EFFECTS OF CEREAL/LEGUME INTERCROPPING ON NITROGEN LEACHING: LYSIMETRIC FIELD EXPERIMENT

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Abstract: The paper presents a year’s summary (third year) of results of a long-term experiment which was established to examine the hypothesis that cultivation of a mixed culture of winter wheat (Triticum aestivum L.) and white clover (Trifolium repens L.) affects leaching of mineral nitrogen from the soil. The reason for the examination of this hypothesis is the increasing amount of mineral nitrogen in drinking water sources. The mixed culture or intercrop means the growing of two crops at the same time in the same place. In this case, the particular mixture consists of cereal/legume intercropping. A lysimetric field experiment was established to verify the hypothesis. Six variants were established in three repetitions. In two variants, only winter wheat with fertilisation (140 kg of N/ha/yr) and without fertilisation was grown. In the other four variants, winter wheat was grown alongside white clover (Trifolium repens L.) with the use of varying doses of mineral (DAM 390) and organic (Lignohumate B) fertilisers. Percolate leaching from the experimental lysimeters was collected and traps with ion exchange resin (IER) were placed in each container. The amount of ammonia (NH₄⁺-N) and nitrate (NO₃⁻-N) nitrogen in the water was measured regularly. The amount of trapped mineral nitrogen into the structure of from IER was also measured. A statistically significant difference (ANOVA; P < 0.05) was found between variant CY and all other variants. Of particular importance is the difference between variants CY and A1, since both were fertilised with mineral fertiliser only. From this, we can deduce that intercrop/mixed culture reduced the leaching of ammoniacal nitrogen from the system. A statistically significant difference (ANOVA; P < 0.05) was found between variant CY and A2 in the capturing of ammoniacal nitrogen (NH₄⁺-N) from traps.

Key Words: winter wheat, white clover, ground water, topsoil, subsoil

INTRODUCTION

Winter wheat is the second most important cereal in the world and plays an indispensable role in human nutrition. In recent years, modern agriculture’s methods of cultivating winter wheat have resulted in high doses of nitrogen entering into the soil through mineral nitrogenous fertilisers. Long-term application of mineral fertilisers has negative impact which leads to the deterioration of the soil. One of the consequences of using mineral fertilisers is also the leaching of mineral forms of nitrogen into ground water and subsequent contamination of drinking water sources. Field ecosystems subjected to intensive agricultural processes leach 10–40% of nitrogen from fertilisers into the ground water on loamy soil and 25–80% of nitrogen on sandy soils (Howarth et al. 1996). One of the ever more frequently discussed options of limiting the leaching of nitrogen into ground water is the use of intercrops or mixed cultures. Mixed culture in the context of this paper means the growing of two crops in one area at the same time. Currently, the predominant intercrops crown are stubble crops for green manuring. Intercrops enrich the soil with easy-to-decompose organic matter which increases the microbial activity in the soil. Organic matter from roots and aboveground portion of plants improves the physical conditions in the soil (primarily the structure of the soil), contributes to protecting the soil against water and wind erosion and allows better utilisation of rainfall in the inter-vegetational period.

Cereal/legume intercropping can be an effective strategy to reduce N leaching losses and fertiliser inputs (Mariotti et al. 2015). Positive effects on the loss of nitrogen are also presented, for instance, by
The use of legume–barley intercropping also stimulates microbial activity and as stated by (Scalise et al. 2015), increased mineral N made available in soil allows a comparable grain yield with a reduced N-fertiliser use. Aside from the positives listed, the use of mixed cultures also allows more effective use of nutrients, and the symbiotic bacteria of legumes fixate air N\(_2\) and enrich the soil with it. The main aim of the present study was to verify the hypothesis that mixed cultures of winter wheat (\textit{Triticum aestivum} L.) and white clover (\textit{Trifolium repens} L.) affect the leaching of mineral nitrogen from the soil in the sense to reduce the leaching of mineral forms of nitrogen into ground water and prevent contamination of drinking water sources with nitrogen which results from intensive agricultural activity.

**MATERIAL AND METHODS**

To confirm the hypothesis, a lysimetric experiment was established. Eighteen cylindrical PVC (polyvinyl chloride) containers of the same size were built, 50 cm tall and 30 cm wide, which were then sunk into the ground. The containers were filled with 25 kg of subsoil and 25 kg of arable topsoil. The lysimeters and the area of interest were described, inter alia, in (Elbl et al. 2013b). The arable soil used to fill the containers was taken from fields surrounding the area of interest. Soil samples were sifted through a sieve with mesh size of 10 mm and subsequently homogenised. The topsoil and subsoil were sifted and homogenised separately. All seeping water from each lysimetric container was led through a drain into a plastic pipe into a collecting plastic container. The containers were located in an inspection shaft. The inspection shaft was regularly checked several times a week and if seeping water (percolate) was found in the collecting containers, the percolate was taken for evaluation. The entire site is located in a II. class water source protection zone in Březová nad Svitavou. All the sites of the water source protection zone fall within vulnerable areas (according to the so-called Nitrate directive, implementing regulation of government regulation 262/2012 Coll.) The total of average annual rainfall in the area is 600 mm with average annual temperature of over 7.6 °C. The local soil suffers from long-term lack of organic matter which is not supplied to the soil in sufficient amounts. Fertilisation and planting of individual variants is described in (Table 1).

<table>
<thead>
<tr>
<th>Variant</th>
<th>Plant</th>
<th>Fertiliser</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY</td>
<td>Winter wheat</td>
<td>Application of 140 kg of N·ha⁻¹·yr⁻¹</td>
</tr>
<tr>
<td>CN</td>
<td>Winter wheat</td>
<td>No fertiliser</td>
</tr>
<tr>
<td>A1</td>
<td>Winter wheat + white clover</td>
<td>80% of the recommended dose of N for winter wheat.</td>
</tr>
<tr>
<td>A2</td>
<td>Winter wheat + white clover</td>
<td>50% of the recommended dose of N and 100% of the recommended dose of C(_{\text{org}}) for winter wheat.</td>
</tr>
<tr>
<td>A3</td>
<td>Winter wheat + white clover</td>
<td>50% of the recommended dose of N and 50% of the recommended dose of C(_{\text{org}}) for winter wheat.</td>
</tr>
<tr>
<td>A4</td>
<td>Winter wheat + white clover</td>
<td>No fertiliser</td>
</tr>
</tbody>
</table>

All variants were prepared in three repetitions. Variants CY and CN were thus only planted with winter wheat (\textit{Triticum aestivum} L.). Variants A1–A4 were planted with a mixed culture of winter wheat (\textit{Triticum aestivum} L.) and white clover (\textit{Trifolium repens} L.). The experimental containers were planted and fertilised every year of this long-term experiment. The composition of the fertilisers used was already published in (Elbl et al. 2013b, Kintl et al. 2014): Nitrogen was applied as liquid fertiliser DAM 390. DAM 390 is a solution of ammoniacal 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 1/2 is in the form of urea). One hundred litres of DAM 390 contain 39 kg of nitrogen. Organic carbon (C\(_{\text{org}}\)) was applied as organic fertiliser Lignohumate B (LG B). Lignohumate is a product of chemical transformation of lignosulfonate. This material is completely transformed into the final product: a solution containing 90% of humic salts (1 : 1 ratio of humic and fulvic acids). Two 15 cm long nylon stocking traps with ion exchange resin (IER) were installed in the soil in each experimental container. IER traps were prepared
as a nylon stockings from Uhelon filled with IER. Uhelon is a thick nylon mesh woven from polyamide fibres using technology which ensures the anchoring of the individual fibres in the mesh, thus providing constant mesh size. Both probes were filled with IER. One trap was filled with IER (CER type C100E), the other with IER (AER type A520E) made by company Purolite. The purpose of these was to collect the ammoniac (NH$_4^+$-N) and nitrate (NO$_3^-$-N) nitrogen leaching from the system.

**Determination of Mineral Nitrogen**

In the event that the collecting containers located in the inspection shaft contained water seeping from the experimental containers, the water was taken for determining mineral nitrogen content (NH$_4^+$-N and NO$_3^-$-N). The amount of percolate from each collecting container was measured separately, and a sample was taken. IER traps were dried at laboratory temperature. The IE resins were then transferred to plastic containers. Ammoniacal (NH$_4^+$-N) a nitrate (NO$_3^-$-N) nitrogen was extracted from the structure of the IER using concentrated NaCl solution (10%). The containers with an exact amount of NaCl added were then shaken for one hour in a laboratory shaker so that the desired extraction occurs. The amount of mineral nitrogen in the individual extracts from the traps, as well as from the leaching water collected from the experimental containers was determined using a distillation-titration method in accordance with (Peoples et al. 1989). Distillation was performed on a Behr S3 device and titration on automatic burette Titronic 96.

**Statistical Analysis**

All results were analysed using Statistica 12 software. Potential differences in the results were analysed using single-factor ANOVA analysis in combination with the post-hoc Tukey’s test.

**RESULTS AND DISCUSSION**

The study presents the results of the last year of measurements from an experiment initiated in 2012. The measurements are focused primarily on the ammoniacal and nitrate form of nitrogen, since these forms of mineral nitrogen have the greatest value for plants. Ammoniacal nitrogen is not very mobile in the soil profile, while nitrate nitrogen is very mobile. Nitrate nitrogen can therefore endanger the quality of the drinking water due to its leaching from the fields into the groundwater (Elbl et al. 2013).

Figure 1 shows the amount of ammoniacal nitrogen recalculated for one m$^2$, as measured from seeping water captured in collecting containers located in the shaft. A statistically significant difference (ANOVA; P < 0.05) was found between variant CY and all other variants. No other statistically significant differences were found. Of particular importance is the difference between variants CY and A1, since both were fertilised with mineral fertiliser only. From this, we can deduce that intercrop/mixed culture reduced the leaching of ammoniacal nitrogen from the system.

Figure 2 shows the amount of nitrate nitrogen recalculated for one m$^2$, as measured from seeping water captured in collecting containers located in the shaft. A statistically significant difference (ANOVA; P < 0.05) was found between variants CY and A2, A2 and A1, A4 and A1. Especially important is the difference between variants A2 and CY. The application of organic carbon in the form of LG B in variant A2 along with intercrop/mixed culture cultivation likely encouraged microbial activity in the soil. The differences between variants A2 and A1 show that the difference in mineral nitrogen leaching was caused primarily by the application of LG B in variant A2. The positive effect of intercropping on leaching of mineral nitrogen was presented by (Szumigalski and Van Acker 2006, Pappa et al. 2011).

Figure 3 shows the graph of ammoniacal (NH$_4^+$-N) and nitrate (NO$_3^-$-N) nitrogen captured from traps located in the soil. A statistically significant difference (ANOVA; P < 0.05) was found between variant CY and A2 in the capturing of ammoniacal nitrogen (NH$_4^+$-N). In variant CY, the amount of ammoniacal nitrogen captured was also higher compared to all other variants, but the difference is no longer statistically demonstrable, though it can be observed in the graph. Compared to other variants, the microorganisms as well as the cultivated plants were no longer capable of immobilising ammoniacal nitrogen efficiently in variant CY. The process of mineralisation was likely predominant. This may indicate that the microorganisms and plant communities present began to lose the ability to efficiently...
use the hard-to-access nitrogen. These results are in line with the first year of measurements (Kintl et al. 2014) and with other results which have yet to be published.

**Figure 1 Graph of the amount of ammoniac nitrogen**


**Figure 2 Graph of the amount of nitrate nitrogen**

CONCLUSION

The study presents the results from one year of measurements in a long-term experiment. However, alongside the already published results, the present study points to the possibility of using intercropping/mixed culture cultivation as a promising method of reducing the leaching of mineral forms of nitrogen from the soil. Of particular importance is the difference between variants CY and A1, since both were fertilised with mineral fertiliser only. From this, we can deduce that intercrop/mixed culture reduced the leaching of ammoniacal nitrogen from the system. This is particularly important for areas where mineral nitrogen passes from the soil into the ground water and contaminates the drinking water supplies of the population, which currently presents a significant environmental problem. This also applies to the area of interest in Březová nad Svitavou, where the experiment was performed and which serves as a source of drinking water for the city of Brno.

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REFERENCES


