Biological soil activity when applying liquid manure on rice fields in Kuban

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Abstract. The research was aimed at studying the effect of liquid manure application on biological activity of meadow-chernozem soil (GleyicChernozem) when growing rice on the Maryano-Cheburgol irrigation massif of the rice irrigation system of the Krasnoarmeisky district of the Krasnodar Territory. The experimental scheme included the following options: without fertilizers (control); rice cultivation technology on a farm; autumn manure pumpapplication (30 t/ha); autumn manure pump application (30 t/ha) + Entec FL DMPP nitrification inhibitor; spring manure pumpapplication (30 t/ha); spring manure pumpapplication (30 t/ha) + Entec FL DMPP nitrification inhibitor. It was found that the autumn application of liquid manure into the soil combined with the Entec nitrification inhibitor contributed to an increase in the amount of CO2 emitted from the soil by 0.031–0.038 mg CO2 kg⁻¹ day⁻¹; inactivation of urease in the germination phase and its activation during rice tillering, which ensured a longer preservation of nitrogen in the soil; an increase in catalase activity during the period of maximum peroxide formation (in the rice flowering phase) by 11–29% and invertase activity by 2.3–7.5% compared with other options fertilized with manure.

1. Introduction

The soils used for sowing rice are kept in a flooded state for 3–4 months. Oxidizing conditions change to reduced ones sharply, the nature of the biochemical processes occurring in the soil changes [1–3]. Rice soils flooding causes a sharp change in the water, air and thermal regimes of the soil, and, consequently, in the conditions of life of microorganisms. First of all, oxygen consumption and reduction of NO3-, Mn3+ and Fe3+ takes place, then sulphides are formed, hydrogen and methane fermentation develops [4]. In accordance with this, quantitative changes in microflora and a change in aerobic and anaerobic groups of microorganisms occur.

Biological activity is directly related to the degree of soil moisture. When soil hydromorphism increases, the respiration rate and catalase activity decrease, while the activities of invertase, phosphatase, and ferri-reductase increase. When soils are flooded, dehydrogenase activity is suppressed, and it is activated when the environment is alkaliized [4, 5].

Application of mineral and organic fertilizers effects the activity of soil enzymes [6–8]. According to Y. Liang, organic fertilizers significantly increase the activity of alkaline phosphatase and urease in
the rice fields soils in China, formed on alluvial and marine deposits, and also increase the intensity of its respiration [9]. The positive effect of organic fertilizers on the activity of enzymes in the soil of rice fields is shown in the following works [7, 10, 11].

The abovementioned determined the purpose of the work, i.e. to study the effect of applying liquid manure on the biological activity of meadow-chernozem soil during rice cultivation.

2. Objects and methods
The research has been carried out on the Maryano-Cheburgolsky irrigation area of the rice irrigation system of the Krasnoarmeiskiy district of the Krasnodar Territory. The territory of the region is included in the Kuban delta-floodplain region in terms of geomorphological zoning, and belongs to the Azov-Ciscaucasian soil province of the steppe zone of ordinary and southern chernozems, and the soil district of the lower reaches of the Kuban River with the distribution of meadow-steppe and meadow soils [12].

The soil cover of the territory of the Region is represented mainly by meadow-chernozem soil [13] (corresponding to Gleyic Chernozem in WRB [14]), located on ridges and ridge-like near-river rises of the flooded plain. The soil is light clayey with a well-pronounced differentiation of genetic horizons, a high thickness of the humus horizon (114 cm), the presence of hydromorphic features (streaks of rust, ocher spots, black dots of manganese oxide, sulphide spots), carbonate new formations below the arable horizon. The parent rock is alluvial light clays. Groundwater occurs at a depth of 1.60–2.20 m [12].

Agrochemical characteristics of meadow-chernozem soil are the following: pH\textsubscript{H2O} = 6.6; humus – 3.3%; N\textsubscript{tot} – 0.22%; C\textsubscript{HA} : C\textsubscript{FA} = 1.86; P\textsubscript{2}O\textsubscript{5} – 39.5 mg/kg; K\textsubscript{2}O – 240.0 mg/kg.

The objects of the research are liquid manure and Entec FL DMPP nitrification inhibitor.

Manure pump is a nitrogen-potassium fertilizer with nitrogen content of 0.2–0.3%, potassium of 0.4–0.5% and a small amount of phosphorus – 0.01%. Nitrogen is contained in the form of urea [CO(NH\textsubscript{2})\textsubscript{2}], which is rapidly converted into ammonium carbonate [(NH\textsubscript{4})\textsubscript{2}CO\textsubscript{3}] under the influence of urobacteria, which decomposes into CO\textsubscript{2}, H\textsubscript{2}O and NH\textsubscript{3}.

Entec FL nitrification inhibitor is an inhibitor of DMPP ammonium nitrogen (3,4-dimethylpyrazole phosphate), it slows down the process of nitrification and stabilizes the ammonium form of nitrogen in the soil. The principle of Entec FL action is to block the enzyme of Nitrosomonassoil bacteria – ammonium monooxygenase. When it is blocked, the first stage of the nitrification process, i.e. the oxidation of ammonium nitrogen to nitrate one, is inhibited. The Nitrosomonas bacteria are not affected in this case. As a result, the ammonium nitrogen from fertilizers remains in the soil for a long time. Fertilizer containing Entec inhibitor suppresses the nitrification process from 20 to 65 days and increases the absorption period of ammonium nitrogen by 1.5–2.0 months and to reduce the loss of nitrogen in gaseous form twofold, depending on the method of application, as well as due to the washout of nitrates with water.

To study the biological activity of the soil on the rice irrigation system, a field experiment was laid. Manure pump was applied from autumn and spring in the amount of 30 t/ha. Entec FL nitrification inhibitor was added to the manure pump tank and mixed well. On the day of application, the pump was embedded in the soil with heavy discs to the depth of 8–10 cm. N\textsubscript{37}P\textsubscript{32} ammo phos was used as a main fertilizer; N\textsubscript{36}Carbamide was additionally applied in the technology of rice cultivation adopted on the farm. Fertilizing was carried out with carbamide in the germination and tillering phases of rice at doses of N\textsubscript{36} and N\textsubscript{37}, respectively.

Experimental design:
1. Control (without fertilizers).
2. Technology of rice cultivation on the farm.
3. Manure pump, 30 t/ha (autumn).
4. Manure pump, 30 t/ha + Entec FL nitrification inhibitor (autumn).
5. Manure pump, 30 t/ha (spring).
6. Manure pump, 30 t/ha + Entec FL nitrification inhibitor (spring).

Soil samples were selected before sowing, according to vegetation phases and after harvesting rice from the topsoil (0 ... 20 cm). They determined:
«the intensity» of breathing according to A. Sh. Galstyan. The method is based on taking into account the quantitative changes in the carbon dioxide content in a certain enclosed space. Carbon dioxide released during breathing is captured by an excess of alkali solution during the reaction of CO₂ + 2NaOH = Na₂CO₃ + H₂O. The rest of the alkali is titrated with acid. The resulting value allows one to judge the amount of carbon dioxide that has reacted with the alkali. The count of CO₂ respiration by soil is carried out by setting up a laboratory experiment in four replicates [15].

– the catalase activity according to A. Sh. Galstyan. The method is based on measuring the rate of hydrogen peroxide decomposition during its interaction with the soil by the volume of the released oxygen (the gasometric method) [15].

– the invertase activity according to V. F. Kuprevich and T. A. Shcherbakova. The method is based on accounting reducing sugars formed during the sucrose splitting [15].

– the urease activity according to A. Sh. Galstyan. The method is based on accounting the amount of ammonia formed during the carbamide hydrolysis [15].

The enzyme activity was expressed in units of enzymatic action, which presented the quantitative changes produced by enzymes in the substrate in a certain period of time under strictly defined conditions, such as substrate concentration, pH, temperature, etc. To compare different soils in terms of enzymatic activity, rating scales were used [16].

The measurements of the indicators of the biological activity of the soil were carried out in four replicates. The statistical evaluation of the research results was carried out using Microsoft Excel. The arithmetic mean and standard deviation (σ) were calculated [17].

3. Results and discussion

The soil respiration rate is propelled by its biological activity and determined by the amount of oxygen consumed and the amount of carbon dioxide produced. Vital activity of microorganisms and soil fauna, respiration of roots, enzymatic activity, physicochemical and chemical processes are the main source of CO₂ in the soil [5]. «Microbial respiration» of fungi and bacteria constitute more than 95% of the pure soil source of CO₂ (without roots), respiration of soil animals, primarily earthworms account for the remaining 5% [18].

Before sowing period, the meadow-chernozem soil respiration rate reached 0.249 ± 0.029 mg CO₂ kg⁻¹ day⁻¹. After the soil was flooded, which is typical for the rice vegetation period, this indicator dropped to 0.199 ± 0.023 in the control without fertilizers and 0.206–0.237 (σ = 0.021–0.026) mg CO₂ kg⁻¹ day⁻¹ in options where fertilizers were used. The use of manure pump, both separately and combined with a nitrification inhibitor, increased the biological activity of the soil. Autumn manure application increased the amount of CO₂ emitted from the soil by 0.029 mg compared with the control after harvesting time and by 0.022 mg compared with the application of mineral fertilizers (farming technology). When a nitrification inhibitor was added to the manure, the soil respiration rate increased by 0.031–0.038 mg CO₂ kg⁻¹ day⁻¹, respectively. The spring manure application, both separately and combined with a nitrification inhibitor, also increased the intensity of CO₂ soil respiration, but to a lesser extent than their autumn application. It should be noted that the addition of a nitrification inhibitor to the manure, regardless of the time of application, increased the soil microbiological activity – carbon dioxide respiration rates increased relative to the control by 17–19% (figure 1).

The soil contains various exo- and endoenzymes released after cell lysis. The enzymes secreted in the soil remain active for a long time. They determine the intensity and direction of biochemical processes in the soil. Enzymatic activity can serve as an indicator of biological activity and soil fertility [5].

Anaerobic conditions caused by soil flooding inhibited the reactions of sugar hydrolysis catalyzed by invertase, as well as the reactions of oxidative metabolism carried out by catalase. At the same time, under anaerobic conditions dehydrogenase, nitrate reductase, nitrite reductase and ferri-reductase were activated – the enzymes involved in the oxidation of organic substances, reduction of nitrates, nitrites and iron oxides, respectively. The enzymatic activity was assessed for the topsoils. The catalase was determined from oxidoreductases catalyzing redox processes in soils to play a huge role in biochemical
reactions. The invertase and urease were determined from enzymes belonging to the class of hydrolases to catalyze the reactions of hydrolytic decomposition of high-molecular organic compounds that enrich the soil with mobile nutrients.

The catalase activity is one of the characteristic indicators of soil biological activity. It is widely present in the cells of living organisms, including microorganisms and plants. The catalase is widespread in different types of soils and belongs to the number of respiratory enzymes, inherent in all aerobic and optionally anaerobic microorganisms. The result of its activity is the splitting of hydrogen peroxide into water and molecular oxygen: \(2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2\). Hydrogen peroxide is formed during the respiration of living organisms and as a result of various biochemical reactions of oxidation of organic substances. The role of catalase is to destroy hydrogen peroxide poisonous for rice plants.

The meadow-chernozem soil is characterized by a weak catalase activity \[16\]. Before rice sowing, the amount of catalase reached \(2.8 \pm 0.272 \text{ cm}^3 \text{O}_2 \text{ kg}^{-1}\) in 2 minutes. The creation of anaerobic conditions in the rice field led to the inhibition of its activity. In the seedling phase a sharp decrease in the content of this enzyme was observed, especially in the soils of the option without fertilization. When using mineral and organic fertilizers, the activity of catalase increased twofold relative to the control sample (figure 2).

During tillering period and active production of oxygen by the roots, an increase in the of catalase content was observed. The maximum enzyme activity was observed during rice flowering. In the control option without fertilizers, the catalase activity in the tillering and flowering phases of plants was \(1.4 \pm 0.255 \text{ and } 2.2 \pm 0.200 \text{ cm}^3 \text{O}_2 \text{ kg}^{-1}\) for 2 minutes, respectively. When mineral and organic fertilizers were applied, the enzyme content during these rice growing periods increased to 1.8–3.1 and 3.3–4.9 \text{ cm}^3 \text{O}_2 \text{ kg}^{-1}\), respectively. To a greater extent, the effect of catalase was manifested during the manure application. The highest enzyme activity was provided by the joint autumn application of liquid manure and the Entec inhibitor. Peroxide formation (which is a substrate of catalase) in the rice flowering phase increased by 2.0 and 1.5 times, respectively in relation to the control option and the
option with the use of some mineral fertilizers. The increase in the enzyme activity was 11–29%, depending on the options fertilized with manure.

After the discharge of water from rice fields and the creation of oxidizing conditions in the soil, the values of catalase activity did not increase. The catalase inactivation is associated with the content of ferrous iron in the soil, which accumulated by the end of the rice growing season.

The processes of hydrolysis and conversion of urea nitrogen (carbamide) into an accessible form of urea nitrogen are associated with the action of urease. In the soil, it is formed during the transformation of nitrogenous organic compounds, that is proteins and nucleic acids. The urease hydrolyzes urea to ammonia and carbon dioxide. The NH₃ formed as a result of the urease reaction served as a source of nitrogen nutrition for plants. Therefore, the activity of urease is considered one of the important indicators of the biological activity of soils [5].

The urease activity of the meadow-chernozem soil is low and is classified as poor in terms of the degree of enrichment [16]. Before sowing rice, its activity was 0.35 ± 0.038 mg N-NH₃ kg⁻¹ day⁻¹. Soil flooding and creation of restored conditions in it led to urease activation, which increased in the germination phase to 0.45 ± 0.047 mg N-NH₃ in the control option and to 0.53–0.65 (σ = 0.026–0.051) mg N-NH₃ kg⁻¹ day⁻¹ when mineral and organic fertilizers were applied. Application of liquid manure with the addition of a nitrification inhibitor, regardless of the time of their application, slowed down the enzymatic decomposition of urea in comparison with other experiment options. At the same time, the greatest urease inactivation was observed with the manure and Entec inhibitor autumn application, which ensured a longer preservation of nitrogen in the soil. This was important for the nutrition of rice with nitrogen, since its greatest losses occurred in the first two weeks after the application of nitrogen fertilizers.

In the subsequent rice growing phases, the urease activity decreased, which was associated with the intensive consumption of nitrogen compounds by plants. However, suppression of urease activity was not observed in all options. In the rice tillering phase, activation of urease activity was observed during the autumn application of liquid manure and a nitrification inhibitor, which was due to the mobilization of organic nitrogen fixed in the fertilizer (figure 3).

![Figure 3. Dynamics of urease activity of meadow-chernozem soil: 1–6 are experimental options indicated in the methods section.](image)

After discharging water from rice fields and harvesting rice, when soil aeration improved and oxidative processes resumed in it, the urease activity decreased. The initial level of enzyme activity was restored when organic fertilizers together with a nitrification inhibitor were applied, and soil nitrogen losses were minimal.

The invertase is widespread in nature and occurs in almost all types of soil. It is considered a characteristic indicator of the genetic affiliation and biological activity of the soil. The presence of the enzyme invertase in the soil, which takes part in the carbon cycle, is closely related to the content of organic matter in it. The level of invertase activity reflects the content of easily hydrolyzable carbohydrates in the soil, which serve as an energy material for all soil heterotrophs. Easily hydrolyzable
carbohydrates such as sucrose and starch enter the soil along with plant waste and root exudates. Their mobilization occurs with the participation of two enzymes – the invertase and the amylase [19, 20].

Meadow-chernozem soil is provided with the invertase to a medium extent [16]. Before rice sowing, the invertase activity reached 18.2 ± 1.436 mg glucose kg\(^{-1}\) day\(^{-1}\). Soil flooding increased the invertase content up to 19.0 ± 1.361 mg of glucose in the control option and 20.2–24.3 (σ = 1.251–1.312) mg glucose kg\(^{-1}\) day\(^{-1}\) when using mineral and organic fertilizers, which was due to mobilization of organic matter in the restored conditions of the rice field. As the flooding period increased and carbohydrate reserves decreased, the inverting capacity of the soil decreased as well. By the rice flowering phase, the invertase activity was suppressed and the hydrolysis of sugars was very weak. During this period the activity of the enzyme relative to the germination phase decreased by 3.8 mg of glucose in the control option without fertilization, by 4.2 mg of glucose when using some mineral fertilizers and, to a lesser extent, by 2.9–3.0 mg glucose kg\(^{-1}\) day\(^{-1}\) when applying liquid manure only or combined with Entec inhibitor. After the harvesting period, the invertase activity increased when the hydrothermal conditions improved (figure 4).

**Figure 4.** Dynamics of invertase activity of meadow-chernozem soil: 1–6 are experimental options indicated in the methods section.

The inclusion of the nitrification inhibitor in the manure pump and the autumn application of the mixture promoted the intensification of microbiological processes and the invertase activation. The invertase activity was 28 and 20% higher in the germination phase, by 32 and 25% in the tillering phase and by 40 and 33% in rice flowering, respectively regarding the control option and the option with the use of some mineral fertilizers (farming technology). The inverting capacity of the soil also increased in comparison with other options fertilized with manure – during the growing season by 2.3–7.5% on average.

4. Conclusion

Autumn application of liquid manure with the addition of Entec FL DMPP nitrification inhibitor contributed to an increase in the amount of CO\(_2\) emitted from the soil by 0.031–0.038 mg CO\(_2\) kg\(^{-1}\) day\(^{-1}\); inactivation of urease in the germination phase and its activation during rice tillering, which ensured a longer preservation of nitrogen in the soil; an increase in catalase activity during the period of maximum peroxide formation, in the rice flowering phase, by 11–29% and the invertase activity by 2.3–7.5% compared to other options fertilized with manure.

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References

[1] Bahmanyar M A 2007 The influence of continuous rice cultivation and different waterlogging periods on morphology, clay mineralogy, Eh, pH and K in paddy Soils Pak. J. Biol. Sci. 10
2844–9

[2] Sheudzhen A Kh, Gutorova O A, Shein E V and Romanenkov V A 2019 Agrogenic soil evolution of rice agrolandscapes IOP Conf. Ser. Earth Environ. Sci. 368 012044

[3] Bezuglova O S, Stepovoy V I and Goyvalyova I G 1996 Impact of irrigation on dark-chestnut soil chemical properties Eurasian Soil Sci. 28(9) 97–105

[4] Nikolayeva S A 1996 Stability of delta ecosystems under intensive irrigation for rice cultivation Eurasian Soil Sci. 28(11) 320–8

[5] Kazeev K Sh, Fomin S E, Kolesnikov S I and Valkov V F 2004 Biological properties of locally hydromorphic soils in Rostov oblast Eurasian Soil Sci. 37(3) 310–20

[6] Chen Y, Liu J and Liu S 2018 Effect of long-term mineral fertilizer application on soil enzyme activities and bacterial community composition Plant Soil Environ. 64 571–7

[7] Singh G, Kumar D and Sharma P 2015 Effect of organics, biofertilizers and crop residue application on soil microbial activity in rice – wheat and rice-wheat-mungbean cropping systems in the Indo-Gangetic plains Cogent Geosci. 1 1085296

[8] Tripathi S, Chakraborty A, Bandyopadhyay B K and Chakrabarti K 2008 Effect of long-term application of fertilizers on soil quality and rice yield in a salt-affected coastal region of India Archives Agronomy Soil Sci. 54(4) 439–50

[9] Liang Y, Yang Y, Yang C, Shen Q, Zhou J and Yang L 2003 Soil enzymatic activity and growth of rice and barley as influenced by organic manure in an anthropogenic soil Geoderma 115(1–2) 149–60

[10] Dinesh R, Dubey R P and Prasad G S 1998 Soil microbial biomass and enzyme activities as influenced by organic manure incorporation into soils of a rice-rice system J. Agron. Crop. Sci. 181(3) 173–8

[11] Jing Y, Zhang Y, Han I, Wang P, Mei Q and Huang Y 2020 Effects of different straw biochars on soil organic carbon, nitrogen, available phosphorus, and enzyme activity in paddy soil Sci. Rep. 10 8837

[12] Sheudzhen A and Gutorova O 2020 Changes in the magnetic susceptibility of Kuban soils depending on soil formation conditions Advances Engineering Res. 191 264–8

[13] Egorov V V, Fridland V M, Ivanova E N, Rozov N N, Nosin V A and Friev T A 1977 Classification and soil diagnostics of the USSR (Moscow: Kolos) p 221 (in Russian)

[14] World Reference Base for Soil Resources 2014. Update 2015. International soil classification system for naming soils and creating legends for soil maps World Soil Resources Reports 106 (Rome: FAO) p 192

[15] Khaziev F Kh 1976 Enzymic activity of soils (Moscow: Nauka) p 180 (in Russian)

[16] Zvyagintsev D G 1978 Biological activity of soils and a scale for assessing some of its indicators Soviet Soil Sci. 6(6) 48–54 (in Russian)

[17] Sheudzhen A Kh and Bondareva T N 2015 Agrochemical research methodology and statistical evaluation of their results (Maykop: Polygraph-South) p 664 (in Russian)

[18] Zavarzin G A and Kudayyarov V N 2006 Soil as the key source of carbonic acid and reservoir of organic carbon on the territory of Russia Her. Russ. Acad. Sci. 76(1) 12–26 (in Russian)

[19] Abramyan S A 1993 Variation of enzyme activity of soil under the influence of natural and anthropogenic factors Eurasian Soil Sci. 25 57–73

[20] Dadenko E V, Prudnikova M A, Kazeev K Sh and Kolesnikov S I 2013 Application of indicators of enzymatic activity in assessing the state of soils under agricultural land Izvestia RAS Samara Sci. Center 153(4) 1274–7 (in Russian)