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The influence of biological preparations Azofix and Maxprolin and nitrogen fertilisation on soil mineral nitrogen content in growing season and after spring wheat harvest

Wpływ preparatów biologicznych Azofix i Maxprolin oraz nawożenia azotem na zawartość azotu mineralnego w glebie w okresie intensywnego wzrostu i po zbiorze pszenicy jarej

Summary. The objective of the research reported here was to determine the effect of Azofix and Maxprolin against nitrogen fertiliser on mineral nitrogen content in the soil during the period of intensive growth of spring wheat plants and after its harvest. The following two factors were chosen: I. Biological products: control, Azofix, Maxprolin, Azofix + Maxprolin; II. Nitrogen fertilisation: non-fertilised control, 60 kg N ha^{-1}, 90 kg N ha^{-1}, 90 kg N ha^{-1} + foliar fertilisation. During the period of intensive growth of spring wheat plants and after harvesting, mineral nitrogen content in the soil was determined. The research demonstrated that, during the period of their intensive growth, spring wheat plants had access to the largest amount of mineral nitrogen in the topsoil following treatment with Azofix + Maxprolin and an application of the nitrogen rate of 90 kg N ha^{-1}.

Key words: *Azotobacter vinelandii* bacteria, L-alpha proline amino acid, nitrogen fertilisation

INTRODUCTION

When fertilising spring wheat grown in sustainable agriculture, care should be taken to protect the soil environment using conventional and biological methods. Compared to intensive mineral nitrogen fertilisation, its partial replacement with nitrogen biologically bound by bacteria reduces the risk of mineral nitrogen leaching into the soil profile [Bargaz et al. 2018], surface runoff, and volatilisation or N-gas emissions [Ladha et al. 2016]. Strains of *Azotobacter*, *Rizobium* or *Azospirillum* bacteria are a source of biologically fixed nitrogen for crops, including cereals [Wani et al. 2016]. On the other hand, up to 50% of conventional N-based fertilisers is subject to loss into the soil and the environment [Singh et al. 2014, 2015]. This could substantially impact economic and environmental issues such as increasing greenhouse gas emissions (e.g.: volatilisation of nitrous oxides accounts for an approximately 10-fold higher emission of CO_2-equivalent), soil acidification, depletion of non-renewable resources and nitrate leaching into the groundwater and surface water, which can produce devastating effects such as water eutrophication. Thus, there is an urgent need to introduce more sustainable use of N fertiliser in order to meet agriculture sustainability challenges such as better crop nutrition and productivity necessary to feed the ever-increasing world population. Nitrogen bacteria reduce molecular nitrogen levels in the air, and use the element to build proteins in their bodies. After their death, the protein decomposes into mineral nitrogen forms which become available to crops such as spring wheat, hence the name nitrogen biologically bound by soil microorganisms. Nitrogen bacteria are a source of biological nitrogen for plants and they also improve the chemical, physical and biological properties of soil, a fact which is attracting increasing attention at present [Bargaz et al. 2018]. *Azotobacter* bacteria delay nitrification and increase soil fertility, supplement mineral nitrogen fertilisation, colonise the rhizosphere, transform important nutrients enhancing plant growth, increase the number and biological activity of beneficial soil microorganisms [Singh et al. 2014, 2015]. Microorganisms convert complex organic matter into simple ingredients that the plant can easily take up, and increase root profiling by releasing growth-stimulating hormones. The studies by Sumbul et al. [2020] have shown that *Azotobacter vinelandii* are the most effective bacteria in terms of fixing molecular nitrogen from the air, and providing crops with mineral nitrogen. According to Gauri et al. [2012] and Phyu et al. [2019], *Azotobacter* is the best form of bio-fertiliser used in next-generation agriculture. However, bio-fertilisers cannot completely replace mineral fertilisers – they can only supplement them. The role of biostimulants is to control and enhance plant life processes, increase resistance to stress, and stimulate root and leaf development [Popko et al. 2018]. Moreover, they stimulate plant activity and chlorophyll synthesis. Therefore, the use of ready-to-absorb amino acids allows plants to save energy and increase their growth rate. They also improve bacteria proliferation. Additionally, the use of the amino acid L-alpha proline has a positive effect on the growth and development of spring wheat plants. As a result, the plants take up more nitrogen from the soil environment, which reduces mineral nitrogen leaching into the soil profile [Rutkowska 2014]. Limiting mineral nitrogen fertilisation and introducing microorganisms into the soil ensure continuous access of plant nutrients, including nitrogen for wheat, can reduce soil contamination [Ladha et al. 2016, Aasfar et al. 2021]. The combined use of lower mineral nitrogen fertiliser rates and nitrogen biologically fixed by bacteria results in a constant supply of nitrogen for plants throughout the growing season, hence the need to conduct this type of research. The aim of the research reported here was to determine the effect of Azofix and Maxprolin against nitrogen fertiliser on mineral nitrogen content in the soil during the period of intensive growth of spring wheat plants and after the cereal harvest. It will also make it possible to choose the combinations which result in the highest mineral nitrogen contents within 30 days following an application of biological products.

MATERIALS AND METHODS

Field research was carried out from 2017 to 2019 on a family-owned farm located in Krzymosze (52°03'27"N, 22°33'74"E) near Siedlce, Poland. The experimental soil was Stagnic Luvisol. It was brown soil, a type of fawn soil, made of strong clay sand. Soil samples were taken from a soil layer of 0–30 cm. In spring, prior to the experiment set-up, the following contents were determined: $–NH_4^+$, 4.97 mg kg^{-1} soil, $N\text{-}NO_3^-$, 7.84 mg kg^{-1} soil, and the available forms of P, 0.82 mg kg^{-1} soil (spectrophometric method), and K, 1.87 mg kg^{-1} soil (flame photometry method). Soil reaction was neutral (pH in KCl 6.2) – determinations were made by potentiometry using a pH-meter – and humus content was 18.8 g kg^{-1} as determined by the Tiurin method. The experiment was arranged as a split-block design with three replicates, the plot size was 20 m^2, and 16 m^2 for harvest. It was a system of perpendicular strips. Two factors were examined:

I. Biological products: non-treated control, Azofix 1 dm^3 ha^{-1} + 250 dm^3 water, Maxprolin 2 g ha^{-1} + 250 dm^3 water, Azofix 1 dm^3 ha^{-1} + 250 dm^3 water + Maxprolin 2 g ha^{-1} + 250 dm^3 water;

II. Nitrogen fertilisation: non-fertilised control, 60 kg N ha^{-1} (preplant), 90 kg N ha^{-1} (60 kg N ha^{-1} preplant + 30 kg N ha^{-1} at the stem elongation stage – BBCH 31), 90 kg N ha^{-1} + foliar fertilisation (60 kg N ha^{-1} preplant + 30 kg N ha^{-1} at the stem elongation stage + 30 kg N ha^{-1} – foliar application of 8% urea solution at the stage of initial ear formation – BBCH 49).

Nitrogen fertilisation was applied in the form of 34% ammonium nitrate. Spring wheat cv. Mandaryna was preceded by maize. Phosphorus and potassium fertiliser rates were chosen based on soil availability and amounted to 30.8 and 99.6 kg ha^{-1} of P and K, respectively. Nitrogen rates were as described for factor II above. Spring wheat was sown in early April at the rate of 500 grains per 1 m^2. *Azotobacter vinelandii* bacteria were used in the form of Azofix, and the amino acid L-alpha proline in the form of the Maxprolin biostimulator. The biological products were sprayed with a knapsack sprayer at the stage of spring wheat tillering (BBCH 25) to an area of 104 m^2 (26 m^2 × 4). A strip included 4 combinations of mineral nitrogen fertilisation and 3 paths of 2 m in lenght. Azofix was used at the rate of 10.4 ml per 2.6 l of water whereas the rate of the biostimulator Maxprolin was 20.8 mg after dissolving 10 ml of water taken from a dose of 2.6 l and then added to the remaining amount of water. Before application by means of a knapsack sprayer, the solution was thoroughly mixed in a foliar sprayer. In the plots to which the mixed combination was assigned, Azofix was the first product sprayed and followed by Maxprolin. Biological preparation (Azofix) contains the *Azotobacter vinelandii* MVY-010 (1x10 CFU/l) and micro-elements Mn, Fe, Cu, Mo, Zn, Co and B-group vitamins: B1, B3, B6 (max. 0.02%), and it is a biological product intended for increasing the nitrogen content in soil. It contains the non-symbiotic, free-living soil bacterium *Azotobacter vinelandii*, which effectively assimilates the atmospheric nitrogen and extracts bioactive substances that improve the development of plants, as well as polysaccharide alginates having influence on the formation of water-resistant units in soil. Biological preparation (Maxprolin) contains L-aproline (purity 99.5%), a biostimulator increasing the natural resistance of plants to stress. Both biological preparations should be dissolved in water and sprayed with 250 dm^3 ha^{-1} of water. The distributor of these biological preparations in Poland is PHU Biotel Sp. zoo. Dzikowice 87, 67-300 Szprotawa. During the period of intensive spring wheat growth (BBCH 50), that is 30 days following an application of biological products and after spring wheat harvest, soil samples were collected from two soil layers: 0–30 and 30–60 cm to determine mineral nitrogen content. The ammonium and nitrate contents were determined by colorimetric method [Fotyma 1996]. Soil samples for the determination of mineral nitrogen were collected in 2017 on June 19 and August 7; in 2018 on June 21 and August 11, and in 2019 on June 20 and August 9. The content of mineral nitrogen in the soil was determined at the Chemical and Agricultural Station in Warsaw Wesoła. In the work, the results of the research are presented as three-year averages. Each characteristic tested was subjected to analysis of variance suitable for the split-block design. When significant sources of variation were confirmed, relevant means were separated using Tukey test. Calculations were performed in MS Excel 12.0.

Table 1. Weather conditions in spring wheat growing seasons according to the Zawady Meteorological Station

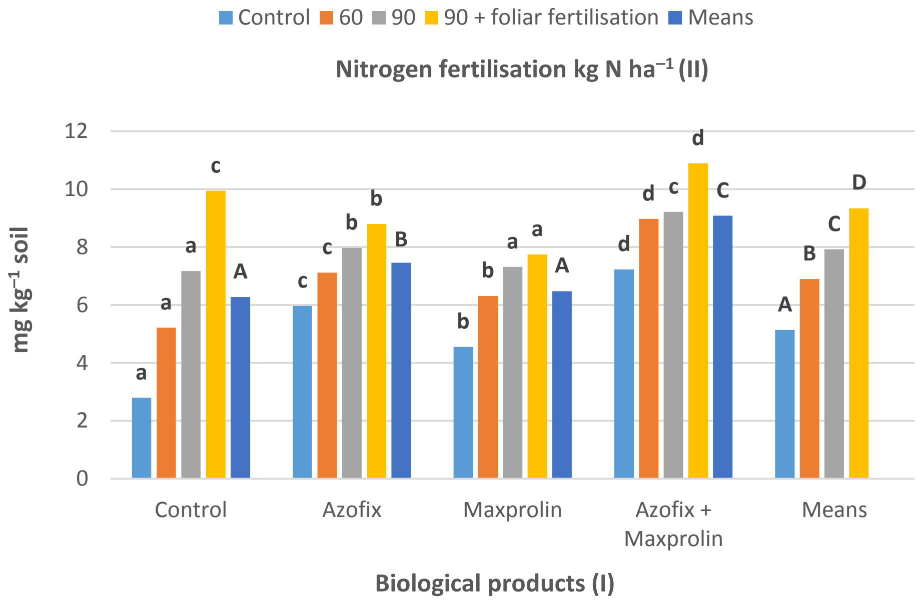
Years	Month					Mean
	IV	V	VI	VII	VIII	
Mean air temperature (°C)						
2017	6.9	13.9	17.8	16.9	18.4	14.8
2018	13.1	17.0	18.3	20.4	20.6	17.9
2019	9.8	13.3	17.9	18.5	19.9	15.9
Long-term (15 yr) mean	8.2	14.2	17.6	19.7	19.1	15.8
Rainfall sum (mm)						
2017	59.6	49.5	57.9	23.6	54.7	245.3
2018	34.5	27.3	31.5	67.1	24.5	184.9
2019	5.9	59.8	35.9	29.7	43.9	175.2
Long-term (15 yr) mean	37.4	47.1	48.1	65.5	43.5	241.6

Weather conditions in the study years were changeable (Tab. 1). The most favorable year for the cultivation of spring wheat was 2017, when the highest amount of rainfall was recorded. Worse weather conditions were recorded in 2018, with a lower total precipitation and an average air temperature higher than the long-term average. The highest rainfall shortage was recorded in 2019. The average air temperature oscillated around the long-term average.

RESULTS

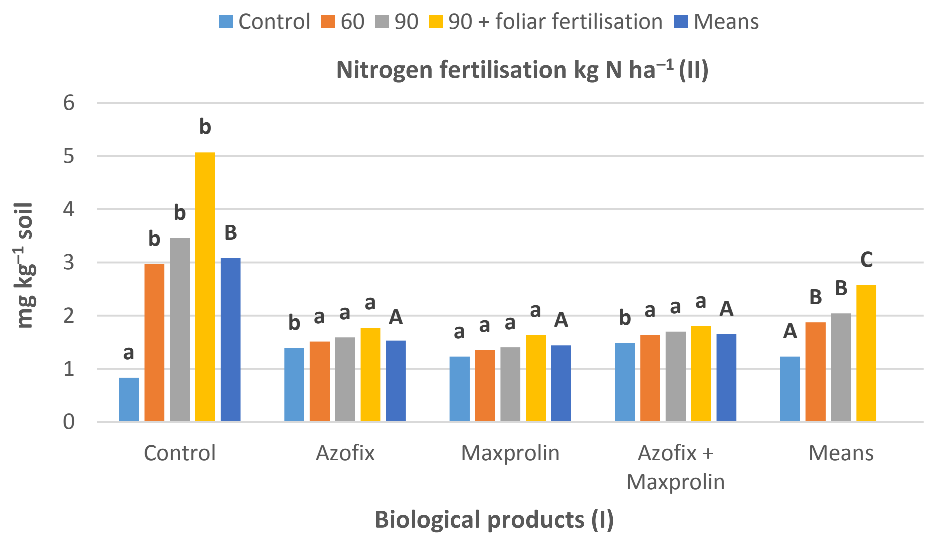
1.1. N-NH_4^+ content in the 0–30 and 30–60 cm soil layers 30 days after an application of biological products

Compared with control fertilised with mineral nitrogen only, an application of biological products contributed to a significant increase in N-NH_4^+ content in the topsoil, the content being significantly lower in the subsoil (Figs 1 and 2). The highest N-NH_4^+ content



Values in biological products for the interaction (I × II) followed by the same small letter (a, b) do not differ significantly at $p < 0.05$. Means followed by the same capital letter (A, B) do not differ significantly at $p < 0.05$.

Fig. 1. NH_4^+ content in the 0–30 cm soil layer 30 days after an application of biological products (means across 2017–2019)



Explanation as in Fig. 1

Fig. 2. NH_4^+ content in the 30–60 cm soil layer 30 days after an application of biological products (means across 2017–2019)

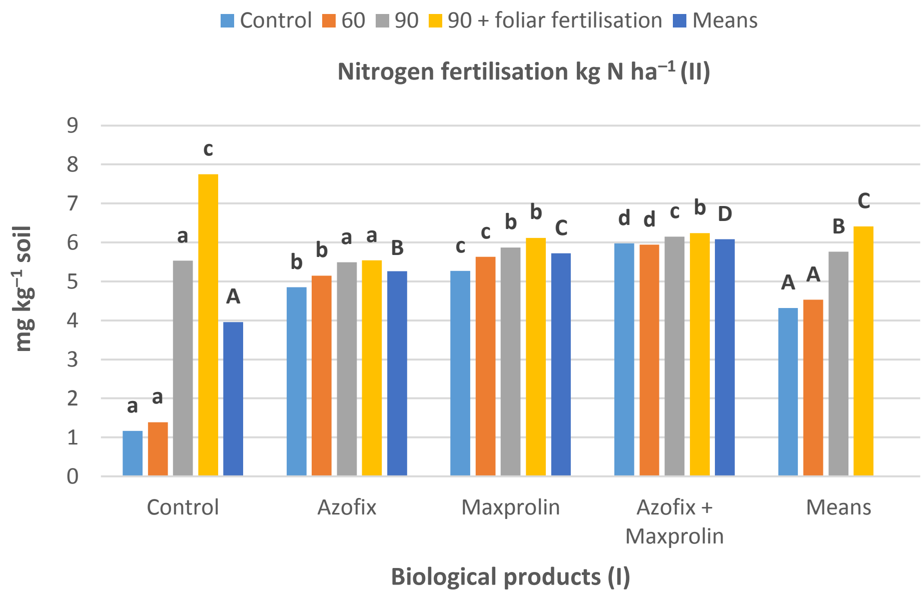
in the 0‒30 cm soil layer was due to an application of Azofix + Maxprolin. $N\text{-}NH_4^+$ content was significantly lower in the soil treated with L-alpha proline amino acid, it being at the same level as in the control unit where only mineral nitrogen had been applied. Mineral nitrogen fertilisation significantly influenced $N\text{-}NH_4^+$ content in two soil layers, it being higher in the topsoil compared with the subsoil. The lowest $N\text{-}NH_4^+$ content was recorded in the control unit unamended with mineral nitrogen. Increasing mineral nitrogen rates contributed to an increase in the soil content of $N\text{-}NH_4^+$. The highest concentration of this component was recorded following an application of the highest mineral nitrogen rate, that is 90 kg N ha^{-1} + foliar fertilisation. An interaction was confirmed indicating that the highest $N\text{-}NH_4^+$ content in the 0–30 cm soil layer was determined in plots treated with Azofix + Maxprolin and fertilised with the rate of 90 kg N ha^{-1} + foliar spraying, and in the 30–60 cm soil layer of the control unit fertilised with the rate of 90 kg N ha^{-1} + foliar application. This indicates that $N\text{-}NH_4^+$ was transferred into deeper soil strata compared with the unit additionally treated with biological products which are environmentally-friendly. In turn, the lowest $N\text{-}NH_4^+$ content in the topsoil and subsoil was found in the non-N fertilised control unit where no biological products had been applied.

1.2. $N\text{-}NH_4^+$ content in the 0–30 and 30–60 cm soil layers after spring wheat harvest

An application of biological products resulted in a significant increase in the topsoil content of $N\text{-}NH_4^+$, it being reduced in the subsoil (Figs 3 and 4). The highest $N\text{-}NH_4^+$ content in the 0–30 cm soil layer was recorded following a combined application of Azofix + Maxprolin, it being lower in the remaining units treated with the biological products, and the lowest in the control unit fertilised with mineral nitrogen. In the subsoil, the relationship was reversed ‒ the lowest $N\text{-}NH_4^+$ content was determined in plots treated with biological products, it being the highest in the control unit fertilised with mineral nitrogen. Mineral nitrogen fertilisation significantly affected $N\text{-}NH_4^+$ content in two soil layers. Increasing mineral nitrogen rates contributed to an increase in $N\text{-}NH_4^+$ content in the 0–30 and 30–60 cm soil layers, a lower content being recorded in the subsoil. An interaction was found indicating that $N\text{-}NH_4^+$ content was the highest in the control topsoil and subsoil after the rate of 90 kg N ha^{-1} + foilar spraying had been applied, which is indicative of extensive leaching of this component. The lowest concentration of $N\text{-}NH_4^+$ in the topsoil was determined in the control unit which was not fertilised or amended with 60 kg N ha^{-1} whereas for the subsoil such result was associated with an application of biological products which prevent mineral nitrogen leaching.

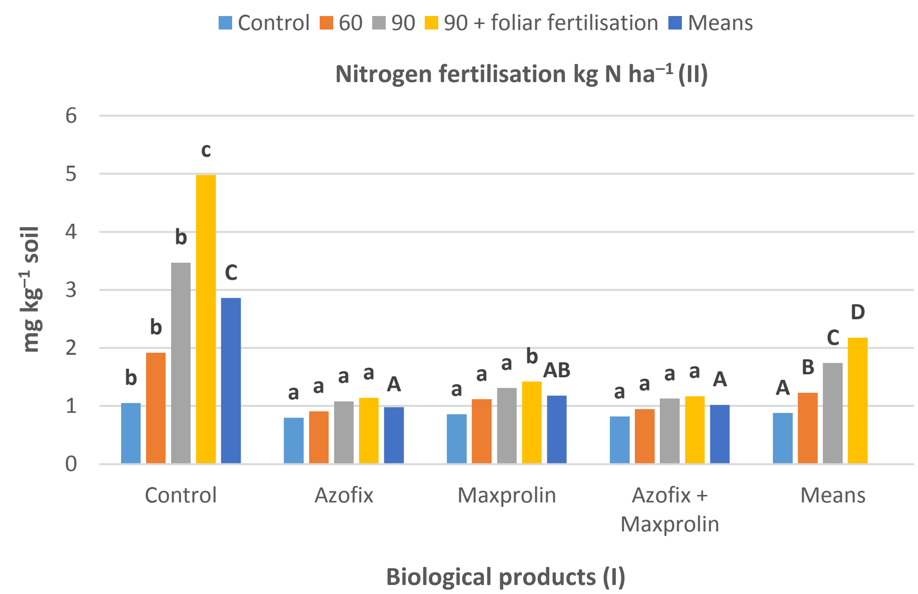
1.3. $N\text{-}NO_3^-$ content in the 0–30 and 30–60 cm soil layers 30 days after an application of biological products

The highest $N\text{-}NO_3^-$ content in the topsoil was recorded in the unit where a combination of two biological products (Azofix + Maxprolin) had been applied (Fig. 5). A high $N\text{-}NO_3^-$ content was also determined following treatment with Azofix. By contrast, an application of Maxprolin resulted in the lowest $N\text{-}NO_3^-$ content in the 0‒30 cm soil layer, it being even lower than for the control fertilised with mineral nitrogen. The reverse was observed for the $N\text{-}NO_3^-$ content in the 30–60 soil layer (Fig. 6). The highest $N\text{-}NO_3^-$ concentration was recorded in the control unit where only mineral nitrogen had been applied. An application of biological products significantly reduced $N\text{-}NO_3^-$ leaching into the subsoil. The lowest $N\text{-}NO_3^-$ content was determined in the unit treated with either



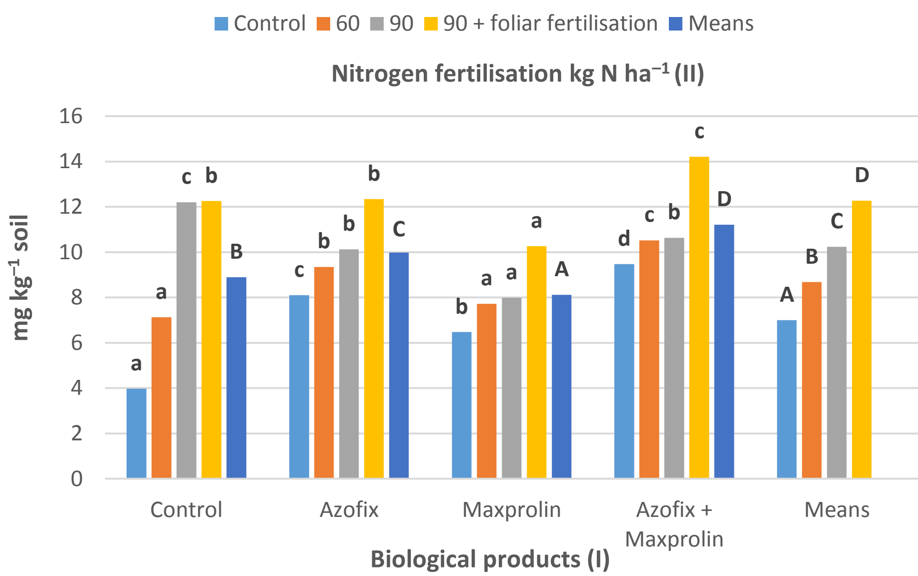
Explanation as in Fig. 1

Fig. 3. N-NH_4^+ content in the 0–30 cm soil layer after spring wheat harvest (means across 2017–2019)



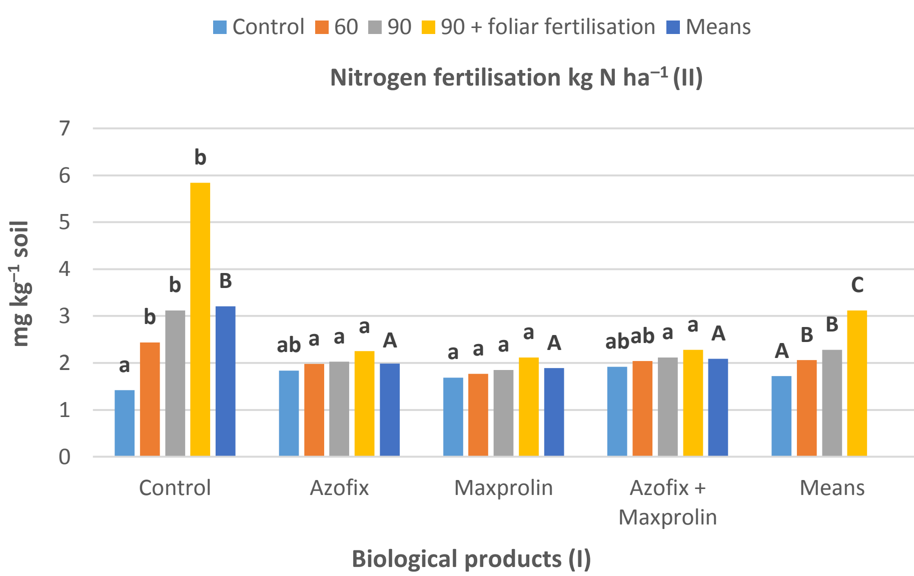
Explanation as in Fig. 1

Fig. 4. N-NH_4^+ content in the 30–60 cm soil layer after spring wheat harvest (means across 2017–2019)



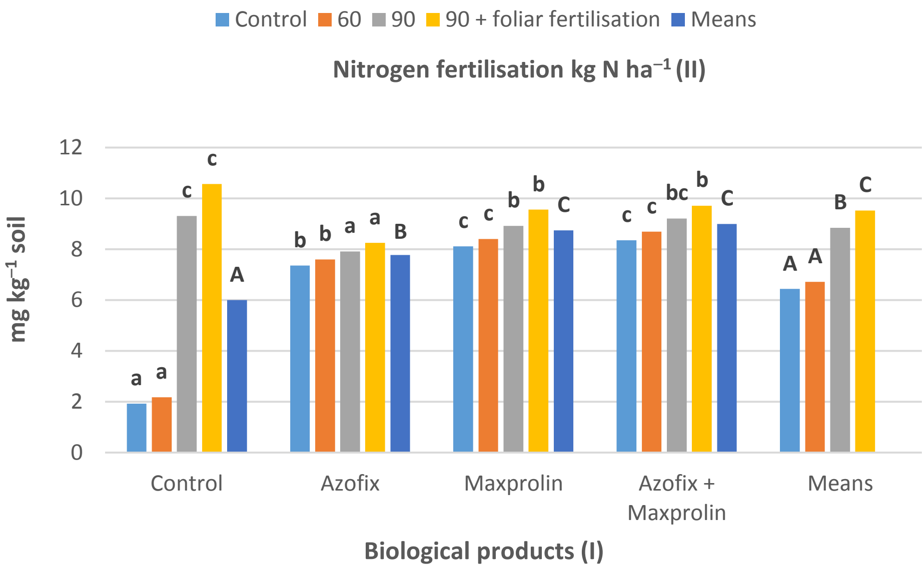
Explanation as in Fig. 1

Fig. 5. N-NO_3^- content in the 0–30 cm soil layer 30 days after an application of biological products (means across 2017–2019)



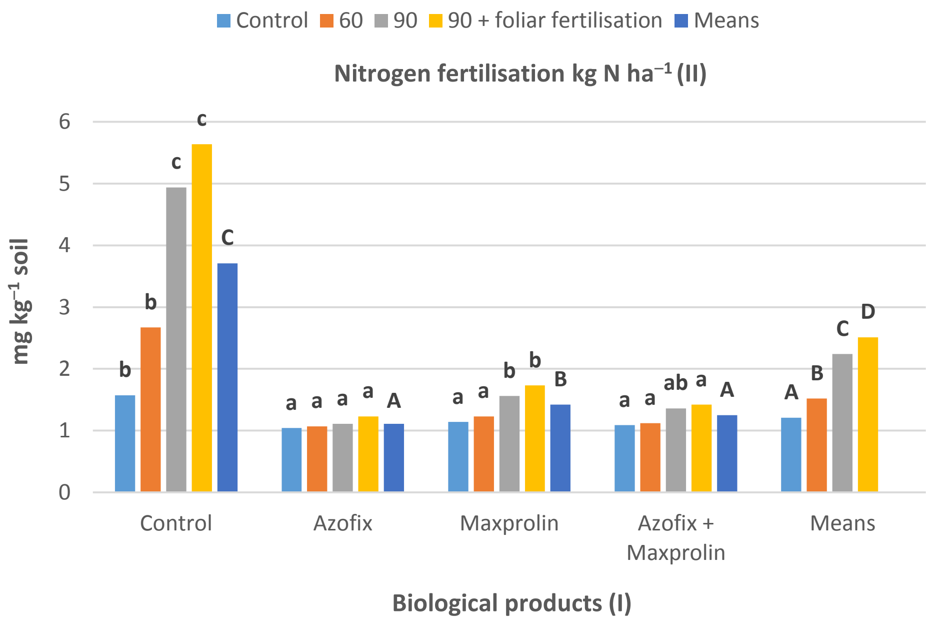
Explanation as in Fig. 1

Fig. 6. N-NO_3^- content in the 30–60 cm soil layer 30 days after an application of biological products (means across 2017–2019)



Explanation as in Fig. 1

Fig. 7. N-NO_3^- content in the 0–30 cm soil layer after spring wheat harvest (means across 2017–2019)



Explanation as in Fig. 1

Fig. 8. N-NO_3^- content in the 30–60 cm soil layer after spring wheat harvest (means across 2017–2019)

Maxprolin or Azofix. The combined application of these producte (Azofix + Maxprolin) contributed to an increase in the subsoil content of N-NO_3^-, the differences being statistically insignificant. Mineral nitrogen fertilisation significantly affected N-NO_3^- content in the topsoil and subsoil. An increase in mineral nitrogen rate was followed by an increase in N-NO_3^- content in the 0–30 soil layer, and increased leaching of N-NO_3^- to the 30–60 layer, in particular for the rate 90 kg N ha^{-1} + foliar spraying. An interaction between the experimental factors was found and it indicated that the highest concentration of N-NO_3^- in the topsoil was recorded in the unit treated with Azofix + Maxprolin combined with the mineral nitrogen rate of 90 kg N ha^{-1} + foliar spraying. In turn, the lowest N-NO_3^- content in the 0–30 cm soil layer was determined in the control unit and Maxprolin – treated unit, without mineral nitrogen fertilisation or fertilised with the rate of 60 kg N ha^{-1}.

1.4. N-NO_3^- content in the 0–30 and 30–60 soil layers after spring wheat harvest

An application of biological products significantly boosted N-NO_3^- content in the topsoil, the reverse being observed for the subsoil, compared with mineral nitrogen application, as it prevents N-NO_3^- leaching into deeper soil strata (Figs 7 and 8). Also mineral nitrogen fertilisation affected N-NO_3^- content in two soil layers. Increasing nitrogen rates produced an increase in N-NO_3^- content both in the topsoil and subsoil. Although the subsoil content of this component was lower, after the highest mineral nitrogen rate had been applied, N-NO_3^- content was 3.51 mg kg^{-1} soil, which is indicative of substantial leaching of this component into deeper layers of the soil profile. An interaction was confirmed indicating that the highest N-NO_3^- content in the topsoil and in the layer directly below it was determined in the unit fertilised with 90 kg N ha^{-1} + foliar spraying. The values for both the layers were similar, which indicates that nearly all the N-NO_3^- amount leached into the subsoil. The lowest N-NO_3^- content in the 0–30 cm soil layer was recorded in the control unit and the unit amended with 60 kg N ha^{-1}. In the subsoil, N-NO_3^- content was the lowest in the control unit where biological products had been applied alone or in combination with 60 kg N ha^{-1}. It should be emphasised that the subsoil content of N-NO_3^- was the lowest following an application of Azofix at all the levels of mineral nitrogen fertilisation.

DISCUSSION

In this study, an application of Azofix + Maxprolin and Azofix, significantly increased and reduced mineral nitrogen content, respectively, in the topsoil and subsoil, compared with an application of only mineral nitrogen during the period of intensive spring wheat growth. This may be explained by the fact that an application of products containing bacteria of the genus *Azotobacter* which reduce atmospheric nitrogen, contributes to an increase in crop plant – available mineral nitrogen, this process taking place regularly throughout the growing season thus preventing nitrogen leaching into the deeper strata of the soil profile [Singh et al. 2010]. In the study reported here, a similar relationship was also observed after an application of Maxprolin containing an amino acid which is a nitrogen source for spring wheat although the supply is lower. An application of increasing mineral nitrogen rates was followed by an increase in mineral nitrogen content and, since it was observed in both the topsoil and subsoil, the phenomenon was very negative from the standpoint of the environment. High mineral nitrogen rates, even when applied during the period of intensive plant growth, result in leaching of the mineral nitrogen into deeper soil layers, which pollutes the environment [Szulc 2013]. In this work, an application of biological products led to an increase in mineral nitrogen content in the topsoil after spring wheat harvest, a decline being observed in the subsoil, compared with nitrogen fertilisation, in particular the highest N rate. *Azotobacter vinelandii* introduced into the soil with biological products reduce elemental nitrogen from the air during the period of about six months after their application. Thus, they are also a biological nitrogen source for the following plants which may include winter oil seed rape or stubble catch crops (white mustard, phacelia). As a result, an application of biological products prevents mineral nitrogen leaching into deeper layers of the soil profile, which occurs when high mineral nitrogen rates are applied. This is in agreement with reports by Mona et al. [2012] and Szulc [2013].

CONCLUSIONS

The results indicated that during the period of intensive growth, that is 30 days after an application of biological products, spring wheat plants had an access to the greatest store of mineral nitrogen available in the topsoil following an application of Azofix + Maxprolin and the mineral nitrogen rate of 90 kg N ha^{-1}. Compared with mineral nitrogen fertilisation, an application of biological products increased mineral nitrogen quantity in the topsoil following spring wheat harvest.

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