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**OPTIMIZATION OF LAWN FERTILIZATION  
WITH NITROGEN. PART III. DYNAMICS  
OF SOIL MICROBIOLOGICAL COMPOSITION  
AND ENZYMATIC ACTIVITY OF DEHYDROGENASES**

**OPTIMALIZACJA NAWOŻENIA AZOTOWEGO TRAWNIKA  
CZ. III. DYNAMIKA SKŁADU MIKROBIOLOGICZNEGO GLEBY.  
AKTYWNOŚĆ ENZYMATYCZNA DEHYDROGENAZ**

**Abstract:** The aim of this study was to determine the effect of increasing nitrogen fertilization of lawns (at doses corresponding to 0, 50, 100, 150 and 200 mg N dm<sup>-3</sup> soil) on dynamics of changes in microbial community composition of soil: total counts of fungi, bacteria, *Actinomycetes*, oligotrophic and copiotrophic bacteria, as well as enzymatic activity of dehydrogenases. No significant effect was found of analyzed levels of nitrogen fertilization on counts of microorganisms and enzymatic activity of soil. A trend, although not confirmed statistically, could be observed for microbial counts to increase in case of the N 150 combination. A factor significantly modifying the microbial community composition and enzymatic activity of soil was sampling date and related atmospheric conditions. A significant increase of total bacterial counts was recorded at the 2nd sampling date (in April). The quantity of *Actinomycetes* increased towards the end of the summer period (August), while the highest enzymatic activity of dehydrogenases was determined in July. The contents of copiotrophs and oligotrophs in soil decreased significantly with the duration of vegetation.

**Keywords:** nitrogen fertilization, dehydrogenases activity, fungi, bacteria, oligotrophic and copiotrophic bacteria

Cultivation, fertilization, protection and contamination of soil modify its physico-chemical properties as well as change its biological activity. A measure of biological activity, comprising all occurring compound and energy conversions, may be enzymatic activity [1] and dynamics of development of selected groups of microorganisms living in the soil. It depends both on the type of soil, the depth of the soil profile, vegetation

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cover, atmospheric conditions as well as cultivation regime and fertilization of soil, and many other factors affecting soil [2–6].

Factors having an effect on the activity of microorganisms in soil, their quality and quantity, include eg contents of organic substances, nitrogen compounds, macro- and microelements, water, oxygen as well as pH and temperature of soil.

The level as well as the manner of soil inhabitation by microorganisms depends to a considerable degree on contents of such readily available nutrients in soil, such as sugars, proteins and fats. The occurrence of microorganisms is also determined by the presence of allelopathic substances in the soil, which are secreted by plant roots, as well as interactions between different groups of microorganisms [7]. The population size of soil microorganisms is considerably influenced by fertilization, including organic and mineral nitrogen fertilization, applied in order to supply plants with nutrients [8].

The aim of the conducted investigations was to determine the effect of increasing lawn fertilization with nitrogen on the dynamics of changes in the microbial community composition of soil (total counts of bacteria, fungi, *Actinomycetes*, oligotrophic and copiotrophic) as well as microbiological activity expressed as dehydrogenases activity.

## Material and methods

Vegetation experiments were conducted in the years 2007–2008 at the ‘Marcelin’ Experimental Station of Departments of the Faculty of Horticulture, the Poznan University of Life Sciences. Analyses were conducted on five increasing nitrogen lawn fertilization levels (in  $\text{mg N dm}^{-3}$ ) of 0, 50, 100, 150, 200 (denoted as N 0, N 50, N 100, N 150 and N 200). The control was combination N 0, in which no fertilization with nitrogen was applied. Contents of phosphorus, potassium and magnesium in all tested combinations were supplemented to standard levels (in  $\text{mg dm}^{-3}$ ): P 100, K 200, Mg 180 (2007) and 300 (2008). Soil samples for microbiological analyses were collected at the following dates: 15.03, 15.04, 12.06, 08.07 and 19.08.2008. Each time from a given combination a total of 14–18 individual samples were collected from the topsoil (0–20 cm), from which a representative mixed sample ( $0.4\text{--}0.5 \text{ dm}^3$ ) was produced after mixing. Air temperature at a height of 5 cm above the ground was recorded using a HOBO Weather Station by ONSET. The course of its changes is presented in Fig. 1.

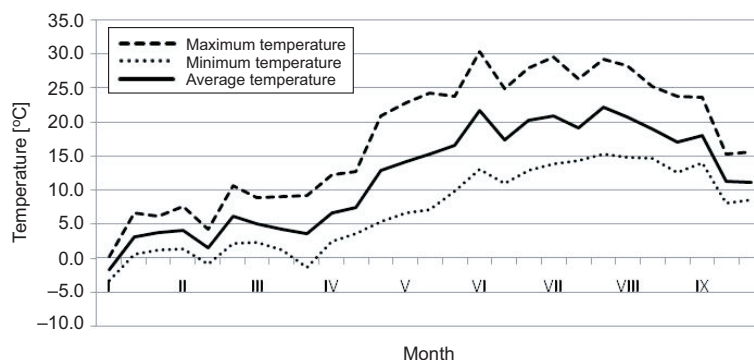


Fig. 1. Dynamics of changes in air temperature at a height of 5 cm above the ground

### Microbiological analyses

In soil samples collected from the radical zone counts of microorganisms were determined using the plate method on respective agar media (in five replications). The mean number of colonies was calculated as converted to 1 g<sup>-1</sup> d.m. of soil:

- The total bacterial counts were determined on a medium from a soil extract after 14-day incubation at 25 °C;
- Fungi were determined on Martin's medium after 5-day incubation at 24 °C [9];
- *Actinomycetes* were determined on a medium according to Pochon after 5-day culture at 25 °C [10];
- Copiotrophs were determined on the NB medium after 5-day incubation at 25 °C [11];
- Oligotrophs were determined on the DN medium after 5-day incubation at 25 °C [12].

### Enzymatic analyses

The analysis of enzymatic activity of soil fertilized with varied doses of nitrogen was based on the determination of dehydrogenases activity using colorimetry, with 1 % TTC (*triphenyltetrazole chloride*) applied as a substrate, after 24-h incubation at 30 °C, at a wavelength of  $\lambda = 485$  nm and it was expressed in [cm<sup>3</sup> · H<sub>2</sub> · kg<sup>-1</sup> · 24 h<sup>-1</sup>].

Dynamics of changes in microbial community composition of soil and enzymatic activity of dehydrogenases were analyzed statistically using the Duncan test. Inference was performed at significance level  $\alpha = 0.05$ .

### Results and discussion

In the conducted experiment a differentiating effect of nitrogen fertilization was found – among other things – on the dynamics of microbiological changes in soil. A trend, although not confirmed statistically, could be observed for the counts of microorganisms in soil to increase with an increase in the intensity of fertilization. Earlier studies showed a similar trend, concerning total bacterial count (eg from genera *Arthrobacter*, *Bacillus* and *Pseudomonas*), total count of fungi (eg from genera *Fusarium* and *Penicillium*) and *Actinomycetes* (from genus *Streptomyces* sp.) [13]. Several studies confirmed a significant differentiating effect of fertilization, particularly with nitrogen, on counts of soil microorganisms as well as their species composition [14–16].

In the analyses conducted by the authors of this study no differentiating effect was found for either nitrogen fertilization or vegetation period on the total population of fungi in soil (Table 1). A trend could be observed, although it could not be proved statistically, for the amounts of this group of microorganisms to increase in case of the combination intensively fertilized with nitrogen (N 150, N 200). Soil fungi, mainly moulds, are capable of nitrogen uptake not only from organic compounds, but also ammonium and nitrate salts. The highest levels of these microorganisms among tested combinations were determined in July at a fertilization level of 150 kg N (56.0

CFU  $\times 10^5$ ). Recorded pH values of soil in this combination were minimally lower than in the other combinations, which could have promoted the development of this group of microorganisms.

Table 1

The effect of varied nitrogen fertilization on fungal count (CFU  $\times 10^5$  g<sup>-1</sup> d.m. of soil)

| N level | Month |       |       |       |        |       |
|---------|-------|-------|-------|-------|--------|-------|
|         | March | April | June  | July  | August | Mean  |
| N 0     | 36.0  | 20.0  | 20.0  | 13.0  | 6.0    | 19.0a |
| N 50    | 23.0  | 30.0  | 23.0  | 33.0  | 20.0   | 25.8a |
| N 100   | 20.0  | 5.0   | 20.0  | 30.0  | 30.0   | 21.0a |
| N 150   | 30.0  | 31.0  | 12.0  | 56.0  | 20.0   | 29.8a |
| N 200   | 36.0  | 10.0  | 26.0  | 20.0  | 40.0   | 26.4a |
| Mean    | 29.0a | 19.2a | 20.2a | 30.4a | 23.2a  |       |

The level of analyzed fungi in soil did not undergo significant periodical changes. However, their biggest mean number was recorded at the 1st sampling date (in March) and at the 4th sampling date (in July), amounting to 29.0 and 30.4 CFU  $\times 10^5$ , respectively. At the other dates of analyses their level was markedly lower. In turn, at the onset of flowering (2nd date) in the control combination (with no nitrogen fertilization) the lowest count of fungi was determined, which could have resulted from an increased soil moisture content and slightly higher pH.

Similarly as in case of fungi, no significant effect was found of tested nitrogen fertilization levels on total bacterial count in soil (Table 2). It ranged from 48.3 (N 50) to 86.5 CFU  $\times 10^6$  (N 150). Vegetation period was the factor significantly modifying the count of this group of microorganisms. A significant increase in bacterial content in soil was found in April (113.4 CFU  $\times 10^6$ ), which could have been connected with atmospheric conditions and an increase with mean soil temperature over 5 °C (Fig. 1), causing an increase in microbiological activity. The highest total bacterial count among all the tested combinations was recorded in April at fertilization N 150 (223.0 CFU  $\times 10^6$ ). Lower bacterial counts in June, July and August could have been caused by an increased level of fungi and *Actinomyces*. Earlier studies confirmed the varied dynamics of microbiological changes during vegetation [17]. Those authors indicated that the highest microbiological activity of soil is observed usually in spring.

Table 2

The effect of varied nitrogen fertilization on total bacterial count (CFU  $\times 10^6$  g<sup>-1</sup> d.m. of soil)

| N level | Term  |        |       |       |        |       |
|---------|-------|--------|-------|-------|--------|-------|
|         | March | April  | June  | July  | August | Mean  |
| N 0     | 63.3  | 92.3   | 46.0  | 12.3  | 38.3   | 50.4a |
| N 50    | 21.3  | 77.2   | 76.3  | 37.3  | 29.3   | 48.3a |
| N 100   | 43.6  | 61.3   | 71.6  | 42.0  | 34.0   | 50.5a |
| N 150   | 63.6  | 223.0  | 24.3  | 56.3  | 65.3   | 86.5a |
| N 200   | 71.3  | 113.0  | 24.0  | 39.0  | 41.3   | 57.7a |
| Mean    | 52.6b | 113.4a | 48.4b | 37.4b | 41.6b  |       |

No differentiating effect of nitrogen fertilization on the count of *Actinomycetes* in soil was shown in this study. Vegetation period was a factor significantly modifying the count of this group of microorganisms (Table 3), similarly as in case of the above-mentioned groups of microorganisms. Their mean count was stable and ranged from 17.5 (N 200) to 23.7 CFU  $\times 10^6$  (N 50). However, their highest number was recorded in summer months in case of combination N-150 (51.4 CFU  $\times 10^6$ ).

Table 3

The effect of varied nitrogen fertilization on *Actinomycetes* count (CFU  $\times 10^6$  g<sup>-1</sup> d.m. of soil)

| N level | Month |       |       |       |        | Mean  |
|---------|-------|-------|-------|-------|--------|-------|
|         | March | April | June  | July  | August |       |
| N 0     | 9.7   | 11.1  | 19.4  | 13.1  | 41.6   | 19.0a |
| N 50    | 7.7   | 14.1  | 20.2  | 42.7  | 33.9   | 23.7a |
| N 100   | 8.3   | 10.3  | 16.9  | 29.9  | 27.5   | 18.6a |
| N 150   | 9.9   | 11.0  | 14.0  | 31.6  | 51.4   | 23.6a |
| N 200   | 12.2  | 12.1  | 12.9  | 21.1  | 29.3   | 17.5a |
| Mean    | 9.6b  | 11.7b | 16.7b | 27.7a | 36.7a  |       |

In fertile soils the level of *Actinomycetes* is usually lower than that of bacteria (the ratio of bacteria and *Actinomycetes* counts is then 60 : 40). In most analyzed samples the determined content of bacteria was higher than that of *Actinomycetes*, except for the 4th and 5th dates in combinations N 0 and N 50, where the population of *Actinomycetes* dominated over bacteria, which could have resulted from the too low nitrogen content in soil.

Another group of microorganisms, which counts were determined in the course of this experiment, comprised oligotrophic bacteria. They are microorganisms, which grow well in a medium with an exceptionally low level of available organic compounds. Optimal concentration of nutrients for oligotrophic bacteria ranges from 1 to 15 mg dissolved C dm<sup>-3</sup>. The term oligotrophy refers to bacteria, which grow on a poor medium, with a low nutrient concentration only at the onset of culture [18]. They are bacteria exhibiting low variability in terms of their population size and activity. These organisms are highly sensitive to amino acids, organic acids, vitamins and inorganic salts, such as NaCl and KCl [19].

In the conducted experiment the amount of oligotrophic bacteria in soil underwent significant periodical changes within a year. It was highest at the beginning of the spring period (in March), and next it decreased significantly (Table 4).

Considerable amounts of root exudates of grasses, which could have contained amino acids, carbohydrates, vitamins, organic acids, enzymes and metal ions most probably could have inhibited the growth and development of analyzed oligotrophic bacteria.

An upward trend could be observed, although not proved statistically, for the count of oligotrophic in soil to increase in case of combinations intensively fertilized with nitrogen. Their mean content ranged from 78.9 (N 0) to 128.9 CFU  $\times 10^6$  (N 150).

Table 4

The effect of varied nitrogen fertilization on oligotrophic bacteria count (CFU  $\times 10^6$  g<sup>-1</sup> d.m. of soil)

| N level | Month  |        |       |       |        |        |
|---------|--------|--------|-------|-------|--------|--------|
|         | March  | April  | June  | July  | August | Mean   |
| N 0     | 232.0  | 63.0   | 32.0  | 11.0  | 56.6   | 78.9a  |
| N 50    | 118.0  | 172.0  | 83.6  | 52.3  | 68.0   | 98.8a  |
| N 100   | 195.0  | 119.0  | 86.3  | 85.3  | 103.3  | 117.8a |
| N 150   | 203.0  | 170.0  | 47.6  | 103.3 | 120.6  | 128.9a |
| N 200   | 229.0  | 129.0  | 25.0  | 69.6  | 132.0  | 116.9a |
| Mean    | 195.4a | 130.6b | 54.9c | 64.3c | 96.1c  |        |

What is more, no significant effect was found of nitrogen fertilization on total count of copiotrophic bacteria in soil (Table 5). Their content ranged from 36.5 (N 100) to 59.6 CFU  $\times 10^6$  (N 150). Similarly as in case of oligotrophic bacteria, the content of copiotrophic bacteria was highest in the spring period (March, April) and next it was significantly reduced with the time of vegetation. It is a specific group of soil microorganisms proliferating intensively during the influx of organic matter to soil, mainly in the form of fresh plant and animal residue. Their nutrient requirements are thus connected with high concentrations of organic components in the substrate, which optimal dose is approx. 1000 mg dissolved C dm<sup>-3</sup> [20]. The population size of oligotrophic bacteria decreases relatively fast after the readily available nutrient substrate is depleted, as a result of autolysis of most cells or the other cells entering the latent phase.

Table 5

The effect of varied nitrogen fertilization on copiotrophic bacteria count (CFU  $\times 10^6$  g<sup>-1</sup> d.m. of soil)

| N level | Month |       |       |       |        |       |
|---------|-------|-------|-------|-------|--------|-------|
|         | March | April | June  | July  | August | Mean  |
| N 0     | 94.0  | 101.3 | 15.3  | 6.0   | 27.6   | 48.8a |
| N 50    | 63.4  | 78.0  | 30.0  | 7.6   | 25.0   | 40.8a |
| N 100   | 77.6  | 42.6  | 22.3  | 10.6  | 29.3   | 36.5a |
| N 150   | 73.0  | 184.6 | 7.0   | 15.3  | 18.0   | 59.6a |
| N 200   | 95.3  | 61.3  | 6.6   | 17.3  | 31.6   | 42.4a |
| Mean    | 80.7a | 93.6a | 16.2b | 11.4b | 26.3b  |       |

Assuming that metabolic activity of microorganisms is manifested in the activity of their enzymes, changes in the activity of dehydrogenases in soil were considered in the experiment. Their level of activity determines the rate of redox changes in the soil medium, which characterizes a given soil, being a measure of its fertility [21]. No significant effect was observed in the conducted analyses of nitrogen fertilization on enzymatic activity of dehydrogenases in soil, which was similar in all tested combinations (Table 6). However, a significant increase in enzymatic activity of dehydrogenases

was recorded in the summer (in July). In the opinion of Sinsabaugh et al [22], activity of enzymes is connected not only with a given plant species or plant development phase, but it also depends on the amount of plant residue, the depth of root systems and temperature. Pawluczuk [23] also confirmed a significant effect of changes in soil temperatures on occurring enzymatic processes. In turn, Amdor et al [24] stressed a strong relationship between activity of enzymes and soil properties (pH, organic carbon content, etc.).

Table 6

The effect of varied nitrogen fertilization on enzymatic activity of dehydrogenases

| N level | Month  |        |        |        |        |        |
|---------|--------|--------|--------|--------|--------|--------|
|         | March  | April  | June   | July   | August | Mean   |
| N 0     | 0.013  | 0.004  | 0.004  | 0.017  | 0.003  | 0.008a |
| N 50    | 0.004  | 0.003  | 0.004  | 0.021  | 0.006  | 0.008a |
| N 100   | 0.012  | 0.003  | 0.007  | 0.099  | 0.005  | 0.025a |
| N 150   | 0.008  | 0.008  | 0.009  | 0.021  | 0.007  | 0.011a |
| N 200   | 0.008  | 0.005  | 0.013  | 0.060  | 0.009  | 0.019a |
| Mean    | 0.009b | 0.005b | 0.007b | 0.043a | 0.006b |        |

Regular microbiological changes in soil, apart from the quantitative composition of the microbial community composition, are also shown by the interrelations between their individual groups. The following microbiological indexes are considered:

- A** the quantitative ratio of bacteria to *Actinomycetes*;
- B** the ratio of total count of bacteria to that of fungi;
- C** the quantitative ratio of oligotrophic to copiotrophic bacteria.

In fertile soils bacteria predominate in terms of their number over *Actinomycetes* (ratio **A** = 60 : 40). In most analyzed combinations the determined count of bacteria was higher than that of *Actinomycetes* (apart from the 4th and 5th date in combinations N 0 and N 50). Based on the above, it may be stated that soil in case of combinations intensively fertilized with nitrogen was characterized by good fertility. An increased number of *Actinomycetes* over bacteria in the above-mentioned combinations could be explained by a reduced amount of nutrients as well as inferior water and air relations. In poor soils higher contents of *Actinomycetes* may be found, since soil quality is one of the factors having a selective effect on them.

In the opinion of some researchers [25, 26] another microbiological index of soil fertility (**B**), based on the ratio of fungi to the total bacterial count, expresses more accurately biological properties of soil than the count of each of these groups separately. Moreover, it indicates compensatory dependencies in the development of communities of bacteria and fungi, also observed by other authors (Weyman-Kaczmarek and Pedziwilk) [27]. Dominance of fungi over bacteria shows a higher ability of fungi, than that of bacteria, to survive under deteriorating environmental conditions. Such an undesirable phenomenon was not recorded in the discussed experiment.

Another significant index of microbiological activity of soil is also the ratio of oligotrophic to copiotrophic bacteria (**C**). It is an index of biological balance, indicating

an appropriate direction of microbiological changes of soil organic matter. In the conducted experiment in all combinations the proportion of oligotrophic bacteria predominated in the soil microflora. The above dominance is appropriate and necessary for the maintenance of a constant level of organic matter.

Mineral nitrogen is utilized by many bacteria and fungi in their metabolism. By uptake of ammonium and nitrate ions they affect the acidity of the external medium. More readily available ammonium salts contribute to the acidification of the substrate, while nitrates cause its alkalization. Organic nitrogen is most readily available in the form of amino acids, which are used as a source of nitrogen and energy material. Certain microorganisms absorbing nitrogen require only those amino acids, which they are not capable of synthesizing (this concerns mainly auxotrophic microorganisms).

## Conclusions

1. No differentiating effect was found of fertilization with nitrogen on total counts of fungi, bacteria, *Actinomycetes*, copiotrophic and oligotrophic bacteria in soil as well as the enzymatic activity of dehydrogenases. A trend for the microbial count to increase could be observed, although not proved statistically, in case of combination N 150.

2. A factor significantly modifying the levels of microorganisms and dehydrogenases activity in soil was sampling date and related atmospheric conditions.

3. A significant increase of the total bacterial count in soil was determined in spring (April), while that of *Actinomycetes* – towards the end of the summer period (August). The highest enzymatic activity of dehydrogenases was recorded for soil in July. With the duration of vegetation the contents of copiotrophic and oligotrophic bacteria in soil decreased.

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CZ. III. DYNAMIKA SKŁADU MIKROBIOLOGICZNEGO GLEBY.  
AKTYWNOŚĆ ENZYMATYCZNA DEHYDROGENAZ**

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**Abstrakt:** Celem przeprowadzonych badań było określenie wpływu wzrastającego nawożenia azotowego pod trawnikiem (w dawkach odpowiadających: 0, 50, 100, 150 i 200 mg N dm<sup>-3</sup> gleby) na dynamikę zmian składu mikrobiologicznego gleby: ogólnej liczby grzybów, bakterii, promieniowców, oligotrofów i koptotrofów, a także aktywność enzymatyczną dehydrogenaz. Nie stwierdzono znaczącego wpływu badanych poziomów nawożenia azotowego na liczebność mikroorganizmów i aktywność enzymatyczną gleby. Zarysowała się, nie udowodniona statystycznie, tendencja do wzrostu liczebności mikroorganizmów w przypadku kombinacji N 150. Czynnikiem istotnie modyfikującym skład mikrobiologiczny i aktywność enzymatyczną gleby był termin pobierania próbek i związane z nim warunki atmosferyczne. Znaczący wzrost ogólnej liczby bakterii

stwierdzono w II terminie pobierania próbek (w kwietniu). Liczba promieniowców znacznie wzrastała pod koniec okresu letniego (sierpień), a największą aktywność enzymatyczną dehydrogenaz oznaczono w lipcu. Wraz z trwaniem wegetacji wyraźnie obniżała się zawartość koptotrofów i oligotrofów w glebie.

**Słowa kluczowe:** żywienie azotem, aktywność dehydrogenaz, grzyby, bakterie, oligotrofy i koptotrofy