Soil Structure Formation: Role of the Soil Amphiphilic Organic Matter

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Abstract
Soil organic matter plays a leading role in the soil structure formation, especially in soil water stability soil properties. This is the most important property of soils on which depend the basic ecological functions of soil in the biosphere. However, so far unknown mechanism of formation of the soil structure, the soil water stability properties and value of soil organic matter in this processes. A physical reasonable hypothesis of formation water stable aggregate structure of chernozemic soils, based on the amphiphilic properties of soil organic matter, is proposed. Water stability of the aggregate structure is directly related to the surface properties of elementary soil particles (ESP) in the solid phase (SP) of the soil. In the case of elementary soil particles (ESP) hydrophilicity, water flows through the capillaries in the dry aggregate and leads to an increase of water pressure in the aggregate and its destruction. When ESP are hydrophobic, water and ions cannot get into the aggregate, and the "dead space" is created. Two forms of organic substances determine ESP architecture of soil aggregate and a combination of hydrophilic and hydrophobic surface properties of solid phase. Organic compounds adsorbed on the surface of mineral ESP provide film moisture migration, and hydrophobic organic ESP localized in the pore space of the aggregate provide the water repellent function and prevent the rapid migration of capillary moisture in the soil pore space.

Keywords: Soil structure; aggregate; soil organic matter; hydrophilic and hydrophobic properties of the soil solid phase.

Introduction
The phenomenon of the aggregate structure of the soil has always held and continues to hold one of the main places in the exploratory research of soil scientists. Origin, formation and stability of aggregates and, conversely, the processes of aggregate structure degradation - are the processes that directly affect the complex fundamental physical-chemical and biological processes in the soil, and at the same time are actually practical. However, there is no universally accepted and comprehensive theory of the aggregate formation and the structure formation control. There are a number of hypotheses on the aggregates formation, based on the works of V.P. Williams on the structure formation.

Unique natural formation that gives the soil a specific form of structural and hierarchical organization underlying all soil functions - soil unit - has a spatial organization, where the main role of "adhesives" is played by humic substances (HS), silt and cations such as Ca, Al, Fe-ions
The mineral skeleton, in most cases represented by elementary particles of mineral (quartz grains, feldspar, etc.). This is how the soil unit is formed: primary mineral particles are connected with each other with "adhesives" of different nature. This is confirmed by the huge amount of meso- and micromorphological observations, granulometric and mineralogical analysis of soil in general and the microaggregate fractions. This structure of the aggregates can be considered an indisputable fundamental fact of soil science [11, 32].

Based on the morphological structure of soil aggregates, the hypotheses of soil structure formation appeared. Among these hypotheses, the three main or most common can be selected. Initially, the coagulation theory of structure formation was dominant; K.K. Gedroyts can be regarded as the founder of this theory, which was subsequently developed by Antipov-Karataev and colleagues [1, 10]. It can be assumed that this hypothesis allowed to develop new schemes of analysis of soil aggregates, to classify and identify their characteristic types.

The most significant contribution to the theory of structure formation was V.R. Williams theory (1936), who emphasized the role of biological factors. He emphasized the importance of the plants root systems as a macro factor of granular structure formation, as well as the role of the so-called "fresh" organic matter, which is formed under anaerobic (!) conditions, and is a metabolic product of anaerobic bacteria functioning. According to the assumptions of VR Williams, anaerobic zones are localized within soil aggregates, and aerobic processes go on their surfaces. This ingenious guess of V.R. Williams was subsequently confirmed experimentally [11, 29, 34], and it was proven that when humidity exceeds 70% of the total moisture, the redox potential inside the unit is 100-200 mV lower as compared with the surface. Moreover, soil microbiologists experimentally established the presence of facultative anaerobic bacteria in soil aggregates [23], which form local anaerobic zones of microcenosis [29]. These facts indirectly indicate that the genesis of stable soil aggregates may be associated with specific properties of organic matter, which is formed in a lack of air and with the participation of specific microflora.

However, the question of the physical and chemical properties, origin and mechanism of structure involving a "fresh organic matter" (by V.R. Williams) is not fully understood as of now.

To date, studies of humic substances (HS) allow to regard them as a multicomponent system. The strategy of the study of such objects is simplification by separation into components and their independent research. This approach involves the consideration of the initial multicomponent system as a mixture of a number of discrete states, the number of which is defined and limited by sufficiency to describe the currently available experimental data. Basic and fundamental problem here is the choice of criteria on which to carry out the separation.

Since the middle of last century to the present day separation of HS system is based on their solubility in acid and alkali. Although this property is never realized in a real natural setting, solubility parameter was adopted in soil science as a genetic characteristic of humus and soils of different genesis. With the widespread use of acid-base HS separation into components, this feature does not reveal the mechanisms of formation of humus soil profile, to explain the reasons for differences in the components of HS in soils of different genesis.

Indeed, most if not all soil processes involve soil water. The nature of interaction of the substance with water depends on the intensity of its hydrophilic or hydrophobic properties. Organic substances, of which HS are formed as a result of humification, have only biological origin. Most biological molecules are amphiphilic compounds, i.e. are capable of exhibiting both hydrophilic and hydrophobic properties [12, 23]. Amphiphilicity is caused by the presence of both hydrophilic (polar) groups and hydrophobic (nonpolar) zones in their structure. The ratio of the hydrophilic and hydrophobic portions in the molecule determines its solubility, spatial organization and diversity of functional properties. Apparently, the have more pronounced hydrophilic properties HS have, the more these substances are mobile in the profile of the soil, the more they act as acidic hydrolysis agents, the more they interact from the aqueous phase with the solid phase surface. Hydrophobic HS will, on the contrary, be fixed at the place of their formation, forming accumulative characteristics of the profile. Apparently, this constitutes the significance and the role of amphiphilic properties in the formation of humus (accumulative, eluvial, eluvial-illuvial) soil profile.

Because mineral components are hydrophilic, organic matter plays the role in the formation of the hydrophobic surfaces in the soil. The degree of surface hydrophobicity of organic particles will depend on their ability to interact with each other through hydrophobic binding and formation
water-stable aggregates, or susceptibility to peptization, due to the formation of hydrogen bonds. In this paper, we adhere to the assumption that the hydrophobic HS cause structural bonds and are responsible for the formation and stability (water resistance) of the soil structure. The formation of aggregates through hydrophobic interaction between elementary HS-coated soil particles is caused by the formation of the energetically most favorable, in an aqueous environment, surface of the resulting aggregate. Strong bonds existing between the water molecules are broken upon "dissolution" of a substance in water. In the case of ionic (hydrophilic) compounds these breakages are compensated by replacing water-water interaction by ion-water interaction. Upon "dissolution" of non-polar (hydrophobic) substances there is no such compensation and dissolution of substance in water does not occur. Association of hydrophobic particles to one another whereby the interaction between the water molecules are broken to the least degree is more energetically favorable (18). In general, the hydrophobic binding can be defined as the interaction between the particles, which is stronger than the interaction of these particles with water, and which can be caused by covalent or hydrogen bonding, electrostatic attraction, or charge transfer. On this basis, it is the severity of hydrophobicity properties of HS will determine water resistance (and, apparently, all resistance to external influences) of soil structure.

It is believed that the main role of organic matter (OM) supplier in soils, particularly in the cultivation of herbs, is played by plant roots waste. OM of the root waste, entering the soil in situ, is subsequently converted by soil biota, passing the step of biotransformation with insufficient oxygen; therefore mainly humic acids and fulvic acids are formed, which, in the modern understanding of the properties of the newly formed soil organic matter, are amphiphilic substances containing hydrophobic and hydrophilic components [3, 11, 18]. All this data indicate that the formation of stable soil aggregates is caused by the special properties of OM, which is produced by specific biota in the lack of air. To date, these hypotheses are supplemented with data about the importance of the fungi hyphae in the primary (first mechanically and then biochemical) formation of aggregates, the data on the role of hydrophobization in the creation of the structure stability, primarily by increasing the duration of moistening, reducing the "discontinuous" action of entrapped air [18, 34], the importance of anaerobic processes in the formation of water-stable aggregates. Aggregate forming function of OM is associated with the acquisition of the hydrophobic properties by SOM components in anaerobic conditions through hydrophobic interactions formation [9,24]. However, this is little to no data on participation of the hydrophilic and hydrophobic components in the formation of the soil aggregate structure and their change upon its degradation. The purpose of this work is to substantiate the value of the amphiphilic properties of soil organic matter in the formation and degradation of the water stable soil structure.

Objectives:
1. To assess the value of the hydrophilic and hydrophobic components of SOM in the formation of the soil structure;
2. To study the mechanism of structure formation with participation of hydrophilic and hydrophobic components;
3. To identify the preferred mechanisms of desaggregation of soil properties (on the case of the typical chernozem) under anthropogenic load.

Material and Methods
The object of study is typical chernozem (Alekhin Central Chernozemic Reserve, Kursk region) which is located under native steppe vegetation and bare (since 1947) fallow. These objects are described in detail in [1, 15].

Humic substances were isolated from mineral genetic soil horizons by 0.1M Na₄P₂O₇ +0.1 M NaOH solution at 1:10 soil:solution ratio. For organic horizons, the ratio of 1:40 was used. Humic substances were purified from the extract of mineral impurities by centrifugation (8000 rpm., 15 min) and filtered through a membrane filter with 0.45μm pores. Chromatography of the hydrophobic interaction was conducted on Octil-Sepharose CL-4B (Pharmacia). Fractioning was conducted for the humic acid preparations, dissolved (5mg/ml) in 0.05M Tris-HCl buffer, pH = 8 and OM directly extracted from the soils. Sample volume was 0.5 ml, filtration rate 1ml/min., eluate detection was conducted at 280 nm, column 1x10 cm.
As a result of chromatographic separation, the chromatograms clearly reveal five fractions differing in hydrophilic-hydrophobic interactions (Fig. 1): fractions 1-3 interacted demonstrated the least hydrophobic interactions and were regarded as hydrophilic fractions, whereas fractions 4-5 were regarded as hydrophobic fractions.

**Results and Discussion**

Long-term bare fallow conditions of chernozem in comparison with chernozem under native vegetation led to a decrease in humus content (Fig. 2) and a significant compaction of the upper layer (0.60-0.75 g/cm$^3$ – native steppe, 1.05-1.31 g/cm$^3$ – bare fallow). A free filtering capability of upper chernozem horizons under native steppe is reduced in the bare fallow to 1.3-0.3 mm/min. Both amplitude and depth of profile freezing and heating increased. Aggregates of >2 mm lost the property of water-stability. According to microaggregate analysis, there was an increase in fraction of physical clay and silt and a significant decrease in content of fractions of medium and coarse sand. Decrease in SOM content within the plow layer is accompanied by accumulation of carbon in the lower horizons of the profile. The migration process of humic substances in the lower horizons of the profile was observed not only for the typical chernozem under bare fallow, but also for ordinary chernozem and dark chestnut soil, which were under long-term agricultural use (Fig. 3).
Significant changes in the structure of the chernozem under plowing, primarily related to the reduction of its water resistance, are characteristic consequences of anthropogenic degradation processes [2, 5, 24]. Although water resistant chernozemic aggregate structure is associated with the humic acids, identification of the humus composition in the virgin and arable chernozems reveals decline in Arable value of nonhydrolyzable residue and a significant reduction of fulvic acids (by 22-46%) as compared to their content in virgin soils. The absolute content of humic acids was less decreased (4-26%). As a result, the index value Chumic / Cfulvic in Arable as compared to Al of virgin soil increases [1, 14, 21]. According to some reports [21] long-cultivated chernozems demonstrate the carbon loss of about 4-5% in silt fractions and of 25% in clay fraction, indicating a more rapid mineralization of organic matter of the clay fraction and of microbiological and chemical stability of the humus composition in silt fractions. At our objects the carbon loss of the clay fraction is about 32% (10.24% in the virgin lands, 6.93% in fallow).

Chromatographic analysis (Fig. 4) of HS from virgin chernozem profile and arable chernozem demonstrated that prolonged exposure of a typical chernozem as black fallow led to a substantial decrease in the absolute content of hydrophilic components (fraction 1 and 2) as part of HS of arable horizon and their increase in the underlying horizons. Content of the hydrophobic components did not change in fraction 3 and increased in fraction 4.

Reduction of hydrophilic HS in A_{arable} with their accumulation in the lower profile horizons indicates lesser stability of these components of HS to chemical and microbiological processes of mineralization on the one hand, and the migration of hydrophilic components through the profile on the other. This data is consistent with well-known facts about changing fractions of HS under degradation of the aggregate structure. Although traditionally chernozemic aggregate structure is associated with humic acids, determination of the humus composition in the virgin and arable chernozems shows a significant decrease in fulvic acids in the latter (22-46%) as compared to their content in virgin soils.
Thus, in conditions of the black fallow, under the deficit of the incoming fresh organic material primarily the mineralization of HS hydrophilic components localized on the surface of the mineral matrix occurs. Obviously, this process is a major cause of loss of water-resistance properties of the soil aggregates.

Accumulation of HS of hydrophobic nature in the SOM of water-stable aggregates is confirmed by results of HIC (Fig. 5). The content of hydrophobic HS in water-stable aggregates is greater (especially the third HS fraction) than in the soil as a whole. HS from clay fraction of aggregates are predominantly hydrophilic in nature (clay fraction from water-stable aggregates was isolated by decantation). We believe that hydrophobic components of water-stable aggregates HS are represented by the products of SOM humification in situ and correspond to particles of organic nature with size of 2-10 µm. HS of hydrophilic nature are mainly localized in clay-organic compounds.

The data obtained allow to create highly schematized model of the structural and functional organization of the hydrophobic-hydrophilic components in the chernozem aggregate formation. In the mineral horizons profile during humification of organic material in situ the heterogeneous system of SOM is formed whose components differ by hydrophobic-hydrophilic properties. Water-soluble (hydrophilic) products are removed from humificated plant residues and, hitting the surface of the mineral particles, form sorption and organo-mineral complexes with this surface, reducing its hydrophilic properties due to mutual locking of polar groups of the mineral matrix and organic molecules, covering her surface. In other words, the surface of mineral solids becomes hydrophobizated. In turn, the hydrophobic products of organic material humification, incapable of water migration, remain at the place of formation. This hydrophobic components are spatially "isolated" from mineral particles, beeing caught between them. Accumulation of hydrophobic components of HS in the internal volume of the aggregate is helped by quasianaerobical conditions of organic material humification. It was described previously that the central part of water-stable chernozem aggregates is an ecological niche for anaerobic microorganisms. Additional 4 species of anaerobes were found in the center zone of aggregates compared to soil in whole and initial aggregates (2 species of clostridia - primary anaerobes, as well as ferroreducers and sulphatereducers - secondary anaerobes). Concentration of clostridia in "nuclei" of aggregates is 10-17 (!) times its content in the original soil [19]. These conditions of the formation of "fresh" humus were described in works of V.R.Williams. Nonpolar molecular fragments, on the one hand, determine their hydrophobic properties, and on the other - their resistance to oxidative, microbiological waste mineralization, especially in quasianaerobical conditions.

Presence of non-polar HS in the aggregate volume causes distortions in the structure of water that can be transmitted over considerable distances along chains of hydrogen bonds and cause long-range hydrophobic interaction. The combined action of HS, which hydrophobize the surface of mineral matrix and isolated in the micro areas, nonpolar molecules, stochastically distributed in
the aggregate, cause its water-stable properties. In the aqueous environment, the non-polar areas gravitate towards each other, as the approaching minimizes their thermodynamically unfavorable contacts with water. The total effect inside the hydrophobic areas of the aggregate is countering the rapid flow of water in the aggregate, reducing its swelling and promoting its stability in a saturated state. The last point is proven by data on water-resistant aggregates. Mineralization of the hydrophilic HS components, localized on the surface of the mineral particles, leads to the exposition of the hydrophilic surfaces of mineral solid soil phase, the efficiency of hydrophobic interactions within the aggregate drops and it is dispersed by water.

One should specifically highlight the role of hydrophilic components in soil humus. The role of hydrophobic interactions in soils, in particular, is emphasized by the fact that the stability of soil aggregates correlates with the content of hydrophobic humus fraction in the soil [7]. Water-stability of aggregates increases by adding nonpolar liquids in the soil [6]. In addition, as a prerequisite for modifying the surface properties of soil solid phase by organic matter, it should be located in the liquid phase of soil, i.e. hydrophilic fraction or water-soluble organic matter.

**Conclusion**

1. Prolonged (from 1947) typical chernozem soil exposure in the black fallow conditions led to a decrease in humus content, the formation of subsoil compaction, compaction of the upper layer and a sharp decrease in water stability of aggregates. In conditions of arable black fallow, with a deficit of fresh-organic material, mineralization and partial migration down through the profile of hydrophilic HS components, localized on the surface of the mineral matrix, primarily occur.

2. Hypothesis of V.R. Williams on the role of "fresh" organic matter in the process of aggregation is confirmed by physico-chemical models of formation of black soil aggregate structure, where the role of sorbed on mineral surfaces matter is played by hydrophilic components of HS, and the links between the particles “mineral surface-hydrophilic components” are formed by hydrophobic HS components. Therefore organo-mineral water-stable aggregate has the following organo-mineral composition: "mineral particle surface – (hydrophilic-hydrophobic components) = (hydrophobic-hydrophilic components) – mineral surface" where the symbol () denotes a molecule of SOM, and the symbol "=" represents hydrophobic interactions.

3. A conceptual model of water stability of soil aggregates associated with the ratio of hydrophilic and hydrophobic components and their spatial localization is proposed. The combined action of hydrophobic components of SOM, which hydrophobize the surface of the mineral matrix and nonpolar molecules, isolated in microzones, stochastically distributed in the aggregate, cause its water-stable properties.

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