# ROLE OF MINERAL SUBSTRATES IN FORMATION

# OF HUMIC COMPOUNDS IN SOIL

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## Abstract

*The effect of mineral matrices of loam, quartz sand, sand + 30% kaolinite, and sand + 15% bentonite on the dynamics of transformation of plant residues (PRs) of corn and red clover was studied. It is shown that the dynamics of PRs transformation has a wave pattern and depends both on the nature of mineral matrices and on the composition and properties of PRs. The kinetic parameters of corn and clover decomposition were studied, using a two-term exponential polynomial. The turnover period for the labile pool of clover and corn in all substrate (8-10 days) is typical for the organic acids and simple saccharides. The turnover time of the stable clover pool (0.95 years) and of the corn (1.60 years) corresponds to the turnover time of plant biomass.*

KEYWORDS: MINERAL MATRICES, HUMIC SUBSTANCES, CORN, CLOVER, DECOMPOSITION DENAMICS

## 1. *Introduction*

The transformation of plant residues (PRs) in soils is complicate due to two opposite processes: mineralization and humification of organic matter (OM). Both processes are complex and contain many successive-parallel stages. These are biochemical decomposition of PRs and partly newly formed organic material, stabilization of the decomposed products on each step of the process, humification of more stable pools of soil organic matter and more slow mineralization of already stabilized humic substances. Thus, a continuous set of organic and organo-mineral compounds different by their resistance to decomposition by soil microorganisms is formed.

A large number of studies have been devoted to investigation of these processes in soils. Most of them are associated with the study of the nature of organic matter of soils, its composition and properties, as well as the biochemical mechanisms for formation of specific humic substances - humic (HA) and fulvic (FA) acids. The participation of mineral components of soils in the formation of humic substances is poorly studied till now.

Mineralization of organic matter (OM) in soils depends on its availability to microorganisms (external factor) and their inherent stability to decomposition (internal factor). The least stable organic compounds are decomposed for several hours or days, the most stable ones remain in the soil for years or even millennia (Alekseeva et al., 2009; Bertrand et al., 2011).

Finely dispersed mineral components, predominantly clay minerals, play an extremely important role in the formation of humic substances and their conservation in soils. They ensure physical and chemical mechanisms for the protection of humic substances from biodegradation. This is due to the decrease in the availability of organic substances for microorganisms as result of formation of stable organomineral compounds on the surfaces of soil microaggregates (Mikutta, 2006; Van Lutzow, 2006). Recent theoretical and experimental works confirm the important role of adsorption on the surface of clay minerals for stabilization of newly formed OM and in synthesis of humic substances (Birkel et al., 2006; Kaiser and Guggenberger, 2003; Lehmann et al, 2007; Pinskiy et al., 2014). In particular, Travnikova et al. (1992) proposed that the condensation of PRs decomposition products to HAs occurs mainly through the formation of mineral–organic compounds, where organic component interacts with the surface of finely dispersed mineral.

The HAs thus formed are similar in composition and molecular weight to HAs and FAs and are bounded with the clay fraction of soils. These studies imply the direct participation of clay minerals in humus formation. Zavarzina (2006) revealed a direct effect of mineral matrix on enzymatic synthesis of humus-like substances from simple decomposition products of PRs. The consideration of soil humus as a complex of organomineral compounds attracts the increasing attention of researchers.

The aim of this work is to study the effect of mineral substrates of different composition on the mineralization and humification of the decomposition products of PRs of corn and clover under controlled conditions.

## 2. *Objects and methods*

In the experiments we used the aboveground mass of corn (*Zéa máys*) and red clover (*Trifolium praténse*), which was preliminary dried and milled to the sizes 3-5 mm. Mineral matrixes were as following: pure quartz sand and carbonate-free covering loam (the soil forming material of gray forest soil from the Experimental Field Station of the Institute of Physico-chemical and Biological Problems in Soil Science Russian Academy of Sciences), the mixtures: sand + 15% bentonite and sand + 30% kaolinite. Mineral content of the loam was 59% quartz, 16% kaolin, 13% mica, 11% feldspar, and 2% smectite. The bentonite contained beidellite, montmorillonite, talk, some quartz, calcite, and mica. Different admixtures in caolinite were less than 2%.

All mineral substrates were thoroughly mixed with the milled aboveground mass of corn and clover in the ratio 1:10, placed into glass vessels (200 cm$^3$), watered to 60% of the water holding capacity (WHC). Soil microorganisms were inoculated into the substrates of each vessel by adding of 1 ml of gray forest soil – water suspension (1:100 w/v). After that, the substrates were incubated at 20$^o$C and 60% WHC. Duration of experiments was 6-19 months. Sampling for the analyses was carried out after 5, 10, 20, 30, 60, 90, and 180 days from the beginning of the experiment with the clover set and after 7, 14, 20, 30, 60, 90, 180, 285 and 570 days – with the corn set. The experiments were run in three replicates. The content of organic carbon was determined by the Tyurin method (The theory..., 2006). Carbon mineralization losses were estimated from the difference between the contents of organic carbon in the beginning of the experiment ($C_0$) and in the respective sampling date. Biochemical composition of PRs is presented in Table 2.

**Table 2.** *The some components in corn and clover biomass*

| Organic substances groups | % of ash-free matter | |
|---|---|---|
| | Corn | Clover |
| Ash | 18 | 13 |
| Wax resins | 19.1 | 6.0 |
| Proteins | 4.9 | 14.8 |
| Hemicellulose | 32.6 | 23.8 |
| Cellulose | 26.0 | 20.4 |
| Lignin | 10.8 | 8.2 |

The elemental composition of the OM (C, N) in the samples of the incubated material was determined on an Elementar Vario EL III CHNS analyzer.

## 3. Results and discussion

### 3.1. Dynamics of plant residue transformation

The temporal changes in the relative content of carbon in PRs of corn and clover are presented in Fig.1. As a whole plant residues of clover are mineralized faster than corn ones. For both cultures the least amounts of mineralized plant residues were observed in the loam, the highest amounts - in the kaolinite (for corn) and sandy (for clover) substrates.
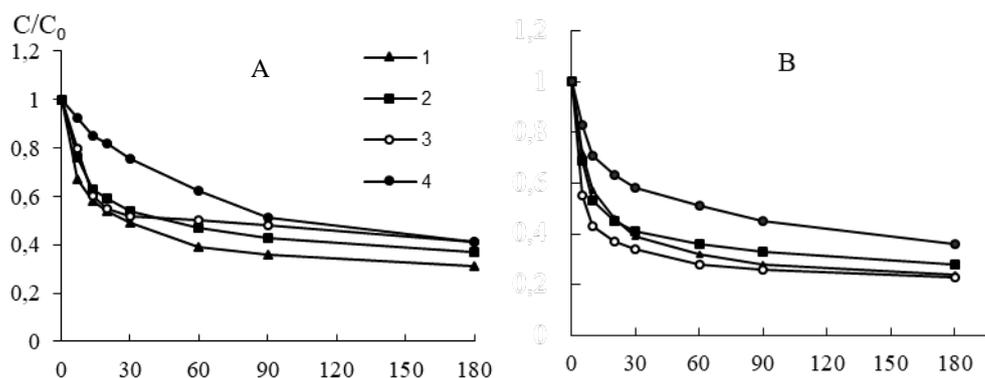


**Fig. 1.** The dynamics of mineralization of PR of corn (A) and clover (B) in the substrates studied: 1 – sand+30% kaolinite, 2 –sand+15% bentonite, 3 –sand, 4 – loam.

Mineralization of PRs of corn and clover (Fig. 1) conditionally includes three stages: fast, transitional, and slow. The fast stage lasts about one month, the transitional stage - from one to three months, the slow stage – more than three months. Decomposition of organic matter in substrates obeys an exponential law in time. In general, the clover residues are mineralized faster than corn ones due to the influence of biochemical composition of plant residues. For both cultures the least amounts of plant residues were mineralized in the loamy substrate, the largest - in the substrates containing kaolinite (corn) and sand (clover) due to the effect of mineral matrices.

During the transformation of the PRs, a change in the populations of microorganisms occurs at each stage in the transformation of OM, due to a change in the composition of nutrient substrates (Rybalkina and Kononenko, 1959). At the same time, a complex of humic substances with undecomposed plant residues and a system of universal succession complexes of microorganisms-destructors is formed (Chertov et al. 2007). It may be one of the causes of the wave pattern of humification of PRs, clearly manifested in the change in the content of humic substances and in the C/N ratio in the process of decomposition in time (Fig. 2).

The effect of mineral substrate on the humification is also clearly manifested in the content of the formed HAs and FAs. On the loam, more significant amounts of HAs are accumulated during the entire period of incubation, except for the first month of the experiment, when the portion of HA on the sand was slightly higher. The dynamics of the HA in the incubated systems copy the dynamics of the humic substances: they have an undulating character with a tendency for a gradual decrease in their content during the experiment. The end of the first humification stage is more clearly indicated by the $C_{HA}/C_{FA}$ ratio. Minimal values of this ratio were observed in both systems after three months of incubation. A decrease in the humification rate, most significant in the sandy substrates, was simultaneously observed. At the next stages of humification, HA are always predominant in the humic substances. The $C_{HA}/C_{FA}$ ratio reaches its maximal values to the end of the experiment, which perfectly coincides with the main law of Orlov's kinetic theory of humification: the selection of thermodynamically and biochemically stable humic substances (Humic substances…, 1993).

Thus, the studies showed that the dynamics of the PRs mineralization and humification in different mineral environments is largely similar and is determined by the undulating development of the microbial community. The rate and qualitative characteristics of the processes at the constant temperature and moisture conditions of the incubation depend on the composition and properties of the mineral matrix; therefore, they are significantly different.
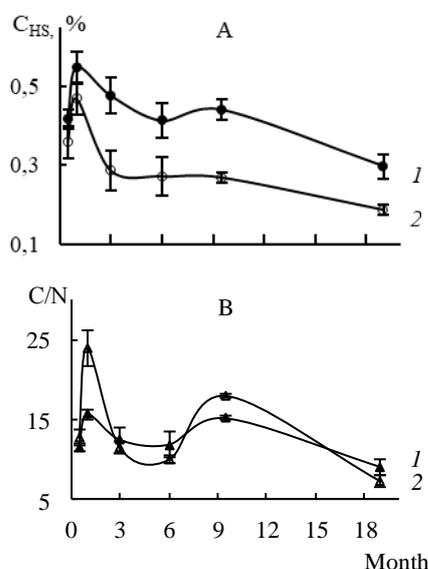


**Fig. 2.** Changes in the content of humic substances (A) and C/N ratio (B) during the humification of corn residues in loam (1) and in sand (2).

**Table 1.** Change in the group composition of humus during the incubation of plant residues of corn in different mineral substrates.

| Time of incubation, month | $C_{HS}$ | $C_{FA}$ | $C_{HS}/C_{FA}$ |
|---|---|---|---|
| | % of $C_0$ | | |
| sand | | | |
| 0.5 | 4.1 | 3.3 | 1.2 |
| 1 | 6.0 | 3.7 | 1.6 |
| 3 | 2.9 | 3.1 | 0.9 |
| 6 | 3.5 | 2.3 | 1.5 |
| 9.5 | 3.7 | 1.9 | 1.9 |
| 19 | 2.7 | 1.0 | 2.7 |
| loam | | | |
| 0.5 | 4.2 | 3.6 | 1.2 |
| 1 | 5.7 | 4.7 | 1.2 |
| 3 | 4.3 | 4.7 | 0.9 |
| 6 | 4.3 | 3.4 | 1.3 |
| 9.5 | 5.1 | 3.2 | 1.6 |
| 19 | 3.4 | 2.1 | 1.6 |

### 3.2. *Kinetics of plant residue decomposition*

The model selected for the quantitative description of plant residue mineralization involved two OM pools in the substrate: labile (LP) and decomposition-resistant ones; the decomposition process follows the first-order kinetic equation (Jenkinson and Rayner, 1977; Kuzyakov, 2006):

$$(1) \qquad \frac{dC}{dt} = -(k_1 C_1 + k_2 C_2),$$

The analytical solution of the linear equation at the known initial content of organic carbon in the system ($C = C_0$ at $t = 0$) is the two-term exponential function:

$$(2) \qquad \frac{C_t}{C_0} = A e^{-k_1 t} + B^{-k_2 t}$$

where $C_t$ is the residual organic carbon maintained in the soil at the time point $t$, $A$ – is the share of labile matter, $B$ – is the share of the resistant substances ($B = 1 - A$). The values of kinetic parameters calculated from equation (2) are presented in Table 2.

The analysis of the data obtained demonstrates that the kinetics of mineralization of PRs of corn and clover is adequately described by the binomial exponential polynomial. With that the share of LP of organic carbon in clover biomass is higher (57-63%) than that in corn one (47-49%). That is connected with the peculiarities of biochemical composition of PRs. In the loam substrate, the share of labile pool in clover biomass decreases to 39% that is explained by higher ability of the loam to stabilize the products of clover decomposition by means specific adsorption.

The rate constants of decomposition of labile and resistant pools in all cases differ: $k_1 \gg k_2$. The least rate of LP decomposition of corn is typical for the loam substrate, and maximal one – for the substrate with kaolinite. In the latter case $k_{1clover} \gg k_{1corn}$. Evidently, labile compounds of corn are stabilized by the loam stronger and are slower decomposed by microorganisms compared to the clover. The highest rate of PRs decomposition of corn and clover is typical for the substrate with kaolinite. Hence, the kaolinite accelerates the decomposition process of PRs of both cultures, and OM of the clover in a higher level.

**Table 2.** *The kinetic parameters of organic matter transformation in mineral substrates with different composition after six month incubation*

| Parameter | Sand + 15% bentonite | Sand + 30% kaolinite | Sand | Loam |
|---|---|---|---|---|
| Corn | | | | |
| A | 0.484±0.012 | 0.490±0.019 | 0.483±0.024 | 0.468±0.030 |
| B | 0.516±0.012 | 0.510±0.019 | 0.517±0.024 | 0.532±0.030 |
| $k_1 \cdot 10^{-1}$, day$^{-1}$ | 0.99±0.13 | 1.29±0.30 | 1.00±0.05 | 0.22±0.03 |
| $k_2 \cdot 10^{-3}$, day$^{-1}$ | 2.02±0.27 | 3.16±0.53 | 1.16±0.18 | 1.61±0.25 |
| $T_1$, day | 10 | 8 | 10 | 46 |
| $T_2$, year | 1.4 | 0.9 | 2.4 | 1.7 |
| Clover | | | | |
| A | 0.567±0.015 | 0.600±0.011 | 0.632±0.018 | 0.385±0.013 |
| B | 0.433±0.015 | 0.400±0.011 | 0.368±0.018 | 0.615±0.013 |
| $k_1 \cdot 10^{-1}$, day$^{-1}$ | 1.56±0.11 | 1.15±0.15 | 2.33±0.14 | 1.12±0.20 |
| $k_2 \cdot 10^{-3}$, day$^{-1}$ | 2.58±0.40 | 3.19±0.32 | 3.11±0.40 | 3.10±0.28 |
| $T_1$, day | 6 | 9 | 4 | 9 |
| $T_2$, year | 1.1 | 0.9 | 0.9 | 0.9 |

The turnover time is related to the mineralization rate by the relationship $T = 1/k$. The turnover period for the labile pool of clover (4-9 days) in all substrates and corn in sand and substrates with kaolinite and bentonite (8-10 days) is typical for the organic acids and simple saccharides. In the loam substrate, the turnover period for the LP of the corn is about 46 days due to stronger stabilization of the LP components. The turnover period of the stable pool of clover (0.95 year) is essentially less than that of corn (1.6 year) and generally corresponds to the turnover period of plant biomass. Calculation of the rate constants of decomposition of labile and resistant carbon pools at incubation during 19 months has shown that $k_1$ values of mineralization of PRs of corn in sand substrate was 0.69 day$^{-1}$ ($T_1 = 15$ days), in loam substrate - 0.032 day$^{-1}$ ($T_1 = 31$ days). The values of $k_2$ were similar to both substrates and were 0.0015 day$^{-1}$ ($T_2 = 1.9$ years) in sand and 0.0012 day$^{-1}$ ($T_2 = 2.3$ years) in loam substrates and practically did not differ from the values obtained at incubation during 6 months. Concluding, the deceleration of the total rate of mineralization with time occurs mainly at the expenses of the labile pool.

### 4. *Conclusion*

It is shown that mineralization of PRs of corn and clover includes three stages: fast, transitional and slow. The fast stage takes about one month, the transitional stage – from one to three months, the slow stage – more than three months. Decomposition of organic matter in substrates obeys an exponential law into time. In general, the clover residues are mineralized faster than corn ones. For both cultures the least amount of plant residues was mineralized in the loamy substrate, the largest amount - in the substrates containing kaolinite (corn) and sand (clover).

The dynamics of HS formation, HA and FA content, their ratios, and the C/N indexes are of wavy pattern. This is the result of changes in the structure of microbial community under the changes in the nutrients composition.

The kinetics of mineralization of PRs of corn and clover in the investigated mineral substrates is satisfactorily described by a two-termed exponential polynomial. The decomposition constants of labile and stable pools are different in all cases: $k_1 \gg k_2$. The lowest decomposition constant of the corn labile pool is observed in the loamy substrate, and the highest rate in the kaolinite-containing substrate, and $k_1$ (clover) > $k_1$ (corn). Apparently, the labile OM components of corn are more strongly stabilized by loam than those of clover and are more slowly decomposed by microorganisms. The highest decomposition rate of corn and clover stable pools is observed for the kaolinite-containing substrate. Hence, kaolinite accelerates the decomposition of OM in both cultures, especially in clover.

The turnover time of the clover labile pool (4–9 days) in all substrates and that of corn in sand and kaolinite- and bentonite-containing substrates (8–10 days) is typical for organic acids, amino acids, and simple sugars. In the loamy substrate, the turnover time of the corn labile pool is about 46 days due to the stronger stabilization of the labile pool components. The turnover time of the clover stable pool (0.95 years) is significantly lower than that of

corn (1.60 years) and largely corresponds to the turnover time of plant biomass.

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