Anthropogenic impacts on organic matter transformation and biological activity in the meadow brown heavy loamy soils

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Abstract. This study aimed to determine the effect of continuous anthropogenic pressure on soil organic matter in three long-term, experimental, rotated crop fields established in 1963–1965. The application of the maximum dose (N\textsubscript{3}P\textsubscript{3}K\textsubscript{3}) of a mineral fertilizer partially neutralized the adverse effects of continuous anthropogenic pressure on soils, by providing plants with essential nutrients and increasing the soil biological activity. The organic matter of soils in which wheat, soybeans and oats were grown was 0.62%, 1.49% and 0.55% less, respectively than that of soils in natural ecosystems. The use of N\textsubscript{3}P\textsubscript{3}K\textsubscript{3} resulted in 1.34 million (oats), 0.60 million (soybean), and 6.18 million (wheat) more ammonifier units than the control. It also increased amylolytic microflora by 19.57 million (oats), 17.86 million (soybean), and 50.61 million (wheat) units compared to control.

1. Introduction

The soils of the meadow-brown heavy loamy are highly vulnerable to changes in hydrothermal conditions and anthropogenic stress caused by, among many factors, agricultural activities [1, 2]. Unsustainable use of agricultural lands can lead to soil organic carbon loss, acidification, salinization and compaction, while sustained anthropogenic pressure contributes to the acceleration of these losses [3, 4]. The main source of replenishment of soil organic matter is organic residues, which can be 2–8 times lower in agroecosystems than natural ones depending on the crop type, yield and fertilization system [5]. Therefore, the form and amount of organic matter play essential roles in sustainable agriculture. The type and nature of organic matter are determined by the continuous activity of soil macro- and microorganisms [6, 7] which are involved in the circulation of compounds, affecting the mineralization of organic residues and turning the insoluble forms of nutrients into elements available for plants [8]. Various chemical processes precede this transformation: ammonification, nitrification and conversion of the minerals within the soil, organic and mineral fertilizers, and other soil system compounds [9]. In the process of microbiological decomposition of fertilizers, a significant amount of diverse interim products of their decay is formed in soil, which is then used in the formation of humic substances [8].

Our study aimed to investigate the effect of different doses of mineral fertilizers on the composition of organic matter, the type of humus formed, and the number of ammonifying and nitrifying microorganisms in the soil.
2. Materials and methods

The study was conducted on three long-term experimental fields of the Far Eastern Agricultural Research Institute (Khabarovsky District, Russia; 48°31’05.9” N, 135°16’25.6” E), established consecutively in 1963–1965. The data was collected during the 8th rotation (comprised of oats, soybean and wheat) of the crop system in 2019. The adjacent meadow was selected as a control, as there was no record of it having been exposed to anthropogenic impact. The soil cover of the territory is represented by meadow-brown heavy loamy soils with an acidic pH and low natural fertility, as determined by high soil acidity, low amounts of nutrients and organic matter, and minimal biological activity. Before the experimental plots were established in 1963–1965, the soil contained up to 4% organic matter, and mobile phosphorus and potassium content was 1.4–4.2mg and 12.5–26.6mg, respectively, per 100g of soil. The pH was 4.2–4.6 and the hydrolytic acidity was 4.7–6.6 mEq per 100g of soil. The crops were grown according to the conventional technology generally accepted within the region, which included ploughing after the previous crop from autumn to winter swelling, cultivation, then harrowing in two tracks.

The experimental design included a control without fertilizers (since 1963) and plots with increasing doses – N₁P₁K₁ and N₃P₃K₃ – of mineral fertilizers. The dose of mineral fertilizers (N₁P₁K₁) for oats was N₁₆P₁₆K₁₆, and for soybean and oats was N₃₂P₃₂K₃₂; the dose of mineral fertilizers (N₃P₃K₃) for oats was N₆₄P₆₄K₆₄, and for soybean and oats was N₆₈P₆₈K₆₈. The experiment was performed using a randomized complete block design with four replications on plots 150–255 m² in size.

Soil samples were collected during the flowering stages of all three crops, which coincided with the maximum soil organic matter mineralization by hydrothermal processes within the region. The amount of organic carbon was determined according to Tyurin [10]: an air-dried soil sample was oxidized with a solution of potassium dichromate in sulfuric acid, and trivalent chromium, equivalent to the amount of organic matter, was determined using a photoelectric colourimeter. The amount of humic and fulvic acids was determined according to the accelerated method of Kononova and Bel’chikova [10]. Briefly, humic substances were extracted with a mixture of sodium pyrophosphate and sodium hydroxide. After filtering and dissolving the precipitate with a hot solution of 0.05N sodium hydroxide, 10ml of the obtained filtrate was evaporated, and the precipitate was dissolved with a solution of potassium dichromate in sulfuric acid. Total carbon was determined on a photoelectric colourimeter, and the amount of humic acids was calculated as a percentage to the total carbon in the original soil. The amount of fulvic acids was calculated as the difference between the total carbon in soil extract and total carbon in humic acids. The type of humus was determined as a ratio of humic acids to fulvic acids. The measurements were conducted in three replicates; the average value was used for additional calculations. Significant differences were calculated using LSD₀₅ values.

Samples for microbiological analysis were collected using aseptic technique. Serial dilutions of 1g of dry soil were used to inoculate agar media by the spread plate method. Nutrient agar (NA) was used for cultivation of ammonifying microorganisms that decompose the nitrogen-containing organic matter in the soil. Starch-ammonia agar (SAA) was used to cultivate amylolytic microflora, capable of degrading oligo- and polysaccharides and immobilizing nitrogen. Plated samples were incubated at 27°C. Colonies were counted after 2–4 days on NA, and starting from day four on SAA (depending on the colony growth). The number of microorganisms in 1g of soil was determined from the number of colonies in Petri dishes using established methods [11].

3. Results

All the most significant processes in soil occur with direct or indirect involvement of organic matter. Fulvic and humic acids play a special role in soil formation [12]. Organic matter interacts with physical, biological and chemical properties of the soil, accumulates reserves of carbon, nutrients and trace elements, and contributes to the creation of optimal soil regimes and structures. Humic compounds...
prevent losses from leaching, the formation of gaseous products and sparingly soluble mineral compounds, and increase the efficiency of mineral fertilizers [13, 14]. The application of mineral fertilizers contributes to the mineralization of soil organic matter [15, 16]. After applying the N\textsubscript{1}P\textsubscript{1}K\textsubscript{1} mineral fertilizer, the decrease in organic matter compared to natural soils was greatest in all studied crops (0.72–1.86%) (table 1). After applying the maximum dose of fertilizer, N\textsubscript{3}P\textsubscript{3}K\textsubscript{3}, the decrease in organic matter was less (0.55–1.49%).

Table 1. The amount of organic matter, fulvic and humic acids in soil.

| Experiment trials | Mass fraction of organic matter, % | Humic acids, % | Fulvic acids, % |
|-------------------|----------------------------------|----------------|----------------|
|                    | oats    | soybean | wheat | oats    | soybean | wheat | oats    | soybean | wheat |
| Ecosystem (meadow) | 4.89    | 4.89    | 4.89   | 0.58    | 0.58    | 0.58   | 1.14    | 1.14    | 1.14   |
| Control            | 3.64    | 2.79    | 3.27   | 0.26    | 0.24    | 0.40   | 0.59    | 0.74    | 0.73   |
| N\textsubscript{1}P\textsubscript{1}K\textsubscript{1} | 4.08    | 3.03    | 4.17   | 0.31    | 0.23    | 0.22   | 0.73    | 0.55    | 0.64   |
| N\textsubscript{3}P\textsubscript{3}K\textsubscript{3} | 4.34    | 3.40    | 4.27   | 0.29    | 0.18    | 0.24   | 0.64    | 0.73    | 0.60   |
| Average            | 4.02    | 3.07    | 3.90   | 0.29    | 0.22    | 0.29   | 0.65    | 0.67    | 0.66   |
| Deviation          | 0.88    | 0.76    | 1.37   | 0.06    | 0.08    | 0.25   | 0.18    | 0.27    | 0.17   |
| LSD\textsubscript{0.05} | 0.41    | 0.20    | 0.19   | 0.05    | 0.07    | 0.04   | 0.05    | 0.06    | 0.06   |

The greatest losses (2.1%, LSD\textsubscript{0.05}=0.20) of organic matter occurred in the control plots in which soybean was grown. In soybean plots with both mineral fertilizer concentrations, the organic matter decreased by 1.86–1.49% (LSD\textsubscript{0.05}=0.20), which could be explained by the crop’s higher nutrient intake. Using the maximum dose of mineral fertilizer (N\textsubscript{3}P\textsubscript{3}K\textsubscript{3}), the smallest decrease (0.62%, LSD\textsubscript{0.05}=0.19) in the amount of organic matter compared to natural soils was observed during the cultivation of wheat.

Fulvic acids were the main organic matter component measured in the soils, composing 1.14%. The cultivation of various crops in rotation has led to a change in the qualitative composition of the humus. The decrease in the amount of humic acids during the cultivation of oats was 0.32–0.27% (LSD\textsubscript{0.05}=0.05), of soybeans, 0.34–0.40% (LSD\textsubscript{0.05}=0.07) and of wheat, 0.18–0.36% (LSD\textsubscript{0.05}=0.04), compared to natural soils.

The humus quality depends on the ratio of humic acid carbon to fulvic acid carbon. All soils with humate–fulvate ratios (C\textsubscript{HA}/C\textsubscript{FA}) of 1.0–0.5 (and especially <0.5), are characterized by an acidic pH and are in an urgent need of liming [10]. In control plots with wheat, the humus type remained unchanged (C\textsubscript{HA}/C\textsubscript{FA}=0.55, LSD\textsubscript{0.05}=0.04) as did the soils of the natural ecosystems (C\textsubscript{HA}/C\textsubscript{FA}=0.51) (figure 1). In the plots to which mineral fertilizers were applied, a change in the type of humus occurred, due to a decrease in the amount of humic acids, and the type of humus became fulvate. The ratio of humic acids to fulvic acids in experimental oat plots was 0.42–0.45 (LSD\textsubscript{0.05}=0.07), and in soybean plots was 0.25–0.42 (LSD\textsubscript{0.05}=0.09).

Continuous anthropogenic pressure on agricultural lands significantly alters the living conditions for soil microorganisms [17-19], affecting their concentration and composition, possibly disrupting the course of microbial transformation processes, and subsequently, a change in soil formation conditions. The critical role of soil microorganisms is determined by their rapid response to external factors, the formation of the humus state of soil which serve as a matrix for the conversion of incoming substances [20, 21].
Figure 1. Change in the ratio of humic and fulvic acids in anthropogenically transformed and natural soils.

The soils of experimental plots were significantly nutritionally inferior to those of the natural ecosystem by the amount of detected microorganisms: the number of ammonifiers under oats was 2.38 million units ($LSD_{0.05}=0.11$), under soybeans, 2.39 million units ($LSD_{0.05}=0.45$) and under wheat, 4.71 million units ($LSD_{0.05}=0.13$), compared to 41.04 million units in the meadow. The amount of amylolytic microflora also decreased for all crops: under oats, there were 17.89 million units ($LSD_{0.05}=1.19$), under soybeans, 15.03 million ($LSD_{0.05}=2.15$), and under wheat, 35.24 million units ($LSD_{0.05}=2.24$), compared to 65.07 million units in the meadow (table 2).

Table 2. The amount (units) of ammonifying and amylolytic microorganisms in experimental and control plots.

| Experiment trials | Number of colony forming units in 1 g of absolutely dry soil, million units | SAA |
|-------------------|--------------------------------------------------------------------------------|-----|
|                   | oats | soybean | wheat | oats | soybean | wheat |
| Ecosystem (meadow) | 41.04 | 41.04 | 41.04 | 65.07 | 65.07 | 65.07 |
| Control           | 1.53 | 2.00 | 1.36 | 5.62 | 5.33 | 6.10 |
| $N_1P_1K_1$       | 2.75 | 2.58 | 5.22 | 22.86 | 16.58 | 42.92 |
| $N_3P_3K_3$       | 2.87 | 2.60 | 7.54 | 25.19 | 23.19 | 56.71 |
| Average           | 2.38 | 2.39 | 4.71 | 17.89 | 15.03 | 35.24 |
| $LSD_{0.05}$      | 0.11 | 0.45 | 0.13 | 1.19 | 2.15 | 2.24 |

The application of the maximum dose ($N_3P_3K_3$) of mineral fertilizers increased the number of ammonifiers compared to the control plot by 1.34 million units under oats ($LSD_{0.05}=0.11$), by 0.60 million under soybeans ($LSD_{0.05}=0.45$) and by 6.18 million under wheat ($LSD_{0.05}=0.13$). The number of amylolytic microorganisms increased by 19.57 million units under oats ($LSD_{0.05}=1.19$), by 17.86 million under soybean ($LSD_{0.05}=2.15$) and by 50.61 million under wheat ($LSD_{0.05}=2.24$). Our data allow us to conclude that the application of increasing doses of mineral fertilizers helps to reduce the depletion caused by many years of anthropogenic activities, thereby slightly increasing the biological activity of soils. While the control soils (without fertilizers) are characterized by poor to medium microflora enrichment [22] (NA...
2.00 million units and SAA 6.10 million units), soils supplied with the maximum doses (N\(_3\)P\(_3\)K\(_3\)) of mineral fertilizers vary from medium to very rich (NA 7.54 million, SAA 56.71 million units). In comparison, soils never exposed to agricultural activities were very rich in both types of microorganisms (NA 41.04 million units and SAA 65.07 million).

Statistical analyses show a very strong correlation (0.76–0.99, \(p<0.05\)) between the amount of humus and the number of both groups of microorganisms involved in mineralization processes. Additionally, there was a strong correlation between the number of amylolytic microflora and the amount of fulvic acids (>0.98, \(p<0.05\)), and the number of ammonifying microorganisms and the amount of humic acids (0.88–0.98, \(p<0.05\)) under oats and soybeans respectively.

4. Conclusions
Continuous anthropogenic pressure on soils has led to a decrease in the amount of organic matter; the largest losses occurred in the control variant under soybeans (by 2.1%). A decrease also was observed in the variants with mineral fertilizers under soybeans (by 1.86–1.94%).

The decline in humic acids under oats was by 0.32–0.27%, under soybeans, 0.34–0.40% and under wheat, 0.18–0.36%, compared to natural soils. The main portion of the organic matter of the meadow-brown heavy loamy soils was fulvic acids; in the soils of natural ecosystems, fulvic acids composed 1.14%.

The soils of experimental plots were significantly nutritionally inferior to those of natural ecosystems in the numbers of ammonifier bacteria: there were 2.38 million units under oats, 2.39 million under soybean and 4.71 million under wheat, versus 41.04 million units in the meadow. The number of amylolytic microflora was 17.89 million units under oats, 45.10 million under soybeans and 35.24 million under wheat, versus 65.07 million under wheat, versus 65.07 million units in the meadow.

We concluded that the application of a maximum dose of mineral fertilizers (N\(_3\)P\(_3\)K\(_3\)) allows for partial remediation of the negative effect of continuous anthropogenic pressure on soils, thereby increasing their biological activity and providing plants with essential nutrients. Using this fertilizer variant, the amount of organic matter declined by 0.62% under wheat, 1.49% under soybeans and 0.55% under oats, compared to soils of natural ecosystems. The number of ammonifiers increased by 1.34 million units under the oats, by 0.60 million under soybeans, and 6.18 million under wheat, compared to the control plot. In addition, amylolytic microflora also increased by 19.57 million units under the oats, 17.86 million under the soybean and 50.61 million under the wheat, compared to the control plot.

References
[1] Aseeva T A and Kim L V 2003 *Ways to increase the effectiveness of scientific research in the far* 2 78–84
[2] Selezevna N A, Aseeva T A and Fedorova T N 2019 *Change in the qualitative composition of organic matter in brown–meadow heavy loamy soils under anthropogenic impact: Sb.nauchn.tr.* (Moscow)
[3] Koch A et al 2013 *Global Policy* 4 434–41
[4] Burdukovsrii M L, Golov V I and Kovshik I G 2016 *Eurasian soil science* 49(10) 1174–79
[5] Kiryushin V I and Lebedeva I N 1984 *Reports of the All-Union Academy of Agricultural Sciences Named after Lenin* 5 51–59
[6] Matson P A, Parton W J, Power A G and Swift M J 1997 *Science* 277 504–9
[7] Six J, Bossuyt H, Degryze S and Denef K 2004 *Soil Till. Res.* 79(1) 7–31.
[8] Cherepukhina I V and Bezler N V 2018 *Microbiological activity of leached chernozem during plowing of straw of cereals in grain-crop rotation: Sb. nauchn.tr.* (Moscow) 136–44
[9] Kozlov A V and Selitskaya O V 2015 *Vestnik of Minin University* 3(11) 27
[10] Sheudzhen A Kh, Neshchadim N N and Onishchenko L M 2007 Organic matter of the soil and methods for its determination (Maykop: Adygea)

[11] Titova V I and Kozlov A V Methods for assessing of the soil microbiocenosis involved in the transformation of organic matter: Scientific and methodological manual (Nizhny Novgorod)

[12] Bassisty V P 2008 Fundamentals of soil science. Soils of the Russian Far East (Khabarovsk)

[13] Kooch Y, Ehsani S and Akbarinia M 2020 Soil & Tillage research 200 1–11

[14] Semenov V M, Kuznetsova T V, Ivannikova L A Semenova N A and Lisova E P 2001 Agrochemistry 7 5–12

[15] Naimi O I 2015 Scientific almanac Biological science 8 1067–72

[16] Purtova L N, Schapova L N and Polokhin O V 2017 Scientific Review Biological science 5 23–27

[17] Kozlov A V and Selitskaya O V 2015 Vestnik of Minin University 3 27–34

[18] Dobrovolskaya T G, Golovchenko A V, Pankratov T A, Lysak L V and Zvyagintsev D G 2009 Eurasian soil science 42(10) 1138–47

[19] Kirchmann H, Thorvaldsson G 2000 European Journal of Agronomy 12 145–61

[20] Sedykh V A and Savich V I 2014 Agroecological assessment of soil-forming processes (Moscow)

[21] Demkina T S, Khomutova T E, Kuznetsova T V, Kontoboitseva A A and Borisov A V 2016 Eurasian soil science 49(1) 56–59

[22] Zvyagintsev D G, Aseeva I V and Babaieva I P 1980 Methods of soil microbiology and biochemistry (Moscow)