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Köln, 25.08.2019

Referee's evaluation of the PhD thesis
"**Importance of ecological stoichiometry in soil development**"
submitted by Ing. Hana Veselá

The trajectories of succession in post-mining sites can be strongly influenced by the composition of the vegetation and the activities of soil fauna, which control decomposition and the recycling of growth-limiting nutrients for plants. We have however, still an uncomplete understanding of factors driving succession on novel soils of reclaimed sites. The availability and limitation of essential elements such as nitrogen and phosphorus is a major determinant of soil fertility and primary production, and potentially can feed back on soil carbon dynamics and plant succession. Ecological stoichiometry is based on the idea that carbon (C), nitrogen (N) and phosphorus (P) are the major elements in all organisms, and constrain their physiology and growth if not available to a sufficient extent, or in unbalanced ratios. Ecological stoichiometry thus is able to integrate very different levels of biological organization and may provide a valuable and novel theoretical framework to better understand the feedbacks of of plant species and soil fauna on nutrient dynamics during succession in post-mining sites.
Considering this theoretical framework, Hana Veselá investigated in her thesis how different plant species and soil fauna influence the ecological stoichiometry of post-mining sites. Her focus was on sites reclaimed with alder, a nitrogen-fixing tree species and on unreclaimed sites, where succession is retarded by *Calamagrostis epigeios*, a grass species with a peculiar influence on nutrient availability in soil.

The PhD thesis of Mrs Veselá consists of a conclusive abstract which sums up the study, a detailed introduction into the theory of ecological stoichiometry, and the research questions addressed. The thesis is based on three subsequent scientific publications in well respected peer-reviewed journals and two scientific manuscripts ready for submission to peer-reviewed journals. A summary with a general discussion of the results obtained in these studies, and general conclusions complete the thesis.

In the first publication Ms Veselá investigated the influence of different tree species on soil formation in terms of ecological stoichiometry. A number of earlier studies reported a preference of earthworms for low C/N ratio litter. Ms Veselá and coworkers significantly expand this knowledge by showing that soil

formation on post-mining sites by the incorporation of litter carbon into the mineral soil A horizon by earthworms correlates with litter C/N stoichiometry of the dominant tree species. Thus soil formation is a function composed of two key elements, tree identity in the succession and presence of earthworm macrofauna. This finding is of great importance for understanding and managing the successional trajectories for soil formation after land reclamation.

The second publication represents a detailed investigation of the life cycle and ecological function of a key-stone member of the soil fauna, the bibionid dipteran *Penthetria holosericea*, in alder forests on reclaimed mining sites in Cesky Krumlov. Soil ecology has a traditional focus on the roles of earthworms and millipedes as major consumers of tree litter in forests. Only a handfull of studies to date investigated the roles of bibionid larvae on soil formation. In an extensive study, Ms Veselá not only provides a very detailed account of the leife cycle of *Penthetria holosericea*, but provides clear evidence that this species with peak densities of > 1000 ind/m² and an annual consumption of at least 40% of the litter layer must be considered as a key player for litter transformation in the alder forests. Surprisingly, the development of the larvae appeared idependent on stoichiometric changes of litter food quality through the year. An important result that calls for further investigations in the future.

Of all subsequent manuscripts, Ms Veselá is first author.
In the third publication Ms Veselá expanded her research to unreclaimed mining areas in order to investigate why the dominant grass species *Calamagrostis epigeios* often retards plant succession on these sites. The study includes a complex experimental design in order to test a hypothesis for a stoichiometric basis for the effects of *C. epigeios* on the neighbouring vegetation. Her results reveal a novel mechanism, by which after senescence, nutrient leachig of the standing biomass of the grass results in grass litter of poor quality, which subsequently is highly efficient in reducing nitrogen losses from soil during the critical winter period, when N losses are usually high. With this strategy, the grass gradually impoverishes the surrounding soil from nutrients for its competitors and maintains its dominance, thereby retarding plant succession.

The fourth study is still presented in manuscript form, although ready to be submitted to a peer-reviewed scientific journal at any time. This study compares nutrient cycling on reclaimed mining sites with alder plantations vs. natural successional forests of unreclaimed mining sites. In extension of the first and second publication, this mansucript gives a whole stoichiometric nutrient balance of alder and natural successional forests. This study shows that the ability of alder to fix atmospheric nitrogen speeds up nutrient cycling, but despite the quick formation of a fertile A-horizon by soil fauna activity, it leads to stoichiometric imbalances with significant N-losses in form of nitrate leaching. Natural successional forests as a whole were surprisingly more efficient in retaining nutrients in the system.

Also the fifth study is presented in manuscript form. It continuous the investigations of reclaimed mining sites with alder plantations in comparison to natural successional forests of unreclaimed mining sites, but the focus is on seasonal variations on chemical and stoichiometric leaf traits between individual tree species of reclaimed and unreclaimed sites.

Overall, the work presented by Mrs Hana Veselá provides new and deep mechanistic insights from a novel stoichiometric perspective into the interactions between vegetation and its feedbacks on soil formation of reclaimed and unreclaimed mining sites.

Without reservation I consider the thesis suitable for the defense by Mrs Veselá. The quality of Mrs Veselás thesis clearly fulfills the criteria necessary for obtaining a Ph.D. degree by the candidate.

Sincerely,

(Prof. Dr. Michael Bonkowski)