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# Application of Crop Residues in Combination with a Mineral Nitrogen Fertilizer to Albic Retisols and Their Effect on the Nitrous Oxide Production

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**Abstract**—A laboratory incubation experiment was conducted to examine the effect from the application of crop residues (rye straw) in combination with a mineral nitrogen fertilizer on the production of nitrous oxide in albic retisols. A linear correlation between the weight of plant residues applied to the soil and the increase in the microbial carbon content in this soil has been identified. An important parameter characterizing the studied system is the C : N ratio in microbial biomass and its dynamics (both determined by changes in the amount of applied crop residues and temporal dynamics). If the C : N ratio in plant residues exceeds 40 at early stages of their decomposition in the soil, a change of predominant process occurs: microbial immobilization of nitrogen is replaced by mineralization of organic nitrogen compounds. It is shown that the maximum nitrogen immobilization 'capacity' of microbial biomass is achieved when the C : N ratio in the substrate is 20–40. A relationship between the nitrous oxide emission from the soil and the amount of crop residues applied to this soil has been identified. It is established that the emission factor (i.e., $N_2O$ amount produced per 1 g of applied C) reaches the maximum values in experimental variants involving the application of substrate enriched with nitrogen (C : N = 7.5–10); apparently, this is because better nitrogen supply conditions contribute to a faster and more complete decomposition of plant residues.

**Keywords:** nitrous oxide, microbial biomass of soils, C : N of microbial biomass, albic retisols

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## INTRODUCTION

The use of mineral nitrogen fertilizers is a prerequisite for sustainably high yields; however their application poses a number of environmental risks [12, 20]. One of the most global problems caused by the use of mineral nitrogen fertilizers is the emission of nitrous oxide ($N_2O$) from agricultural soils. Nitrous oxide is an important greenhouse gas whose effect is 298 times higher in comparison with $CO_2$ [22]; it interacts with ozone in the stratosphere [18] and persists in the atmosphere for up to 150–180 years. Nitrous oxide is formed in soils in the course of a number of microbiological processes; the most important of them are nitrification and denitrification [1, 25].

Forecasts of $N_2O$ emissions from soils of agroecosystems must take into account both nitrogen fertilizer application rates and effects of soil–environmental factors on nitrogen transformation processes in soils. Today, the emphasis must be placed on the assessment of the role played by the organic matter availability in the regulation of nitrous oxide emissions from soils, since changes in the ratio between available carbon and nitrates affect the composition of final products of the denitrification process (i.e., $N_2O$ or $N_2$) and can contribute to the fixation of nitrogen contained in fertilizers in the soil. Application of crop residues is one of the simplest and most common practices used to enhance soil fertility, improve its physical properties, and increase the organic carbon content in the soil. However, assessments of impacts of this practice on nitrous oxide emissions performed to date produce mixed results [5, 7, 21]; this indicates that mechanisms behind the effects exercised by straw on the $N_2O$ formation and gaseous nitrogen losses from soils still remain poorly understood. A good understanding of the correlation between the organic matter availability in soils and $N_2O$ emissions is required to develop an action plan with the purpose to reduce nitrogen losses from soils and ensure sustainable yields.

The purpose of this study was to examine the effect from the application of straw and a mineral nitrogen fertilizer on nitrous oxide formation processes in albic retisols.

**Table 1.** Basic properties of the studied albic retisol

| Parameter | Value |
|---|---|
| Granulometric composition | Medium-textured loam |
| Soil density (g $cm^{-3}$) | $1.15 \pm 0.05$ |
| $C_{tot}$ (%) | $2.0 \pm 0.2$ |
| $N_{tot}$ (%) | $0.3 \pm 0.1$ |
| C:N | $7.1 \pm 0.6$ |
| $pH_{H_2O}$ | $6.0 \pm 0.2$ |

## MATERIALS AND METHODS

The study was performed using albic retisol samples collected in the agroecosystem of Chashnikovo Training and Experimental Soil Ecological Center of the Moscow State University, Solnechnogorsk district, Moscow oblast (55°59′21″ N, 37°24′17″ E). Soil samples (0–10 cm) were collected on five sites randomly selected within one field. The basic properties of soil samples are provided in Table 1. Rye (*Secale cereale* L.) straw with an initial C : N ratio of $110 \pm 2$ was used in the experiment as plant residues. Potassium nitrate was used as a nitrogen fertilizer; it was applied to the soil at a rate of 200 kg N/ha.

Prior to the laboratory incubation experiment, the soil was sifted through a sieve with a mesh diameter of 2 mm, and visible plant residues were removed. In accordance with recommendations published earlier [2, 4], the study was conducted using fresh soil samples that were neither dried nor stored for a long time. Soil samples weighing 300 g were placed in 1-L glass flasks and covered with the Parafilm film to prevent moisture losses.

The experiment involved the following variants: (1) control 1 (soil + bidistilled water); (2) control 2 (soil + $KNO_3$ (at a rate of 200 kg N/ha)); (3) control 3 (soil + plant residues (16.1 g/kg)); and (4) variants with the application of both $KNO_3$ (at a rate of 200 kg N/ha) and plant residues (from 1.61 to 32.2 g/kg) to obtain mixtures with the following resultant C : N ratios: 5, 7.5, 10, 20, 40, 50, 75, and 100. The experiment was conducted with a threefold replication for each variant. Potassium nitrate was applied to the soil in the form of an aqueous solution in small portions and mixed thoroughly for homogeneous distribution. Plant residues were ground prior to the application using a laboratory mill. The 1–2-mm plant residue fraction isolated on a system of sieves was used in the experiment. The prepared plant residue quantities were applied to the soil in small portions and mixed thoroughly for homogeneous distribution.

The laboratory incubation experiment was conducted for 30 days at a temperature of +22°C; the soil moisture content was maintained at 60% of the maximum field moisture capacity. The concentration of nitrous oxide emitted by the soil was registered every 2 days using a gas chromatograph with an electron capture detector (column length: 1 m, diameter: 3 mm, adsorbent: Porapak N 80/100, column temperature: 60°C, detector temperature: 200°C, evaporator temperature: 100°C, and carrier gas ($N_2$) flow rate: 90 mL/min). Soil respiration intensity was measured in the samples prior to the beginning of the experiment and 7 and 30 days after its beginning using a gas chromatograph with a thermal conductivity detector (Haye Sep N column, inner diameter: 2 mm, length: 2000 mm, adsorbent: 80/100 Porapak Q, column temperature: 60°C, carrier gas (helium) flow rate: 20 mL/min, and injected gas sample volume: 1 mL). To determine concentrations of produced nitrous oxide and $CO_2$, the flasks with soil were sealed with ground rubber stoppers and incubated for 6–8 hours. Prior to the beginning of the experiment and 7 and 30 days after its beginning, the content of microbial carbon and nitrogen was determined using the fumigation–extraction method [6, 23] with modifications [3]. For this purpose, soil specimens were collected from experimental flasks using a soil borer with an inner diameter of 5 mm (5–6 specimens for each sample). For all experimental variants involving the application of plant residues, the $N_2O$ emission factors (i.e., nitrous oxide amounts produced over the 30 days of incubation per 1 g of C contained in the applied plant residues) were computed.

The table and graphs provide mean values ± errors of mean; all calculations were performed for absolute dry soil weight (105°C, 8 hours). Statistical data processing was performed using Statistica 10.0 and Microsoft Excel 2007.

## RESULTS

One-way analysis of variance (ANOVA) has identified a statistically significant ($p < 0.05$) effect of the weight of plant residues applied to the soil on the content of microbial carbon and nitrogen, both at early incubation stages (day 7) and at the end of incubation (day 30). The increase in the content of microbial carbon correlates ($r = 0.85–0.92$, $p < 0.05$) with the weight of applied plant residues (Figs. 1A and 1B). At early incubation stages, the microbial biomass present in the soil becomes enriched with nitrogen at C : N = 5–10 in the applied substrate. By contrast, at C : N = 20 in the substrate, this parameter does not change; while starting from C : N = 40, the rate of microbial biomass enrichment with nitrogen (i.e., C : N ratio) decreases and virtually does change after subsequent increases in the weight of applied plant residues (Fig. 1C).

One-way analysis of variance has identified a statistically significant ($p < 0.05$) effect of the weight of plant residues applied to the soil on the microbial res-

piration rate (Fig. 2). The stimulating effect of plant residues was noted both at early (day 7) and later (day 30) incubation stages. However, by the end of incubation, the respiratory activity of microorganisms decreased by 2.5–6.0 times. The most significant drop in respiration intensity was observed in variants involving high initial plant residue application rates (Fig. 2).

It was found that the nitrous oxide emission dynamics follows a similar pattern regardless of the applied plant residue amounts: the maximum $N_2O$ emission rate is typical for the first few days and then it gradually decreases; the larger is the weight of applied crop residues, the slower the $N_2O$ emission rate goes down. One-way analysis of variance has identified a statistically significant ($p < 0.05$) effect of the weight of plant residues applied to the soil on the total amount of nitrous oxide emitted from the soil sample over the 30 days of incubation (Fig. 3). The correlation between these parameters is strong ($r = 0.88$, $p < 0.05$).

It was established (Fig. 4) that the maximum values of the $N_2O$ emission factor are typical for experimental variants involving the application of substrate enriched in nitrogen (C : N = 7.5–10). The emission factor decreases after the application of substrates with C : N = 5 and C : N = 20. The minimum emission factor values were registered in experimental variants involving the application of substrates with C : N = 40–100; these values do not differ from those in control variants with the application of plant residues only (i.e., without the mineral fertilizer).

## DISCUSSION

The increase in the content of microbial carbon, as well as the increase in the respiratory activity of soil microorganisms, closely correlate with the weight of plant residues applied to the soil and serving as available carbon and energy sources. The strong correlation between these parameters indicates that the availability of carbon is a limiting factor for soil microorganisms.

An important parameter characterizing the studied system is the C : N ratio in microbial biomass and its dynamics (both determined by changes in the amount of applied crop residues and temporal dynamics) (Fig. 1). According to earlier studies [16], the combined use of crop residues and mineral nitrogen fertilizers has a positive effect on the fixation of nitrogen contained in fertilizers in the soil due to its more intense microbial immobilization in the first days after the application. However, our data indicate a decrease in the enrichment of microbial biomass with nitrogen at C : N > 40 in the substrate. This pattern can be explained by the relation between C : N ratios in the consumed substrate and in the microbial biomass. If the substrate is more enriched in nitrogen than the microbial biomass (at the beginning of the experiment, the C : N ratio in microbial biomass was equal to 15), then microbial immobilization of nitrogen takes place. Earlier, it was theoretically computed based on meta-analysis of data that at C : N = 41 in plant residues at early stages of their decomposition in the soil, a change of predominant process occurs: microbial immobilization of nitrogen is replaced by mineralization of organic nitrogen compounds [24]. Thus, the obtained results experimentally confirm these computations. At a later stage of plant residue decomposition in the soil, the relationship between the microbial immobilization of nitrogen and the initial C : N ratio in the substrate changes: if the C : N ratio in the substrate is up to 20, then the microbial biomass is depleted in nitrogen; if the C : N ratio in the substrate exceeds 40, then the value of this parameter does not change. Apparently, this occurs due to an increase in fungal biomass capable of decomposing plant residues with a low nitrogen content and typical for later plant residue decomposition stages. Therefore, the maximum nitrogen immobilization 'capacity' of microbial biomass in albic retisols is achieved when the C : N ratio in the substrate is 20–40.

The identified correlation between nitrous oxide emissions from soils and the amount of applied crop residues originates from the fact that both nitrification and denitrification (i.e., the main processes producing $N_2O$) consume energy, and labile organic matter contained in plant residues can serve as its source [8, 17, 19]. Higher nitrous oxide formation rates observed in the first few days of incubation can be explained inter alia by the consumption of rapidly mineralized components contained in plant residues (amino sugars, amino acids, and hemicellulose) [11, 13]. The subsequent decrease in nitrous oxide emissions occurs because microorganism have to metabolize compounds that are more resistant to microbiological destruction (cellulose and phenolic components) to produce the required energy. One of the possible reasons for higher $N_2O$ emissions from samples with a high content of plant residues may be a decrease in the partial pressure of oxygen that is consumed in the course of active microbial respiration (Fig. 2), thus creating favorable conditions for the activity of enzymes catalyzing the denitrification process. In addition, the application of mineral N can accelerate the decomposition of labile carbon compounds in crop residues [14]. In turn, an increase in the content of soluble organic carbon in the presence of mineral N stimulates the denitrification process [15], thus, resulting in an increase in $N_2O$ emissions. Earlier data [9, 10] also indicate that $N_2O$ emissions increase after the application of plant residues in combination with a mineral nitrogen fertilizer. Concurrently, an increase in the $N_2O$ emission factor with a decrease in the C : N ratio

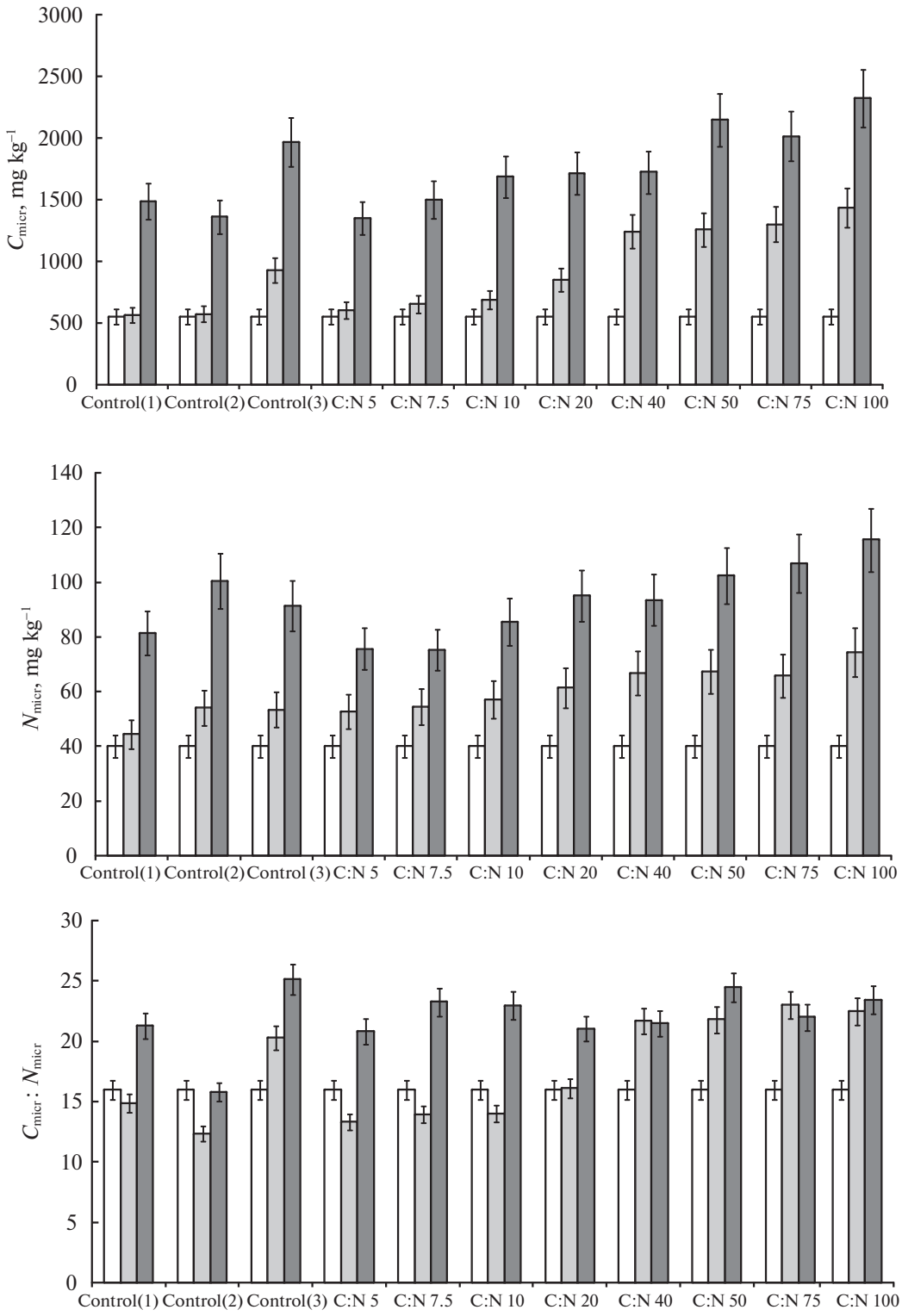


**Fig. 1.** Dynamics of microbial carbon content (A), microbial nitrogen content (B), and C : N ratio in microbial biomass in the course of incubation.

in the substrate can be caused by a more complete decomposition of plant residues under better nitrogen supply conditions. Overall, the application of plant residues enriched in nitrogen (C : N ≈ 10) to soils provides the most favorable conditions for the production of nitrous oxide.

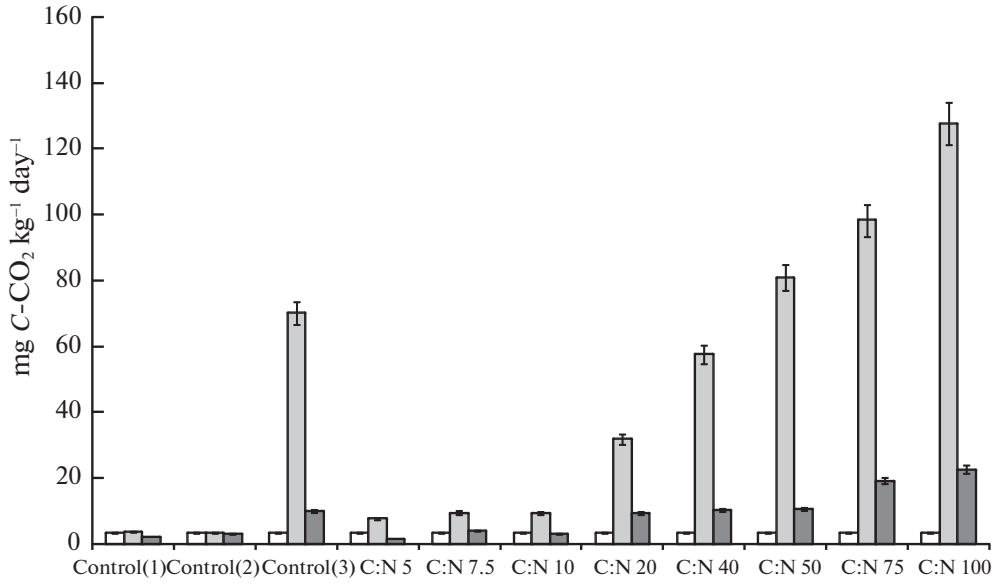


**Fig. 2.** Respiratory activity of soil microorganisms (mg $kg^{-1}$ of absolute dry soil $day^{-1}$).

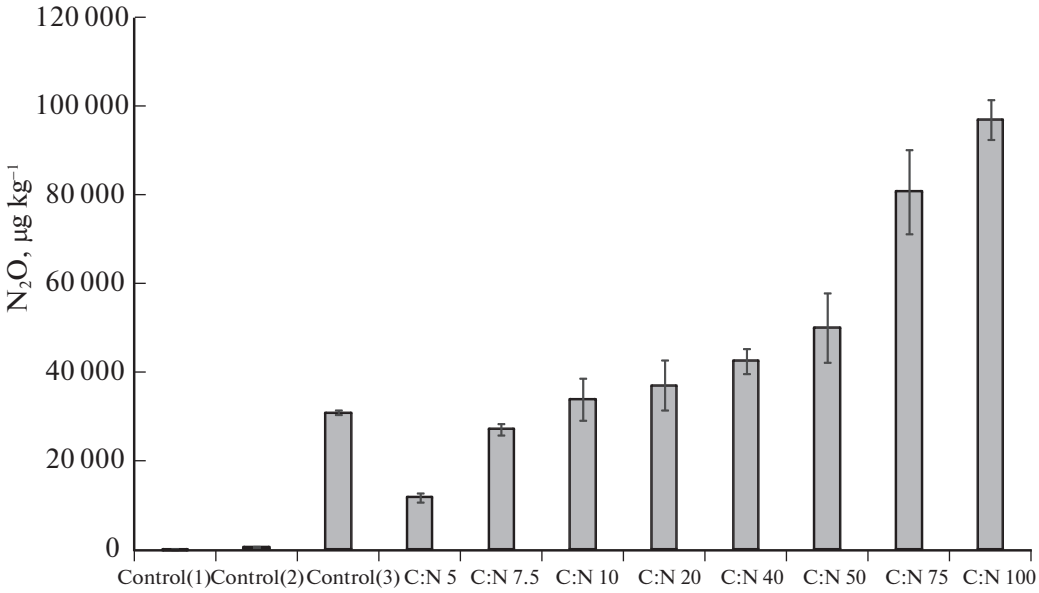


**Fig. 3.** Total $N_2O$ amounts emitted over the incubation period (μg $kg^{-1}$ of absolute dry soil).

## CONCLUSIONS

Application of plant residues in combination with mineral nitrogen fertilizers to soils affects both the content and activity of microbial biomass. The nitrogen enrichment degree of the substrate applied to the soil determines its main transformation path in the soil. The maximum nitrogen immobilization 'capacity' of microbial biomass in albic retisols is achieved when the C : N ratio in the substrate is 20–40. The nitrous oxide production intensity in the soil increases with higher crop residues application rates. Apparently, this is due to the better provision of microorganisms with energy sources and formation of anaerobic zones as a result of intense microbial respiration; such zones are optimal for enzymes involved in the denitrification process. It is established that the $N_2O$ emission factor reaches the maximum values when the substrate is enriched in nitrogen (C : N = 7.5–10). Therefore, to reduce losses of nitrogen fertilizers in the form of nitrous oxide and ensure the maximum nitrogen fixation in microbial biomass, nitrogen fertilizers

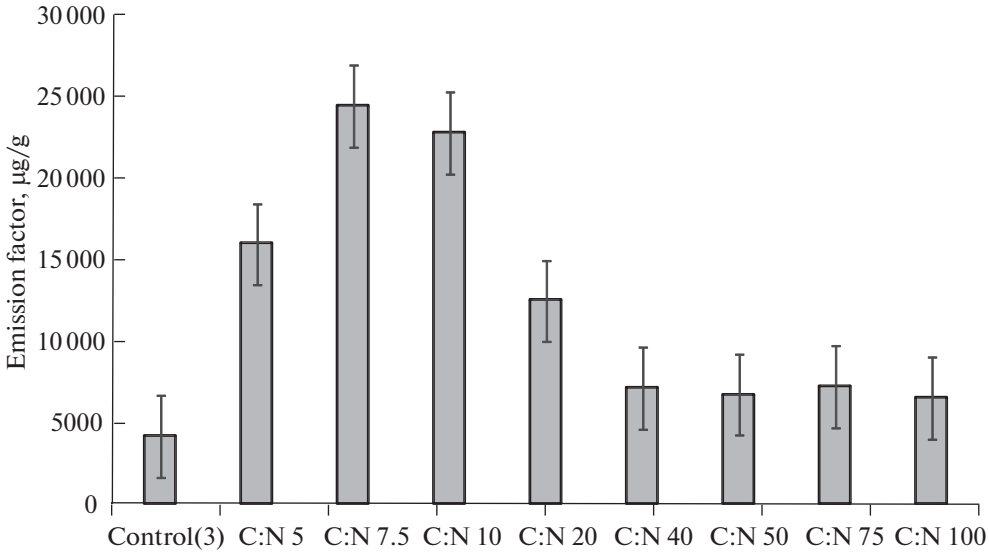


**Fig. 4.** Emission factor values in different variants of the incubation experiment (μg $N_2O$ per 1 g of C contained in plant residues).

should be applied to soil in combination with plant residues; the optimal C : N ratio in the substrate is 20–40.

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of Interest*

The authors declare that they have no conflicts of interest.

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