

Effect of new lines of winter wheat on microbiological activity in Luvisol

S. Jezierska-Tys¹*, L. Rachon², A. Rutkowska³, and G. Szumilo²

¹Department of Agricultural Microbiology, University of Life Sciences, Leszczyńskiego 7, 20-069 Lublin, Poland

²Department of Detailed Cultivation of Plants, University of Life Sciences, Akademicka 15, 20-069 Lublin, Poland

³Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland

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A b s t r a c t. The study presented in this paper was conducted under the conditions of a field experiment. Microbiological analyses were made at various stages of winter wheat plants development *ie* heading, milk ripeness and full ripeness. The objective of the study was to acquire knowledge on the effect of cultivation of various lines of winter wheat on the numbers of bacteria and fungi with proteolytic capabilities, on protease and urease activity, and on the rate of the processes of ammonification and nitrification.

The results of conducted study demonstrated that the number of proteolytic bacteria and fungi, as well as the activity of protease and urease, and the intensity of ammonification and nitrification processes in soil depended on both the development stage and cultivated line of winter wheat.

K e y w o r d s: soil, bacteria, fungi, protease, urease, ammonification, nitrification

INTRODUCTION

Soil is inhabited by huge number of microorganisms – bacteria, fungi, actinomycetes and algae. In the soil environment microorganisms tend to concentrate in the plant roots zone and the humus horizon, due to the presence of available source of nutrients (Gajda, 2010).

In the root zone mutual interactions take place between plants and microorganisms (Singh *et al.*, 2004; Stottmeister *et al.*, 2003). Enzymes secreted out of and into cells are catalysts of biochemical processes in soil, and play an important role in the circulation of nutrients and mineralization of soil organic matter (Böhme and Böhme, 2006; Simon, 2002; Trásar-Cepeda *et al.*, 2000). Therefore, both the size of microbial populations and their enzymatic activity are good indicators of changes caused by biotic and abiotic factors (Acosta-Martínez *et al.*, 2007; Alkorta *et al.*, 2003; Colombo, 2002;

Emmerling *et al.*, 2002; Janvier *et al.*, 2007). Soil microorganisms play a highly important role in the transformation of nitrogen compounds, including the processes of ammonification and nitrification, among others (Nannipieri *et al.*, 2003; Sorensen, 2001). The intensity of these processes, is considered to be a very important indicator of occurred disturbances in soil biological activity (Quilchano and Maranon, 2002; Shi *et al.*, 2004). The content of nitrate and ammonium ions constitutes an important link in the balance of nitrogen in the soil environment (Simon, 2002).

The study was aimed to determined the effect of cultivation of new lines of winter wheat on populations of proteolytic bacteria and fungi, the activity of enzymes-protease and urease and the intensity of ammonification and nitrification processes in Luvisol.

MATERIAL AND METHODS

A field experiment for comparing of the effect of cultivation of various lines of winter wheat on selected groups of soil microorganisms and their activity was set up the random blocks design, on Luvisols developed from loess formations, with the particle size distribution of medium loam, classified in the good wheat complex. Experiments were carried out in 2009-2010. The model of the experiment, established at the Felin Experimental Farm of the University of Life Sciences in Lublin, comprised 5 treatments in 4 replications. The experimental treatments were as follows:

1. Common winter wheat (*Triticum aestivum ssp. Vulgare*): cv. Tonacja.
2. Line of durum winter wheat (*Triticum durum* Desf.): STH 716.

*Corresponding author's e-mail: stefania_tys@op.pl

3. Line of durum winter wheat (*Triticum durum* Desf.): STH 717.
4. Line of spelt winter wheat (*Triticum spelta* L.): STH 3.
5. Line of spelt winter wheat (*Triticum spelta* L.): STH 715.

The common winter wheat cultivar Tonacja (treatment 1) was constituted as the control treatment in the experiment.

As Table 1 shows the monthly rainfall and monthly mean air temperatures during the growing season. In the experiment NPK fertilization was used according to Polish cultivation requirements. The chemical prophylactic protection of wheat plants against the most common dicotyledonous weeds and pathogenic fungi was applied. Soil samples for analyses were taken from the arable horizon periodically on every experimental plot during the chosen wheat plants development stages-heading, milk ripeness and full maturity.

Microbiological analyses of soil samples included: determination of number of proteolytic bacteria and fungi on the Frazier medium with gelatin (Rodina, 1968); the intensity of ammonification and nitrification processes according to Polish Standard PN-ISO (2000)- the determination of N-NH_4^+ concentration using the Nessler method, the determination of N-NO_3^- concentration using the brucine method (Nowosielski, 1981); the measurement of protease activity using the Ladd and Butler (1972) method as modified by Alef and Nannipieri (1995), and urease activity using the Zantua and Bremner (1975) modified method.

Two-factor analyses of variance (ANOVA) were performed to examine the effect of the new winter wheat lines and the terms of analyses on the values of microbiological and biochemical parameters studied. Mean values calculated for the examined parameters for the experimental treatments and the terms of analyses were compared using Tukey 95% intervals of confidence at significance level $P = 0.05$.

Table 1. Monthly rainfall and mean air temperatures during the growing season. The source data: Data from the meteorological station in Felin

Month	Rainfall (mm)	Mean air temperatures (°C)
2009		
September	21.0	15.3
October	103.6	6.9
November	43.1	5.5
December	37.7	-1.7
2010		
January	35.6	-8.2
February	34.6	-2.0
March	18.6	3.2
April	24.5	9.4
May	156.7	14.5
June	65.6	18.0
July	101.0	21.6
August	132.8	20.6

RESULTS AND DISCUSSION

Data presented in Fig. 1a show that the greatest numbers of proteolytic bacteria were found in the control soil (treatment 1) under winter wheat cv. Tonacja sampled during the plant development stage of heading. Also, in the control soil sampled during the stage of full maturity of wheat plants the numbers of the microbial groups studied were the greatest. Analysis of the mean values calculated for the numbers of proteolytic bacteria for the particular treatments (Fig. 1b) reveals that treatment 1 (cv. Tonacja) was characterized by the highest value of that parameter. The lowest number of proteolytic bacteria was recorded in treatment 3 (line STH 717). This trend was supported by results obtained in analyses performed in the heading, milk ripeness and full ripeness stages of wheat plants development (Fig. 1a). In the other experimental treatments the numbers of proteolytic bacteria oscillated around similar values, which were confirmed by the mean values obtained for these particular treatments (Fig. 1b).

Changes in the numbers of proteolytic fungi in soil under winter wheat during the period of the experiment are presented in Fig. 2a. The highest numbers of proteolytic fungi were observed in the soil under common winter wheat cv. Tonacja (treatment 1) sampled during the stage of milk ripeness of wheat plants (2nd term of analysis). The lowest numbers of proteolytic fungi were recorded in treatment 3 (line STH717) in the stages of heading and full maturity. Analysis of the mean value calculated for the population of fungi for the experimental treatments (Fig. 2b) revealed the lowest value of the parameter in treatments 3 (line STH717) and 5 (line STH715), and that value was significantly lower than that obtained in the control treatment (cv. Tonacja). In the other experimental treatments the numbers of proteolytic fungi oscillated around a similar value, though notably higher than that in treatments 3 and 5, which was confirmed by the mean values obtained for the other treatments.

The results of the study indicate that changes in the numbers of proteolytic bacteria and fungi in soil were strongly influenced by the wheat plants development stage. Similar results were reported by the other authors *ie* Fraç and Jezierska-Tys (2008).

Analysis of the activity of soil enzymes related to the biological processes taking place in soil provides an information concerning the specific metabolic activity and the function of the assemblage of soil microorganisms. Measurements of the activity of soil enzymes can be utilised for the assessment of changes in soil quality under various land use conditions, also.

Proteolytic activity, the preliminary phase of mineralization of organic nitrogen, is significant from the viewpoint of nitrogen nutrition of plants.

Changes in protease activity in the experimental treatments studied are presented in Fig. 3a. Analysis of the results indicated that over the whole vegetation period a variation in

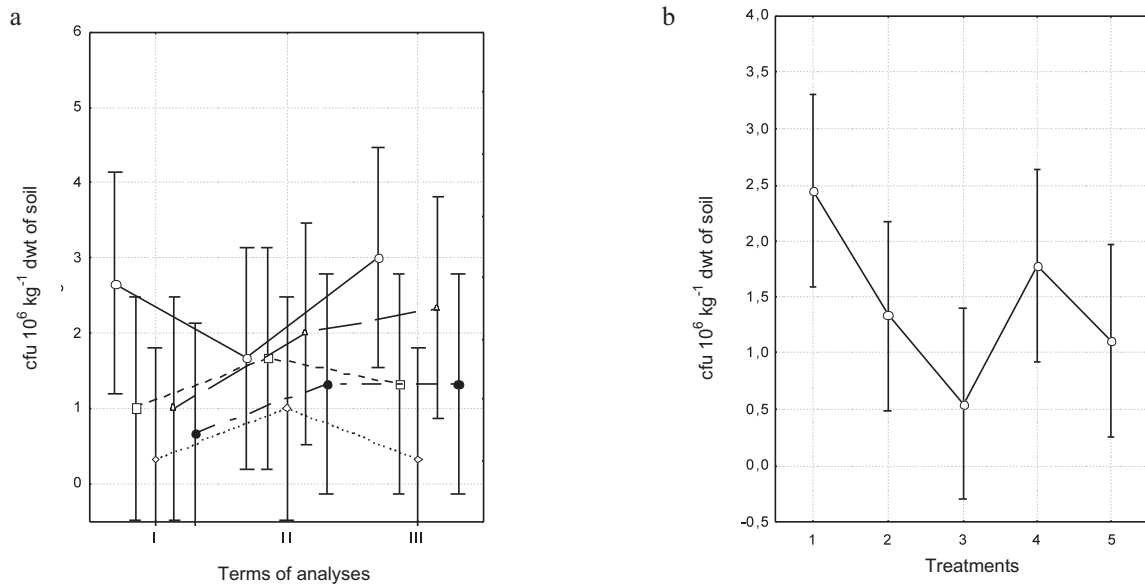


Fig. 1. Numbers (a) and mean numbers (b) of 'proteolytic' bacteria: ○ – treatment 1, □ – treatment 2, ◇ – treatment 3, △ – treatment 4, ● – treatment 5. Terms of analyses: I - heading stage, II - milk stage, III - full maturity stage of wheat plants development. Experimental treatments: 1- Winter cultivar of common wheat (*Triticum aestivum* ssp. *Vulgare*): Tonacja, 2- Line of durum winter wheat (*Triticum durum* Desf.): STH 716, 3- Line of durum winter wheat (*Triticum durum* Desf.): STH 717, 4- Line of spelt winter wheat (*Triticum spelta* L.): STH 3, 5- Line of spelt winter wheat (*Triticum spelta* L.): STH 715. Vertical columns denote 0.95 confidence interval.

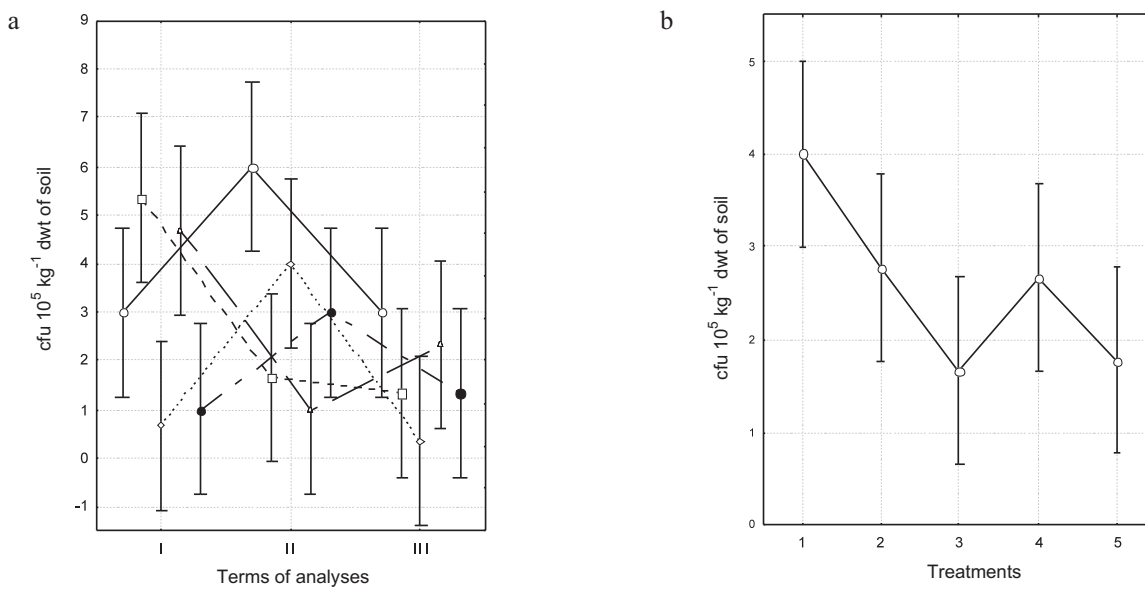


Fig. 2. Numbers (a) and mean numbers (b) of 'proteolytic' fungi. Explanations as in Fig. 1.

protease activity could be observed. The highest activity of the protease was found in soil under the winter wheat line STH715 (treatment 5) sampled during the development stages of milk and full ripeness. Analysis of the mean values for the particular experimental treatments confirmed the highest activity of protease in treatment 5. Also in the soil under wheat lines STH716 (treatment 2) and STH3

(treatment 4) the protease activity was significantly higher as compared to treatments 1 and 3. Analysis of mean values confirmed the lowest activity of protease in the treatment 3 (Fig. 3b). In the other experimental treatments with the wheat lines STH716 and STH3 measurements of proteolytic activity were on a similar level but significantly higher than proteolytic activity measured in treatments 1 and 3.

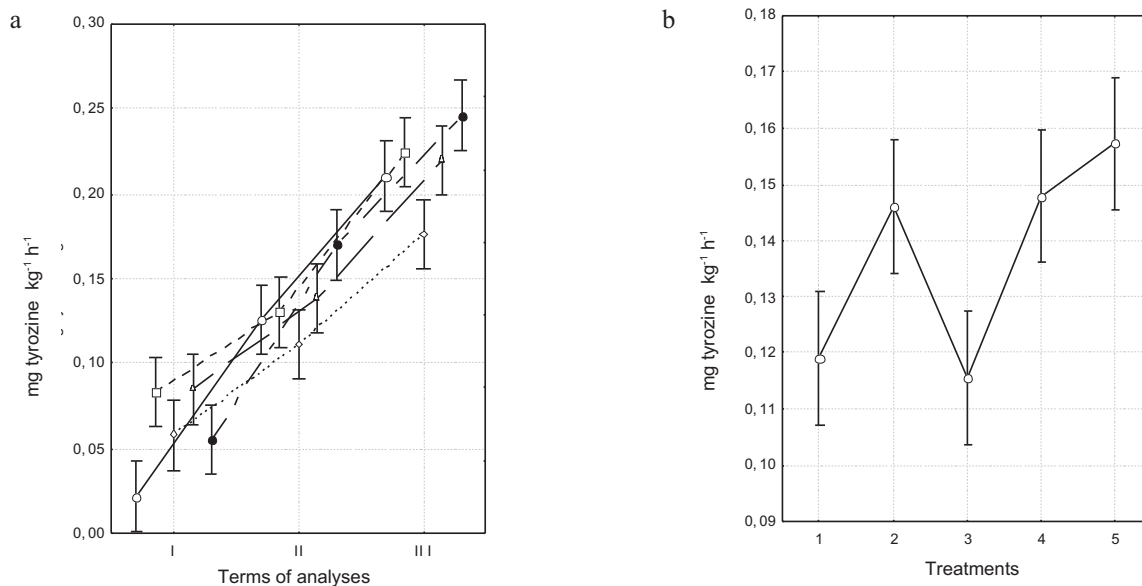


Fig. 3. Protease (a), mean proteolytic (b) activities. Explanations as in Fig. 1.

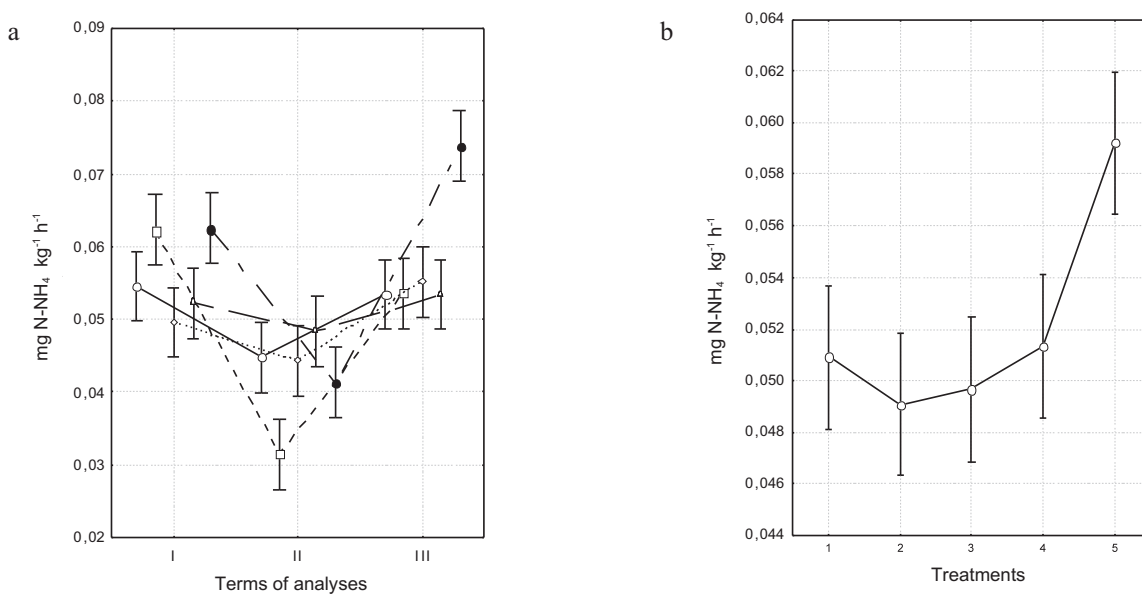


Fig. 4. Urease activity (a), mean activity of urease (b). Explanations as in Fig. 1.

The results obtained clearly indicate that proteolytic activity in the experimental treatments displayed a permanent increasing tendency over the duration of the experiment. This was probably related to the availability of organic matter in soil for proteolytic microorganisms. The enzyme urease belongs to the group of hydrolases. It is an enzyme that is characterized by high specificity, as it catalyses only one reaction – the decomposition of urea to ammonia and carbon dioxide.

The results of the study concerning urease activity are presented in Fig. 4a. The highest activity of urease was observed in soil under wheat line STH715 (treatment 5) sampled during

the stage of full ripeness and the lowest in soil under wheat line STH716 sampled in the stage of milk ripeness (treatment 2). Analysis of mean values for all experimental treatments (Fig. 4b) confirmed the highest urease activity in the soil under wheat line STH715 (treatment 5), while in the other four experimental treatments the activity of the enzyme was at a similar level, though significantly lower than in treatment 5. The data obtained indicated that changes in urease activity in soil were strongly affected by wheat plants development stages, and by the cultivated lines of winter wheat. Also Yang *et al.* (2008) observed the relation between the activity of urease in soil and plant development stage.

The high level of activity of the enzymes in the spring season may be attributed to atmospheric conditions which may have had a favourable effect on the biological activity of the soil. Other authors *eg* Gianfreda and Ruggiero (2006) reported that the enzymatic activity of soil can be affected by natural environmental factors.

The end-products of proteolysis – amino acids – are substrates for further microbiological transformations that involve the released ammonia. Ammonium nitrogen, both formed in the process of ammonification and introduced into soil with mineral fertilizers, constitutes an easily available source of nitrogen for plants and auto- and heterotrophic bacteria. Under aerobic conditions, ammonium nitrogen is oxidized to nitrates by nitrifying bacteria.

Figure 5a presents the intensity of the process of ammonification in soil under winter wheat. Analysis of the data showed that in the stages of heading and milk ripeness

of wheat development the rate of the process of ammonification in the soil was at a fairly uniform level in all experimental treatments.

The highest intensity of ammonification was observed in the stage of full maturity in the soil under the common winter wheat cultivar Tonacja (treatment 1) and under the durum winter wheat line STH717 (treatment 3). Analysis of mean values for the particular experimental treatments also confirmed the highest intensity of the process in treatments 1 and 3. In the other three treatments the rate of ammonification was at a similar level, lower than in treatments 1 and 3.

Data presented in Fig. 6a indicate that the highest intensity of the nitrification process was observed in soil under winter wheat line STH3 (treatment 4) in all plants development stages. Analysis of the mean values for the process confirmed that treatment 4 was characterized by the highest intensity of nitrification.

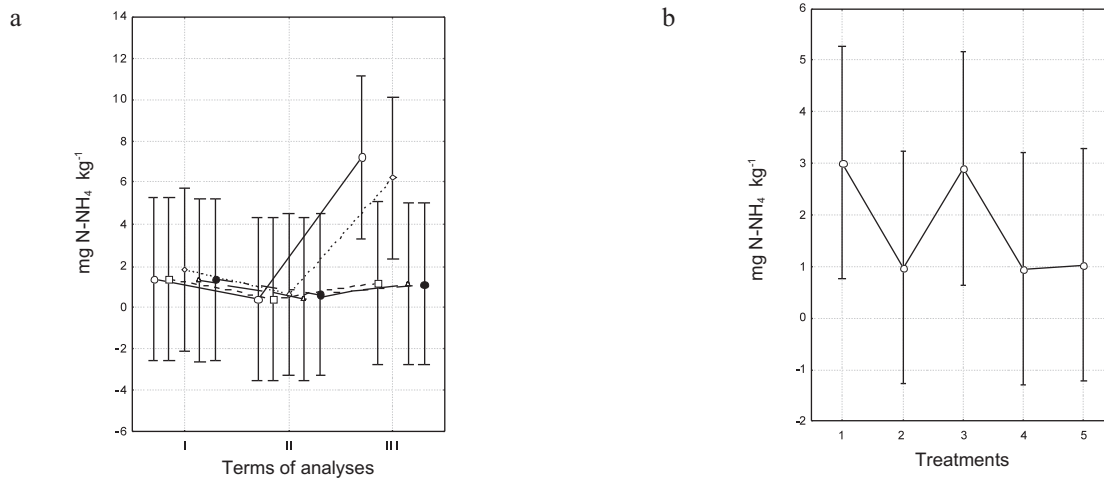


Fig. 5. Intensity (a) and analysis of mean values (b) of the process of ammonification. Explanations as in Fig. 1.

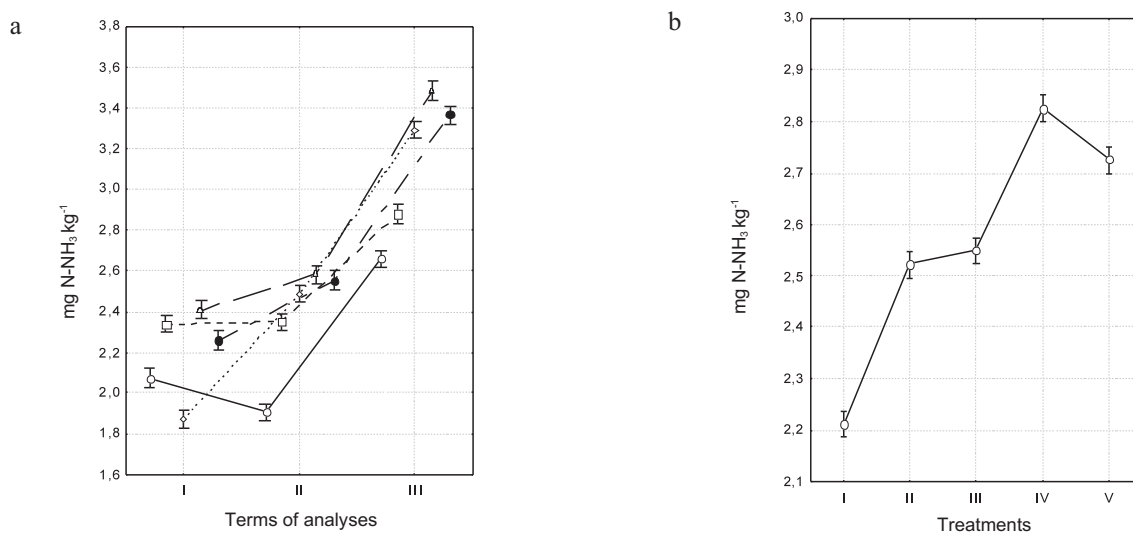


Fig. 6. Intensity (a) and analysis of mean values (b) of the process of nitrification. Explanations as in Fig. 1.

The obtained results for nitrification clearly indicated higher intensity of the process in soil under cultivated lines of winter wheat as compared to the control soil under common winter wheat cv. Tonacja (treatment 1) in all plants development stages.

CONCLUSIONS

1. The changes of the number of proteolytic bacteria and fungi number in Luvisol depended on both the wheat plants development stage and cultivated line of winter wheat.

2. The activity of protease and urease as well as the intensity of ammonification and nitrification processes displayed a high seasonal variability.

3. The highest protease activity was noted in soil under winter wheat lines STH716, STH3 and STH715 (treatments 2, 4, 5), and urease activity under the line STH715 (treatment 5).

4. No interference with the transformation of mineral nitrogen in the soil was observed. It was no significant influence of winter wheat cultivars observed on transformation processes of mineral nitrogen in experimental treatments.

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