

## Mesofauna Influence on Humification Process of Vegetable Oddments with Participation Microarthropod

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### ABSTRACT

Relevance of the studied problem is caused by the fact that stability of natural ecosystems strongly depends on functioning of their destructive block which closes a biological circulation. The organisms that ensure functioning of the destructive block are very different and numerous. All of them partly supplement, partly duplicate functions of each other that is an important factor of reliability of this ecosystem block. Shallow arthropods are important regulators of fossils mineralization and immobilizations of various biogens. Experiments demonstrate that if there are microarthropods, humification processes amplify and the optical density of humic acids raises. The aim of the article is to show degree and depth of change of mineralization and humification processes at the interaction of microarthropods and some mesofauna representatives in the processes of transformation of organic substance of vegetable oddments on the basis of experimental research. The most popular method to a research of this problem is the creation of laboratory microecosystems which have a clear boundary, which are easily reproduced and convenient for experimenting with subsequent instrumental determination of quantitative and qualitative humification parameters. In the course of experimental work it is revealed that the activity of soil-forming invertebrates results in strengthening humification processes. Humification rates, transformation depth of organic substance directly depend on cenotic organization of destructors. It is obvious that, the more complete the composition of biodegradative agents, the more intensive the decomposition process, the stronger the humification processes prevail over mineralization, the higher their maturity degree. All above-mentioned has a huge value to preserve a long-term fertility and high agronomical soil value, both in agrophitocenosis, and in natural biogeocenosis.

### KEYWORDS

Mesofauna, vegetable Oddments, microarthropod

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## Introduction

There is a lot of materials about food activity of soil invertebrates, their roles in a regulation of decomposition processes of organic substance and

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environmental-forming ("engineering") activity and it emphasizes important functional value of these animals and enables to consider complexity and variety of their participation in ecosystem processes (Anderson, 2000; Cassagne, Gers & Gauquelin, 2003; Wardle, 2002). However, the lack of quantitative assessments of activity of soil invertebrates reflects in underestimation of the functional activity of animals-destroyers at the creation of quantitative models of basic processes of a biological circulation (Byzova, 1987).

The complex of soil invertebrates includes different functional cenotic groups that differ in a feeding way, food digestion and an activity form (Perel & Karpachevsky, 1968). A saprophage is the most typical for them, which comprises most soil animals. The role of a saprophile complex in biogeocenosis is direct biochemical and physical impact on fossils, and also stimulation of saprophytic complex's activity.

The transformation of a dead soil cover proceeds as a uniform soil and biological process submitting to particular regularities. The main factor of this process is the activity of microorganisms and soil micro and mesofauna (Wallwork, 1976).

The most fissile degradative agents of a laying are earthworms and wood lice, which are defined as nitro - and carboliberants or humificators and mineralizers.

The main role of earthworms in a forest laying is the acceleration of a decomposition process, the increase of humic substances, the increase of abundance and a ratio of functional microorganisms groups (Satchell, 1967). The function of wood lice in a destruction is mechanic destruction of a leaf fall and increase the number of microorganisms in it as the decomposition processes of proteins and other nitrogen-containing substances in their intestines are poor (Reves & Tiedje, 1976).

Microarthropod is a consumer of the second order regulating microbiological activity and a ratio of humification and mineralization processes (Bengtsson & Rundgren, 1983; Cassagne, Gers & Gauquelin, 2003; Visser, Whittaker & Parkinson, 1981).

It is possible to assume that at the interaction and various combination of biodegradative agents there are considerable changes in mineralization and humification processes of vegetable debris, knowledge about which will enable to predict and operate soil agronomical value.

## **Methods**

### ***Research methods***

In the course of the research the following methods were used: theoretical (studying of domestic and foreign literature on the problem); diagnostic (collecting primary information, method of a comparative analysis); empirical (experiment, measurement, comparison); experimental (definition of cause and effect relationships); methods of mathematical statistics and graphic results representation.

### ***The experimental research base***

Pilot and experimental research base:

1. Theoretical justification of a scientific method. As natural ecosystems are difficult and dynamic, it is difficult to study them by means of traditional scientific methods. Therefore it is necessary to address the laboratory microecosystems that have a clear boundary, which are easily reproduced and convenient for experiments.

If we take into consideration biological components, then it is possible to allocate two types of microecosystems:

- microecosystems taken immediately from nature by seeding the cultural medium by organisms from various natural habitats;
- systems created by a combination of the types which are grown up in the axenic cultures.

The systems of the first type are nature "simplification" that leads to the creation of communities of those organisms which can exist in the conditions created by an experimenter. Such systems are created to model a particular natural situation.

At the second approach certain systems are created by selecting isolated and well studied components. Such systems are possible to call "gnotobiotic" as the structure and quantity of organisms in is precisely known. Besides, in such systems almost all its abiotic parameters are precisely known. Such systems allow studying trophics, biochemistry and many other aspects of activity of certain types and the whole group of organisms.

We used both approaches, as well as something between them as not only precise quantitative answers are important to us, but also searching for features and regularities of a microarthropod's role in the system of elementary soil-forming processes in various conditions.

2. Creation of laboratory microecosystems. We estimated a contribution of various ecological groups of biodestructors into a power stream of a top humus in laboratory conditions.

The technique of microecosystems' creation, and also technique of physical and chemical analyses, was offered in the 1980s (Simonov & Gorlova, 2014) and is applied recently (Simonov, 1984a; Simonov, 2013a; Simonov, 2013b).

The experiment assumed the following options:

1 option – monitoring: decomposition of vegetable oddments with participation of only microorganisms.

Experienced options:

2 option: microorganisms and Annelidas (*Lumbricus rubellus* Hoffmeister, 1843).

3 option: microorganisms and wood louse (*Porcellio scaber* Latreille, 1804),

4 option: microorganisms, microarthropods (*Collembola*) and worms (*Lumbricus rubellus* Hoffmeister, 1843),

5 option: microorganisms, microarthropods (*Collembola*) and wood louse (*Porcellio scaber* Latreille, 1804).

Microarthropods of experienced vessels belonged to the 4th ecological groups and 8 types of collembolans. Initial density in a dead soil cover was equal to 10 pieces/g of a dead soil cover.

The specific structure included a collembolan:

1. *Entomobrya* sp., *Isotoma viridis* – surface forms.
2. *Isotoma* sp.gr. *olivacea* – upper-litter forms.
3. *Isotoma notabilis*, *Folsomia quadrioculata*, *Pseudosinella alba* – hemiedaphic forms.
4. *Folsomia fimetaria*, *Onychiurus* sp.gr. *armatus* – euedaphic forms.

Earthworms and wood lice are placed in the vessels by 2 copies, weighed beforehand. Average loading was equal to 0,086 g/g of dead soil cover (worms) and 0,00531 g/g of dead soil cover (wood louse).

The simulated leaf fall was created not from fallen oak leaves, a linden and a filbert collected in the winter from trees and bushes. As experiments do not include studying specific features of chemical composition of vegetable debris, this set of leaf fall can be considered as optimum. The structure of a leaf fall can be variable depending on research objectives and biota structure.

According to numerous research the quantity of a simulated leaf fall is optimum when there are 10 g of air-dry weight in microcosms of 1 liter (Simonov, 2002).

Leaf fall was humidified to 60% of its aggregate moisture capacity. The temperature of the vessels is 20-22 ° C. Vessels were closed by a double-layer gauze bandage. The frequency of leaf fall humidification is once a week.

The differentiation of activity of shallow arthropods and microorganisms in microcosms was implemented by heat treatment of a leaf fall at 60-70 C for 1-2 hours. This mode keeps viable all basic groups of microorganisms, but eliminates both layings, and diapausing invertebrates.

For an experiment correctness in experienced and control options leaf fall was placed on an inert substratum. It can be a basis for the formation of organic-mineral complexes. As such substratum in our experiments is river sand which is washed out sequentially by water, a sodium pyrophosphate, water, muriatic acid and again water. The prepared substratum allows noting vertical penetration of mineralization and humification products with gravitational moisture. A five centimetric sand layer in vessels of 1 liter is experimentally confirmed.

To create a group of microarthropod in microcosms we used a floatation method of extraction of shallow arthropods from a substratum or an electronic method of distillation into water with the subsequent transfer of selected types of soil-forming animals on the experimental substratum by dissecting needles.

3. Instrumental inventory. In the analysis of output and accumulation of humic substances Dyakonova K. V. technique was applied in Simonov's modification. (Simonov, 1984b; Simonov, 2013a; Simonov & Gorlova, 2014; Simonov, 2012). The optical density of extracts was defined on CPP-3 (a concentrated photo-electric photometer) with a length of waves of 465 and 650 nanometers.

Receiving extracts was made in polyethylene vessels up to 550 ml for 15-17 hours. A ratio of water and leaves 1:1, for a sodium pyrophosphate the ratio is 2:1. An average shot for a leaf fall is 3 grams. After stirring at LE-203 installation centrifugation was made at 4000 rpm for 15 minutes with afterfiltration on a membrane filter of "Sinpor-8".

Carbon content was determined by amount of chrome which was used for oxidizing reaction at 590 nanometers on CPP-3 with recalculation on carbon on a gage scale.

Carbon oxidation by chromic mixture was made in a filtrate which was evaporated beforehand on a water bath. Then chromic mixture of 5 ml 0,4 N and calcinated pumice was added (for equal boiling) and then combusted it on an ethernite stove for 5 minutes from the moment of boiling.

After cooling water was added to make 15 ml of mixture, centrifugated and left for a night. The Pirophosphate extract was mixed to 100 ml.

The rest of a filtrate was spilled on centrifugal test tubes and pelleted by a strong hydrochloric acid in drops till PH-1 according to a universal indicator. In 12 hours mixture was centrifuged at 8000 rpm for 15 minutes. The fulvic acid was drained off. In the gel of humic acids acidified water was poured and again centrifuged till peptization. Supernate was drained off, and the gel was transferred to crystal watch glasses and dried up.

The chromacity coefficient of humic acids was defined on CPP-3 with a length of waves of 465 and 650 nanometers.

### **Investigation stages**

The research of the problem was carried out in 3 steps:

At the first, information retrieval stage scientific approaches and concepts were analyzed, scientific literature was studied, Russian and foreign experience of studying humification process was generalized.

At the second, forming stage the model and technology of conducting experimental work are developed, the experimental work was organized on the definition of quantitative-qualitative indicators of participation of meso - and microfauna in transformation processes of organic substance of a top humus.

At the third, analytical-synthetical stage processing, analysis, interpretation of experimental data were carried out, materials of theoretical and empirical research were generalized.

### **Results and Discussions**

In an experiment during all exposition term there was no increase in number of earthworms and wood lice, while our experiment of 2002 (Simonov & Pinayeva, 2002) showed that the availability of microarthropods, their activities on the transformation of organic substance of a leaf fall affected vegetal functions of mesofauna representatives positively.

Research of absorbency and consumption speed of a leaf fall by earthworms showed that feeding depends on a type of food. Filbert, oak, linden are the most preferable (Striganova, 1980).

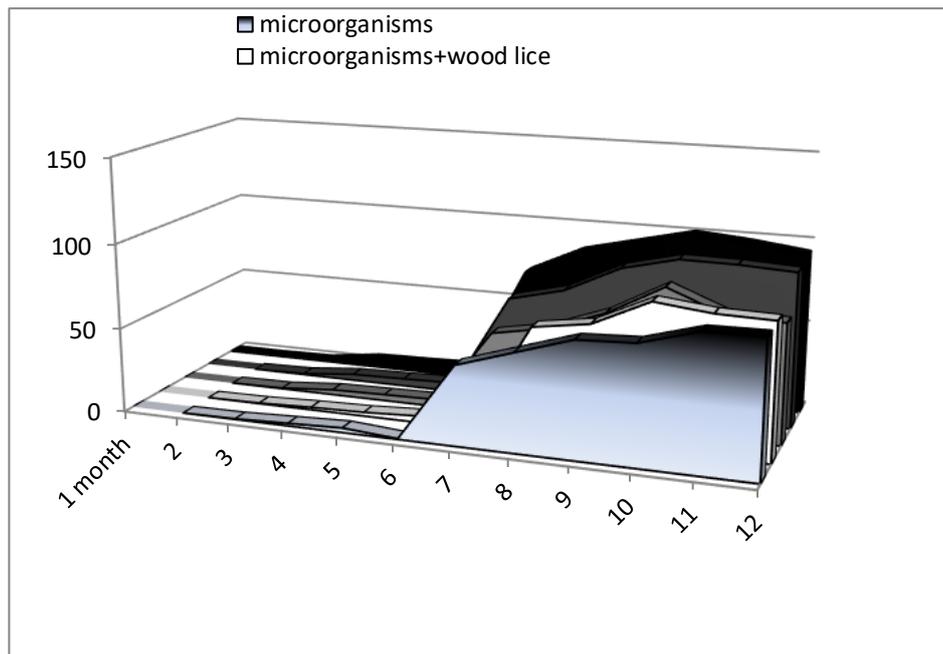
Within a year the mass of earthworms provided the availability of microarthropods changed from 857,01 mg / a vessel (the beginning of an experiment) to 781,06 mg / a vessel (the end of an experiment) with a maximum of 931,01 mg / a vessel on the fifth month of an exposition.

In experimental conditions wood lice experienced delayed biotic processes judging by their biomass dynamics: from 53,11 to 56,02 mg / a vessel at the

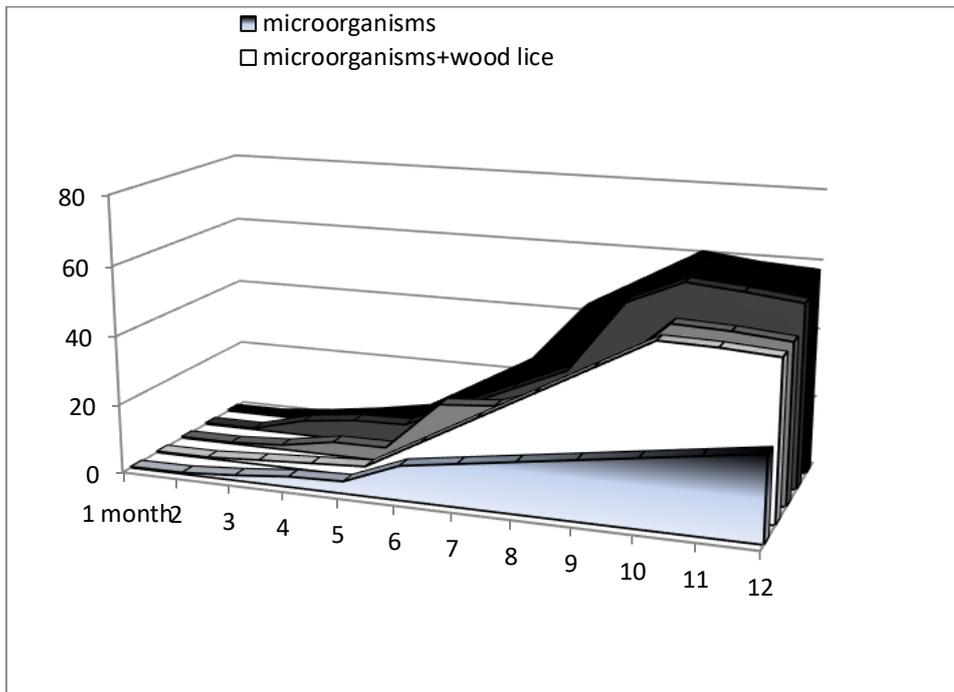
beginning and at the end of an experiment with a maximum of 61,41 mg / a vessel for the 5th month of an exposition with microarthropods.

The deficiency of collembolans negatively affected biomass of mesofauna representatives. So, biomass of earthworms during an experiment fluctuated from 879,04 to 750,23 mg / a vessel with a maximum of 895 mg / a vessel on the 5th month, and with wood lice these indexes looked as follows: from 51,01 to 52,87 mg / a vessel with a maximum of 58,46 mg / a vessel for the 5th month.

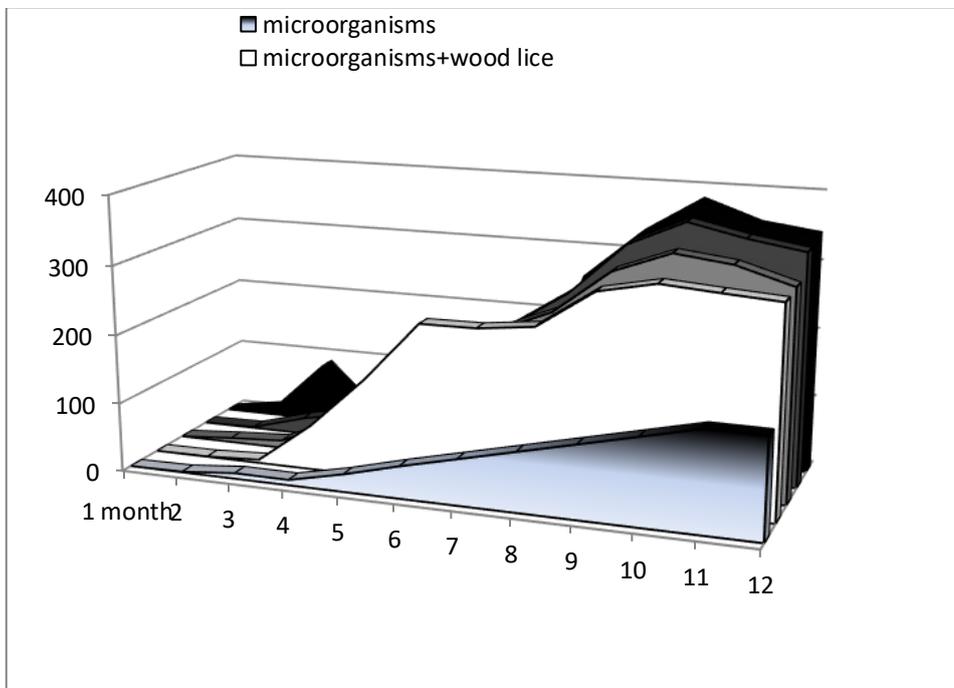
In the real experiment the content of humic substances in sand extracts exceeds those indicators in extracts from leaves (fig. 1,2,3,4).



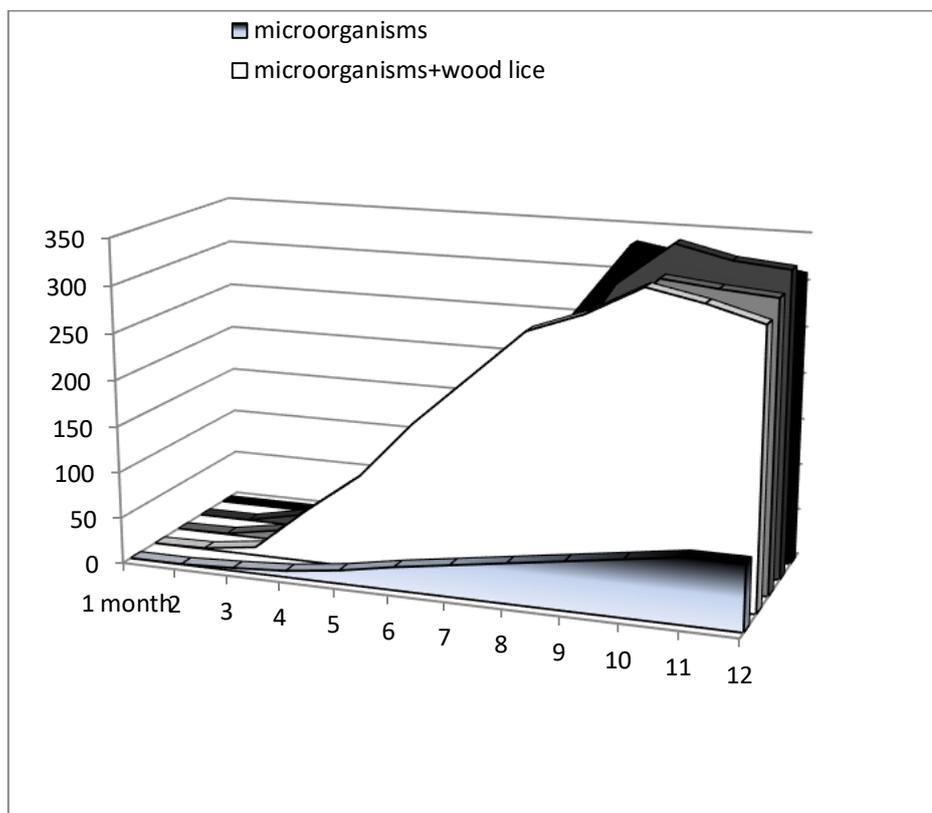
**Figure 1.** Total content of organic carbon in a sand-water extract, the mg of C  $\times 10^{-6}$ /g of dry substance (characteristic results of this research)



**Figure 2.** Total content of organic carbon in leaf - water extract, mg of  $Cy10^{-6}/g$  of dry substance (characteristic results of this research)



**Figure 3.** Total content of organic carbon in a pyrophosphate sand extract, the mg of  $Cy 10^{-6}/g$  of dry substance, (characteristic results of this research)



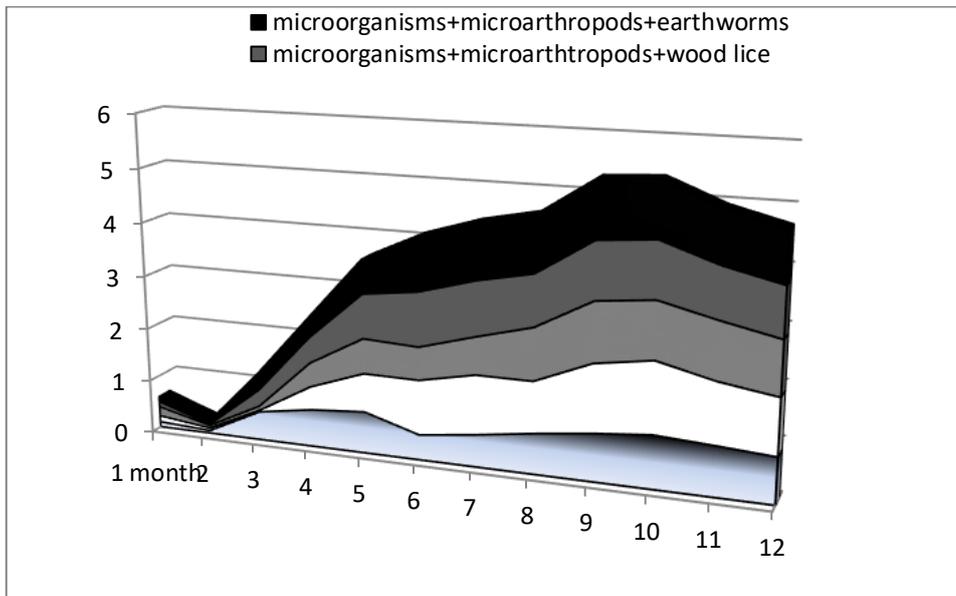
**Figure 4.** Dynamics of total Corg in a pyrophosphate leaf extract (mg of Cy  $10^{-6}$ /g of dry substance) (characteristic results of this research)

In the option of leaf fall destruction with the participation of microorganisms any noticeable deviations from earlier obtained (Simonov, 2002) data did not happen: noticeable increase of humic substances is marked on the border of 3-4 months of an exposition. In the next months, in comparison with the first peak, the amount of humic substances increases by 1,3-1,5 times. On the border of 3-4 months there was an increase in a share of humic acids in comparison with fulvic acids. From this point there was an increase in humic substances due to the growth of fulvic acids.

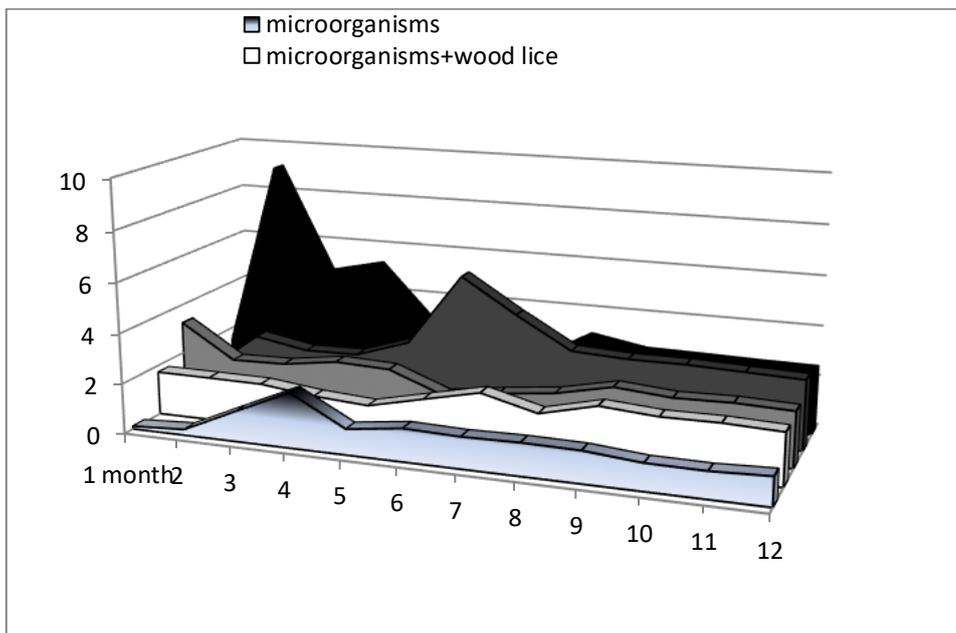
In the analysis of content of organic carbon in total extracts (fig. 1,2,3,4) the following features clearly are shown:

1) Activities of microarthropods and mesofauna representatives stimulate all destruction processes of a leaf fall in comparison with activities of microorganisms .

2) More complete groups of soil fauna shift transformation processes of organic substance towards humification strengthening (fig. 5,6).



**Figure 5.** A ratio of C humic acids / C fulvic acids in a pyrophosphate sand extract (characteristic results of this research)



**Figure 6.** A ratio of C humic acids / C fulvic acids in a pyrophosphate leaf extract (characteristic results of this research)

Correlation coefficient of indexes of humic substances' output between the options where leaf fall was decayed by earthworms with microarthropods and without is 0,96, and in the options with wood lice is 0,97. It is explained by a total action of bioagents on a humification process. The ratio of C humic acids / C fulvic acids in these terms displays a considerable prevalence of humification

processes over mineralization processes. Some shift of coefficient C humic acids / C fulvic acids in the following terms, apparently, is explained by increasing a role of microorganisms.

3) The deficiency of microarthropods in a leaf fall with worms lowered humification quantitative and qualitative indicators of vegetable debris.

One of the features of conducted experiment was an invariable biomass of earthworms and wood lice. It is known that the main food resource of earthworms is carbon nitrogen bonds. In the conditions of simulated microcosms reserves of these substances in a substratum peter out that results in a fertility minimum, and slow rates of individual body height.

The majority of land wood lice are phitosaprophages. The main energy resource is the cellulose which stocks in experimental conditions are limited (Striganova, 1980). The trophic relations with a heterotrophic microbial flora are not characteristic of wood lice which consume vegetable oddments. Wood lice have an increased need for Ca, and in the conditions of a microcosm animals need additional sources of calcium. The factor that limits biotic activity is humidity, for the majority of wood lice humidity less than 86% is disastrous though *Porcellio scaber* are more hardy due more complex structure of pseudotracheas.

In the options where mesofauna representatives were kept separately from microarthropods, there is a biomass decrease. Microarthropods as regulators of microbial activity in a leaf fall create more favorable biochemical and microbial situation that positively affects physiological processes of earthworms and wood lice, and microarthropods' deficiency is negative.

In the experiment the content of humic substances in sand extracts exceeds those indicators in leaf extracts. Earlier we displayed that the content of humic substances is much less in case of availability of microarthropods in the sand underlying a leaf fall, than in a leaf fall itself, as well as a ratio of C humic acids / C fulvic acids (Simonov, 1984a, 1984b).

Possibly, it is connected with nonpercolative regime of a substratum in an experiment and with formation of water insoluble organ and mineral complexes.

It is apparent that the depth of leaf fall destruction in this experiment is much more considerable that results in intensification of migratory processes for molecules of humic substances in a leaf fall. Besides, excrements of wood lice and worms promote changes in sand content and structure that leads to the a significant amount of organic-mineral complexes.

There are unusual results of humification activity of earthworms: by all indexes of accumulation of humic substances they concede to primary destroyers: wood lice.

Some prevalence of common carbon in the option microarthropos + wood lice is connected with an increase of maceration rates, as land wood lice are mostly phitosaprophages. In intestines of wood lice there is stimulation of microbial growth. High microbial activity remains some time in the excrement in the soil and then fades (Striganova, 1980). It is probable that as a result of earthworms' activity there is an increase in abundance and in a ratio of microorganisms (Striganova, 1989) which cause formation of fragile compounds of humic substances and also participation in their decomposition.

Rather close values of Corg as a part of extracts with worms and wood lice points to the considerable stimulation of microbial activity of destruction process. The stimulation mechanism is various, also the fate of humic substances is various. There is no expected output of humic substances in the options with worms, probably, because worms can stimulate the activity of microorganisms - humus-consumers. As microorganisms consume easily decomposed fulvic acids therefore a ratio of C humic acids / C fulvic acids and coefficient of a chromacity of humic acids in the options with worms is higher, than in options with wood lice. Proceeding from the analysis of these figures 5 and 6, activity of microarthropods obviously stimulates this process.

Digestion features of structural components of a leaf fall and their transformation in worms intestines (Kozlovskaya, 1984; Perel & Karpachevsky, 1968; Striganova, 1989) forwarded decomposition process towards humification strengthening, however quantitative values are slightly less, than in options 2 and 3. Apparently, the excluding of microarthropods as the main agents stimulating microbial activity (Simonov, 2002), reduces formation process of humic substances in the conditions of this option.

We observe a similar effect also in the option with a layer destruction with the participation of only wood lice. Activity of wood lice stimulates an increase in the surface area of a macerative layer, but deficiency of microarthropods, increasing selective colonization of a dead soil cover due to microorganisms (Simonov, 1989; Bengtsson & Rundgren, 1983; Visser, Whittaker & Parkinson, 1981) leads to decrease in quantitative indicators of an output of humic substances. The coefficient C humic acids / C fulvic acids demonstrates strengthening of mineralization processes (fig. 5,6).

In all total extracts the content of organic carbon in the options with microarthropods is higher, than in monocultures. The quantitative indicators of humic substances which are formed at collaborative activity of microarthropods and large invertebrates are higher, in comparison with the options of their separate keeping.

Trophic activity of microarthropods not only influences humification process (Simonov, 1984a; Naglitsch & Grabert, 1968), but also leads to the intensity of this process (Simonov, 1984b). Output increase and accumulation of humic substances provided microarthropod's availability can be explained in such way that feeding by microorganisms (melaniferous) leads to more complete spontaneous process of a granules release of "humous" substance from cells (Reisinger & Kilbertus, 1973; Vegter, 1983). Apparently, it promotes the shift of time of including these substances in a humification process that affects maturity degree of humic substances.

The combination of microorganisms, microarthropods and mesofauna representatives positively affects not only the quantity of formed GV, but also their quality (a ratio of humic acids / fulvic acids). The most optimum combination of bioagents in experimental conditions is microarthropods activity with nitrogen liberator.

The analysis of an optical density both "total" extracts, and extracts of humic acids in water and pyrophosphatic extracts from sand and leaves showed that micro and mesofauna influence on humification process is unequal. The inclined planes of an optical density in the range of lengths of waves differ by

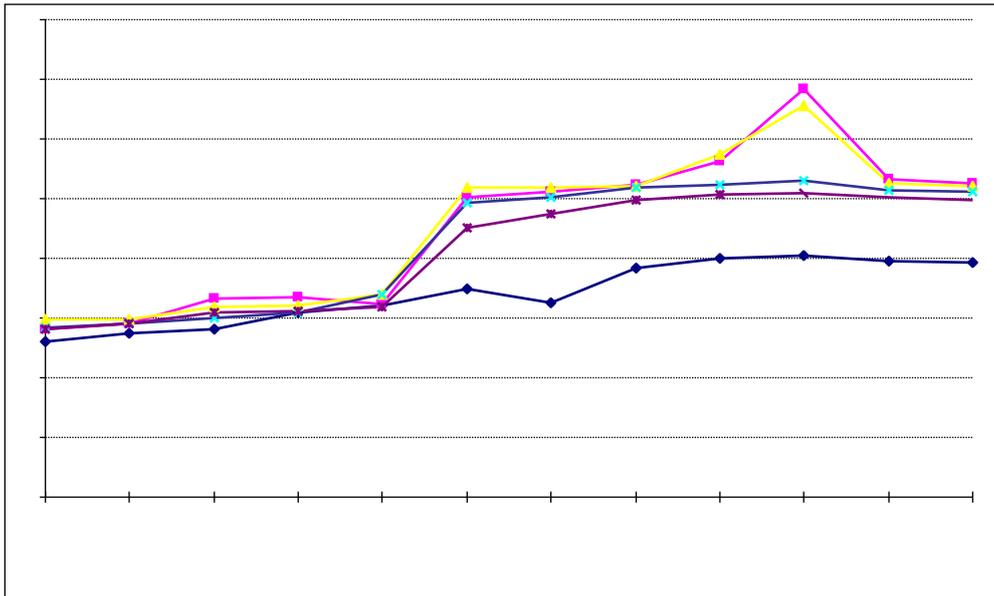
location and falling steepness that gives an idea of changes of humic substances concentration.

The maximal value of an inclination angle was necessary for 5-6 months and for 9-10 months that corresponds to available data (Chernova, 1989).

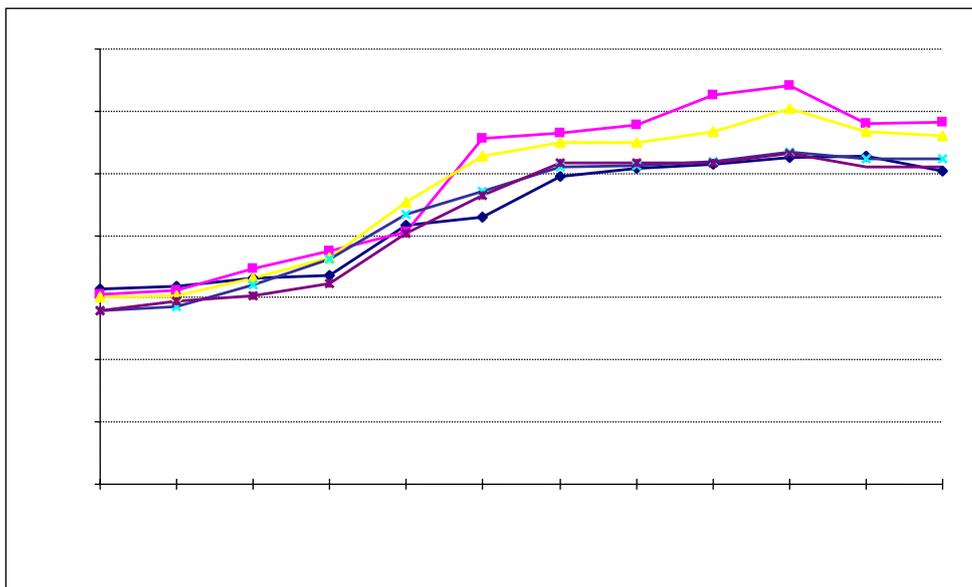
Availability of earthworms displaces peaks of microarthropods for earlier terms in comparison with the activity of wood lice. These data are rather contradictory. On the one hand, activity of primary destroyers increases a total surface of dead soil layer, so it increases the abundance of microorganisms and microarthropods, but it does not occur. It is apparent that the activity of a humificator (earthworms) creates more favorable conditions for the development of a collembolan community .

In indexes dynamics of humic substance's output and their maturity degree (humic acids / fulvic acids) one common regularity is noted: the highest values of these parameters are late in terms in comparison with the periods of maximal weight loss of a dead soil layer, and this indicator is shifted to the right regarding microarthropods abundance (in options 2 and 3). It is apparent that variety increase of biodegradative agents strengthens humification and mineralization processes.

In this regard it is interesting to analyse the data of optical density of humic substance received from pyrophosphatic extracts of experimental options (fig. 7,8)



**Figure 7.** Dynamics of angle of inclination of lines of an optical density of humic substances of a leaf pyrophosphatic extract (results of characteristic research)



**Figure 8.** Dynamics of angle of inclination of lines of an optical density of a pyrophosphatic extract from sand (results of characteristic research)

The analysis of values of an optical density of humic substance and a corner size of inclined planes of an optical density with various lengths of waves in a water extract from sand displayed the analogy of conclusions that we made for a water extract from leaves.

It is apparent that forming, condensating and maturing of water-soluble materials GV in leaf layer dictates proportional nature of condensity, maturing and fixing of humus substance in underlying substratum.

The analysis of indexes of an optical density of GV and their angle of inclination with various lengths of waves in a pyrophosphatic extract from leaves is similar to indexes a water extract from leaves, both in terms of maximal values, and in size of angle of inclination.

Inclined planes of an optical density in pyrophosphatic extracts from leaves in all options differ in quieter nature of "running-off" that says that processes of maturing, condensation and other possible processes (disintegration of GV under the microorganisms influence) of more complex humus substance taken by Na pyrophosphate proceed more quietly, more systematically, than in simple humic substance (water-soluble).

Earlier increase in an angle of inclined planes of an optical density of humus substance in a pyrophosphatic extract from sand at similar parameters (see above) indicates that humus substance with mineral particles of underlying substratum begin to form an organic-mineral complex at earlier stages, than condensation processes of humic substance in leaf litter.

The maximal optical density was noted in the options with full structure of destructors.

More high optical density of humic substances in the options gives the chance to speak about some changes in their condensation degree. It is known that in coloring formation of humic substances not only the grid of aromatic carbon, but also structure of a peripheral part of molecules matters (Orlov, 1974;

Orlov, 1990). It was shown that factors like microbial activity affect the content change of this part of molecules. It is possible that the regulating activity of microarthropods, earthworms, wood lice and their combination in relation to microorganisms affects a peripheral part's structure of molecules of humic substances. The Increase in an angle of inclined planes of an optical density coincides with the growth of carbided humic substances, i.e. with the loss of side chains. Lower optical density in the options without microarthropods is caused, apparently, by the availability of less mature humic substances, that is with more developed peripheral chains in molecules.

Thus, the activity of soil-forming invertebrates leads to strengthening of humification processes. Activity of earthworms is more efficient in comparison with wood lice, and it is connected with transformation processes of organic substance in worms intestines where there is a polymerization of low-molecular weight compounds in more complicated and mature forms of humic substances, however disintegration of humic substances in worms intestines is not excluded due to the stimulation of certain groups of microorganisms (Tiunov, 2003; Tikhonov et al., 2010). The molecular mass of humic acids is one of the parameters with which their biological activity correlates (Naglitsch & Grabert, 1968; Vaughan & Malcolm, 1985).

The interaction of microarthropods with earthworms is more efficient. It is obvious that, the fuller the content of biodegradative agents, the more intensive the decomposition, the stronger humification processes prevail over a mineralization, the more condensed the humic substance, the higher the maturity degree.

### Conclusion

Thus, as a result of conducted experiment in the conditions of artificially created microecosystems on the basis of quantitative data it is revealed that:

1. Microarthropods' activity positively affects a ratio of humification processes of top humus (shift towards humification) at keeping with the representatives of soil mesofauna.
2. Activity of earthworms and wood lice changes the course and rates of transformation process of organic substance, defining it as deep and intensive.
3. Collateral activity of microarthropods and mesofaunae promotes more considerable output of humic substances, maturity degree (chromacity coefficient, corbidity degree) of which is more considerable, than separate functioning of soil biota representatives.

### Implications and Recommendations

The received results have huge practical value for creation and preservation of long-term fertility and high agronomical soil value, both in agrocenosis, and in natural biogeocenosis.

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