



Centrum biológie rastlín a biodiverzity

SLOVENSKÁ AKADEMIA VIED



Mgr. Miroslav Cabon, Ph.D., Laboratórium Molekulárnej Ekológie a Mykológie, Centrum Biológie Rastlín a Biodiverzity, Slovenská Akadémia Vied, Dúbravská cesta 9, 845 23 Bratislava miroslav.cabon@savba.sk, tel. +421 948 422 577

V Bratislave
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Review of PhD. thesis: Sujun Balami: Fungi at land use gradient in temperate region of central Nepal

Studies of effective land use and land management are tightly connected with topics of global food security and as such, they are nowadays highly desirable. Presented thesis focuses on effects of land abandonment, succession and forest restoration on soil fungal communities. Such effects are well-known in case of aboveground vegetation, however it's impact on belowground soil communities needs yet to be clarified. Sujun Balami accepted the challenge and he explored the topic combining traditional methods such as root and fruitbodies sampling with modern metabarcoding approach focusing on both general and mycorrhizal fungal community composition. Moreover, he established the main area of interest in the highly underexplored region of Nepal, which makes precise identification of the retrieved units/OTUs exceptionally challenging.

Thesis is well-structured, composed of traditional chapters (introduction, summary, bibliography) and four first-authored papers – 2 already published manuscripts and 2 drafts, submitted or prepared for submission. All those studies complement each other and together they provide detailed insight to the land management in Nepal.

Elaboration of the particular manuscripts in points

Chapter I - Soil fungal communities across land use types

First manuscript is a review of the state-of-the-art with subsequent metaanalysis of already published studies. Sujun proved that he is able to perform a good analysis of selected topic, summarise them in a comprehensive conclusion. Review also nicely pointed to the lack of global standards for soil sampling and conducting metabarcoding studies, which makes comparison with other studies quite challenging. In the study, I am missing more insight into how the summary of the studies was conducted. It raises a question, **whether you take into account the fact that sequencing of AM fungi is strongly biased by used primers and also that widely used ITS2 primers for Illumina sequencing favors more EcM fungi?**

In addition, I would avoid subjective terminology whether changes in the diversity are good or bad. Observed and commented changes are mostly connected with succession, thus returning to the climax state of the land. Rhetorically, we can ask whether it is bad or good?

organizačná zložka
Botanický ústav
Dúbravská cesta 9
845 23 Bratislava
tel.: +0421 2 59426111

Centrum biológie rastlín a biodiverzity SAV
Dúbravská cesta 9
845 23 Bratislava 4
tel.: +421 2 59426111
e-mail: botuinst@savba.sk
IČO: 00679089, DIČ: 2020831054

organizačná zložka
Ústav genetiky a biotechnológií rastlín
Akademická 2
P.O.Box 39A
950 07 Nitra 1
tel.: +421 37 6943409

Chapter II - Soil fungal communities in abandoned agricultural land has not yet moved towards the seminatural forest

This chapter represents a nicely conducted metabarcoding study, which focuses on the species composition of both AM and ECM fungi under different land changes. Study is polished to the smallest details and published in the reputable journal Forest Ecology and Management. Authors did the golden standard and also something more, thus I have no further comment here.

I have only one small question – **Did have all of the analysed samples approx. same land history (same fertilization, land management etc?). Do you expect that you will get the same results if you compare lands with different histories?**

Chapter III - Land use effect on the root-associated fungi of *Alnus nepalensis* and *Schima wallichii*

Once again, nicely conducted study with particular focus on unravelling mycorrhiza and its dynamic of two targeted host trees *Alnus nepalensis* and *Schima wallichii*. As such, it nicely complements previous study. I have only a few questions there.

At p. 77 you hypothesized that „after abandonment, the diversity of mycorrhizal (AM and ECM) fungi and total fungi would increase due to gradual changes in soil properties and a reduction in disturbance “. I am uncertain whether soil properties and disturbance play a major role in shaping EcM communities. **Would it not be possible that the major driver of shaping EcM communities is the presence of EcM hosts, which appear during succession?**

You also hypothesized *The community composition on abandoned land will be different from that of regenerated forests (used as a reference) due to differences in the tolerance of individual fungal species to disturbance, availability of propagules, and the effect of neighbouring plants*. Did you also take into the account succession of the fungal communities? Trees of the different ages are preliminary colonized by different fungal species. **Would it not be possible that community composition on AL will be at least partially different from RF not due to differences in the tolerance of fungal species to disturbance, but also due to the succession stage of the host tree?**

At p. 78 you classified Land use to the categories, one of them is regenerated forest. In the abstract, you are mentioning seminatural regenerated forest. What is the difference?

Chapter IV - Three new ectomycorrhizal fungi from central Nepal: *Alnicola alni-nepalensis*, *Cortinarius nepalensis*, and *Lactarius alni-nepalensis*

It is quite challenging to describe 3 species from three unrelated families within a single paper. I was concerned that due to limited pagination there would not be enough space to properly discuss and put all information in the right context. Regretfully, I must say that my concerns meet with reality. I found several mistakes in morphological measurements, lack of proper discussion and also

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some relevant references missing. Needless to say, I am more critical in this case especially because this is a draft before submission and I believe my comments will help to improve the overall quality of the manuscript.

Lactarius alni-nepalensis

Spores presented on figures do not appear to be globose as indicated by lower Q value. Also, you presented a minimum Q value 0.8 which appears not to be correct, since Q represents the ratio of length and width. It is more likely that you include to the measurement some improperly oriented spores, which also results in quite a wide Q value variation from 1.0 to 1.2. Also, the reticulum does not appear to be nearly complete. Figure 7e does not represent basidia. Those are shown in 7f, but you drew 3-spored basidium, despite the fact you are stating only 4-spored basidia.

In the discussion for Lactarius, you mentioned “*The most similar species L. atrii (ITS sequence similarity 95.42%)*”. Similarity of the species should not be based on BLAST similarity, while BLAST does not recognize phylogenetically informative positions.

Cortinarius nepalensis

Basal clamps are not shown on the drawings. Some of the pictures are uninformative and higher magnification (incl. oil immersion) should be used. Overall, oil-immersion should be used at least for high-precision measurements of the spores, but it is not mentioned in methods. This applies also for Lactarius. **Could you please specify, which magnification was used for the measurement of each element?**

For the phylogenetic placement of the Cortinarius, placement of the targeted species is not supported by molecular analysis. Since last year's division of the genus Cortinarius s.lat. into several genera (Liimatainen et al. 2022 – not mentioned in manuscript), it is quite important to show that described species is firmly placed within Cortinarius s.s. and thus generic name Cortinarius is correct. Lack of reference sequence should not be an excuse (as mentioned in discussion) and authors should rather build their own reference dataset.

Final remarks

Despite my little criticism and in accordance with formal requirements, I conclude that in the submitted dissertation S. Balami demonstrated his scientific skills and profound literature knowledge. The objectives mentioned at the beginning were clearly achieved. The results also have already found their way into scientific publications. Finally, I propose the acceptance of the dissertation with preliminary Grade A “excellent”.

Thank you for giving me the opportunity to review this thesis, I definitely enjoyed reading it

Mgr. Miroslav Caboň, PhD.

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