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Interactions of soil fauna and plants during succession on spoil heaps after brown coal mining

Ph. D. Thesis

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Prohlášení

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Declaration

I claim that I have written this thesis individually, using only the literature cited.
I also claim that the results published here haven’t been used to gain other scientific degrees.

In Prague 2013 ....
Alena Roubíčková

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List of publications

The thesis is based on following book chapter and 3 papers:

a. **Roubíčková A**, Mudrák O, Frouz J, Soil Fauna Plant Interactions during Succession at Post-mining Sites. in Frouz J (ed) Soil biota and ecosystem development in post mining sites, CRC press Tailor and Francis group, Bota Racon (in press)

Alena Roubíčková was responsible for running and statistically evaluating the three experiments that are published only in the book chapter and participated in writing of the book chapter

b. **Roubíčková A**, Mudrák O, Frouz J (2009) Effect of earthworm on growth of late succession plant species in postmining sites under laboratory and field conditions. Biology and Fertility of Soils 45:769-774.

Alena Roubíčková was responsible for running two of the three experiments and participated in their statistical evaluation; participated in writing the manuscript.

c. **Roubíčková A**, Frouz J, Survival and performance of earthworms on unrecultivated spoil heaps at different successional stages. Manuscript.

Alena Roubíčková was responsible for running both experiments and their statistical analysis and was responsible for writing the manuscript.

d. **Roubíčková A**, Mudrák O, Frouz J (2012) The effect of belowground herbivory by wireworms (Coleoptera: Elateridae) on performance of *Calamagrostis epigejos* (L) Roth in post-mining sites. European Journal of Soil Biology 50:51-55.

Alena Roubíčková was responsible for the greenhouse experiment and its statistical analysis and participated in collecting data and statistical analysis of the field experiment, was responsible for writing the manuscript.

Abstract

Earlier research on the spontaneous succession on spoil heaps near Sokolov, NW Bohemia, has documented major changes in vegetation during middle successional stages, that correspond with occurence of some groups of soil macrofauna (earthworms and wireworms - Elaterid beetle larvae). We have tested the interactions between these soil animal groups, plants and soil in several experiments:

In two laboratory and two field experiments earthworms positively affected growth of late successional plants (*Arrhenatherum elatius, Agrostis capillaris, Centaurea jacea, Festuca rubra, Plantago lanceolata, Lotus corniculatus,* and *Trifolium* spp.), during the laboratory experiments a decrease in soil pH and increase in microbial respiration, oxidable C, total N, and exchangeable P, K, and Ca content was detected in presence of earthworms. In a laboratory experiment earthworms also negatively affected germination of small seeds in comparison with large seeds. Concurrently, earthworm performance is affected by the biotic and abiotic conditions at the site; their biomass increased when kept in pots at sites 28 and 48 years old with high tree coverage and decreased in pots at younger sites.

Wireworms negatively affected biomass of *Calamagrostis epigejos* in both laboratory and field experiment, their reduction in field also caused a change in whole plant community composition; in the laboratory experiment wireworms positively affected growth of late successional grass *F. rubra*.

This infers that colonization of spoil heaps by soil macrofauna during natural succession may affect on the whole plant community and consequently development of the whole ecosystem.

Abstrakt

Při předchozím výzkumu spontánní sukcese na sokolovských výsypkách byly zaznamenány významné změny ve vegetaci během středních fází sukcese; tyto změny korelují s výskytem některých skupin půdní makrofauny (žížal a drátovců – larev kovaříků, Elateridae). Zkoumali jsme interakce těchto skupin živočichů s rostlinami a půdou v několika pokusech:

Ve dvou laboratorních a dvou terénních pokusech žížaly pozitivně ovlivnily růst pozdně sukcesních druhů rostlin (*Arrhenatherum elatius, Agrostis capillaris, Centaurea jacea, Festuca rubra, Plantago lanceolata, Lotus corniculatus,* a *Trifolium* spp.), v laboratorních pokusech také způsobily pokles pH a nárůst mikrobiální respirace, oxidovatelného C, celkového N a výměnného P, K, a Ca. V laboratorním experimentu žížaly také negativně ovlivnily vzcházení malých semen; na velká semena neměly vliv. Zároveň bylo zjištěno, že přežívání a prospívání žížal na výsypce je podmíněno biotickými i abiotickými podmínkami – žížaly přibyly na váze pouze na plochách starých 28 a 48 let, na plochách mladších došlo k úbytku váhy i jedinců. Drátovci měli negativní vliv na třtinu *Calamagrostis epigejos* v laboratorním i terénním pokuse, v laboratorním pokuse navíc podpořili růst pozdně-sukcesní trávy kostřavy *Festuca rubra*; jejich potlačení insekticidem mělo za následek změnu celého rostlinného společenstva.

Domníváme se, že kolonizace výsypek půdní makrofanou v průběhu spontánní sukcese může mít zásadní vliv na rostlinná společenstva a potažmo na celý ekosystém.

Contents

General Introduction

Plant-animal interactions during sucession

It is generally accepted that plants play a key role in the environment and that plant community composition determines the composition of animal and soil microorganism communities (Spehn et al. 2000, Frouz et al. 2001, Milcu 2004, Frouz et al. 2008, Frouz et al. 2013). Plant succession is determined mainly by abiotic site conditions, the species pool and interspecific competition (Grime 1979). Hovewer, soil organisms can dramatically affect both the soil environment, on which plants are dependent, and also directly interact with plant roots which may consequently influence the plant community composition (Brown and Gange 1989, 1992, Thompson et al. 1993, De Deyn et al. 2003, Frouz et al. 2008). A good example of how soil fauna can change the whole ecosystem can be seen in North American broadleaf temperate forests, where the introduction of European earthworms (of the Lumbricidae family) caused radical changes in soil (loss of fermentation layer, mixing soil profiles and faster mineralization) and consequently in composition of the understory vegetation and tree seedling recruitment (Bohlen et al. 2004, Hale 2004, Frelich et al. 2006, Eisenhauer et al. 2007). De Deyn et al. (2003) and Kardol et al. (2006) found that there is a growing positive feedback between soil community and plants during succession and that growth of late-successional plants is positively influenced by soil fauna. Studies on the effect of pollution by heavy metals on soil and the whole ecosystem pointed out an increase in accumulation of organic matter on top of the soil due to a dramatic reduction of soil fauna abundance (Gillet and Ponge 2002).

As mentioned above, soil organisms affect soil and plants through several different mechanisms, depending on their size, life strategy and feeding ecology (e.g. Anderson 1988, Lavelle et al. 1997, Brussaard 1998). In principal two major pathways of soil biota effect on plants can be distinguished (Wardle et al., 2004). The first one is related to modification of soil conditions by litter consumption and bioturbation. The second pathway includes organisms interacting directly with roots. Those include symbiotic interactions between roots and microorganisms, mainly mycorrhisae and symbiotic nitrogen fixation. There are many other important interaction in rhisosphere, among them the most important are interaction between bacteria and their predators, namely protozoa, that may effect nutrient availability for plants (Bonkowski et al., 2000). Amongst macrofauna related to plant roots, herbivoty belongs to the most important (Brown and Gange 1992). As this study was focusing on macrofauna I have payed more attention to herbivores than other rhizospere interactions.

As concern soil fauna influence on soil properties already Darwin (1881) has noted that earthworms have a detrimental effect on soil and his results have been supported by many recent studies (Lee 1985, Thompson et al. 1993, Lavelle 1997, Marashi and Scullion 2003, Wurst 2004, Frouz et al. 2006).

By creating burrows through soil horizons, earthworms affect soil physical properties – especially in heavy soils rich in clay, where earthworm burrows help aeration of soil, infiltration of water and also create space for root growth (Curry 1987, Killham 1994, Brown and Gange 1996, Sveisbup et al. 1997, Frouz et al. 2006). In earthworm guts and excrements mineralization of organic matter is enhanced and the outcome can be directly utilized by plant roots (Bohlen and Edwards 1995), microaggregates created in earthworm guts have a great surface area which hosts a diverse community of soil microflora and micro- and meso-fauna (Loranger a kol. 1998, Migge-Kleian a kol. 2006, Mummey et al. 2006), increase water holding capacity of soil (Frouz et al. 2006) and serve as carbon storage thanks to their high stability (Bossyut et al. 2004). By mixing plant litter and mineral soil and increasing nutrient availability earthworms also influence the activity and diversity of soil microflora (Brown 1995, Lavelle 1997, Frouz et al. 2006). In addition to that earthworms can affect plant community directly – by consumption of small seeds (generally < 2 mm), their digestion or redistribution in the soil profile (Thompson et al. 1993, Willems and Huijsmans 1994, Decaens et al. 2003, Milcu et al. 2006).

But mainly the extent of these changes in soil has earned earthworms the name ecosystem engineers - they consume in average 1.0–2.5 g dry mass per gram of fresh biomass a day (Scheu 1987, Curry et al. 1995), which means around 20 kg of dry soil mass per m² per year, with an average earthworm density 100 ind./m² (for nutrient rich deciduous forest or extensive grassland (Duvigneaud 1988)), and there are estimates that earthworms process more than ¼ of the surface layer of soil every year (Pižl 2002).

Herbivores remove above- or belowground biomass of plants which negatively affects plant health and makes them invest more energy into compensational growth instead of reproduction (Andersen 1987, Haag a kol. 2004) and increases their vulnerability to pathogens and other herbivores, environmental stress as well as competitive exclusion by other plants (Brown and Gange 1992, Coffin et al.. 1998, Jonášová a Prach 2004). Their effect is dependant on the degree of specialization of each herbivore, plant defence strategy and specific plant response to herbivory, which changes throughout succession (Fenny 1975, Grime 1979, Rassmann et al. 2011).

Belowground herbivory has been less explored than aboveground herbivory, due to the hidden life and difficulties in experimental assesment of root herbivore effect on plants; nevertheless a couple of studies have shown that their effect on plant communities is detrimental (Andersen 1987, Brown and Gange 1989, 1990, Blossey and Hunt-Joshi 2003, van Ruijven et al. 2005). Generalist root herbivores play an important role in the early stages of succession, because they selectively feed on poorly protected and/or nutrient rich belowground parts, belonging mainly to early-successional plant species, and cause a decrease of their abundance in plant community (Brown and Gange 1989, 1992, De Deyn et al. 2003). Blossey and Hunt-Joshi (2003) have pointed out the high success of using root herbivores, especially Coleoptera larvae, in biological control of weeds and invasive plant species.

I have focused mainly on soil macrofauna, specifically on earthworms and wireworms, because both groups can have a dramatic effect on vegetation and consequently affect the whole ecosystem (e.g. Brown and Gange 1989, Blossey and Hunt-Joshi 2003, Bohlen et al. 2004; Frouz et al. 2008) and are abundant in certain phases of succession on the studied spoil heaps.

By understanding the interactions during spontaneous succession on spoil heaps we could help planning management practices of directed succession with only minor effort, supporting the occurence of desired species, to form an ecosystem with good production as well as environmental functions.

Spontaneous succession on the Sokolov post-mining soils

Sites that have been affected by mining represent excellent model system to study succession. In particular where open cast mining has occured, a large volume of materials (overburden) covering targeted resources has had to be removed and placed elsewhere. Overburden differs substantially from recent soils and represents a starting point for primary succession. Mining can occur in one area over many years and simmilar technology has been used to move the overburden which means that new fresh overburdens have been created over time in a simmilar manner to that created decades ago. Moreover, the mining companies usually have good evidence about age of individual plots, which makes mining sites excellent for a chronosequential research aproach. In comparison with other primary succession, such as lava fields, mining sites have relatively fast succession which allows us to observe successional changes in a reasonable time. As the sites were created by large scale man-made disturbances, expriments of a distructive nature towards the envirment, which would be considered questionable in other areas, are allowed to occur on these sites.

At the Sokolov brown coal mining district there is a history of more than three centuries of brown coal mining, at first underground and since 1950 open pit mining (Fejlková 2009). The geological layer containing coal is about 100m deep and to get to it, the whole overburden is removed and piled on so-called spoil heaps without regard to the original position in the geological profile. At the surface we can find mainly inert geological material, forming longitudinal rows of depressions and elevations with 1 – 2 elevation difference. In the case of Sokolov area this material contains mainly tertiary cypriss clay sediments which consisting caolinite, illite and montmorilonite and are often be impregnated by calcite, siderite and by fossil organic matter. It has a slightly Alcaline pH (around 8) and relatively high cation exchange capacity; the character of the substrate is an important determinant of succession (Frouz et al. 2001, Prach 2003).

The law dictates rehabilitation of the mining areas, therefore most of the spoil heaps are subject to technical recultivations, where the surface is ploughed, leveled (with only mild slopes) and tree seedings are planted into the raw substrate. During this process some soil animals and microorganisms (e.g. mycorhitic fungi, earthworms) are introduced (Topp et al. 2001, Pižl 2002), soil is more homogeneous, free of solid blocks of clay and there is an input of organic material in the form of tree litter, therefore succession on freshly recultivated spoil heaps is initially faster than on sites that are left without intervention.

Research on the spontaneous succession in the Sokolov and Most mining districts (Prach 1987, Prach and Pyšek 2001, Hodačová and Prach 2003, Frouz et al. 2008) has shown that natural succession has the potential to restore the ecosystem functions and services and that in a 30 – 40 years time it produces more diverse plant and animal communities than the succession on technically recultivated sites. In addition to that it gives us unique opportunities to follow the mechanisms of primary succession in field, which is important for making decisions regarding restoration of post mining soils in the future (Bradshaw 1997, Prach et al. 2001, Prach 2003).

The alkaline substrate first colonized by bacteria, fungi and algae, whose spores are brought by the wind and partly also by deep subsurface microflora that was recorded in (30-150 m deep) overburden layer (Frouz et al., 2011). These organisms form biotic crusts on the surface, utilizing high concentrations of mineral ions and possibly also fossil organic carbon, causing chemical and biological weathering of the substrate and producing the first recent organic material (Lukešová 2001, Bowker 2005, Helingerová et al. 2010). Plant seeds distributed mainly by wind (anemochoric), to a smaller extent by animals (zoochoric) are also present from the first moments after deposition, but only a small percentage is able to germinate and proliferate on the raw anorganic material with high pH, which is subject to wide fluctuations of temperature and moisture (Bazzaz 1979, Prach 1987, Wiegleb and Felinks 2001). Seeds of pioneer plant species (e.g. *Chenopodium* spp., *Hordeum jubatum, Arenaria serpillyfolia, Erucastrum gallicum, Atriplex* spp. etc) are better adapted to germination on bare soil substrate and so these prevail in the initial succession stages on spoil heaps. (Bazzaz 1979, Prach 1987, Mudrák, unpublished data). Perennial plants and shrubs (e.g. *Cirsium arvense, Calamagrostis epigejos, Tanacetum vulgare, Salix caprea, Populus tremula* etc.) are more sensitive to abiotic conditions during seed germination and seedling recruitment, but once established they develop a larger root system reaching the deeper and wetter layers of the substrate, which helps them survive droughts and around the 15th year they start dominating the plant community (Prach 1987, Frouz et al. 2008). Amongst these *C. epigejos* often prevails and at some sites creates monospecific stands and retards further succession (Prach 1987, Wiegleb and Felinks 2001, Frouz et al. 2008, Mudrák et al. 2010). After 15 years vegetation can reach 100 % coverage at the more environmentally suitable sites. Generally, succession, especially in early stages, runs faster in the depressions of the spoil heaps, due to higher moisture, accumulation of litter and mineral nutrients (Prach 1987, Topp et al. 2001, Frouz et al. 2008).

Succession of the soil community generally follows succession of the plant community. In early stages when there is no organic matter on top of the soil, we can find only several groups, including bacteriophagous and mycofagous nematodes, Tardigrada and microsaprophagous diptera larvae, around 10 years after deposition root feeding beetle larvae (i.e. wireworms, Elaterid beetle larvae) start appearing (personal obsrevation). On 15-year old sites plant litter starts accumulating on the soil surface (production is higher than disappearance) and a permanent fermentation layer starts forming, a home for large populations of soil saprophagous fauna (Oribatida, Collembola, Testaceae, Enchytraeidae) (Jedlička and Frouz 1999, Frouz et al. 2008).

When trees (mainly *S. caprea, P. tremula, Betula pendula*) start dominating the spoil heaps, a thick fermentation layer covers the depressions. At this stage, some epigeic earthworm species (*Dendrobaena octaedra, Dendrodrilus rubidus, Lumbricus rubellus*), and then later endogeic (*Aporrectodea caliginosa*) start occuring. As a result of eartworm colonization of the soil, the whole soil profile starts changing, fermentation layer is reduced and an organomineral humus layer with a high content of earthworm casts and burrows is formed. Consequently, around the 25th year after deposition, typical forest and meadow herbs (e.g. *Achillea millefolium, Rubus fruticosus, Daucus carota, Poa angustifolia, Festuca rubra, Fragaria vesca, Lotus corniculatus, Viola riviniana, Veronica officinalis* etc.) start appearing in the forest undergrowth (Frouz et al. 2007, 2008). The presence of earthworms first at this stage may be caused by low migration ability of these animals, but at the same time it is most probably facilitated by vegetation; a thick litter layer prevents soil from drying out and provides enough food supply, which are prime conditions for earthworm establishment (Rushton 1986, Edwards and Bohlen 1996, Dworschak 1997)

In this study we have been trying to prove that soil fauna can affect plant community composition and the direction of seccession on the spoil heaps. This included 3 partial hypotheses:

a) earthworms affect plants by changing soil conditions and consumption of plant seeds and this (effect) action gives a competitive advantage to late-successional plant species

b) Earthworms colonize the spoil heaps due to establishment of favourable soil conditions with contribution of the plant community

c) wireworms can affect plant community composition by preferential feeding on poorly protected roots of some plant species

The hypothesis have been tested in a series of greenhouse and field experiments and presented in 3 papers and one book chapter.

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Book chapter

Soil Fauna Plant Interactions during Succession at Post-mining Sites

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Introduction

Studying succession in ecosystems is of vast theoretical and practical importance (Odum 1969, Luken 1990, Frouz et al. 2001, Prach and Pyšek 2001). Most succession studies are focused on plants. Plants are considered to be the most important ecosystem component because they form the bottom of the trophic cascade and strongly affect the physical arrangement of habitats for other organisms including soil invertebrates (Dunger 1968, 1991, Frouz 1997, Pižl 2001). Plant succession is driven mainly by abiotic conditions of the environment, the species pool and interspecific competition or facilitation (Odum 1969, Glenn-Lewin et al. 1992, van Andel et al. 1993), but other trophic levels can affect succession as well. Many studies have reported about the influence of herbivores on plant succession (Brown et al. 1987, Brown and Gange 1989 b, 1992, Bach 1994, Fagan and Bishop 2000). Less is known about the effect of belowground herbivors and other soil fauna even though they can have a substantial effect on plant succession (De Deyn et al. 2003). Soil fauna can affect plants via trophic and non-trophic interactions or via influencing the microbial soil community and physical or chemical properties of the soil (Anderson 1988).

Studies of the effect of the soil biota on plants and other ecosystem properties are difficult because soil organisms live cryptically in the soil, and changes belowground are associated with changes aboveground in a complex way. If a disturbance is not extremely large, so as to limit migration from the surroundings, changes in the below- and aboveground part of the ecosystem are usually closely associated, so it is difficult to separate causes from effects at a given moment. To assess the role of a group of soil fauna in the ecosystem, one can manipulate this group experimentally (Brown and Gange 1989 a and b). The soil is a very complex environment, however, which is difficult to manipulate. Many attempts to manipulate certain soil organism often affect other soil organisms or physical soil properties and/or create a severly artificial situation. Another possibility is to use situations in which certain members of the soil fauna are naturally missing. An example of such a situation is the introduction of European earthworms to other continents (Bohlen et al. 2004, Hale 2004, Frelich et al. 2006, Eisenhauer et al. 2007). In this case, however, we are dealing with invasive species that are not native to the given environment. This imposes certain limitations as to the interpretation of findings concerning the role of soil fauna in ecosystem development under natural conditions. Furthermore, some experimental approaches, such as experimental inoculation of non-native species, can be ethically questionable. Another possibility is to study primary succession following large scale disturbances in places where the soil develops de novo and where colonization by plants and soil-dwelling animals may be decoupled. The study of primary succession on post-mining spoil heaps offers such an opportunity. Mining disturbances may be large, and the migration distance from surrounding habitats can vary from zero to several kilometres, which can decouple fast and slow migrants. This creates areas at similar stages of succession in which a certain organism can be present or absent based on a migration barrier, which makes such sites very suitable for various manipulative exclusion or introduction experiments (Frouz et al. 2006, Roubíčková et al. 2009, 2012). During ecosystem development, individual groups of organisms colonize sites gradually, allowing us to test their effect on soil development and other organisms (Frouz et al. 2001, 2008). Moreover, post-mining sites are excellent for chronosequence studies because similar post-mining habitats are created repeatedly over the course of the mining process and their history is usually well documented. The aim of this chapter is to summarize our studies about interactions between the soil fauna and plants at post-mining sites and put them into a wider context. We focus on a single trophic interaction (herbivory) and mechanisms by which soil invertebrates alter the conditions for plants and, vice versa, how plants alter the conditions for the soil fauna.

Root Herbivory

Belowground plant tissues contribute 50% – 90% to the total plant biomass (Andersen 1987) and are responsible for uptake of water and mineral nutrients from the soil (roots), storage of assimilates during periods of dormancy and vegetative reproduction (underground stems). Roots are crucial organs for plants, so reduction of root biomass negatively affects plant fitness, and root damage can also increase plant susceptibility to various diseases (Andersen 1987). The role of root herbivores in ecosystems is often underestimated because they are generally elusive (Andersen 1987, Brown and Gange 1989b, 1992). Specialized root feeders have a major impact on individual plants as well as on the entire plant community (Brown and Gange 1989 a, Blossey and Hunt-Joshi 2003, van Ruijven. 2005). Many groups of root herbivores can be used in biocontrol programs more successfully than leaf herbivores (Blossey and Hunt-Joshi 2003). Other soil animals that occasionally consume small roots together with the surrounding soil usually do not significantly affect the total root biomass (Gange 1993, Ostle et al. 2007).

Research on belowground herbivory is methodologically complicated because of difficulties with assessing herbivores and roots as well as with quantifying the impact of herbivores on plants. Belowground herbivores have complicated reproduction cycles and do not rapidly migrate to new areas (Andersen 1987, Brown and Gange 1989b, 1992). Plants can deal with effects of root herbivory when no other environmental stresses such as competition occur (Masters et al. 2001). A certain level of belowground herbivory can even stimulate plant growth aboveground followed by compensation growth of roots (Strauss and Agrawal 1999, Wurst and Van der Putten 2007). The effect of root herbivory on plants is often cumulative, which means that long-term experiments (3 years or longer) are needed (Maron 1998, Blossey and Hunt-Joshi 2003).

Generalist root herbivores mainly occur in early successional stages because they feed on nutrient-rich roots of certain ruderal plant species which are abundant in this successional stage thanks to their fast growth and reproduction attributable to the fact that they do not invest as much into antiherbivore defence (Andersen 1987, Brown and Gange 1989b, 1992, De Deyn et al. 2003). It seems that belowground herbivores, especially beetle larvae, can speed up succession by suppressing growth of prevailing forb species, thus giving space to grasses and woody species, which have tough and less palatable roots (Prach 1987, Brown and Gange 1989b, 1992, van Ruijven 2005, Roubíčková et al. 2012).

We found that during initial stages of succession at post-mining sites, wireworms (click-beetle larvae, Elateridae) potentially reduce the competitive ability of *Calamagrostis epigejos*, the dominant plant species in initial stages of succession (Frouz et al. 2008). In a laboratory experiment, wireworms reduced both the aboveground and belowground biomass of *C. epigejos* when grown together with *Festuca rubra* – a grass from later successional stages with tougher roots. In a field experiment in which we reduced the biomass of wireworms with an insecticide, only the belowground biomass of *C. epigejos* was significantly reduced, but wireworms also had an effect on some other early successional plants (e.g. *Hieracium piloselloides*) (Roubíčková et al. 2012).

In later successional stages, the prevailing antiherbivore defence strategy is to produce large amounts of “inedible” or slightly toxic (e.g. tannins) compounds, which do not allow any resistance to be evolved against them (Fenny 1975). Root feeders are therefore usually specialized on one or a couple of similar plant species whose populations they regulate (Coley 1985, Blossey and Hunt-Joshi 2003). It has been shown that root herbivores can influence aboveground herbivores by stimulating production of defence compounds that get transported into leaves and which would otherwise be produced in lower concentrations (Bezemer et al. 2003). This is contradicted by the results of Masters et al. (2001) showing that root herbivores enhance the nutrient quality of plants, resulting in the aboveground herbivore (tephritid) preferentially feeding on plants whose roots had previously been attacked.

Herbivory and Seeds and Seedlings

Plant seeds and seedlings are generally rich in nutrients and are a food source favoured by many animal species. Large seeds cannot be easily consumed by soil invertebrates but are a preferred food source for rodents and birds (Thompson 1987). Seeds that are small and poorly protected are usually consumed and destroyed in the digestive tract of soil animals. Small seeds are numerous, which increases the probability that some will stay untouched and will find good conditions for germination. Various soil invertebrates contribute to post-dispersal seed predation. Among them certain ants and beetles specialize on seed predation, especially of seeds lying on the surface (Thompson 1987). Some ants are able to consume whole seeds, and certain plant species produce specific tissues (elaisomes) attractive to ants, so ants eat these and leave the rest of the seeds intact (Pemberton and Irving 1990). Either way, some seeds get transported by ants and then germinate, which can aid plant dispersal (Pemberton 1988, Hughes and Westoby 1992a, b).

Other soil invertebrates, such as earthworms, can consume seeds together with soil or litter.

Earthworms belong to the most important seed consumers in the soil (Willems and Huijsmans 1994, Milcu et al. 2006). They have been found to prefer seeds of some plant species, depending on their shape and surface (Willems and Huijsmans 1994, Eisenhauer et al. 2009), but generally consume all seeds that have less than 1 – 3 mm in diameter, depending on the earthworm species (Tiunov and Scheu 1999, Milcu et al. 2006, Zaller and Saxler 2007, Eisenhauer et al. 2009). Seeds are often not digested but transported to greater depths, where they can germinate (Milcu et al. 2006). Seeds moved into deep soil layers by earthworms contribute to the formation of a persistent seed bank (Thompson 1987, Thompson et al. 1993). When buried in earthworm burrows, large seeds have relatively good conditions for germination, but depending on the depth and the size of the seeds, the seedlings might not be able to reach the surface (Thompson et al. 1994, Traba et al. 1998, Eisenhauer et al. 2007). Germination of small seeds is usually suppressed at greater depths because they often need high temperatures or light to induce germination (Milberg 1992). Seed germination may be reduced in worm casts, however (Decaëns et al. 2003). Gut transit can cause damage to seeds, and earthworm casts, which are rich in ammonia and relatively impervious to gases and water, do not provide suitable conditions for seed germination (McRill and Sagar 1973, Blanchart et al. 1993). On unrecultivated spoil heaps, earthworms – especially the epigeic species *Lumbricus rubellus* – tend to suppress the germination of small seeds (weight of thousand seeds < 0.1 g) without affecting large seeds (weight of thousand seeds > 1 g) Fig. 1.

Besides seeds, seedlings also often fall victim to herbivory. Seedling consumption is assumed to be one of the major reasons for successional changes induced by tipulid larvae in spoil dumped by the building industry, for example (Frouz 1998). Despite numerous reports about seed and seedling herbivory, there are insufficient data about their effect on plant community development during succession.

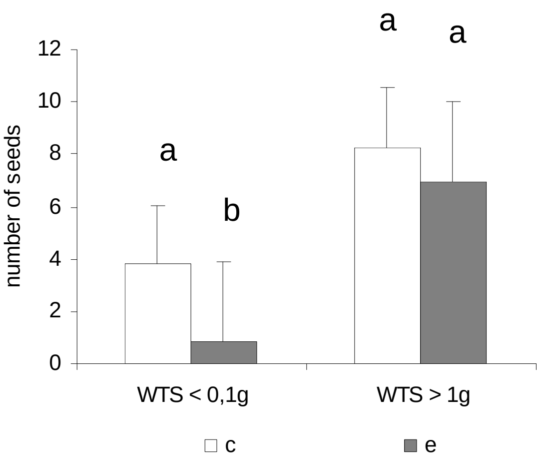

Fig. 1 Mean number of large (WTS > 1 g) and small (WTS < 0.1 g) seeds in control treatments (c) and treatments with earthworms (e), where different letters show significant differences ($p < 0.05$). Error bars indicate the standard error of the mean.

Indirect Effects of the Fauna on Plants

Main species of the soil fauna feed on dead organic matter. These animals greatly differ in size – from 1µm (Protozoa) to 2 meters (giant earthworms) – and have different effects on the soil depending on their size (Bradford et al. 2002, Frouz et al. 2007, Frouz 2008). They contribute in various ways to mineralization of organic matter mostly through regulation of microbial populations in the soil (Adejuyigbe et al. 2006) and at the same time affect the physical arrangement of the soil (Ponge 2003, Frouz et al. 2007). Plants are indirectly influenced by these organisms via the availability of nutrients, due to changes in soil structure (formation of pores and aggregates) that affect water availability and many other soil properties. The soil fauna can increase the availability of nutrients to plants through speeding up the mineralization process by fragmenting litter and increasing the surface that can be settled by bacteria (Alphei et al. 1996). It can also decrease it by competing for nutrients with plant roots (Alphei et al. 1996) or decreasing the microbial biomass in the soil (Tiunov and Scheu 1999, Eisenhauer et al. 2007).

Some effects of the soil fauna on the soil, namely on its structure, may have a cumulative effect over time, as pores and aggregates produced by some animals can last several years and can stay in the soil a long time even after their creators are gone (Killham 1994, Barot et al. 2006).

Certain groups of soil animals can play an important role in disseminating certain species of soil fungi, of which VAM fungi are essential for nutrition of a majority of plant species (Harinikumar and Bagyaraj 1994, Klironomos and Kendrick 1995). This may be important especially in disturbed areas such as spoil heaps from brown coal mining, where the soil is poor in propagules of mycorrhizal fungi (Loree and Williams 1987, Gange et al. 1993, Prach et al. 1997).

As shown by micromorphological studies conducted at post-mining sites near the town of Sokolov (Frouz et al. 2007), many groups of soil animals, such as earthworms, millipedes, diptera larvae or various groups of the soil mesofauna, can have a significant effect on soil formation. In terms of biomass, earthworms are among the most important detritivores in terrestrial ecosystems (Edwards and Bohlen 1996). They are true ecosystem engineers with a dramatic impact on soils (Lavelle 2002, Jouquet et al. 2006, Birkhofer et al. 2011). They affect soil formation and structure, soil pH, organic mater transformation, nitrogen mineralization, nutrient availability and other soil properties and processes (Killham 1994, Alphei et al. 1996, Scheu et al. 2002, Ma et al. 2003). Earthworms have a large potential in restoration of post-mining soils (Boyer and Wratten 2010). Frouz et al. (2008) demonstrate that colonization of unreclaimed post-mining sites by earthworms and consequent changes in the soil are associated with dramatic changes in plant community composition, namely an increase in diversity and the appearance of more demanding forest and meadow species. A series of laboratory and field experiments was therefore organized to test whether earthworm activity can alter the condition of post-mining substrates in such a way that can promote the establishment and growth of plants, especially late-successional species. Under both laboratory and field conditions, earthworms supported the growth of the grass *Festuca rubra.* In one field experiment, the presence of earthworms caused a decrease in biomass of the leguminous plant *Trifolium medium*, but in one long-term laboratory experiment, earthworms promoted the growth of this species. This biomass increase corresponded to a significant decrease in pH and an increase in oxidable C, total N, and exchangeable P, K and Ca content (Roubíčková et al. 2009). Another laboratory experiment showed higher biomass production of a late-successional plant community in the soil from a late-successional stage (already affected by earthworms) compared to early succession soil, which had not yet been affected by earthworms. However, the introduction of earthworms into the soil from an early successional stage increased biomass production of these species to the level observed in the late succession soil (Roubíčková et al. 2009). Mudrák et al. (2012) present a laboratory experiment showing that earthworms increased plant biomass, especially that of the large-seeded grass *Arrhenatherum elatius*, but reduced the number of plant individuals, mainly those of the small-seeded species *Agrostis capillaris* and the legumes *Lotus corniculatus* and *Trifolium medium* with nutrient-rich tissue. In particular, earthworms clearly improved the growth of late-successional plants. By contrast, field experiments at post-mining sites near the town of Sokolov (Mudrák, unpublished data) in large pots filled with early-successional soil unaffected by worms, half of which were inoculated with earthworms (*Lumbricus rubellus* and *Aporrectodea caliginosa*), show that earthworm presence affected only the early successional species *H. piloselloides*, which doubled its cover and biomass in the presence of earthworms (Fig. 4 and Table 1, respectively). Another early successional species, *C. epigejos,* and the late-successional species *F. vesca* remained unaffected by earthworms (Table 1, Fig. 4). Some early successional plant species can therefore also take advantage of earthworm activity, and other factors are responsible for the decrease in their abundance over the course of succession. *Hieracium piloselloides* has, for example, been found to be sensitive to root herbivory by wireworms (see above). In this experiment, earthworms also increased the total belowground biomass although the total plant biomass remained unaffected (Table 1).

Table 1 The effect of earthworms on plant biomass at the end of the field experiment lasting three growing seasons. Three plant species (*Calamagrostis epigejos*, *Fragaria vesca* and *Hieracium piloselloides*) were replanted from surrounding vegetation into pots with and without earthworms (*Lumbricus rubellus* and *Aporrectodea caliginosa*). Presented are means ± standard errors of the means and p values of the t-test, which compares pots with and without earthworms. Significant or marginally significant p values are in bold.

Biomass [g]	Earthworms	No earthworms	p
Total aboveground	9.4 ± 0.9	8.0 ± 0.8	0.299
Total belowground	16.6 ± 0.9	13.0 ± 1.3	**0.070**
Calamagrostis epigejos	6.6 ± 0.8	4.8 ± 0.5	0.130
Fragaria vesca	1.5 ± 0.2	1.7 ± 0.3	0.439
Hieracium piloselloides	1.1 ± 0.1	0.6 ± 0.1	**0.009**

Even though not all of the plants responded to earthworm presence, manipulative experiments have generally confirmed the positive effect of earthworms on the growth of late-successional plant species, as assumed based on field observations (Frouz et al. 2008).

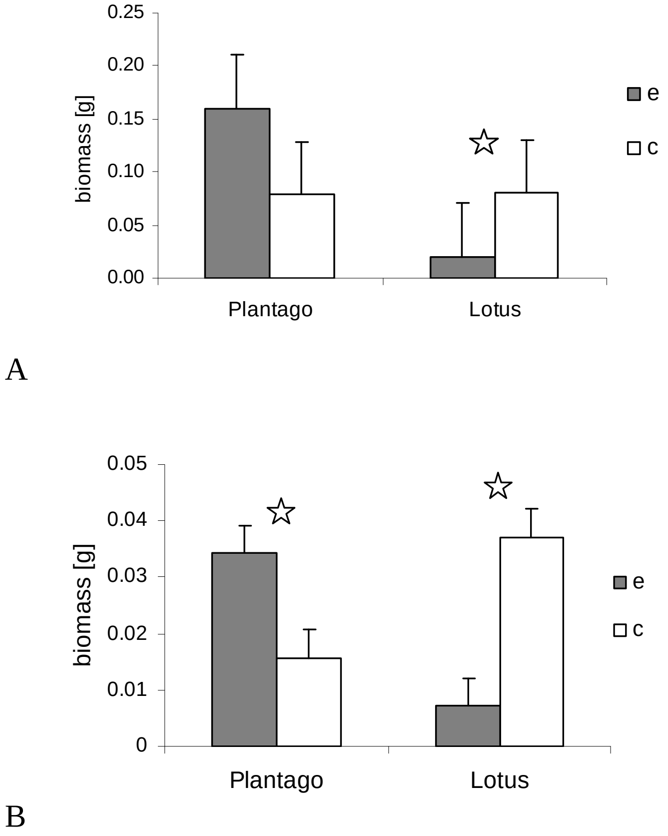

Fig. 2 Mean aboveground (A) and belowground (B) biomass of plant species *Plantago lanceolata* and *Lotus corniculatus* in a treatment with earthworms (e) and in control treatments (c). Stars mark significant differences between treatments ($p < 0.05$). Error bars indicate the standard error of the mean.

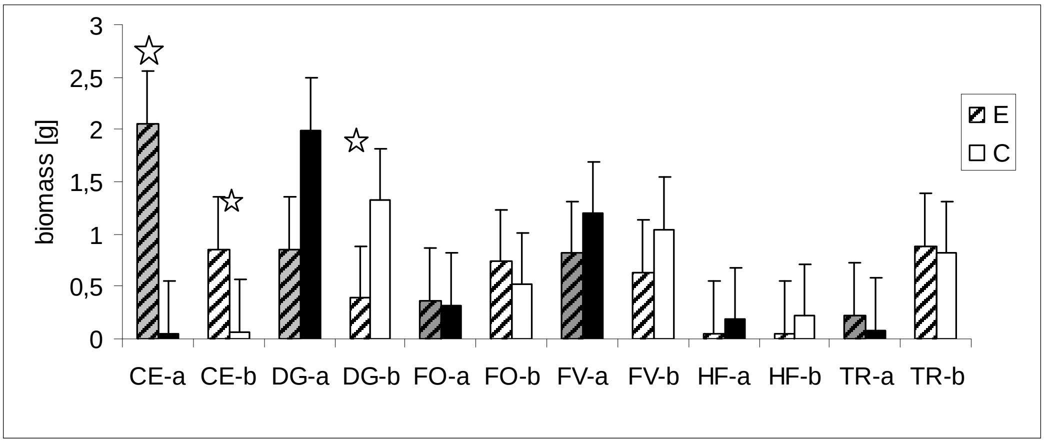

Fig. 3. Biomass of aboveground (a) and belowground (b) parts of individual treatments – with earthworms (E) and without earthworms (C). Stars mark significant differences between treatments (p = 0.05). Acronyms of plant species: CE = *Calamagrostis epigejos*, DG = *Dactylis glomerata*, FO = *Festuca ovina*, FV = *Fragaria vesca*, HF = *Hieracium piloselloides*, TR = *Taraxacum* sect. *Ruderalia*. Error bars indicate the standard error of the mean.

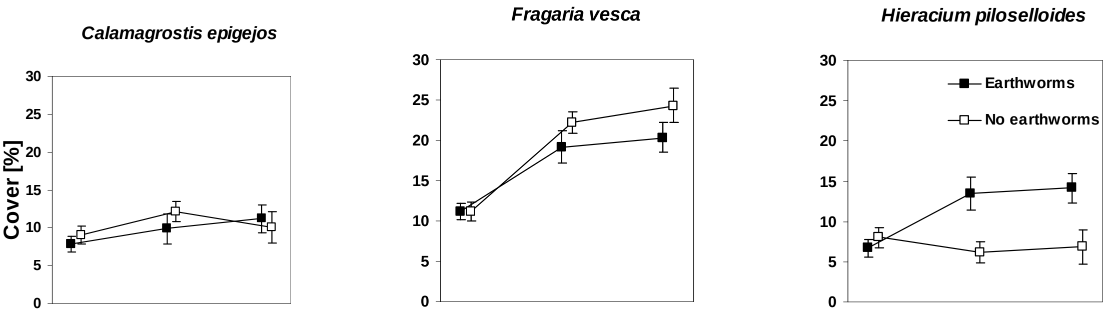

Fig. 4, Cover of the *Calamagrostis epigejos*, *Fragaria vesca*, and *Hieracium piloselloides* planted in pots with and without earthworms. Repeated measures analysis of variance (rANOVA) found the effect of earthworms to be significant only in the case of *H. piloselloides* (p = 0.003 for the main effect of earthworms; p = 0.014 for the interaction of the effects of earthworms and year).

Effects of Plants on Soil Fauna Establishment during Succession

As already mentioned, plants substantially contribute to the formation of the physical structure of the habitat and, consequently, affect species composition of the soil fauna (Spehn et al. 2000, Frouz et al. 2001, Milcu 2005, Frouz et al. 2008, Hendrychová et al. 2008). This is true also for post-mining sites (Dunger et al. 2001). These effects may be related to the nutritional value of plants for the soil fauna. Cornelissen et al. (1999), Wardle et al. (2002), and Curry and Schmidt (2007) found a positive feedback relationship between plants and the soil fauna involving palatability of plant litter and the rate of its decomposition, not only at former mining sites. As mentioned above, Tipulidae depend on the presence of small seedlings in the ground layer, which are their favourite food (Frouz 1997) Similarly, mosses and algal crust are an important food source for larvae of terrestrial Chironomidae during early stages of succession (Frouz 1997b).

Besides a direct effect on habitat structure, animals produce dead organic matter, which forms the litter layer and later also other soil layers. An abundance of easily decomposible litter supports a rich macrofaunal community and facilitates the formation of mull or a modern type of humus (Ponge 2003). Conversely, hard-to-decompose litter results in low participation of the soil fauna in decomposition and, consequently, leads to the formation of a moor type of humus. The thicknesses of the litter, fermentation and humus layer are important soil parameters that determine the appearance of individual species of soil fauna during succession (Frouz et al. 2001, 2008). It is difficult, however, to determine the direct importance of a particular habitat or succession stage for individual groups of the soil fauna. Roubíčková (unpublished) introduced the earthworm *Aporrectodea caliginosa* into various succession stages at post-mining site in mecocosms (5L barrels) consisting of defauned soil of the same succession stage surrounded by mesh to avoid emigration or immigration of worms. In this experiment, earthworms were able to survive in all succession stages, but their population decreased at sites younger than 20 years and their abundance and biomass only grew at older sites (Fig. 5).

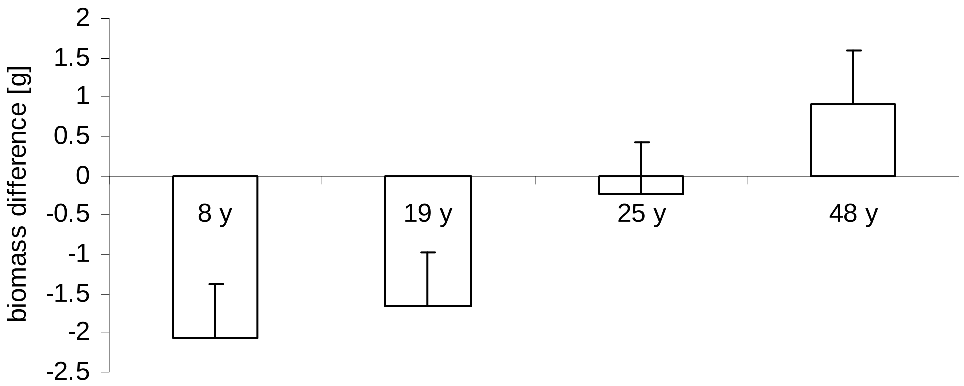

Fig. 5, Mean biomass difference in earthworm biomass between the beginning and end of the experiment under different treatments – on 8, 19, 25 and 48 years old sites of the spoil heap. ANOVA (multiple comparison test) showed significant differences among all the treatments (p = 0.05).

In many cases, however, the performance of invertebrates is more related to the physical arrangement of the habitat, its microclimatic conditions etc. The establishment of young nests of the ant *Lasius niger*, for example, is much more common in open patches with scarce vegetation than under a dense vegetation cover (Holec and Frouz 2005). Similarly, open vegetation patches are preferred for flight and oviposition by terrestrial chironomids (Frouz 1997). It is interesting that in both cases mentioned above the dominant plant species were similar in both open and dense vegetation. Rather than species composition, height and density of vegetation were important for the fauna.

Animals also often use more than one vegetation patch, and the relationships between the fauna and the vegetation structure may not be straightforward. As already mentioned, nests of *Lasius niger* ants are more frequently found in open vegetation patches, apparently because they may benefit from better insulation there; however, foraging workers prefer dense vegetation, particularly during summer. This may be caused by better food supply in dense vegetation but also by the fact that open surfaces gets too hot for foragers during the day. Similarly, as already mentioned, chironomid larvae prefer open patches in early succession stages, in which mosses and algae are abundant. These open patches, however, may be subjected to summer drought, potentially causing the whole population to go extinct. Nevertheless, these sites get recolonized by a subsequent generation from surrounding dense vegetation (Frouz and Kindlmann 2001). We can say that soil animals very often require several different vegetation patches, which have to be available in certain proportion, rather than a single habitat type.

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Paper I

Effect of earthworm on growth of late succession plant species in postmining sites under laboratory and field conditions

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Abstract

Earlier studies of postmining heaps near Sokolov, Czech Republic (0–46 years old) showed that massive changes in plant community composition occur around 23 year of succession when the heaps are colonized by the earthworms *Lumbricus rubellus* (Hoffm.) and *Aporrectodea caliginosa* (Savigny). The aim of the current study was to test the hypothesis that the introduction of earthworms into a postmining soil enhances growth of late succession plant species. In a laboratory experiment, earthworms significantly increased biomass of *Festuca rubra* and *Trifolium hybridum* grown in soil from a 17-year-old site. The biomass increase corresponded to a significant decrease in pH and an increase in oxidable C, total N, and exchangeable P, K, and Ca content. A second laboratory experiment showed higher biomass production of late successional plant community (*Arrhenatherum elatius*, *Agrostis capillaris*, *Centaurea jacea*, *Plantago lanceolata*, *Lotus corniculatus*, *and Trifolium medium*) in soil fromlate successional stage (46 years old); the introduction of earthworms into soil from an early successional stage (17 years old) increased biomass production. In a field experiment, introduction of *L. rubellus* to enclosures containing a 17-year-old soil not colonized by earthworms significantly increased the biomass of grasses after 1 year. The results support the hypothesis that colonization of postmining areas by earthworms can substantially modify soil properties and plant growth.

Keywords

Earthworms . Plant succession . Plant growth . Postmining soils

Introduction

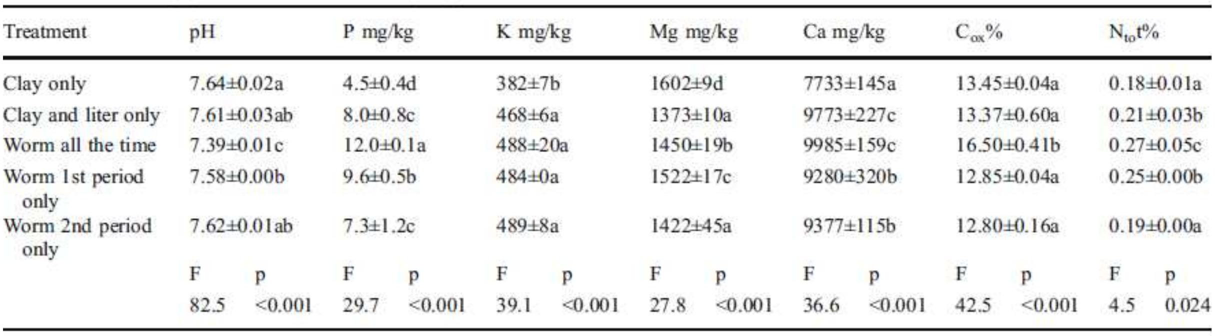
Earthworms are important soil organisms that can affect many important soil properties (Lee 1985), such as soil aggregate formation (Marashi and Scullion 2003), water holding capacity (Frouz et al. 2006), activity of microflora by mixing plant litter and mineral soil (Frouz et al. 2006), and nutrient availability (Lavelle et al. 1997). Because of their effects on these properties, earthworms often affect plant growth (Scullion and Malik 2000; Thomas et al. 1993; Yu et al. 2008; Wang et al. 2006; Wurst 2004). For example, introduction of European earthworms to North American forests has been reported to change soil conditions and plant growth (Bohlen et al. 2004). Because earthworms are slow migrants and sensitive to disturbance (Tondoh et al. 2007), sites that have experienced large-scale disturbances may lack earthworms even if environmental conditions are otherwise suitable for them (Dunger 1991; Huhta and Raty 2005; Frouz et al. 2008). Previous studies of primary succession of vegetation in postmining sites near Sokolov (Czech Republic) revealed sudden and marked changes in plant community composition around year 23 of the succession (Frouz et al. 2008). These changes corresponded with the colonization of sites by earthworms and the appearance of an organomineral humus layer, which consist mainly of earthworm casts (Frouz et al. 2007, 2008). This suggested that the initial plant community facilitates site colonization by earthworms (by providing litter and habitat modification) and that earthworm colonization and consequent soil changes then affect the establishment and growth of latter successional plant species (Frouz et al. 2008). The aim of this research was to study the effect of earthworms on growth of latter succession plants in postmining sites. We used two earthworm species, *Lumbricus rubellus* and *Aporectodea caliginosa*, which are important colonizers of postmining heaps in Sokolov (Pižl 2001), under both laboratory and field conditions and eight plant species that commonly grow on postmining heaps during latter succession stages (Frouz et al. 2008; Prach unpublished data).

Materials and methods

Sites

All materials used in the laboratory experiments were collected from unreclaimed postmining sites located in one large heap (c 1.5 km wide and 10 km long) near Sokolov at the altitude of 500–600 m asl. The properties of the used materials are shown in Table 1. The mean annual precipitation was 650 mm, the mean annual temperature was 6.8°C, and the mean temperature during the vegetation season was 13.0°C (Frouz et al. 2001, 2008). This heap was formed by the dumping of alkaline tertiary clays (mixture of kaolonite, illite, and montmorillonite with traces of quartz and calcium carbonate) and during time C and N gradually accumulated in the soil and pH decreased (Šourková et al. 2005). Only scarce herbs and grasses (mainly *Tussilago farfara* and *Calamagrostis epigeios*) could grow on 2–14-year-old heaps, whereas shrubs (*Salix caprea*) could grow on 15–25-year-old heaps and trees (*Populus tremuloides* and *Betula* spp.) on 25–41-year-old heaps (Frouz and Nováková 2005; Frouz et al. 2008). Earthworms colonized the unreclaimed postmining sites near Sokolov after about 23 years of the succession (Frouz et al. 2008).

Table 1 Chemical properties of soil in laboratory experiment 1



Treatment	pH	P mg/kg	K mg/kg	Mg mg/kg	Ca mg/kg	C_{ox}%	N_{to}t%
Clay only	7.64±0.02a	4.5±0.4d	382±7b	1602±9d	7733±145a	13.45±0.04a	0.18±0.01a
Clay and liter only	7.61±0.03ab	8.0±0.8c	468±6a	1373±10a	9773±227c	13.37±0.60a	0.21±0.03b
Worm all the time	7.39±0.01c	12.0±0.1a	488±20a	1450±19b	9985±159c	16.50±0.41b	0.27±0.05c
Worm 1st period only	7.58±0.00b	9.6±0.5b	484±0a	1522±17c	9280±320b	12.85±0.04a	0.25±0.00b
Worm 2nd period only	7.62±0.01ab	7.3±1.2c	489±8a	1422±45a	9377±115b	12.80±0.16a	0.19±0.00a
	F p	F p	F p	F p	F p	F p	F p
	82.5 <0.001	29.7 <0.001	39.1 <0.001	27.8 <0.001	36.6 <0.001	42.5 <0.001	4.5 0.024

Values are means±standard deviation. *F* and *p* values refer to one-way ANOVA. Values in the same column marked by the same letter are not significantly different (LSD post hoc test, $p>0.05$)

First laboratory experiment

The first laboratory experiment compared growth of two late succession plant species on spoil clay material with or substrate without earthworms in spring and summer 2005. Laboratory microcosms consisted of plastic boxes (11×13×10 cm) containing 200 g of spoil clay material collected from 17-year-old site, whichwere not yet colonized by earthworms. Litter (dominated by *S. caprea* and *Calamgrostis epigeios*) was collected from the same site. There were five treatments: clay only, clay and litter only, clay and litter with earthworms present during the first half of the experiment, clay and litter with earthworms present during the second half of the experiment, and clay and litter with earthworms present during the whole experiment. Each treatment was replicated four times. Litter was added to the surface of appropriate microcosms to form a 2-cm-thick layer. Two specimens of earthworm *L. rubellus* and one specimen of *A. caliginosa* were added to microcosms at the start of the experiment. Earthworms were collected by digging and hand sorting from 30 to 45-year-old unreclaimed sites. Microcosms were covered by an air-permeable lid that helped to maintain original soil moisture and were kept in a dark place at 15°C. After 4 months, earthworms were removed from microcosms of which half were kept without earthworms (treatment with earthworm only during first half of experiment), whereas earthworms were returned in second half (treatment with earthworms during whole experiment). Earthworms were added in the same numbers and species composition to microcosms that did not previously contain earthworms (earthworms during second half of experiment). Microcosms without earthworms were manipulated 4 months after start of experiment to mimic disturbance caused by worm removal; then, all microcosms were sown with 40 seeds each of *Festuca rubra* and *Trifolium hybridum*. The microcosms were incubated for 3 months at 20°C with 12-h dark/12-h light and were watered every third day to maintain soil moisture constant. The number of plants in each microcosm was counted 12, 17, 23, 34, and 45 days after seeding. At the end of experiment, aboveground plant parts were harvested, dried at 35°C for 5 days, and weighed. The substrate in the microcosms was dried at 60°C for 24 h and chemically analyzed. Oxidizable carbon content (Cox) was determined by the wet acidified dichromate oxidation method (Jackson 1958), and total nitrogen (Nt) was measured by Kieldal mineralization according to Bremner and Mulvaney (1982). Extractable P, K, Mg, and Ca were extracted by the Mehlich III extractant (Mehlich 1984), cations were then quantified by flame spectrophotometer, and P was quantified according to Murphy and Rieley (1962). Soil pH (1:5 soil to water ratio) was measured by glass electrode.

Second laboratory experiment

This experiment compared the growth of late succession plants in substrates with different successional ages as effected by presence of earthworms in young succession soil. In April 2007, soil was collected from 17-, 23-, and 46-year-old postmining sites. In the 17-year-old site, soil was formed by broken mudstone; in the 23-year-old site, a thick fermentation layer had developed; and in the 46-yearold site, the organomineral humus horizon was about 6 cm thick (Frouz et al. 2007, 2008). The 17- and 23-year-old sites had not been colonized by earthworms, while the 46- year-old site had been naturally colonized by earthworms (Frouz et al. 2008). Intact soil monoliths (14×14×10 cm) were collected from the sites and placed in pots of matching size; ten pots each were established with monoliths from the 23- and 46-year-old sites and 20 pots with monoliths from the 17-year-old site. The pots were surrounded by a 40-cm-high rim of fine mesh (0.5 mm mesh size), preventing movement of earthworms into and out of pots. Existing aboveground vegetation was removed, and visible roots were removed without disturbing the original soil structure. The pots were dried at 30°C for 1 month to remove any earthworms, then kept in an outdoor plastic shelter and watered twice each weekly for 1 month; during this period, any emerging seedling was removed. On 1 July 2008, 1 g of seed-free litter of C. epigeios was added to each pot. Two mature *L. rubellus* and two mature *A. caligimosa* per pot were added to each of the ten 17-year-old pots, while the remaining ten 17-year-old pots were not treated by earthworms. All pots were then planted with seeds of six plant species (*Arrhenatherum elatius, Agrostis capillaris, Centaurea jacea, Plantago lanceolata, Lotus corniculatus, Trifolium medium*) that are common in latter succession stages on the postmining sites near Sokolov (Frouz et al. 2008); ten seeds per species were added to each pot. Emerging seedlings were counted 19, 30, and 130 days after seeding. Above- and belowground biomass of plants was collected 130 days after seed planting. Biomass was dried as described above and weighed. Earthworms were found in all replicated pots to which they had been added but not in the untreated ones.

Field experiment

A field experiment was conducted from September 2005 to September 2006 on a 17-year-old unreclaimed site uncolonized by earthworm. Ten field microcosms were constructed from 12-L plastic buckets (diameter 30 cm) with six drainage holes in the bottom. To prevent earthworms from entering or leaving the microcosms, drainage holes were covered by fine (0.2 mm) mesh. The same mesh also extended vertically about 50 cm above the sides of each microcosm. The microcosms were buried in the field so that the surface of soil in the microcosms was at the same level of the surface of the surrounding field soil. Each microcosm was filled with a soil monolith (30 cm in diameter) from the same location. The soil used in microcosms was not overgrown by plants but was covered by a litter layer about 2 cm thick. Five specimens of *L. rubellus* and two specimens of *A. caliginosa* were added to five microcosms but not to the other five ones. All microcosms were then planted with seeds of *F. rubra* and *T. hybridum* (0.25 g each). At the end of experiment (350 days after seeding), above- and belowground plant parts were harvested. Because surrounding plants had grown into the microcosms, we determined yields of Fabaceae, *Trifolium* (mainly *T. hybridum*), Poaceae (mainly *F. rubra*), and other herbs. Plant dry mass was determined as described earlier.

Statistical analysis

One-way ANOVAwas used to compare individual parameters among treatments of laboratory experiments. Redundancy analysis (RDA) was used to compare community composition of plants growing in soil collected from individual sites. In the field experiment, plant biomass of treatments with and without earthworms was analyzed by a nonpaired t test. Statistica 6.1. software (StatSoft, USA) was used for the t test and ANOVA, and Canoco 4.0 was used for RDA (Ter Braak and Smilauer 1998).

Results

Plant numbers were greater in clay than in the clay treated with earthworms (Fig. 1). The biomass of *T. hybridum* or *F. rubra* were significantly the greatest when earthworms were present during the whole experiment (Fig. 1). The changes in plant growth corresponded to changes in clays chemistry. Clay without litter and earthworms had the lowest concentration of nutrients except Mg. Soil pH was significantly lower, and content of available P, Ntot, and Cox were significantly the highest if earthworms were present during the whole experiment (Table 1). Seedling numbers did not differ significantly among soils with a different age (Fig. 2). Both above- and belowground biomass at the end of experiment were significantly higher in the 46-year-old monoliths than in those from the 17-year-old monoliths (Fig. 2). Addition of earthworms to 17-year-old monoliths increased both aboveand belowground biomass, and the ANOVA shows that the earthworm treatment on 17-year-old sites did not differ significantly from 46-year-old soil and from the noearthworm treatment (Fig. 2). However, when earthworm and no-earthworm treatment of the 17-year-old site were compared separately, then both above- and belowground biomass was significantly higher in the worm treatment (one side t test, p=0.026 and 0.031 for above- and belowground biomass, respectively). RDA of plant community composition, based on aboveground biomass of individual species, indicated a significant difference (F= 3.657, p=0.0260, Monte Carlo permutation test) between treatments without earthworms (Fig. 3, right side) and those with native (46-year-old site) or experimentally (17-yearold site with earthworms added) colonized by earthworms (Fig. 3, left side). All plant species grew better in these treatments (Fig. 3). The biomass of grasses, mainly *F. rubra*, was significantly greater with earthworms than without earthworms (Fig. 4). The total biomass of all plants was also greater with earthworms than without earthworms but the difference was only marginally significant (p=0.090).

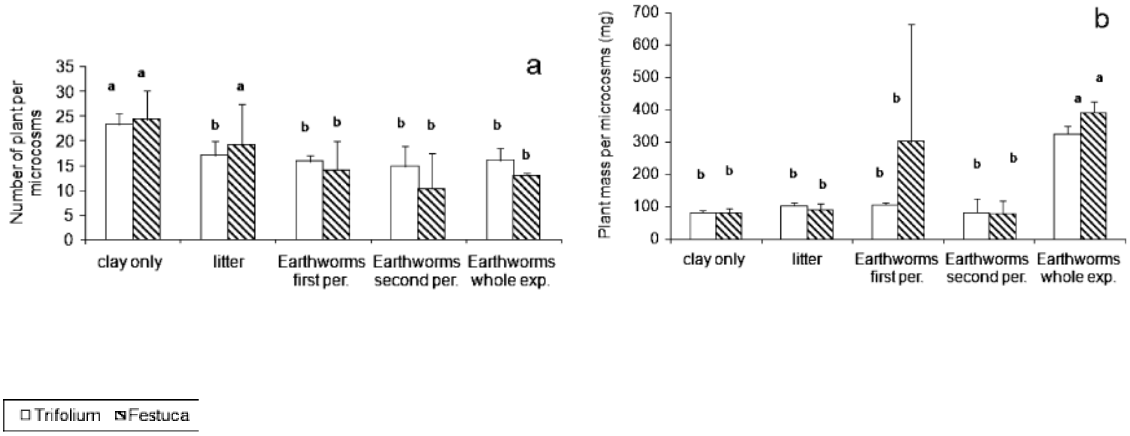


Fig. 1 Number of plants per microcosm (a) and plant dry mass per treatment (b; mean plus standard deviation shown as bars) during the first laboratory experiment. Bars with different letters are significantly different (LSD test, p<0.05; in the repeated measurement ANOVA; F=6.213, p=0.0002 and F=8.872, p<0.0001 for plant number of Trifolium and Festuca, respectively, and F=5.865, p=0.003 and F=16.000, p<0.0001 for biomass per one plant of Trifolium and Festuca, respectively). Earthworms were present 3 months before seeds were planted but not after seeds were planted (earthworms first experiment), only after seeds were planted (earthworms second experiment), or before and after seeds were planted (earthworms whole experiment)

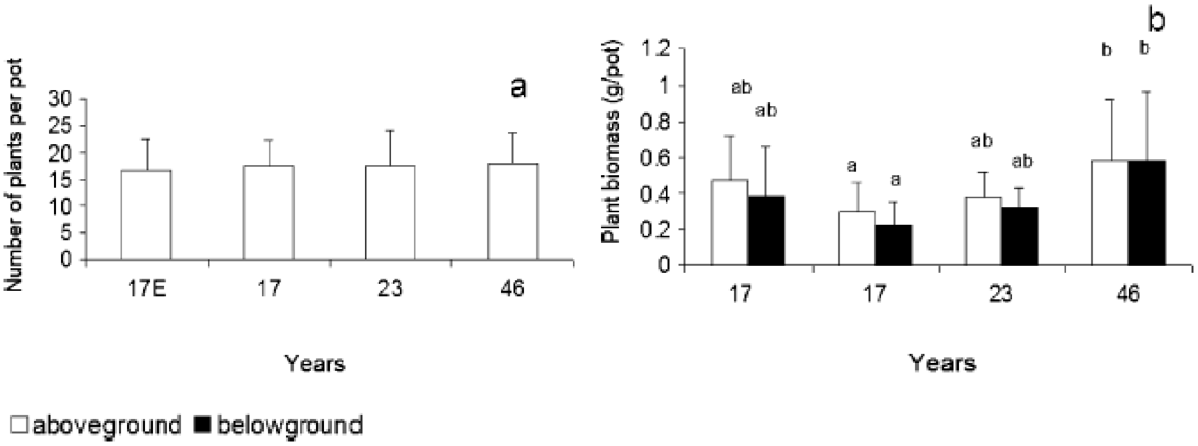


Fig. 2 Number of plants per microcosm during the second laboratory experiment (a) and above- and belowground plant biomass at the end of the second laboratory experiment (b; mean plus standard deviation shown as bars). Differences between treatments were not significant for plant number (in the repeated measurement ANOVA F=0.218 and p=0.927). Biomass bars with different letters are significantly different (LSD test, p<0.05; in the ANOVA F=2.640 and p=0.064 for aboveground biomass and F=3.413 and p=0.028 for belowground biomass). Numbers on the x axis indicate age of sites where soil was collected.

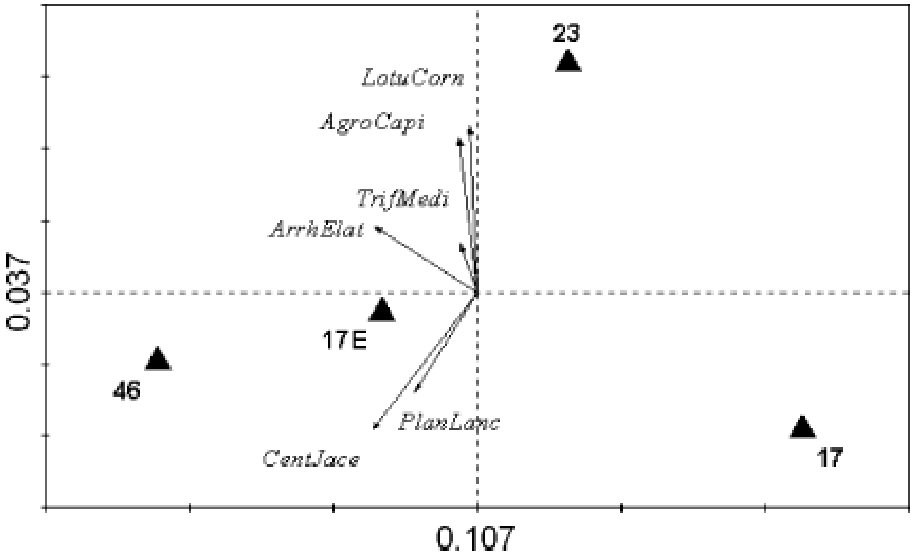


Fig. 3 RDA of plant community (based on aboveground biomass) growing in microcosms containing soil from different successional stages. Numbers represent successional age of field site where soil was collected. E indicates treatment with earthworm addition. Differences between individual treatments are statistically significant (F=2.13, p=0.04, Monte Carlo permutation test). *ArrhElat A. elatius, AgroCapi A. capillaris, CentJace C. jacea, PlanLanc P. lanceolata, LotuCorn L. corniculatus, TtrifMedi T. medium*. Numbers near the axes represent axis eigenvalues.

Discussion

This study showed that substrate quality increased during succession, and earthworm activity contributed to this process. Earthworms significantly altered the spoil substrate and increased the capacity of spoil to support plant species typical of latter successional stages in the Sokolov area in both the field and laboratory experiments. This finding confirms that earthworms affect plant growth as indicated before in different ecosystems (Stockdill 1982; Thomas et al. 1993; Bonkowski et al. 2001; Wurst 2004). The previous study of plant succession in Sokolov postmining sites found sudden and market change in plant community composition, which correlated with colonization of the sites by earthworms (Frouz et al. 2008). This study supports the hypothesis that earthworm colonization contributes to this sudden massive changes in plant community. The laboratory experiments indicated that earthworms increased plant growth but not seed germination. Numbers of emerging seedlings were reduced (Fig. 1a), probably because seeds were consumed and transported to deeper soil layers, as reported previously (Thompson et al. 1993; Milcu et al. 2006). The emergence of *T. hybridum* seedlings was also reduced by the *S. caprea* litter, probably due to allopathic effect by S. caprea litter (Schutt and Blaschke 1980). The increase in plant growth in the laboratory experiment 1 corresponded to the increase in soil nutrient content (Table 1). This agrees with Bohlen et al. (2004), who indicated that earthworms increased nutrient availability. Earthworms may increase nutrient availability by enhancing mineralization of plant litter and also by modifying soil pH (Haimi and Boucelham 1991; Araujo et al. 2004). The pH of the original spoil material was alkaline and was decreased by earthworms. In agreement with Frouz et al. (2006, 2007), we suspected that the decrease in pH is caused by the incorporation of organic matter in alkaline soil. This decrease in pH may increase availability of some nutrients, especially P, which becomes more available in neutral or slightly acidic conditions (Brady and Weil 1996). In both laboratory and field experiments, growth of the grass *F. rubra* was more stimulated than growth of the legume *T. hybridum* by earthworms. This confirms that grass respond faster than legumes to worm activity (Scullion and Malik 2000; Wurst 2004). This difference in plant response is probably caused by the faster response of grasses than legumes to changes in nutrient supply (Wurst 2004). Better growth of latter successional plants in defaunated soil from late successional stages probably depends on long-term changes of soil. Earthworms probably contribute to these long-term changes (Lavelle and Martin 1992; Lavelle et al. 1997), but their effects are probably gradual and may accumulate only slowly over time (Lavelle et al. 1997). In the first laboratory experiment, plants grew better if earthworms were present during the whole experiment than in the only half of the experiment. This indicates that the earthworm effect increases with the duration of their presence. Plant growth of 46-year-old soil, which is affected by earthworms during the last 20 years, was higher than plant growth of 17-year-old soil. Earthworms' addition, in the 17-year-old soil increased plant growth, when earthworms were present, but this affect disappeared when worm were removed. This suggests that the persistent effect of earthworms on plant growth requires long period of time.

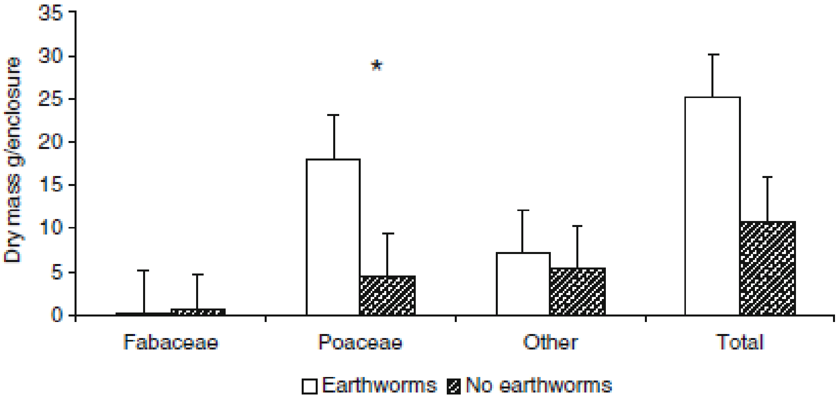


Fig. 4 Biomass of plants in field microcosms with and without earthworms. The asterisk indicates a statistically significant difference between the treatments with and without earthworms (t test, $p<0.05$)

Acknowledgements

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Paper II

Survival and performance of earthworms on unrecultivated spoil heaps at different successional stages

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Manuscript

Abstract

Earlier studies of postmining soils near Sokolov, Czech Republic (0–48 years old) showed that massive changes in plant community composition and soil structure and quality occur around 23 year of succession when the heaps are colonized by earthworms. The aim of the current study was to test the hypothesis that earthworm occurence on spoil heaps is affected by the character of vegetation, litter and soil at the individual sites. We have constructed 2 field experiments; in the first experiment we tested performance (number and biomass change) of earthworms that were kept in pots (10 l) at 4 sites of increasing successional age (8, 15, 25, 48 years) for 6 months; earthworm biomass and numbers were lowest at the youngest site and increased with increasing age of site. In the second experiment we tested performance of earthworms in pots at mid-successional stages (19 and 25 years) with different types of litter for 12 months; earthworms performed better at site 25 years old in pots with herbaceous litter. The results show that earthworm survival, growth and reproduction is highly dependant on the vegetation cover and corresponding quality of soil and litter, but facilitation of soil conditions by earthworms can play an important role in their colonization of spoil heaps.

Keywords

Earthworm performance, Aporrectodea caliginosa, post mining soils, spontaneous succession

Introduction

Earthworms are important soil organisms that can affect many crucial soil properties, plant growth and plant community composition (Scullion and Malik 2000; Thompson et al. 1993; Yu et al. 2008; Wang et al. 2006; Wurst 2004). They also play an important role during succession on post mining soils (Frouz et al. 2006, Roubíčková et al. 2009, Boyer and Wratten 2010).

Because earthworms are slow migrants and sensitive to disturbance (Tondoh et al. 2007), sites that have experienced large-scale disturbances may lack earthworms even if environmental conditions are otherwise suitable for them (Dunger 1991; Huhta and Raty 2005; Frouz et al. 2008). Earthwom distribution is dependent on soil conditions – soil texture, humidity, pH and vegetation chracteristics such as rooting depth or litter palatability (Dunger 1969, Rushton 1986, Edwards and Bohlen 1996, Pižl 2001, Eijsackers 2011, Piotrowska et al.2013). Migration abilities are dependant on the ecological group of earthworm – epigeic species generally migrate faster into new areas, whilst endogeic species, as *Aporrectodea caliginosa*, are slow migrants and facilitation of soil conditions plays an important role in their dispersal (Lavelle 1988, Mathieu et al. 2010, Caro et al. 2013)

The spoil heaps near Sokolov, NW Bohemia, are formed by tertiary clays, that are brought to surface from depth of 100 – 200 m and are therefore free of organic matter, apart from fossil organic matter. Some parts of the spoil heaps undergo the process of recultivation, some are left to spontaneous primary succession. On unreclaimed spoil heaps first earthworms occur 20 – 25 years after deposition. The first colonizers are commonly epigeic species (*Dendrobaena octaedra*, *Dendrodrillus rubidus*), later the species *Lumbricus rubellus*, *Aporrectodea caliginosa* and *Allolobophora chlorotica* (Frouz et al. 2001, 2006, 2008). These are generally pioneer earthworm species that are able to quickly colonize new areas and survive harsh conditions of spoil heaps, as are drying-out of the upper soil horizons, low palatability and unfavourable chemical composition of litter (Rushton 1986, Pižl 1992, Dworschak 1997, Pižl 2001, 2002).

We have constructed two field experiments to test soil suitability of individual sucessional stages and particular vegetation types for establishment of *Aporectodea caliginosa* during spontaneous sucession on post mining sites.

Material and methods

Site description

The study was carried out at a post-mining area in the Sokolov brown-coal mining district in the Czech Republic (50°14‘N, 12°39‘E). The average altitude of the spoil heaps is about

500 - 600 m a.s.l. Mean annual precipitation is 650 mm, and the mean annual temperature is 6.8 °C. The spoil heaps originated from open-cast brown-coal mining and consist mainly of tertiary clays of the so-called cypric series, which are well supplied with mineral nutrients (Štýs 1981). The pH of the substrate in initial successional stages is 8 – 9 and gradually decreased with sucesion age. The sites are characteristic by longitudinal rows of depressions and elevations formed during the heaping process. The top of a wave or row is about 1-2 m above the base of the depression, and individual rows are about 6 m apart. The studies were conducted on unreclaimed plots that were in different stages of succession (8 – 48 years after deposition):

The youngest site (8 years) is characterized by a sparse herbaceous cover of pioneer plant species (*Tussilago farfara, Hordeum jubatum, Calamagrostis epigejos*), litter layer is very thin or absent.

On the site 15 years old vegetation is much denser, in depressions dominated by *C. epigejos*, on elevations is a mixture of pioneer herb and grass species (*Taraxacum ruderalia*, *Hieracium piloselloides*, *Tussilago farfara*, *Poa compressa*), with small trees of species *Salix caprea*, *Populus tremula* and *Betula pendula*. The litter layer is thin (0 – 3 cm)

Site 19 years old is very similar to site 15 years old, with slightly higher trees and often dominated by *Calamagrostis epigejos*.

The site 25 years old is covered by a dense tree canopy with dominant *S. caprea* and less common *P. tremula* and *B. pendula*. Herbaceous cover is present only on some elevations and is dominated by *Fragaria* spp. *Hieracium* spp., *Poa compressa* etc. The litter + fermentation layer is 5 – 15 cm thick in depressions and 1 – 3 cm on elevations.

The site 48 years old is characterized by a forest dominated by *B. pendula*, with occasional *P. tremula* mixed in. Sparse herbaceous cover is present, dominated by *Festuca rubra* and *F. ovina*, *Fragaria* spp, *Plantago* spp etc. Litter + fermentation layer is thinner than on II (1 – 5 cm), but a 5 – 15 cm thick humus layer with high earthworm activity is present.

Experimental design, data processing

Experiment n.1

In April 2008 on all sites described above, an area of lower plant coverage was chosen and 4 pots with diameter 25 cm and volume 12 l were buried, filled with autochtonous soil and litter that was previously frozen at -70°C fo 24 hours to eliminate presence of earthworms or their cocoons. These pots had 2 drainage holes in the bottom, covered by 1 mm mesh to prevent earthworm escape and at the top of the pot a 40-cm high mesh column, which was tied together at the top by a string, was glued and taped on, preventing earthworms from escaping over the top. To each pot 4 specimen of *A. caliginosa* were added, each earthworm being weighed first.

After 6 months (in October 2008) all pots were collected and earthworms were extracted by hand-sorting, counted by species and weighed. Using program ANOVA, multiple comparison in program S-Plus (Insightful, 1988-1997), change in biomass for both species was compared between sites of different age.

Experiment n. 2

In October 2010 on sites 19 and 25 years old 10 pots were buried, five of them were with autochtonous litter + fermentation layer, to five of them the litter + fermentation layer from the other site was transferred. Site 25-year old litter was dominated by *Salix caprea*, the C/N ration was 44, while litter from site 19 was dominated by *Calamgrostis epigeios* and other herbs ad grasses, the C/N was about 32. The soil was previously frozen at -70°C fo 24 hours to eliminate presence of earthworms or their cocoons. All the pots were secured against earthworm escape as in Experiment n.1. To each pot 4 specimen of *A. caliginosa* were introduced, all earthworms being weighed prior to that. After 12 months (in October 2011) all pots were collected and earthworms were extracted by hand-sorting, counted by species and weighed. Using program ANOVA, factorial, and t-test in program S-Plus (Insightful, 1988-1997), change in biomass and numbers was compared between the two sites and two types of litter.

Results

Experiment n.1

Individuals of *A. caliginosa* were found in 15 out of total 16 pots, although increase in earthworm biomass (by 40%) was recorded only from pots at the oldest site (48-year old). At younger sites there was a decrease in earthworm biomass – 11 % at site 25-year old, 73 % at site 15-year old and 80 % at site 8 years old, as can be seen on Fig.1.

Sites also differed in numbers of individuals and especially in numbers of juveniles, as shown on Fig. 2. At the two oldest sites numbers of specimen of *A. caliginosa* increased in most pots with a high percentage of juveniles – 67% on oldest site and 43 % on site 25-year old. At site 15 years old there was 1 juvenile found in total and the number of adults decreased by 50 – 75 % and at site 8 years old there were no juvenile individuals found and numbers of specimen decreased by 50 – 100 %.

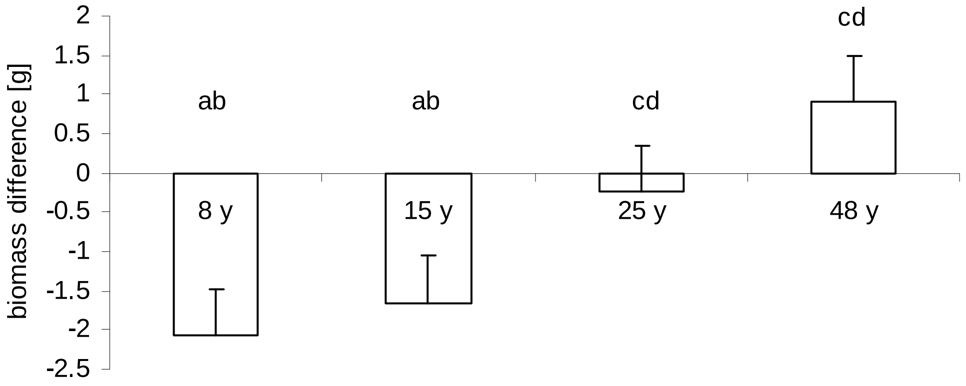

Fig. 1. Change in biomass of earthworm *A. caliginosa* in pots buried on 4 sites, the biomass difference was changing with the age of site - from negative 80% and 73% at the two youngest sites, respectively, negative 11% at the 25-year old site to increase by 40% at the oldest site (8 y - 8 years old, 15 y – 15 years old, 25 y – 25 years old, 48 y – 48 years old). Different numbers sign significant difference between treatments (p = 0,0004). Error bars show standard error (SE).

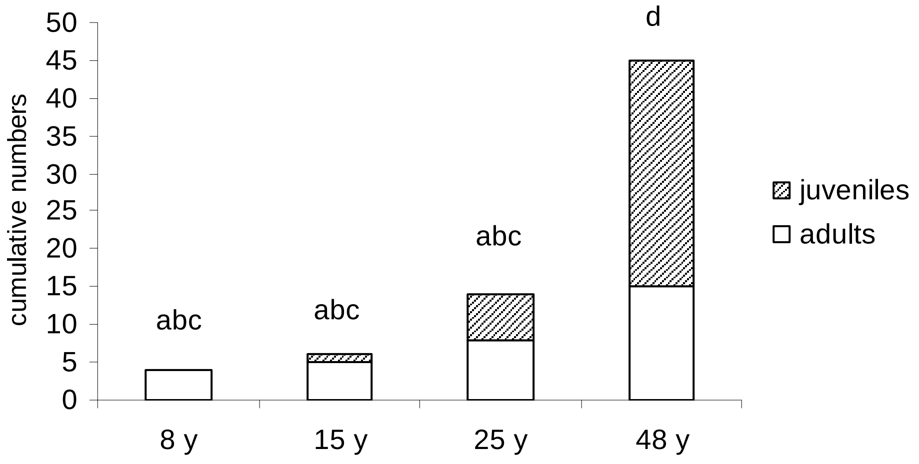

Fig. 2. Numbers of juveniles and adult earthworms (*A. caliginosa*) found at the end of experiment at the 4 sites. Different letters sign significant difference between treatments (p = 0,00001). Error bars show standard error (SE).

Experiment n. 2

Survival of earthworms was generally lower on site 19 years old – an average of 30% of original earthworm biomass. At site 25 years old about 85 % of biomass of earthworms remained at the end of experiment. At the 19-year old site there was no difference in earthworm survival between pots with different type of litter.

At the site 25 years old, earthworm survival was more successful in pots with herbaceous litter from the younger site (increase in biomass by 36 %); the litter from site 25 years old, containing mainly *S. caprea* leaves, had a strong negative effect on earthworm biomass (biomass decreased by 70%), as shown on Fig. 3, although the difference was only marginally significant (p = 0,08).

More pronounced is the combined effect of soil (+ site) and litter on numbers of juvenile earthworms – the highest number of individuals (total of 32 in 5 pots) was in pots with soil from site 25 years old with litter from the younger site and there was generally a higher number of juveniles in pots on site 25 years old (10 in total), whilst on the younger site there were only 4 juvenile individuals found in total.

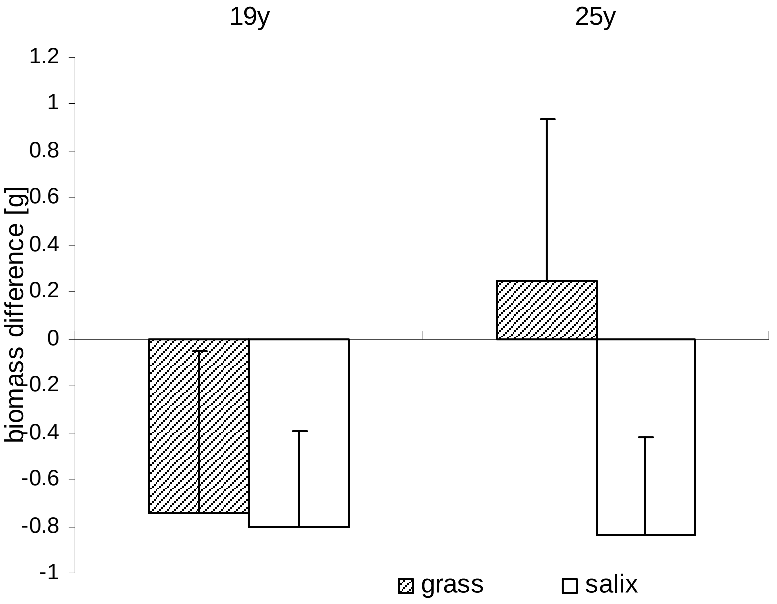

Fig. 3. Earthworm biomass change on 19-year old site (19y) and 25-year old site (19y) with grass and *salix* litter after 12 months of experiment duration. Error bars show standard error of mean (SE).

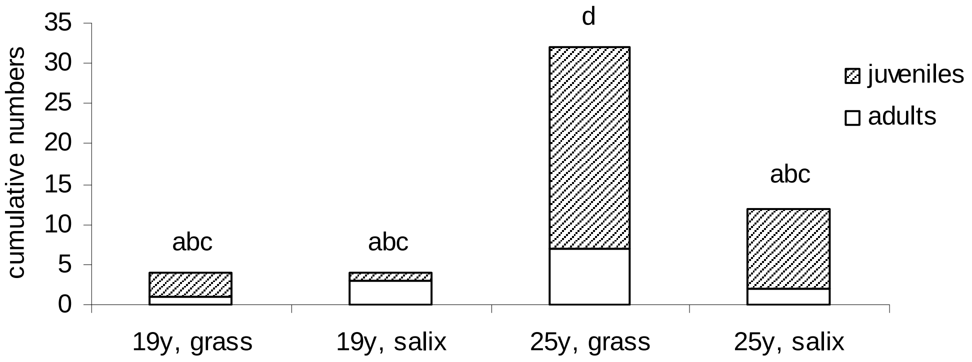

Fig. 4. Numbers of juvenile (striped) and adult (white) earthworms at sites 19 years old (19y) and 25 years old (25y) with different litter (grass = litter from site 19 years old, salix = litter from the site 25 years old). Different letter signs significantly different total number of individuals (p = 0,022). Error bars show standard error (SE).

Discussion

The first experiment showed a positive relationship between site age and earthworm (*A. caliginosa*) biomass, which can be explained by improving biotic and abiotic conditions at individual sites according to the successional stage – especially by the increase in vegetation cover, litter layer thickness, shift pH to neutral value (Frouz et al., 2008) as well as gradual weathering of mudstones (Kuráž et al. 2012).

The endogeic species *A. caliginosa* is generally an euryecic species, that is often found in ecosystems under disturbance, such as mine colliery deposits, ash deposits and arable fields and has a relatively high tolerance to drought (Holmstrup et al. 1998, Pižl 1992, 2002, Eijsackers 2011).

At sites younger than 25 years, both biomass and number of earthworms decreased in comparison with number of earthworms introduced in to the pots and no or little juveniles were found (Fig 1 and 2). This indicates that not only migration barrier but also habitat suitability limit earthworm establishment on these sites. In 25-year old site a large number of juveniles was found, which indicates some population growth. This is consistent with observation that earthworms colonise sites around 25 year old (Frouz et al. 2008). In general, accumulation of litter on soil surface and a canopy closure which buffer the temperature and moisture fluctuations (Brady and Weil 2000) belong to major factor that may be responsible for improvement of site conditions. Although the litter, created mainly by *S. caprea* leaves, is highly acidic and of unfavourable chemical composition (Schutt and Blaschke 1980). The fact that endogeic earthworm species haven’t been found at this site before could be caused by their lower migration ability (then that of epigeic species that occur at this site) (Rushton 1986, Dworschak 1997, Suárez et al. 2006), because the closest site where they are present is more than 1km away and earthworms would have to migrate over some sites in younger successional stages.

The highest population growth was found at the oldest site, which signs that conditions were optimal for this earthworm species. This is consistent with the fact that earthworms already colonised the site and most probably affected the plant community composition (Frouz et al. 2008, Roubíčková et al. 2009). The litter from the tree species growing here (*B. pendula, P. tremula*) and especially grass and herb litter of a well developed herb layer (Frouz et al., 2008) is favourable fo earthworms. Previous experiments show that earthworms, as ecosystem engineers, promote formation of this well developed herb layer (Frouz et al. 2008, Roubíčková et al. 2009, Mudrák et al., 2012) which supports the idea that earthworms can cause changes in ecosystem and make the conditions more favourable for themselves (Lavelle et al. 1997, Jouquet et al. 2006, Mathieu et al. 2010).

Earthworm performance in experiment 2, after a year of duration, was generally worse than after 6 months in the first experiment, which is obvious when comparing survival at 25 year old site with autochnonous litter with survival during Experiment n.1 at this site. This may be caused by the fact that most of the adult individuals that were introduced the previous year, have died at the end of the vegetation season and there weren't enough juvenile individuals to compensate the weight loss (Edwards and Bohlen 1996). In agreement with results of experiment 1, at the 15-year old site there was a generally lower number of earthworms at the end of experiment, which we explain by harsher conditions due to absence of a tree canopy.

Larger number of surviving adults and juveniles in the pots at 25-year old site with herbaceous litter (with prevailing *Calamagrostis epigeios*) suggests that *S. caprea* litter is less palatable then grassy litter, most likely due to the high levels of lignin and tannins (Frouz et al. 2013). This corresponds with the results of Šlapokas and Granhall (1991) who found that leaves of a willow species with the highest content of tannins had the lowest decomposition rate, irrespectable of the nutrient content. As already mentioned, the earthworms promote development of herb and grassy vegetation in understory (Frouz et al. 2008, Roubíčková et al. 2009, Mudrák et al. 2012), which means this is a positive feedback - they establish better in vegetation they promote, and promote vegetation in which they grow better. Despite willow (*Salix caprea*) peaked in 20-25 year old sites some small patches without willow canopy still exist. These patches are covered by more dense herb and grassy vegetation including *C. epigeios* and they may benefit from more advanced site conditions and vegetation development, but at the same time they offer more suitable liter as a food source. This sugests that successionally delayed patches of vegatation at sites that are in a more advanced stage of succession may be crucial for establishment of successiomaly more advanced species and may help moving succession forward.

The conditions inside the pots though, thanks to the absence of communication with surrounding soil with no possibility of horizontal migration to find moist patches (Edwards and Bohlen 1996), may have been more extreme than natural conditions in the surrounding soil and may have negatively affected survival of earthworms in general.

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Paper III

The effect of belowground herbivory by wireworms (Coleoptera: Elateridae) on performance of *Calamagrostis epigejos* (L) Roth in post-mining sites

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Abstract

The effect of belowground herbivory by the wireworm *Agriotes lineatus* L. (Coleoptera: Elateridae) on the grass *Calamagrostis epigejos* was studied in greenhouse and field experiments. In the greenhouse, *C. epigejos* and *Festuca rubra* were grown together in pots with or without wireworms; wireworms decreased the above- and belowground biomass of *C. epigejos* but increased the above- and belowground biomass of F. rubra. In a field experiment at a post-mining site, repeated insecticide application reduced a wireworm population dominated by *A. lineatus* by 70% and significantly (p Ľ 0.0317) increased the belowground biomass of *C. epigejos*; redundancy analysis (RDA) indicated a significant difference in plant community composition. Both the field and greenhouse experiment indicated that wireworms negatively affect *C. epigejos* and therefore can speed up succession and help establishment of a more diverse plant community on spoil heaps.

Keywords

Belowground herbivory, Wireworms, *Calamagrostis epigejos*, Post-mining soils

Introduction

Understanding spontaneous vegetation succession in postmining sites is important for both practical and theoretical reasons. From a practical perspective, natural succession is fundamental to restoring these highly disturbed areas. From a theoretical perspective, post-mining sites represent a unique opportunity to document ecosystem processes and interactions. It is generally accepted that vegetation changes during succession are crucial for the development of the whole ecosystem (Frouz 1997, Frouz et al. 2001, 2008). At the same time, other organisms can influence plant performance and the composition of the plant community by changing the environment or by direct consumption of plant tissue (Brown and Gange 1989, 1992, De Deyn 2003, Roubíčková et al. 2009, Thompson et al. 1993, Wurst 2004). Herbivory in general reduces plant biomass and causes plants to invest more resources in compensatory growth or defense against herbivory rather than in reproduction or growth (Andersen 1987, Brown and Gange 1992, Coley et al. 1985). The role of root herbivores in ecosystems is often underestimated because they are generally unseen, have often complicated reproduction cycles and donot rapidly migrate to new areas (Andersen 1987, Brown and Gange 1989, 1992). The fact that these organisms are difficult to observe and manipulate complicates research on them. Root herbivores, however, can substantially reduce the abundance of some plant species (Blossey and Hunt-Joshi 2003). Some studies have shown that preferential feeding on early-successional plants by generalist root herbivores can speed up succession and result in faster establishment of late-successional plants with better protected roots Andersen 1987, Brown and Gange 1989, 1992, De Deyn et al. 2003, Hemerik et al. 2008, Rassman and Agrawal 2008). Wireworms are click-beetle (Elateridae) larvae. Some species are predaceous but most feed on plant roots and seeds. Wireworms in the genus *Agriotes* are generalist root herbivores that can be abundant in initial stages of succession (Jedlička and Frouz 1999, 2007) and can be important pests in agroecosystems (Chaton et al. 2008, Johnson et al. 2010, Rychterová 2009). In many areas, the grass *Calamagrostis epigejos* is a dominant plant species during initial successional stages in disturbed ecosystems (Prach 1987, Somodi et al. 2008, Wiegleb and Felinks 2001), including post-mining sites. This fast growing plant often outcompetes all other plant species and creates monospecific stands in soils rich in nitrogen. It is an important ecosystem engineer which alternates condition of soil surface. Dominance of this species is caused besides very rapid vegetative reproduction also by very tough, slowly decomposable litter, which inhibits the growth of other plant seedlings (Massey et al. 2007, Mudrák et al. 2010, Rebele and Lehmann 2001). Even though the autecology of *C. epigeios* has been intensively studied (Gloser 2002, Kavanová and Gloser 2004, Rebele and Lehmann 2001), little is known about its interaction with belowground herbivores. It has fleshy rhizomes that serve as vegetative reproductive organs and that also play a role in nutrient transport from source organs of mature tillers to young tillers (Gloser 2002, Kavanová and Gloser 2004). Roots of *C. epigejos* store nitrogen-rich compounds during the winter, which enables very fast re-growth of the aboveground parts in early spring (Gloser 2002). On Sokolov spoil heaps *C. epigejos* occurs in monospecific stands on many sites from 5 to 45 years (the oldest part of the heap) after deposition. *Festuca rubra* is another grass species that commonly grows on post-mining sites, but in contrast to C. *epigejos*, *F. ru*bra is more typical of late rather than early successional stages (Deyl and Hísek 2002), commonly found on spoil heaps about 40 years after deposition. There it can often grow in mixed stands together with *C. epigejos* but reaches much higher coverage when the latter is rare and is suppressed in *C. epigejos*-dominated communities. This clonal grass is common in soils with moderate fertility and relatively rich in water (Deyl and Hísek 2002, Rychterová 2009). The objectives of the current study were to determine how belowground herbivory by the larvae of *Agriotes lineatus* (wireworms) affect: i) *C. epigejos* growth; ii) competition between *C. epigejos* and other plant species including *F. rubra*; and iii) plant community composition at a post-mining site. We hypothesized that wireworms negatively influence *C. epigejos* and some other early-successional plant species by preferential feeding on their roots.

Materials and methods

Site description

The study was carried out at a post-mining area in the Sokolov brown-coal mining district in the Czech Republic (50°14‘21‘‘N, 12°39‘24‘‘E). The average altitude of the spoil heaps is about 500-600 m a.s.l. Median annual precipitation is 650 mm, and the median annual temperature is 6.8°C. The spoil heaps originated from open-cast brown-coal mining and consist mainly of tertiary clays of the so-called cypric series, which are well supplied with mineral nutrients (Šourková et al. 2005). The pH of the substrate in initial successional stages is 8 - 9. The study was conducted in an unreclaimed area that was about 15 years old (i.e., the spoil heapswere deposited 15 years earlier) and was characterized by longitudinal rows of depressions and elevations formed during the heaping process. The top of a wave or row was about 1 - 2 m above the base of the depression, and individual rows were about 6 m apart (see Ref. (Frouz et al. 2001) for more site details.

Greenhouse experiment

In this experiment we were testing the hypothesis that wireworms can change competition between C. epigejos and *F. rubra* by preferential feeding on *C. epigejos* roots. It was begun in September 2008 in an un-heated greenhouse. Soil was collected from the plot described in Section 2.1, from 5 to 20 cm depth, both from tops of the waves and the bases and the soil was mixed afterwards. It was defaunated by drying (14 days of air drying at mean temperature 25°C), and then ground, passed through a 6-mm sieve, mixed, and placed in 14 ceramic pots (10 cm diameter and containing 0.5 L of soil weighing approximately 300 g). In August 2008, clumps, containing in average 9 ramets of *F. rubra* (shoots, rhizomes, roots, and associated soil) were collected from a 48-year-old unreclaimed plot in a post-mining area in the Sokolov brown-coal mining area and clumps, containing in average 3 ramets of C. epigejos were taken from the site described in Section 2.1. Three clumps of *F. rubra* (with leaf length from 15 to 20 cm) and three clumps of *C. epigejos* (with leaf length from 40 to 60 cm) were planted in each pot. Two larvae of *A. lineatus* in 2nd and 3rd stage of development were added to seven pots, and the other seven pots were used as controls. The temperature in the greenhouse was ranging from 2° C to 25° C resembling the outside temperatures only with milder fluctuations. Plants were watered once every 3 days with 20 – 50 ml of water. The light regime was not regulated, only natural daylight was present. At the end of May 2009 (8 months after the experiment started), plants were harvested, air dried for 10 days at mean temperature 20° C, separated into aboveground and belowground parts by species, and weighed.

Field experiment

A field experiment was conducted from June 2009 to June 2010 in the 15-year-old spoil heap described in Section 2.1. The area was covered with herbaceous vegetation and also had scattered shrub patches; these shrub patches were omitted from the experiment. As noted, the soil surface is undulating (it has awave-like character) because the spoil heaps were deposited in rows with an east-to-west orientation. Five pairs of plots were designated. Each plot was 2 – 5 m, and adjacent plots were 1 m apart. Each plot included the northern and southern slope of the wave (extending to the centre of the wave base). Before the experiment began, the vegetation cover was visually estimated (according to the Braun- Blanquet method) on the centre of the southern and northern slope of the wave and at the wave base of each plot. One randomly selected plot of each pair was not treated, and the other plot was treated with the insecticide Dursban 10G (containing 10% chlorpyrifos) at 10 g/m². Dursban 10G is a contact insecticide used for controlling soilborne herbivorous insects and has a low toxicity against other soil organisms and does not directly influence plants [5]. At the start of the experiment, in June 2009, pesticide granules were applied to narrow slits (about 3 cm wide, 5 cm deep, and 10 cm apart) that were made in the soil with a spade. The same slits without pesticide were made in control plots. This application was then repeated four times: in August 2009, October 2009, March 2010, and in May 2010, to give five applications in total. In June 2010, the vegetation survey was repeated, and one cylindrical soil core (area 625 cm² and depth 10 cm) was collected per plot. Each core (including the above- and belowground plant material) was immediately placed on a large plastic sheet. After the aboveground biomass of *C. epigejos* associated with the core was collected, the core was broken apart, and roots of *C. epigejos* and visible wireworm larvae were collected. The remainder of the core was transported to the laboratory, where the soil was placed in Tullgren funnels to extract remaining wireworm larvae. Wireworms from each slit were counted and weighed, aboveground and belowground biomass of *C. epigejos* was air dried (20 _C for 14 days) and weighed as well.

Data processing

For comparison of the aboveground and belowground biomass of both species in the two treatments (± wireworms) in the greenhouse experiment, a standard parametric t-test was used. The data set had a normal distribution. The aboveground and belowground biomass of the wireworms and *C. epigejos* in the field experiment were compared in control and insecticide-treated plots with a standard paired t-test. Plant species cover (in %) in the community was compared using redundancy analysis (RDA) using the Canoco program [36]. In addition, coverage of dominant species was compared with a paired t-test. All univariate analyses were performed in the program S-Plus (Insightful 1988-1997).

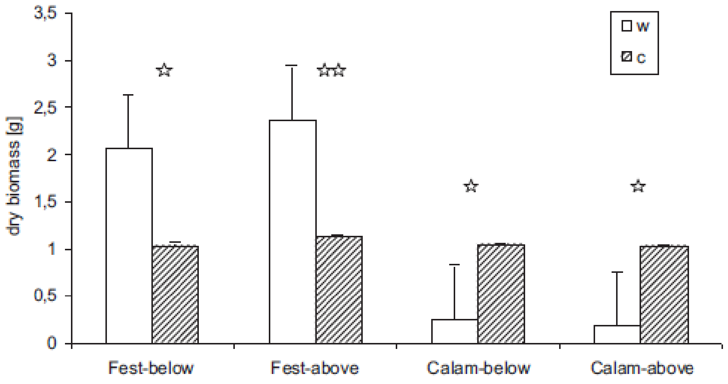

Fig. 1. Aboveground and belowground biomass of Festuca rubra and Calamagrostis epigejos grown together with wireworms (w) and without wireworms as control (c) in the greenhouse experiment. Stars indicate significant differences between treatments with and without wireworms: (one star = $p < 0.05$, two stars = $p < 0.01$). Error barsindicate the standard error of the mean.

Results

Greenhouse experiment

Here the addition of wireworms brought about a reduction in the aboveground and belowground biomass of *C. epigejos* but increased the biomass of *F. rubra* (Fig. 1). The differences were more pronounced in the aboveground biomass (p Ľ 0.0065 for *F. rubra*, p Ľ 0.012 for *C. epigejos*), the biomass of roots was less influenced, especially for *F. rubra* (p Ľ 0.044 for *F. rubra*, p Ľ 0.024 for *C. epigejos*).

Field experiment

Here the application of an insecticide for 1 year significantly reduced wireworm biomass by about 70% (Fig. 2). All undamaged last instar larvae were identified and determined to be *A. lineatus*. Aboveground biomass of *C. epigejos* was slightly but not significantly higher in insecticide-treated plots but belowground biomass of *C. epigejos* was significantly higher in treated plots (Fig. 2). According to redundancy analysis (RDA) in which insecticide treatment was used as the only explanatory variable, coverage of individual plant species differed between treated and untreated plots (Fig. 3). The same analysis made the previous year, just before insecticide application, indicated no significant difference in plant community between these plots (data not shown). Other species typical for initial succession stages (*Hieracium piloselloides, Hordeum jubatum, Poa compressa, Sinapis arvensis, Tanacetum vulgare, and*

Taraxacum ruderalia) tended to have higher coverage in insecticide-treated than in untreated plots (Fig. 3), but the difference was significant only for H. piloselloides (p = 0.016).

Discussion

By reducing the biomass of *C. epigejos* in the greenhouse experiment, belowground herbivory by wireworms (larvae of *A. lineatus*) gave a competitive advantage to *F. rubra. C. epigejos* was evidently a preferred food source or was more susceptible to wireworms attack than *F. rubra*. This seems to be connected with *C. epigejos* morphology, namely presence of thick fleshy shoots (Rassmann et al. 2010) that may be better food source for wireworms than hairy roots common in many other grasses including *F. rubra*. Important is also chemical composition because the roots and belowground shoots of *C. epigejos* are rich in amino acids, nitrate, and proteins (Andersen 1987, Gloser 2002, Kavanová and Gloser 2004). Differential effects of wireworms on these two plant species became obvious in early spring (April, only optical observation), during the sixth month of the greenhouse experiment. This suggests that feeding on underground shoots and roots by wireworms reduces the fast spring re-growth of *C. epigejos*, which is an important trait of this species (Gloser 2002). The timing of the growth suppression is also consistent with data showing that wireworms are active during spring and autumn, when the temperature in upper soil layers is moderate (Chaton et al. 2008, Karren 2002). In the absence of herbivory or other limiting factors, the ability to grow rapidly in spring could help *C. epigejos* competitively exclude other plants, especially relatively slow-growing grasses such as *F. rubra*, which are characteristic of later successional stages. We infer that roots of *F. rubra* are tougher and contain less nitrogen than those of *C. epigejos*, and are therefore not preferred by wireworms (Johnson et al. 2010). This inference is supported by Hemerik et al.(2003) , who reported that *Agriotes obscurus* and *Athous haemorrhoidalis* wireworms rejected *F. rubra* roots in a food-preference experiment. Because both plant species were grown close to each other, nutrients leaking from the damaged roots of *C. epigejos* and wireworm excrements were easily accessible to roots of *F. rubra* and could have supported growth of the latter as well (Bardgett et al. 1999, Dromph et al. 2005).

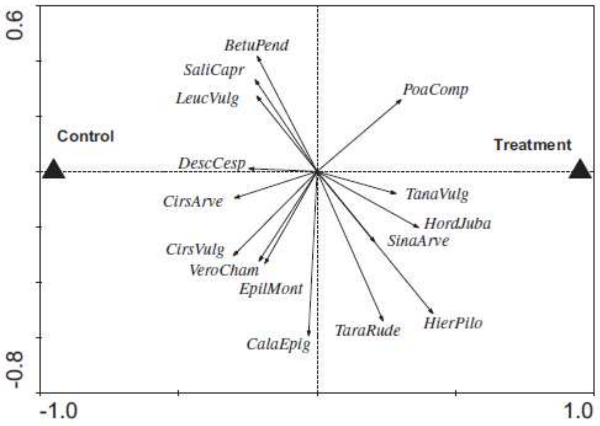

Fig. 3. RDA based on coverage of plant species in insecticide-treated plots (treatment) and in untreated plots (control) in the field experiment; control and treatment were used as the only explanatory variables. The canonical axes explained 7.8% of the variability (F =Ľ 2.605, p = 0.0360, Monte Carlo permutation test). Species acronyms: *BetuPend = Betula pendula; CalaEpig = Calamagrostis epigejos; CirsArve = Cirsium arvense; CirsVulg = Cirsium vulgare; DescCesp = Deschampsia cespitosa; EpilMont = Epilobium montanum; HierPilo = Hieracium pilosella; HordJuba = Hordeum jubatum; LeucVulg = Leucanthemum vulgare; PoaComp = Poa compressa; SaliCapr = Salix caprea; SinaArve = Sinapis arvensis; TanaVulg = Tanacetum vulgare; TaraRude = Taraxacum ruderalia; VeroCham = Veronica chamaedrys.*

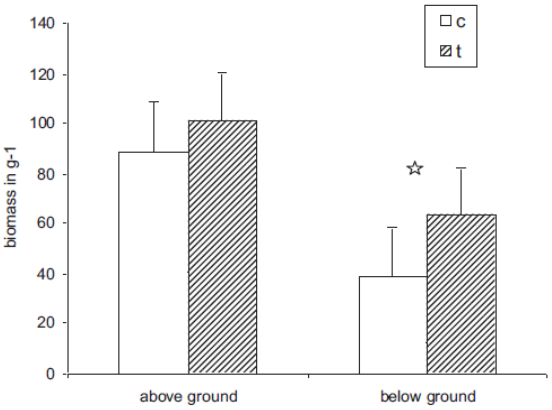

Fig. 2. Biomass of aboveground and belowground parts of *C. epigejos* in control plots (c) and insecticide-treated plots (t) in the field experiment. Star indicates a significant difference. Error bars indicate the standard error of the mean.

In the field experiment, insecticide treatment increased *C. epigeios* root biomass presumably because it reduced wireworm biomass. The insecticide treatment tended to increase *C. epigeios* aboveground biomass but the effect was not statistically significant perhaps because heterogeneous field conditions increased variances and because wireworms had other vulnerable food sources as well. That pesticide treatment increased the abundance of other plant species typical of initial succession stages shows that wireworms influence the whole plant community. *Hieracium pilloselloides* has shown significant increase in coverage in insecticide treatment, which might be explained by the fact, that its thick, fleshy roots are a favourite food source for wireworms. This is consistent with the hypothesis that root herbivores can accelerate succession by feeding preferentially on early-successional plants that lack defensive compounds (Brown and Gange 1989, 1992, De Deyn et al. 2003). The insecticide Dursban eliminates soil dwelling, root feeding insects, but has been reported to negatively influence fungivorous Collembola, which can result in better nutrient supply to plants due to enhanced growth of mycorrhizal fungi (Brown and Gange 1989). *C. epigejos* could potentially benefit from this effect, but the fact that the greater difference was in biomass of belowground parts indicates, that the major impact is that of wireworms. We found that wireworms have the potential to reduce biomass of *C. epigejos*, but further investigations are needed to examine whether they can remarkably affect the coverage of *C. epigejos* in ecosystems. Their influence on plants on spoil heaps can be modified by the harsh abiotic conditions of the unrecultivated spoil heaps e the tops of the mounds are exposed to severe draught and extreme temperatures, which can eliminate the wireworm population, and compact soil probably reduces migration of these soil animals (Blossey and Hunt-Joshi 2003, Brown and Gange 1990). On our experimental site *C. epigejos* grew in community with some other ruderal plant species, that serve as another food source for wireworms, which probably decreased herbivore pressure on the single species. As already mentioned, *C. epigejos* is an important ecosystem engineer which can by fast growth and changes in soil and litter environment outcompetes many other species (Massey et al. 2007, Rebele and Lehmann 2001). On the contrary its reduction results in increase of plant species diversity and in increase of coverage of plants with more palatable litter (Massey et al. 2007, Rebele and Lehmann 2001, Somodi et al. 2008), which then induces changes in the soil and the whole community changes into a more diverse one. Alternatively, wireworms and other root herbivores can sometimes decrease the diversity of plant communities, probably by feeding on seeds and seedlings of plants (Brown and Gange 1989, Chaton et al. 2008), which has not been noticed in this study. This might be caused by the character of the post-mining soils that might restrict wireworm foraging for food sources, which is different from sites where the other studies were carried out (Brown and Gange 1989, Chaton et al. 2008) .

Conclusions

The results from both experiments indicate that wireworms can reduce the growth and competitive ability of *C. epigejos*. The reduction of *C. epigejos* coverage would affect the rest of the plant community by promoting growth of other plant species (Ryser et al. 1996, Van der Putten et al. 2003). Further investigation, such as insecticide treatment on experimental plots for a couple of years, may be required to determine whether wireworms can reduce the coverage of *C. epigejos* in spoil heaps and other disturbed areas and how such herbivory affects succession over the long term.

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Summary and conclusions

Primary succession on Sokolov spoil heaps is a complex process that is mainly directed by the initial inputs – substrate quality, climate and microclimate and species pool, but modified by interactions of plants, animals and soil. Amongst soil animals, earthworms are a group that can radically affect soil conditions (Lavelle et al. 1997, Bohlen et al. 2004) and seems to play an important role in ecosystem development on Sokolov spoil heaps (Frouz et al. 2006, 2008, Roubíčková et al., 2009). Our experiments support the hypothesis that changes in soil caused by earthworm activity have different effect on different plant species and that these changes gradually accumulate through time (Roubíčková et al., 2009). Earthworms directly negatively affect germination of plants with small seeds (The book chapter) by consumption of seeds, which corresponds with results obtained by other authors (i.e. Thompson et al. 1993, Willems and Huijsmans 1994, Milcu et al. 2006). This may contribute to the general trend of growing size of plant seeds during succession (Grime 1979). Indirectly earthworms affect plant growth via improving the substrate quality followed by higher nutrient availability (Thompson et al. 1993, Scullion and Malik 2000, Wurst 2004, Roubíčková et al. 2009). Due to different life strategies of early- and late-successional plant species, earthworm activity seems to positively affect mainly late-successional plant species (Roubíčková et al. 2009).

These results confirm the hypothesis that earthworms can remarkably influence vegetation succession on spoil heaps and indicate that the findings of Frouz et al. (2008), about concurrent appearance of earthworms and some plant species typical for late-successional communities of meadows and forrests, aren’t purely coincidential, and stress the importance of the detritivore trophic level in ecosystem function.

On the other hand, establishment of favourable soil conditions by plant communities during succession is an important factor in earthworm distribution on the spoil heaps; earthworms showed a low survival on sites with sparse vegetation cover and thin litter layer, which means that their occurence in certain stages of succession isn‘t determined only by migration abilities or passive dispersal (Paper II). More field experiments are needed to test if earthworms could be used in directed succession management practices to speed up the natural rate of succession. Preliminary results from an experiment with introduction earthworms to a 20- year old, earthworm-free site indicate that colonization of this site from a single deposition of about 100 specimen of epigeic and 100 endogeic earthworms is slow and not very efficient.

Research on the effect of wireworms on performance of *C. epigejos* and its interaction with other plant species has confirmed the hypothesis that abundance of this plant can be reduced by a generalist root feeder (Roubíčková et al. 2012). This result could be utilized in ecosystem management, because *C. epigejos* is an undesired expansive species, known to decrease species numbers where dominant (Prach 1987, Somodi et al. 2008), although further research on wireworm ecology and its interaction with host plants in a long term is needed to establish whether introduction of these root herbivores could be used at a larger scale.

Wireworms as generalist root herbivores also have the potential to affect the whole plant community composition, which was documented in the field experiment and supported by results of other authors (Blossey and Hunt-Joshi 2003, Chaton et al. 2008, Roubíčková et al. 2012). The exact mechanisms of their effect and selectivity though are still unclear and need further investigations.

The present studies point out the importance of studying the above- and below-ground interactions to better understand the mechanisms of succession.

To conclude, major contribution of this thesis to better understanding of the role of soil fauna in succession are:

a) pointing out that plant community composition isn’t purely determined by the substrate quality, climate and competition between species; soil fauna can affect it as well

b) soil fauna affects plants through different mechanisms – modification of the soil conditions or by consumption of some parts of the plant, i.e. roots or seeds

c) different plant species respond differently to the activity of soil fauna; late successional plants usually benefit from the presence of soil macrofauna, whether it be earthworms or wireworms, whilst early successional plants are unaffected or affected negatively by soil fauna

d) plant community composition reversely influences the soil community composition, although presence of soil fauna can also be affected by their migration abilities

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