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Summary of the Ph.D. thesis

Development of soil nematode communities during primary and secondary succession

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Abstract

Biodiversity loss is one of the biggest threats of our era. Habitats with all the unique species linked with them disappear due to the landscape changes and various mankind activities, namely obtaining of resources. Open-cast mining and intensive agriculture are an example of human activities that vastly degrades soil and diversity of its fauna. Therefore, ecosystem restoration is a way how to mitigate those losses and conserve or even bring back unique habitats. Despite the fact that most of restoration projects aim at plant communities, interest of soil fauna communities restoration increased amongst scientists lately.

Nematodes serve as a very useful tool for a soil food web quality assessment. Their characteristics, such as various body length or different feeding habits makes them fit into almost all important ecological niches within the soil fauna group. This together with the fact that they are amongst first to colonize new habitats allows us to track changes in soil food web during primary or secondary succession. Moreover, using similar methodology and specific nematode indices developed over years enable comparison between different habitats and stages of succession.

Restoration of soils and soil processes represent serious challenge in many restoration projects. Various restoration practices has been used to speed soil development and related interactions with plants and ecosystem development. Introduction of soil, litter, hay or similar materials is often used. These introduced biotic material serve as a source of biota. These techniques are intended to support the development of soil and complex soil food web. However, empirical evidence proving this is rare.. Testing these approaches was major aim of my thesis.

In this thesis, three different habitats with different amount of introduced biotic material are presented. I explored the succession and success of nematodes to colonize new space in the terms of quantity (total abundances) and quality (proportion of trophic groups). First two manuscripts map first seven years of soil fauna succession of de novo heathlands in the Netherlands in different biotic as well as abiotic conditions. Soil material spreading onto the bare substrate boosted nematode colonization in the first four years. However, water regime of the heathlands was overall more important factor than addition of material or pH manipulation. The third manuscript is a case study from former limestone

quarry that has been afforested and later on enriched with various soil or litter amendments. Also in this study soil material affected nematode communities, however, this effect was mitigated by other factors such as already established plant and soil fauna communities or position of study sites. The very last manuscript presented in this thesis compares nematode community right after the introduction of a meadow soil blocks into an exposed substrate with the community sampled twenty-one years after at the same sites. Our results showed that despite the fact that quantitatively there were no significant differences between meadow soil and soil developed spontaneously from the overburden, even twenty one years and large blocks of undisturbed material were not enough to enable colonisation of meadow nematodes throughout the whole spoil heap.

This thesis brings closer look on the dynamics of colonization of various substrates by nematodes in different habitats. At the same time, assessment of application of different biotic materials into soil can provide valuable insight for future restoration projects.

1. Introduction

Humanity is facing many problems nowadays, loss of biodiversity as a result of change of landuse being one of them. Intensive agriculture changes physico-chemical properties of soil, which has a negative impact on soil fauna. Open cast mining destroys ecosystems and vastly modifies conditions of topsoil and its content by excavation, translocation and stockpiling. Restoration ecology knows processes to at least partly return lost ecosystems, however, restoration practitioners tend to focus on plant communities (Bakker et al., 2003) and do not pay attention to soil communities. Fortunately, this trend shifts with an increasing knowledge about belowground processes and importance of a healthy soil as precursor to a successful restoration (Farrell et al., 2020).

Intensive agriculture brings excessive amount of nutrients and other harmful substances into soil and therefore by leaking into water sources as well. Thus, restoration of valuable oligotrophic habitats from this land is merely impossible without huge and costly removal of topsoil by heavy machinery. Open cast mining destroys soil communities on several levels: complete removal of ecosystem from the landscape disrupts historical and ecological continuity of the place, heavy machinery compacts substrate and rest of the soil left in the mine, last but not least topsoil excavated from the mine is stockpiled or directly buried by substrate in spoil heaps, which both has large effect on soil fauna composition. The substrate has often extreme pH and may be contaminated by heavy metals or other toxic compound and this alters target soil ecosystem development (Bradshaw, 1997). However, spoil heaps with milder condition can provide mosaic patches important for occurrence of rare endangered species (Tropek et al., 2012).

Restoration of soil is important for returning of ecosystem services provided by soil into the landscape. One of these services is provision of shelter and food for soil organisms that are part of soil food web. Soil food web is crucial for nutrient cycling, sequestration of carbon, or formation of soil aggregates that, by the extent, affects plants growth. Common restoration practices involves an application of topsoil, which should be most beneficial in restoring target communities, as it contains whole soil communities. However, several obstacles have to be taken into consideration. When topsoil right from the mine is to be applied, usually it has to be transported and stored. Both is costly, time and space consuming, long storage also degrades soil communities inside the pile (Johnson et al., 1991). Topsoil for spreading can also be taken from target natural or semi-natural habitats, however, there is a risk in destruction of these (protected) habitats. Before the topsoil application, sometimes physical or chemical properties of substrate have to be improved by tillage, application of organic or inorganic fertilizers, liming, or acidification. Getting rid of excessive nutrients (in case of restoration of oligotrophic habitats) usually means removal of topsoil, while depth of removal depends on nutrients content (Frouz et al., 2009).

Soil trophic web is a fundamental part of terrestrial ecosystems and nematodes are a fundamental part of soil trophic webs. Their abundancy, presence in almost all ecosystems, and position in almost every level within the trophic food web makes them a great tool for an assessment of soil health (Boag and Yeates, 1998). Many nematode-based indices were developed over the years to assess maturity of soil food webs or progress of restoration efforts, e.g. Maturity index by Bongers (1990).

Restoration of degraded soils is supposed to be a base for ecosystem recovery if we want to halt the rapid loss of biodiversity. Some human activities produce bare substrates, therefore soils have to be built from scratch. However, processes driving substrate colonization by soil fauna are not yet fully understood and there is a lack of knowledge for optimization of restoration practice.

2. Aims of the study

This thesis deals with assessment of progression of colonisation of soil fauna into developed and undeveloped soils.

Main objectives are:

- assessment of success of soil fauna, namely nematodes, in colonisation of new substrates, with or without the help of soil transplant,
- assessment of establishment of soil fauna, namely nematodes, in the close and farther surroundings of transplanted soil,
- assessment of the effect of soil addition into already established soil in shifting nematode assemblages towards target communities,
- assessment of the effect of various environmental conditions on the establishment of soil fauna.

3. Material and methods

For easier comparison of all experiments we used similar protocols for sampling and handling of soil fauna:

Nematoda

Three Kopecki's rings of soil were taken and mixed together as a composite sample. 20-30 g of soil was then taken to modified Baerman funnels and extracted according to Háněl (1995) for 36 hours. Nematodes were then killed and fixed with hot formaldehyde and transported onto permanent slides. Nematodes were identified according to morphological characteristics into genera and then sorted into trophic groups according to Yeates et al. (1993) under the light microscope.

Nematode-based indices were computed using NINJA platform (Sieriebriennikov et al., 2014).

Mesofauna

Three Kopecki's rings of soil were taken and mixed together as a composite sample. The whole sample was placed in the Tullgren's apparatus for a week under the heat source. Mesofauna was then sorted and identified under dissection microscope.

Macrofauna

Three cores of the surface of 625 m^2 each were taken and mixed together. The soil was then exctracted in Tullgren's apparatus under a heat source for a weak and organisms were then identified under the dissection microscope.

Data handling

Statistical analyses were performed in Statistica software and multivariate analyses were performed in CANOCO software.

4. Results and discussion

Major results of the study are summarised in four published papers. The major idea of individual papers can be summarised as follows:

Publication 1: A.U. van der Bij, M.J. Weijters, R. Bobbink, J.A. Harris, M. Pawlett, K. Ritz, P. Benetková, J. Moradi, J. Frouz, R. van Diggelen, Facilitating ecosystem assembly: Plant-soil interactions as a restoration tool, Biological Conservation, Volume 220, 2018, Pages 272-279, https://doi.org/10.1016/j.biocon.2018.02.010.

Publication 2: P. Benetková, R. van Diggelen, L. Háněl, F. Vicentini, R. Moradi, M. Weijters, R. Bobbink, J. A. Harris, J. Frouz, Soil fauna development during heathland restoration from arable land: Role of soil modification and material transplant, Ecological Engineering, Volume 176, 2022, 106531, https://doi.org/10.1016/j.ecoleng.2021.106531.

Both published papers collected data mapping the effect of different biota introduction or pH manipulation in recreated heathland in Dwingelderveld national park, the Netherlands. These heathlands were created instead of former agricultural land in the centre of the national park and prior to the experiment, top 40 cm of soil was removed. The first publication was more focused on heather plant communities restoration in first three years after the experiment setup, however, nematode assemblages showed some significant differences between treatments as well. High numbers of omnivores were confirmatory sign of ongoing succession. Also total nematode densities were highest in the treatments with soil addition, and, at the same time, there was a big difference between wet and dry heathlands. Still, numbers were at least 15 times lower than in local reference heathlands.

The second publication aimed at the assessment of seven years of soil fauna colonisation in the very same experiment. Nematodes showed different development in wet and dry heathlands. Structure index (SI), which measures complexity of trophic structure in nematode assemblages, differed in experimental plots according to the water regime: lowest SI was in year 2013 and highest in 2017 in wet plots, whereas in dry plots SI was lowest in 2017 and highest in 2014. Square diagram combining Structure and Enrichment (amount of nutrition in the soil) indices (MS2 Fig 3) shows different course of development in time in experimental plots. Enrichment index below 50 at both sites means that substrate stripped from agricultural topsoil kept oligotrophic characteristics that are crucial for heathlands. At the same time, in case of wet site we can see developmental trajectory of communities, whereas dry sites are les distinguished and quite structured for the whole time, probably due to high proportion of omnivores and predators to lower proportion of bacterial feeders from the beginning of experiment .Whereas total numbers of nematodes were still significantly lower than in local reference heathlands, trophic groups showed a trend to similarity in relative abundance of trophic groups between experimental assemblages and assemblages at local reference sites. Particularly in wet heathlands, there were almost no significant differences between experimental and reference heathlands throughout the years, whereas in the dry heathlands the trophic structure was more differentiated, especially in the case of omnivores that were more than four times higher throughout the whole observation period than in the references. Together our results from both publications support the hypothesis that wet heathlands will develop faster due to the relatively stable conditions. Results from both studies suggest that soil addition promotes development in soil fauna communities, however, water content in soil probably plays more important role in heath habitats.

Publication 3: P. Benetková, L. Tichý, L. Háněl, J. Kukla, F. Vicentini, J. Frouz, The effect of soil and plant material transplants on vegetation and soil biota during forest restoration in a limestone quarry: A case study, Ecological Engineering, Volume 158, 2020, 106039, https://doi.org/10.1016/j.ecoleng.2020.106039.

This study of afforested quarry explored the ability of various biota amendments to shift the vegetation and soil biota towards the target habitat assemblages. Plant and soil material was added onto soil of 14-year-old forest. Total abundances of nematodes were slightly lower in plots with no addition in both fully and partially (only tree trunks covered) fenced treatments. However, we did not detect any significant differences between experimental plots and samples from reference forests. Plant feeding nematodes were higher in the plots with soil material, however, relative abundances as well as total densities of plant feeders at the reference sites was overall smaller. This experiment showed that soil addition can shift soil organisms towards the target communities, however, in this particular case there were other stronger factors affecting the succession, like maturity of soil communities at the site as well as in the close surroundings.

Publication 4: P. Benetková, L. Háněl, J. Frouz, Nematode Assemblages Development Twenty-One Years after the Introduction of Meadow Soil into Bare Post Mining Spoil Heap. *Diversity*. 2022; 14(7):567. https://doi.org/10.3390/d14070567.

This study assess the nematode assemblages in an experiment, where six large blocs of meadow soil were inserted into bare spoil substrate exposed after the landslide. Soil samples were taken in the blocs, in their close surroundings (2 m from the blocs) and in the distant part of the spoil heap (30 m from the blocs). Sampling was done in the beginning of the experiment and 21 years after the soil introduction. Mean abundance of nematodes was overall lower in the beginning than in the late phase. In the same time frame? the highest abundance was measured in the close surroundings of the blocks, then in the transplanted soil and the lowest abundances were in the distant spoil substrate. There were differences in trophic groups as well: for example, predators decreased in the initial phase with the distance from transported blocks, however, they increase in the late phase along the same gradient. Notable differences were also in the plant feeding nematodes. Whilst they were highest in the blocks from the beginning, they increased rapidly in the close surroundings in the late phase. Ongoing succession from the beginning was displayed by the two most abundant genera from the trophic group of omnivores: *Aporcelaimellus* being present in all samples and *Ecumenicus*, abundant in spoil heaps and completely missing in the transplanted blocks. As I mentioned before, plant feeders were dominant in the late phase at all three sites, therefore *Helicotylenchus* was also the most abundant nematode genus in the blocks as well as in the spoil heap. There was also a change in specific nematode indices showing complexity of soil food web. With the time going they decreased in the blocks (indication of near-climax stage), but remained the same or increased in the spoil heap, proving continuity of succession in these sites. In this experiment, transplanted blocks did serve as an important refugium and inoculum of nematodes and enabled their spread into the spoil heap substrate.

5. Conclusions

In all conducted experiments soil transplant lead to faster establishment of soil fauna, namely nematode communities in transplanted soil, however, in most cases the established community had lower density that in the target ecosystem. The outcome is context dependent and is affected by substrate quality, substrate modification and natural variation. The effect of vegetation transplant was less conclusive. The effect of soil transplant in close vicinity of transplanted soil, however, was less pronounced and mostly not significant, this suggest that transplanted soil has its role not only to overcome migration barrier for soil biota but primarily to form islands od suitable soil condition. Initial migration to the substrate can be fast but further development of communities is probably limited by adverse environmental conditions of surrounding ecosystem. To conclude, this thesis provides a further insight into mechanisms of primary and secondary succession of soil fauna, with a focus on nematodes. Testing the influence of various amendments can serve as a knowledge for restoration practitioners for effective planning of further restoration projects.

6. References

Bakker, J.D., Wilson, S.D., Christian, J.M., Li, X., Ambrose, L.G., Waddington, J., 2003. Contingency of grassland restoration on year, site, ad competition from introduced grasses. Ecol. Appl. 13, 137–153.

Boag, B., Yeates, G.W., 1998. Soil nematode biodiversity in terrestrial ecosystems. Biodivers. Conserv. 7, 617–630.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19.

Bradshaw, A.D., 1997. Restoration of mined lands - using natural processes. Ecol. Eng. 8, 255–269.

Farrell, H.L., Léger, A., Breed, M.F., Gornish, E.S., 2020. Restoration, soil organisms, and soil processes: emerging approaches. Restor. Ecol. 28, S307–S310. https://doi.org/10.1111/rec.13237

Frouz, J., van Diggelen, R., Pižl, V., Starý, J., Háněl, L., Tajovský, K., Kalčík, J., 2009. The effect of topsoil removal in restored heathland on soil fauna, topsoil microstructure, and cellulose decomposition: Implications for ecosystem restoration. Biodivers. Conserv. 18, 3963–3978. https://doi.org/10.1007/s10531-009-9692-5

Háněl, L., 1995. Secondary successional stages of soil nematodes in cambisols of South Bohemia. Nematologica 41, 197–218.

Johnson, D.B., Williamson, J.C., Bailey, A.J., 1991. Microbiology of soils at opencast coal sites. I. Short-and long-term transformations in stockpiled soils. J. Soil Sci. 42, 1–8. https://doi.org/10.1111/j.1365-2389.1991.tb00085.x

Sieriebriennikov, B., Ferris, H., de Goede, R.G.M., 2014. NINJA: An automated calculation system for nematode-based biological monitoring. Eur. J. Soil Biol. 61, 90–93. https://doi.org/10.1016/j.ejsobi.2014.02.004

Tropek, R., Kadlec, T., Hejda, M., Kocarek, P., Skuhrovec, J., Malenovsky, I., Vodka, S., Spitzer, L., Banar, P., Konvicka, M., 2012. Technical reclamations are wasting the conservation potential of post-mining sites. A case study of black coal spoil dumps. Ecol. Eng. 43, 13–18. https://doi.org/10.1016/j.ecoleng.2011.10.010

Yeates, G.W., Bongers, T., de Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding Habits in Soil Nematode Families and Genera - An Outline for Soil Ecologists. J. Nematol. 25, 315–331.