Evolution of soil organic matter qualitative composition under different land-use types in the Yaroslavl oblast (European Russia) over longstanding period

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Abstract. The paper describes the postagrogenic transformation of soil organic matter (SOM). For this purpose, the results of chemodestructive fractionation (CDF), the content of chlorophylls a and b, pheophetins and carotenoids were used for the first time. To assess the state of soil algae, Margalef’s pigment index (PI) was calculated. Soils humus horizons of arable lands and fallow ones of the Poshekhonsky district of the Yaroslavl oblast of the Russian Federation were selected as objects of research. The objects were grouped into two groups that differ in the type of land use. In one of them the soil for 30–40 years remained arable (arable-arable group), other soils arable land over the same time period were transferred to the fallow (arable-fallow group). It was found that the conversation of arable soils to fallows led to: improving the state of soil algocenosis (a value of Margalef’s PI decreased), increasing of both total organic carbon and amount of soil organic matter (SOM) easily oxidized fraction, strengthening of hydrolytic processes that reduced content of carotenoids and chlorins (in particular, pheophetins and chlorophylls a and b) in SOM. When the content of total organic carbon did not exceed 1%, the conversion of arable soils to the fallows did not cause a noticeable change in the studied characteristics of soil organic matter qualitative composition.

1. Introduction

Soil organic matter (SOM) is a complex mixture consisting of both specific substances (especially humic substances), and individual (nonspecific) organic compounds, as well as the products of interaction between each other and with the mineral part of soil [1]. In the practice of Russian soil scientists, an assessment of SOM qualitative composition is usually carried out on basis of humus state of soils [2–5], which is based on alkaline extraction and acid fractionation of humic acids (HAs) and fulvic acids (FAs). However, in accordance with the opinion of a lot of researchers [6–9], isolation of humic substances (HSs) by of some sort alkaline solutions is not correct.

One of the most important characteristics of SOM is content of its easily, medium and hard oxidizing parts. Various components of organic material and varied parts of organic macromolecules have different resistance to oxidation and play different roles in the ecological functioning and manifestation of certain soil properties. To characterize the qualitative composition of organic matter in postagrogenic soils against the background of modern climate changes, the method of chemodestructive fractionation (CDF) can be used. This method makes it possible to determine the content of SOM components with different oxidation stability [10].
Soils there is a certain reserve of chlorins (chlorophyll $a$ and $b$, and pheophytins), which are capable of being preserved without destruction [11]. The main sources of photosynthetic pigments in soils are plant residues and algae. Content of chlorophylls in soils is connected with their water regime [12]. Carotenoids are the other widely distributed class of natural pigments of isoprenoid nature in SOM, they are synthesized de novo by prokaryotes, algae, fungi, higher plants and other living organisms [13, 14]. It is worthy of note that most plants are characterized by low levels of carotenoids in the roots [15]. Chlorins and carotenoids can be preserved in buried soils for up to several thousand years [16, 17]. This may be connected with that carotenoids have high antioxidant activity [18]. Due to this property carotenoids can preserve in SOM. Besides photosynthetic pigments are able to solubilize in micelles of HSS too [19]. Initially [3, 5] only presence or absence of chlorophylls in alcohol-benzene distribution from soils was included as indicators of humus state of soils. The presence of chlorophylls in SOM was most often determined based on increase of alcohol-benzene solution absorbance in the range $\lambda = 660$–$670$ nm [20]. The content of chlorins (chlorophylls $a$ and $b$, pheophytins) and carotenoids in SOM was practically not determined. Later, D S Orlov and co-authors [4] considered that the presence of chlorophylls in SOM should not be included in additional indicators of humus state of soils. In our opinion, it is important to use the content of photosynthetic pigments, when studying SOM qualitative composition.

The exploration objective – to characterize the postagrogenic transformation of soils on base of CDF of SOM and photosynthetic pigments content.

2. Objects and methods of research
Humus horizons of soils of arable lands and fallow areas of the Poshekhhonsky district of the Yaroslavl oblast of the Russian Federation were selected as objects of research. The soil classification of both Russian (2004) [21] and World Reference Base (WRB) for Soil Resources 2014, update 2015 [22], is shown in table 1. All the selected sites were duplicated by archival soil samples taken in the same places 30–40 years ago. The research objects were divided into two groups that differ in the type of land use. In one group the arable soil remains arable – arable-arable group, other group arable soils were transferred to fallow land – arable-fallow group. The residence time of some soils in the fallow state are shown in table 1 too.

In the researched soil samples, pH(H$_2$O) was determined by the potentiometric method [23], the total organic carbon content (C$_{org}$) was determined by wet oxidation of $K_2Cr_2O_7$ with photometric determination of Cr$^{3+}$ [24, 25], most often the excess dichromate is titrated with FeSO$_4$ [23, 25, 26]. In addition the content of SOM components with different oxidation stability was determined by the method of CDF [10]. For CDF, oxidation of SOM with $K_2Cr_2O_7$ solutions, a series of three solutions with the same concentration of oxidizer ($K_2Cr_2O_7$), but with linearly increasing oxidizing capacity was prepared. Oxidizing capacity of solutions depended on the concentration of hexoxoromium ions. This capacity is specified by different volumes of sulfuric acid (H$_2$SO$_4$) and is described with Hammett’s acidity function. Labile (easily oxidizing) SOM compounds were oxidized with the solutions with low oxidizing capacity, and relatively stable compounds (for example, medium and hard oxidizing parts) were oxidized with the solutions with high oxidizing capacity. The technique of determination the qualitative composition of SOM with the help of CDF was the following [10]: weighed portions of soil 50–100 to 500–800 mg (the same as for determining the carbon of organic compounds with Tyurin’s method [23]) were placed into three 50-mL heat resistant test tubes; then 5 mL of 0.8 N solution of $K_2Cr_2O_7$ were added in all tubes, then 5 mL of 30% H$_2$SO$_4$ were added into the first tube, 5 mL 60% H$_2$SO$_4$ were added into the second tube, and 5 mL of concentrated H$_2$SO$_4$ were added into the third tube. Then all tubes were placed into a thermostat heated to 100 °C and incubated during one hour; the volume of oxidized organic matter was determined with photometric method (by green color caused by Cr$^{3+}$ ions). Before the measurement, 40 mL of cold distilled water were added into every tube. The schedule was made using glucose or saccharose solutions [24].
Table 1. Index of soil profile, soil name, land use, depth of sampling and value of pH_{H_2O}.

| Index of soil profile | Soil classification                                                                 | WRB classification                                    | Land use               | Depth of sampling, cm | pH_{H_2O} |
|-----------------------|--------------------------------------------------------------------------------------|-------------------------------------------------------|------------------------|-----------------------|-----------|
| ZI-223-19             | Light agrozem loamy sandy on lacustrine sandy loams underlain by lacustrine coarse-silty loams | Chromic Cambisol (Geoabruptic, Arenic, Aric)          | Modern arable land     | 0–30                  | 6.2       |
| ZI-233                | Same                                                                                 | Same                                                  | Old arable land        | 0–30                  | 5.5       |
| ZI-21-19              | Light agrozem loamy sandy on lacustrine sandy loams underlain by lacustrine coarse-silty loams | Glossic Retisol (Abruptic, Silic, Aric, Cutanic, Raptic) | Modern arable land     | 0–20                  | 4.9       |
| ZI-21                 | Same                                                                                 | Same                                                  | Arable land            | 0–20                  | 6.5       |
| S-175–19              | Agrosoddy-podzolic gleyed postagrogenic silty loamy on cover loams underlain by moraine | Glossic Stagnic Retisol (Abruptic, Silic, Anoaric, Cutanic, Raptic) | Fallow land (20 years) | 0–28                  | 5.7       |
| S-175                 | Agrosoddy-podzolic silty loamy on cover loams underlain by moraine                    | Glossic Retisol (Abruptic, Silic, Aric, Cutanic, Raptic) | Old arable land        | 0–30                  | 5.4       |
| V-161-19              | Agrozem texturally differentiated gleyed postagrogenic silty loamy on carbonate cover loams | Glossic Stagnic Endocalcaric Retisol (Siltic, Anoaric, Cutanic) | Fallow land (15 years) | 0–32                  | 5.8       |
| V-161                 | Agrozem texturally differentiated silty loamy on carbonate cover loams                | Glossic Endocalcaric Retisol (Siltic, Aric, Cutanic)  | Old arable land        | 0–30                  | 5.2       |
| S-41-19               | Agrosoddy-podzolic gleyed postagrogenic silty loamy on cover loams underlain by carbonate moraine | Stagnic Endocalcaric Retisol (Abruptic, Silic, Anoaric, Cutanic, Raptic) | Fallow land (15 years) | 0–23                  | 5.9       |
| S-41a-19              | Agrosoddy-podzolic postagrogenic silty loamy on cover loams underlain by carbonate moraine | Glossic Endocalcaric Retisol (Abruptic, Silic, Anoaric, Cutanic, Raptic) | Fallow land (20–25 years) | 0–28                  | 5.1       |
| S-41                  | Agrosoddy-podzolic loamy on cover loams underlain by carbonate moraine                | Endocalcaric Retisol (Abruptic, Silic, Aric, Cutanic, Raptic) | Old arable land        | 0–30                  | 5.7       |
| Z-37-19               | Agrozem texturally differentiated gleyed postagrogenic silty loamy on cover loams underlain by carbonate moraine | Stagnic Endocalcaric Retisol (Abruptic, Silic, Anoaric, Cutanic, Humic, Raptic) | Fallow land (7–10 years) | 0–30                  | 6.5       |
| Z-37                  | Agrozem texturally differentiated silty loamy on cover loams underlain by carbonate moraine | Endocalcaric Retisol (Abruptic, Silic, Aric, Cutanic, Humic, Raptic) | Old arable land        | 0–30                  | 6.9       |
| I-79-19               | Agrosoddy-podzolic gleyed postagrogenic silty loamy on carbonate cover loams          | Stagnic Endocalcaric Retisol (Silic, Anoaric, Cutanic, Humic) | Fallow land (12–15 years) | 10–30                 | 6.1       |
| I-79                  | Agrozem texturally differentiated silty loamy on carbonate cover loams                | Endocalcaric Retisol (Silic, Aric, Cutanic, Humic)    | Old arable land        | 10–30                 | 6.6       |
| R-131-19              | Light agrozem postagrogenic loamy sandy on glaciolacustrine sandy loams              | Cambisol (Arenic, Anoaric, Humic)                     | Fallow land (20–25 years) | 0–20                  | 6.4       |
| R-131                 | Light agrozem loamy sandy on glaciolacustrine sandy loams                            | Cambisol (Arenic, Aric)                               | Old arable land        | 0–20                  | 6.1       |
Chlorophylls, pheophytins, carotenoids and some other compounds were isolated from SOM of research soils by 90\% acetone (dimethyl ketone) solution; ratio soil : solution was 1 : 10 [27]. It was used two-isolation [28]. Content of photosynthetic pigments was determined with VIS-spectrophotometer (model UV–9600, Rayleigh, Beijing, China) by standard method according to GOST 17.1.4.02-90.

In addition, the Margalef’s pigment index \( \left( \frac{E_{436}}{E_{664}} \right) \) was calculated to characterize the soil algocenosis. Usually this index was used for bioindication purposes of water bodies [29–31]. It is considered [30], that in the red part of the Vis spectrum \( (\lambda = 663–664 \text{ nm}) \) the absorption is due to chlorophyll \( \alpha \), and in the blue part of the spectrum \( (\lambda = 430–480 \text{ nm}) \) – mainly common carotenoids.

The 3-fold replication was used for all tests. Methods of variation statistics were used for mathematical processing of experimental data [32, 33]. If the data was obtained as a percentage, the Fisher’s angular transformation was used before performing the variance analysis.

3. Results and discussion

As a result of SOM qualitative composition grading by CDF, it was revealed (table 2) that long intense agricultural use of soils in one case (ZI-223 to ZI-223-19) led to a significant both increase of hard oxidizing fraction (HOF) and decrease of medium oxidizing fraction (MOF) of SOM, and in second case (ZI-21 to ZI-21-19) to a significant decrease in the content of total organic carbon. Such phenomenon is mostly connected with the disturbance of SOM transformation (the decrease of the rate of new humic substances formation and renewal of old humic substances (HSs) present in the soil), and also changes of 3D-spatial structure of HSs colloidal micelles (irreversible shrinkage of these molecular associates by drying). It was established (table 2), both increase of SOM HOF and decrease of SOM MOF were found in soils of arable-fallow group with a total organic carbon content not exceeding 1\% (S-175 to S-175-19, V-161 to V-161-19, S-41-S to 41-19). In other words, the conversion of arable soils to fallow land ones did not change the direction of SOM transformation. The transformation remained the same as if these soils would continue to plow. This fact can be explained by the fact that the conditions that contribute to the very strong destruction of SOM (high redox potential and acidity, predominance of microorganisms and fungi producing hydrolyase in soil biota, etc.) could not change over a 20–25-year period. As one of the confirmations, we can cite the fact that the acidity in these objects (table 1) was the same.

If the total organic carbon content in arable soils of arable-fallow group was higher than 1\% (table 2), then their conversion to the fallow lend ones made for increase of content both total organic carbon and SOM easily oxidizing fraction (EOF). This can be explained by the fact that the conversion of arable soils to fallow lend soils occurs the development of reducing processes, and these processes resulted in domination of EOP in SOM and increase in total organic carbon.

It was revealed (table 3) if in soils of both groups the content of \( C_{\text{OX}} \) did not exceed 1\%, then quantity of pheofetins and carotenoids decreased (ZI-223 to ZI-223-19, S-175 to S-175-19, V-161 to V-161-19, S-41-S to 41-19). A reliable decrease of chlorophyll \( b \) amount was observed in two objects (ZI-223 to ZI-223-19 and ZI-21 to ZI-21-19) arable-arable group and in one object arable-fallow group (V-161 to V-161-19). Note that all of these objects had the lowest total organic carbon content. It was found too that in four out of six sites in the arable-fallow group, the conversion of soils from arable to fallow contributed to an improvement of algocenoses state. In this case Margalef's pigment index decreased.

The chlorophyll \( a \) content in the dry mass of green algae can reach 1\%, which makes it possible to use as indicator of algae biomass [34]. The presence of even a small amount of chlorophyll \( b \) indicates the development of small flagellate (green) and blue-green algae [35]. Under unfavorable conditions for algae, chlorophyll \( a \) is primarily destroyed and carotenoids that are more resistant to destruction accumulate [13]. Decomposition of \(^{14}\text{C}-\text{labelled} \) chlorophylls and \( \beta \)-carotene in soil is slower compared to decomposition of glucose, hemicellulose and cellulose [36]. Herewith under the aerobic conditions of the forest floor the carotenoids are less resistant to decomposition than pheophytin \( a \), the dominant chlorophyll derivative in soils [37]. As shown by a series of model experiments [38], the destruction of chlorophylls takes place in two stages: the first, very fast stage occurs in the tissues of plants themselves,
the second, slower-in soils under the influence of soil biota. The intensity of microbial decomposition is regulated by conditions such as soil acidity, humidity and temperature, as well as the presence of toxic substances, etc. Microorganisms decomposed both chlorophylls \(a\) and \(b\) in two to four months in field soils; chlorophyll \(a\) was attacked most. Of the chlorophyll-type compounds, pheophytin, the most closely related derivative of chlorophylls, resisted decomposition largest [38].

**Table 2.** Content of total organic carbon and SOM fractions with different resistance to oxidation.

| Index of soil pit | Content of total CO\(_x\), % | Content, g/kg of soil | Part, % to SOC (CO\(_x\)) |
|------------------|-------------------------------|-----------------------|--------------------------|
|                  | Arable-arable group           |                       |                          |
| ZI-223-19        | 0.77                          | 3.10                  | 1.23                     |
|                  |                               | 3.41                  | 40.0                     |
|                  |                               | 15.9                  | 44.1                     |
| ZI-223           | 0.73                          | 2.51                  | 2.10                     |
|                  |                               | 2.68                  | 34.4                     |
|                  |                               | 28.8                  | 36.8                     |
| \(t_a\)          | 0.75                          | 2.58                  | 6.20                     |
| \(t_{05}\)       | 2.78                          | 2.78                  | 2.78                     |
| ZI-21-19         | 1.08                          | 3.61                  | 2.48                     |
|                  |                               | 4.73                  | 33.4                     |
|                  |                               | 22.9                  | 43.7                     |
| ZI-21            | 1.37                          | 3.70                  | 2.37                     |
|                  |                               | 5.84                  | 31.1                     |
|                  |                               | 19.9                  | 49.0                     |
| \(t_a\)          | 2.89                          | 0.54                  | 0.54                     |
| \(t_{05}\)       | 2.78                          | 2.78                  | 2.78                     |
|                  | Arable-fallow group           |                       |                          |
| S-175-19         | 0.90                          | 3.11                  | 1.52                     |
|                  |                               | 4.39                  | 34.5                     |
|                  |                               | 16.9                  | 48.6                     |
| S-175            | 0.81                          | 1.96                  | 2.79                     |
|                  |                               | 3.33                  | 24.3                     |
|                  |                               | 34.5                  | 41.2                     |
| \(t_a\)          | 9.23                          | 5.40                  | 6.92                     |
| \(t_{05}\)       | 2.78                          | 2.78                  | 2.78                     |
| V-161-19         | 0.79                          | 3.02                  | 1.22                     |
|                  |                               | 3.72                  | 37.9                     |
|                  |                               | 15.3                  | 46.8                     |
| V-161            | 0.80                          | 2.72                  | 2.28                     |
|                  |                               | 2.91                  | 34.4                     |
|                  |                               | 28.8                  | 36.8                     |
| \(t_a\)          | 0.45                          | 1.25                  | 7.15                     |
| \(t_{05}\)       | 2.78                          | 2.78                  | 2.78                     |
| S-41-19          | 0.99                          | 3.18                  | 1.26                     |
|                  |                               | 5.47                  | 32.1                     |
|                  |                               | 12.7                  | 55.2                     |
| S-41a-19         | 0.95                          | 3.06                  | 2.49                     |
|                  |                               | 3.91                  | 32.3                     |
|                  |                               | 26.3                  | 41.4                     |
| S-41             | 0.93                          | 2.71                  | 2.38                     |
|                  |                               | 4.21                  | 29.1                     |
|                  |                               | 25.6                  | 45.3                     |
| \(F_{u}\)        | 0.31                          | 2.01                  | 31.12                    |
| \(F_{05}\)       | 5.14                          | 5.14                  | 5.14                     |
| LSD\(_{05}\)     | 0.19                          | 0.60                  | 0.42                     |
| Z-37-19          | 1.32                          | 5.23                  | 2.84                     |
|                  |                               | 5.39                  | 38.9                     |
|                  |                               | 21.1                  | 40.0                     |
| Z-37             | 1.20                          | 4.13                  | 2.52                     |
|                  |                               | 5.36                  | 34.4                     |
|                  |                               | 21.0                  | 44.6                     |
| \(t_a\)          | 9.61                          | 2.87                  | 1.46                     |
| \(t_{05}\)       | 2.78                          | 2.78                  | 2.78                     |
| I-79-19          | 1.49                          | 6.26                  | 1.90                     |
|                  |                               | 6.01                  | 44.2                     |
|                  |                               | 13.4                  | 42.4                     |
| I-79             | 1.20                          | 4.37                  | 1.17                     |
|                  |                               | 6.46                  | 36.4                     |
|                  |                               | 9.8                   | 53.8                     |
| \(t_a\)          | 13.37                         | 4.27                  | 5.67                     |
| \(t_{05}\)       | 2.78                          | 2.78                  | 2.78                     |
| R-131-19         | 1.54                          | 6.47                  | 2.81                     |
|                  |                               | 6.18                  | 42.0                     |
|                  |                               | 17.9                  | 40.1                     |
| R-131            | 1.38                          | 4.75                  | 2.76                     |
|                  |                               | 6.24                  | 34.4                     |
|                  |                               | 20.4                  | 45.2                     |
| \(t_a\)          | 9.30                          | 3.72                  | 0.19                     |
| \(t_{05}\)       | 2.78                          | 2.78                  | 2.78                     |

\(^a\) EOF – easily oxidizing fraction.

\(^b\) MOF – medium oxidizing fraction.

\(^c\) HOF – hard oxidizing fraction of SOM.

\(^d\) \(t_a\) – statistics Student criterion.

\(^e\) \(t_{05}\) – Student criterion critical values.

\(^f\) \(F_{u}\) – statistics Fisher one.

\(^g\) \(F_{05}\) – Fisher criterion ones for the significance level of 0.05.
Table 3. The content of chlorophylls $a$ and $b$, pheofetins, carotenoids in SOM and value of Margalef’s pigment index.

| Index of soil pit | Content, ng/kg of soil | Margalef’s pigment index |
|------------------|------------------------|--------------------------|
|                  | Chlorophyll $a$ | Chlorophyll $b$ | Pheofetins | Carotenoids |
| Arable-arable group | | | | |
| ZI-223-19 | 0 | 92 | 261 | 91 | 7.4 |
| ZI-223 | 0 | 341 | 560 | 202 | 6.2 |
| ZI-21-19 | 70 | 75 | 314 | 164 | 6.1 |
| ZI-21 | 34 | 125 | 452 | 144 | 7.0 |
| t<sub>st</sub> | 12.2 | 8.38 | 8.67 |
| t<sub>05</sub> | 2.78 | 2.78 | 2.78 |
| S-175–19 | 0 | 32 | 43 | 46 | 10.0 |
| S-175 | 0 | 26 | 96 | 111 | 21.5 |
| t<sub>st</sub> | 2.48 | 8.78 | 9.37 |
| t<sub>05</sub> | 2.78 | 2.78 | 2.78 |
| V-161-19 | 65 | 96 | 96 | 48 | 4.7 |
| V-161 | 61 | 210 | 365 | 261 | 8.6 |
| t<sub>st</sub> | 0.83 | 8.52 | 12.33 |
| t<sub>05</sub> | 2.78 | 2.78 | 2.78 |
| S-41–19 | 0 | 84 | 152 | 72 | 9.3 |
| S-41 | 116 | 107 | 92 | 95 | 6.9 |
| S-41 | 68 | 97 | 216 | 142 | 7.7 |
| S<sub>F</sub> | 169.33 | 4.38 | 44.17 | 33.52 | 5.09 |
| LSD<sub>05</sub> | 15.5 | – | 32.4 | 21.4 | – |
| Z-37-19 | 24 | 89 | 213 | 53 | 3.7 |
| Z-37 | 32 | 79 | 247 | 64 | 6.7 |
| t<sub>st</sub> | 3.4 | 1.48 | 1.79 | 2.29 |
| t<sub>05</sub> | 2.78 | 2.78 | 2.78 |
| I-79-19 | 77 | 126 | 425 | 193 | 8.0 |
| I-79 | 68 | 110 | 165 | 338 | 15.6 |
| t<sub>st</sub> | 1.68 | 1.57 | 9.86 |
| t<sub>05</sub> | 2.78 | 2.78 | 2.78 |
| R-131-19 | 49 | 230 | 109 | 207 | 12.2 |
| R-131 | 122 | 271 | 6 | 250 | 13.8 |
| t<sub>st</sub> | 9.68 | 1.96 | 16.33 |
| t<sub>05</sub> | 2.78 | 2.78 | 2.78 |

4. Conclusion
After 30–40 years the conversation of arable soils to fallows led to increasing of both total organic carbon and amount of soil organic matter (SOM) easily oxidized fraction, improving the state of soil...
algocenosis and strengthening of hydrolytic processes that reduced content of carotenoids and chlorins (in particular, pheophitens and chlorophylls a and b) in SOM. When the content of total organic carbon did not exceed 1%, the conversion of arable soils to the fallows did not cause a noticeable change in the studied characteristics of soil organic matter qualitative composition.

Acknowledgments
This work was supported by Russian Foundation for Basic Research (grant no. 19-29-05243).

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