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Examiners report on the PhD thesis of Sujan Balami entitled
"Fungi at land use gradient in temperate region of central Nepal"

Dear Sir or Madam

This PhD thesis by Sujan Balami deals with land use change in Nepal. In this region, there are large gaps in knowledge about the many different land uses and their changes. It seems that people are abandoning areas and thus intensively used land such as agricultural land is gradually changing into forested areas. In this context, it is of interest to know how soil fungal communities change and whether these changes have consequences for the ecosystems, as fungi fulfill a variety of soil functions. Fungi are involved in many ecosystem processes, either directly through mutualistic or antagonistic symbioses with plants, or indirectly through litter decomposition and nutrient cycling.

The thesis consists of a "General Introduction", followed by four research papers ("Chapters I to IV") and a final "Summary". The first two research papers have already been published in international journals, the third research paper has been submitted and the fourth research paper is about to be submitted. All four papers are of high quality and meet the scientific standards for writing and publishing research results.

The "General Introduction" describes in detail how intensively used land goes through different stages: from abandoned arable land to annual grassland, then to areas with perennial plants, to areas with shrubs, to areas with young forests and finally to old forests. Fungi play a particularly important role, as they are mutualistic symbionts for crops, grasses, perennials, shrubs, and trees through arbuscular mycorrhizal fungi. However, some trees, such as *Alnus*, harbor ectomycorrhizal fungi, which is particularly

evident from the appearance of fungal fruiting bodies when ectomycorrhizal trees colonize the land.

In “Chapter I”, a total of 190 studies worldwide were reviewed and evaluated. A wide variety of fungal parameters were used to investigate the effects of land use change. In general, the results of the studies analyzed often showed a negative response of quantitative fungal parameters after a change in land use from less intensive to intensive management. In most cases, the land use change led to significant shifts in the composition of the fungal community. However, the current state of knowledge is not sufficient to conclude on general effects of land use on soil fungi, as the evaluated studies are fragmented and limited by the local context of land use change.

In “Chapter II”, it has been assessed whether soil fungi can affect the succession of abandoned agricultural lands. To achieve this goal, the authors analyzed soil fungal communities in agricultural lands, abandoned agricultural lands, and regenerating seminatural forests. Although there were significant differences observed in the fungal diversity among land use types, the fungal species compositions of abandoned lands, agricultural land, and seminatural forests were clearly differentiated in the ordination analysis. Especially ectomycorrhizal fungi associated with trees of the genus *Alnus*, such as *Russula* and *Lactifluus*, appeared on the long-term abandoned land and regenerating forests. However, fungal succession was not as straightforward as the change in vegetation.

In “Chapter III”, the diversity and species composition of arbuscular and ectomycorrhizal fungi including total fungi associated with fine roots of the trees *Alnus nepalensis* and *Schima wallichii* were studied. Both tree species are economically important and occurred in abandoned land and seminatural regenerated forest. The results did not show any differences in the diversity of arbuscular fungi and total fungi in either tree species among land use types. However, land use has a significant effect on species composition, which is more pronounced than tree species identity. The arbuscular mycorrhizal fungus *Acaulosporaceae* showed a lower relative abundance in short-term abandoned land than in other land types. In contrast, ectomycorrhizal fungi occurred where *Alnus* was present. In particular, the fungal genera *Alnicola*, *Tomentella* and *Russula* appeared preferentially in short-term abandoned areas, while *Cortinarius* sp. was found more frequently in rejuvenating forests.

In “Chapter IV”, three ectomycorrhizal fungi, *Alnicola alni-nepalensis, Cortinarius nepalensis* and *Lactarius alni-nepalensis*, found as fruiting bodies, were described as new to science. All three species grew under the canopy of *Alnus nepalensis* on abandoned agricultural land and in regenerated forests. A description of the macroscopic and microscopic features together with a careful and extensive genetic

analysis and a comparison with similar taxa confirmed the distinctness of these species.

Overall, this study demonstrated the impact of land use change on soil and root-associated fungal communities of abandoned agricultural land in the temperate region of central Nepal based on regional but also global data. The study has also shown the need to describe new fungal species and to study their ecology. With the description of three new species associated with *Alnus nepalensis*, this work has achieved a very high level of attention. The work has also shown that the combination of land use change and fungal taxonomy at the community and taxon level can provide deep insights into the ecological processes of ecosystems in regions that have not yet been well studied.

To conclude, I recommend this PhD thesis to be defended.

Yours sincerely

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Questions from I. Brunner

1)

Why was the tree genus *Alnus* used in this study? *Alnus* is not a typical ECM tree, as it is only associated with a limited number of ECM species. *Alnus* also harbours AM fungi and *Frankia* actinobacteria (N-fixers). Thus, both AM fungi and *Frankia* of *Alnus* would directly or indirectly influence soil status, e.g. soil fungal communities and soil N status, apart from the effects of ECM fungi of *Alnus*.

An alternative pioneer tree would be *Castanopsis*, which is associated with a large variety of ECM fungi. Why was this tree not chosen?

2)

In Chapter 2, the fungal genus *Lactifluus* was mentioned? In the other chapters 1, 3 and 4, only the genus *Lactarius* is mentioned. What is the difference between these two genera? And why *Lactifluus* appeared only in chapter 2?

3)

In Chapter 3, three different land use types with different ages (6 years; 15-20 years; 30-40 years) were studied on which the tree species *Alnus* and *Schima* grow. Consequently, the trees in the three different land use types also had different ages. It is therefore to be expected that the ECM fungi associated with *Alnus* are different due to fungal succession. Has this fact been sufficiently considered and discussed in this Chapter 3? The term "succession" only appears in the Summary and Introduction, but not in the Discussion or Conclusion.

In addition, I miss some typical fungal genera associated with *Alnus* in the fungal list, such as *Alpova* and *Paxillus*. What could be the reason for this?

4)

Chapter 4 deals with fungal communities associated with the genus *Alnus* worldwide. However, I miss here one of the first and most fundamental studies on subalpine *Alnus* forests: the study on subalpine forests in the Swiss National Park region written by Jules Favre (1960) in the region of the Swiss National Park? And furthermore, my own work on the fungal communities in the *Alnus* forests in Alaska has not been considered? Is there a specific reason why these studies were not considered? By considering them, the list of *Cortinarius* and *Lactarius* associated with *Alnus* could be supplemented by many more *Cortinarius* and *Lactarius* species.

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