Combined effect of intercrop cultivation and method of fertilization on mineral nitrogen leaching: Lysimetric experiment

ANTONIN KINTL, JAKUB ELBL, LUKAS PLOSEK, JAROSLAV ZAHORA
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

antonin.kintl@mendelu.cz

Abstract: Under the term of mixed culture or intercrop, 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. Legumes have always been important crops, which have represented a source of food for humans, feed for animals and atmospheric nitrogen for soil. At present the importance of legumes is ignored, despite the fact that the cultivation of these plants brings a lot of benefits.
The aim of this study is to describe the influence of intercrop cultivation and method of fertilization on leaching of mineral nitrogen from arable soil. This effect was tested by lysimetric experiment. Six variants (C1 a C2, A1 – A4) of the experiment were prepared, each one was prepared in three repetitions. These variants were divided into two groups. First group consists of two variants: C1 (Winter Wheat - Sole crops, control without addition of fertilizers) and C2 (Winter Wheat - Sole crops, 140 kg of N per hectare and year were applied here). Second group consists of four variants A1 – A4, mixed culture of Winter Wheat (*Triticum aestivum*) and White Clover (*Trifolium repens*) were cultivated here. These variants were fertilized by mineral and organic fertilizers.
Significant differences (ANOVA; $P<0.05$) in leaching of mineral nitrogen were found between variants with intercrop (White Clover; A1-A4) and variants without (C1-C2). The significant lowest values were always found in variant A2 with intercrop. Based on these results we conclude that cultivation of intercrop/mixed culture represents new opportunity to mitigate the negative influences of extensive agriculture – leaching of mineral nitrogen.

Key-Words: winter wheat, white clover, intercrops, leaching of mineral nitrogen

Introduction
Under the term of mixed culture or intercropping 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 [1][2],[3]. 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 agroecosystem sustainability [4]. For farming systems to remain productive, it will be necessary to replenish the reserves of nutrients which are removed or lost from the soil. In the case of nitrogen (N), inputs into agricultural systems may be derived from atmospheric N_2 via biological N_2 fixation [5]. Biological nitrogen fixation is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity [6].

Crop yield depends on ability to extract sufficient amount of nutrients (especially Nitrogen) and water from soil. Uptake of nutrients and water is dependent on the availability of nutrients in rhizosphere. Nitrogen is a key element for all living organisms because it is an essential component of proteins and nucleic acids. Although the element nitrogen is extremely abundant, making up 78 % of the Earth's atmosphere, it exists mainly as unreactive di-nitrogen (N_2). By contrast, to be useable by most plants and animals, reactive nitrogen forms are needed. These include oxidized and reduced nitrogen compounds, such as nitric acid, ammonia, nitrates, ammonium and organic nitrogen compounds, each of which is normally scarce in the natural environment. The most important form of reactive nitrogen in the soil is the mineral nitrogen which is formed by nitrate and ammonium nitrogen [7]. Leaching of nitrate-N (NO_3^--N) from intensive agro-systems is a main environmental problem in many countries [8]. The use of legumes grown in rotations or intercropping is now regarded as an alternative and sustainable way of introducing N into lower input into agro-systems [9].

The nitrates are most dangerous in comparison with ammonium nitrogen (NH_4^+-N) because they are very mobile in soil. They have a negative charge and soil sorption complex has minimal affinity for negatively charged particles. Leaching of nitrate-N from arable soil is a major threat to the quality of drinking water from underground reservoirs [10].

Grain leguminous can cover cereal nitrogen demand from biological fixation of atmospheric N_2 [11][12] and therefore, they compete less for soil N_{min} in intercropping with cereals [13]. The success of intercrop farming systems depends initially on effective nitrogen fixation, and more importantly, on subsequent transfer of nitrogen to the non-legume [14]. Lupwayi & Rice [15] states that legume-based crop rotations are more sustainable crop management systems. Moreover Jarvis et al. [16] reported: “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”.

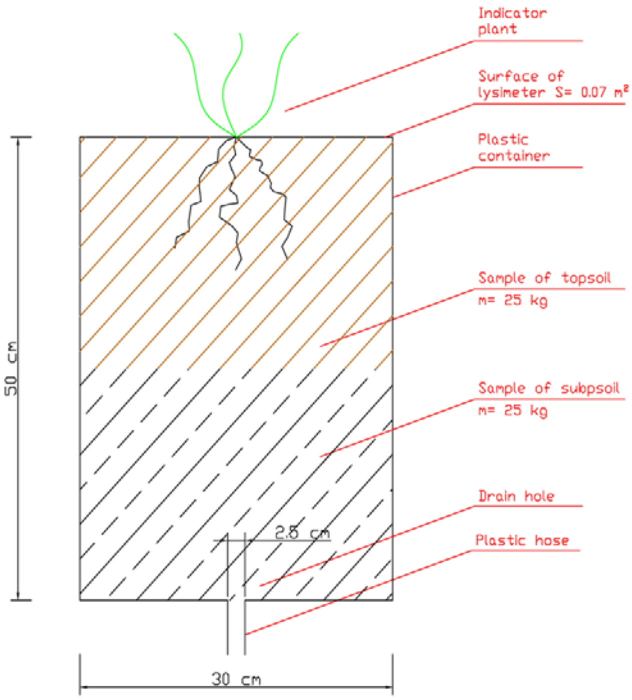
Therefore, we focused on impact of mixed culture cultivation on leaching of mineral nitrogen (N_{min}); this hypothesis was tested: cultivation of Winter Pea together with Winter Wheat has positive effect on reducing the loss of N_{min} from arable soil.

Material and Methods

Design of experiment

Lysimetric experiment was used to test the above hypotheses. Eighteen lysimeters was used as experimental containers and located in the area of our interest. Lysimetric experiment and area of our interest and was described by Elbl et al [17]: The experiment was conducted in the protection zone of underground source of drinking water Březová nad Svitavou where annual climatic averages (1962-2012) are 588.47 mm of precipitation and 7.9 °C mean of annual air temperature. The lysimeters were made from PVC (polyvinyl chloride). Each lysimeter was the same size and was filled with 25 kg of subsoil and 25 kg of topsoil (arable soil). See the Fig 1.

Fig. 1 Lysimeter – experimental container [17]



Moreover Elbl et al. [17] state that: Topsoil and subsoil were collected from a field in the area. Soil samples were sieved through a sieve (grid size of 10 mm) and homogenized. Topsoil and subsoil were prepared separately. Each Lysimeter had one drain hole and plastic hose for collecting soil solution. Hose leads into the plastic bottle. All lysimeters were buried into the ground (Fig. 2). Collection of soil solution and monitoring of the lysimeters was carried out in the control shaft. Lysimeters were completed and filled in October 2012.

Fig. 2 Location of lysimeters

Six variants (C1 a C2, A1 – A4) of the experiment were prepared. Each one was prepared in three repetitions. These variants were divided into two groups. First group consists of two variant:

- C1: Winter Wheat - Sole crops (SC), control without fertilizers
- C2: Winter Wheat - Sole crops (SC), application of 140 kg of N· ha^{-1}· yr^{-1}.

Second group consists of four variants (A1 – A4), mixed culture (IC) was cultivated there – Winter Wheat (*Triticum aestivum*) in combination with White Clover (*Trifolium repens*):

- A1: 80% of recommended doses of N for Winter Wheat.
- A2: 50% of recommended doses of N and 100 % of recommended doses of C_{org} for Winter Wheat.
- A3: 50% of recommended doses of N and 50% of recommended doses of C_{org} for Winter Wheat.
- A4 (without fertilizers).

Comment for all variants: Seeds of Winter Wheat/Winter Pea were sown mixed in the rows in the same depth on the 9th of October 2012.

Information on the applied fertilizers was published in Elbl et al [17] and [18]: Nitrogen was applied as a liquid fertilizer DAM 390. DAM 390 is a solution of ammonium nitrate and urea with an average content of 30 % nitrogen (1/4 of nitrogen is in the form of ammonium, 1/4 is in the nitrate form and ½ is in the form of urea). One hundred liters of DAM 390 contain 39 kg of nitrogen. Organic carbon (C_{org}) was applied as organic fertilizer Lignohumate B (LG B). Lignohumate is a product of chemical transformation of lignosulfonate. This material is completely transformed into the final product: solution containing 90% of humic salts (1:1 ratio of humic and fulvic acids).

Determination of mineral nitrogen leaching

Concentration of mineral nitrogen was measured in soil solution which was collected from each lysimeter into plastic bottles. These bottles were placed in the control shaft. The procedure for sampling of soil solution was described by Elbl et al. [17]: The amount of the solution was monitored three times per week. If a solution was found in a bottle, it was taken for the determination. Samples were stored at 4 °C before the determination. Moreover, Elbl et al. [17] reported that: Concentration of mineral nitrogen (N_{min}) was measured using distillation-titration method by Peoples [19]. Ammonium nitrogen was determined by distillation-titration method in an alkaline solution after the addition of MgO. Nitrate nitrogen was determined in the same manner using Devard´s alloy. Concentration of $NH4_{+}$-N and NO_3^--N was calculated according formula in Figure 3:

Fig. 3 The value of N_{min} was calculated as the sum of the detected ammonium and nitrate forms [17]

$$\text{mg } NH_4^+ \text{ or } NO_3^- - N =$$

$$\left(\frac{\text{normality of standart HCl}}{0.03571}\right) \times 0.5 \times \text{titration}$$

Statistical analysis

Potential differences in leaching of N_{min} were analyzed by the one-way analysis of variance (ANOVA) in combination with the post-hoc Tukey´s test. All analyses were performed using Statistica 10 software.

Results and Discussion

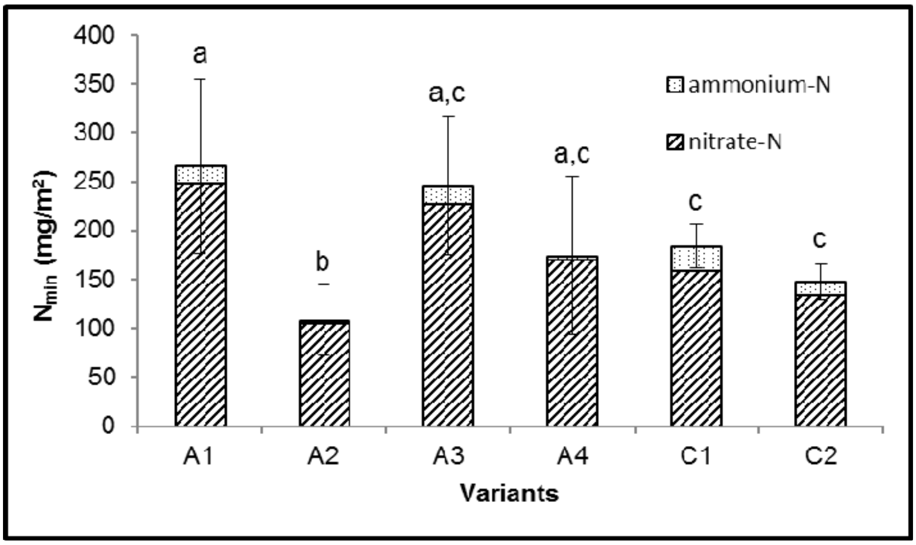
The content and quality of nitrogen in the soil is the result of soil historic development formed by natural soil forming factors and more or less by human´s activity. Therefore, every present's content of nitrogen in the soil corresponds with the present development stage of the soil and its previous commercial utilization [20].

This work presents the first results from the field/lysimetric experiment which is focused on possibilities of IC cultivation and its impact on N_{min} leaching. This experiment was established in 2012 and will continue for the next three years. The following Figures 4 – 6 present values of N_{min}

concentrations and it's both forms (ammonium-N and nitrate-N) which were measured in soil solution from January 2013 to September 2013. Moreover, Figures 5 and 6 show the loss of two most important forms of N_{min}: ammonium-N and nitrate-N.

Nitrogen is a key element for all living organisms because it is an essential component of proteins and nucleic acids. The most important form of reactive nitrogen in the soil is the mineral nitrogen which is formed by nitrate and ammonium nitrogen [7].

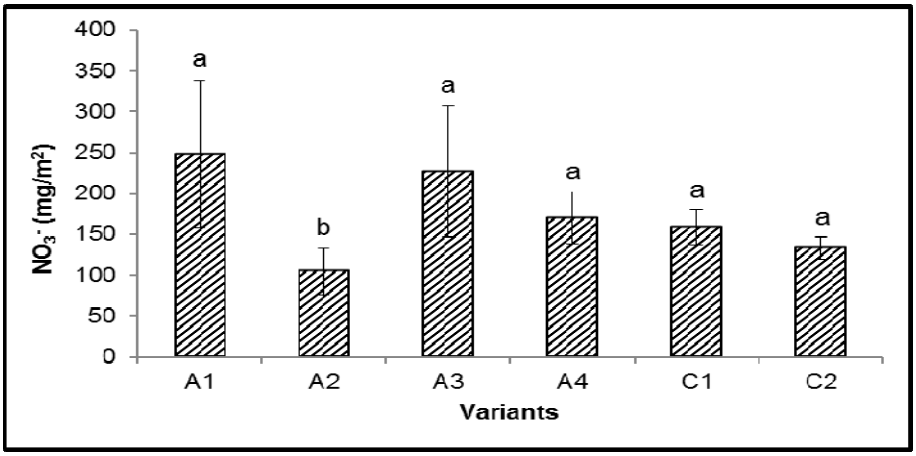
Fig. 4 Loss of N_{min} from experimental containers



Legend for Figures 5 – 7: A1 Intercrops with 80% of N; A2 Intercrops with 50% of N and 100% of LG B; A3 Intercrops with 50% of N; A4 Intercrops without addition of N or LG B; C1 Sole crops without addition of N or LG B; C2 Sole crops with 100% of N. Different small letters indicate significant differences (P<0.05) between individual variants; means values are presented n=3 with ± SD.

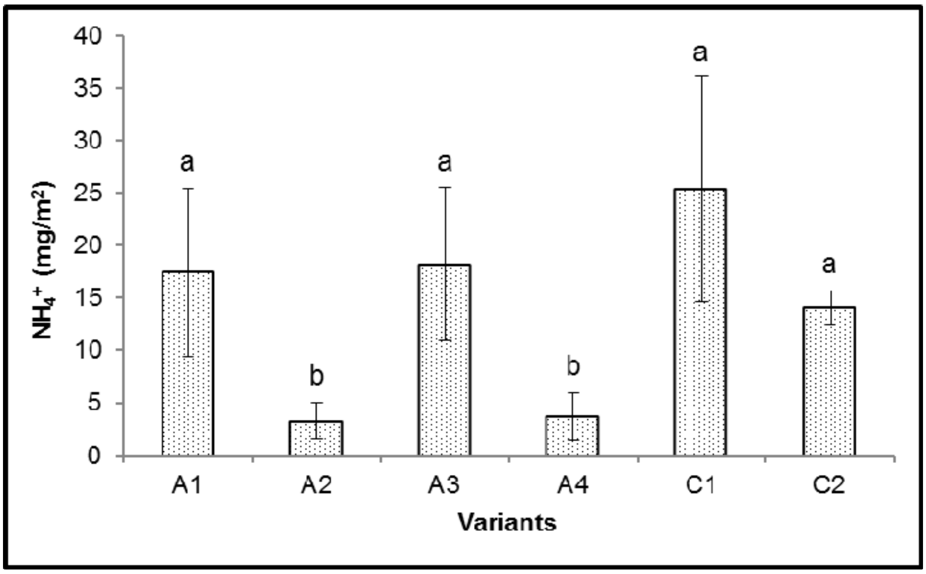
The above and following Figures (4 – 6) show significant differences between individual variant of experiment. Values of N_{min} leaching (Figures 4) indicate a positive effect of IC cultivation and reduced dose of fertilizers on loss of N_{min} from arable soil. The significant (ANOVA; $P<0.05$) lowest loss of N_{min} was found in variants A2 (108.20 mg/m^2) in comparison with variants A1; A3; A4; C1 and C2. Dubach & Russelle [21] state that legumes can transfer significant amounts of symbiotically fixed N_2 to neighbouring plants, and a putative pathway for N transfer is decomposition of fine roots and nodules. Moreover, according to Mukerji [22], legumes produce root exudates which contain a large amount of C_{org}. This fact together with application of LG B into this variant was the main reason for differences in N_{min} leaching between variant A2 and others. Impact of roots exudates from leguminous crops on soil microbial activity was confirmed by Fustec [9].

Fig. 5 Loss of N_{min} in form of nitrate-N from experimental containers



Consider values from Figures 6 and 7. The significant lowest values of ammonium-N loss were found in variants A2 (3.25 mg/m^2). We conclude that cultivation of IC and application of LG B has positive effect on soil microbial community. This community is responsible for utilization of N_{min} forms in soil. Böhme and Böhme [23] published that soil microbial biomass is fundamental for maintaining soil functions because it represents the main source of soil enzymes regulating transformation processes of elements in soils. It also controls the build-up and breaks down of organic matter, the decomposition of organic residues, and it is an early indicator of changes in soil management.

Fig. 6 Loss of N_{min} in form of ammonia-N from experimental containers



There are big differences between loss of ammonium-N and nitrate-N; the loss of nitrate-N was about 300 % higher than the loss of ammonium-N. The ammonium form of N_{min} is immobile and it may be degraded by soil microorganisms and plants. Conversely, nitrates are most dangerous in comparison with ammonium nitrogen (NH_4^+-N) because they are very mobile in soil. Elbl [10] state that nitrates are very mobile in the soil environment. Therefore, we found such great difference between these forms of nitrogen and leaching of nitrate-N from arable soil is a major threat to the quality of drinking water from underground reservoirs.

Conclusion

Our work presents first results of lysimetric experiment which was established in September 2012; therefore we must interpret these results with caution. The above results indicate that cultivation of these crops has positive influence on a decrease in leaching of mineral nitrogen. Based on these results we conclude, that cultivation of intercrop/mixed culture represents a new opportunity to mitigate the negative influences of extensive agriculture – leaching of mineral nitrogen.

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