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NO-TILL TECHNOLOGY AS A FACTOR OF ACTIVITY OF SOIL INVERTEBRATE IN AGRICULTURAL CHERNOZEMS OF STAVROPOL REGION

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Abstract

Soil macro- and mesofauna is highly sensitive to various methods of agricultural cultivation, therefore soil invertebrates are used as bioindicators of agrocenoses ecological condition. Since the macro- and mesofauna to a largely extent control the water balance of the soil and participate in the formation of humus, special attention should be paid to soil fauna in arid regions, primarily in the Cernozem Region. In this work, for the first time, an integrated estimation of the population density and ecological and functional diversity of macro- and mesofauna of Vorony-Calcic Chernozem in the Stavropol region is given. The possibility of using these groups of invertebrates as bioindicators of the ecological status of agrocenoses has been shown. It is proved that the use of no-till technology stimulates the activity and number of all groups of macro- and mesofauna. The purpose of the work is to estimate the numbers and taxonomic diversity of ecological and functional groups of macro- and mesofauna with various technologies of soil cultivation (traditional plowing and no-till) with and without mineral fertilizers on the agrochernozems of the Stavropol region. Experiments on research no-till technology were carried out in 2012-2019 in an experimental farm of the North Caucasus Federal Scientific Agrarian Center (Shpakovsky district of the Stavropol region). In 2019 we studied plots of fields with three types of factors: tillage (plowing and no-till technology); presence/absence of fertilizers; agricultural crops. The soil is Vorony-Calcic Chernozem. Crop rotation: maize (*Zea mays* L.) variety Mashuk, soybean (*Glycine max* L.) variety Duniza (until 2018), which was replaced later by peas (*Pisum sativum* L.) variety Phaeton, winter wheat (*Triticum aestivum* L.) variety Deya, sunflower (*Helianthus annuus* L.) variety Bagrat. Deposit soil near the experimental fields served as a control. Fertilizers were applied at the time of sowing ($N_{160}P_{90}K_{60}$ for winter wheat, $N_{72}P_{58}K_{32}$ for sunflower, $N_{80}P_{48}K_{48}$ for corn, and $N_{60}P_{60}K_{60}$ for soybeans and peas). The soil macrofauna was registered by the method of excavation of areas 25×25×30 cm and manual analysis of soil samples. Soil mesofauna was isolated from the soil monolith by the method of eklectors, identified and counted using a microscope Biomed-5 PR LUM (Russia) at a magnification of ×40. Most abundant among the macrofauna were centipedes (*Myriapoda*), adults and larvae of coleopterans (*Coleoptera*), spiders (*Araneae*) and earthworms (*Lumbricidae*). *Aporrectodea caliginosa* dominated among earthworms, while single of *A. rosea* were found only in deposit lands. The minimum number of *A. caliginosa* (32 ind/m^2) was recorded under peas and sunflower with traditional plowing, the maximum — under corn on no-till plots and on plowed plots (556 and 512 ind/m^2, respectively). In general, the number of earthworms was higher in no-till fields under all crops (excluding sunflower) in comparison with plowed plots. Among other groups of soil macrofauna, the most numerous were centipedes (up to 1500 ind/m^2), as well as spiders (up to 500 ind/m^2) and beetles (up to 500 ind/m^2). Woodlice (*Oniscidea*) and molluscs (*Gastropoda*) were also encountered. The density of centipedes, spiders, coleoptera and earthworms was always higher for no-till options than for plowed fields, regardless of crop. The application of mineral fertilizers, as a rule, reduced the number and diversity of the macrofauna representatives. Among the mesofauna, ticks (*Acari*) and collembolans (*Collembola*) prevailed in terms of abundance and diversity. Mesofauna of no-till fields was taxonomically more diverse than plowed plots. The minimum number of mesofauna representative was found under peas and corn, the maximum — under winter wheat and sunflower. In general, the distribution of soil invertebrates (macro- and mesofauna) was significantly influenced by the method of soil cultivation, however, the agricultural culture often influenced the abundance indicators. The use of fertilizers reduced the biodiversity of macrofauna and decreased its number in all plots, regardless of the method of soil cultivation.

Keywords: no-till, plowing, chernozems, soil invertebrates, *Lumbricidae*, macrofauna, mesofauna, bioindication

Intensive agriculture based on conventional tilling (plowing) often degrades not only fertility [1, 2] but also the ecology of agricultural biocenoses (agrocenoses) [3]. Irrational farming drastically alters the physical properties of soil [2, 4] and kills soil macrofauna and mesofauna en masse; the geobionts it destroys are critical for agriculture as they additionally loosen, mix, and structure the soil, disintegrate and homogenize plant residues, convert matter, deoxidize the soil, and kill phytopathogens [5-7]. Since invertebrates largely control the water balance of soils by making runs and redistributing pore space [3], geobionts are crucial for arid regions, in particular Chernozem areas. Notably, macrofauna and mesofauna are directly involved in the formation of humus [8-10], and a severe decline in, or nullification of, detritophage population may cause uncontrolled soil degradation [4-6]. Unfortunately, some farms fail to see that intensive agriculture needs to be not only cost-effective but also reasonably eco-friendly [8]. However, some farms have undertaken to protect the environment by minimizing the tilling, in particular by adopting a no-till approach [11, 12]. Data on the benefits or hazards of both plowing and no-till is contradictory and needs to be verified [11]. Since invertebrate geobionts are the quickest to respond to tilling [5, 13, 14], a comprehensive "census" of macrofaunal and mesofaunal communities is imperative in order to project the effects of this or that soil tillage method [6, 15, 16].

This paper is the first to present an integrated density and ecofunctional diversity assessment of macrofauna (earthworms, spiders, beetles, myriapods, woodlice, and mollusks) and mesofauna (mites, springtails, proturans, nematodes) found in typic chernozem within the experimental farm in the Stavropol Territory. The paper further demonstrates these groups of invertebrates can indicate the ecological status of agrocenoses. No-till is shown to boost the activity and population of all macrofauna and mesophauna.

The goal hereof was to evaluate the population and taxonomic diversity of macrofaunal and mesofaunal ecofunctional groups in southern chernozems of the Stavropol Territory as affected by different tillage methods (conventional plowing vs no-till) with and without mineral fertilizers.

Materials and methods. No-till experiments with/without mineral fertilizers were run in 2012-2019 at an experimental farm operated by North Caucasus Federal Agricultural Research Center (45°07′48″N 42°01′39″E, Shpakovsky District of the Stavropol Territory). In 2019, land plots were tested by three factors: plowing vs. no-till, fertilized vs. unfertilized, and sown crops (pea, corn, sunflower, and winter wheat). Each plot was 300 m^2 (50×6 m), 90 m^2 the declared area. Experiments were run three times.

The soil was typic medium low-humus heavy-loam chernozem upon lцss-like carbonate loams [17]. The arable horizon was relatively low on humus (3.87%) and nitrate nitrogen (11.9 mg/kg), had medium concentrations of labile phosphorus (18.7 mg/kg, Machigin's test) and exchangeable K (245 mg/kg); pH 6.32. Deeper sampling was associated with a reduction in all the parameters. Horizon C located at 126-175 cm down had 0.65% humus, 0.5 mg/kg N-NO_3, 3.4 mg/kg P_2O_5, 155 mg/kg K_2O; pH 8.3 [17]. In some plots, the soil was subject to conventional plowing (with overturning); other plots utilized a no-till approach. Such distinct practices were applied over the course of eight years.

Crop rotation was corn (*Zea mays* L.) variety Mashuk, soybean (*Glycine max* L.) variety Duniza (until 2018) later replaced with pea (*Pisum sativum* L.) variety Phaeton, winter wheat (*Triticum aestivum* L.) variety Deya, and sunflower (*Helianthus annuus* L.) variety Bagrat. For control, the research team tested soils from near-field long fallows. Mineral fertilizers were applied by sowers when sowing. The estimated dosage was: $N_{160}P_{90}K_{60}$ for winter wheat, $N_{72}P_{58}K_{32}$ for sunflower, $N_{80}P_{48}K_{48}$ for corn, $N_{60}P_{60}K_{60}$ for soybean and pea.

Pre-harvest soil density was 1.23 g/cm^3 in conventionally tilled land plots, 1.24 g/cm^3 in no-till plots within 10 cm of depth. Macrofauna estimates relied on sampling from sites sized 25×25×30 cm that were manually sorted into layers (0-10, 10-20, and 20-30 cm) [13]. Samples were kept in 96% ethanol prior to testing. Earthworms were classified by species per Kasprzak [18]; other groups were classified by supra-species taxa [19].

Mesofauna was extracted from monoliths (cylinders sized 101.1 cm^3) sampled from upper soil (down to 5.1 cm in depth) by means of eclectors; estimation involved a Biomed-5 PR LUM microscope (Biomed, Russia) at a magnification of ×40.

Diagrams were plotted in Microsoft Excel by calculating the means (*M*) and standard deviations (±SD). Cluster analysis was run in Statistica 10 (StatSoft Inc., US). A dendrogram was plotted by Ward's method based on analysis of variance for inter-cluster distance measurement.

Results. In soil macrofauna, we found and recorded earthworms (family *Lumbricidae*), spiders (order *Araneae*), larvae and imagoes of beetles (order *Coleoptera*), myriapods (subphylum *Myriapoda*), woodlice (suborder *Oniscidea*), and mollusks (class *Gastropoda*). The most numerous taxa were the subphylum *Myriapoda*, the orders *Coleoptera* and *Araneae*, and the family *Lumbricidae*.

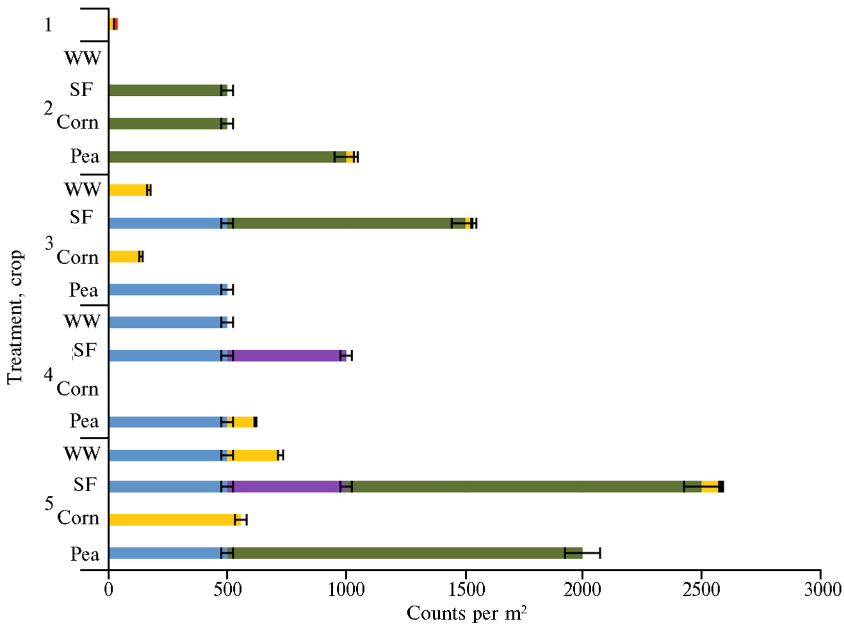

Fig. 1. Population of core macrofaunal groups as a function of soil tillage method (1 for fallows, 2 and 3 for plowing, 4 and 5 for no-till)**, use** (2, 4) **or non-use** (3, 5) **of fertilizers, and the crop:** WW — winter wheat *Triticum aestivum* L. cv. Deya, SF — sunflower *Helianthus annuus* L. cv. Bagrat, Corn — *Zea mays* L. cv. Mashuk, Pea — *Pisum sativum* L. cv. Faeton; ■ — order *Coleoptera,* ■ — order *Araneae*, ■ — superclass *Myriapoda*, ■ — *Aporrectodea caliginosa*, ■ — *A. rosea* (Shpakovsky District, the Stavropol Territory, 2019).

The taxonomy of earthworms in the experimental soils had only one endogeic species — *A. caliginosa*. Only fallows contained another endogenic species, the *A. rosea* (Fig. 1).

The smallest population of *A. caliginosa* (32 ind/m^2) was observed under fertilized pea and unfertilized sunflower in tilled soils; the largest populations were observed under corn on non-till plots, as well as in plowed unfertilized soils (556 and 512 ind/m^2). In general, the earthworm population was higher in no-till fields for any crop except sunflower. It was low at 40 ind/m^2 in fallows; however, only fallows contained *A. rosea*, the relative abundance of which was as high as 40%. The general earthworm population in the Center's fields was on the order of data collected in other studies carried out in the steppes of Central Fore-Caucasus [13].

Such low earthworm diversity might be due to two factors. First, soil was sampled in late May, when Stavropol soils are relatively dry [20]. Invertebrates usually tend to be more prevalent and diverse in wet soils [10]. Second, agricultural activity (plowing in particular) is known to reduce the diversity and population density of earthworms [5]. Fallows, on the other hand, are transitioning to natural biogeocenosis that is more diverse in terms of invertebrate population compared to fields [10, 21, 22].

In some cases, the crop and fertilization had a greater effect on the worm population than the tillage method. This pattern has also been observed in other zoological studies [8, 10, 23]. No-till plots had a greater earthworm population due to lesser mechanical agitation of soil and the abundance of plant residues, which contribute to the well-being of all *Lumbricidae* species [15, 23]. Earthworms were not found under fertilized winter wheat, corn, or sunflower regardless of the tillage method. Mineral fertilizers are well-known to kill macrofauna [24], especially earthworms, since they lack chitin or lime carapaces that most other invertebrates have [25]. Only pea plots had earthworms even when fertilized. Adding legumes to crop rotation is known to boost worm population [26]. *A. caliginosa* were found in nearly all unfertilized plots under most crops, whether tilled or not tilled.

A. caliginosa being the single dominant endogeic species is not untypical for agrocenoses [27-29]. *A. caliginosa* is less vulnerable to drought compared to other earthworms [27]. Adult and juvenile *A. caliginosa* are capable of "taking" a summer diapause, whereby they dehydrate their tissues, adult specimens shed their tubercula pubertatis, and worms “curl up” alone or together with others to mitigate moisture loss [30]. This sustainability mechanism is especially relevant in the Stavropol Territory, where spring and summer droughts are not unheard of [20].

Myriapods, spiders, and beetles constituted other populous macrofaunal groups. Woodlice and mollusks were rather rare.

Unfertilized no-till fields had as diverse as possible macrofauna. Beetles were found in all but corn-sown plots at up to 500 ind/m^2; however, spiders were only found on sunflower plots at up to 500 ind/m^2, see Fig. 1. Myriapods on unfertilized no-till plots had populations of up to 1,500 ind/m^2), but they were detected only under pea and sunflower. Beetle and spider populations were the same in fertilized no-till soil as on unfertilized no-till plots; however, myriapods were not found in such soil, see Fig. 1.

Plowed unfertilized fields were devoid of spiders; however, sunflower and pea fields had beetles (up to 500 ind/m^2) and myriapods (up to 1,000 ind/m^2), see Fig. 1. Plowed fertilized fields totally lacked any macrofauna except myriapods (up to 500 ind/m^2) found under corn and sunflower, see Fig. 1.

Beetles had a higher population density in no-till fields compared to plowed fields, where they were only numerous on unfertilized pea and sunflower plots, which might be due to the abundance of plant mulch in no-till fields [31]. Ecologically, these fields are closer to steppe biogeocenoses as they preserve litter (a horizon that provides habitat to predators and myxophages) and plant residues, which are a trophic resource for saprophages, phytophages, and myxophages [32].

Dense spider populations of up to 500 ind/m^2 were only found in sunflower fields. Perhaps the biotope affinity of these obligate carnivores to sunflower was due to the abundance of potential prey, in particular *Coleoptera*, in such fields [33].

Myriapods were the most common group in unfertilized no-till fields of sunflower and pea ind/m^2). They also were the only group that inhabited fertilized conventionally tilled fields. Since the subphylum Myriapoda has saprophages (*Diplopoda*) and predators (*Chiplopoda*), their density depends on the quality and quantity of post-harvest plant residues and potential invertebrate prey [34].

In general, winter wheat and corn fields were the least favorable habitats for soil macrofauna. In some cases, these fields did not contain any macrofauna at all when fertilized. It is only logical to assume that post-harvest cereal residues are harder to decompose due to their wider carbon-to-nitrogen ratio as compared to legumes such as pea or soybean, or composite flowers such as sunflower; this makes such fields less attractive for saprophages. Decomposition rates of cereals are only half those of legumes: wheat only loses 23.8% of its post-harvest weight over a year, whilst alfalfa loses 45.5% [35].

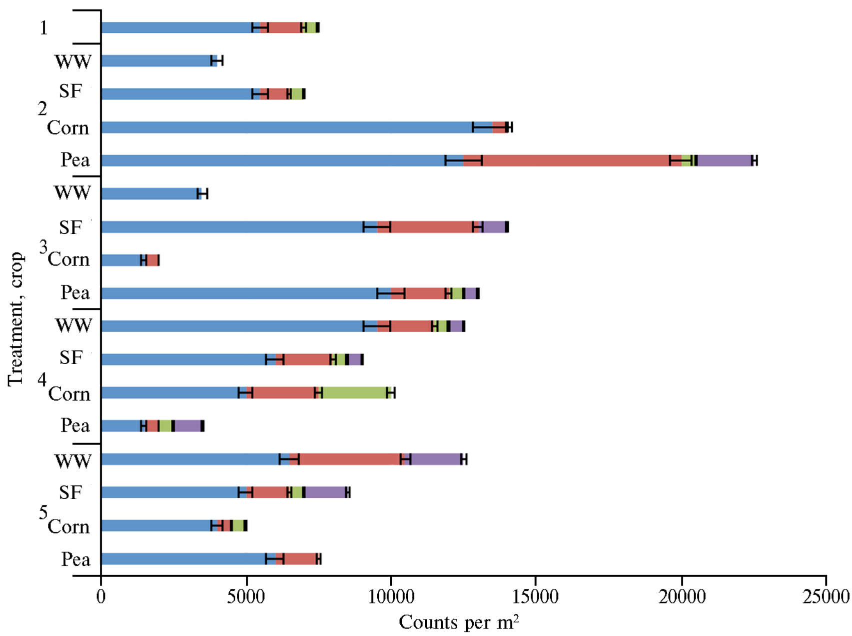

Fig. 2. Population of core mesofaunal groups in soil as a function of soil tillage method (1 for fallows, 2 and 3 for plowing, 4 and 5 for no-till)**, use** (2, 4) **or non-use** (3, 5) **of fertilizers, and the crop:** WW — winter wheat *Triticum aestivum* L. cv. Deya, SF — sunflower *Helianthus annuus* L. cv. Bagrat, Corn — *Zea mays* L. cv. Mashuk, Pea — *Pisum sativum* L. cv. Faeton; ■ — subclass *Acari,* ■ — subclass *Collembola*, ■ — type *Nematoda*, ■ — order *Protura* (Shpakovsky District, the Stavropol Territory, 2019).

Mesofauna was dominated by oribatid mites and gamasina (subclass Acari) and springtails (subclass *Collembola*), see Fig. 2. Proturans (order *Protura*) and nematodes (type *Nematoda*) were found as well. No-till fields had a far richer mesophauna (total population density of 9.5×10^3 ind/m^2 and 12 morphotypes) compared to plowed plots (4.0×10^3 ind/m^2 and 7 morphotypes). Pea was associated with a minimum mesofaunal population whilst winter wheat had the maximum numbers.

As for unfertilized no-till fields, corn was associated with the smallest population and lowest diversity (up to 4.0×10^3 ind/m^2 for mites, up to 500 end/m^2 for nematodes and springtails), whilst sunflower and winter wheat had the largest populations and the best diversity (up to 6.5×10^3 ind/m^2 for mites, up to 4.0×10^3 ind/m^2 for springtails, up to 500 ind/m^2 for nematodes, up to 2.0×10^3 ind/m^2 for proturans), see Fig. 2. Proturans were only found under sunflower and winter wheat, whilst nematodes were only found under sunflower and corn. The proportionate population of springtails depended on the crop, increasing in the following order: corn, sunflower, pea, fallow, winter wheat.

In fertilized no-till fields, the mesofaunal population was minimum at up to 5.0×10^3 ind/m^2 for mites, up to 2.5×10^3 ind/m^2 for springtails and nematodes under corn; maximum at 9.5×10^3 ind/m^2 for mites, up to 2.0×10^3 ind/m^2 for springtails, up to 500 ind/m^2 for nematodes and proturans under winter wheat, see Fig. 2. Regardless of the crop, mites were the most common mesofaunal group followed by springtails, nematodes, and proturans (the latter were only absent in corn fields).

As for plowed unfertilized fields, corn was associated with minimum mesofauna (mites at up to 1.5×10^3 ind/m^2, springtails at up to 500 ind/m^2), and so was winter wheat (mites at up to 3.5×10^3 ind/m^2), whilst maximum mesofauna was associated with pea (mites at up to 1.2×10^4 ind/m^2, springtails at up to 7.5×10^3 ind/m^2, nematodes at up to 500 ind/m^2, proturans at up to 2.0×10^3 ind/m^2) and with sunflower (mites at up to 9.5×10^4 ind/m^2, springtails at up to 3.5×10^3 ind/m^2, proturans at up to 1.0×10^3 ind/m^2), see Fig. 2. Proturans were only found under pea and sunflower, nematodes were only found in pea fields. Wheat fields only had mites. Springtails accounted for 33% of the total mesofaunal population of pea and corn fields, 25% in sunflower fields.

Plowed fertilized fields had the least diverse taxonomy. Wheat was associated with minimum population and diversity of mesofauna at up to 4.0×10^3 ind/m^2 for mites, whilst pea was associated with maximum figures at up to 1.25×10^4 ind/m^2 for mites, 7.5×10^3 ind/m^2 for springtails, 500 ind/m^2 for nematodes, and 2.5×10^3 ind/m^2 for proturans, see Fig. 2. Wheat fields were found to only contain mites. Proturans were only found in pea fields, and nematodes only in pea and sunflower fields. Pea fields were most springtail-populous at up to 7.5×10^3 ind/m^2.

The general mesofaunal population in the Center's fields was on the order of data collected in other studies carried out in the steppes of Central Fore-Caucasus [36]. The dominance of mites in the tested soils was due to the use of eclectors, a method specifically designed to count oribatids [13].

The more numerous and diverse mesofaunal taxonomy of no-till fields was mainly due to the abundance of plant residues and the lack of mechanical impact on soil, which is known to kill invertebrates [24]. The low population of mesofauna under pea and the maximum figures under winter wheat and corn could be due to the better preservation of post-harvest cereal residues thanks to their slower decomposition by saprophagous invertebrates and microorganisms [35]. This preserves a larger horizon that provides habitat to mesofauna, especially the one found in litter, i.e., mites and springtails [37, 38]. The use of mineral fertilizers also had a negative impact on mesofaunal population and diversity, which is in line with what Prasanthi *et al.* reported [39].

The fact that the full taxonomic spectrum of mesofauna was found in unfertilized no-till fields confirms what other researchers reported: plowing and abundance of mineral fertilizers depopulate agricultural landscapes of invertebrates [8, 23, 26]. At the same time, fertilizers may actually boost the growth of some soil invertebrates in plowed fields. The increased mesofaunal population and diversity in fertilized plowed fields might be due to the boosted growth of microorganisms that benefit from the excess of nitrates and phosphates [14].

Springtails are common in agrocenoses, as are mites. In this particular study, springtails were abundant on virtually any plot. Other authors have also shown springtails to be highly adaptable to various agricultural techniques in no-till, plowed, fertilized/unfertilized fields [40].

Proturans are fairly rare and have a rather specific ecology [41]. Their substantial presence in all the tested fields might be due to the good porosity of soil, since proturans themselves are incapable of digging and instead occupy the airspace between soil aggregates that larger invertebrates create. An early study showed a positive correlation between the density of endogeic worms and that of proturans [42].

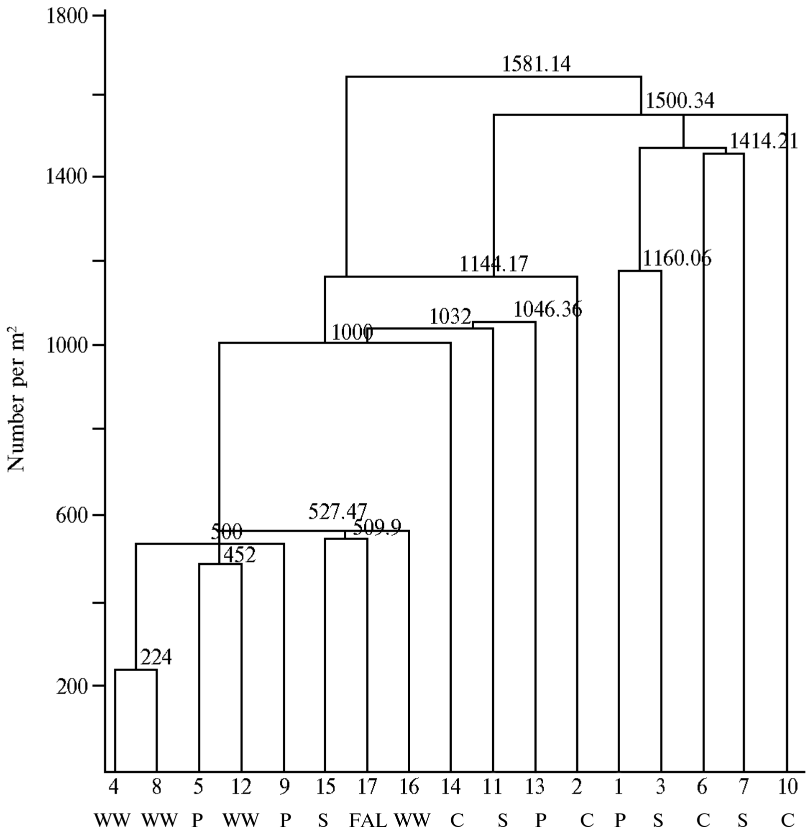

Fig. 3. Dendrogram of similarity between experimental fields in terms of macrofaunal and mesophaunal density as affected by tillage, presence or absence of fertilizers, and the crop (data based on population figures): 1 to 4 for unfertilized no-till fields; 5 to 8 for fertilized no-till fields; 9 to 12 for unfertilized plowed fields; 13 to 16 for fertilized plowed fields; 17 for fallows; WW — winter wheat (*Triticum aestivum* L.) cv. Deya, S — sunflower (*Helianthus annuus* L.) cv. Bagrat, C — corn (*Zea mays* L.) cv. Mashuk, P — peas (*Pisum sativum* L.) cv. Faeton; FAL for fallows (Ward's method) (Shpakovsky District, the Stavropol Territory, 2019).

When clustering plots by the density of macrofauna and mesofauna (Fig. 3), plots that were both plowed and fertilized formed a separate cluster (Nos. 14 to 16). The same cluster also included a fallow field (No. 17) that had similar macrofaunal and mesofaunal density. This could be due to the fact that this fallow was young and had earlier been used as plowed fertilized land. The cluster of highest invertebrate density contained no-till plots, both fertilized and unfertilized (Nos. 1, 3 and 6, 7), where sunflower and pea were farmed. The smallest invertebrate populations were observed on fertilized no-till pea and winter wheat plots (Nos. 5 and 8), as well as on plowed unfertilized pea and winter wheat fields (Nos. 9 and 12). The same cluster contained an unfertilized no-till plot with winter wheat (No. 4). Therefore, single-crop wheat cultivation prevented invertebrates from populating even no-till fields, i.e., had the same effect as fertilization or plowing. In general, the distribution of soil invertebrates (macrofauna and mesofauna) was greatly affected by the tillage method; however, the crop itself often influenced the population figures, too.

Thus, this paper presents an integrated assessment of population density and taxonomic diversity of macrofauna and mesofauna in agricultural chernozems in the Shpakovsky District of the Stavropol Territory, as affected by tillage method, use of fertilizers, and the crop of choice. It shows that the energy-saving no-till technology had a positive effect on macrofaunal and mesofaunal biodiversity whilst mitigating the dominance of specific groups and lowering the risks of pest outbreaks. Tested macrofaunal groups had their numbers increased by a factor of 2.5; the populations of earthworms, myriapods, spiders, and beetles rose. Mites and springtails constituted the most populous mesofaunal groups, whilst proturans were the least populous group. Therefore, these groups of soil invertebrates could serve as biological indicators of the ecofunctional status of agrocenosis. Mineral fertilizers were found toxic for macrofauna, having a negative impact on its biodiversity and population regardless of the tillage method. Fertilized land plots had no earthworms but a larger myriapod population. Fertilization did not have a profound impact of mesofauna; rather, it was affected by the crop in rotation. Winter wheat was associated with the largest mesofauna population.

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