Earthworm (Lumbricidae) assemblages of forest ecosystems in the anthropogenically disturbed area of the eastern Krušné hory Mts. (Czech Republic)

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ABSTRACT: Assemblages of earthworms were evaluated in spruce and beech stands in the Kienhaida Nature Reserve (Krušné hory Mts., Czech Republic) and its immediate surroundings, where site conditions were modified by the soil preparation for forest regeneration. The beech stands of the reserve showed low diversity but higher earthworm abundance than did the spruce stands, which in turn showed the lowest Lumbricidae abundance (18 individuals·m^{-2}). The highest abundance of earthworms (124 individuals·m^{-2}) was found in the soil of mounds created 30 years prior to sampling from the organic soil of the A_h horizon. The highest species diversity and low abundance were characteristic of areas between the mounds, the soil surface of which was greatly disturbed after removal of the A_h horizon to create those mounds. The degree to which the reforested clear-cuts created due to air pollution were overgrown with weeds contributed positively to the diversity and abundance of Lumbricidae.

Keywords: forest ecosystems; Kienhaida Nature Reserve; Krušné hory Mts.; Lumbricidae; reforestation procedures; site conditions

Forest ecosystems with site conditions significantly modified by long-term anthropogenic stress (air pollution; clear-cutting; creation of microclimates; changes in pH and in the herb and tree layers) are typical of the Krušné hory Mts. in the Czech Republic (Slodičák et al. 2008). The regeneration of clear-cut areas devastated by the effects of air pollution is carried out by establishing stands of substitute species (Kubelka et al. 1992) and involves a somewhat controversial site-preparation technique using bulldozers (Šach 1992, 1995). Revitalization of the soil environment is achieved by the use of soil-improving trees species (*Betula, Alnus, Sorbus*) (Balcar et al. 2008) and liming (Podrázský 2001, 2006). Nevertheless, original spruce and beech stands such as the Kienhaida Nature Reserve (NR) have even remained in the area of the Krušné hory Mts. exposed to air pollution (Smejkal 2000).

Out of 52 species and subspecies of earthworms recorded in the Czech Republic (Pižl 2002a), nine species were previously reported in the Krušné hory Mts. (Kula, Matoušek 2004). Earthworms of mountain forest ecosystems in the Bohemian Forest Mts. were studied and described by Pižl (2001, 2002b), who reported assemblages poor in species (just 4–5 species) in spruce stands of the Beskids and Krkonoše Mts. (Pižl 1991a,b). Existing studies from similar ecosystems have proved the dominance of an acid-tolerant species, *Dendrobaena octaedra* (Savigny), accompanied by *Dendrodrilus rubidus* (Savigny) and *Lumbricus rubellus* (Hoffmeister), and in some cases also by *Aporrectodea rosea* (Savigny) and *Aporrectodea caliginosa* (Savigny) (Abrahamsen 1972; Huhta et al. 1986).

Assemblages of earthworms represent an important element in the soil function and contribute to an improvement in the soil quality while mitigating

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the effects of site disturbance. At present, there is a lack of information about the earthworm assemblages and their response to soil acidification in the Krušné hory Mts. during and after the period of severe acid deposition (Houšková 1991; Pižl 2002a).

Wallwork (1976) stated that earthworms hardly survive in anthropogenically acidified forest soils, and especially sensitive are those of endogeic and anecic species.

There is a negative relationship between soil acidity and organization of earthworm communities (Abrahamsen 1972; Nordström, Rundgren 1974). Acidity affects the earthworm abundance, activity, growth, and reproduction (Bengtsson et al. 1986). Generally, the number of species and the fertility of earthworms are limited in conditions of low soil pH. Both decreasing earthworm abundance (Persson et al. 1987) and lower species diversity have been shown to occur as a direct consequence of soil acidification (Nordström, Rundgren 1974; Enckell, Rundgren 1988). Unnatural levels of soil acidification under coniferous stands can severely affect the earthworm species requiring high soil quality. Prior to acidification, assemblages were composed of 2–4 species of earthworms. Due to changes in acidity, 1-species assemblages tend to occur in the affected areas (Rundgren 1994).

Several authors have addressed the effects of forest management and forest stand regeneration on earthworms (Huhta et al. 1967; Huhta 1976; Heliovaara, Vaisanen 1984; Tajovský, Pižl 2003; Pontégnie et al. 2005). It is known that litter decomposition may be slowed due to a lower soil temperature beneath closed stands. Increased litter input after opening up a stand by thinning can result in positive effects on the coenosis of earthworms (Castin-Buchet, André 1998). However, the effects of unconventional measures of forest soil preparation, such as large-scale site preparation by bulldozer and subsequently the outplanting of stands of substitute tree species, on populations of earthworms have not been known very well until now.

MATERIAL AND METHODS

Sites

The Kienhaida NR is situated in the Krušné hory Mts. near the village of Načetín (50°34'27"N, 13°17'20"E) at an altitude of 780–820 m a.s.l. It consists of indigenous, well-regenerating beech stands which were preserved through the period of air pollution disaster. Site conditions are characterized by the mean annual temperature of 5.2°C and long-term total precipitation of 917 mm·year^{-1}. The occurrence of drought episodes does not exceed 10% of days each year, and the growing season is 120–140 days long (Smejkal 2000).

In the vicinity of the Kienhaida Nature Research (NR), dead spruce stands are replaced by stands of larch *Larix decidua* Mill., birch *Betula pendula* Roth, and blue spruce *Picea pungens* (Engelm.). Prior to the establishment of these stands, bulldozer and excavator site-preparation techniques were used to create topsoil mounds. Thirty sampling sites were selected (Table 1) at sites with the presence of such mounds. The selected stands created a dense network within an area of about 2 km^2. Sampling was carried out in the area between mounds and on the mounds of piled organic material. With the exception of closed beech stands in the reserve, all sites are characterized by severe weed pressure (e.g. *Calamagrostis* sp., *Carex* sp.) (Table 1).

Sampling and measurements

Soil samples were cut out with a spade as compact 25 × 25 cm blocks to a depth of 10–15 cm. They were sampled in a linear transect of the sampling plot whereby 4 samples 20 m apart were taken in spring (May, 2009) and again in late autumn (September, 2009). Each of the soil samples (240 in total) was placed separately into a polyethylene bag, marked for identity, and transported to the laboratory. Worm extraction from the soil samples was carried out in the Tullgren apparatus (Novák et al. 1969) as later modified by Tuf and Tvardík (2005) and by Kula (2009). The extraction began within 72 hours after field sampling. The extraction time was 21 days, killing medium was 0.5% formaldehyde, and the captured earthworms were preserved in 75% ethanol.

The earthworm biomass was measured by weighing after rapid desiccation on blotting paper. No corrections were made for the gut content or to account for the preservation (Pižl 1995). Additionally, individual collection of earthworms was carried out according to the methodology of Pižl et al. (2004) in summer 2009. Earthworms were sought out in moist soil, under stones and fallen stems, in moss vegetation, in places with accumulated organic residues, etc. This supplementary earthworm sampling was carried out with equal intensity at each of the sites for a period of 20 min. At each of the sites, soil samples were taken in order to measure active soil reaction (pH/$H_2$0), potential

Table 1. Characteristics of stands in the Kienhaida NR and in its surroundings with the differentiated preparation of soil

Tree species	*N*	Age	Altitude (m)	Soil preparation		Soil pit	Forest weed (%)
Fagus sylvatica L.	5	148	800–820	without		in stand	0
Picea abies (L.) Karst.	1	61	810	without		in stand	0
Picea abies (L.) Karst.	1	78	780	without		in stand	40
Picea abies (L.) Karst.	3	120	770–780	without		in stand	40
Betula pendula Roth	2	30	740–780	B		in stand	100
Larix decidua Mill.	2	22	780–800	B		in stand	50
Picea pungens Engelm.	2	19	740	B		in stand	100
Betula pendula Roth	2	0	800	VR		open area	100
Larix decidua Mill.	2	0	800	VR		open area	100
Picea pungens Engelm.	1	0	800	VR		open area	100
Betula pendula Roth	3	25	780–800	mounds	site of mound	in stand	100
					MV	in stand	
Larix decidua Mill.	3	20	790–810	mounds	site of mound	in stand	50
					MV	in stand	
Picea pungens Engelm.	3	19	780–810	mounds	site of mound	in stand	100
					MV	in stand	

B – site preparation using an excavator; MV – area between mounds (V – mounds created from the soil of A_h horizon); VR – mounds spread to the area between mounds

exchange soil reaction (pH/KCl), and humus content in the A_h horizon.

Juvenile and adult individual earthworms were identified by RNDr. Václav Pižl, CSc., from the Institute of Soil Biology, Biology Centre of the Academy of Sciences of the Czech Republic in České Budějovice.

Soil pH was determined in a laboratory using a pH-meter with a combined glass and calomel electrode. The proportion of humus substances was determined by annealing pulverized earth (ČSN 72 1110 1959; ISO/DIS 10390 1992).

Data analysis

Based on the results of the Tullgren method, the abundance (individuals·m^{-2}) and biomass (g·m^{-2}) of earthworms were calculated for each sampling plot. The dominance and structural characteristics (diversity) of the earthworm community were calculated according to Shannon and Weaver (1963) in Losos et al. (1984).

Statistical evaluation was done at the levels of soil preparation type and particular tree species, and the numbers of samples were merged from the two collection times (Table 1). Results were processed using nonparametric ANOVA (Kruskal-Wallis test) in STATISTICA 8 (StatSoft 2007). Significance was tested at the level $\alpha = 0.05$ (Meloun et al. 2005). With respect to the considerable variance and occurrence of outlying and extreme data, the Box-Cox transformation was used to adjust the values of mean and standard deviation. We tested the influence of site disturbance on the Lumbricidae community using canonical correspondence analysis (CCA) and tests for the significance of ordinations by Monte Carlo permutation test (with 999 permutations per analysis) using Canoco for Windows 4.5 (TerBraak, Šmilauer 2002).

RESULTS

In the territory of the Kienhaida NR and in its vicinity, a total of 1,135 earthworms (643 in spring and 492 in late summer) of eight species were captured using the Tullgren method. Another 250 individuals of 13 species were obtained by individual collection. While in this area *Dendrobaena attemsi* was superdominant in the spring season (representing 57.5% of all worms captured), it was not captured by the Tullgren method at the end of the growing season.

Dendrobaena vejdovskyi progressively increased its dominance between spring and summer (14.9% vs 57.9% of the total). Similar changes were found

Table 2. The dominance of species of the family Lumbricidae caught by the method of tullgrens and individual collection (2009)

Species	Method of tullgrens								Individual collection (average)
	Canoco	B	V	MV	VR	ZBK	ZSM	average	
Aporrectodea caliginosa (Savingi)	A_cal			2.82				0.39	2.00
Aporrectodea rosea (Savingi)	A_ros								0.40
Dendrobaena attemsi (Michaelsen)	D_att	29.68	47.06	16.90	20.89	44.83	19.61	32.91	17.20
Dendrobaena illyrica (Cogneti)	D_ill	0.71	1.31	3.52	1.27		2.94	1.46	3.60
Dendrobaena octaedra (Savingi)	D_oct	33.92	21.24	34.51	44.94	1.38	23.53	26.67	9.20
Dendrobaena vejdovskyi (Černosvitov)	D_vej	29.68	27.45	33.80	22.78	51.72	52.94	33.52	13.20
Dendrodrilus rubidus (Eisen)	D_rub		0.33	2.82	1.90	2.07		1.01	39.20
Dendrodrilus rubidus subrubicundus (Eisen)	D_rub_sub								2.00
Dendrodrilus rubidus tenuis (Eisen)	D_rub_ten								0.40
Eiseniella tetraedra tetraedra (Savingi)	E_tet_tet								0.40
Lumbricus rubellus (Hoffmeister)	L_rub	6.01	2.29	2.11	8.23		0.98	3.45	6.40
Lumbricus terrestris (Linnaeus)	L_ter								1.60
Octolasion lacteum (Savingi)	O_lac		0.33	3.52				0.59	4.40
Abundance		283	306	158	145	102	142	1135	250
Index of diversity		1.29	1.22	1.55	1.36	0.84	1.15	1.23	1.59

B – site preparation using an excavator; V – mounds created from the soil of A_h horizon; MV – area between mounds; VR – mounds spread to the area between mounds; ZBK – preserved stands of *Fagus sylvatica*; ZSM – preserved stands of *Picea abies*

for *Dendrobaena octaedra* (20.8% vs 35.2%). Nevertheless, the latter species was not captured very successfully by individual collections (representing just 9.2% of that total). *Dendrodrilus rubidus* was captured the most frequently of all types by individual collection (39.2%), while by the Tullgren method it was a non-dominant species (1.01%) (Table 2).

Seasonal changes in the earthworm abundance are characterized by a decline of adult individuals. In spring, the ratio of adults to juveniles was 169:474 (i.e. 36%) while in autumn it was 71:421 (i.e. 17%). Although the abundance of *D. vejdovskyi* increased from 12.8 to 38.0 individuals·m^{-2} between spring and late summer, a decline in the overall earthworm density from 85.7 to 65.6 individuals·m^{-2} was caused by the complete disappearance of *D. attemsi* individuals in late summer vs the high spring abundance of 49.3 individuals·m^{-2}. We did not determine the reasons for the continual decline in earthworms.

The earthworms captured in the Kienhaida NR and its vicinity affected by acidification can be characterized by three superdominant species (*D. attemsi, D. vejdovskyi* and *D. octaedra*), all occurring in approximately balanced proportions (26.7–33.5%).

The remaining species in the community (0.39–3.45%) increase the overall earthworm diversity in a different way in particular stand conditions (Table 2).

In the monitored area, the 148-years-old beech stand in the NR is regarded as a comparative basis representing long-term stable stand conditions. It had a very poor earthworm community (diversity index H' 0.84) and was characterized by two species generally distributed in the area (*D. attemsi* and *D. vejdovskyi*).

The spruce stands (61–120 years of age), which developed during the period with air pollution impacts, had a spectrum of 5 earthworm species. In common with the beech stands, they showed a dominance of *D. vejdovskyi*, while the lower occurrence of *D. attemsi* in spruce stands was offset by the greater presence of *D. octaedra* (23.53%), which was a non-dominant species in the beech stands.

The species diversity (H' 1.15) approached its mean in the monitored area. The clear-cut areas were originally characterized by dominant spruce stands and the earthworm species which would be expected as mentioned above.

Ranking among the relatively more environmentally friendly procedures for renewal the site preparation using an excavator is done only where the localized disturbance of the soil surface occurs.

The earthworm community there (H' 1.29) comprised the most balanced proportions of superdominant species (*D. attemsi, D. vejdovskyi* and *D. octaedra*, in the range of 29.7–33.9%) and one dominant species *L. rubellus* (6%). The bulldozer preparation, whereby the A_h soil horizon is gathered into mounds, had a marked impact on site conditions. In the soil of mounds rich in organic matter, the entire spectrum of the earthworm coenosis (H' 1.22) was present, with the exception of *A. caliginosa. D. attemsi* (47.1%), together with *D. octaedra* and *D. vejdovskyi,* responded to this treatment especially positively. The greatest species diversity (H' 1.56) was observed in areas between mounds, where the fundamental disturbance of the soil surface occurred 30 years ago. Of the eight species captured there, *D. octaedra* and *D. vejdovskyi* showed identical dominance and *D. attemsi,* which was concentrated in the mounds, was reduced. The actual regeneration and stand establishment are carried out on soils from pre-existent mounds which are mechanically spread. This results in the relatively high species diversity after five years (H' 1.36). *D. octaedra,* accompanied by *D. vejdovskyi* and *D. attemsi,* responded to this treatment positively. *L. rubellus* showed a relatively high proportion where the mounds were spread (Table 2).

The effect of site conditions on the average abundance of earthworms was significant [H(5, N = 240) = 11.32554; P = 0.0231], as manifested between the earthworm coenosis in mounds (124 individuals·m^{-2}) and that at other sites. The average abundance on plots with the "excavator-style" site preparation (78 individuals·m^{-2}) was significantly higher than that in spruce and beech stands of the Kienhaida NR. Differences in the average earthworm abundance between beech (46 individuals·m^{-2}) and spruce (18 individuals·m^{-2}) stands were also statistically significant (Fig. 1).

The average biomass of earthworms – notable for its wide standard deviation – was highest in mounds (2.83 ± 2.0 g·m^{-2}) and in stands with the excavator-style site preparation (2.63 ± 2.24 g·m^{-2}). Higher earthworm biomass was typical of the areas where mounds had been spread out (2.12 ± 3.52 g·m^{-2}). A similar level of earthworm biomass was determined in the beech stands (1.60 ± 1.59 g·m^{-2}) and in the between-mound areas (1.62 ± 1.41 g·m^{-2}). Spruce stands appeared to be poor in earthworm biomass (0.99 ± 1.19 g·m^{-2}). The statistical analysis showed no significant influence of the type of soil preparation on earthworm biomass [H(5, N = 240) = 1.011464; P = 0.3852].

No significant influence of the particular types of replacement trees on earthworm abundance was observed [H(2, N = 160) = 1.8999005; P = 0.3869]. Nor did the tree type affect earthworm biomass [H(2, N = 160) = 0.6578789; P = 0.7197]. This was also demonstrated by minimum differences, for example, between stands of birch (82 individuals·m^{-2}, 2.22 g·m^{-2}), larch (75 individuals·m^{-2}, 1.98 g·m^{-2}) and blue spruce (73 individuals·m^{-2}, 1.91 g·m^{-2}).

For earthworms captured by the Tullgren method, CCA by the type of site disturbance (Fig. 2) corroborated a significant effect of forest weeds at localities with the excavator-style site preparation and at sites with organic soil spread from the mounds. Under these conditions, *D. octaedra* and *L. rubellus* were dominant species of the coenosis. *D. attemsi* preferred mounds while the areas between mounds were populated by *D. illyrica, D. rubidus, A. caliginosa* and *O. lacteum. D. vejdovskyi* is a characteristic species both in original beech and spruce stands (Fig. 2). Soil acidity (pH_{KCl}, 2.75–4.22) appears to be a factor affecting only *D. illyrica* and *D. attemsi* (Fig. 2) in the studied area. Localities with mounds are characterized by the increased humus content (Fig. 3), which can positively affect the abundance of earthworms (Fig. 1). Areas between mounds showed low humus content (Fig. 3; Table 1) and low earthworm abundance (Fig. 1; Table 2). *L. rubellus* was an important species affecting the total biomass of earthworms (for example, on the areas with spread mounds) (Fig. 3).

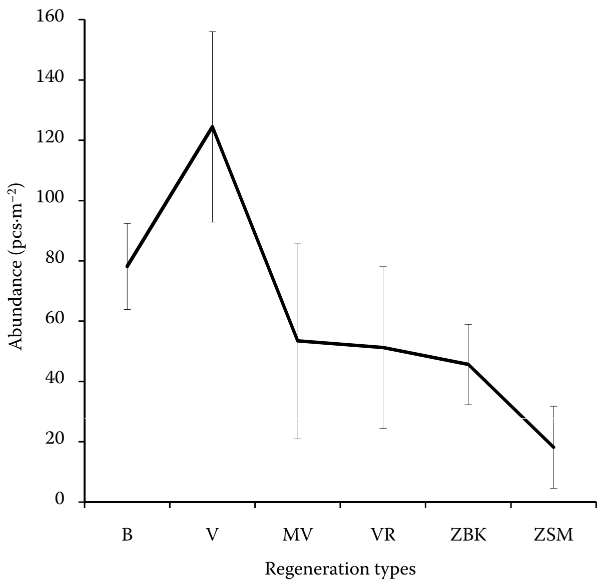

Fig. 1. Mean abundance of earthworm species in forest stands of the Kienhaida Nature Reserve and at sites affected by the site preparation (Legend see Table 2)

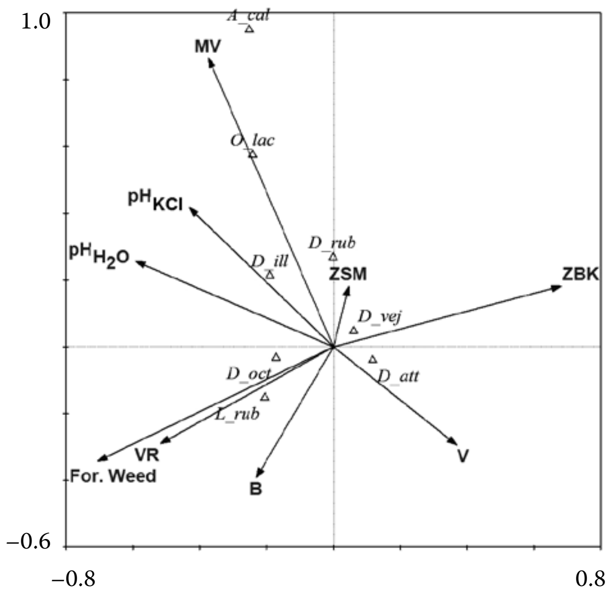

Fig. 2. Canonical Correspondence Analysis (CCA) for the earthworm species depending on the type of site disturbance, pH and forest weed infestation (Legend see Table 2)

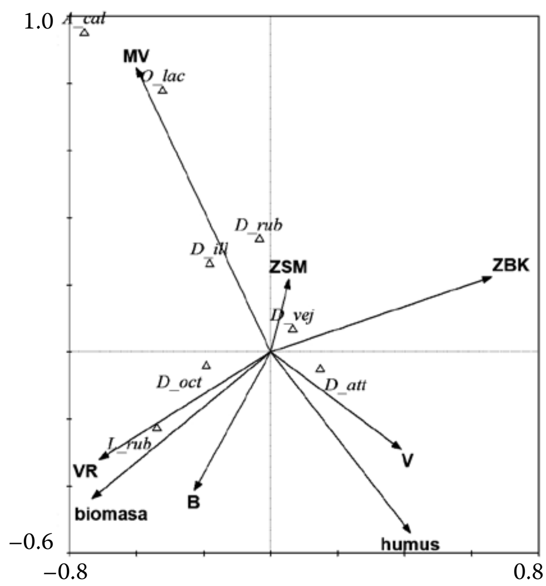

Fig 3. Canonical Correspondence Analysis (CCA) for the earthworm species depending on the type of site disturbance, humus content and earthworm biomass (Legend see Table 2)

DISCUSSION

Data on relatively poor assemblages of earthworms from mountain forest ecosystems are known from the Bohemian Forest, Beskids and Krkonoše Mts. (Wilcke 1940; Pižl 1991a,b, 2001, 2002b). In the Krušné hory Mts., the occurrence of earthworms was previously evaluated in relation to forest vegetation zones (Kula, Matoušek 2004). By the method of individual collection, in grid mapping square number 5,445 the earthworms *Eiseniella tetraedra tetraedra* (Savigny), *Dendrodrilus rubidus subrubicundus* (Eisen) and *Dendrodrilus rubidus tenuis* (Eisen) occurred which were not reported there before. Based upon the findings, the range of species was expanded from 6 to 13 and the known earthworm fauna of the eastern Krušné hory Mts. also increased.

For the area as a whole, the earthworm assemblages were generally composed of three superdominant species (*D. octaedra*, *D. attemsi* and *D. vejdovskyi*) and of the accompanying species *L. rubellus.* The species *D. attemsi* and *D. vejdovskyi* reached on average 66.4% combined total dominance in the stands of the Kienhaida NR. It is noteworthy that the earthworm community of old beech and spruce stands changed positively in its species diversity under conditions of disturbed forest ecosystems. It is likely that the site preparation and establishment of stands of substitute tree species offered different and more abundant food.

Pižl (1995) reported that the density and biomass of earthworms markedly increased at locations with a higher degree of damage to spruce stands and on clear-cuts created due to air pollution. Probable reasons included food of lower quality due to the litter from spruce needles being difficult to digest at localities that suffered little damage. In open stands, the greater herb cover created organic matter more favourable for the development of earthworms. The above-mentioned author came to similar conclusions in the Krkonoše Mts., where the highest abundances of earthworms occurred in a clear-cut area created due to air pollution and in a meadow biotope (Pižl 1998). In the monitored area of the reserve, a qualitatively and quantitatively very poor community of earthworms was found in spruce stands in spite of their partial colonization by the weed species *Avenella flexuosa* (L.) Drejer and *Carex* sp. In beech stands free of undergrowth with sufficient litter, abundance was similar to that in stands of substitute species growing in the soil with the A_h horizon removed but with long-term 50% weed infestation (*Calamagrostis* sp., *Carex* sp., *Senecio* sp., Bryophyta). In forest soils one may expect to see decreased numbers of earthworms in soil during the first years after the site preparation. Earthworms are thereby damaged and lifted towards the soil surface. Thus, the earthworms become the prey of predators and, last but not least, the burrows of earthworms are disturbed, and particularly those of the species living in deep soil layers (Vrba, Huleš 2007). In subsequent years, the weed infestation of the locality serves to increase the food offer (Theenhaus, Schaefer 1995).

Spreading the material in the mounds is a reliable method for improving the soil environment (Vavříček 2007), mainly because its content of mineral nitrogen is 2–4 times higher than that in shallow humus horizons of the original "bulldozer-type" areas (Vavříček 2003). Areas between mounds newly covered by the layer of organic material from mounds showed 100% cover with forest weeds after 5 years (*Scrophularia nodosa* L., *Eupatorium cannabinum* L., *Senecio* sp., *Veronica* sp.). Areas between mounds have stabilized in the course of 30 years and the coenosis of earthworms is characterized by high diversity there.

After spreading the mounds with the originally high abundance of earthworms, the decline in earthworm numbers and the disruption of their assemblages probably occurred due to their damage, predation, and compaction of soil layers before reforestation.

Earthworm assemblages in mounds with 100% overgrowing by *Calamagrostis* sp. and/or *Carex* sp. attained increased abundance and biomass due to the high content of organic matter. Moreover, the potentially large supply of organic matter in mounds with favourable carbon-to-nitrogen ratios creates a condition for the increased occurrence of earthworms (Huhta 1976), as the insufficiency of soil nitrogen seems to be a limiting factor for earthworm populations (Satchell, Lowe 1967; Hendrix et al. 1992). Over the long-term (e.g. 25 years), weed colonization adds to the supply of dead organic matter that constitutes the main source of food for earthworms. In the case of the "excavator-style" site preparation, the abundance of earthworms was higher because the soil surface was not disturbed very much. In broadleaved stands of substitute tree species, forest litter is shown to have favourable soil remediation effects (Kulhavý et al. 2008) while coniferous stands accelerate the acidification process (Kooijman et al. 2000).

The specific conditions of a locality are influenced both by weeds and by the stand itself, with its litterfall and effects on light or shade. Consequently, dense larch stands were the cause of decreased development of the herb layer.

It is interesting that the late summer collection failed to capture the species *D. attemsi,* inasmuch as Eggleton et al. (2009) reported the epigeal species *D. octaedra* and *L. rubellus* as being particularly sensitive while *D. attemsi* is not ranked among the sensitive species. It is known that changes in earthworm populations depend upon warm and dry periods. These are overcome by the epigeal species in the cocoon stage while the endogeic species slip into diapause.

CONCLUSION

The occurrence of 13 species of earthworms was confirmed in the territory of the Kienhaida NR in the Krušné hory Mts. and its vicinity that are strongly disrupted by anthropogenic impacts. Beech and spruce stands of the Kienhaida NR are distinguished by a poor earthworm community (just 3–5 species). In the vicinity of the reserve, stands of replacement tree species were established using two different types of soil preparation which caused differentiation in the earthworm communities. The highest species diversity was observed in the space between the mounds and in those areas where the mounds were spread out and then became overgrown with vegetation. A balanced community of superdominant species was created where the excavator-style soil preparation was used. After spreading of the mounds, the abundance of earthworms decreased. The biomass of earthworms trended downward from being the highest at the level of the mounds through the excavator-prepared areas through the areas of the spread mounds to the lowest level at the between-mound areas of the beech and spruce stands of the reserve. The tree species used for renewal was not shown to influence the earthworm abundance.

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