Uptake and Utilization of Nitrogen from Organic Fertilizers Influenced by Different Doses of Copper

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Abstract: Copper is a microelement involved in the metabolism of nitrogen compounds in plants. Good utilization of nitrogen from soil and fertilizers by plants requires an adequate supply of copper. The aim of the study was to determine the effect of increasing levels of copper (100, 200, and 300 mg Cu·kg$^{-1}$ of soil) applied together with various organic fertilizers (cattle manure, chicken manure, and spent mushroom substrate) on nitrogen content and uptake by cocksfoot (Dactylis glomerata L.) and the coefficient of nitrogen utilization from organic fertilizers. The pot experiment was carried out in three growing seasons (May–September) in greenhouse, and in this cocksfoot was grown and harvested four cuts in each year. Copper and organic fertilizers were applied once in the first year before sowing cocksfoot, and the after-effect was investigated in the second and third years. Application of different amounts of copper did not influence the nitrogen content in the biomass of cocksfoot. At the same time, soil application of this micronutrient in the amount of 100 mg Cu·kg$^{-1}$ of soil caused an increase in nitrogen uptake in the biomass of cocksfoot. Application of 100 and 200 mg Cu·kg$^{-1}$ of soil caused an increase in the coefficient of nitrogen utilization from the organic fertilizers, which was highest effect in the case of cattle manure. All of the organic materials used increased the content of nitrogen and its uptake by cocksfoot, but the greatest effect was noted following application of chicken manure. The study showed no synergistic or antagonistic relationships between copper and nitrogen.

Keywords: cattle manure; chicken manure; heavy metals; nitrogen utilization coefficient; spent mushroom substrate; synergism; antagonism

1. Introduction

Pollution of the natural environment with heavy metals is a major threat to human life and health [1–6]. The increase in the content of these elements in the water, soil and air is due mainly to the development of heavy industry, improper waste management, fertilizers, and plant protection products [7,8]. The mobility and availability of heavy metals for plants depend on numerous soil properties, such as grain-size distribution, pH, organic matter content, content of iron and manganese oxides, adsorption capacity, and type of heavy metal [9,10]. One heavy metal whose content in the environment changes dynamically is copper, which has both natural and anthropogenic sources [11–14]. It is well known that organic matter plays a very important role in the binding of copper, which has a major impact on its availability and toxicity [15–19]. It is an essential microelement for the plants but can be toxic to them if taken up in excessive amounts [20–23]. Copper in plants functions as an activator of numerous enzymes that catalyze oxidation reactions involving molecular oxygen. It is a component of oxidoreductase (polyphenols, cytochrome and ascorbic acid), takes part in photosynthesis, respiration, and cell wall metabolism, protects against oxidative stress, and is involved in seed reproduction and protein formation [24–26]. It is also involved in metabolism of nitrogen compounds; plants require an adequate supply of copper for optimal utilization of mineral nitrogen from the soil and fertilizer [27]. This is especially important...
because nitrogen is one of the most important elements for plant growth and development. At the same time, the efficiency of its utilization from fertilizer is low, on average 25–50% for mineral fertilizers [6]. Despite the major physiological role of copper in plants, it has a tendency to accumulate. In excessive amounts it causes metabolic disturbances [20]. Excess copper interferes with electron transport and chlorophyll synthesis, causes chlorosis, necrosis, and leaf discoloration, and inhibits root growth, thus limiting the development of the plant and reducing yield [28–31]. The phytotoxicity of copper for plants can be reduced by introducing organic material to the soil to chelate it [32,33]. Both the chelation reaction and the effectiveness of soil organic matter in the chelating process are pH-dependent, and generally a more alkaline soil will be less affected by high Cu levels. In addition to organic matter application, lime may also be added in order to raise the pH and aid the Cu removal process by which Cu is removed [33].

Proper plant growth and development require not only an appropriate concentration of copper in the tissues, but also suitable proportions of copper and other elements, due to antagonistic and synergistic interactions. Antagonism occurs most often between copper and zinc, copper and iron, copper and phosphorus, and copper and calcium [34].

Although copper takes part in metabolism of nitrogen compounds and potentially can affect the content and uptake of nitrogen by plants, the literature contains little information on the antagonistic and synergistic interactions between these elements, and the information available is often conflicting [35–37]. Data are also lacking on effect of copper application on nitrogen utilization from organic fertilizers. Therefore, a study was conducted to determine the effect of various amounts of copper applied together with cattle manure, chicken manure, or spent mushroom substrate on nitrogen content and uptake by cocksfoot and the efficiency of nitrogen utilization from these fertilizers. It was hypothesized that there are antagonistic and/or synergistic relationships between copper and nitrogen, and that copper influences the efficiency of nitrogen utilization from organic fertilizers.

2. Materials and Methods
2.1. Field Experiment

A three-year pot experiment was conducted in a vegetation hall in three years: 2014, 2015, 2016. To the pots with a capacity of 10 dm³, 12 kg of Luvisols soil consisting of 71% sand, 24% silt, and 5% clay were introduced. Two factors were tested in an experiment with a completely randomized design. The first factor was the copper application rate: no copper application—control (0) and 100, 200 and 300 mg Cu·kg⁻¹ of soil. Copper was introduced to the soil once, prior to sowing of the test plant, only in the first year, in the form of an aqueous solution of CuSO₄·5H₂O. The second factor was organic fertilizer: control without organic fertilizer (CO) and cattle manure (CM), chicken manure from laying hens (ChM), and spent mushroom substrate (MS). These fertilizers were applied separately, once, in the first year, two weeks before sowing of the cocksfoot, in the dose amounted 2 g Corg·kg⁻¹ of soil. Selected properties of the organic substances used are presented in Table 1. The nitrogen content in the cattle manure and mushrooms substrate was similar, and in the chicken manure it was lower. The soil used in the experiment was characterized by pH = 6.7, total nitrogen 1.48 g·kg⁻¹, organic carbon 16.10 g·kg⁻¹, available phosphorus 189 mg·kg⁻¹, available potassium 110 mg·kg⁻¹, and total copper content 12.93 mg·kg⁻¹. Cocksfoot (*Dactylis glomerata* L.) of the Amera variety was grown in all treatments in each year of the study. The seeds were sown every year in the first decade of May. Their aboveground parts were harvested four times in each year (4 cuts) at 30-day intervals.
Table 1. Chemical composition of organic fertilizers used in pot experiment.

| Organic Fertilizers | Dry Matter (%) | C<sub>org</sub> | N<sub>tot</sub> | P | K | Ca | Mg | S | C<sub>org</sub>:N<sub>tot</sub> | Cu mg kg<sup>-1</sup> dm<sup>-1</sup> |
|---------------------|----------------|----------------|----------------|---|---|----|----|---|-----------------------------|-----------------------------|
| Cattle manure       | 20.0           | 394.5          | 23.70          | 6.50 | 17.02 | 11.28 | 3.24 | 3.68 | 16.6:1                     | 5.97                        |
| Chicken manure      | 29.0           | 160.3          | 14.10          | 8.74 | 9.10  | 13.59 | 2.43 | 3.07 | 11.4:1                     | 42.98                       |
| Mushroom substrate  | 31.0           | 315.7          | 24.50          | 6.14 | 17.20 | 45.18 | 3.12 | 26.20 | 12.9:1                     | 15.61                       |

2.2. Laboratory Analyses

Laboratory analyses were performed according to the methods presented by Krzywy-Gawronska [38]. In each organic fertilizer sample the following were determined: dry matter (dm at 105 °C), total nitrogen content by the CHN method (CHN autoanalyzer with IDC detector, Series II 2400, Perkin-Elmer, CA, USA), and the content of P, K, Ca, Mg, S and Cu by the ICP-AES method (Optima 3200 RL, Perkin-Elmer, Waltham, MA, USA) after dry mineralization of samples at 500 °C. The following were determined in the soil: pH (1 mol dm<sup>-3</sup> KCl), C organic by the Tiurin method, N total content by CHN analysis, as above, Cu total by ICP-AES as above, following mineralization in a mixture of HCl and HNO<sub>3</sub> (3:1), and available phosphorus and potassium by the Egner–Riehm method. Nitrogen content in cocksfoot was determined by elemental analysis in a CHN autoanalyzer, as above.

2.3. Calculations

The results for nitrogen content in cocksfoot and the data pertaining to its yield, published in Kuziemska et al. [39], were used to calculate nitrogen uptake (NU<sub>N</sub> and NU<sub>ON</sub>) and the nitrogen utilization coefficient (NUC) from organic fertilizers, according to the following equations:

\[
\text{NU}_N = Y \cdot C_N
\]

where:

- \(\text{NU}_N\) — nitrogen uptake by cocksfoot fertilized with CM, ChM, MS;
- \(Y\) — yield (dry matter) of cocksfoot fertilized with CM, ChM, MS;
- \(C_N\) — nitrogen content in cocksfoot dry matter fertilized with CM, ChM, MS.

\[
\text{NU}_0N = Y_0 \cdot C_{0N}
\]

where:

- \(\text{NU}_0N\) — nitrogen uptake by cocksfoot not fertilized with organic material (control treatment);
- \(Y_0\) — yield (dry matter) of cocksfoot not fertilized with organic material (control treatment);
- \(C_{0N}\) — nitrogen content in cocksfoot dry matter not fertilized with organic material (control treatment).

\[
\text{NUC} = \frac{(\text{NU}_N - \text{NU}_0N)}{N_{\text{amt}}} \times 100\%
\]

where:

- \(\text{NUC}\) — nitrogen utilization coefficient (%);
- \(\text{NU}_N\) — nitrogen uptake by cocksfoot fertilized with CM, ChM, MS;
- \(\text{NU}_0N\) — nitrogen uptake by cocksfoot not fertilized with organic material (control treatment);
- \(N_{\text{amt}}\) — amount of nitrogen introduced into soil with CM, ChM, MS.
2.4. Statistical Analyses

Statistical analysis of the results was performed by three-way ANOVA:

\[ y_{ijk} = \mu + a_i + b_j + c_k + ab_{ij} + ac_{ik} + bc_{jk} + abc_{ijk} + e_{ijkp} \]  

(4)

where: \( \mu \) — mean from all treatments; \( a_i \) — effect of Cu level; \( b_j \) — effect of organic fertilizer type; \( c_k \) — effect of year (third source of variation); \( ab_{ij} \) — interaction of Cu level and fertilizer type; \( ac_{ik} \) — interaction of Cu level and year; \( bc_{jk} \) — interaction of fertilizer type and year; \( abc_{ijd} \) — interaction of Cu level and fertilizer type and year; \( e_{ijkp} \) — sampling error.

The significance of the experimental factors was determined using the Fisher–Snedecor distribution, and LSD values for comparison of means were calculated by the Tukey test at \( \alpha = 0.05 \). The calculations were made in Statistica 13 PL (StatSoft, Tulsa, OK, USA). In addition, Pearson’s linear correlation coefficient was calculated for some of the examined traits.

3. Results

3.1. Nitrogen Content

Copper application at all doses (100, 200, 300 mg Cu kg\(^{-1}\)) was not shown to affect the nitrogen content in the dry mass of cocksfoot (Table 2).

Table 2. Nitrogen content in cocksfoot biomass, g N kg\(^{-1}\) dm (mean ± SD).

| Organic Fertilizer | Year | Copper Dose (mg Cu kg\(^{-1}\) of Soil) | Mean |
|--------------------|------|----------------------------------------|------|
|                    |      | 0                                      | 100  | 200  | 300  |      |
| Without organic fertilization | 1st  | 17.07 ± 0.45 | 17.57 ± 0.45 | 18.23 ± 0.53 | 19.85 ± 0.24 | 18.26 ± 1.14 B |
|                    | 2nd  | 15.23 ± 0.26 | 16.57 ± 0.46 | 17.14 ± 0.20 | 16.24 ± 0.51 | 16.30 ± 0.79 A |
|                    | 3rd  | 17.81 ± 0.58 | 17.56 ± 0.37 | 17.48 ± 0.44 | 17.54 ± 0.80 | 17.60 ± 0.58 B |
| mean               |      | 16.70 ± 1.17 A | 17.23 ± 0.64 AB | 17.72 ± 0.72 AB | 17.88 ± 1.60 B | 17.38 ± 1.19 a |
| Cattle manure      | 1st  | 18.25 ± 0.55 | 20.43 ± 0.55 | 21.90 ± 0.55 | 19.87 ± 0.29 | 20.11 ± 1.40 C |
|                    | 2nd  | 17.60 ± 0.98 | 17.42 ± 0.72 | 17.25 ± 0.79 | 16.60 ± 0.44 | 17.22 ± 0.85 A |
|                    | 3rd  | 18.65 ± 0.59 | 18.52 ± 1.11 | 18.68 ± 1.31 | 18.14 ± 0.77 | 18.50 ± 1.01 A |
| mean               |      | 18.17 ± 0.85 A | 18.79 ± 1.49 AB | 19.28 ± 2.16 B | 18.20 ± 1.44 A | 18.61 ± 1.62 b |
| Chicken manure     | 1st  | 21.73 ± 0.60 | 22.12 ± 0.69 | 22.78 ± 0.55 | 23.10 ± 1.22 | 22.43 ± 0.97 C |
|                    | 2nd  | 18.26 ± 0.67 | 17.50 ± 1.33 | 16.90 ± 1.08 | 16.72 ± 0.94 | 17.35 ± 1.20 A |
|                    | 3rd  | 20.65 ± 0.61 | 20.28 ± 0.62 | 19.54 ± 0.80 | 19.15 ± 1.43 | 19.91 ± 1.10 B |
| mean               |      | 20.21 ± 1.58 A | 19.97 ± 2.12 A | 19.74 ± 2.55 A | 19.66 ± 2.90 A | 19.89 ± 2.35 c |
| Mushroom substrate | 1st  | 17.82 ± 0.55 | 19.94 ± 0.28 | 19.07 ± 0.46 | 21.66 ± 0.44 | 19.62 ± 1.47 C |
|                    | 2nd  | 18.03 ± 0.79 | 17.02 ± 0.28 | 16.70 ± 0.52 | 16.38 ± 0.60 | 17.03 ± 0.85 A |
|                    | 3rd  | 19.00 ± 0.32 | 18.40 ± 0.30 | 18.00 ± 0.56 | 17.84 ± 0.91 | 18.31 ± 0.73 B |
| mean               |      | 18.28 ± 0.78 A | 18.45 ± 1.23 A | 17.92 ± 1.10 A | 18.63 ± 2.33 A | 18.32 ± 1.50 b |
| Mean for Cu dose   |      | 18.34 ± 1.69 a | 18.61 ± 1.73 a | 18.66 ± 1.99 a | 18.59 ± 2.25 a | 18.55 ± 1.94 a |
| Mean for years     | 1st  | 18.72 ± 1.89 A | 20.02 ± 1.71 B | 20.57 ± 1.88 BC | 21.12 ± 1.52 C | 20.11 ± 1.96 c |
|                    | 2nd  | 17.28 ± 1.41 A | 17.13 ± 0.88 A | 17.00 ± 0.76 A | 16.49 ± 0.68 A | 16.97 ± 1.02 a |
|                    | 3rd  | 19.03 ± 1.16 A | 18.69 ± 1.20 A | 18.43 ± 1.14 A | 18.17 ± 1.18 A | 18.56 ± 1.21 b |

\( a, b, c \) — means for investigated factors indicated by different small letters (in the columns for organic fertilizers and for years but in the row for copper doses) are significantly different. \( A, B, C \) — means for the interaction of the studied factors indicated by different capital letters in the rows and in the column ‘Mean’ of the table are significantly different.

All of the organic materials used caused an increase in the amount of nitrogen in the test plant. The highest content of this macronutrient was recorded in the grass fertilized with ChM, in which it was 14.4% higher than in the control plants and 6.9% and 8.6% higher than following application of CM and MS, respectively. At the same time, application of 300 mg Cu kg\(^{-1}\) of soil caused an increase in nitrogen content in the control plants, and 200 mg Cu kg\(^{-1}\) of soil increased nitrogen content in the plants fertilized with cattle.
manure. Application of various levels of copper together with ChM and MS did not significantly affect the content of nitrogen in cocksfoot. Comparison of the years of research showed the highest nitrogen content in cocksfoot grown in the first year of the experiment and the lowest in the second year, which may have been due to differences in the rate of conversions of nitrogen compounds in the soil.

3.2. Nitrogen Uptake

Nitrogen uptake by cocksfoot (Tables 3 and 4) was significantly dependent on the Cu application dose, organic fertilizer, and the year of research. After application of 100 mg Cu·kg⁻¹ of soil, nitrogen uptake by cocksfoot (Tables 3 and 4) was greater than in the control treatment and after application of 300 mg Cu·kg⁻¹ of soil. On average during the experiment, grass fertilized with 100 Cu·kg⁻¹ of soil accumulated 5.9% more nitrogen than plants from the control treatment and 3.9% more than after application of 300 mg Cu·kg⁻¹ of soil. Cocksfoot fertilized with cattle manure, chicken manure, or spent mushroom substrate took up more nitrogen than plants in the control treatment. In addition, plants fertilized with chicken manure took up more nitrogen than those fertilized with cattle manure (by 7.4%) or spent mushroom substrate (by 16.9%). Application of copper at 100 and 200 mg Cu·kg⁻¹ of soil caused an increase in nitrogen uptake by the plants fertilized with cattle manure, while application of 100 mg Cu·kg⁻¹ of soil increased nitrogen uptake by plants fertilized with spent mushroom substrate. Different doses of copper applied together with chicken manure were not shown to affect accumulation of nitrogen in the grass. Nitrogen uptake by cocksfoot significantly decreased in successive years of the study; in the second and third years it was 45.6% and 53.7% lower, respectively, than in the first year. The correlation coefficients (Table 5) showed no significant relationship between nitrogen content (Table 2) and copper content in cocksfoot presented by Kuziemska et al. [39], but showed a significant relationship linking nitrogen content and uptake (Tables 3 and 4) with the yield of the crop reported in the study cited.

### Table 3. Nitrogen uptake by cocksfoot, g N·pot⁻¹ (mean ± SD).

| Organic Fertilizer | Year | Copper Dose (mg Cu·kg⁻¹ of Soil) | Mean |
|--------------------|------|----------------------------------|------|
|                    |      | 0  | 100 | 200 | 300 |      |
| Without organic fertilization | 1st | 0.267 ± 0.001 | 0.221 ± 0.006 | 0.202 ± 0.015 | 0.251 ± 0.006 | 0.235 ± 0.027 B |
|                      | 2nd | 0.197 ± 0.017 | 0.239 ± 0.013 | 0.291 ± 0.013 | 0.185 ± 0.014 | 0.213 ± 0.027 B |
|                      | 3rd | 0.161 ± 0.014 | 0.159 ± 0.002 | 0.147 ± 0.007 | 0.132 ± 0.003 | 0.150 ± 0.014 A |
|                      | mean | 0.208 ± 0.046 A | 0.206 ± 0.036 A | 0.193 ± 0.037 A | 0.189 ± 0.049 A | 0.199 ± 0.043 A |
| Cattle manure       | 1st | 0.456 ± 0.022 | 0.571 ± 0.051 | 0.535 ± 0.005 | 0.403 ± 0.013 | 0.491 ± 0.072 B |
|                      | 2nd | 0.237 ± 0.013 | 0.258 ± 0.015 | 0.258 ± 0.008 | 0.229 ± 0.008 | 0.246 ± 0.017 A |
|                      | 3rd | 0.222 ± 0.019 | 0.216 ± 0.014 | 0.267 ± 0.033 | 0.279 ± 0.011 | 0.246 ± 0.035 A |
|                      | mean | 0.305 ± 0.108 A | 0.349 ± 0.161 B | 0.353 ± 0.130 B | 0.304 ± 0.074 A | 0.328 ± 0.124 C |
| Chicken manure      | 1st | 0.586 ± 0.009 | 0.579 ± 0.027 | 0.635 ± 0.040 | 0.562 ± 0.029 | 0.591 ± 0.039 C |
|                      | 2nd | 0.273 ± 0.017 | 0.252 ± 0.027 | 0.240 ± 0.011 | 0.243 ± 0.018 | 0.259 ± 0.023 B |
|                      | 3rd | 0.213 ± 0.005 | 0.235 ± 0.016 | 0.201 ± 0.022 | 0.204 ± 0.020 | 0.213 ± 0.022 A |
|                      | mean | 0.357 ± 0.164 A | 0.356 ± 0.160 A | 0.358 ± 0.198 A | 0.336 ± 0.162 A | 0.352 ± 0.172 d |
| Mushroom substrate  | 1st | 0.402 ± 0.003 | 0.463 ± 0.017 | 0.456 ± 0.018 | 0.472 ± 0.026 | 0.448 ± 0.033 C |
|                      | 2nd | 0.262 ± 0.022 | 0.267 ± 0.005 | 0.251 ± 0.009 | 0.215 ± 0.004 | 0.249 ± 0.024 B |
|                      | 3rd | 0.204 ± 0.002 | 0.220 ± 0.014 | 0.206 ± 0.008 | 0.195 ± 0.012 | 0.206 ± 0.014 A |
|                      | mean | 0.289 ± 0.084 A | 0.317 ± 0.106 B | 0.304 ± 0.110 AB | 0.294 ± 0.127 AB | 0.301 ± 0.108 b |
| Mean for Cu dose     |     | 0.290 ± 0.121 ab | 0.307 ± 0.140 c | 0.302 ± 0.148 bc | 0.281 ± 0.124 a | 0.295 ± 0.134 e |
| Mean for years      | 1st | 0.428 ± 0.115 A | 0.459 ± 0.147 B | 0.457 ± 0.162 B | 0.422 ± 0.116 A | 0.441 ± 0.138 c |
|                      | 2nd | 0.242 ± 0.034 B | 0.254 ± 0.020 B | 0.245 ± 0.015 B | 0.218 ± 0.025 A | 0.240 ± 0.028 b |
|                      | 3rd | 0.200 ± 0.026 A | 0.208 ± 0.032 A | 0.205 ± 0.047 A | 0.203 ± 0.054 A | 0.204 ± 0.041 A |

a,b,c,d—means for investigated factors indicated by different small letters (in the columns for organics fertilizers and for years but in the row for copper doses) are significantly different. A,B,C—means for the interaction of the studied factors indicated by different capital letters in the rows and in the column ‘Mean’ of the table are significantly different.
Table 4. Total nitrogen uptake by cocksfoot in three years, g N·pot⁻¹ (mean ± SD).

| Organic Fertilizer          | Copper Dose (mg Cu·kg⁻¹ of Soil) | Mean   |
|-----------------------------|----------------------------------|--------|
|                             | 0      | 100   | 200   | 300   |
| Without organic fertilization | 0.625 ± 0.027 A | 0.618 ± 0.019 A | 0.579 ± 0.017 A | 0.568 ± 0.014 A | 0.598 ± 0.031 a |
| Cattle manure               | 0.914 ± 0.016 A | 1.046 ± 0.062 B | 1.060 ± 0.034 B | 0.912 ± 0.008 A | 0.983 ± 0.079 c |
| Chicken manure              | 1.072 ± 0.023 A | 1.067 ± 0.053 A | 1.079 ± 0.027 A | 1.009 ± 0.028 A | 1.056 ± 0.044 d |
| Mushroom substrate          | 0.868 ± 0.023 A | 0.951 ± 0.019 B | 0.913 ± 0.030 AB | 0.882 ± 0.018 AB | 0.903 ± 0.039 b |
| Mean                        | 0.870 ± 0.132 ab | 0.920 ± 0.185 c | 0.907 ± 0.201 bc | 0.843 ± 0.166 a | 0.885 ± 0.182   |

a,b,c,d—means for investigated factors indicated by different small letters (in the column for organic fertilizers and for years but in the row for copper doses) are significantly different. A,B—means for the interaction of the studied factors indicated by different capital letters in the rows are significantly different.

Table 5. Linear correlation coefficients between selected properties of cocksfoot.

| Specification | Cocksfoot Yield | N Uptake | Cu Content | Cu Dose |
|---------------|-----------------|----------|------------|---------|
| N content     | 0.639 *         | 0.768 *  | 0.188      | 0.033   |
| N uptake      | 0.979 *         | -        | 0.279      | -0.025  |

*p < 0.05.

3.3. Nitrogen Utilization Coefficient

An essential aspect of assessment of the efficiency of organic fertilizers and waste materials is the coefficient of utilization of nutrients. The coefficients of nitrogen utilization from cattle manure, chicken manure, and spent mushroom substrate (Tables 6 and 7) depended on the level of copper applied. The total nitrogen utilization coefficient for the three-year experiment was highest after 200 mg Cu·kg⁻¹ application (24.6%), somewhat lower following 100 and 300 mg Cu·kg⁻¹ application (22.6% and 20.2%, respectively), and lowest in the control treatment (17.8%).

Table 6. Nitrogen utilization from organic fertilizers by cocksfoot, % (mean ± SD).

| Organic Fertilizer | Year | Copper Dose (mg Cu·kg⁻¹ of Soil) | Mean   |
|--------------------|------|----------------------------------|--------|
|                    |      | 0      | 100   | 200   | 300   |
| Cattle manure      | 1st  | 13.1 ± 1.6 | 24.3 ± 3.2 | 23.1 ± 0.9 | 10.6 ± 1.2 | 17.8 ± 6.3 C |
|                    | 2nd  | 2.8 ± 1.5 | 1.3 ± 1.6 | 1.9 ± 1.4 | 3.1 ± 1.3 | 2.3 ± 1.6 A  |
|                    | 3rd  | 4.2 ± 0.5 | 4.0 ± 1.1 | 8.4 ± 1.9 | 10.2 ± 0.9 | 6.7 ± 2.9 B  |
| Mean               |      | 6.7 ± 4.7 A | 9.9 ± 10.5 B | 11.1 ± 9.0 B | 8.0 ± 3.6 A | 8.9 ± 7.7 c  |
| Chicken manure     | 1st  | 14.8 ± 0.3 | 16.6 ± 1.4 | 20.0 ± 1.6 | 14.4 ± 1.6 | 16.5 ± 2.6 B |
|                    | 2nd  | 3.5 ± 1.3 | 0.6 ± 1.9 | 0.4 ± 1.2 | 2.7 ± 1.1 | 1.9 ± 1.9 A  |
|                    | 3rd  | 2.4 ± 0.8 | 3.6 ± 0.8 | 2.5 ± 1.0 | 3.3 ± 1.0 | 2.9 ± 1.0 A  |
| Mean               |      | 6.9 ± 5.7 A | 6.9 ± 7.1 A | 7.7 ± 8.9 A | 6.8 ± 5.5 A | 7.1 ± 6.9 b  |
| Mushroom substrate | 1st  | 7.0 ± 0.1 | 12.6 ± 1.1 | 13.3 ± 1.7 | 11.5 ± 1.1 | 11.1 ± 2.7 B |
|                    | 2nd  | 3.4 ± 0.8 | 1.4 ± 0.9 | 1.1 ± 0.5 | 1.5 ± 0.7 | 1.9 ± 1.2 A  |
|                    | 3rd  | 2.2 ± 0.7 | 3.2 ± 0.6 | 3.1 ± 0.4 | 3.2 ± 0.8 | 2.9 ± 0.8 A  |
| Mean               |      | 4.2 ± 2.1 A | 5.8 ± 5.0 A | 5.8 ± 5.4 A | 5.4 ± 4.4 A | 5.3 ± 4.5 a  |
| Mean for Cu dose   |      | 5.9 ± 4.6 A | 7.5 ± 8.1 bc | 8.2 ± 8.3 c | 6.7 ± 4.7 ab | 7.1 ± 6.7    |
| Mean for years     | 1st  | 11.6 ± 3.5 A | 17.9 ± 5.3 B | 18.8 ± 4.4 B | 12.2 ± 2.1 A | 15.1 ± 5.1 c |
|                    | 2nd  | 3.3 ± 1.3 B | 1.1 ± 1.6 A | 1.1 ± 1.2 A | 2.4 ± 1.2 AB | 2.0 ± 1.6 a  |
|                    | 3rd  | 2.9 ± 1.1 A | 3.6 ± 0.9 B | 4.6 ± 2.9 BC | 5.6 ± 3.4 C | 4.2 ± 2.6 b  |

a,b,c—means for investigated factors indicated by different small letters (in the columns for organic fertilizers and for years but in the row for copper doses) are significantly different. A,B,C—means for the interaction of the studied factors indicated by different capital letters in the rows and in the column ‘Mean’ of the table are significantly different.
Table 7. Total utilization of nitrogen from organic fertilizers by cocksfoot in three years times, % (mean ± SD).

| Organic Fertilizer   | Copper Dose (mg Cu·kg⁻¹ of Soil) | Mean   |
|----------------------|----------------------------------|--------|
|                      | 0                                | 100    | 200    | 300    |
| Cattle manure        | 20.1 ± 3.0 A                     | 29.7 ± 3.0 B | 33.4 ± 1.2 B | 23.8 ± 1.1 A | 26.7 ± 5.6 c |
| Chicken manure       | 20.7 ± 1.5 A                     | 20.8 ± 3.3 A | 22.0 ± 1.7 A | 20.4 ± 1.9 A | 21.2 ± 2.5 b |
| Mushroom substrate   | 12.6 ± 0.4 A                     | 17.3 ± 2.0 A | 17.4 ± 2.0 A | 16.3 ± 1.0 A | 15.9 ± 2.5 a |
| Mean                 | 17.8 ± 4.1 A                     | 22.6 ± 5.9 bc | 24.6 ± 6.8 c | 20.2 ± 3.4 ab | 21.3 ± 5.8   |

a,b,c—means for investigated factors indicated by different small letters (in the column for organics fertilizers and for years but in the row for copper doses) are significantly different. A,B—means for the interaction of the studied factors indicated by different capital letters in the rows are significantly different.

The significantly highest nitrogen utilization coefficient (for the entire three-year experiment) was noted for cattle manure. It was 25.9% and 67.9% higher than in the case of chicken manure and spent mushroom substrate, respectively. At the same time, copper introduced into the soil at doses 100 and 200 mg Cu·kg⁻¹ of soil together with cattle manure increased the coefficient of nitrogen utilization from this fertilizer. Copper, irrespective of the application rate, applied together with chicken manure and spent mushroom substrate, did not affect the coefficients of nitrogen utilization from these fertilizers. In successive years of the experiment, the coefficient of nitrogen utilization by cocksfoot was small, and in the second and third years of the study it was 13.2% and 27.8% of the value obtained in the first year.

4. Discussion

Uptake of nutrients by plants is mediated by proteins located in the cell membranes of the roots. Cations and anions with similar structure compete to bind carrier proteins. Some of the identified cell membrane transporters are specific to an ion or group of ions, while others do not display this trait [36]. At the same time, antagonistic and synergistic interactions between nutrients are most likely one of the factors influencing the yield and chemical composition of crop plants [34]. Our study showed no significant influence of varied copper application on the content of nitrogen in cocksfoot and no relationships between the content of nitrogen and copper in the plants. Meller and Jarnuszewski [26] also found that soil application of copper did not affect the content of nitrogen in various plant species, which may indicate the absence of antagonistic and synergistic interactions between these elements. A lack of antagonistic and synergistic interactions between copper and nitrogen is also pointed out by Rietra et al. [36], who analyzed this question based on publications by various authors. Kumar et al. [35], however, in a study of the effect of various levels of nitrogen and copper in the soil on their content in wheat (Triticum aestivum L.), found that nitrogen and copper are antagonists. In contrast, Alhasany et al. [37], in a study determining the effect of varied copper application rates on selected traits of broad bean, reported a synergistic effect of the two elements. Snowball et al. [40] also showed an increase in the content of total and protein nitrogen in subterranean clover (Trifolium subterraneum L.) after application of varied amounts of copper. The various nitrogen levels obtained in the present study in cocksfoot following application of organic substances may be due to the introduction of different amounts of this macroelement with organic fertilizers (0.12 mg N·kg⁻¹ of soil with cattle manure, 0.18 mg N·kg⁻¹ of soil with chicken manure, and 0.16 mg N·kg⁻¹ of soil with spent mushroom substrate) and to different rates of mineralization in the soil. Assessment of the fertilization value of organic materials should take into account not only the effect on yield and nutrient content, but also the accumulation of nutrients in the biomass of plants. Our study showed that uptake of nitrogen by cocksfoot was significantly correlated with its yield and its content in the plant biomass and depended on the amount of copper in the soil and on application of organic fertilizers. Total nitrogen uptake during the three years of experiment was highest after 100 mg Cu·kg⁻¹ of soil application and following chicken manure application. Geng et al. [41] also reported a positive effect of organic fertilizer on nitrogen accumulation in plants.
At the same time, many authors emphasize that chicken manure has high content of nutrients and a high potential to increase yield [42,43]. Cocksfoot accumulated the most nitrogen in the first year of the study and the least in the third year, which is linked to removal of this element from the soil. In comparison with other important minerals in plant nutrition, nitrogen is a highly mobile element, which makes it susceptible to losses from ecosystems due to leaching and volatilization [44]. Research by Rutkowska [45] showed that the degree of nitrogen utilization from mineral and organic fertilizers is small. The availability of nitrogen for plants from various organic substances and the use of this macronutrients is determined, among others, by the ratio of carbon to nitrogen contained in them [46]. The organic fertilizers used in the study had a small C:N ratio (11.4–16.6:1), which determines their rapid mineralization and high nitrogen availability in the first year after application to the soil. This observation is confirmed by the results of own research and in the literature [47,48]. Both, uptake and utilization of nitrogen were significantly higher in the first year of the study than in the following years. The results of own research and information from the literature on the highest rate of mineralization of organic fertilizers with a small C:N ratio in the period up to 150 days after their introduction into the soil [46] indicate that such fertilizers can be applied to the soil every year, directly before sowing plants. The literature lacks reports on the effect of varied copper application rates on the coefficient of nitrogen utilization from organic fertilizers. In our study, copper application at 100 and 200 mg Cu·kg⁻¹ of soil caused an increase in the coefficient of nitrogen utilization from CM, ChM and MS.

There was no significant influence of 300 mg Cu·kg⁻¹ on the value of the nitrogen utilization factor in organic fertilizers, however, the value obtained in this combination was slightly higher than in the control object. This proves the potential stimulating effect of copper on the mineralization processes of organic fertilizers in the soil. Premi and Cornfield [49] using copper in the dose of 1000 mg Cu·kg⁻¹ obtained its stimulating effect on nitrogen mineralization in the soil. At the same time, this dose was not high enough to induce nitrification. It also did not affect the ammonification processes. In the light of these reports, the doses of Cu used in our study, not exceeding 300 mg Cu·kg⁻¹, probably also did not intensify the nitrification process. Thus, they did not contribute to increasing the loss of easily migrating forms of nitrogen.

In all years of experiment, the highest utilization of nitrogen was noted in the case of cattle manure, which had slightly highest carbon to nitrogen ratio, and the slightly lowest in the case of spent mushroom substrate. Wiśniewska and Jankowski [50] presented other results, in which showed that spent mushroom substrate was more efficient than cattle manure in the first and second year of application. In addition, they found that the effect of spent mushroom substrate was short-lived. It requires supplementation with mineral fertilizers to improve its efficiency. Rao et al. [51] and Polat et al. [52] have also pointed out high variability and imbalance of the chemical composition of spent mushroom substrates. According to the authors cited, this is a clear disadvantage that necessitates continued research on their composition and supplementation with deficient elements to improve the value of fertilizer.

The effect of varied levels of copper applied together with cattle manure on the content and accumulation of nitrogen in cocksfoot and the value of the coefficient of utilization of this element from cattle manure may indicate that copper is involved in the metabolism of nitrogen compounds [27].

The obtained results of own research concern cocksfoot grass and should be continued with the use of other plants, differing e.g., in the structure of the root system, the length of the vegetation period, tolerance to soil contamination with heavy metals, etc.

5. Conclusions

Copper application at 100, 200 and 300 mg Cu·kg⁻¹ of soil did not significantly affect the content of nitrogen in cocksfoot, but application of 100 mg Cu·kg⁻¹ of soil increased its uptake. The lack of significant relationships between copper and nitrogen content in
the grass indicates the absence of antagonistic or synergistic interactions between these elements. The highest content and accumulation of nitrogen in the plants was observed following application of chicken manure. Copper applied at 100 and 200 mg Cu·kg⁻¹ of soil increased the coefficient of nitrogen utilization from the organic fertilizers. The coefficient was highest in the case of cattle manure. The highest uptake of nitrogen by cocksfoot was obtained by applying copper at 200 mg Cu·kg⁻¹ of soil in combination with chicken manure, while its utilization from organic fertilizer was highest after application of 200 mg Cu·kg⁻¹ of soil together with cattle manure. The results of our research indicate the possibility of increasing the nitrogen utilization from organic fertilizers after the use of small doses of copper.

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