gradual exhaustion of substrate for microbial growth in soil. The results of SIR confirm that the use of Winter Pea (in crop rotation) has a beneficial effect on the microbial activity in the Rhizosphere.

Index of Nitrogen availability

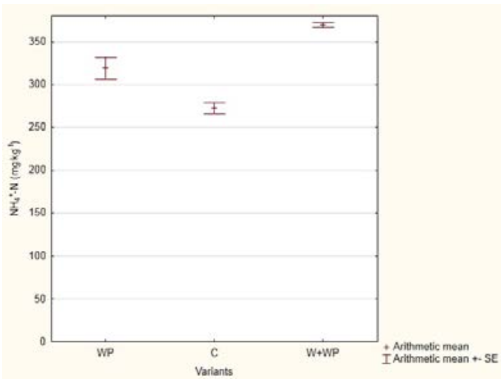

Fig. 3 Content of NH_4^+-N in microbial biomass ($\overline{x}$ from each variant; n = 3; ± σ)

The effect of use of Winter Pea (WP) in crop rotation is illustrated in Figure 3. The measured values indicated a positive effect of WP to nitrogen deposits in microbial biomass. This situation can perhaps be explained by the fact that WP in cooperation with soil microorganisms can capture nitrogen from the air. Subsequently, this nitrogen allows the development of microbial communities in soil. The highest content of NH_4^+-N was found in variant W+WP (369.5 mg·kg^{-1}).

CONCLUSIONS

This contribution presents the first results of a long-term field experiment. Therefore, these results must be interpreted with caution. Based on these results, we can conclude that the addition of Winter Pea to crop rotation has a positive effect on microbial activity in the rhizosphere soil.

ACKNOWLEDGMENT

The work was supported by the project NAZV, registration no.: QJ 1220007.

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