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Restoration of Earthworms Community (Oligochaeta: Lumbricidae) at Sand Quarries (Smolensk Oblast, Russia)

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Abstract. The earthworm community formation studies have been carried out during the primary succession at the sand quarries formed during the sand mining. It has been established that the earthworm colonization during the 7-50 years sand quarry overgrowth is slow and the species composition is not fully restored therewith. The quarries are mainly occupied with the endogeic species. The results of the experiment on the introduction of the species of earthworms of different morpho-ecological groups have been described for the quarries at different stages of restoration. It has been established that the endogeic species colonization is advisable at the initial stages of the quarry overgrowth and the epi-endogeic, epigeic and anecic species colonization – at the later stages.

Key words: introduction of earthworms, morpho-ecological groups of earthworms, technogenic disturbances, community restoration.

1. Introduction

The restoration of soil ecosystem functions after technogenic disturbances largely depends on the soil macrofauna restoration rate (Dunger, 1968; Hüttl & Weber, 2001; Majer et al., 2007; Topp et al., 2001; Boyer & Wratten, 2010; Roubickova & Frouz, 2014; Moradi et al., 2018). The activity of earthworms, pot worms, ants, woodlice, termites, larvae of flies, beetles and other invertebrates is fundamental in the creation and structuring of the soil profile of the disturbed areas, which contributes to the succession of plant community (Mudrak et al., 2012; Roubickova et al., 2012).The earthworms of the *Lumbricidae* family play the key role in the soil formation processes in the central part of Russia (Perel, 1979; Striganova, 1980; Bulavincev, 1979; Geraskina, 2016). There are many factors that prevent the natural colonization and expansion of the soil fauna in the industrial areas: lack of vegetation and organic matter, unstable water regime and substrate acidity, unfavorable microclimatic conditions (Dunger, 1968, 2006; Dunger et al., 2004; Hüttl & Weber, 2001; Topp et al., 2001; Pizl, 2001; Curry, 2004; Majer et al., 2007; Eijsackers, 2010; Roubickova & Frouz, 2014). Substrate toxicity is a serious problem for settling soil invertebrates (Frouz & Vinduskova, 2018). Vegetative recultivation of the disturbed areas (Langmaid, 1964; Abbott, 1989; Pizl, 2001; Topp et al., 2001; Dunger & Voigtländer, 2002; Dunger et al., 2004) and the introduction of soil invertebrates, earthworms, in particular (Brun et al., 1987; Ma & Eijsackers, 1989; Lee, 1995; Butt, 2008; Snyder & Hendrix, 2008; Moradi et al., 2018) are the most important soil formers which contribute to a more rapid development of the substrates.

Purpose of the study – estimation of the lumbricofauna restoration rate at sand quarries of different stages of overgrowing and the search for the most appropriate species of earthworms for introduction into the sandy soils of these quarries.

2. Study Area

The studies have been conducted on the outskirts of the city of Smolensk (Russian Federation) in the quarries formed as a result of sand extraction for construction works. Three quarries have been surveyed: the initial overgrowth stage quarry (Quarry 1), the middle overgrowth stage quarry (Quarry 2) and the late overgrowth stage quarry (Quarry 3). Coordinates of the objects: Quarry 1 – N 54.807028, E 32.968778; Quarry 2 – N 54.800211, E 31.964002, and Quarry 3 – N 54.801289, E 31.959546. Quarries are located 30 km from the central point of the city. The distance between quarries from 1 to 3 kilometers. Quarry 1 and Quarry 2 are located from 300 meters from small forest areas. Quarry 3 are located more far – 1500 meters from forest area. Until the middle of the 20th century this was a forest territory with a mixed conifer broad-leaved forests complexes. In the middle of the 20th century (60s-70s), in this area have been started the construction of residential buildings and the extraction of sand for construction.

3. Materials and Methods

The quarry overgrowth period at the start of the studies (2007) totaled 7 years (Quarry 1), 18 years (Quarry 2), and 46 years (Quarry 3). The surface form of all quarries was concave. At the first quarry (Quarry 1), the height of the slopes was 1-1.5 meters, the slope angle was 10-15°. The vegetation cover at the has been represented by the annual and perennial cereals (*Gramineae*). The projective cover of vegetation at the bottom of quarry was 95%, slopes – 70-80%. At the second quarry (Quarry 2) as well the height of the slopes was 1-1.5 meters, the slope angle was 10-15°. Among the herbaceous plants, *Gramineae* prevailed at the 18-year-old quarry; there are also the representatives of the *Fabaceae*, *Rosales*, *Compositae*, *Cruciferae*, *Umbelliferae*, *Chenopodioídeae* families; in addition, there were the shrub and woody forms of vegetation: *Corylus avellana*, *Alnus incana*, *Populus tremula*, *Betula pendula*. The

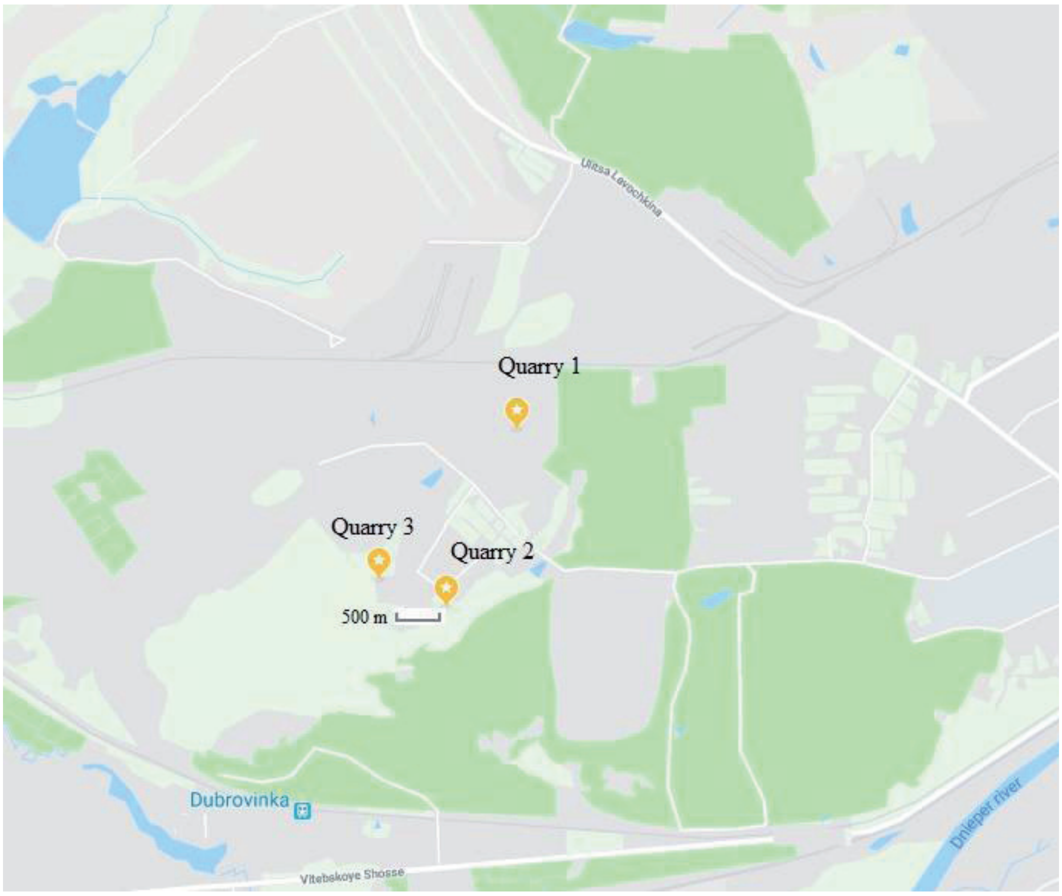

Figure 1. The localizations of sand quarries, Smolensk region, Russia

projective cover of vegetation at the bottom and slopes of quarry was 95%. The third quarry (Quarry 3) is the deepest of the three quarries: the height of the slopes was 2-2.5 meters; the slope angle was 30°. Among the herbel vegatation prevailed tall grasses (*Umbelliferae*, *Compositae*, *Urticaceae*). *Corylus avellána* prevails among the shrubs, and *Alnus incana*, *Betula pendula* – among the woody forms. The projective cover of vegetation at the bottom and slopes of the oldest quarry was total.

At all the sites, the soil was sandy, the humus horizon was 0.5 cm at the 7 year old quarry, 1-1.5 cm – at the 18 year old quarry and 3 cm – at the 46 year old quarry.

The quantitative surveys of earthworms have been carried out in the quarries in spring 2007 and the faunal collections – in the vicinity of the quarries. The method of excavation and manual separation of soil samples (Gilyarov, 1987) has been used. Sixteen samples have been taken at random at each site at approximately equal distance from each other. One sample area – 25 x 25 cm^2, depth – up to 30 cm. Studied area at each quarry – 0.5 ha. The earthworms were fixed in the 4% formalin solution. The species composition (Vsevolodova-Perel, 1997), abundance, occurrence, age structure, and biomass of the fixed worms upon full intestines have been determined in the samples. The life forms of the earthworms have been presented according to the Perel T.S. classification (Perel, 1979).

3.1. Earthworm Introduction Experiment

The earthworm introduction was made at three quarries in September 2007: 25 *Lumbricus rubellus*, 25 *L. castaneus*, 30 *Aporrectodea caliginosa* and 10 *L. terrestris* individuals were introduced at the depth of 15 cm into three sites of Quarry 1 of 1 m^2 each (the early stage of overgrowing), 25 *L. rubellus*, 25 *L. castaneus* and 10 *L. terrestris* individuals have been introduced in the same way at three sites to Quarry 2 (the middle stage of overgrowing). Only 45 *L. terrestris* individuals have been introduced into Quarry 3 (the late stage of overgrowing), since other species had already settled this territory (Fig. 2). We were unable

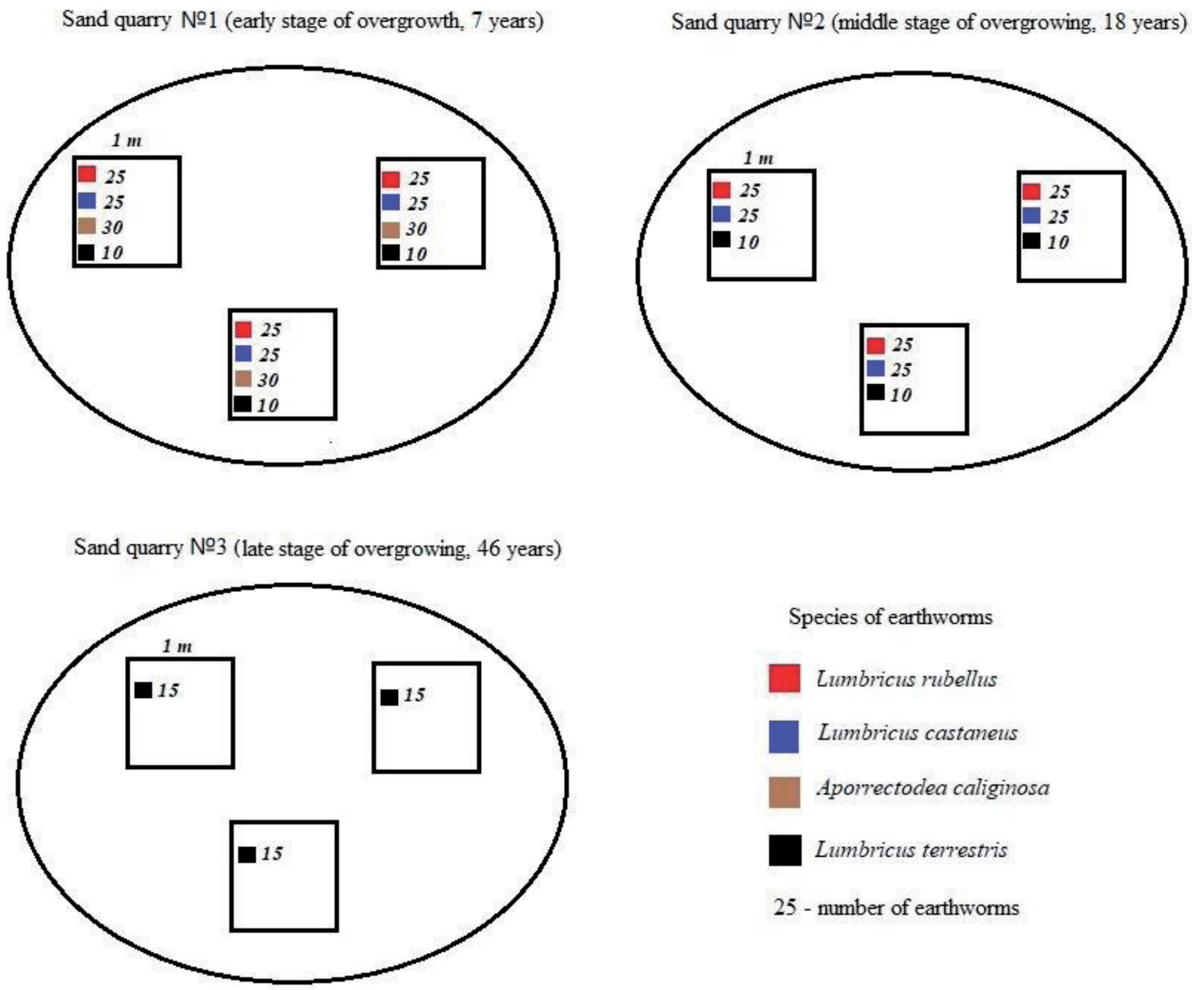

Figure 2. Experiment design of introduction of earthworms

to introduce *L. castaneus* to the third quarry due to the lack of collected material of this species for introduction. *L. terrestris* was added to the third quarry, despite the fact that it already lived there, but its numbers before the introduction was very low (4 ind./m^2). And we have to check how the introduction of an anecic species of worm will occur in three different quarries, including the old quarry, where it was shown that *L. terrestris* can live, and haw this can affect at the dynamics of biomass and the number of earthworms in these conditions. The points, to which earthworms were introduce, were surveyed earlier in the course of lumbricofauna counts in 2007. All the introduced worms were sexually mature. The repeated quantitative recording of earthworms (16 soil samples) was made in 2008 and 2012.

The statistical data processing was performed with the use of MS Excel 2016 software packages. The following parameters have been determined in the calculations: mean value (X), standard error of the mean (SE), median (M), quartiles (Q1, Q3). The non-parametric Mann-Whitney test ($p < 0.05$) was used in the sampling comparison and identification of the statistically significant differences.

4. Results

4.1. Pre-Introduction Earthworm Population

The earthworms have not been detected in Quarry 1 formed 7 years ago. 4 species of earthworms (*A. caliginosa*, *A. rosea*, *L. rubellus*, *L. terrestris*) have been found in the faunistic collections in the territory adjacent to the quarry. Only two species of earthworms (*A. caliginosa* and *A. rosea*) have been found in Quarry 2 formed 18 years ago. Four species of *Lumbricidae* (*A. caliginosa*, *A. rosea*, *L. rubellus*, *L. terrestris)* have been found in Quarry 3 formed 46 years ago. The number of earthworms is significantly higher in the late overgrowth stage quarry as compared to the middle overgrowth stage quarry (Table 1). Seven species of earthworms (*A. caliginosa*, *A. rosea*, *L. rubellus*, *L. terrestris*, *L. castaneus*, *D. octaedra*, *E. fetida*) have been found in the forest and meadow areas adjacent to Quarries 2 and 3.

The endogeic *A. caliginosa* species is dominant in terms of abundance and biomass in the 18 and 46 year old quarries. The relative abundance of another representative of the endogeic group of the *A. rosea* species is not high and almost the same in two (Table 1). Epi-endogeic *L. rubellus* and anecic worm *L. terrestris* have been found in small numbers only in the 46 year old quarry (Table 1).

4.2. After 1 Year Introduction Earthworm Population

Earthworms have been found in the quarry of the early stage of overgrowing one year after the introduction (Table 1, Fig. 3). Only three out of four introduced species of earthworms (*A. caliginosa*, *L. rubellus*, *L. castaneus)* have been found. Neither a single *L. terrestris* individual, nor any traces of its vital activity have been found.

The maximal number of worms has been revealed in the samples at a distance of 1 m from the place of introduction. Singular individuals of the epi-endogeic *L. rubellus* and epigeic *L. castaneus* species have been found only in the uppermost soil layer in the root zone of the plants, not more than 1.5 meters from the place of introduction.

Table 1. Species composition and number of earthworms in overgrown sand quarries before and after the introduction of earthworms (m±SE)

Species of earthworms	Sand Quarry 1 (the early stage of overgrowing)			Sand Quarry 2 (the middle stage of overgrowing)			Sand Quarry 3 (the late stage of overgrowing)		
	Pre-introduction	Post-introduction		Pre-introduction	Post-introduction		Pre-introduction	Post-introduction	
		1 year	5 years		1 year	5 years		1 year	5 years
A. caliginosa	0	8±2.0	12±3.0	35±5.6	38±9.6	32±4.5	51±1.2	48±4.6	44±6.0
A. rosea	0	0	0	4±0.6	5±1.6	6±2.5	7±0.4	5±1.5	8±0.2
L. castaneus	0	4±1.0	5±1.0	0	4±1.5	8±1.6	0	0	0
L. rubellus	0	3±1.6	6±1.5	0	**5±0.6**	**12±3.5**	3±0.7	5±1.2	6±1.5
L. terrestris	0	0	0	0	0	0	**4±0.6**	**8±1.0**	**12±1.5**
Total:	0	**15±3.5***	**23±4.9**	**39±7.5**	52±11.5	**58±10.5**	65±1.5	66±10.5	70±8.5

* bold type points out statistically significant differences between the samplings on the same sample area at different years of counting, Money-Whitney test, $p < 0.05$.

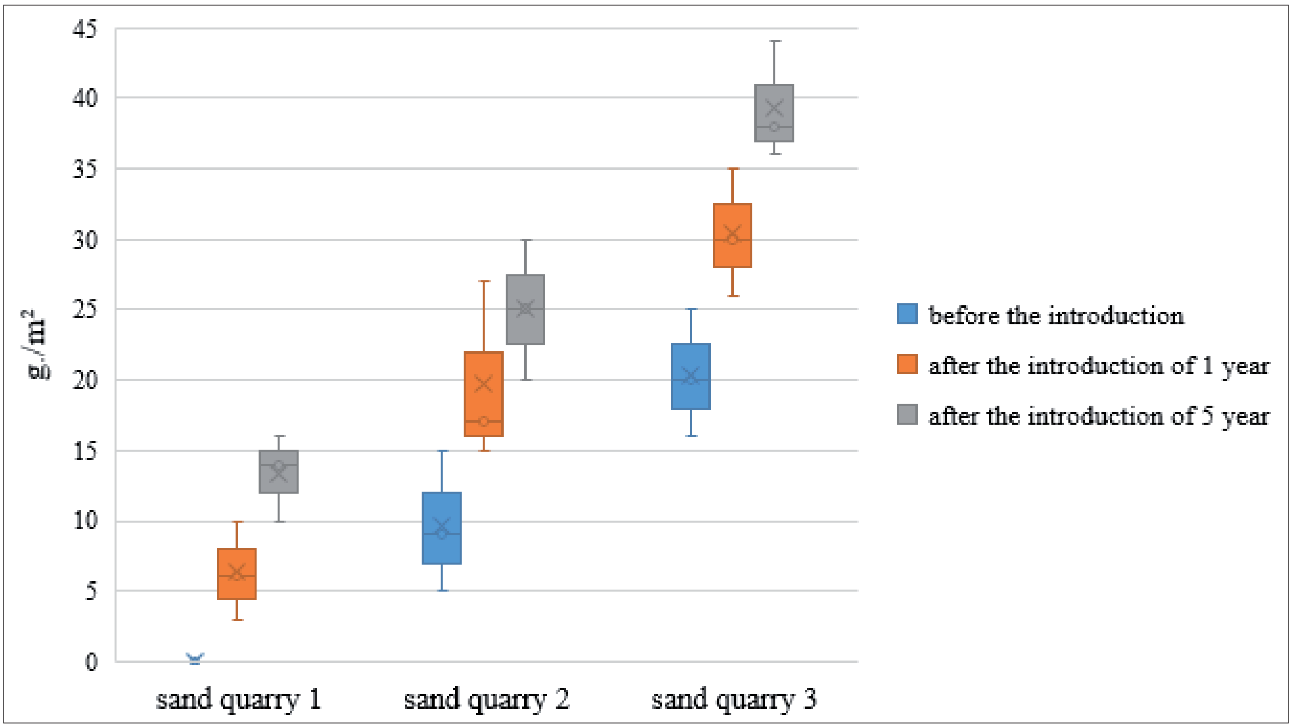

Figure 3. Total biomass of earthworms in sand quarries

The found worms were sexually mature. The endogeic species of *A. caliginosa* accounted for half the biomass of the total population of earthworms (Fig. 4). This species has spread within a radius of 2.5 m from the places of introduction. The juvenile individuals made up for almost 50% of the found worms which indicates the start of the reproduction process of this species under the new conditions. All the worms of this species were in the upper level of the soil at 1-6 cm.

Only two species – epigeic *L. castaneus* and epi-endogeic *L. rubellus* of the three introduced species have been found in the quarry of the middle stage overgrowth (Quarry 2) a year after the introduction. As in the previous quarry, the anecic species of *L. terrestris* has not been found. The epigeic species of *L. castaneus* has been found at a maximal radius of 1.5 m from the place of introduction, *L. rubellus* – not farther than 2.5 m. The number of endogeic species (*A. caliginosa* and *A. rosea*) previously encountered in this quarry has not significantly changed (Table 1). However, the share of these species in biomass decreased two times rather due to colonization of other species (Fig. 4). The total biomass of all the earthworms significantly increased over the year in statistical terms (Fig. 3).

The number of *L. terrestris* species per 1 m^2 a year after its introduction doubled in the quarry of the late stage of overgrowing (Quarry 3). In a year, the biomass of earthworms in Quarry 3 has significantly increased due to an increase in the number of large (Fig. 3). The number of other species previously noted in this quarry has not significantly changed (Table 1). The biomass dominance structure has changed: the share of species doubled, endogeic *A. caliginosa* almost halved (Fig. 4).

4.3. Post 5 Year Introduction Earthworm Population

The number and biomass of the worms has significantly increased in the quarry of the early stage of overgrowing five years after the introduction (Table 1, Fig. 3) as compared with the previous numbers. All the introduced species that had been discovered a year after the introduction have survived. The ratio of species by biomass and the main dominant endogeic species of *A. caliginosa* have not significantly changed over five years.

In the quarry of the middle stage of overgrowing (Quarry 2) the total number and biomass of worms has significantly increased five years after the introduction, as compared to the pre-introduction counts (Table 1; Fig. 3) and the number and biomass of epi-endogeic *L. rubellus* significantly increased over the 5-year period (Table 1; Fig. 4).

The abundance and biomass of the introduced anecic *L. terrestris* species has significantly increased at the quarry of the late stage of overgrowing (Quarry 3) in five years (Table 1; Fig. 4). In general, the species composition and lumbricids population remained the same. The relative pro-

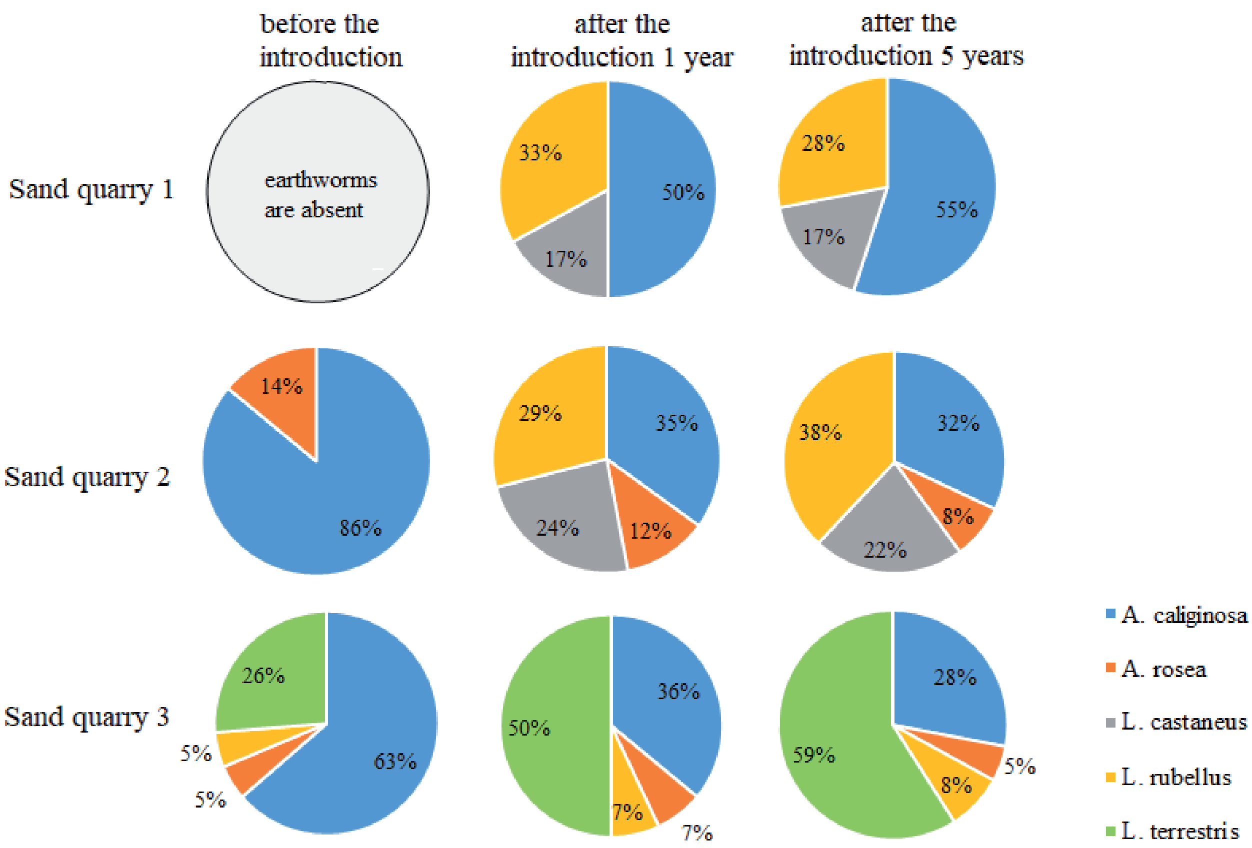

Figure 4. Changes in share (participation) of species in total biomass during the overgrowing of sand quarries

portion of the biomass of the endogeic *A. caliginosa* species (Fig. 4) has decreased.

5. Discussion and conclusion

A number of studies on the population of earthworms in the anthropogenically disturbed areas and the dynamics of the lumbricofauna during the primary soil formation indicate that the earthworms appear 5-8 years after the end of the anthropogenic interference. The endogeic species of *A. caliginosa*, *A. rosea* (Bulavincev, 1979; Dunger & Voigtländer, 2002; Dunger et al., 2004) are most often the first to populate the soil. According to other sources, the earthworms may be absent in the dumps overgrowing during 8-10 years (Pizl, 2001; Roubickova & Frouz, 2014). The earthworms are absent in our research conducted 7 years after the sand quarry formation, despite the meadow vegetation development and the possibility of colonization by the earthworms from the neighboring territories.

A very poor variety (only two species of earthworms) and low amount of the earthworms (about 40 ind./m^2) has been found at the quarry that had been overgrown for circa 20 years. According to the V.I. Bulavincev (1979) data, the number of earthworms on the technogenic territories of the Tula Oblast overgrown with meadow vegetation can reach more than 200 ind./m^2, and the species diversity – not less than 5 species. At the same time, according to Dunger et al. (2004), the number of earthworms in the dumps of Germany during the first succession accounts for not more than 20 ind./m^2 even 23 years later, and the forest species of earthworms can be found on dumps not earlier than 23 years. The slower recovery of the earthworms among all macrofauna groups was shown even after transplanted blocks of topsoil from a well-developed meadow into the bare postmining heap in Sokolov coal mining region (Czech Republic); 20 years after the experiment, the number of earthworms was 30 ind./m^2 (earthworms were absent before the experiment) (Moradi et al., 2018). Our studies have demonstrated that the forest species of earthworms: epi-endogeic, epigeic and anecic inhabit the areas that are close to the studied quarries. However, the epigeic species have not been found in the studied quarries, and a single most important soil-former *L. terrestris* has been found only in the 46 year old quarry. The *L. terrestris* activity contributes to a significant intensification of the plant decline decomposition, humus formation, soil profile restoration, water regime optimization (Topp et al., 2001).

Many authors note the succession changes in the earthworm complex with the forest vegetation devlopment: decrease in the proportion of endogeic species and increase in the number of other groups of *Lumbricidae* – epigeic and anecic species (Bulavincev, 1979; Pizl, 2001; Dunger & Voigtländer 2002; Dunger et al., 2004). Our study has not observed any succession dynamics. The endogeic species continue to develop the territory with the 18 to 46 year old quarry overgrowth with the increase in their number and biomass. The epi-endogeic group and anecic worm representatives recorded only in the oldest quarry are developing there due to favorable conditions with the respect to the natural development of the shrub and woody vegetation and the formation of a plant litter horizon.

Our experiment on the earthworms (main ecological group representative) introduction demonstrated that *A. caliginosa* is the most successful colonizer at the initial stages of soil formation in sandy substrates. This species belongs to the endogeic species of *Lumbricidae* group and lives at a depth of 10-30 cm (Bouche, 1977; Perel, 1979). However, *A. caliginosa* a year after its introduction was found only in the upper soil layer among the roots of herbaceous plants under the conditions of our experiment. Probably, the habitat for this species still remains unsuitable at a greater depth in the sandy substrate. *A. caliginosa* is known to most successfully populate the clay soils, rather than the sandy ones (Eijsackers, 2010), but, nevertheless, it is one of the few the species that most effectively and sustainably explored the territory of the sandy pit of the early stage of overgrowing.

The epi-endogeic *L. rubellus* and epigeic *L. castaneus* most intensively explore the quarry territory overgrowing for about 20 years with the more suitable conditions for them as compared to the quarry at the early stage of overgrowing. Despite the fact that *L. rubellus* colonizes the sandy soils well, according to the reference data (Eijsackers, 2010), the introduction of this species into the young quarry was less successful than into the middle stage of vegetation with a higher humus horizon thickness. *L. rubellus* and *L. castaneus* maximally spread to 2 – 2.5 m yearly. According to the reference data, *L. rubellus* settles on the reclaimed dumps after its introduction at the distance of 4 – 9 m yearly (Ma & Eijsaaskers, 1989); settlement in the arable soil occurs at the rate of up to 14 meters yearly (Marinissen & Van den Bosch, 1992).

The anecic worm introduction was successful only in the oldest quarry. *Lumbricus terrestris* goes down to a depth of 2 m while being the primary consumer of plant material and bringing the plant waste deep into the soil; therefore, its livelihood is associated with the permanent vertical migrations. The anecic worms are better adapted for the toleration of the periodic soil drying, but can only live in the well-drained soil (Perel, 1979). There is a probability that the extreme conditions of the sandy substrate, such as high density, sharp fluctuations of humidity were the main factors preventing the development of this species into the first two quarries. The soil profile is formed on the 46 year old quarry. The rhizosphere of the woody plants is well developed, and *L. terrestris* was detected there only once before its introduction. After the introduction, its number markedly increased during the first year within a radius of up to 6 m; the uniform distribution throughout the quarry (0.5-1 ha) occurs within 5 years.

Thus, according to the results of the study the following conclusions can be made: the earthworm complex formation in the explored sand quarries of the City of Smolensk starts not earlier than 10 years after the quarry formation. The restoration of a full-fledged complex of earthworms does not occur over a period of almost 50 years. The endogeic species settle first during the primary succession with the *A. caliginosa* more successful exploration of the territory. The representatives of other morpho-ecological groups (epi-endogeic and anecic species: *L. rubellus*, *L. terrestris*) are found sporadically. The makrofauna introduction contributes to the formation of a sustainable complex of earth worms. The endogeic species should be populated at the initial stages of the overgrowing of quarries, and epigeic, epi-endogeic and anecic species – at the later stages.

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